Harvey I. Pass
Nicholas J. Vogelzang
Michele Carbone *Editors*

Malignant Mesothelioma

Advances in Pathogenesis, Diagnosis, and Translational Therapies

Springer

Malignant Mesothelioma

Malignant Mesothelioma

Advances in Pathogenesis, Diagnosis, and Translational Therapies

Harvey I. Pass, MD
Chief, Thoracic Oncology, Barbara Ann Karmanos Cancer Institute, Harper University Hospital; Professor of Surgery and Oncology, Wayne State University School of Medicine, Detroit, Michigan

Nicholas J. Vogelzang, MD
Director, Nevada Cancer Institute; Clinical Professor, Department of Internal Medicine, University of Nevada School of Medicine, Las Vegas, Nevada

Michele Carbone, MD, PhD
Professor of Pathology, Director, Thoracic Oncology, Cardinal Bernardin Cancer Center, Loyola University Chicago, Stritch School of Medicine, Maywood, Illinois

Editors

With 178 Illustrations

Springer

Harvey I. Pass, MD
Chief, Thoracic Oncology,
Barbara Ann Karmanos Cancer Institute,
Harper University Hospital;
Professor of Surgery and Oncology,
Wayne State University School of Medicine,
Detroit, MI 48201
USA

Michele Carbone, MD, PhD
Professor of Pathology,
Director, Thoracic Oncology,
Cardinal Bernardin Cancer Center,
Loyola University Chicago,
Stritch School of Medicine,
Maywood, IL 60153
USA

Nicholas J. Vogelzang, MD
Director, Nevada Cancer Institute;
Clinical Professor,
Department of Internal Medicine,
University of Nevada School of Medicine,
Las Vegas, NV 81935
USA

ISBN 10: 0-387-22949-3 Printed on acid-free paper.
ISBN 13: 978-0387-22949-2

Printed in the United States of America. (MVY)

9 8 7 6 5 4 3 2 1 SPIN 10941027

springeronline.com

Dedications are insights into the personal lives and motivations of the editors of a book. Because each of us has dedicated so many years of our professional and personal lives to studying and grappling with mesothelioma, we collectively decided to dedicate this book to the amazing and wonderful patients with mesothelioma and their families who have changed each of our lives for the better. Their humanity, compassion, humor, and courage during their unique and heroic battles are beacons that will forever illuminate the path forward.

Personally, we each dedicate the book to special people in our lives:

To Helen, Ally, and Eric Pass, who put up with Poppy becoming completely overwhelmed but still provide him with the love he always needs.

Harvey I. Pass, MD

To my father Reverend Nicholas Vogelzang who at age 85 continues to have intense curiosity, a keen sense of humor, love of family, and dedication to the welfare of others. I love you Dad.

Nicholas J. Vogelzang, MD

To my father, Carmine Carbone, Professor of Orthopedics and sixth generation physician in my family, who inspired and in a way forced me to become the seventh generation physician.

Michele Carbone, MD, PhD

Preface

Malignant mesothelioma remains one of the sentinel malignancies of oncology. It has a breathtakingly rapid natural history with a median survival of 6 to 8 months when untreated, is environmentally related, and has such economic and social impact that attorneys specialize in representing only mesothelioma patients. Expert witnesses devote full time to testifying, and governments are forced to consider not only the banning of the environmental agent but also a reappraisal of the whole tort system for compensation to injured victims. Furthermore, its presence in certain populations has changed the mindset of whole communities, such as Libby, Montana, Cappadocia, Turkey, Sarnia, and Ontario.

Because of its infrequent occurrence, malignant mesothelioma is considered an orphan disease and managed in an anecdotal fashion in most oncologic practices. Yet this disease has set new scientific paradigms—in the clinic, laboratory, and community.

This book has been assembled to correct an information "disconnect" about this orphan disease and to raise awareness among scientists everywhere about new concepts in the molecular genetics, epidemiology, and carcinogenesis of mesothelioma. We, as editors and authors, work to spread knowledge about mesothelioma and reverse the disproportionately low amount of NCI funding committed to the study of this cancer. Furthermore, we believe that study of this fascinating disease, while occurring in the context of litigation concerns, should proceed along the same paths that all science takes, following the trail of discovery. Legal issues should have no influence—but sadly often do have—on the direction taken by science and medicine.

Over the last ten years, data have accumulated indicating that mesothelioma is a cancer caused by the environmental carcinogens asbestos and erionite, which interact with genetic predisposition and viral infection during cardinogenesis. The outcome of these complex interactions determines who among exposed individuals will develop malignancy. Moreover, mesothelioma has become the ideal model to study how genetics and viral infection influence environmental

carcinogenesis, as well as to discover novel targets for early detection and therapy.

Few cancers have caused so much controversy as mesothelioma. For more than 40 years scientists have argued whether chrysotile asbestos does or does not cause mesothelioma. As if the chrysotile controversy was not enough, a new controversy developed in the field of mesothelioma when two of the editors of this book (HP and MC) reported that SV40, a DNA tumor virus that causes mesothelioma in animals, was present in some human mesotheliomas. Besides these important causality issues, conflict exists regarding the best surgical therapy for the disease and the interpretation of novel trials for mesothelioma. All these volatile issues, including the economic, legal, and most important of all, the scientific aspects, are addressed in various chapters in this book. We encourage the reader to not only digest these topics but to follow these controversies in mesothelioma prospectively as new data are introduced.

The proliferation of mesothelioma-specific knowledge has led to an increase in the number of global conferences devoted to mesothelioma, at which scientists present new and exciting findings. A sufficient quantity of mesothelioma-specific research now stands strong and is no longer the stepchild at meetings devoted to lung cancer or sarcoma. Clinicians and scientists alike are being identified as "mesothelioma experts," and their advice in preventing and detecting the disease early, as well as in the treatment of the disease, is being solicited not only by other physicians, but by a growing number of E-mails directly from patients and their families.

The editors envisioned a comprehensive text that described the controversies and facts in order to heighten awareness of the mesothelioma epidemic and to aid both clinicians and bench scientists in their efforts to either treat the disease or design new therapeutic options. The complexity of mesothelioma has only recently been realized, and this complexity demands that the disease "graduate" from being just another chapter in an oncology text. Therefore, this book is intended to be used as an authoritative guide by PhDs, primary care physicians, pulmonologists, medical oncologists, radiation oncologists, and surgical oncologists, as well as by fellows in training in these subspecialties. Moreover, because of the economics and legal impact of mesothelioma, this book will have a significant impact in courts of law.

This was truly an international effort, and the North American, European, Middle Eastern, and Australian perspectives on both the clinical and translational aspects of mesothelioma are represented. This fact, in itself, reinforces the global nature of this smoldering epidemic, and emphasizes that a reference source that can potentially be expanded in future editions should be launched at this time. The editors are grateful to all of the authors who took time from their incredibly busy schedules to contribute to this first effort. Their enthusiasm and patience in providing the most up-to-date information regarding their areas of expertise are reflected in their chapters, and the editors are convinced that their efforts will be rewarded with a newer

generation of oncologists and investigators who will approach mesothelioma with knowledge instead of apathy.

Finally, the editors wish to thank Springer for having the foresight to recognize the void in the literature regarding mesothelioma by publishing this book. When the publishing house was first approached about this project, there was never any hint of too small a market or population to endorse or support the project, and Springer has been a wholehearted working partner in this effort. Special thanks go to Beth Campbell, Stephanie Sakson, Barbara Chernow, Brian Drozda, and Laura Gillan diZerega, all of whom stood by this undertaking with unwavering support.

Harvey I. Pass, MD
Nicholas J. Vogelzang, MD
Michele Carbone, MD, PhD

Contents

Contributors

Steven M. Albelda, MD
Department of Medicine, University of Pennsylvania Medical Center, Philadelphia, PA, USA

H. Richard Alexander, MD
Surgical Metabolism Section, National Cancer Institute/National Institutes of Health, Bethesda, MD, USA

Shahriyour Andaz, MD, MBBS
Department of Surgery, Division of Thoracic Surgery, David Geffen School of Medicine at The University of California, Los Angeles, Los Angeles, CA, USA

Karen Antman, MD
Departments of Medicine and Pharmacology, Columbia University, College of Physicians and Surgeons, New York, NY, USA

Sinoula Apostolou, PhD
Human Genetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA

Samuel G. Armato III, PhD
Department of Radiology, Pritzker School of Medicine at The University of Chicago, Chicago, IL, USA

Paul Baas, MD, PhD
Department of Thoracic Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Binaifer R. Balsara, PhD
Human Genetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA

Izzetin Y. Baris
Güren Hospital, Ankara, Turkey

Maurizio Bocchetta, PhD
Department of Pathology, Loyola University Medical Center, Maywood, IL, USA

Raphael Bueno, MD
Division of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA, USA

Paola Cacciotti, PhD
Department of Medical Sciences, University of Piemonte Orientale, Novara, Italy

Robert B. Cameron, MD, FACS
Department of Surgery, Division of Thoracic Surgery, David Geffen School of Medicine at The University of California, Los Angeles, Los Angeles, CA, USA

Michele Carbone, MD, PhD
Cardinal Bernardin Cancer Center, Loyola University Chicago, Stritch School of Medicine, Maywood, IL, USA

Nancy M. Carroll, MD
Surgery Branch, National Cancer Institute/National Institutes of Health, Bethesda, MD, USA

Alfonso Catalano, MD
Institute of Experimental Pathology, University of Ancona, Ancona, Italy

Paolo G. Cerrano, MD, PhD
Department of Environmental Epidemiology, National Cancer Research Institute, Genoa, Italy

John A. Chabot, MD
Division of General Surgery, Department of Surgery, Columbia University Medical Center, New York, NY, USA

A. Philippe Chahinian, MD
Departments of Medicine and Oncological Sciences, Mount Sinai School of Medicine, New York, NY, USA

Katherine D. Crew, MD
Department of Oncology, Columbia University Medical Center, New York, NY USA

Charles L. Croteau, DO
Department of Radiology, Pritzker School of Medicine at The University of Chicago Chicago, IL, USA

Richard M. DeMay, MD
Director of Cytopathology, Department of Pathology, Pritzker School of Medicine at The University of Chicago, Chicago, IL, USA

A. Umran Dogan, PhD
Department of Geological Engineering, Ankara University, Ankara, Turkey

Meral Dogan, PhD
Department of Geological Engineering, Hacettepe University, Beytepe Campus, Ankara, Turkey

Dean A. Fennell
Department of Medical Oncology, St. Bartholomew's Hospital and Medical College, London, UK

Rosangela Filiberti, PhD
Department of Environmental Epidemiology, National Cancer Research Institute, Genoa, Italy

Susan Gross Fisher, PhD
Division of Epidemiology, Department of Community and Preventive Medicine, University of Rochester Medical Center, Rochester, NY, USA

Raja Flores, MD
Department of Thoracic Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Karen Fountain, MD
Mesothelioma Center, Columbia University College of Physicians and Surgeons, New York, NY, USA

Raoul Fresco, MD, PhD
Department of Pathology, Loyola University Medical Center, Maywood, IL, USA

Joseph S. Friedberg, MD
Division of Thoracic Surgery, Department of Surgery, Presbyterian Medical Center, Philadelphia, PA, USA

Giovanni Gaudino, PhD
Department of Medical Sciences, University of Piemonte Orientale, Novara, Italy

Adi Gazdar, MD
Department of Pathology, The University of Texas, Southwestern Medical Center at Dallas, Dallas, TX, USA

J. Bernard L. Gee, MD
Department of Medicine, Yale University School of Medicine, New Haven, CT, USA

Allen R. Gibbs, TD, MBChB, FRCPath
Department of Histopathology, Llandough Hospital, South Glamorgan, Wales, UK

Giovan Giacomo Giordano, MD, PhD
Institute of Pathology, Second University of Naples, Naples, Italy

Donald G. Guinee, Jr., MD
Department of Pathology, Virginia Mason Medical Center, Seattle, WA, USA

Stephen M. Hahn, MD
Department of Radiation Oncology, University of Pennsylvania Health System, Philadelphia, PA, USA

Philip Harber, MD
Department of Family Medicine, David Geffen School of Medicine at The University of California, Los Angeles, Los Angeles, CA, USA

Raffit Hassan, MD
Laboratory of Molecular Biology, National Cancer Institute/National Institutes of Health, Bethesda, MD, USA

Dan Hauzenberger, MD, PhD
Department of Clinical Immunology, Karolinska University Hospital at Huddinge, Stockholm, Sweden

Mary Hesdorffer, RN
Department of Medical Oncology, Columbia University Medical Center, New York, NY, USA

Michael T. Jaklitsch, MD
Lung Transplant Program, Division of Thoracic Surgery, Department of Surgery, Brigham and Women's Hospital, Boston, MA, USA

Pasi A. Jänne, MD, PhD
Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

Bharat Jasani, BSc (Hons.), PhD, MBChB, FRCPath
Department of Pathology, Cardiff University Hospital, Cardiff, Wales, UK

Elliot Kagan, MD, FRC Path
Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Larry Kaiser, MD
Department of Surgery, University of Pennsylvania Health System, Philadelphia, PA, USA

Agnes B. Kane, MD, PhD
Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA

Mary Louise Keohan, MD
Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Charles R. Key, MD, PhD
Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM, USA

Oliver S. Kim, MD
Department of Pathology, Advocate Lutheran General Hospital, Park Ridge, IL, USA

Julius Klominek, MD, PhD
Department of Lung Medicine, Karolinska University Hospital at Huddinge, Stockholm, Sweden

Robert A. Kratzke, MD
Department of Medicine, University of Minnesota; Division of Hematology, Oncology, and Transplant, Minneapolis VA Medical Center, Minneapolis, MN, USA

Thomas Krausz, MD
Director of Anatomic Pathology, Pritzker School of Medicine at The University of Chicago, Chicago, IL, USA

Joyce A. Lagnese, JD
Danaher, Lagnese and Neal, P.C., Hartford, CT, USA

Richard A. Lake
University Department of Medicine, Queen Elizabeth II Medical Centre, Nedlands, Australia

Heber MacMahon, MD
Department of Radiology, Pritzker School of Medicine at The University of Chicago, Chicago, IL, USA

Gian Pietro Marchetti, MD
Pulmonology Department, Spedali Civili Di Brescia, Brescia, Italy

Marcella Martinelli, PhD
Department of Pathology, The University of Vermont College of Medicine, Burlington, VT, USA

Alison McDonald, MD
University of London; Department of Occupational and Environmental Medicine, Brompton National Heart and Lung Institute, London, UK

J. Corbett McDonald, MD
University of London; Department of Occupational and Environmental Medicine, Brompton National Heart and Lung Institute, London, UK

Markku Miettinen, MD
Department of Soft Tissue Pathology, Armed Forces Institute of Pathology, Washington, DC, USA

Faheez Mohammed, MBChB, MRCS
The Washington Cancer Institute and Washington Hospital Center, Washington, DC, USA

Brooke T. Mossman, PhD
Department of Pathology, The University of Vermont College of Medicine, Burlington, VT, USA

Luciano Mutti, MD, PhD
Department of Medicine, Local Health Unit #11, Piedmont, Vercelli, Italy

Oscar Nappi, MD
Institute of Anatomic Pathology, Histopathology and Diagnostic Cytology, Naples, Italy

Alfred I. Neugat, MD, PhD
Department of Medicine and Epidemiology, Columbia University Medical Center, New York, NY, USA

Anna K. Nowak, MBBS, FRACP
Department of Medicine, University of Western Australia, Perth, Australia

Geoffrey R. Oxnard
Department of Radiology, Pritzker School of Medicine at The University of Chicago, Chicago, IL, USA

Bruno Pasquotti, MD
Department of Surgical Oncology, Centro Riferimento Oncologico-IRCCS, Aviano, Italy

Harvey I. Pass, MD
Barbara Ann Karmanos Cancer Institute, Harper University Hospital, Department of Surgery and Oncology, Wayne State University School of Medicine, Detroit, MI, USA

Nick Pavlakis, BSc, MBBS, MMed (Clin. Epi.), FRACP
Department of Medical Oncology, Royal North Shore Hospital, St. Leonards, New South Wales, Australia

Roman Perez-Solar, MD
Department of Oncology, Montefiore Medical Center, Bronx, NY, USA

Betta Pier-Giacomo, MD
Department of Oncology, Pathology Unit, Azienda Sanitaria Ospedaliera, Alessandria, Italy

Bilal Piperdi, MD
Department of Medicine (Hemato-Oncology), University of Massachusetts Medical School, Fitchburg, MA, USA

Camillo Porta, MD
Department of Medical Oncology, IRCCS San Matteo University Hospital, Pavia, Italy

Amy Powers, MD
Department of Pathology and Laboratory Medicine, The Cleveland Clinic Foundation, Cleveland, OH, USA

Antonio Procopio, MD
Department of Molecular Pathology and Innovative Therapies, Polytechnic University of Marche, Ancona, Italy

Riccardo Puntoni, PhD
Department of Environmental Epidemiology, National Cancer Research Institute, Genoa, Italy

Marc Ramael, MD, PhD
Departments of Surgical Pathology and Molecular Pathology, Saint Elisabeth Hospital, Herentals, Belgium

Maria E. Ramos-Nino, PhD
Department of Pathology, The University of Vermont College of Medicine, Burlington, VT, USA

Bruce Robinson, MD, PhD
University Department of Medicine, Queen Elizabeth II Medical Centre, Nedlands, Australia

Evelio Rodriguez, MD
Department of Surgery, Thomas Jefferson University Hospital, Philadelphia, PA, USA

Kenneth Rosenzweig, MD
Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Katie Ross, MD
Department of Pathology, Cardiff University Hospital, Cardiff, Wales, UK

Valerie W. Rusch, MD
Department of Thoracic Surgery, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Umberto Saffiotti, MD
Center for Cancer Research, National Cancer Institute/National Institutes of Health, Bethesda, MD, USA

Luca Scapoli, PhD
Department of Pathology, The University of Vermont College of Medicine, Burlington, VT, USA

Dong M. Shin
Division of Hematology and Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

Dorsett D. Smith, MD
Division of Respiratory Diseases and Critical Care, University of Washington Medical Center, Seattle, WA, USA

Ryan P. Smith, MD
Department of Radiation Oncology, University of Pittsburgh Cancer Center, Pittsburgh, PA, USA

Jeremy P.C. Steele, MD
Mesothelioma Unit, St. Bartholomew's Hospital and Medical College, London, UK

Daniel H. Sterman, MD
Pulmonary, Allergy, and Critical Care Division, University of Pennsylvania Health System, Philadelphia, PA, USA

Luigi Strizzi, MD, PhD
Department of Oncology and Neurosciences, G. D'Annunzio University, Chieti, Italy

David J. Sugarbaker, MD
Division of Thoracic Surgery, Department of Surgery, Harvard Medical School; Brigham and Women's Hospital, Boston, MA, USA

Paul H. Sugarbaker, MD
Program in Peritoneal Surface Malignancy, The Washington Cancer Institute, Washington Hospital Center, Washington, DC, USA

Gian Franco Tassi, MD
Department of Pulmonology, Spedali Civili Di Brescia, Brescia, Italy

Robert N. Taub, MD, PhD
Mesothelioma Center, Professor of Clinical Medicine, Department of Medicine, Columbia University Medical Center, New York, NY, USA

Joseph R. Testa, PhD
Human Genetics Program, Senior Member, Population Science, Department of Population Science, Fox Chase Cancer Center, Philadelphia, PA, USA

William D. Travis, MD
Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology, Washington, DC, USA

Eric Vallieres, MD
Lung Cancer Program, Department of Surgery, Swedish Cancer Institute, Seattle, WA, USA

Claire Verschragen, MD
Cancer Research and Treatment Center, University of New Mexico School of Medicine, Albuquerque, NM, USA

Nicholas J. Vogelzang, MD
Nevada Cancer Institute, Department of Internal Medicine, University of Nevada School of Medicine, Las Vegas, NV, USA

Jennifer A. Wagmiller, MD
Department of Medicine, University of Rochester Medical Center, Rochester, NY, USA

Anil Wali, PhD
Departments of Surgery and Pathology, Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Daniel Wiener, MD
Department of General Surgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

1

The History of Mesothelioma

Dorsett D. Smith

The story of the discovery of this rare tumor and of the subsequent controversies that arose about its causation by specific forms of commercial asbestos is long and complex. It could fill an entire book. This chapter focuses on the early history of the discovery, from 1767 to 1900; on the histologic controversies, from 1900 to 1942; and on the diagnostic controversies and the role of asbestos, from 1943 to 1973 (Table 1.1). The period from 1972 through the 1980s and 1990s could be characterized by advances in the industrial hygiene assessment of exposures, case-control studies, and other major epidemiologic studies concerning health effects in asbestos end-product users, paraoccupational exposures, household exposures, school and building exposures, and the role of specific asbestos fiber types, fiber characteristics, and lung fiber burden analysis. The 1970s to 1990s was also the period when the role of environmental exposure to erionite, tremolite, and ceramic fibers was discovered, and molecular and cellular biology focused on the characteristics of fiber carcinogenicity. In the final period, from the late 1990s to the present, the focus has been on the viral contribution to pathogenesis such as SV40 and human genetics and treatment strategies. The history of the discoveries after 1973 is covered by other authors in other chapters in this book.

Early Discovery, 1767 to 1900

The history of the term *mesothelioma* has entailed more than 100 years of controversy. The earliest mention of a possible tumor of the chest wall was by Joseph Lieutaud (1), generally regarded as the founder of pathologic anatomy in France according to Wolf (2), as quoted by Robertson (3). Lieutaud published a study of 3000 autopsies, among which were two cases of "pleural tumors." The published account mentions a boy who suffered from marked dyspnea following trauma, who at postmortem showed fleshy masses adherent to the pleura and the ribs. Laennec (4) in 1819 is also said by Robertson to have suggested that there was an entity of primary malignancy of the pleura based on

Table 1.1. Important historical events between 1767 and 1972

Year	Researcher	Event
1767	Lietaud	Report of first possible case of pleural mesothelioma
1854	von Rokitansky	First pathologic description of peritoneal mesothelioma
1870	Wagner	First pathologic description of pleural mesothelioma
1890	Biggs	First American case
1920	Du Bray, Rosson	First use of the term *mesothelioma*
1924	Robertson	Best review of literature up to that time
1942	Stout, Murray	Further evidence on histogenesis
1953	Weiss	Association with pleural mesothelioma made in Germany
1954	Leichner	Association of asbestosis with peritoneal mesothelioma
1957	Godwin	Clear pathologic criteria for pleural mesothelioma
1960	Winslow, Taylor	Clear pathologic criteria for peritoneal mesothelioma
1960	Wagner	Mesothelioma associated with northwest Cape crocidolite
1964	Enticknap, Smither	Association of asbestos and peritoneal mesothelioma
1965	Selikoff	New York Academy of Science Symposium, report on U.S. insulators
1969	Wagner	Animal model further perfected
1972	Stanton, Wrench	Stanton hypothesis on the importance of fiber size/length

the epithelial nature of these pleural cells. In 1843, von Rokitansky (5) actively opposed the idea of primary cancer of the pleura, and stated that pleural cancer always was secondary to a primary focus elsewhere. Ironically von Rokitansky in 1854 described what were called primary tumors of the peritoneum, which he called "colloid cancer" and most likely were peritoneal mesotheliomas. This strong opinion on the metastatic origin of pleural mesotheliomas by the German pathologists was to remain the opinion of many pathologists up through the mid-20th century as stated by Willis (6). There were further reports in the early 19th century of what could be considered pleural-based cancers. It was Wagner in 1870 who first described a lesion, which he classified as "Das Tuberkelähnliche Lymphadenom." He felt this was a primary malignancy of the pleura in a 69-year-old woman in whom an epithelial-based malignancy was found. Wagner had described lymph channels filled with tumor. Schultz (7) in 1875 reexamined the preparations of Wagner and emphasized the neoplastic nature of the process and renamed it endothelial cancer. The tumor was thought to arise from the lymph vessels and was commonly called an endothelioma. This was not questioned until 1891, when Engelbach (8) first raised the question of whether these tumors arose from the endothelium of the lymph vessels or from the surrounding serosal surfaces.

During the late 19th century and early 20th century, there was general acceptance that some sarcomas arose from the pleura when there was no evidence of a primary elsewhere, and it was generally accepted that the only tumor that might be primary to the pleura or the subpleura was a primary sarcoma. This was generally the Italian view as summarized by De Renzi (9). In 1890 Biggs (10) was the first American to report two cases of "endothelioma of the pleura" at the New York Pathological Society. Primary fibrous sarcomas of the pleura were generally accepted as arising from the fibroblast but not the pleural tissue itself. The fact that the pleural lining was capable of producing tumors that were both epithelial and of connective tissue origin was first pointed out by Paltauf (11), Borst (12), and Kaufmann (13). By 1909 Patterson (14) found 96 cases in the literature and added two of his own. The disease occurred twice as frequently in men than in women, and the greatest number of cases was found in patients between the ages of 40 and 60 years.

Histologic Controversy, 1900 to 1942

Miller and Wynn (15) were the first to advance the opinion that a peritoneal neoplasm was able to present both epithelial and fibroblastic characteristics because of the embryologic relationship of these cells to the mesoderm. Later, Maximow (16) was able to demonstrate via tissue culture direct transitions from the mesothelioma cell to fibroblast.

In 1924 Robertson's (3) article on endothelioma of the pleura was probably the most thorough review of the literature that had been done up until that time. At the time of that publication, endotheliomas or primary pleural malignancies were certainly rare, in that Clarkson (17) in 1914 stated that out of 10,829 postmortem exams performed in Munich, Germany, there were only two cases of primary endothelioma of the pleura, although he could find records of only 41 cases. Later, Robertson quotes Keilty (18), who reviewed the records of the pathology department at the University of Pennsylvania and found nine cases of primary endothelioma of the pleura in 5000 postmortem examinations.

Bayne-Jones (19) described a 16-year-old boy with a pleural-based malignancy that Bayne-Jones thought was a primary neoplasm of the lining cells of the pleura and an epithelial tumor, which he described as a carcinoma of the pleura. Bayne-Jones thought this tumor was not an endothelioma or it did not arise from the endothelium of the lymphatics but from the mesothelial cells and therefore was an epithelial carcinoma. In 1920 Du Bray and Rosson (20) proposed the term *primary mesothelioma of the pleura*. They thought the term *pleural carcinoma* or *endothelioma* was not appropriate, but that the term *mesothelioma* was most appropriate. In 1921 Eastwood and Martin (21) agreed that the term should be *mesothelioma*. Zeckwer (22) also used the term *mesothelioma* in his report of 1928. The issue as to whether there was such a thing as a primary endothelial malignancy arising from the pleura was carefully discussed by Robertson (3) in his seminal paper, and he

rejected the idea that the epithelial tumors were primary tumors of the mesothelium; he thought that these tumors were most likely metastatic tumors of some other origin. He thought that only sarcomas could be classified as primary malignant tumors, and that all other types of growth were secondary tumors with implementations or metastasis from unrecognized, latent primary malignancies elsewhere.

In 1931 Paul Klemperer and Coleman Rabin (23) published a report of five cases from Mt. Sinai Hospital in New York City, including one case with both epithelial and mesenchymal characteristics. They thought that diffuse neoplasms of the pleura arose from the surface lining cells, the mesothelium, and should be designated mesothelioma as previously suggested by others.

In 1933 S. Roodhouse Gloyne (24) reviewed his series of asbestosis cases and stated, "Of the complications unrelated to the asbestosis the following have been noted: (a) abdominal carcinoma; (b) mitral stenosis; (c) cerebral hemorrhage, and (d) cholelithiasis. There has been one case of squamous carcinoma of the pleura. There is no evidence at the moment that this was in any way related to asbestosis." It is open to speculation as to whether these were the earliest cases of mesotheliomas in asbestos-exposed workers!

Ewing (25) in 1940 raised the question of the influence of chronic irritation or trauma and low grades of inflammation in causing connective tissue changes in the pleura, and wondered if some of the cases of pleural malignancy were connected with tuberculosis. Many of the previously reported cases had evidence of coexistent tuberculosis, in several attacks of pleurisy on the involved side. The trauma and chronic inflammation as a cause of pleural transformation were reviewed by Ewing (25). Ewing's comments were amplified by an excellent review of the literature by Andrea Saccone and Aaron Coblenz (26) from New York City in 1943. The authors were able to identify 41 cases in seven published series between 1910 and 1938 from a total of 46,000 autopsies or 0.09% mesotheliomas. They concluded from their review of the case reports that some of these tumors were misdiagnosed and were metastatic from other sites. Certainly the confusion in making the pathologic diagnosis would continue for many years. From 1960 to 1968 only one half of Canadian mesothelioma cases on death certificates could be confirmed by an expert panel (27).

Further support for the idea that these tumors arose from the mesothelium rather than from the endothelium was provided by Stout and Murray (28) of New York City in 1942. They used their studies on tissue cultures to support the idea that malignant cells arose primarily from the mesothelial cell. Their concept of histogenesis was so controversial at that time that their Department of Pathology chairman required them to publish a statement of his disbelief in their paper. Stout was later to become professor of pathology at Columbia University in New York City. He was able to accumulate pathologic material on 156 mesotheliomas between July 1919 and June 1964. This was the largest series from a single institution in the world as of 1964 and yet Stout (29) later commented that in retrospect he was unaware of a single case associated with asbestosis.

Further support for Stout's theory of histogenesis came from Canada in a paper by Postoloff (30) entitled "Mesothelioma of the Pleura," in which he concluded that, indeed, the mesothelioma is capable of transforming into both an epithelioid malignancy and a sarcomatous malignancy. He emphasized the importance of an osteoid matrix in the histologic features of mesothelioma. He also mentioned that his team found only seven mesotheliomas out of 7878 consecutive autopsies covering a 20-year period between 1923 and 1942.

By 1946 Arnold Piatt (31), a radiologist at the Newark Hospital, reviewed the radiologic aspects of primary mesothelioma or endothelioma of the pleura. By then over 200 authors had discussed and offered opinions on the entity, which at that time was called primary mesothelioma or endothelioma of the pleura. Piatt points out that it was a very difficult diagnostic problem for pathologists, who argued among themselves as to the type and histologic origin of the neoplasm. By then there were as many as 30 different terms used to describe this clinical entity, including *endothelioma*, *mesothelioma*, *endothelial carcinoma*, *pleural carcinoma*, *primary papillary endothelioma of the pleura*, *adenoendothelioma*, *sarcoendothelioma*, *pleural sarcoma*, *round cell sarcoma*, *spindle cell sarcoma*, *angiosarcoma*, *lipomyxosarcoma*, *giant cell sarcoma of the visceral pleura*, *sarcomatous malignancy of the pleura*, *malignant tumor of the pleura*, *mesothelial carcinoma*, *perithelioma*, *endothelioma*, *carcinomatodes*, *lymphangioendothelioma*, *fibroendotheliosis of the pleura*, *lymphangitis proliferans*, *pleuroma*, *abdominal colloid tumor*, and *tubercle-like lymphadenoma* (32).

Definition and Suspicion, 1943 to 1960

In the confusion about whether mesothelioma was truly a separate clinical entity, there were five different opinions as to the source of the tumor: (1) an aberrant nest of lung epithelium became malignant within the lining of the pleura; (2) the endothelial lining of the subpleural lymphatics was the source of the tumor, hence the name endothelioma; (3) the tumor arose from the pleural capillary endothelium or endothelial lining of the subpleural lymphatics, or both; (4) the tumor arose from the mesothelial lining of the pleura itself, or was a mesothelial-derived tumor or a mesothelioma; (5) those tumors of epithelial origin always arose from a primary tumor elsewhere that had metastasized to the pleura. These primary tumors could be so small that they were easily missed on a routine autopsy. A sarcoma was a primary from the subpleural connective tissue. It is because of the differences in opinion about the origin of the tumor that there was such a large number of terms used to describe the same process.

In this setting of confusion, early reports began to filter out that some patients with asbestosis developed an unusual form of pleural malignancy. The first report was by Wedler (33), who reported the results of 30 autopsies on asbestos workers in Germany. He excluded one case, and of the 29 remaining autopsies, four had bronchial cancers, and two others had a malignant pleural growth. He commented about his own

impression that the incidence of cancer, which was 20% for malignant tumors in this population, was much too high to be by chance, and that the lung cancer was due to the asbestos exposure. He reviewed all the known studies at that time, and pointed out that the first mention of a lung cancer associated with asbestosis was made in 1933 by Gloyne (34), who stated, "There has also been one case of squamous cancer of the pleura. There is no evidence at the moment this was in any way related to asbestosis." In 1935 Gloyne (35) was able to report two additional patients with lung cancer and asbestosis. Wedler did not discuss whether the pleural cancers he found were true mesotheliomas or were related to an underlying lung cancer; he simply reported these findings and called them pleural growths of epithelial origin. He stated that lung cancer was the most common complication encountered in cases of asbestosis.

While the report of Wedler was readily accepted in Germany, the information was generally ignored elsewhere. In retrospect, Harrington (36) stated, "Of particular interest is the apparent influence of politics, given that the earliest published accounts emanated from Nazi Germany, thus received less attention and credence than was their due. Furthermore, there was the skepticism—presumably natural rather than biased—on the part of many early scientific observers in both the United States and Britain." In 1947 a patient with a mesothelioma of the pleura and pericardium who worked with asbestos cutting insulation board was reported as chronic pulmonary congestion (CPC) by the Massachusetts General Hospital, but the association with the asbestos exposure was not made (37). In 1952 Cartier (38) reported in a scientific meeting via an abstract of a discussion of a paper by W.E. Smith seven cases of respiratory cancer in 4000 asbestos workers working in the Quebec chrysotile mining and milling industry, and included in the cohort were two cases of pleural mesothelioma. Cartier thought that since the two mesothelioma cases did not have asbestosis, causation from asbestos exposure could not be made. The details of these cases were never published.

A year later, in 1953, Weiss (39) added a third case to the two malignant tumors of the pleura described by Wedler, that of a man with asbestosis and pleural mesothelioma who had done insulation work in a naval dockyard from 1920 until 1935. Weiss believed that the association between asbestosis and pleural mesothelioma was strong, and therefore he recommended that the German government accept this as a work-related condition. Von Rokitansky (40) in 1854 described what were called primary tumors of the peritoneum, which he called "colloid cancer." While this tumor was mentioned in the English literature, first by Miller and Wynn (15) in 1908, the association between peritoneal tumors and possible asbestos exposure was not made until 1954 when another German, Leichner (41), described an autopsy done 2 years earlier on a 53-year-old man who worked in an asbestos factory primarily as a spinner. Leichner reported that the patient had asbestosis and tuberculosis, but had what appeared to be an incidental finding of a peritoneal mesothelioma. Leichner found evidence of asbestos fibers in the tumor, and felt that this peritoneal mesothelioma was

again work related. A short time later, in 1955, Bonser et al (42) reported 72 autopsies of patients with asbestosis in which four were found to have abdominal neoplasms consistent with a peritoneal mesothelioma, but the authors never made the association that these were asbestos-induced peritoneal mesotheliomas.

In 1956 Ackerman (43) wrote that it was the majority opinion that primary mesotheliomas were rare but do exist. A year later, in 1957, Godwin (44) wrote a very important paper that laid down strict diagnostic criteria for the diagnosis of pleural mesotheliomas. In 1958 Van der Schoot (45) reported two mesotheliomas in insulation workers.

In 1958 McCaughey (46) from Belfast, Ireland, reported 11 diffuse and two localized pleural mesotheliomas. He felt there was strong evidence to support the belief that diffuse pleural mesothelioma was a clinical entity in spite of opposition to this idea. He did not make the association in this study to asbestos exposure, but he would do so in retrospect a few years later (47). This paper was a response to an article published by Smart and Hinson (48) of the London Chest Hospital who reported 24 cases of pleural neoplasm and concluded that the occurrence of a true neoplasm of pleura could not really be denied, that the lesion is produced from known primaries, and that there was no need to postulate an origin from that site (49). In 1956 Eisenstadt (50) of Port Arthur, Texas, reported a patient who worked in a refinery who developed what appeared to be a malignant mesothelioma of the pleura. He pointed out that very experienced pathologists denied the existence of such a tumor, but he felt impelled to report the case anyway.

A good example of the confusion about what to do with the diagnosis of mesothelioma is the discussion of the condition by Sir Richard Doll (51) in his classic 1955 study of the association between lung cancer and asbestosis. In Table II of the article he describes 15 patients with asbestosis and some type of lung cancer, but only uses 11 of the 15 in his analysis. Two of the patients are recorded as having either an endothelioma of the pleura or epithelial carcinoma. Three additional patients with lung cancer were found, but they did not have asbestosis. The association between the asbestos exposure and the endothelioma of the pleura was not made, and, evidently, was excluded from this statistical analysis.

The seminal year for making the association between asbestos exposure and mesothelioma is 1960. The seminal paper is that by Wagner et al (52), entitled "Diffuse Pleural Mesothelioma and Asbestos Exposure in the Northwestern Cape Providence." The paper was very controversial because it described 33 cases of diffuse pleural mesothelioma with exposure to only one type of asbestos, so-called Cape Blue asbestos mined in the asbestos hills west of Kimberly in the northwest Cape Providence of South Africa. Wagner et al said the tumor was rarely seen elsewhere in South Africa. This means the tumor seemed to be rather specific to a certain geographic area and a specific type of crocidolite asbestos. The data were considered suspect by many pathologists, in that only four of the patients had full autopsies, the rest having had simple pleural biopsies that were recognized by many as being unreliable in making the diagnosis of mesothelioma. The other

problem was that previously reported patients had heavy industrial exposure and usually asbestosis, and the majority of Wagner et al's cohort did not have asbestosis or heavy industrial exposure. The general consensus at that time was that a true mesothelioma diagnosis could not be made unless there was a complete autopsy excluding some primary tumor elsewhere in the body that had metastasized to the pleura and unless there also was concomitant asbestosis. The initial response was muted, as so eloquently stated by Elliott McCaughey (53) because of "the lack of experimental animal evidence, rejection or lack of knowledge of science conducted outside of the United States, and reluctance of individual writers to change their minds." In an editorial written in South Africa in 1968, the relationship between crocidolite exposure and mesothelioma was still thought to be unproven (54).

In 1960 Eisenstadt and Wilson (55) published a paper describing two patients with pleural mesothelioma. The second patient had a long-term history of exposure to asbestos, and there were asbestos bodies in the lung biopsy specimen. The authors felt there was an association between the asbestos exposure and the subsequent development of this unusual pleural malignancy.

Association and Causation, 1960 to 1973

Also in 1960 Keal (56) reviewed the records of an English hospital and found 23 women with asbestosis. Four had carcinomatosis of the peritoneum without a known primary, one had ovarian cancer, and four others had peritoneal malignancy possibly of ovarian origin. The association with asbestosis is glaring, but the connection between asbestos exposure and peritoneal malignancy was not strongly suggested until 4 years later. Winslow and Taylor (57) published a series of 12 cases of peritoneal mesothelioma in 1960 and reviewed 13 previously reported cases found in the world literature. No association with asbestos exposure was mentioned in their paper. However, the association between asbestos exposure and diffuse abdominal tumors was established in the English literature by the paper of Enticknap and Smither (58) in 1964. Here again, the Germans made the association between asbestos exposure and this rare tumor earlier than other investigators. While attempts to define the tumor mesothelioma were made by earlier investigators such as Klemperer and Rabin (23) in 1931, there was no general agreement among pathologists that such an entity really existed. In 1957 Godwin (44) published strict criteria for the diagnosis of pleural mesotheliomas that placed the pathologic identification on a more firm scientific footing. It was not until 1960 that Winslow and Taylor did the same thing for peritoneal mesothelioma tumors. After Wagner's discovery of the association between Cape Blue crocidolite asbestos and the increased risk of mesothelioma in South Africa, the question arose as to whether this was a unique problem limited to South Africa or whether this was a problem occurring in the United States. The American Medical Association Council on Occupational Health (59) published an article on Pneumoconioses in the *Archives of*

Environmental Health in 1963, in which there is a section on asbestosis. The panel of experts concluded:

> The relationship between cancer of the lung and asbestosis constitutes a problem of great current interest. There is no doubt that the two diseases appear in the same lung. Whether that occurrence is one of mere coincidence, or of direct cause–effect, the relationship cannot be resolved on the basis of a single case. The total body of evidence favors a relationship, especially as it involves certain kinds of asbestos and possibly only those that contain specific chemical substances have the capacity to cause cancer. Attention is invited to experiences in the union of South Africa where pleural mesotheliomas have been discovered in appreciable numbers of persons exposed to the inhalation of crocidolite-amosite asbestos. Certainly detailed epidemiologic clinical and experimental studies are required for the ultimate resolution of the problem. [p. 37]

In 1962 Wagner (60) was able to produce mesothelial tumors of the pleura by direct implantation of asbestos dusts in laboratory animals. In 1963 Wagner reported at the 14th International Congress of Occupational Health on 120 cases of mesothelioma, but curiously less than one half of the patients directly worked with asbestos; they just lived in the area where there was environmental exposure. The question at that time was whether this was a localized group of mesothelioma patients or the forerunner of an international epidemic. This question was answered at the International Meeting on Biological Effects of Asbestos held at the New York Academy of Sciences in New York City in October 1964 but not published until December 31, 1965 (61). Reports at the New York meeting from Newhouse and Thompson in London, Elmes and Wade in Ireland, Jacob and Anspach in Germany, Hammond, Selikoff, and Churg in the United States, and Viliani and coworkers in Italy confirmed the global extent of the problem.

Selikoff et al (62) reported their working experience with the relationship between asbestos exposure and mesothelioma in the *New England Journal of Medicine* in 1965, further cementing the relationship between asbestos exposure and mesothelioma and raising the question of whether others types of asbestos might also cause mesotheliomas. The authors did not believe that American workers had significant exposure to crocidolite. They thought that the emergence of mesotheliomas in their cohort of asbestos insulators represented mainly exposure to chrysotile and amosite. All patients had heavy exposure and asbestosis. This article was followed by an editorial in the *New England Journal of Medicine* on March 18, 1965 (63). The editorial mentions that amosite, the third commercially used form of asbestos, has yet to be incriminated, but there are no definitive studies to date to confirm or deny such a connection.

Sluis-Cremer (64) of the Miner's Medical Bureau in Johannesburg, South Africa, gave a report to the New York Academy of Science in 1965. Sluis-Cremer in his discussion of mesotheliomas pointed out that his epidemiologic studies found mesotheliomas only in the northwest cape area of South Africa. The Transvaal amosite deposits had been actively developed for longer than this period, and he mentioned that

in the 1940s amosite was produced in three times the amount of the northwest crocidolite, yet no mesotheliomas were seen in the northwest area related to amosite exposure.

Of particular interest was the case control study of Newhouse and Thompson (65). They diagnosed 83 patients, 41 men and 43 women, with mesothelioma in association with a Cape Blue asbestos factory that opened in London in 1913. There were 27 peritoneal tumors and 56 pleural tumors. The factory used Cape crocidolite exclusively until 1926, when small amounts of amosite and chrysotile were added. Eighteen patients were employed in the asbestos factory and eight as insulators and laggers. An additional nine patients lived in the same house as an asbestos worker. Particularly distressing was the discovery of 36 patients with no known work or domestic exposure to asbestos. Eleven of these patients lived within one-half mile of the asbestos factory, suggesting neighborhood exposure. This case-control study and one by Elmes et al (66) were the first two case-control studies to confirm the earlier report of Wagner from South Africa. The concern about neighborhood exposure was echoed by Lieben and Pistawka (67) of the Pennsylvania Health Department, who reported that of 42 patients with mesothelioma only 20 had occupational exposure, eight lived within the vicinity of an asbestos plant, and three had family exposure.

The general medical community had believed that if asbestosis could be avoided by reducing exposure to friable asbestos, then asbestos-related malignancy would also be avoided. The early mesothelioma cases were generally heavily exposed in the early 1900s prior to the promulgation of dust control measures. Selikoff (68) stated in 1969, "I have yet to see a mesothelioma in a man who began work after 1930 or a case of lung cancer in an asbestos worker who had worked in that industry less than twenty years." However, the data of Wagner, Newhouse and Thompson, Lieben, and others challenged this. Thompson (69,70) reported in 1963 asbestos bodies in the lungs of people who were not asbestos workers and called it a modern urban hazard.

In 1968 Utidjian et al (71) reported that almost 100% of urban dwellers had asbestos bodies in their lungs. By 1970 Thompson's original observations were widely confirmed in Montreal, Milan, London, Newcastle, Glasgow, Belfast, Dresden, Pittsburgh, Miami, and New York (68). A paradigm shift had occurred; by 1970 it was generally accepted that low-level exposure to northwest Cape Blue crocidolite was capable of causing mesothelioma. By 1966 the importation of crocidolite asbestos had been voluntarily abandoned in England, and new asbestos regulations accepting the relationship between asbestos and mesothelioma were adopted in 1969. The standard for asbestos exposure in England was set at 0.2 f/mL (F is the degree of fineness of abrasive particles) for crocidolite or one-tenth the acceptable level of exposure to other forms of commercial asbestos at 2 f/mL (72). The question remained how much exposure was too much. The next 30 years would be focused on the role of other types of commercial asbestos and noncommercial asbestiform materials. Wagner and Berry (73) by 1969 had perfected an animal model that would help answer

many of these questions. Stanton and Wrench (74) had demonstrated in 1972 that the carcinogenic potential of asbestos was related to its diameter and length.

In 1965 Sir Bradford Hill (75) proposed criteria for assessing causation in chronic diseases. His seminal paper presented at the Royal Society of Medicine provided a systematic approach to evaluate the association between asbestos exposure and mesothelioma. The main requirements were strength of association, consistency of association, dose-response relationship, and biologic plausibility. The acceptance of new ideas moves slowly. Biologic plausibility of carcinogenesis is meant primarily to be based on animal and cell tissue modeling or by analogy to other human tumors. Unfortunately, biologic plausibility for many in the 1960s and early 1970s meant that if I can't understand it, I don't believe it.

The Doubters and the Role of Other Forms of Asbestos

It seems that every advance in science has its naysayers who are pulled along screaming and kicking. Garrett Schepers (76), then working as an American pathologist, was originally from South Africa. He related his own experience at the New York Academy of Sciences meeting:

> As a boy, I lived not far from Kuruman for a number of years. One could not imagine a more healthy territory. However, there is a particular irritating type of grass in the area (Klitsgras), whose seeds burrow into every garment they cling to, as these seeds are armed with fine barbs. Surely, when the wind blows, as often it does in Kuruman, some of these minute barbs may be inhaled. I wonder whether some of these fiber structures reported in the lungs of persons in that area may not represent reactions to grass barbules. I offer this Klitsgras theory of Kuruman mesotheliomatosis in order to clear the hurdle created by the discovery of this rare disease in such abundance in persons with such little meaningful exposure to asbestos. Perhaps the South African pathologists will now have their turn to make mincemeat of my theory. [p. 599]

Also at that meeting Schepers stated:

> My first impression is that there is now less certainty that asbestos inhalation is associated with pulmonary neoplasia than there was 10 or 20 years ago. Perhaps this is due to greatly reduced dust exposures. Asbestos may after all prove to be carcinogenic only in overwhelming dosage. Thus, the high prevalence of neoplasia which was reported several decades ago may be a function of the severity of exposure rather than an indication of high carcinogenic potency. I suspect that in the final analysis the carcinogenicity of asbestos will be rated as of low order. Perhaps carcinogenicity will prove to be a correlate of asbestosis rather than a specific biological function of the mineral asbestos. This may be the crux of the matter. In all cases of asbestos-associated lung cancer that I have personally studied (the number now exceeds two dozen), there invariably was well-established asbestosis. Not only was the asbestosis of marked degree in the areas where the cancer arose, but there generally was evidence from serial chest x-rays that asbestosis had been present in the lungs for a protracted period. [p. 595]

Ian Webster (77), who was J.C. Wagner's brother-in-law and a well-respected pulmonary pathologist, still stated that there were unsolved problems in the relationship between asbestos and malignancy in a paper he published in February 1973 in the *South African Medical Journal*. Webster remained skeptical as to why this previously rare tumor seemed to be found primarily only in direct relationship to crocidolite exposure. Webster suggested that some other factor, possibly mineral, must be present to explain the high incidence of mesothelioma in a very localized area of South Africa. He looked at exposure to asbestos and the association with 232 cases of pleural mesothelioma. Almost all the individuals had been exposed to Cape Blue asbestos and only two miners had been exposed to amosite as far as could be discerned. Thirty-two cases occurred where there was no evidence of any asbestos exposure, presumably having environmental exposure. There were only two cases related to exposure to amosite out of 232 confirmed cases of mesotheliomas. He stated, "Furthermore, it is difficult to conceive of amosite in the intermediate group of asbestos fibers causing malignancy, as suggested by Wagner et al when there are so few cases in the employees of the amosite mines." He goes on to say, "The production of amosite far exceeded that of Cape Blue asbestos. It is suggested that more attention should be paid to the determination of the nature of the substance of the Cape Blue areas and not in the Transvaal Blue, and apparently limited to the areas where amosite is mined." The same opinion had been offered earlier, in 1969, by George Wright (78), one of America's most respected investigators in occupational pulmonary disease, who in his review, "Asbestos and Health in 1969," stated, "That something other than, or in addition to, asbestos plays a role in mesothelioma formation seems inescapable." Wright accepted asbestos as a cause of mesothelioma but felt there was a "tolerable level of airborne asbestos fiber which does not cause an undue risk of development of mesothelioma." He later states that "the tolerable level was substantial."

The Role of Amosite and Chrysotile

In 1965 the polarization of expert opinion began between Irving Selikoff and his Mount Sinai co-investigators, and the British and largely European view on the role of chrysotile asbestos in causation of mesothelioma versus crocidolite asbestos. Selikoff et al (62) stated,

> American asbestos utilization differed to some extent from British and South African experience in at least one important respect. Crocidolite is a relative newcomer to the American asbestos-industry scheme. Thus American imports of crocidolite (none mined here) were less than 500 tons in 1935 and reached a level of only 20,000 tons even in 1962. In contrast, chrysotile, the type of asbestos fiber widely used in the American asbestos industry, was imported at a level of 165,000 tons in 1935 and 650,000 tons in 1962.

Later the authors stated, "If mesothelioma could be found with increased frequency in association with asbestos in this country, it would demonstrate that this tumor was another neoplastic hazard of

asbestos exposure in general and not limited to one area or to one type of asbestos." How much of the incidence of mesothelioma in America was due to amosite was unclear.

J.S. Harington (79) wrote a chapter on mesothelioma in the book *The Prevention of Cancer*, edited by Raven and Rowe in 1967. He stated, "The results of animal experimentation so far available suggests that crocidolite and chrysotile may be more active in inducing mesotheliomas than amosite. If the present trend is confirmed, substitution in mining and industry of amosite (for example, for the more dangerous types of asbestos where they cannot be safely used) may be a practical and important preventative measure."

In an unsigned editorial in *Lancet* published on March 5, 1966, the author stated, "A possible important clue to prevention was just uncovered by Wagner in South Africa, where after showing association between mesothelial tumors and exposure to the crocidolite form of asbestos, he and his colleagues were unable to find any tumors in those exposed only to the amosite or chrysotile-types of fiber. The position in South Africa remains the same, despite the continuing intensive search in the amosite and chrysotile mining areas." He goes on to say, "Mesothelioma tumors have been seen in a few individuals apparently exposed only to chrysotile in the United States and Canada, and other populations, either industrial or residential, exposed only to one type of fiber must now be investigated. This can be achieved only by international cooperation, because such exposures are almost entirely limited to those engaged in mining and milling of the fiber, which is done in the countries where the different types of asbestos are found." Selikoff et al (80) reviewed the results of a study of an asbestos insulation manufacturing plant in Paterson, New Jersey, and published their results in the *Archives of Environmental Health* in September 1972. In this paper the authors pointed out "few data exist concerning the comparative neoplastic potential in man of the several kinds of asbestos, and particularly there has been no evidence concerning whether amosite variety is carcinogenic. . . . Whether or not amosite is carcinogenic is of some practical importance. Because this variety of asbestos has not been reported to cause cancer, there has been a tendency in Great Britain, for example, to substitute it for other types of asbestos, especially crocidolite." Selikoff and coworkers went on to report an increased incidence of lung cancer and mesothelioma in this plant, where it was thought to be just a pure exposure to amosite asbestos. While it was generally accepted in the United States that pure amosite caused a high incidence of mesotheliomas and lung cancers, the paper by Selikoff and coworkers was not well accepted abroad. McCullagh (81) published a paper in the *Journal of the Society of Occupational Medicine* in 1980, "Amosite as a Cause of Lung Cancer and Mesothelioma in Humans." He pointed out that many of the Paterson, New Jersey, cohorts studied by Selikoff had previous exposure to asbestos. He felt that rather than one fiftieth of the group, it seemed more likely that one third of the group or 300 members of the Paterson cohort had been occupationally exposed to asbestos before entering the cohort. This is of import since crocidolite was being used in large quantities in

asbestos factories in the same area. Selikoff had felt that very little crocidolite had been used in the shipyards and in the United States, and therefore, if a mesothelioma developed, it was most likely related to amosite since there was very little crocidolite exposure. In fact the monthly trade journal *Asbestos* mentioned the use of crocidolite and amosite asbestos in July 1919.

John Harington and Neil McGlashan (82) reviewed the destination of South African exports of crocidolite and amosite asbestos as well as chrysotile from 1959 until 1993, and the studies indicate that the United States received a considerable amount of crocidolite asbestos up until 1992. This study and others have suggested there was more crocidolite asbestos used in the United States than had been previously recognized, and that the use of crocidolite asbestos is a major reason why there was an increased risk of mesothelioma.

J.C. Wagner recapitulated his overview of the association between blue asbestos and mesotheliomas in an article published in the *British Journal of Industrial Medicine* in 1991. He reviewed his story of the discovery of the association between asbestos and mesothelioma, and concluded that there was evidence that all types of commercial asbestos except anthophyllite may be responsible for a mesothelioma. He went on to state, "The risk is greatest with crocidolite, less with amosite, and apparently less with chrysotile. With amosite and chrysotile there appears to be a higher risk in the manufacturing than in mining and milling. . . . There is overwhelming evidence that crocidolite is a main fiber associated with mesotheliomas." This has primarily been the British view, and Raymond Parkes (83), in his classic book *Occupational Lung Disorders*, 2nd edition, published in 1982, stated about mesothelioma causation, "On present evidence its occurrence appears to be closely, but not uniquely, related to crocidolite alone or a mixture of fiber types in the distant past" (p. 276).

The most recent article relating to the historical crocidolite exposure issue in the United States was by Langer and Nolan (84) entitled, "Asbestos in the Lungs of Persons Exposed in the USA" and published in *Monaldi Archives of Chest Disease* in 1998. In their appendix of crocidolite consumption in the United States, they pointed out that blue asbestos for boiler and steam covering for locomotives was advertised in trade journals, such as *Engine*, as early as 1897. The data from the U.S. Department of Commerce reveals significant crocidolite importation in the 1920s and 1930s, and it included the spraying of crocidolite in the form of Limpet up until 1966. The paper goes on to mention that all three major fiber types were permanently used on ships, and crocidolite was extensively applied in warships in the United Kingdom. International investigators outside the United States have interpreted this to mean that crocidolite was also used aboard American ships, and if mesotheliomas occurred among American insulation workers who worked in military shipyards, this was indirect evidence of crocidolite exposure. The authors went on to state, "Still other investigators suggested that British ships were re-outfitted in U.S.A. ports during the war, and they have been the source of crocidolite exposure to

American shipyard workers. This most certainly occurred and citations in the literature support this."

It seems ironic that 100 years earlier in 1870 the distinct pathologic entity of pleural mesothelioma was postulated by one Wagner, and then greatly advanced 100 years later by another physician named Wagner whose contributions have propelled science into the next millennium.

Selikoff in hindsight also reviewed the literature on mesothelioma and stated, "During the 1950s there were several reports of deaths in asbestos workers caused by these diffuse tumors of the mesothelial surfaces. These isolated cases would have received little notice had it not been for the fact that the tumor has always been considered extraordinarily rare. It is no longer rare amongst asbestos workers. Indeed, it is so common a cause of death amongst them now. While still rare amongst individuals not known to be exposed to asbestos—it almost constituted tumor specific to asbestos exposure." Furthermore, writing in 1988, Dr. Selikoff and coworkers (85) stated, "Nevertheless only in the past 25 years has malignant mesothelioma been widely accepted as an independent diagnostic entity." These workers found 175 deaths from mesothelioma occurred among 2221 men who died between 1967 and 1976, and 181 more deaths in the next 8 years for a total of 356 deaths from mesothelioma out a total of 3500 deaths from all causes by 1984; 134 of these were pleural and 222 were peritoneal mesotheliomas.

The history of the early years of mesothelioma discovery are an example of how slowly the medical community accepts new discoveries. Acceptance was in part slowed by the lack of specific mesothelial cell markers such as are available today to assure proper diagnosis; experts disagreed among themselves as to the proper classification of these tumors. As the frequency of these tumors increased, pathologists made the diagnosis with more confidence and, as noted by Selikoff, there was general acceptance of not only the criteria for diagnosis but also the clear association with asbestos exposure by 1973. The role of specific fiber types would have to await the results of further studies, particularly lung fiber analysis by electron microscopy over the next 30 years.

References

1. Lieutaud J. Historia anatomico-medica, etc. Paris 1767;2:86.
2. Wolf. Die Lehre von der Krebskrank. 1911;2:834.
3. Robertson HE. Endothelioma of the pleura. J Cancer Res 1924;8:317–375.
4. Laennec R. Traite de l'auscultaton medicale. 1819;2:368.
5. Rokitansky C von. Lehrbuch der pathol. Anatomie. 1843;3.
6. Willis RA. Pathology of Tumors, 4th ed. London: Butterworth, 1967.
7. Schultz. Endothelcarcinoma. Arch Heilk 1876;17:1.
8. Engelbach. Ein Beitrag Zur Differential Diagnose Pleuritischer Exudate Und Neubildungen Der Pleur Im Anschluss Daren Ein Fall Von Endothelcarinom Der Pleur. Inaug-Diss., Freiburg, 1891.
9. De Renzi. La Riforma Med 1893;9(1):188.

10. Biggs H. Proc NY Pathol Soc 1890;119.
11. Paltauf R. Ueber Geschwülsten Der Glandula Carotica. Beitr Z Pathol Anat U Z Allg Pathol 1892;11:277.
12. Borst M. Dielehre von Den Geschwülsten, vol 1. Wiesbaden: JF Bergmann, 1902:287.
13. Kaufmann E. Spezielle pathologische Anatomy, vol 1. Berlin: W de Gruyter, 1922:385.
14. Patterson HS. A case of endothelioma of the pleura with a review of 96 cases. J Med Soc NJ 1909;5:373.
15. Miller J, Wynn WH. A malignant tumor arising from the endothelium of the peritoneum and provoking a mucoid acidic fluid. J Pathol Bact 1908; 12:267–280.
16. Maximow A. Ueber Das Mesothelium (Deckzellen Der Serösen Haute) Und Die Zellen Der Serösen Exudate. Arch Exp Zelforsch 1927;4:1.
17. Clarkson. Canad Med Assoc J 1940;4:192.
18. Keilty. Am J Med Sci 197;153:888.
19. Bayne-Jones. Johns Hopkins Hosp Rep 1919;18:213–227.
20. Du Bray, Rosson. Arch Intern Med 1920;26:715–737.
21. Eastwood, Martin. Lancet 1921;201:172.
22. Zeckwer IT. Mesothelioma of the pleura. Arch Intern Med 1928;34:191.
23. Klemperer P, Rabin CR. Primary neoplasms of the pleura. Arch Pathol 1931; 11:385–412.
24. Gloyne SR. The morbid anatomy and histology of asbestosis. Tubercle 1933; 14:550–558.
25. Ewing J. Neoplastic Diseases, 4th ed. Philadelphia: WB Saunders, 1940: 355–359.
26. Saccone A, Coblenz A. Endothelioma of the pleura. Am J Clin Pathol 1943; 13:186–207.
27. Ducic S. L'exactacttitude des cases de dececes. Une comparison avec les diagnostics a' l'autopsie dans une serie de mesotheliomas et autres tumeurs malignes du poumon. Can J Pub Health 1971;62:395–402.
28. Stout AP, Murray MR. Localized pleural mesothelioma. Arch Pathol 1942;34:951–964.
29. Stout AP. Comments. In: Selikoff IJ, Churg J, eds. Biological Effects of Asbestos. Annals of the New York Academy of Sciences 1965;132:680.
30. Postoloff AV. Mesothelioma of the pleura. Arch Pathol 1944;37:286–289.
31. Piatt AD. Primary mesothelioma (endothelioma) of the pleura. Case report. AJR 1946;55:173–180.
32. Krumbein C. Uber die Natur der Deckzellen der Serosen Haute-Untersucht an Hand eines primaren Pleuracarcinoms. Virchows Arch Pathol Anat 1924;249:400.
33. Wedler HW. Lung cancer in asbestosis patients. Dtsch Arch Klin Med 1943; 191:189–209.
34. Gloyne SR. Tubercle 1933;14:550–557.
35. Gloyne SR. Tubercle 1935;17:5.
36. Harrington JM. Commentary re: attitudes and opinions regarding asbestos and cancer, 1934–1965. Am J Ind Med 1993;23:505–506.
37. Mallory PB, Castleman B, Parris EE. Mesothelioma of the pleura and pericardium. N Engl J Med 1947;236:407.
38. Cartier P. In discussion of paper by Smith WE entitled, "Survey of Some Current British and European Studies of Occupational Tumor Problems." Arch Ind Hyg Occup Med 1952;5:262–263.
39. Weiss A. Pleurakrebs Bei Lungenaspestose. In Vivo Morphologish Gesichert. Medizinische 1953;3:93.

40. Rokitansky C von. Manual of Pathological Anatomy. London: Sydenham So. Trans., 1854:265.
41. Leichner F. Primary mesothelial-cell tumor of the peritoneum in asbestosis. Arch Gewerbepathol Gewerbehyg 1954;13:382.
42. Bonser GM, Faulds JS, Stewart MJ. Occupational cancer of the urinary bladder in dyestuffs operatives and of the lung in asbestos textile workers and iron ore miners. Am J Clin Pathol 1955;25:126–133.
43. Acherman LV. Atlas of Tumor Pathology, section 6, part 23. Washington, DC: Armed Forces Institute of Pathology, 1956:100.
44. Godwin MC. Diffuse mesotheliomas with comment on their relation to localized fibrous mesotheliomas. Cancer 1957;10:298–319.
45. Van der Schoot HCM. Asbestosis en pleuragezwellen. Ned Tijdschr Geneeskd 1958;102:1125.
46. McCaughey WTE. Primary tumors of the pleura. J Pathol Bact 1958; 76:517–529.
47. McCaughey WTE, Wade OL, Elmes PC. Exposure to asbestos dust and diffuse pleural mesotheliomas. Br Med J, 1962:1397.
48. Smart J, Hinson KFW. Pleural neoplasms. Br J Tuberc 1957;51:319–330.
49. Willis RA. Pathology of Tumors. Washington, DC: Butterworth, 1960:185.
50. Eisenstadt HB. Malignant mesothelioma of the pleura. Dis Chest 1956;30:549–556.
51. Doll R. Mortality from lung cancer in asbestos workers. Br J Ind Med 1955; 12:81–86.
52. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in northwestern Cape Providence. Br J Ind Med 1960; 17:260–271.
53. McCaughey WTE. Asbestos and cancer, 1934–1965. Am J Ind Med 1993; 23:503–504.
54. Editorial: Van Die Redaksie, Asbestos and Neoplasia. S Afr Med J 1968; 6:325.
55. Eisenstadt HB, Wilson FW. Primary malignant mesothelioma of the pleura. Lancet 1960;2:511–514.
56. Keal EE. Asbestosis and abdominal neoplasms. Lancet 1960;2:1211.
57. Winslow DJ, Taylor HB. Malignant peritoneal mesotheliomas. A clinicopathological analysis of 12 fatal cases. Cancer 1960;13:127.
58. Enticap JB, Smither WJ. Peritoneal tumors in asbestosis. Br J Ind Med 1964; 21:20.
59. Mayer E, ed. The pneumoconioses. Arch Environ Health 1963;7:14–55.
60. Wagner JC. Experimental production of mesothelial tumors of the pleura by implantation of dusts in laboratory animals. Nature (London) 1962; 196:180.
61. Selikoff IJ, Churg J, ed. Biological effects of asbestos. Ann NY Acad Sci 1965;132:1–766.
62. Selikoff I, Churg J, Hammond E. Relation between exposure to asbestos and mesothelioma. N Engl J Med 1965;272:560–565.
63. Editorial: asbestosis and malignant disease. N Engl J Med 1965;272:590–591.
64. Sluis-Cremer GK. Asbestos in South Africa—certain geographical and environmental considerations. Ann NY Acad Sci 1965;132:215–234.
65. Newhouse ML, Thompson H. Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. Br J Ind Med 1965; 22:261–269.
66. Elmes P, McCaughey W, Wade O. Diffuse mesothelioma of the pleura and asbestos. Br Med J 1965;1:350–353.

67. Lieben J, Pistawka H. Mesothelioma and asbestos exposure. Arch Environ Health 1967;14:559–563.
68. Selikoff IJ. Asbestos. Environment 1969;11:3–7.
69. Thompson JG. Exposure to asbestos dust and diffuse pleural mesotheliomas. Br Med J 1963;123.
70. Thompson JG, Kaschula O, MacDonald RR. Asbestos as a modern urban hazard. S A Tydskrif Vir Geneeskunde 1963;77–81.
71. Utidjian MD, Gross P, de Treville R. Ferruginous bodies in human lungs. Arch Environ Health 1968;17:327.
72. Murray R. Asbestos: a chronology of its origins and health effects. Br J Ind Med 1990;47:361–365.
73. Wagner JC, Berry G. Mesotheliomas in rats following inoculation with asbestos. Br J Cancer 1969;23:567–581.
74. Stanton MF, Wrench C. Mechanisms of mesothelioma induction with asbestos and fibrous glass. J Natl Cancer Inst 1972;48:797–821.
75. Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965;58:295–300.
76. Schepers GWH. Comments at New York Academy of Sciences meeting October 1964. Ann NY Acad Sci 1965;132:599.
77. Webster I. Asbestos and malignancy. S Afr Med J 1973;47:165–171.
78. Wright GW. Asbestos and health in 1969. Am Rev Respir Dis 1969; 100:467–479.
79. Harington JS. In: Raven, Rowe, eds. The Prevention of Cancer. 1967.
80. Selikoff I, Hammond E, Churg J. Carcinogenicity of amosite asbestos. Arch Environ Health 1972;25:183–186.
81. McCullagh S. Amosite as a cause of lung cancer and mesothelioma in humans. J Soc Occup Med 1980;30:153–156.
82. Harington JS, McGlashan ND. South African asbestos: production, exports, and destinations, 1959–1993. Am J Ind Med 1998;33:321–326.
83. Parks WR. Occupational Lung Disorders, 2nd ed. London: Butterworths, 1982.
84. Langer, Nolan. Asbestos in the lungs of persons exposed in the USA. Monaldi Arch Chest Dis 1998.
85. Ribak J, Lilis R, Suzuki Y, Penner L, Selikoff IJ. Malignant mesothelioma in a cohort of asbestos insulation workers: clinical presentation, diagnosis, and causes of death. Br J Ind Med 1988;45:182–187.

2

Asbestos-Induced Mesothelioma

Maria E. Ramos-Nino, Marcella Martinelli, Luca Scapoli, and Brooke T. Mossman

Asbestos, a group of chemically and physically distinct fibers, is one of the most notorious carcinogens in the lung and pleura. The National Institutes of Health in 1978 estimated that approximately 11 million individuals had been exposed to asbestos in the United States since 1940 (1). Although widely employed in World Wars I and II, the use of asbestos has undergone major changes in recent decades, with severe restrictions in most countries on amphiboles. In developed countries, with the exception of Japan, asbestos production is controlled or banned, while in developing countries, consumption has leveled off or increased (2). Between the 1940s and 1970s, asbestos was utilized extensively in insulation applications (primarily in the building construction industry), and in asbestos-cement pipes. Current usage is generally confined to chrysotile in four products: asbestos cement, friction materials, roof coating and cements, and gaskets. In 1992 approximately 28 million tons of asbestos-cement products were produced in approximately 100 countries (3).

Properties of Asbestos Fibers

Asbestos is a naturally occurring group of fibers, each with its own unique structure and chemical composition (Table 2.1). There are two subgroups: (1) the serpentine group, consisting of chrysotile; and (2) the amphiboles, a group of rod-like fibers including crocidolite, amosite, tremolite, anthophyllite, and actinolite (4). Asbestos fibers are ubiquitous in certain geographic areas and become problematic to human health when they are inhaled. It is unclear how they get to the pleura to cause mesothelioma.

Epidemiology of Asbestos-Induced Mesotheliomas

The most important causal factor for the development of human mesothelioma is exposure to asbestos, primarily the amphiboles crocidolite and amosite. Malignant mesothelioma is presently a worldwide

Table 2.1. Types, composition and characteristics of asbestos fibers

Type	Composition	Source	Morphology
Chrysotile*	$Mg_6Si_4O_{10}(OH)_8$	Northern hemisphere (U.S. and Canada)	Curly, pliable
Crocidolite	$Na_2(Fe_{3+})_2(Fe_{2+})_3Si_8O_{22}(OH)_2$	South Africa, Western Australia	Rodlike, durable
Amosite	$(Fe, Mg)_7Si_8O_{22}(OH)_2$	South Africa	Rodlike, durable
Anthophyllite	$(Mg, Fe)_7Si_8O_{22}(OH)_2$	Finland	Rodlike, durable
Tremolite	$Ca_2Mg_5Si_8O_{22}(OH)_2$	Exists in some deposits of Canadian chrysotile	Rodlike, durable
Actinolite	$Ca_2(Mg, Fe)_5Si_8O_{22}(OH)_2$	Not mined	

* Only member of the "serpentine" family. Other types of asbestos are classified as "amphiboles."

problem (5). Although mesothelioma is a rare disease, with an annual incidence in the United States of 2000 to 3000 cases, a steady rise in cases has been reported (6). In Europe, the incidence of malignant pleural mesothelioma has risen for decades and is expected to peak between the years 2010 and 2020 (7). In Germany, a study conducted on 1605 patients in the mesothelioma register (1987–1999), found that 70% had a history of exposure to asbestos (8). In the United Kingdom, asbestos reportedly accounts for some 600 cases of mesothelioma and 100 cases of bronchial carcinoma per year (9). The incidence of mesothelioma has been rapidly increasing and is expected to increase even more from the present total of 1300 to more than 3000 cases per year. Exposure to fibers is associated with most of these cases (10).

The link between amphibole asbestos exposure and pleural mesothelioma is the result of the pioneering work of Wagner and colleagues (11), who found a relationship between the high incidence of the disease and people working at or living near crocidolite (blue) asbestos mines, with intermediate levels of disease near amosite mines, and no tumors in chrysotile miners.

Lung burden studies (see Chapter 1) have also confirmed that the amphibole subgroup of asbestos (crocidolite, amosite) is the one more strongly associated with the development of both malignant mesothelioma and lung cancers (12). In a recent study on 1445 cases of mesothelioma in the United States, it was determined that commercial amphiboles were responsible for most of the mesothelioma cases observed (13). Chrysotile asbestos may produce mesothelioma in humans, but the number of cases is small and the required exposures large (12). Heavy exposures to chrysotile asbestos alone, or with negligible amphibole contamination, can cause malignant mesothelioma and other lung cancers in humans (14), but studies evaluating worker populations that are transient and may be exposed to different types of fibers over a lifetime are difficult to interpret.

Some studies have implicated tremolite fibers as the likely etiologic factor in mesotheliomas associated with chrysotile exposure (15–17). However, others suggest that chrysotile does cause mesothelioma, although it may be far less potent than amphibole asbestos (18).

Although the association between amphibole asbestos exposure and the development of malignant mesothelioma is well documented (19), available information suggests that other factors contribute to its etiol-

ogy. Some studies suggest that genetic factors may play an important role in the etiology of the disease (20,21). Also, compelling multiinstitutional studies suggest that SV40 tumor (T)-antigen (Tag) is present in a large percentage of human mesotheliomas. Approximately 60% of mesotheliomas in the United States are positive for SV40 Tag (22,23), and possible mechanisms are discussed in other chapters of this volume (see Chapter 3).

Properties of Asbestos Associated with Carcinogenic Potential

The carcinogenic potential of asbestos fibers has been linked to their geometry, size, and chemical composition. Because of the increased potential of long (>5 μm) fibers to cause mesothelioma and fibrosis after intrapleural or intraperitoneal administration to rodents (24), health concerns for long respirable fibers [World Health Organization (WHO) criteria: length >5 μm, diameter <3 μm] are considerable (25).

In addition to size, the chemical composition of fibers plays an important role in determining the durability, biopersistence, and biodegradability of asbestos types. The greater durability of amphiboles compared to chrysotile appears to be one of the principal reasons for their greater carcinogenic potential. Amphibole fibers persist at sites of tumor development and may serve as stimuli for neoplastic growth of cells (26,27). Studies on the retention of asbestos fibers in lung tissues of asbestos workers show that concentrations of amphibole fibers increase with durations of exposure, whereas chrysotile concentration does not (28). Studies also indicate that the lung fiber content of amphiboles is less than that required for chrysotile in the induction of mesothelioma (29). The persistence of the amphibole fibers at the site of tumor formation is important to both tumor induction and promotion because the mean latency period between initial exposure to asbestos and the development of mesothelioma is around 30 to 40 years (19,30).

Role of Reactive Oxygen and Nitrogen Species (ROS/RNS) in Asbestos Bioreactivity

An important unresolved issue is whether asbestos fiber carcinogenicity is through direct effects of asbestos on mesothelial cells or through indirect mechanisms involving oxidative stress (31,32). A ramification of interaction of long (>5 μm) fibers with cells is frustrated phagocytosis and a prolonged oxidative burst (Fig. 2.1) (33).

The increased durability and high iron content of the amphiboles crocidolite and amosite also may contribute to their higher carcinogenic potential through oxidants catalyzed by iron or surface reactions occurring on the fiber. Iron-rich durable fibers such as crocidolite, which contain as much as 36% iron by weight, also may have increased reactivity because of the oxidation state of iron, i.e., increases in ferrous iron, aiding in its chelation (34). The cytotoxicity of crocidolite fibers in

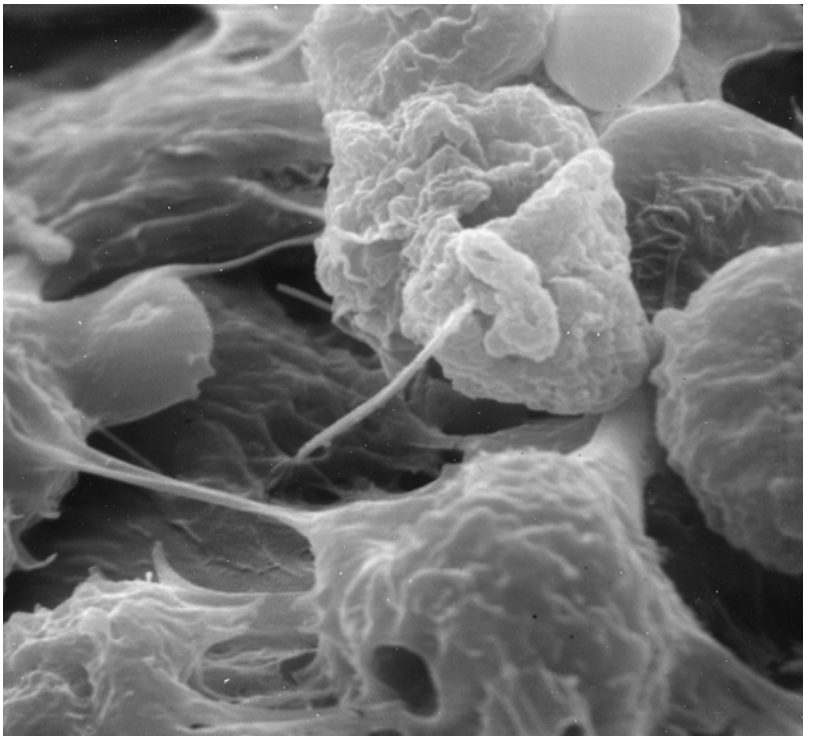


Figure 2.1. Scanning electron microscopy showing phagocytosis of long asbestos fibers by alveolar macrophages.

human lung carcinoma cells is directly linked to iron mobilization and is followed by increased ferritin synthesis, a perpetual feedback system for uptake of iron by cells (35,36).

Studies on animal models and cell cultures have confirmed that asbestos fibers generate ROS and RNS (19,32,37), and these effects may be potentiated by the inflammation associated with fiber exposures (38). Asbestos also activates redox-sensitive transcription factors such as nuclear factor kappa B (NF-κB) (39) and activator protein-1 (AP-1) (40), which lead to increased cell survival, inflammation, and, paradoxically, the upregulation of antioxidant enzymes such as manganese superoxide dismutase (38). This enzyme is also overexpressed in asbestos-related mesotheliomas (41,42), rendering them highly resistant to oxidative stress in comparison to normal mesothelial cells. Moreover, its overexpression prevents cell injury by asbestos (43). In human pleural mesothelial cells in vitro, crocidolite asbestos causes oxidative stress and DNA single-strand breaks (44), but these are not exacerbated by pretreatment with inhibitors of antioxidant enzymes.

Other studies have demonstrated overexpression of enzymes related to oxidative stress, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (NOS-2) (45,46), and endothelial nitric oxide synthase

(eNOS) in malignant mesotheliomas (47). Thioredoxin, a small redox-active protein reduced by the selenoprotein thioredoxin reductase and reduced nicotinamide adenine dinucleotide phosphate (NADPH), is associated in other models of cancer with cell growth and differentiation and is also overexpressed in mesothelioma cells. This protein might be a factor governing the poor prognosis of mesotheliomas and their reduced responsiveness to conventional therapies (48). Overexpression of gamma-glutamylcysteine synthetase, a rate-limiting enzyme in glutathione-associated pathways, could also play an important role in the primary drug resistance of mesotheliomas (49). Catalytically active 5-lipooxygenase could also be involved in the regulation of proliferation and survival in mesotheliomas via a vascular endothelial growth factor (VEGF)-related circuit (50).

Cytogenetic Changes by Asbestos Fibers in Mesothelial Cells and Mesotheliomas

Chromosomal changes and cytogenetic responses to asbestos have been observed in rodent and human mesothelial cells in culture (51–53). Although human mesothelial cells may be more sensitive to the cytotoxic effects of asbestos than bronchial epithelial cells or fibroblasts (52), it is unclear whether individual sensitivity to asbestos fibers is due to specific genetic traits. For example, the glutathione-S-transferase M1 (*GSTM1*) genotypes of patients with mesothelioma suggest that the lack of the *GSTM1* gene does not render human mesothelial cells more sensitive to chromosomal damage by amosite asbestos fibers. However, *GSTM1* null cells are more susceptible than *GSTM1*-positive cells to growth inhibitory effects of fibers (54).

A complex profile of somatic genetic changes has been revealed in human malignant mesotheliomas. These changes implicate a multistep process of tumorigenesis. The occurrence of multiple, recurrent cytogenetic deletions suggests that loss or inactivation of tumor suppressor genes are critical to the development and progression of mesothelioma. Deletions of specific regions in the short (p) arms of chromosomes 1, 3, and 9 and long (q) arms of 6, 13, 15, and 22q are repeatedly observed, and loss of a copy of chromosome 22 is the single most consistent numerical change (55).

Relatively little is known about the early changes in the genesis of mesothelioma. Of the known cytogenetic changes, the most frequent is loss of p16/CDKN2A-p14^{ARF} at 9p21(by homozygous deletion) (56), adversely affecting both Rb and p53 pathways, respectively. NF2 (merlin), a tumor suppressor located at 22q12 (by an inactivating mutation coupled with allelic loss) is also frequently altered in mesotheliomas (57–60). Other conventional proto-oncogenes and tumor suppressor genes have been investigated including N-*ras* (61), Ha- and Ki-*ras* (62), and the tumor suppressor gene *p53*, but no consistently frequent mutations have been found (61–63).

Cell Signaling Pathways, Growth Factors, and Early Response Proto-Oncogenes

The studies cited above suggest that cell proliferation by asbestos may play a more critical role in the promotion and progression of mesotheliomas. Carcinogenesis was classically thought to be a proliferation-driven process. However, it is now recognized that neoplastic growth is an imbalance between apoptosis and proliferation. In support of this concept, a dynamic balance between apoptosis and cell proliferation is observed in mesothelial cells exposed to crocidolite asbestos (64). Studies in vitro indicate that asbestos can induce apoptosis in mesothelial cells through formation of ROS (65,66) and mitochondrial pathways (31,67).

Malignant mesothelioma (MM) routinely expresses the antiapoptotic protein Bcl-xl and the proapoptotic proteins Bax and Bak. Moreover, antisense oligonucleotides against Bcl-xl engender apoptosis in mesothelioma cell lines (68). Inhibitor of apoptosis protein-1 (IAP-1) promotes mesothelioma cell survival, whereas reduced IAP-1 results in increases in apoptotic pathways and reduced resistance to chemotherapeutic drugs (69).

Cell signaling pathways induced by asbestos through receptors on the cell surface trigger early-response proto-oncogenes, activation of transcription factors such as AP-1, and AP-1–dependent gene expression (40,70).

Studies in our group have found that the epidermal growth factor receptor (EGFR) is an important target of asbestos. This growth factor is required for proliferation of human mesothelial cells (71), and is produced in an autocrine fashion in mesotheliomas (72). Autophosphorylation of the EGFR occur in mesothelial cells after in vitro exposures to asbestos. Moreover, aggregation and phosphorylation of the EGFR by long fibers initiates cell signaling cascades linked to asbestos-induced injury and mitogenesis (73,74). Increased expression of EGFR in rat pleural mesothelial cells correlates with the carcinogenicity of mineral fibers (75).

We have also shown that the EGFR is causally linked to activation of the mitogen-associated protein kinase (MAPK) cascade and increased expression of the proto-oncogenes c-*fos* and c-*jun* (73,76). Expression of both Fos and Jun family members (components of the transcription factor AP-1 complex) is required for transition through the G1 phase and entry into the S phase of the cell cycle (70). Moreover, overexpression of c-*jun* induces cell proliferation and transformation (77). Most recently, extracellular signal-regulated kinase (ERK-1/2)–induced activation by asbestos has been linked to the induction of Fra-1, an important component of the AP-1 complex that is causally related to anchorage-independent growth in mesothelioma (41). Complementary DNA (cDNA) microarray analyses have shown increased expression of c-*myc*, *egfr*, and *fra*-1 in rat mesotheliomas (78).

Other growth factors and their receptors also are important in malignant mesothelioma including transforming growth factor-α (TGF-α),

which binds to the EGFR (79). Although normal mesothelial cells, asbestos-transformed mesothelioma cells, and spontaneously transformed mesothelial cells express functional EGFR (55), only cell lines derived from asbestos-induced mesotheliomas express and secrete TGF-α, which binds to the EGF receptor with high affinity. In addition, TGF-α acts as an autocrine growth factor for asbestos-induced mesotheliomas, and their growth is inhibited with use of a neutralizing TGF-α antibody (79). Insulin-like growth factor-II, which functions as an autocrine growth factor in normal mesothelial and mesothelioma cells (71,80), and its corresponding receptor also are important in proliferation of mesothelioma cells (81).

Platelet-derived growth factor (PDGF) (82) may also be an autocrine growth factor for human mesothelioma cells as both PDGF A- and B- chain messenger RNAs (mRNAs) are expressed at higher levels in mesothelioma as opposed to normal mesothelial cell lines (83), and PDGF-like mitogenic activity is observed using mesothelioma cell line–conditioned medium (84). Transforming growth factor (TGF)-β_1, responsible for regulatory functions in many pathologic processes including pleural fibrosis, increases pleural fluid formation in part by stimulating production of VEGF, a regulator of pleural inflammation and cell proliferation (85); VEGF is important in vascular permeability and pleural effusion formation as well as growth of mesothelioma cells (86,87).

Increased levels of hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF), known growth factors for mesothelial cells, have been detected in pleural lavage fluids of patients (88). Although HGF is produced in general by mesenchymal cells, recent work by Cacciotti and colleagues (87) shows that the HGF receptor Met, a proto-oncogene product whose activation leads to cell growth and altered morphogenesis, is activated in SV40-positive human mesothelioma cells. Also, high expression levels of c-*met* have been detected in rat mesothelioma cells and are *fra*-1 dependent (89).

Effects of Asbestos on Extracellular Matrix

Malignant mesotheliomas exhibit elevated amounts of hyaluronan, and hyaluronan synthesis enhances cell proliferation, anchorage independent growth and cell migration in a number of tumor types (90). The hyaluronan receptor gene *cd44* is detected in high amounts by oligonucleotide microarray analysis of human and rat mesothelioma cell lines and may play a role in mesothelial cell motility and migration (89). Other extracellular components such as fibrin deposition via increased expression of tissue factor (TF) may play a role in pleural injury or neoplasia (91). In a study on 16 patients in whom matrix metalloproteinases (MMP)-1, -2, -3, -7, and -9 and tissue inhibitors -1 and -2 were evaluated, MMP-1 and -2 were related directly to invasion and spread of pleural malignant mesothelioma (92).

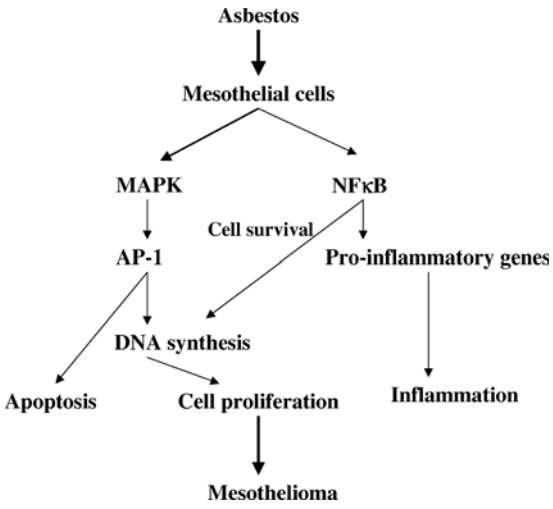


Figure 2.2. Hypothetical schema of cell signaling in mesothelial cells by asbestos.

Conclusion

The reports cited in this chapter provide much insight into mechanisms of asbestos-induced mesotheliomas and the properties of amphibole asbestos fibers that initiate injury and compensatory mesothelial cell hyperplasia. The chemical composition of these fibers and their durability at sites of tumor development may induce chronic activation of cell signaling pathways and transcription factors linked to expression of a number of genes critical to tumor initiation, promotion, progression, and angiogenesis (Fig. 2.2). Many of these pathways have been reported after infection of human mesothelial cells with SV40 (72). Regardless of their etiology, since human mesotheliomas appear to have a number of autocrine growth factor pathways governing proliferation, a focus on common downstream signaling molecules is merited in prevention and therapy of mesotheliomas.

References

1. Manning C, Vallyathan V, Mossman B. Diseases caused by asbestos: mechanisms of injury and disease development. Int Immunopharmacol Cancer Res 2002;2–3:191–200.
2. Algranti E: Asbestos: current issues related to cancer and to uses in developing countries. Cad Saude Publica 1998;14(suppl 3):173–176.
3. Pigg B. The uses of chrysotile. Ann Occup Hyg 1994;38:453–458.
4. Guthrie GT, Mossman BT. Health effects of mineral dusts. In: Ribbe PH, ed. Reviews in Mineralogy. Washington, DC: Mineralogical Society of America, 1993;28:1–584.

5. Bocchetta RP, Powers A, Foddis R, Stekala E, Pass HI, Carbone M. SV40 and the pathogenesis of mesothelioma. Semin Cancer Biol 2001;11:63–71.
6. Grondin S, Sugarbaker D. Malignant mesothelioma of the pleural space. Oncology 1999;13:919–926.
7. Boutin C, Schlesser M, Frenay C, Astoul P. Malignant pleural mesothelioma. Eur Respir J 1998;12:972–981.
8. Neumann V, Gunthe S, Mulle K, Fischer M. Malignant mesothelioma—German mesothelioma register 1987–1999. Int Arch Occup Environ Health 2001;74:383–395.
9. Coggon D. Occupational cancer in the United Kingdom. Environ Health Perspect 1999;107(suppl 2):239–244.
10. Statement on malignant mesothelioma in the United Kingdom. Thorax 2001;56:250–264.
11. Wagner J, Sleggs C, Marchand P. Diffuse pleural mesotheliomas and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960;17:260–271.
12. Churg A. Chrysotile, tremolite, and malignant mesothelioma in man. Chest 1988;93:621–628.
13. Roggli VL, Sharma A, Butnor KJ, Sporn T, Vollmer RT. Malignant mesothelioma and occupational exposure to asbestos: a clinicopathological correlation of 1445 cases. Ultrastruct Pathol 2002;26:55–65.
14. Yano E, Wang Z, Wang X, Wang M, Lan Y. Cancer mortality among workers exposed to amphibole-free chrysotile asbestos. Am J Epidemiol 2001;154: 538–543.
15. Churg A, Wiggs B. Fiber size and number in amphibole asbestos-induced mesothelioma. Am J Pathol 1984;115:437–442.
16. Roggli V, Vollmer R, Butnor K, Sporn T. Tremolite and mesothelioma. Ann Occup Hyg 2002;46:447–453.
17. MacDonald J, McDonald A. Chrysotile, tremolite, and mesothelioma. Science 1995;267(5199):776–777.
18. Steenland K, Stayner L. Silica, asbestos, man-made mineral fibers, and cancer. Cancer Causes Control 1997;8:491–503.
19. Mossman B, Bignon J, Corn M, Seaton A, Gee J. Asbestos: scientific developments and implications for public policy. Science 1990;247:294–301.
20. Roushdy-Hammady I, Siegel J, Emri S, Testa J, Carbone M. Genetic susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 2001;357(9254):444–445.
21. Huncharek M. Non-asbestos related diffuse malignant mesothelioma. Tumori 2002;88:1–9.
22. Carbone M. Simian virus 40 and human tumors: it is time to study mechanisms. J Biol Chem 1999;76:189–193.
23. Klein G, Powers A, Croce C. Association of SV40 with human tumors. Oncogene 2002;21:1141–1149.
24. Health Effects Institute. Asbestos in Public and Commercial Buildings: A Literature Review and Synthesis of Current Knowlegdge. Cambridge, MA: Health Effects Institute–Asbestos Research, 1991.
25. Vu V, Lai D. Approaches to characterizing human health risks of exposure to fibers. Environ Health Perspect 1997;105(suppl 5):1329–1336.
26. Woodworth C, Mossman B, Craighead J. Induction of squamous metaplasia in organ cultures of hamster trachea by naturally occurring and synthetic fibers. Cancer Res 1983;43:4906–4912.
27. Jaurand M, Gaudichet A, Halpern S, Bignon J. In vitro biodegradation of chrysotile fibres by alveolar macrophages and mesothelial cells in culture: comparison with a pH effect. Br J Ind Med 1984;41:389–395.

28. Albin M, Pooley FD, Stromberg U, et al. Retention patterns of asbestos fibres in lung tissue among asbestos cement workers. Occup Environ Med 1994;51:205–211.
29. Churg A, Wiggs B, Depaoli L, Kampe B, Stevens B. Lung asbestos content in chrysotile workers with mesothelioma. Am Rev Respir Dis 1984;130: 1042–1045.
30. Mossman B, Kamp D, Weitzman S. Mechanisms of carcinogenesis and clinical features of asbestos-associated cancers. Cancer Invest 1996;14:466–480.
31. Shukla A, Jung M, Stern M, et al. Asbestos induces mitochondrial DNA damage and dysfunction linked to the development of apoptosis. Am J Physiol Lung Cell Mol Physiol 2003;285(5):L1018–L1025.
32. Kamp D, Graceffa P, Pryor W, Weitzman S. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 1992;12:293–315.
33. Hansen K, Mossman B. Generation of superoxide (O_2^-) from alveolar macrophages exposed to asbestiform and nonfibrous particles. Cancer Res 1987;47:1681–1686.
34. Gulumian M, Bhoolia D, Du Toit R, et al. Activation of UICC crocidolite: the effect of conversion of some ferric ions to ferrous ions. Environ Res 1993;60:193–206.
35. Chao C, Lund L, Zinn K, Aust A. Iron mobilization from crocidolite asbestos by human lung carcinoma cells. Arch Biochem Biophys 1994;314: 384–391.
36. Fang R, Aust A. Induction of ferritin synthesis in human lung epithelial cells treated with crocidolite asbestos. Arch Biochem Biophys 1997;340: 369–375.
37. Janssen Y, Van Houten B, Borm P, Mossman B. Cell and tissue responses to oxidative damage. Lab Invest 1993;69:261–274.
38. Kinnula V. Oxidant and antioxidant mechanisms of lung disease caused by asbestos fibres. Eur Respir J 1999;14:706–716.
39. Janssen Y, Barchowsky A, Treadwell M, Driscoll K, Mossman B. Asbestos induces nuclear factor kappa B (NF-kappa B) DNA-binding activity and NF-kappa B-dependent gene expression in tracheal epithelial cells. Proc Natl Acad Sci USA 1995;92:8458–8462.
40. Ramos-Nino M, Haegens A, Shukla A, Mossman B. Role of mitogen-activated protein kinases (MAPK) in cell injury and proliferation by environmental particulates. Mol Cell Biochem 2002;234–235:111–118.
41. Ramos-Ninos M, Timblin C, Mossman B. Mesothelial cell transformation requires increased AP-1 binding activity and ERK-dependent Fra-1 expression. Cancer Res 2002;62(21):6065–6069.
42. Kahlos K, Pitkanen S, Hassinen I, Linnainmaa K, Kinnula V. Generation of reactive oxygen species by human mesothelioma cells. Br J Cancer 1999; 80:25–31.
43. Mossman B, Surinrut P, Brinton B, et al. Transfection of a manganese-containing superoxide dismutase gene into hamster tracheal epithelial cells ameliorates asbestos-mediated cytotoxicity. Free Radic Biol Med 1996;21: 125–131.
44. Ollikainen T, Linnainmaa K, Kinnula V. DNA single strand breaks induced by asbestos fibers in human pleural mesothelial cells in vitro. Environ Mol Mutagen 1999;33:153–160.
45. Marrogi A, Pass H, Khan M, Metheny-Barlow L, Harris C, Gerwin B. Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2): in vitro antiproliferative effects of a COX-2 inhibitor. Cancer Res 2000;60:3696–3700.

46. Edwards J, Faux S, Plummer S, et al. Cyclooxygenase-2 expression is a novel prognostic factor in malignant mesothelioma. Clin Cancer Res 2002; 8:1857–1862.
47. Soini Y, Puhakka A, Kahlos K, et al. Endothelial nitric oxide synthase is strongly expressed in malignant mesothelioma but does not associate with vascular density or the expression of VEGF, FLK1 or FLT1. Histopathology 2001;39:179–186.
48. Sun X, Dobra K, Bjornstedt M, Hjerpe A. Upregulation of 9 genes, including that for thioredoxin, during epithelial differentiation of mesothelioma cells. Differentiation 2000;66:181–188.
49. Jarvinen K, Soini Y, Kahlos K, Kinnula V. Overexpression of gamma-glutamylcysteine synthetase in human malignant mesothelioma. Hum Pathol 2002;33:748–755.
50. Romano M, Catalano A, Nutini M, et al. 5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. FASEB J 2001;15:2326–2336.
51. Jaurand M, Kheuang L, Magne L, Bignon J. Chromosomal changes induced by chrysotile fibres or benzo-3,4-pyrene in rat pleural mesothelial cells. Mutat Res 1986;169:141–148.
52. Lechner JF, Tokiwa T, LaVeck M, et al. Asbestos-associated chromosomal changes in human mesothelial cells. Proc Natl Acad Sci USA 1985;82: 3884–3888.
53. Health Effects Institute. Asbestos in Public and Commercial Buildings: A Literature Review and Synthesis of Current Knowlegdge. Cambridge, MA: Health Effects Institute–Asbestos Research, 1991.
54. Pelin K, Hirvonen A, Taavitsainen M, Linnainmaa K. Cytogenetic response to asbestos fibers in cultured human primary mesothelial cells from 10 different donors. Mutat Res 1995;334:211–218.
55. Murthy S, Testa J. Asbestos, chromosomal deletions, and tumor suppressor gene alterations in human malignant mesothelioma. J Cell Physiol 1999; 180:150–157.
56. Hirao T, Bueno R, Chen C, Gordon G, Heilig E, Kelsey K. Alterations of the p16(INK4) locus in human malignant mesothelial tumors. Carcinogenesis 2002;23:1127–1130.
57. Bianchi A, Mitsunaga S, Cheng J, et al. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc Natl Acad Sci USA 1995;92:10854–10858.
58. Sekido Y, Pass H, Bader S, et al. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. Cancer Res 1995;55:1227–1231.
59. Lee H, Chang T, Tebalt MR, Senderowicz A, Szabo E. Induction of differentiation accompanies inhibition of Cdk2 in a non-small cell lung cancer cell line. Int J Oncol 1999;15:161–166.
60. Lechner J, Tesfaigzi J, Gerwin B. Oncogenes and tumor-suppressor genes in mesothelioma—a synopsis. Environ Health Perspect 1997;105(suppl 5): 1061–1067.
61. Papp T, Schipper H, Pemsel H, et al. Mutational analysis of N-ras, p53, p16INK4a, p14ARF and CDK4 genes in primary human malignant mesotheliomas. Int J Oncol 2001;18:425–433.
62. Kitamura F, Araki S, Suzuki Y, Yokoyama K, Tanigawa T, Iwasaki R. Assessment of the mutations of p53 suppressor gene and Ha- and Ki-ras oncogenes in malignant mesothelioma in relation to asbestos exposure: a study of 12 American patients. Ind Health 2002;40:175–181.

63. Mayall F, Jacobson G, Wilkins R. Mutations of p53 gene and SV40 sequences in asbestos-associated and non-asbestos-associated mesotheliomas. J Clin Pathol 1999;52:291–293.
64. Goldberg J, Zanella C, Janssen Y, et al. Novel cell imaging approaches show induction of apoptosis and proliferation in mesothelial cells by asbestos. Am J Respir Cell Mol Biol 1997;17:265–271.
65. Broaddus V, Yang L, Scavo L, Ernst J, Boylan A. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. J Clin Invest 1996;98:2050–2059.
66. BeruBe K, Quinlan T, Fung H, et al. Apoptosis is observed in mesothelial cells after exposure to crocidolite asbestos. Am J Respir Cell Mol Biol 1996; 15:141–147.
67. Kamp D, Panduri V, Weitzman S, Chandel N. Asbestos-induced alveolar epithelial cell apoptosis: role of mitochondrial dysfunction caused by iron-derived free radicals. Mol Cell Biochem 2002;234–235:153–160.
68. Smythe WR, Mohuiddin I, Ozveran M, Cao XX. Antisense therapy for malignant mesothelioma with oligonucleotides targeting the bcl-xl gene product. J Thorac Cardiovasc Surg 2002;123:1191–1198.
69. Gordon GJ, Appasani K, Parcells JP, et al. Inhibitor of apoptosis protein-1 promotes tumor cell survival in mesothelioma. Carcinogenesis 2002;23: 1017–1024.
70. Reddy S, Mossman B. Role and regulation of activator protein-1 (AP-1) in toxicant-induced responses of the lung. Am J Physiol (Lung Cell Mol Physiol) 2002;283(6):L1161–L1178.
71. Laveck M, Somers A, Moore L, Gerwin B, Lechner J. Dissimilar peptide growth factors can induce normal human mesothelial cell multiplication. In Vitro Cell Dev Biol 1988;24:1077–1084.
72. Mossman B, Gruenert D. SV40, growth factors, and mesothelioma—another piece of the puzzle. Am J Respir Cell Mol Biol 2002;26:167–170.
73. Zanella C, Posada J, Tritton T, Mossman B. Asbestos causes stimulation of the ERK-1 mitogen-activated protein kinase cascade after phosphorylation of the epidermal growth factor receptor. Cancer Res 1996;56:5334–5338.
74. Pache J, Janssen Y, Walsh E, et al. Increased epidermal growth factor-receptor (EGF-R) protein in a human mesothelial cell line in response to long asbestos fibers. Am J Pathol 1998;152:333–340.
75. Faux S, Houghton C, Hubbard A, Patrick G. Increased expression of epidermal growth factor receptor in rat pleural mesothelial cells correlates with carcinogenicity of mineral fibres. Carcinogenesis 2000;21:2275–2280.
76. Heintz N, Janssen Y, Mossman B. Persistent induction of c-*fos* and c-*jun* expression by asbestos. Proc Natl Acad Sci USA 1993;90:3299–3303.
77. Timblin C, Janssen Y, Mossman B. Transcriptional activation of the protooncogene c-jun by asbestos and H_2O_2 is directly related to increased proliferation and transformation of tracheal epithelial cells. Cancer Res 1995;55:2723–2726.
78. Sandhu H, Dehnen W, Roller M, Abel J, Unfried K. mRNA expression patterns in different stages of asbestos-induced carcinogenesis in rats. Carcinogenesis 2000;21:1023–1029.
79. Walker C, Everitt J, Ferriola P, Stewart W, Mangum J, Bermudez E. Autocrine growth stimulation by transforming growth factor α in asbestos-transformed rat mesothelial cells. Cancer Res 1995;55:530–536.
80. Tubo R, Rheinwald J. Normal human mesothelial cells and fibroblasts transfected with the *EJras* oncogene become EGF-independent, but are not malignantly transformed. Oncogene Res 1987;1:407–421.

81. Rutten AA, Bermudez E, Stewart W, Everitt JI, Walker CL. Expression of insulin-like growth factor II in spontaneously immortalized rat mesothelial and spontaneous mesothelioma cells: a potential autocrine role of insulin-like growth factor II. Cancer Res 1995;55:3634–3639.
82. Metheny-Barlow LJ, Flynn B, van Gijssel HE, Marrogi A, Gerwin BI. Paradoxical effects of platelet-derived growth factor-A overexpression in malignant mesothelioma. Antiproliferative effects in vitro and tumorigenic stimulation in vivo. Am J Respir Cell Mol Biol 2001;24:694–702.
83. Gerwin B. Cytokine signaling in mesothelial cells: receptor expression closes the autocrine loop. Am J Respir Cell Mol Biol 1996;14:505–507.
84. Sekhon H, Wright J, Churg A. Effects of cigarette smoke and asbestos on airway, vascular and mesothelial cell proliferation. Int J Exp Pathol 1995; 76:411–418.
85. Lee YC, Lane KB. The many faces of transforming growth factor-beta in pleural diseases. Curr Opin Pulmon Med 2001;7:173–179.
86. Strizzi L, Catalano A, Vianale G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol 2001;193:468–475.
87. Cacciotti P, Strizzi L, Vianale G, et al. The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. Am J Respir Cell Mol Biol 2002;26:189–193.
88. Adamson IY, Bakowska J. KGF and HGF are growth factors for mesothelial cells in pleural lavage fluid after intratracheal asbestos. Exp Lung Res 2001;27:605–616.
89. Ramos-Nino ME, Scapoli L, Martinelli M, Land S, Mossman BT. Microarray analysis and RNA silencing link *fra*-1 to *cd44* and c-*met* expression. Cancer Res 2003;63(13):3539–3545.
90. Li Y, Heldin P. Hyaluronan production increases the malignant properties of mesothelioma cells. Br J Cancer 2001;85:600–607.
91. Bajaj MS, Pendurthi U, Koenig K, Pueblitz S, Idell S. Tissue factor pathway inhibitor expression by human pleural mesothelial and mesothelioma cells. Eur Respir J 2000;15:1069–1078.
92. Hirano H, Tsuji M, Kizaki T, et al. Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinase, collagens, and Ki67 antigen in pleural malignant mesothelioma: an immunohistochemical and electron microscopic study. Med Electron Microsc 2002;35:16–23.

3

SV40-Mediated Oncogenesis

Maurizio Bocchetta and Michele Carbone

Simian virus 40 (SV40) was first isolated in 1958 among other simian viruses from contaminated polio vaccine preparations, which were inadvertently administered to millions of people in different countries from 1954 to 1963. Soon after SV40 was introduced to the scientific community (1) its capabilities to induce different forms of cancer in experimental animals were recognized (2,3). However, epidemiology failed to establish a conclusive link between the administration of SV40-contaminated polio vaccines to humans and the development of cancer (4–8). Because epidemiology was inconclusive, SV40 has been considered for many years to be harmless to humans. From the 1970s, throughout the 1980s, and until recently, SV40 has been utilized mainly as a tool to understand key molecular processes such as DNA replication, splicing, and translation in mammalian cells. It has also been widely used to uncover the process of the cell cycle control because of the interaction of its major oncogenic protein products with critical tumor suppressor gene pathways of the cell. Indeed, the SV40 oncogenes have probably been the most commonly used tools to experimentally immortalize or transform rodent and human cells, mainly fibroblasts. Occasional screening of human tumors suggested that SV40 could participate in the development of human cancer (9–15).

The interest concerning the association of SV40 with certain human malignancies (specifically, malignant mesothelioma, tumors of the brain and bone, and non-Hodgkin's lymphoma) and the possible causative role of SV40 in the onset of these forms of cancer has vigorously resurfaced during the past decade (reviewed in refs. 16 and 17). This has been caused by the development of new molecular techniques that now allow investigators to better study the presence and the biologic effects of viruses in infected cells. The wealth of experimental data accumulated over the recent past conclusively associates SV40 with human tumors, especially with malignant mesothelioma (16,18,19). A recent meeting of the National Academy of Science, Institute of Medicine (IOM) concluded that SV40 is a "strong" carcinogen, and that there is "moderate evidence" that SV40 causes some human tumors (20). This was emphasized by the recognition that previous epidemiologic

studies were flawed and could not provide any conclusive indication regarding the potential oncogenicity of SV40-contaminated poliovaccines. The IOM has also concluded that the experimental evidence available so far suggests that SV40 may be transmitted among humans, and that SV40 can cause cancer in humans under natural conditions.

This chapter reviews the virology of SV40, discusses the association of SV40 with human malignant mesothelioma, and describes the interactions that SV40 establishes with human mesothelial cells, since these cells are uniquely susceptible to SV40 transformation and immortalization (21).

SV40 Genomic Organization, Gene Transcription, and Cycle of Infection

The genome of SV40 is a small, circular double-stranded DNA molecule. The genome of SV40 strain 776 (also called reference strain, or wild-type SV40) is composed of 5243 base pairs (bp). Different strains of SV40 exist, all sharing a very high level of DNA sequence conservation, with the exception of the transcriptional enhancer region, the very C-terminal portion of the SV40 major oncoprotein (22), and the intron of the early transcripts (23). Aside from differences in the enhancer region, the following description of the genomic organization applies to all SV40 strains. At least six virally encoded protein products are translated in permissive host cells through alternative splicing and translation of overlapping reading frames of the SV40 messenger RNAs (mRNAs). The SV40 genome is organized in three regions: a regulatory region (that includes the viral origin of replication and a bidirectional promoter), a region including the early genes, and a region comprising the late genes. The early and late genes extend in opposite directions with respects to the regulatory regions (Fig. 3.1). The denominations

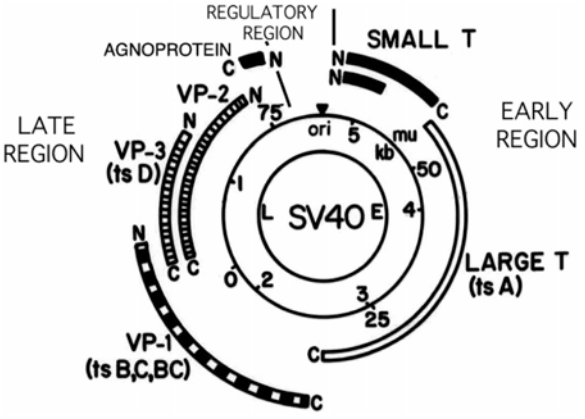


Figure 3.1. Genomic map of simian virus 40 (SV40).

"early" and "late" reflect the order of their transcription/synthesis in the host cell after SV40 infection. SV40 enters the cells after interaction with its receptor [major histocompatibility complex (MHC) class I] (24, 25) on the plasma membrane, and it is internalized within the cytoplasm through a specific endocytosis pathway (caveole) (26). It is then trafficked into the nucleus by means of the interaction of the importin $\alpha 2/\beta$ heterodimer with an SV40 capsid protein (VP-3) (27). It is still unclear where the viral genome is released from its protein envelope. Once within the nucleus, the SV40 early genes are transcribed. The early mRNAs (the precise transcription initiation sites of both the early and late mRNAs vary over a number of positions) contain a 347 bases intron that can be alternatively spliced giving rise to two classes of mRNA (28). Translation of these two types of mRNAs produce two proteins: the large-tumor antigen (or Tag), and the small-tumor antigen (or tag). Overall, the early SV40 mRNAs represent a small fraction of the total RNAs in infected cells, and early genes mRNAs and protein products can be detected in freshly infected cells using only very sensitive methods (29). The ratio between Tag-encoding mRNAs and tag-encoding mRNAs varies in different cell types, and it has been studied only in vitro. In HeLa cell extracts the Tag:tag mRNA ratio is about 100:1 (30). To summarize, the SV40 early genes exert their function even though they are synthesized at very low levels in infected cells.

The SV40 Tag and tag interact with a number of cellular proteins (these activities are discussed below), and the end result of these concerted activities is driving the host cell into the S phase so that the viral genome can be replicated. Tag is required for the replication of the SV40 circular chromosome. Tag binds as a double hexamer to the SV40 origin of replication (31) where it interacts with the host's DNA polymerase α-primase to initiate DNA replication (32). Tag also has DNA helicase activities that play an important role in the SV40 chromosome replication process (33).

As previously stated, the SV40 regulatory region contains a bidirectional promoter. This means that the regulatory region can promote transcription of both the early and late genes. During the early stages of infection, however, transcription of the late genes does not take place because of the binding of transcriptional repressors at sites located in the proximity of the late mRNAs transcription initiation site. These transcriptional repressors belong to the steroid-thyroid hormone receptor superfamily (34,35). During SV40 DNA replication these repressors are progressively titrated-off from the late promoter, so that transcription of the late genes can take place. The binding of Tag to the regulatory region also enhances the latter process, since Tag represses the transcription of its own mRNA and promotes transcription of the late gene mRNAs (36). Efficient synthesis of the late gene mRNAs marks the beginning of the late SV40 cycle of infection during which large amounts of the viral capsid proteins are produced. The late mRNAs also arise from alternative splicing of the same family of transcripts. Two major classes of late mRNAs are produced: the 16S and 19S mRNAs [the classification derives from the migration of these molecules in sucrose gradients (29)]. The 16S mRNAs code for VP-1, which

is the major SV40 capsid protein, while the 19S mRNAs code for the agnoprotein, VP-2 and VP-3. VP-2 and -3 are less abundant capsid proteins but play an essential role in the SV40 packaging process (37,38). VP-3 has also been shown to interact with basal cellular transcriptional factors that repress transcription of the early genes and enhance the completion of the SV40 infection cycle (39). The function of the small agnoprotein is still unclear. Its perinuclear localization suggests that it may participate in nuclear trafficking of SV40 capsid proteins (40, 41). Recent studies have indicated that during JCV infection (JCV is a human polyomavirus closely related to SV40) the agnoprotein may interact (directly or indirectly) with Tag and contribute to the transcription regulation of JCV (42). Furthermore, JCV agnoprotein can inhibit cell cycle progression by binding to cellular p53 and thus increasing the expression of the cyclin-dependent protein kynase (CDK) inhibitor $p21^{WAF}$ (41). Whether the SV40 agnoprotein has similar biologic activities has not been investigated.

The late phase of SV40 infection is characterized by a massive production of capsid proteins that accumulate in the nuclei of infected cells. A large number of viral particles are assembled, and the host cell is eventually lysed, with consequent release in the extracellular environment of infectious SV40 particles (Fig. 3.2). Therefore, SV40 manipulates the host's cell cycle to ensure replication of its own DNA genome. Malignant transformation of the host is not required for

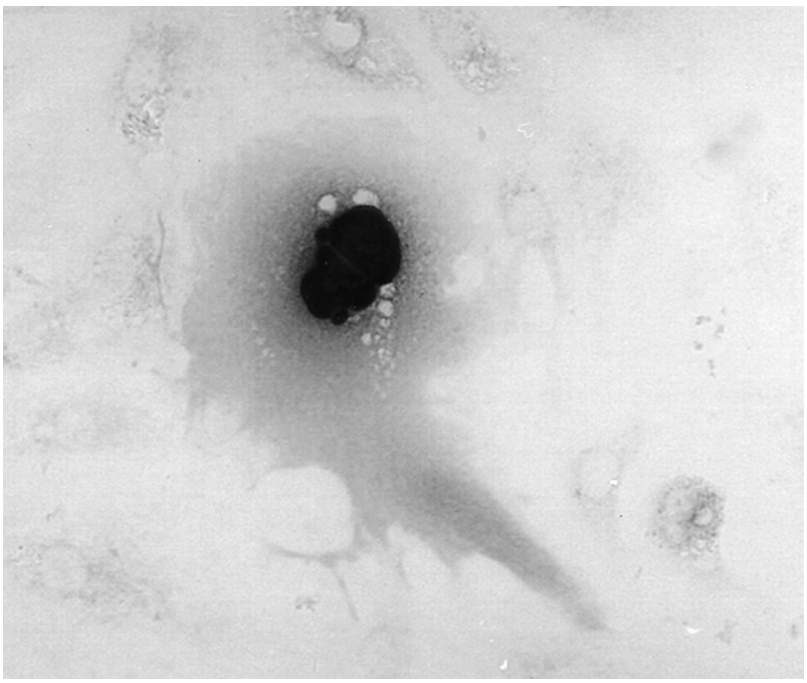


Figure 3.2. Large perinuclear vacuoles in an African green monkey kidney cell infected with SV40. The cell was immunostained using an antibody specific for the SV40 Tag. Original magnification: 200×.

SV40's life cycle, and the most common outcome of SV40 infection is the lysis of the infected host. However, SV40 uses two extraordinarily powerful oncoproteins to undermine the host's cell cycle checkpoints, and, if anything goes wrong with the SV40 lytic pattern of infection, any mammalian cell containing SV40 may undergo malignant transformation. The entity of such risk varies between different cell types. As a whole, in vitro SV40-infected human cells from different tissues display a mixture of cytopathic and transformed phenotypes. This characteristic pattern of infection of human cells by SV40 led some investigators to call SV40 infection of human cells "semipermissive" (43).

Susceptibility to SV40 Infection and SV40-Mediated Transformation

The in vitro outcome of SV40 infection critically depends on the species and cell type of the host. Traditionally, cells are classified as permissive, nonpermissive, and semipermissive to SV40 infection (reviewed in ref. 16). Prototypes of permissive cells are those derived from African green monkey kidneys. These cells are uniformly infected by SV40, synthesize large amounts of viral particles, and display a typical pathologic morphology after SV40 infection characterized by large perinuclear vacuoles (Fig. 3.2) when the SV40 titer reaches about 10^7 median tissue culture infective dose $(TCID)_{50}$/mL in the tissue culture medium (44). SV40-infected African green monkey kidney cells invariably undergo cell lysis in vitro, and SV40-mediated malignant cell transformation in these populations, although theoretically possible, must be an extremely rare event. During the past decade we have infected a substantially large number of African green monkey kidney cells with different strains of SV40 and we have never observed cell survival after SV40 infection. Cell lysis, of course, prevents malignant transformation.

Nonpermissive cells, such as rodent cells, on the other hand, do not allow the replication of the SV40 genome. In rodent cells the SV40 Tag does not properly interact with the host's DNA polymerase α primase, and thus it is unable to initiate the replication of the SV40 chromosome (45). Nevertheless, the SV40 oncoproteins are still capable of driving the host cell into the S phase and eventually into mitosis, but the outcome of this process is a sort of abortive transformation, since the SV40 DNA cannot replicate and is not propagated in the dividing cells. Therefore, SV40 can transform rodent cells only after integration of its genome in the host's chromosomes in such a way that the integrity of the SV40 early genes and their expression are preserved. Integration is rather infrequent. For example, the average rate of cell transformation of mouse fibroblasts after SV40 infection is about 10^{-7} (46). This relatively low frequency of transformation mainly reflects the infrequency of proper integration of the SV40 genome into the host's genome, and does not imply that SV40 is a poor oncogenic factor in rodent systems. In fact, nearly 100% of artificially engineered transgenic mice express-

ing the SV40 oncoproteins develop tumors. If the SV40 regulatory region drives the expression of the SV40 oncoprotein in these animals, mice develop brain tumors (47). However, tissue-specific expression of the SV40 early genes in transgenic mice has led to the development of in vivo tumor models for virtually all tissues (48–50). Altogether, these experimental evidences demonstrate that the SV40 tumor antigens are exceedingly potent cancer-inducing agents in rodents.

Human cells are traditionally described as semipermissive to SV40 infection (43). This term has been used to emphasize different features of human cells infected with SV40, and it reflects the variance in susceptibility to SV40 infection of human cells derived from different tissues (21,51–54). SV40 grows efficiently in some human cells, such as newborn kidney cells (55) and spongioblasts (54), but it grows poorly in cells from other tissues (56). Despite these differences, a unifying feature of human cells exposed to SV40 in vitro is that only a fraction of the cell population supports SV40 infection at any given time, while a substantial percentage of exposed cells is apparently unaffected (reviewed in ref. 16). The molecular basis of this situation is still rather undefined. Early studies indicated that SV40 enters all human cells after exposure, but that the cellular environment plays a pivotal role in determining whether SV40 will produce a productive infection or not (57,58). Therefore, only a fraction of human cells exposed to SV40 in vitro express the SV40 early genes, replicate SV40 DNA, produce viral particles, and undergo SV40-mediated cell lysis at any given time. Accordingly, only a small percentage of human fibroblasts exposed to SV40 expresses Tag 48 hours after infection, actively replicates the SV40 chromosome, and assembles viral particles (Fig. 3.3), an event that

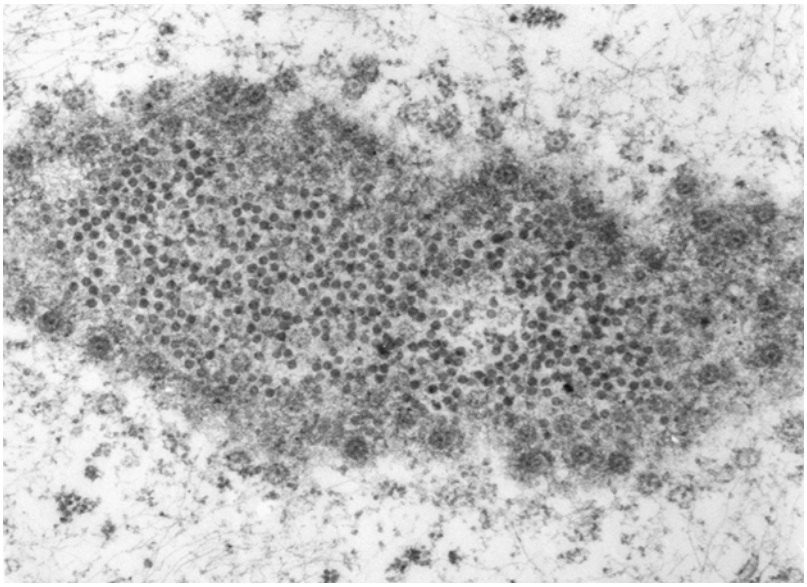


Figure 3.3. Electron microscopy displaying complete SV40 viral particles accumulating in an infected human fibroblasts. Original magnification: 20,000×.

eventually leads to SV40-induced cell lysis, with consequent release of SV40 viral particles in the medium (Fig. 3.4). This equilibrium seems to be maintained in human fibroblast cultures over several weeks after SV40 introduction into the cell population (21). SV40 does not transform human fibroblasts because the viral cytotoxic effects are predominant, and SV40 lyses the cells that support its transcription and replication. The notion that SV40-mediated cell death represents the reason why human fibroblasts are not permanently transformed after SV40 infection is supported by transfection experiments. Introduction into human fibroblasts of SV40 genomes mutated in their origin of replication gives rise to the production of malignantly transformed clones. In the latter scenario, the rodent nonpermissibility to SV40 is artificially reproduced in human cells because of mutations in the SV40 origin of replication that do not allow replication of the SV40 chromosome. In these conditions the frequency of cell transformation of human fibroblasts closely approaches that of mice fibroblasts (rate of transformation of about 10^{-7}) (21). Because of the difficulty of transforming human cells by natural infection, most of the studies concern-

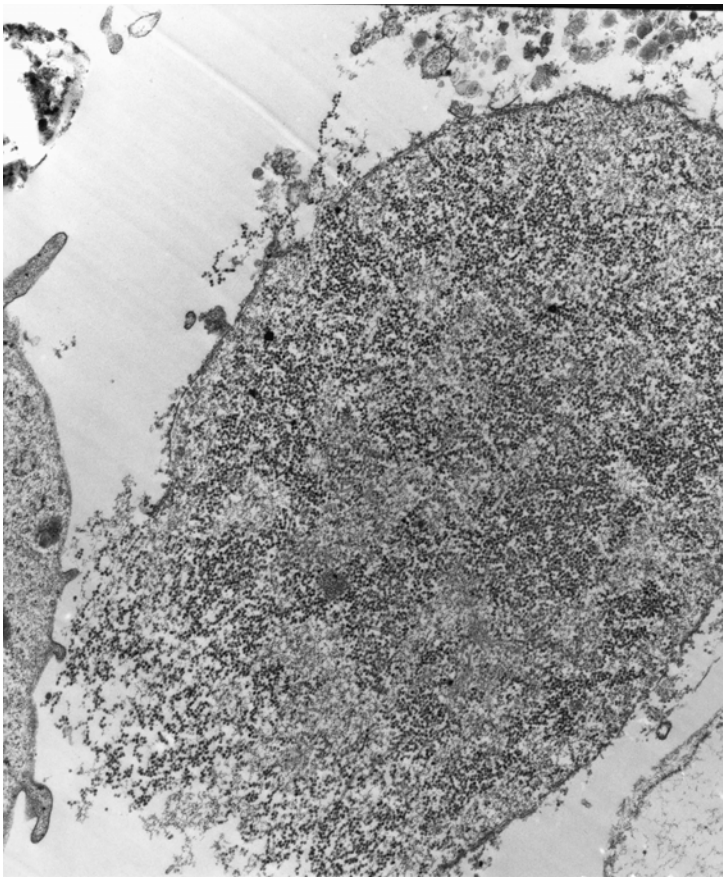


Figure 3.4. Electron microphotograph showing a SV40-infected human fibroblast undergoing SV40-mediated cell lysis. Note the small darker particles representing complete SV40 viruses. Original magnification: 7000×.

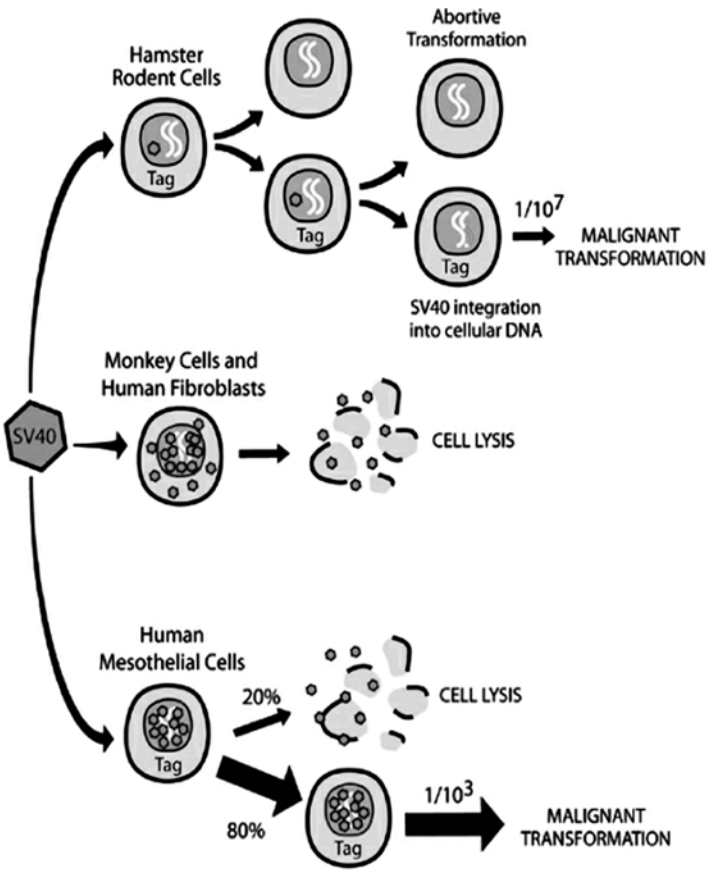


Figure 3.5. Different patterns of SV40 infection. Top: Nonpermissive rodent cells. In these cells SV40 DNA cannot be replicated, thus SV40 DNA can only be retained in dividing rodent cells if integrated into the host's genome. Integration of the SV40 DNA preserving the early genes integrity and expression may lead to cell transformation. Middle: Permissive monkey and human cells. In these cells SV40 DNA is mainly in episomal form; it replicates and leads to the formation of a high number of SV40 viral particles that kill the host. Bottom: Human mesothelial cells. In these cells SV40 DNA is in episomal form; it replicates, but viral particle formation is compatible with survival of the host. In these conditions SV40 causes a very high frequency of cell transformation without the need of the integration of the SV40 DNA in the host's genome.

ing SV40-mediated transformation of human cells have been conducted using transfection of replication defective SV40 genomes. Since stable transfection requires, similarly to the nonpermissive host, integration into the host's genome, it was erroneously assumed that SV40-mediated transformation of human cells required the integration of the SV40 genome in the human cellular DNA (Fig. 3.5).

The SV40 Oncoproteins

As mentioned above, the SV40 genome is rather small. For this reason SV40 must rely mainly on cellular genes to complete its life cycle. Nevertheless, SV40 needs to manipulate the host's cell cycle to ensure

the replication of its DNA. Despite its intrinsic "economy" limitations, SV40 has evolved effective subversive proteins that target both nuclear and cytoplasmic activities. Deregulation of nuclear activities is achieved by Tag, while deregulation of cytoplasmic activities is left to the SV40 tag. Tag is probably one of the most multifunctional proteins in nature, since it participates in DNA, RNA, and protein–protein interactions to ensure SV40 replication and transcription. Tag also engages in a number of interactions with host's cell proteins aimed at the deregulation of the host cell cycle. Tag binds and inactivates the two major tumor suppressor pathways of the cell, p53 (59,60) and pRb protein family (59,61,62). Through these interactions Tag simultaneously knocks out the two most critical cellular networks controlling G1/S transition and possibly G2/M checkpoints (63–65). Through the inhibition of p53, Tag also impairs the major cellular control of genomic stability and apoptosis program (reviewed in ref. 62). Furthermore, the SV40 Tag binds and affects the functions of a number of cellular proteins all involved in protein folding, transcriptional regulation, cell cycle progression, and stability of the genome. So far, the known cellular proteins that interact with Tag include (besides p53 and pRb protein family) the transcriptional coactivator p300 and its closely related p400 (66,67), the mammalian homologue of heat shock protein (Hsc)70 (reviewed in ref. 68), the DNA binding protein kin17 (69), TATA binding protein (TPB) and TFII-D complexes (70), cyclin A/p33CDK2 complexes (71), and possibly others. It is reasonable to anticipate that the list of cellular proteins interacting with the SV40 Tag will expand with further investigations. Probably, we still have an insufficient interpretation of the net result of all these interactions. However, the known outcomes of SV40 introduction in a host cell include induction of the insulin-like growth factor (IGF)-I and its receptor (72,73), induction of cyclin A (74) and the cyclin-dependent kinase cdc2 (75), and promotion of chromosomal instability (76,77). More recent studies (see below) have indicated that Tag participates (at least in some human cell systems) in the induction of *met* signaling (78), telomerase activation (79), RASSF1A repression (80), and Notch-1 induction (81). To summarize, Tag affects nuclear functions through transcriptional transactivation, protein inhibition, and direct binding of Tag to the host DNA. The outcomes are aimed both at the inhibition of cell cycle checkpoints (p53 and pRbs interactions) and at the induction of proteins involved in the promotion of cell cycle (cyclin A, cdc2). At the same time, Tag indirectly provides critical survival signals to the cell through the IGF-I pathway, which may play an essential role for circumventing apoptosis during the early phases of SV40 infection.

Tag has a J-domain in its N-terminal region that mediates its binding to Hsc-70. Mutational analyses have revealed that the majority of Tag functions require an intact J-domain, suggesting that Hsc-70 may play a critical role in the execution of Tag activities (82). In mouse cells, the minimal fragment of Tag sufficient for cell transformation includes the J-domain and a region (amino acids 102–115) responsible for Tag binding to pRb protein family (82,83). On the other hand, mutant Tag molecules missing the N-terminal J-domain are virtually inactive.

These observations have suggested that the SV40 Tag may function essentially as a molecular chaperone through its interaction with Hsc-70 mediated by the Tag J-domain (82,84). It is possible that Tag may interfere with a number of cellular protein functions by modifying their conformation, thus operating as a sort of nuclear chaperone specific for a number of substrates. This would explain why Tag profoundly affects many cellular pathways though being expressed at very low levels in early-infected cells (29).

The functions of Tag are implemented in the cytoplasm by the SV40 tag. The latter is a small protein consisting of 174 amino acids. The first 82 residues of tag are identical to those of Tag; therefore, both SV40 oncoproteins share the same J-domain (reviewed in ref. 68). Downstream from the J-domain there are sequences that mediate tag interaction with cellular protein phosphatase 2A (PP2A) (85). Functions of the SV40 tag include transactivation (and transrepression) of cellular and viral promoters and inhibition of PP2A (reviewed in ref. 86). The latter represents the best known function of tag. PP2A dephosphorylates a number of cellular proteins including components of the mitogen activated protein kinases (MAPKs). The latter proteins are involved in the phosphorylation cascade that mediates the signal transduction pathway common to several growth factor receptors. PP2A exists as a heterotrimer of B, A, and C subunits. The SV40 tag binds PP2A trimer and displaces the B subunit, with consequent reduction of PP2A activity (86). Thus, through its inhibition of PP2A, tag indirectly reinforces mitogenic stimuli by intensifying MAPK signaling, an event that leads to activating protein (AP)-1 induction (87), and transcriptional induction of the key cell cycle regulators cyclin D1 (88). The resulting effect of the SV40 tag is to amplify the activities of Tag, especially during the first phase of SV40 infection (reviewed in ref. 86). Nevertheless, tag is capable of inducing S-phase entry independently from Tag in certain cell systems and, more importantly, its functions are required for SV40-mediated transformation of human cells (86).

SV40 and Apoptosis

The mechanisms through which SV40-containing cells escape apoptosis are still controversial. In theory, Tag should possess both pro- and antiapoptotic activities. Tag binds and inhibits p53; thus it should suppress p53-dependent apoptosis. On the other hand, binding of Tag to pRb causes the release of E2F-1, which is a potent inducer of apoptosis (89,90). However, the experimental data gathered so far complicate this probably oversimplistic model of Tag interaction with proteins that regulate apoptosis. In fact, Tag promotes p53-dependent apoptosis in rat embryonic fibroblasts exposed to genotoxic chemicals (91). A Bcl2-like domain has been identified in Tag, and this domain works in protecting certain cells from apoptosis (92). These observations suggest that Tag may affect the intrinsic pathway of apoptosis directly by means of its Bcl2-like domain. Yet, the actual significance of this domain is rather

obscure. As an example, Tag-induced enhancement of p53-dependent apoptosis induced in rat embryo fibroblasts after exposure to genotoxic chemicals appears completely unaffected by mutations in the Bcl2-like domain (91). We have recently shown that SV40 infection induces Notch-1 (81). Notch-1 is a highly pleiotropic protein that regulates critical cell fate decisions during development and differentiation in a wide spectrum of Methazoan (93). Among other functions, Notch-1 acts as an antiapoptotic protein in murine erythroleukemia cells (94). Thus, it is possible that activation of Notch-1 signaling may participate in prevention of apoptosis in SV40-containing cells, and this hypothesis is now being tested in our laboratory.

Tag seems to protect liver cells apoptosis mediated by Fas. However, in this system Tag apparently does not affect the expression levels of Fas itself; rather, it enhances a protective mechanism involving the protein kinase C signaling pathway (95), thus implicating tag in this process. One study supports this interpretation and indicates that expression of the tag alone in transgenic mice is sufficient to confer liver cells resistance to CD95-mediated apoptosis (96). Furthermore, tag protects from apoptosis rat embryo fibroblasts harboring mutations in the Tag (97). Indeed, the SV40 tag is potentially an antiapoptotic protein. By means of its inhibition of PP2A, tag should reinforce survival signals acting through the protein kinase B/Akt signaling pathway. Akt phosphorylates and inhibits a number of components of the cell death machinery, including BAD, pro-caspase 9, and MDM2 (reviewed in ref. 98). Nevertheless, the outcomes of tag functions on apoptosis are rather controversial as well. In fact, tag induces apoptosis in human osteosarcoma cells through a p53-independent mechanism (99).

Overall, it is probably reductive to clearly identify pro- or antiapoptotic functions of any of the SV40 oncoproteins. All cellular oncogenes share activities that induce cell proliferation and apoptosis. It is now recognized that the proliferative and apoptotic pathways are coupled, and that the role played by survival factors in completing proliferation versus programmed cell death choices can be critical (100). In this light, induction of the IGF and hepatocyte growth factor (HGF) pathways by SV40 may be the key in understanding the overall avoidance of apoptosis in SV40-infected cells (which express the entire SV40 genome and not just portions of the SV40 genome, as most of the systems described above). The recent finding that Tag translocates the IGF-I receptor substrate in the nucleus (101) further underscores the intertwined relation between SV40 and survival signals acting through the IGF pathway.

SV40 Transformation of Human Cells

Human cells from diverse tissues have been transformed using SV40 or the SV40 oncoproteins (59). Among cells of different tissue origin, fibroblasts are the most thoroughly investigated human cell type for SV40-mediated transformation in vitro. Most of the studies have been performed using replication-defective SV40 mutants to avoid SV40-

induced cell death. In these stable transfections, SV40 increases the fibroblasts' proliferative potential of 20 to 30 cell doublings, without conferring tumorigenicity in immunocompromised mice. Then the transformed population enters the so-called crisis characterized by cell growth arrest and senescence that ultimately causes apoptosis in most of the cell population (102). Occasional cells escape senescence and give rise to immortal clones (103,104). In conclusion, in the conditions described above (transfection of the SV40 early region), SV40 inhibition of the p53 and pRb pathways is insufficient to prevent crisis.

The discovery of telomerase and its role in cellular immortalization has provided a better understanding of the process of malignant transformation of the cell (reviewed in ref. 105). Telomerase is an RNA-protein complex that ensures the maintenance of the length of chromosome ends (telomeres). One of the protein components of telomerase (TERT) is a reverse transcriptase that uses the RNA component of the telomerase complex as a template to add tandem repeats of a short sequence (TTAGGG in mammals) at the chromosome ends (105). Human somatic cells do not express TERT. In the absence of telomerase activity the ends of chromosomes shorten at every cycle of cell division because of the intrinsic mechanism of DNA replication. The cell has evolved a sensitive network controlling the length of telomeres. This is necessary because progressive shortening of telomeres eventually leads to dramatic chromosomal instability. Therefore, telomeres function as a sort of "hourglass" for cell division: when telomeres approach a critical size, the cell deploys an irreversible pathway that leads to cell cycle arrest, senescence, and apoptosis (102,106). As a consequence, all tumor cells need to induce some mechanism to ensure the maintenance of telomere length in order to proliferate indefinitely. The experimental evidence available indicates that the majority of human tumors achieve de novo expression of TERT as a result of mutational events. Human tumors in which TERT is not expressed have developed an alternative process of telomeres elongation through recombination, termed ALT, characterized by unusually long telomeres (107). A recent study has shown that SV40 is unable to induce telomerase activity in human fibroblasts (79). Therefore, immortal fibroblasts can arise only after sporadic activation of telomerase due to chromosomal rearrangements or mutations. This explains the infrequency of malignant transformation of fibroblasts after exposure to SV40. A similar situation is repeated in most human cell systems. For example, mammary gland epithelial cells transformed by SV40 undergo senescence, which is not circumvented by the introduction of oncogenic H-*ras*. However, sporadic cells escape crisis and form tumorigenic lines in mice (108). In synthesis, these studies have shown that the introduction of the SV40 oncoproteins, in conjunction with the expression of a chronic mitogenic stimulus represented by oncogenic H-*ras*, is insufficient for malignant transformation of human cells. In these settings, malignant transformation still requires random mutagenic events that affect an undefined number of genetic elements. Recent studies have demonstrated that in the conditions described above, activation of TERT is the critical requirement for malignant

transition. Indeed, the minimal genetic elements for normal human cells to become transplantable tumors in experimental animals are represented by an oncogenic H-*ras* allele, the SV40 early region and active telomerase (109). These apparently limited requirements have led some researchers to the conclusion that the transition to a fully malignant state is achieved in human cells through activation of telomerase, the induction of a chronic mitogenic stimulus, inhibition of both p53 and pRb pathways, and inactivation of PP2A. The SV40 early gene products provide the latter three functions (110).

This recapitulation of the carcinogenetic process in human systems is probably oversimplified. The SV40 oncoproteins do not simply inhibit p53, pRb protein family, and PP2A, but participate in a number of interactions that profoundly affect the entire cell fate program of the host. In other terms, researchers tend to underestimate the extent of multifunctionality and the complexity of operation of the SV40 tumor antigens. A good example of this is provided by the interaction of SV40 with the Notch signaling pathway (see below). Immortal, SV40-transformed human mesothelial cells are completely growth-arrested if activation of Notch is impaired through chemical inhibition (81), implicating Notch-1 as another essential player in the process of malignant transformation of at least some human cell types. Accordingly, interference with Notch-1 using either chemical inhibition or antisense technology suppresses the malignant phenotype of human fibroblasts expressing TERT, oncogenic *ras*, and SV40 oncoproteins (111). This evidence suggests that our interpretation of the molecular circuitry of cancer is still rather incomplete, and additional genetic elements may soon be included among those required for the malignant transformation of human cells.

SV40 and Malignant Mesothelioma

SV40 is highly oncogenic in the hamster, where it induces tumors of different types. The pattern of tumor formation is dependent on the site of administration. When injected intracardially, so that it can spread to all organs, SV40 induces malignant mesothelioma (MM) in about 60% of animals after a latency of 3 months (112). The remaining 40% of hamsters develop lymphomas, osteosarcomas, and myxomas (112). The induction of mesotheliomas in these animals is strictly dependent on the expression of tag, since hamsters injected with SV40 mutants unable to synthesize tag developed lymphomas (the relevance of tag in the process of mesothelial cells transformation is further discussed below). If SV40 is injected directly into the pleural space, 100% of hamsters develop MM (112). Intracranial injection of SV40 causes brain tumors, while subcutaneous injection causes lymphomas and sarcomas at the site of injection (reviewed in ref. 16). This pattern of tumor formation suggests that the hamster mesothelium is particularly susceptible to SV40-mediated oncogenesis.

In the past decade, SV40 has been detected by different laboratories worldwide in a number of human tumors using a variety of techniques

pointed at the detection of SV40 DNA, RNA, and oncoproteins in the tumor specimens (16,17). The wealth of evidence linking SV40 to specific human cancers is such that now three different panels of scientists have conclusively linked SV40 to human cancer (18–20). Strikingly, the panel of human tumors in which SV40 has been detected perfectly matches that induced by SV40 in hamsters, suggesting that SV40 may play a causative role in the onset of these malignancies (Fig. 3.4). Malignant mesothelioma represents the human tumor in which SV40 association and putative oncogenicity have been more extensively studied. The overall consensus is that 50% to 60% of MMs in the United States contain SV40. Geographic differences in the prevalence of SV40 in MM have been described, and the possibility that such variance may originate from differences in SV40 contamination of poliovaccine preparations used in various countries has been proposed (113–118). The association of SV40 with MM is highly specific, since SV40-positive MM specimens contain SV40 only in the tumor cells, while the surrounding stromal tissue is SV40-free (119,120). SV40 is biologically active in MM, because its major oncoprotein Tag has been demonstrated to bind cellular p53 (121) and pRb protein family members (122). The functions of the SV40 oncoproteins are required for the maintenance of the malignant phenotype in MM, since targeting Tag through antisense techniques causes growth arrest and apoptosis in SV40-positive MM cell lines (123). Moreover, human mesothelial cells are uniquely susceptible to SV40-mediated transformation and immortalization in vitro (see below), and evidence of co-carcinogenicity between asbestos and SV40 has been described (21). All this evidence strongly implicates SV40 as a causative agent in MM.

In theory, SV40 may be implicated in the origin of more MMs than those in which it is detected. Some investigators have proposed that SV40 may contribute to cancer formation according to a "hit-and-run" mechanism (reviewed in ref. 124). According to this model, SV40 may be required for the initial stages of tumor formation. During proliferation of the tumor a fraction of the cell population may acquire a number of mutations so that the functions of the SV40 oncoproteins may become disposable. In such a scenario it is conceivable that the SV40-containing cancer cells may be counterselected, since the SV40 oncoproteins are immunogenic, and because SV40 (in these settings) would represent just a metabolic burden. Furthermore, SV40 DNA does not preferentially integrate into the genome of human cancer cells (22), a situation that mirrors that of Epstein-Barr virus (EBV)-mediated carcinogenicity (125). In conclusion, the hit-and-run model could potentially take place in certain cases, and some in vitro experimental data have been produced supporting it (126–130). Some in vivo SV40-driven tumor models also support the hit-and-run mechanism. Transgenic mice expressing Tag under the control of inducible promoters display proliferative disorders and tumors that are dependent on Tag, since suppression of Tag expression causes reversion of the malignant phenotype. However, these tumors become Tag-independent (or partially Tag-independent) if the expression of Tag is silenced after longer periods of time (48,131). In spite of these experimental models, there is

insufficient knowledge on the extent of the hit-and-run process in human cancer, or whether it occurs at all in natural conditions.

Interaction of SV40 with Human Mesothelial Cells in Vitro

In the past 10 years we have studied the molecular effects produced by infection of primary human mesothelial (HM) cells with live SV40 virus. These studies are critically important to understand the pathogenesis of MM, since SV40 is present in MM as a complete virus and not as a nonreplicating, molecularly engineered, transfected plasmid. We found that the SV40 pattern of infection of HM cells is substantially different from the traditional semipermissive SV40 infection of human cells. SV40-infected HM cells express the SV40 early genes, replicate SV40 chromosomes, and synthesize capsid proteins and complete viral particles. However, the majority of infected cells do not undergo SV40-mediated cell lysis, because HM cells synthesize amounts of SV40 compatible with cell survival (21). Survival in the presence of potentially oncogenic SV40 tumor antigens causes a very high rate of cell transformation in SV40-infected HM. A few weeks after the introduction of SV40 in the population, three-dimensional foci of HM cells that have lost contact-inhibition arise. The frequency of focus formation in HM cells is on average 0.2×10^{-4} in different primary cultures (21). Nearly 100% of these foci can be established in culture as cell lines that display a completely transformed phenotype in vitro and are immortal. These results are unique under several perspectives. No other cells have been described to acquire a completely transformed phenotype with such high frequency after SV40 infection (about 1 of 5000 infected HM cells). Moreover, less than 5% of foci developing after SV40 transformation of cells of different origin can be successfully established as cell lines in culture (reviewed in ref. 132). Instead, about 90% of SV40-transformed HM cell lines appear immortal from the start because they never develop a crisis. Recent data have provided a rationale for this unprecedented result. We found that SV40 induces telomerase activity in HM cells as early as 72 hours after infection, and this activity increases with cell passage in vitro (79). This indicated that SV40-transformed HM cells do not need additional mutational events to become immortal, since telomerase activation is a process intimately connected with SV40 infection. SV40-mediated induction of telomerase activity may be specific for HM cells, because telomerase activity was not detected in SV40-infected primary fibroblasts (79). Besides its role in cell immortalization, induction of telomerase by SV40 may be a process connected with focus formation, since TERT has been shown to possess transforming activities (106). SV40 also specifically induces HGF and promotes HGF receptor (*met*) phosphorylation in HM cells, and these cellular effects appear to be a consequence of Tag interaction with pRb (78). SV40-dependent activation of the *met* pathway in HM cells has several implications in the process of HM malignant transformation. Since the proto-oncogene *met* is upstream from *ras*, chronic activation

of the *met* pathway may reproduce, at least in part, the consequences of a constitutively active *ras* allele. Collectively, SV40 infection of HM cells provides a human cell with telomerase activation, *met*, and IGF pathways activation, and (obviously) with the expression of the SV40 oncoproteins. Taking into account the minimal genetic requirements for oncogenic transformation of human cells (109), SV40 appears to be a complete carcinogen for HM cells, with the immune system acting as the ultimate fail-safe for MM development after SV40 infection of the human mesothelium. However, the in vitro data would not support the latter interpretation, since (on average) 1 out of 5000 HM cells will give rise to an immortal cell line after SV40 infection (21). This entails that *met* and IGF pathways activation do not fully complement an oncogenic *ras* allele, and that the event of focus formation in HM is determined by any mutational occurrence that reproduces the cellular outcomes of oncogenic *ras* expression. Taking into account the extremely high frequency of malignant transformation of HM cells after SV40 infection, the latter situation must be achieved fairly frequently. SV40 induction of the *met* signaling pathway in HM cells has additional implications in the pathogenesis of MM. SV40-containing HM cells produce increased amounts of HGF, implying that SV40 can operate also as a landscaper factor in MM, and that SV40 may exert its oncogenic properties even though being expressed only in a fraction of the tumor cells population.

The study of the interactions between SV40 and HM cells in vitro provides a number of mechanistic explanations concerning typical phenotypes characteristic of SV40-positive MMs. In the latter tumors the suppressor gene *RASSF1A* is commonly silenced through methylation of its promoter. The latter instance appears directly mediated by SV40, because SV40 specifically promotes *RASSF1A* promoter methylation during the process of SV40 HM cell transformation (80). Moreover, in SV40-positive MM specimens the expression of the *Notch-1* gene is specifically induced, while SV40-negative MMs do not display upregulation of *Notch-1* (81). As previously indicated, *Notch* genes are proteins regulating crucial cell fate decision during development and differentiation. Such regulation is achieved through *Notch*-dependent control of cell proliferation and survival (reviewed in ref. 93). Deregulated or aberrant expression of Notch proteins is currently being studied in a number of human tumors (133), and appears to critically sustain the malignant phenotype (111). The characteristic *Notch-1* overexpression in SV40-positive MMs is also mirrored and mechanistically explained in the SV40-HM in vitro system. SV40 induces *Notch-1* expression early after HM infection. This induction is maintained through the process of SV40-dependent transformation of HM (and in cell lines derived from SV40-positive MMs), and *Notch-1* activation is necessary for the growth of SV40-containing transformed HMs (81).

As mentioned above, SV40 preferentially induces mesotheliomas in hamsters. However, SV40 mutants unable to produce a functional tag only occasionally induce mesotheliomas in these animals, while, in similar settings, the development of tumors of other origin appear to

be only partially dependent on the function of tag (112). This in vivo evidence indicates that tag functions must play critical roles in the hamster mesothelium. The interaction between hamster mesothelial cells and SV40 has been insufficiently studied so far. However, in human mesothelial cells tag is required for telomerase induction (79), *Notch-1* induction (81), and focus formation in vitro (21). These data underscore the pivotal role of the SV40 tag in the process of HM cell transformation in vitro.

Interactions of live virus with HM cells has also evidenced that gene dosage or expression levels of the SV40 oncoproteins can produce effects that, ultimately, are qualitatively different from transfection experiments. In fact, SV40 induces the *Notch-1* signaling pathway in HM cells only as a replication competent virus, and not as a replication-defective mutant. This situation can be circumvented if the SV40 oncoproteins are artificially expressed in HM cells under the control of a strong promoter (such as the cytomegalovirus promoter).

Crossing the Species Boundaries: SV40, a Human Virus?

SV40 shares with its closest homologues Jamestown Canyon virus (JCV) and BKV the property of exerting powerful oncogenic activities when it is introduced in other species; JCV and BKV are polyomaviruses that commonly infect humans (134,135). Their putative implication in certain human cancers has been proposed (136–139). More often JCV and BKV produce asymptomatic infections and latently persist in human tissues, but they can be reactivated in immunocompromised individuals and consequently cause lethal diseases. Likewise, SV40 establishes in monkeys a similar life cycle to that of polyomaviruses in humans. However, JCV is highly oncogenic in owl and squirrel monkeys (140), BKV causes different types of cancers in hamsters (141) and rats (142), and SV40 is highly oncogenic in hamsters and other rodents, and it is associated with different human cancers in which the experimental data available indicate that SV40 plays a pathogenic role (reviewed in ref. 16). We are not aware of any evidence that human polyomaviruses have propagated in other species under natural conditions. On the other hand, SV40 has been massively introduced into the human population during the early poliomyelitis vaccination program. Furthermore, SV40 seems to be capable to infect humans under natural or pseudonatural conditions. Up to 10% of individuals working in close contact with monkeys develop SV40-specific antibodies (143), and different percentages of laboratory personnel working with SV40, with monkeys, or with monkey cells display immunologic evidences of previous SV40 infections (reviewed in ref. 22). In conclusion, there is evidence that SV40 has entered the human population in different circumstances. The issue of possible human-to-human transmission of SV40 infections has been poorly investigated to date. However, SV40-specific antibodies are

detectable in children born many years after the administration of SV40-contaminated poliovaccines, and the prevalence of SV40-specific antibodies appears to increase with age (22). In some of the children possessing SV40-specific antibodies, SV40 DNA has been detected in tissue specimens using polymerase chain reaction (22). Furthermore, SV40 has been recently retrieved from sewage waters of Calcutta (India) where monkeys are absent (144). These findings strongly suggest that SV40 is being propagated in the human population, and that human-to-human transmission of SV40 is taking place at least in some areas of the world. More studies are needed to assess the extent of SV40 diffusion in human. However, the evidence available so far indicates that SV40 has permanently crossed the barrier between monkeys and humans, and that SV40 is now also a human virus.

Conclusion

The studies of SV40-mediated malignant transformation of cells of different origin, including human cells, underscore the extraordinarily powerful oncogenic potential of SV40. Many aspects of the SV40 natural distribution, prevalence, and life cycle are still largely unknown. The incidence of the human malignancies associated with SV40 is constantly rising, especially mesothelioma. Malignant mesothelioma was virtually unknown before the second half of the 20th century (145). The increase of mesothelioma cases from practically zero to the current 2000 to 3000 new cases per year in the United States alone has been attributed to the widespread use of asbestos during the first half of the 20th century. However, we also know that between 1954 and 1963 about 32 million individuals were injected with various amounts of infectious SV40 in the United States alone (145). Human mesothelial cells are uniquely susceptible to SV40-mediated transformation (21). SV40 is specifically linked to a substantial percentage of mesothelioma cases (16), and there is evidence that asbestos and SV40 can act as co-carcinogens (21). All the above facts indicate that SV40 is at least co-responsible for the rise of the incidence of mesothelioma. The evidence indicating that SV40 is presently propagating in the human population implies that human contact with this virus is not a mere accident circumscribed in time, but that SV40 represents a hazard for human health for some time to come. Therefore, there is an evident need for a broad and detailed study of SV40-mediated carcinogenesis in order to prevent and treat SV40-associated human diseases, including mesothelioma.

Acknowledgments

This work was supported by National Institutes of Health (NIH) grants RO1 CA092657, Riviera Country Club CRFA to Michele Carbone, and by NIH R21 CA91122 to Maurizio Bocchetta.

References

1. Sweet BH, Hilleman MR. The vacuolating virus, S.V.40. Proc Soc Exp Biol Med 1960;105:420–427.
2. Eddy BE. Tumors produced in hamsters by SV40. Fed Proc 1962;21:930–935.
3. Eddy BE, Borman GS, Grubbs GE, Young RD. Identification of the oncogenic substance in Rhesus monkey kidney cell cultures as simian virus 40. Virology 1962;17:65–75.
4. Fisher SG, Weber L, Carbone M. Cancer risk associated with simian virus 40 contaminated polio vaccine. Anticancer Res 1999;19:2173–2180.
5. Heinonen OP, Shapiro S, Monson RR, Harts SC, Rosenberg L, Slone D. Immunization during pregnancy against poliomyelitis and influenza in relationship to childhood malignancy. Int J Epidemiol 1973;2:229–235.
6. Innis MD. Oncogenesis and poliomyelitis vaccine. Nature 1968;219:972–973.
7. Mortimer EA Jr, Lepow ML, Gold E, Robbins FC, Burton GJ, Fraumeni JF Jr. Long-term follow-up of persons inadvertently inoculated with SV40 as neonates. N Engl J Med 1981;305:1517–1518.
8. Strickler HD, Rosenberg PS, Devesa SS, Hertel J, Fraumeni JF Jr, Goedert JJ. Contamination of poliovirus vaccines with simian virus 40 (1955–1963) and subsequent cancer rates. JAMA 1998;279:292–295.
9. Krieg P, Amtmann E, Jonas D, Fischer H, Zang K, Sauer G. Episomal simian virus 40 genomes in human brain tumors. Proc Natl Acad Sci USA 1981;78:6446–6450.
10. Krieg P, Scherer G. Cloning of SV40 genomes from human brain tumors. Virology 1984;138:336–340.
11. Meinke W, Goldstein DA, Smith RA. Simian virus 40-related DNA sequences in a human brain tumor. Neurology 1979;29:1590–1594.
12. Scherneck S, Rudolph M, Geissler E, et al. Isolation of a SV40-like papovavirus from a human glioblastoma. Int J Cancer 1979;24:523–531.
13. Soriano F, Shelburne CE, Gokcen M. Simian virus 40 in a human cancer. Nature 1974;249:421–424.
14. Tabuchi K, Kirsch WM, Low M, Gaskin D, Van Buskirk J, Maa S. Screening of human brain tumors for SV40-related T antigen. Int J Cancer 1978; 21:12–17.
15. Weiss AF, Portmann R, Fischer H, Simon J, Zang KD. Simian virus 40-related antigens in three human meningiomas with defined chromosome loss. Proc Natl Acad Sci USA 1975;72:609–613.
16. Gazdar AF, Butel JS, Carbone M. Opinion: SV40 and human tumours: myth, association or causality? Nat Rev Cancer 2002;2:957–964.
17. Jasani B, Cristaudo A, Emri SA, et al. Association of SV40 with human tumours. Semin Cancer Biol 2001;11:49–61.
18. Klein G, Powers A, Croce C. Association of SV40 with human tumors. Oncogene 2002;21:1141–1149.
19. Wong M, Pagano JS, Shiller JT, Tevethia SS, Raab-Traub N, Gruber J. New associations of human papillomavirus, simian virus 40, and Epstein-Barr virus with human cancer. J Natl Cancer Inst 2002;94:267–273.
20. Stratton K, Almario DA, McCormick M, eds. IOM report: immunization safety review. SV40 contamination of poliovaccine and cancer. National Academy of Sciences, www.nap.edu, 2002.

21. Bocchetta M, Di Resta I, Powers A, et al. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. Proc Natl Acad Sci USA 2000;97:10214–10219.
22. Butel JS, Lednicky JA. Cell and molecular biology of simian virus 40: implications for human infections and disease. J Natl Cancer Inst 1999; 91:119–134.
23. Carbone M, Rizzo P, Procopio A, et al. SV40-like sequences in human bone tumors. Oncogene 1996;13:527–535.
24. Atwood WJ, Norkin LC. Class I major histocompatibility proteins as cell surface receptors for simian virus 40. J Virol 1989;63:4474–4477.
25. Breau WC, Atwood WJ, Norkin LC. Class I major histocompatibility proteins are an essential component of the simian virus 40 receptor. J Virol 1992;66:2037–2045.
26. Anderson HA, Chen Y, Norkin LC. MHC class I molecules are enriched in caveolae but do not enter with simian virus 40. J Gen Virol 1998;79: 1469–1477.
27. Nakanishi A, Shum D, Morioka H, Otsuka E, Kasamatsu H. Interaction of the Vp3 nuclear localization signal with the importin alpha 2/beta heterodimer directs nuclear entry of infecting simian virus 40. J Virol 2002; 76:9368–9377.
28. Berk AJ, Sharp PA. Spliced early mRNAs of simian virus 40. Proc Natl Acad Sci USA 1978;75:1274–1278.
29. Cole CN. Polyomavirinae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, et al, eds. Fields' Virology, 3rd ed. Philadelphia: Lippincott-Raven, 1996.
30. Noble JC, Prives C, Manley JL. In vitro splicing of simian virus 40 early pre mRNA. Nucleic Acids Res 1986;14:1219–1235.
31. Mastrangelo IA, Hough PV, Wall JS, Dodson M, Dean FB, Hurwitz J. ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication. Nature 1989;338:658–662.
32. Collins KL, Kelly TJ. Effects of T antigen and replication protein A on the initiation of DNA synthesis by DNA polymerase alpha-primase. Mol Cell Biol 1991;11:2108–2115.
33. Dean FB, Bullock P, Murakami Y, Wobbe CR, Weissbach L, Hurwitz J. Simian virus 40 (SV40) DNA replication: SV40 large T antigen unwinds DNA containing the SV40 origin of replication. Proc Natl Acad Sci USA 1987;84:16–20.
34. Wiley SR, Kraus RJ, Zuo F, Murray EE, Loritz K, Mertz JE. SV40 early-to-late switch involves titration of cellular transcriptional repressors. Genes Dev 1993;7:2206–2219.
35. Zuo F, Mertz JE. Simian virus 40 late gene expression is regulated by members of the steroid/thyroid hormone receptor superfamily. Proc Natl Acad Sci USA 1995;92:8586–8590.
36. Keller JM, Alwine JC. Analysis of an activatable promoter: sequences in the simian virus 40 late promoter required for T-antigen-mediated transactivation. Mol Cell Biol 1985;5:1859–1869.
37. Dean DA, Li PP, Lee LM, Kasamatsu H. Essential role of the Vp2 and Vp3 DNA-binding domain in simian virus 40 morphogenesis. J Virol 1995;69: 1115–1121.
38. Gordon-Shaag A, Ben-Nun-Shaul O, Roitman V, Yosef Y, Oppenheim A. Cellular transcription factor Sp1 recruits simian virus 40 capsid proteins to the viral packaging signal, ses. J Virol 2002;76:5915–5924.
39. Gordon-Shaag A, Ben-Nun-Shaul O, Kasamatsu H, Oppenheim AB, Oppenheim A. The SV40 capsid protein VP3 cooperates with the cellular

transcription factor Sp1 in DNA-binding and in regulating viral promoter activity. J Mol Biol 1998;275:187–195.
40. Carswell S, Alwine JC. Simian virus 40 agnoprotein facilitates perinuclear-nuclear localization of VP1, the major capsid protein. J Virol 1986;60: 1055–1061.
41. Darbinyan A, Darbinian N, Safak M, Radhakrishnan S, Giordano A, Khalili K. Evidence for dysregulation of cell cycle by human polyomavirus, JCV, late auxiliary protein. Oncogene 2002;21:5574–5581.
42. Safak M, Khalili K. Physical and functional interaction between viral and cellular proteins modulate JCV gene transcription. J Neurovirol 7:288–292.
43. Sack GH Jr. Human cell transformation by simian virus 40—a review. In Vitro 1980;17:1–19.
44. Meyer HM Jr, Hopps HE, Rogers NG, et al. Studies on simian virus 40. J Immunol 1962;88:796–806.
45. Wobbe CR, Weissbach L, Borowiec JA, et al. Replication of simian virus 40 origin-containing DNA in vitro with purified proteins. Proc Natl Acad Sci USA 1987;84:1834–1838.
46. Shay JW, Van Der Haegen BA, Ying Y, Wright WE. The frequency of immortalization of human fibroblasts and mammary epithelial cells transfected with SV40 large T-antigen. Exp Cell Res 1993;209:45–52.
47. Brinster RL, Chen HY, Messing A, van Dyke T, Levine AJ, Palmiter RD. Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. Cell 1984;37:367–379.
48. Ewald D, Li M, Efrat S, et al. Time-sensitive reversal of hyperplasia in transgenic mice expressing SV40 T antigen. Science 1996;273:1384–1386.
49. Maroulakou IG, Anver M, Garrett L, Green JE. Prostate and mammary adenocarcinoma in transgenic mice carrying a rat C3(1) simian virus 40 large tumor antigen fusion gene. Proc Natl Acad Sci USA 1997;91: 11236–11240.
50. Tzeng YJ, Guhl E, Graessmann M, Graessmann A. Breast cancer formation in transgenic animals induced by the whey acidic protein SV40 T antigen (WAP-SV-T) hybrid gene. Oncogene 1993;8:1965–1971.
51. Clayson ET, Brando LV, Compans RW. Release of simian virus 40 virions from epithelial cells is polarized and occurs without cell lysis. J Virol 1989; 63:2278–2288.
52. O'Neill FJ, Carroll D. Amplification of papovavirus defectives during serial low multiplicity infections. Virology 1981;112:800–803.
53. O'Neill FJ, Carney H, Hu Y. Host range analysis of simian virus 40, BK virus and chimaeric SV40/BKV: relative expression of large T-antigen and Vp1 in infected and transformed cells. Dev Biol Stand 1998;94:191–205.
54. Shein HM. Transformation of astrocytes and destruction of spongioblasts induced by a simian tumor virus (SV40) in cultures of human fetal neuroglia. J Neuropathol Exp Neurol 1967;26:60–76.
55. Stein HM, Enders JF. Multiplication of cytopathogenicity of simian vacuolating virus 40 in cultures of human tissues. Proc Soc Exp Biol Med 1962;109:495–500.
56. O'Neill FJ, Xu XL, Miller TH. Host range determinant in the late region of SV40 and RF virus affecting growth in human cells. Intervirology 1990; 31:175–187.
57. Aaronson SA. Susceptibility of human cell strains to transformation by simian virus 40 and simian virus 40 deoxyribonucleic acid. J Virol 1970;6: 470–475.

58. Carp RI, Gilden RV. A comparison of the replication cycles of simian virus 40 in human diploid and African green monkey kidney cells. Virology 1966;28:150–162.
59. Bryan TM, Reddel RR. SV40-induced immortalization of human cells. Crit Rev Oncol 1994;5:331–357.
60. Staufenbiel M, Deppert W. Different structural systems of the nucleus are targets for SV40 large T antigen. Cell 1983;33:173–181.
61. Hansen R, Reddel R, Braithwaite A. The transforming oncoproteins determine the mechanism by which p53 suppresses cell transformation: pRb-mediated growth arrest or apoptosis. Oncogene 1995;11:2535–2545.
62. Testa JR, Giordano A. SV40 and cell cycle perturbations in malignant mesothelioma. Semin Cancer Biol 2001;11:31–38.
63. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 1992;89:7491–7495.
64. Lomazzi M, Moroni MC, Jensen MR, Frittoli E, Helin K. Suppression of the p53- or pRB-mediated G1 checkpoint is required for E2F-induced S-phase entry. Nat Genet 2002;31:190–194.
65. Wang B, Matsuoka S, Carpenter PB, Elledge SJ. 53BP1, a mediator of the DNA damage checkpoint. Science 2002;298:1435–1438.
66. Avantaggiati ML, Carbone M, Graessmann A, Nakatani Y, Howard B, Levine AS. The SV40 large T antigen and adenovirus E1a oncoproteins interact with distinct isoforms of the transcriptional co-activator, p300. EMBO J 1996;15:2236–2248.
67. Lill NL, Tevethia MJ, Eckner R, Livingston DM, Modjtahedi N. p300 family members associate with the carboxyl terminus of simian virus 40 large tumor antigen. J Virol 1997;71:129–137.
68. Ali SH, DeCaprio JA. Cellular transformation by SV40 large T antigen: interaction with host proteins. Semin Cancer Biol 2001;11:15–23.
69. Miccoli L, Biard DS, Creminon C, Angulo JF. Human kin17 protein directly interacts with the simian virus 40 large T antigen and inhibits DNA replication. Cancer Res 2002;62:5425–5435.
70. Damania B, Alwine JC. TAF-like function of SV40 large T antigen. Genes Dev 1996;10:1369–1381.
71. Adamczewski JP, Gannon JV, Hunt T. Simian virus 40 large T antigen associates with cyclin A and p33cdk2. J Virol 1993;67:6551–6557.
72. Baserga R, Sell C, Porcu P, Rubini M. The role of the IGF-I receptor in the growth and transformation of mammalian cells. Cell Prolif 1994;27:63–71.
73. Porcu P, Grana X, Li S, Swantek J, De Luca A, Giordano A, Baserga R. An E2F binding sequence negatively regulates the response of the insulin-like growth factor 1 (IGF-I) promoter to simian virus 40T antigen and to serum. Oncogene 1994;9:2125–2134.
74. Oshima J, Steinmann KE, Campisi J, Schlegel R. Modulation of cell growth, p34cdc2 and cyclin A levels by SV-40 large T antigen. Oncogene 1993;8:2987–2993.
75. Chen H, Campisi J, Padmanabhan R. SV40 large T antigen transactivates the human cdc2 promoter by inducing a CCAAT box binding factor. J Biol Chem 1996;271:13959–13967.
76. Mekeel KL, Tang W, Kachnic LA, Luo CM, DeFrank JS, Powell SN. Inactivation of p53 results in high rates of homologous recombination. Oncogene 1997;14:1847–1857.
77. Ray FA, Meyne J, Kraemer PM. SV40 T antigen induced chromosomal changes reflect a process that is both clastogenic and aneuploidogenic and

is ongoing throughout neoplastic progression of human fibroblasts. Mutat Res 1992;284:265–273.
78. Cacciotti P, Libener R, Betta P, et al. SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. Proc Natl Acad Sci USA 2001;98:12032–12037.
79. Foddis R, De Rienzo A, Broccoli D, et al. SV40 infection induces telomerase activity in human mesothelial cells. Oncogene 2002;21:1434–1442.
80. Toyooka S, Carbone M, Toyooka KO, et al. Progressive aberrant methylation of the RASSF1A gene in simian virus 40 infected human mesothelial cells. Oncogene 2002;21:4340–4344.
81. Bocchetta M, Miele L, Pass HI, Carbone M. Notch-1 induction, a novel activity of SV40 required for growth of SV40-transformed human mesothelial cells. Oncogene 2002;22:81–89.
82. Sullivan CS, Pipas JM. T antigens of simian virus 40: molecular chaperones for viral replication and tumorigenesis. Microbiol Mol Biol Rev 2002; 66:179–202.
83. Wang JK, Knudsen ES, Welch PJ. The retinoblastoma tumor suppressor protein. Adv Cancer Res 1994;64:25–85.
84. Kim HY, Ahn BY, Cho Y. Structural basis for the inactivation of retinoblastoma tumor suppressor by SV40 large T antigen. EMBO J 2001;20:295–304.
85. Mungre S, Enderle K, Turk B, et al. Mutations which affect the inhibition of protein phosphatase 2A by simian virus 40 small-t antigen in vitro decrease viral transformation. J Virol 1994;68:1675–1681.
86. Rundell K, Parakati R. The role of the SV40 ST antigen in cell growth promotion and transformation. Semin Cancer Biol 2001;11:5–13.
87. Frost JA, Alberts AS, Sontag E, Guan K, Mumby MC, Feramisco JR. Simian virus 40 small t antigen cooperates with mitogen-activated kinases to stimulate AP-1 activity. Mol Cell Biol 1994;14:6244–6252.
88. Watanabe G, Howe A, Lee RJ, et al. Induction of cyclin D1 by simian virus 40 small tumor antigen. Proc Natl Acad Sci USA 1996;93:12861–12866.
89. Kowalik TF, DeGregori J, Schwarz JK, Nevins JR. E2F1 overexpression in quiescent fibroblasts leads to induction of cellular DNA synthesis and apoptosis. J Virol 1995;69:2491–2500.
90. Qin XQ, Livingston DM, Kaelin WG Jr, Adams PD. Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. Proc Natl Acad Sci USA 1994;91:10918–10922.
91. Cole SL, Tevethia MJ. Simian virus 40 large T antigen and two independent T-antigen segments sensitize cells to apoptosis following genotoxic damage. J Virol 2002;76:8420–8432.
92. Conzen SD, Snay CA, Cole CN. Identification of a novel antiapoptotic functional domain in simian virus 40 large T antigen. J Virol 1997;71:4536–4543.
93. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999;284:770–776.
94. Shelly LL, Fuchs C, Miele L. Notch-1 inhibits apoptosis in murine erythroleukemia cells and is necessary for differentiation induced by hybrid polar compounds. J Cell Biochem 1999;73:164–175.
95. Rouquet N, Allemand I, Molina T, Bennoun M, Briand P, Joulin V. Fas-dependent apoptosis is impaired by SV40 T-antigen in transgenic liver. Oncogene 1995;11:1061–1067.
96. Gillet R, Cavard C, Grimber G, Briand P, Joulin V. Hepatic expression of SV40 small-T antigen blocks the in vivo CD95-mediated apoptosis. Biochem Biophys Res Commun 2001;284:369–376.

97. Kolzau T, Hansen RS, Zahra D, Reddel RR, Braithwaite AW. Inhibition of SV40 large T antigen induced apoptosis by small T antigen. Oncogene 1999;18:5598–5603.
98. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase-Akt pathway in human cancer. Nat Rev Cancer 2002;2:489–501.
99. Gjoerup O, Zaveri D, Roberts TM. Induction of p53-independent apoptosis by simian virus 40 small t antigen. J Virol 2001;75:9142–9155.
100. Evan G, Littlewood T. A matter of life and cell death. Science 1998; 281:1317–1322.
101. Prisco M, Santini F, Baffa R, et al. Nuclear translocation of insulin receptor substrate-1 by the simian virus 40 T antigen and the activated type 1 insulin-like growth factor receptor. J Biol Chem 2002;277:32078–32085.
102. Macera-Bloch L, Houghton J, Lenahan M, Jha KK, Ozer HL. Termination of lifespan of SV40-transformed human fibroblasts in crisis is due to apoptosis. J Cell Physiol 2002;190:332–344.
103. Huschtscha LI, Holliday R. Limited and unlimited growth of SV40-transformed cells from human diploid MRC-5 fibroblasts. J Cell Sci 1983; 63:77–99.
104. Ozer HL, Banga SS, Dasgupta T, et al. SV40-mediated immortalization of human fibroblasts. Exp Gerontol 1996;31:303–310.
105. Weinberg RA. Telomeres. Bumps on the road to immortality. Nature 1998; 396:23–24.
106. Stewart SA, Hahn WC, O'Connor BF, et al. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. Proc Natl Acad Sci USA 2002;99:12606–12611.
107. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med 1997;3:1271–1274.
108. Bartek J, Bartkova J, Kyprianou N, et al. Efficient immortalization of luminal epithelial cells from human mammary gland by introduction of simian virus 40 large tumor antigen with a recombinant retrovirus. Proc Natl Acad Sci USA 1991;88:3520–3524.
109. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. Nature 1999;400:464–468.
110. Hahn WC, Dessain SK, Brooks MW, et al. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Mol Cell Biol 2002;22:2111–2123.
111. Weijzen S, Rizzo P, Braid M, et al. Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. Nat Med 2002;8:979–986.
112. Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. Am J Pathol 1993;142:1524–1533.
113. De Rienzo A, Tor M, Sterman DH, Aksoy F, Albelda SM, Testa JR. Detection of SV40 DNA sequences in malignant mesothelioma specimens from the United States, but not from Turkey. J Cell Biochem 2002;84:455–449.
114. Emri S, Kocagoz T, Olut A, Gungen Y, Mutti L, Baris YI. Simian virus 40 is not a cofactor in the pathogenesis of environmentally induced malignant pleural mesothelioma in Turkey. Anticancer Res 2000;20:891–894.
115. Heinsohn S, Scholz RB, Weber B, et al. SV40 sequences in human osteosarcoma of German origin. Anticancer Res 2000;20:4539–4545.
116. Hirvonen A, Mattson K, Karjalainen A, et al. Simian virus 40 (SV40)-like DNA sequences not detectable in Finnish mesothelioma patients

not exposed to SV40-contaminated polio vaccines. Mol Carcinog 1999;26: 93–99.
117. Leithner A, Weinhaeusel A, Windhager R, et al. Absence of SV40 in Austrian tumors correlates with low incidence of mesotheliomas. Cancer Biol Ther 2002;1:375–379.
118. Priftakis P, Bogdanovic G, Hjerpe A, Dalianis T. Presence of simian virus 40 (SV40) is not frequent in Swedish malignant mesotheliomas. Anticancer Res 2002;22:1357–1360.
119. Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994;9:1781–1790.
120. Shivapurkar N, Wiethege T, Wistuba II, et al. Presence of simian virus 40 sequences in malignant mesotheliomas and mesothelial cell proliferations. J Cell Biochem 1999;76:181–188.
121. Carbone M, Rizzo P, Grimley PM, et al. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. Nat Med 1997;3:908–912.
122. De Luca A, Baldi A, Esposito V, et al. The retinoblastoma gene family pRb/p105, p107, pRb2/p130 and simian virus-40 large T-antigen in human mesotheliomas. Nat Med 1997;3:913–916.
123. Waheed I, Guo ZS, Chen GA, Weiser TS, Nguyen DM, Schrump DS. Antisense to SV40 early gene region induces growth arrest and apoptosis in T-antigen-positive human pleural mesothelioma cells. Cancer Res 1999;59: 6068–6073.
124. Wiman KG, Klein G. An old acquaintance resurfaces in human mesothelioma. Nat Med 1997;3:839–840.
125. Ohshima K, Suzumiya J, Kanda M, Kato A, Kikuchi M. Integrated and episomal forms of Epstein-Barr virus (EBV) in EBV associated disease. Cancer Lett 1998;122:43–50.
126. Ambinder RF. Gammaherpesviruses and "hit-and-run" oncogenesis. Am J Pathol 2000;156:1–3.
127. Inozemtseva LS, Manuilova ES, Marshak MI, Nikolaeva NP, Gnedoi SN, Grivennikov IA. Isolation and characterization of immortalized human fibroblasts. Mol Gen Mikrobiol Virusol 1997;3:27–33.
128. Nevels M, Tauber B, Spruss T, Wolf H, Dobner T. "Hit-and-run" transformation by adenovirus oncogenes. J Virol 2001;75:3089–3094.
129. Smith KT, Campq MS. "Hit and run" transformation of mouse C127 cells by bovine papillomavirus type 4: the viral DNA is required for the initiation but not for maintenance of the transformed phenotype. Virology 1988;164:39–47.
130. Tzeng YJ, Zimmermann C, Guhl E, Berg B, Avantaggiati ML, Graessmann A. SV40 T/t-antigen induces premature mammary gland involution by apoptosis and selects for p53 missense mutation in mammary tumors. Oncogene 1998;16:2103–2114.
131. Sepulveda AR, Finegold MJ, Smith B, et al. Development of a transgenic mouse system for the analysis of stages in liver carcinogenesis using tissue-specific expression of SV40 large T-antigen controlled by regulatory elements of the human alpha-1-antitrypsin gene. Cancer Res 1989;49: 6108–6117.
132. Rizzo P, Bocchetta M, Powers A, et al. SV40 and the pathogenesis of mesothelioma. Semin Cancer Biol 2001;11:63–71.
133. Jang MS, Zlobin A, Kast WM, Miele L. Notch signaling as a target in multimodality cancer therapy. Curr Opin Mol Ther 2000;2:55–65.
134. Padgett BL, Walker DL. Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J Infect Dis 1973;127:467–470.

135. Shah KV, Daniel RW, Warszawski RM. High prevalence of antibodies to BK virus, an SV40-related papovavirus, in residents of Maryland. J Infect Dis 1973;128:784–787.
136. Del Valle L, Gordon J, Enam S, et al. Expression of human neurotropic polyomavirus JCV late gene product agnoprotein in human medulloblastoma. J Natl Cancer 2002;94:267–273.
137. Enam S, Del Valle L, Lara C, et al. Association of human polyomavirus JCV with colon cancer: evidence for interaction of viral T-antigen and beta-catenin. Cancer Res 2002;62:7093–7101.
138. Imperiale MJ. The human polyomaviruses, BKV and JCV: molecular pathogenesis of acute disease and potential role in cancer. Virology 2000; 267:1–7.
139. Krynska B, Del Valle L, Croul S, et al. Detection of human neurotropic JC virus DNA sequence and expression of the viral oncogenic protein in pediatric medulloblastomas. Proc Natl Acad Sci USA 1999;96:11519–11524.
140. Houff SA, London WT, DiChiro G, et al. Neuroradiological studies of JCV-induced astrocytomas in nonhuman primates. Prog Clin Biol Res 1983; 105:253–259.
141. Corallini A, Altavilla G, Cecchetti MG, et al. Ependymomas, malignant tumors of pancreatic islets, and osteosarcomas induced in hamsters by BK virus, a human papovavirus. J Natl Cancer Inst 1978;61:875–883.
142. Noss G, Stauch G, Mehraein P, Georgii A. Oncogenic activity of the BK type of human papova virus in newborn Wistar rats. Arch Virol 1981;69: 239–251.
143. Shah KV. Evidence for an SV40-related papovavirus infection of man. Am J Epidemiol 1972;95:199–206.
144. Vastag B. Sewage yields clues to SV40 transmission. JAMA 2002;288: 1337–1338.
145. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002;29:2–17.
146. Stewart SA, Weinberg RA. Senescence: does it all happen at the ends? Oncogene 2002;21:627–630.

4

Mesothelioma Carcinogenesis: In Vivo Models

Umberto Saffiotti

In Vivo Models of Mesothelioma, Their Purposes, and Roles

Knowledge of the factors and mechanisms responsible for cancer induction rests largely on the development of animal models. In these models, both benign and malignant tumors, as well as preneoplastic lesions, can be induced by proper experimental designs and with appropriate controls, and their analogy to human pathology can be determined. The animal models can be used to test and identify agents (chemical, physical, or biologic) that are capable of carcinogenic activity, and to investigate their mechanisms. In addition, species and strains of laboratory animals with specific genetic susceptibility to specific types of spontaneous or induced tumors can be identified. Recently, genetic manipulation has given rise to transgenic or gene-deleted (knockout) animals, which can reveal selective molecular pathways to carcinogenesis.

The study of in vivo animal models of carcinogenesis has sometimes followed the indications of the evidence derived from studies of human epidemiology, especially for occupational and environmental carcinogens. On the other hand, with the systematic development of animal bioassays, many experimentally identified active carcinogens have been subsequently shown to be human carcinogens in epidemiologic studies.

Bioassays of potential carcinogenic agents in animal models, previously carried out on a smaller scale, became more systematic in the past 40 years, and they provided a basis for public health measures and primary cancer prevention. The choice of animal biossays depends on our knowledge of the underlying mechanisms of carcinogenesis for different classes of carcinogens. For example, knowledge of the biochemical pathways of metabolic activation of many organic carcinogens in human and animal tissues determined the selection of metabolically competent animal models and corresponding modes of administration. In turn, the animal models have provided an indispensable tool for the study of pathogenetic mechanisms of carcinogenesis, and for the investigation of inhibitory factors (chemoprevention).

In the case of mesothelioma, its association with human exposure to asbestos was first suggested by some case reports in the 1940s, was substantiated by occupational epidemiology studies in the 1960s (1–4), and was confirmed by many subsequent reports.

The pathology of human mesothelioma is presented in Chapter 31. The microscopic patterns of mesothelioma are numerous, but they can be grouped into epithelial, sarcomatous (or fibrosarcomatous), and mixed types (5,6).

The first experimental reports of mesothelioma induction, obtained by intrapleural administration of asbestos, were published in the 1960s and 1970s (7–9), and showed that the epithelial, fibrous, and mixed types of mesotheliomas could be induced experimentally by the same treatments. Numerous experimental results subsequently provided increasingly extensive evidence of the carcinogenicity of various asbestos types and other fibrous minerals, as is discussed below.

Criteria for the evaluation of in vivo models need to be considered. Traditionally, animal models have been evaluated for their analogy to the corresponding human tumors in terms of target organs, tumor morphology, histologic types, and histopathogenesis. Their response to etiologic agents known to be carcinogenic in the human has also been an important criterion for their evaluation and acceptance. Mesothelioma animal models, therefore, have been aimed at the induction of tumors by direct exposure of serosal cells, using intrapleural and intraperitoneal administrations of potential carcinogenic agents; in addition, other methods have been used to explore the induction of mesothelial cell proliferation and transformation following indirect exposures, such as inhalation and intratracheal administration, intravenous (intracardiac) injection, and even dietary intake.

With the development of carcinogenesis studies in animal models, new criteria of analogy with the human counterpart evolved on the basis of mechanism studies, which revealed common pathways of metabolic activation of organic carcinogens and specific mechanisms of activation of mineral particles and fibers, leading to reactive oxygen formation on their surface. Recent advances in the methods of molecular biology now provide a wide range of new opportunities for the investigation of molecular mechanisms in the induction and development of experimental tumors, and for the identification of pathways of signal transduction and gene activation.

Spontaneous Mesotheliomas

Ilgren and Wagner (10) and Ilgren (11) reviewed the "background" incidence of mesotheliomas in humans and animals, and cited references to their sporadic occurrence in a variety of animal species in non-experimental settings, including lower vertebrates, fish, birds, rodents (rats of various strains and wild; hamsters; *Mastomys*), bovines (adult, neonatal and fetal), domestic and wild dogs, felines, marsupials, ovines, and pigs. They also reviewed reports of mesotheliomas in untreated, historical, concurrent, and sham-operated control cohorts in

rats of seven inbred strains. Untreated rats showed the following incidences at different sites: pleura 0.3% to 1.5%; peritoneum 0% to 11%; tunica vaginalis of testes 1.3% to 22%; ovarian serosa 0.1% to 0.2%; unspecified sites 0.1% to 1.7%. Vehicle controls showed incidences of 2.0% to 7.8% for intraperitoneally treated rats, and 2% to 2.5% for intrapleurally treated rats.

Mesotheliomas from the tunica vaginalis of the testes or the epididymis in Buffalo and Fischer rats were described by Morris et al (12). Among spontaneous neoplasms in F344 rats observed for life span, five peritoneal mesotheliomas (from omentum, spleen, liver, pancreas, and intestine) were found in 160 males (3.1%), but none in 192 females (13). In lifetime studies in NEDH rats, no mesotheliomas were found in 793 controls; in parabiont rat pairs (rats surgically prepared to share the peritoneal cavities), no pleural mesotheliomas and only one peritoneal mesothelioma were found in 624 rats (14). Tanigawa et al (15) reported 17 mesotheliomas in 395 untreated male Fischer 344 rats (4.3%), only one of which was in the pleura, and all others in the genital serosa or peritoneum. Pott et al (16) reported one mesothelioma in 204 female Wistar rats injected intraperitoneally with saline, and six mesotheliomas in 394 rats injected with titanium dioxide (anatase) or corundum. Minardi and Maltoni (17) examined untreated Sprague-Dawley rats kept until spontaneous death and found, among 1179 males, three peritoneal and one pericardial mesotheliomas, and among 1202 females, only one pleural mesothelioma. Two historical control groups of male Fischer 344 rats, reported by the National Toxicology Program (NTP) (18), showed mesotheliomas of abdominal and tunica vaginalis origin, respectively, in 16/752 (2.1%) and 12/416 (2.9%). No mesotheliomas were reported in all the NTP studies on female Fischer 344 rats, or in any of the tests in mice. The International Agency for Research in Cancer (IARC) in its reviews on tumors in animals, reported that, in rats, naturally occurring mesotheliomas of the pleura were "virtually unknown" (19), but those of testicular origin were cited by several authors (20–23). In the IARC volume on tumors in hamsters, only induced mesotheliomas were reported (24), and in the volume on mice (25), mesotheliomas were not even mentioned.

About "spontaneous" cancers, the present writer remembers W.C. Hueper emphatically saying, some 50 years ago, "Spontaneous? Let's call them cryptogenetic!"

Induction by Fibrous Minerals

Experimental induction of mesothelioma by mineral fibers has been obtained in several species, including rats, hamsters, and mice, and by different routes of administration. The active fibrous materials include the two types of asbestos: amphiboles (amosite, crocidolite, anthophyllite, tremolite) and serpentine (chrysotile); the zeolite mineral erionite; and man-made mineral fibers and vitreous fibers, including refractory ceramic fibers, glass wool, glass filaments, rock (stone) wool, slag wool, and other recently developed, less biopersistent fibers. Their activity varies considerably, in different test methods, depending on

several characteristics, such as fiber dimensions and durability or biopersistence. Ilgren and Wagner (10) and Ilgren (11) reviewed mesothelioma induction by several nonasbestiform fibrous agents, either naturally occurring or synthetic. Reports of mesotheliomas induced by intrapleural or intraperitoneal injections of various asbestos samples were reviewed and their mechanisms discussed (26–29). Comparisons of routes of administration are reported below.

Detailed reports on the characteristics of tested materials and on carcinogenicity data from human and animal studies, with extensive references, are given in the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, for asbestos (30), for erionite (31), for man-made mineral fibers (32), and for man-made vitreous fibers (33). The 2002 volume (33) partly revises the classifications given in 1988 (32), on the basis of more recent data.

Intrapleural Administration

In 1962 J.C. Wagner reported the induction of a few mesotheliomas in rats by intrapleural injection of asbestos. In a larger study, Wagner (34) described a successful technique for intrapleural injection in SPF Wistar rats, through a needle attached to a two-way tap connected to a capillary manometer, which gave a negative reading once the needle reached the pleural cavity, at which point a suspension of the test dust (which had been ultrasonically dispersed) was injected. Each rat received 0.4 mL of saline containing 20 mg of one of the test dust. The reported preliminary results showed mesotheliomas in rats treated with natural crocidolite (29/50), extracted crocidolite from which organic contaminants had been extracted by cyclohexane (37/62), amosite (8/26), and Canadian chrysotile (55/75). No mesotheliomas were found in 30 rats treated with crystalline silica and in 19 controls that received saline alone. The induced mesotheliomas showed either a large mass or multiple nodules (in similar proportions of animals) and their histologic pattern was tubular epithelial, or spindle-celled, or, most frequently, mixed.

Comparison of SPF and conventional Wistar rats given intrapleural injections of various asbestos samples showed analogous percentages with mesothelioma in both types of rats; the large majority of mesotheliomas were of the mixed type, with lower numbers of either fibrous or epithelial types (35). Wagner et al (36,37) induced mesotheliomas in CD Wistar rats with a single intrapleural injection of 20 mg of five reference asbestos samples from the International Union Against Cancer (UICC); mesotheliomas were induced in 66% of rats with a "superfine" Canadian chrysotile, 61% with crocidolite, 36% with amosite, 34% with anthophyllite, 30% with Canadian chrysotile, 19% with Rhodesian chrysotile, and also with a fine glass fiber (12%), a ceramic fiber (10%), and a glass powder (3%); none with a coarse glass fiber. Pylev and Shabad (38) induced 37.5% mesotheliomas in rats with three intrapleural doses of 20 mg of a Russian chrysotile.

Acid leaching of chrysotile rapidly dissolves magnesium (Mg) from the fibers and alters their structure: in intrapleural tests in Sprague-Dawley rats, oxalic acid-leached chrysotile lost most of its carcinogenic

activity as the proportion of leached Mg was increased from 10% to 89%; hydrochloric acid leaching abolished the activity (39).

In Sprague-Dawley rats injected intrapleurally with 25 mg of asbestos of different types, pleural mesotheliomas were found in males in 65% with crocidolite, 70% with Canadian chrysotile, and 40% with asbestos cement, and in females in 25%, 60%, and 30%, respectively, thus indicating a higher susceptibility in males (17). For control incidences, see Spontaneous Mesotheliomas, above.

Stanton and Wrench (9) developed a technique for intrapleural implantation in female Osborn-Mendel rats of gelatin-coated fiberglass pledgets, on which a dose (usually 40 mg) of the test sample was spread and placed in contact with the visceral pleura; mesotheliomas were obtained in about 58% to 75% of the animals in each of the groups given amosite, chrysotile, and four samples of crocidolite. Samples of fibrous glasses of diverse types and dimensions, implanted in the pleura, induced different incidences of malignant neoplasms, called "pleural sarcomas," but described as identical to mesotheliomas, including cases with acinar or papillary epithelioid configuration; mesothelioma incidences ranging from 5% to 100% were reported for 15 samples (40), and for 33 other samples (41). Analysis of 72 experiments with fibrous minerals of widely different structure showed a correlation with the number of fibers $\leq 0.25\,\mu m$ in diameter and $\geq 8\,\mu m$ in length (42).

Erionite is a fibrous zeolite structured as a framework of aluminosilicate tetrahedra $(Si,Al)O_4$, in which each oxygen is shared between two tetrahedra; it is mined in several countries (31). It was found to induce mesotheliomas in Sprague-Dawley rats of both sexes after intrapleural injection of 25 mg (43). Wagner et al (44) injected 20 mg samples intrapleurally in Fischer 344 rats of both sexes and obtained pleural mesotheliomas in 40/40 rats injected with Oregon erionite, and 38/40 with erionite from Karain, Turkey; for comparison, mesotheliomas developed in 19/40 rats treated with chrysotile and in 1/40 saline controls. In non-inbred rats given three intrapleural injections at 1-month intervals, fibrous erionite from Georgia, in the former USSR, induced mesotheliomas in 39/40 males and 43/48 females with none in controls (45). In a dose-response study of Oregon erionite by single intrapleural injection in Porton rats, Hill et al (46) obtained mesotheliomas in 5/10 rats at a dose of 0.1 mg erionite, 9/10 at doses of 1 and 10 mg, and 8/10 at 20 mg. Thus, erionite appears to have the highest activity in the induction of mesotheliomas in rats by intrapleural injection.

In hamsters, the first experimental evidence of mesothelioma induction was obtained by Smith et al (8) by intrapleural injection of 25 mg of asbestos in groups of 15 male Syrian golden hamsters, to which the same type of asbestos was also fed in the diet; two mesotheliomas were obtained with a sample of "harsh" crysotile, and three mesotheliomas with a sample of amosite. Of these five mesotheliomas, two were of the epithelial type and three of the fibrous type (controls and a sample of "soft" crysotile gave no tumors). In a subsequent experiment on groups

of 50 hamsters treated with a single intrapleural injection of asbestos, Smith and Hubert (47) obtained mesotheliomas with 10 or 25 mg of chrysotile (4/50 and 9/50 mesotheliomas, respectively), with 10 mg of amosite (4/50), with 1 or 10 mg of crocidolite (2/50 and 10/50, respectively), and with 10 mg anthophyllite (3/50).

Intraperitoneal Administration

Effective induction of mesotheliomas was obtained by intraperitoneal administration of various fibrous minerals, usually at fairly high doses. By intraperitoneal administration in female Wistar and Sprague-Dawley rats, Pott et al (16) tested a wide variety of samples and obtained extensive evidence of carcinogenicity by many fibrous minerals; the induced tumors were reported as sarcoma, mesothelioma, or carcinoma in the abdominal cavity, without a separate histologic classification. In subsequent reports of intraperitoneal tests in female Wistar rats, high incidences were reported for tumors in the abdominal cavity described as "mesothelioma or sarcoma" (48), and later specifically as mesotheliomas in a dose-response study of different samples, with mesothelioma incidences up to 97% (49,50). Histopathologic analysis of the mesotheliomas induced by intraperitoneal injection of various mineral fibers in Han:WIS female rats by Pott and coworkers was subsequently reported (51) as follows: 45% epithelioid, 34% sarcomatoid, 37% mixed, 18% mixed with bone/cartilage, and 5% sarcomatoid with bone/cartilage.

Several samples of man-made mineral fibers were tested in groups of 18 to 24 male SPF Wistar rats by single intraperitoneal injection of an estimated dose containing 10^9 fibers >5 µm in length; in comparison with an amosite sample, four fiber types were more active and four less active in inducing mesotheliomas (one ceramic fiber type produced by extreme heating induced no mesotheliomas). The results pointed to a link with the number of fibers >20 µm in length and with biopersistence in rat lungs of fibers >5 µm long (52).

In Sprague-Dawley rats injected intraperitoneally with 25 mg of asbestos of different types, peritoneal mesotheliomas were found with crocidolite in 95% of males and 100% of females, with Canadian chrysotile in 90% of males and 70% of females, with Rhodesian chrysotile in 80% of males and 85% of females, with California chrysotile in 75% of males and 70% of females, with amosite in 90% of both sexes, with antophyllite in 80% of males and 85% of females, with asbestos cement in 45% of males and 60% of females, and none in water controls of both sexes. In the same study, seven samples of modified chrysotiles induced peritoneal mesotheliomas with various incidences (30–85% in males and 25–80% in females) and one other sample in only 15% of males and none in females (17). This study does not report a clear sex difference in susceptibility for peritoneal mesotheliomas, whereas the groups treated with intrapleural injection showed a higher susceptibility in males for pleural mesotheliomas (see above; and for control incidences, see Spontaneous Mesotheliomas, above). The finding of a higher susceptibility for peritoneal than for pleural

mesotheliomas with asbestos of various types was not confirmed with tests on a sedimentary erionite, which induced incidences of 50% peritoneal and 87.5% pleural mesotheliomas (data given for both sexes together) (53). The administration of a single large intracavitary dose, such as 25 mg, may not be appropriate to detect susceptibility differences in the target mesothelial cells.

Dose-response relationships in mesothelioma induction by UICC samples of chrysotile, crocidolite, and amosite and by Oregon erionite, following a single intraperitoneal injection in SPF rats of the AF/Han strain, were analyzed in relation to doses (from 0.005 to 25 mg), fiber dimensions (length and diameter), and number of fibers per milligram (54); this study confirmed the importance of fiber length and pointed out that of the two fibers with highest carcinogenic activity, erionite included a fraction of relatively thick fibers, whereas chrysotile fibers separate largely into individual fibrils in tissues (and therefore have low durability), but include a high number of long fibers. A previous intraperitoneal injection study showed the higher activity of long versus short fibers of chrysotile in terms of mesothelioma incidence and mean induction period (55).

Several samples of tremolite, an amphibole type of asbestos, were tested by single intraperitoneal injection of 10 mg in 2.0 mL saline, in rats of the AF/HAN strain; all induced mesotheliomas, with different incidences, ranging from 100% to 5.5% (56).

Repeated intraperitoneal injections in rats were used for tests of biosoluble synthetic fibers, in groups of 51 female Wistar rats (57); the test materials were administered by intraperitoneal injection to the midabdominal region, with two, eight, or 20 injections, each in 2.5 mL saline, at 1-week intervals, depending on the desired total dose [0.5, 2, and 5×10^9, respectively, of World Health Organization (WHO) fibers]. Glass wool samples B, M, P, and V, of similar density and surface area, but of different solubility, resulted in mesothelioma incidences varying from 0% to 14%, and a sample of stone wool only in 0 and 1%. The incidence of mesotheliomas was correlated with intraabdominal masses, ascites, and chronic peritonitis. Positive controls with crocidolite, administered in a single dose (0.5 and 5.0×10^6 WHO fibers, respectively), resulted in mesothelioma incidences of 27% and 45%, respectively.

In male BALB/c mice, mesotheliomas were induced by single intraperitoneal injection of UICC amosite (a dose of 20 mg induced 26.7% mesotheliomas; 10 mg, 23.5%; 2 mg, 35.1%); UICC chrysotile (20 mg, 12.5%; 2 mg, 0); Calidria chrysotile (2 mg, 25%); erionite I (10 mg, 45.2%); erionite II (10 mg, 37.5%; 2 mg, 54.5%; 0.5 mg, 33.3%); a double injection of 2 mg amosite and 2 mg UICC chrysotile induced mesotheliomas in 60% (58). In this study, a large proportion of mice treated with high doses developed severe peritoneal fibrosis and intestinal obstruction, and many died before 7 months (mesotheliomas were found only after this latent period); this pathology accounts for the reverse dose-response observed with amosite and erionite. Overall, the mesotheliomas included 88% of the fibrous type, 11% of the mixed type, and only 1% of the epithelial type. Peritoneal mesotheliomas were found in

all groups of Swiss mice treated by single intraperitoneal injection with graded doses (5 to 40 mg) of erionite from Karain, Turkey; no evidence of a dose-response was observed in this study (59).

In C57BL/6 mice, the early mesothelial reactions following intraperitoneal crocidolite injection were described as involving cell injury and regeneration, associated with the development of mesotheliomas (60). Following intraperitoneal injection of crocidolite, 25% of BALB/c mice and 45% of CBA mice developed mesothelioma, 7 to 25 months after exposure; cell lines were established from these tumors (61).

As an effective method for mesothelioma induction, A.B. Kane's laboratory adopted weekly intraperitoneal injections of 200 µg of UICC crocidolite in C57Bl/6 mice (62); mesotheliomas were induced after 35 to 66 weeks of treatment. The tumors had the typical histology of epithelial, fibroblastic, or mixed types. This model was used for $p53^{+/+}$, heterozygous $p53^{+/-}$, and homozygous $p53^{-/-}$ mice. Mesotheliomas developed in 37% of the wild-type mice (mean latent period: 67 weeks), and in 76% of the heterozygous *p53*-deficient mice (mean latent period: 44 weeks); the homozygous *p53*-deficient mice developed many other types of tumors early and only one mesothelioma was found in 10 mice, with a latency of only 10 weeks. In the heterozygous mice, 50% of the mesotheliomas showed extensive invasion (63,64).

Inhalation

Following inhalation in SPF Wistar rats of both sexes, for various periods of time, of different samples of asbestos, at doses closely comparable for all samples (as concentrations of mean respirable dust and as cumulative dose), many animals developed lung tumors (adenomas, adenocarcinomas, and squamous cell carcinomas), but only a few developed mesotheliomas. After a 1-day exposure to amosite, 1 of 45 rats at risk developed mesothelioma; 1/28 and 1/18 after 12-month and 24-month exposures to anthophyllite; 1/43, 1/36, and 2/26 after crocidolite exposures, respectively, of 1 day, 3 months, and 12 months; 3/23 and 1/21 after 12-month and 24-month exposures to Canadian chrysotile; and none after exposure to Rhodesian chrysotile and in controls (65). In another asbestos inhalation study in SPF rats of the Han strain, again, lung tumors were induced by UICC amosite, chrysotile, and crocidolite, but only one peritoneal mesothelioma occurred in 42 rats exposed to 2 mg/m^3 chrysotile and one pleural mesothelioma in 43 rats exposed to 5 mg/m^3 crocidolite (66). In a later study (67), long- and short-fiber samples of amosite and chrysotile were tested by inhalation in rats; the long-fiber samples were much more carcinogenic in both cases (inducing both pulmonary tumors and pleural mesotheliomas), whereas the short-fiber samples gave some tumors with chrysotile and none with amosite. Results of many experiments on the induction of lung tumors and mesotheliomas by inhalation of chrysotile and of amphiboles in rats were analyzed by Pott (68) and showed that mesotheliomas were much less frequently induced than lung tumors. Davis et al (54) pointed out that mesotheliomas were induced in rats following inhalation of all asbestos types, but never

with an incidence of more than 10% of exposed animals, even at massive doses (55,65–67).

In contrast, a high incidence of pleural mesotheliomas was observed in Fischer 344 rats exposed to Oregon erionite inhalation: 27/28 rats developed mesotheliomas, with none in controls; in comparison, only 4/124 rats exposed to crocidolite developed mesotheliomas (44). Johnson et al (69) reported that pleural tumors induced in rats by erionite inhalation had similar ultrastructural appearance to mesotheliomas induced by asbestos injection in the pleural and peritoneal cavities. The high incidence of mesotheliomas induced by Oregon erionite is discussed by Davis (70), who draws attention to the fact that its appearance and fiber size distribution are very similar to those of UICC crocidolite, which causes only rare mesotheliomas when inhaled by rats. Johnson (26) observed that the peritoneum seemed more sensitive to crocidolite than the pleura, whereas the pleura was more sensitive to erionite than the peritoneum.

In Syrian golden hamsters (125 males per group, 140 controls), nose-only inhalation tests of amosite showed the induction of inflammation and pulmonary and pleural fibrosis, followed by mesothelial hypertrophy and hyperplasia, and the following incidences of mesotheliomas: 4% with 25 fibers/cm^3, 26% with 125 fibers/cm^3, and 20% with 250 fibers/cm^3. In comparison, only one mesothelioma was found with inhalation of fiberglass sample MMVF33 and none with fiberglass sample MMVF10a of much lower durability (71).

In a comparative inhalation study of a kaolin-based refractory ceramic fiber (RCF-1) in Fischer 344 rats and Syrian golden hamsters, significant pulmonary and pleural inflammation was detected for both species; DNA synthesis by pleural mesothelial cells was higher in hamsters than in rats, and was highest in the parietal pleura; greater collagen deposition was measured in the visceral pleura of hamsters, but was not significantly elevated in rats; and the number of fibers longer than 5 μm per cm^2 of pleural surface was two to three times higher in hamsters than in rats (72). A previous inhalation study in Fischer 344 rats and Syrian golden hamsters, exposed simultaneously to RCF-1, resulted in a high incidence of mesotheliomas in hamsters (43%), but not in rats (1.6%) (73). Both species developed fibrosis and inflammation of the visceral pleura, but hamsters developed greater surface mesothelial cell proliferation and had focal aggregates of mesothelial cells embedded within regions of visceral pleural fibrosis (74).

Comparing the response of different animal models, such as rats and hamsters, is especially interesting, because it may suggest different underlying mechanisms. In studies with crystalline silica, administered either by inhalation or by intratracheal instillation, hamsters developed no fibrogenic or carcinogenic responses, but only macrophagic granulomas, whereas rats showed a high level of pulmonary fibrogenic and carcinogenic responses (75). Silica and asbestos are both active through the formation of reactive oxygen species that produce DNA damage, but particulate materials differ from fibrous materials in physicochemical characteristics and consequently in their transport and localization in target tissues. Both asbestos and silica are carcinogenic in the lungs

of rats, but no mesotheliomas have been induced by silica, even in rats. Wagner et al (76) found that intraperitoneal injection of silica in rats induced malignant histiocytic lymphomas, but no mesotheliomas. For combined exposures to asbestos and quartz, see below.

Intratracheal Instillation

Intratracheal injection of 2 mg Russian chrysotile, together with 5 mg benzo[*a*]pyrene (BP), resulted in pleural mesotheliomas in 6/11 rats, whereas none were induced in rats given BP alone or chrysotile alone at a higher dose (77).

Mohr et al (78) induced pleural mesotheliomas by 8 weekly intratracheal instillations in male Syrian golden hamsters (each of 1 mg dust in 0.15 mL saline) of two types of glass fibers (mesothelioma incidences: 37/136 and 26/138) and of UICC crocidolite (8/142), with 0/142 in TiO_2 controls. These mesotheliomas were all epithelioid, with papillary areas.

I.Y. Adamson and coworkers used intratracheal instillation of crocidolite in mice and rats in a series of short-term studies, to investigate early-phase proliferation of mesothelial cells in vivo. They showed that mesothelial cell proliferation was induced by long fibers in mice. Since this effect was also induced by other agents, such as hyperoxia, bleomycin, and silica, they suggested that it was a response to injury to the lung and probably mediated by the same cytokines that trigger interstitial fibrosis (79). The early proliferative response of mesothelial cells was also seen after intratracheal instillation of crocidolite in rats, and it was found to be blocked by an antibody to keratinocyte growth factor (KGF) (80). Since mesothelial cells share properties of fibroblastic and epithelial cells and they express both vimentin and cytokeratin (81), Adamson et al (80) suggested that KGF, secreted by lung fibroblasts to promote epithelial repair after asbestos, may diffuse to the pleural surface and induce the short-term proliferation of mesothelial cells; in extended exposure periods, this stimulation may be responsible for mesothelial cell proliferation. Further studies by intratracheal instillation of crocidolite in rats showed that during the phase of mesothelial cell proliferation, both KGF and hepatocyte growth factor (HGF) were significantly increased in both bronchoalveolar and pleural lavage fluids (82), and both HGF and KGF stimulated mesothelial cell proliferation (83).

Ingestion

Extensive long-term feeding tests of mineral fibers were reviewed (84). Two hamster studies showed no toxic effects of feeding amosite or taconite tailings, although one mesothelioma was reported in 30 amosite-treated males (none in controls). No mesotheliomas were reported in 10 experimental groups of rats fed amosite or crocidolite. In seven feeding studies with chrysotile, only one showed a peritoneal mesothelioma (in 95 male rats). These tumors were not considered significant. In a large feeding study in rats of chrysotile (alone or mixed with 25% crocidolite, with untreated or palm-oil controls), seven mesotheliomas were reported (two in control groups) and they included three in the thoracic cavity, one peritoneal, one near salivary

glands and two in the testicles (85); again, these mesotheliomas were considered unrelated to treatment, but their origin remains unclear.

Induction by Other Carcinogens

Reports have been published on mesotheliomas in animal models following exposure to a variety of chemicals. The evidence of induction by the test chemicals is often difficult to establish. Ilgren and Wagner (10) and Ilgren (11) listed a large number of tests from the literature in which mesotheliomas were reported, but they did not assess the significance of the findings, and so included tests in which, for example, only one or a few mesotheliomas were reported in a group of animals, compatible with occurrence in control groups. In other cases, the tumors were not clearly diagnosed as mesotheliomas. This section includes only references to studies showing a likely induction of mesotheliomas.

N-nitrosopyrrolidine (fed at 16 mg/kg body weight for 67 weeks) resulted in papillary mesotheliomas of the testis or epididymis in 4/12 (33%) MRC rats, with 0/34 in controls (86). Mesotheliomas and proliferative lesions of the testicular mesothelium (tunica vaginalis and epididymis) were produced by a single intraperitoneal injection of 13 mg/kg body weight of methyl(acetoxymethyl)nitrosamine (DMN-OAc) in male Fischer 344 rats (9/25), Sprague-Dawley rats (4/27), and Buffalo rats (12/27) (87).

N-2-fluorenylacetamide, fed at 0.06% in the diet to male Fischer 344 rats, in three cycles of 3 weeks each, with 1-week intervals on normal diet, resulted in testicular mesotheliomas in 9/25 rats (36%), with none in a group with only one cycle and in controls (88). A previous study of *N*-2-fluorenylacetamide, fed at 0.025% in the diet to Buffalo strain rats, gave mesotheliomas of the testes or epididymis in 3/18 rats, but 2/6 controls also had these tumors (12). Ethylene oxide, tested by inhalation in male Fischer 344 rats, induced significant incidences of peritoneal mesotheliomas at 33 and 100 ppm in one study (89) and at 100 and 300 ppm in another study (90).

Mice of different strains, treated with intragastric administrations of 3-methylcholanthrene (MCA), 20 mg/kg in olive oil, weekly for 10 weeks, developed peritoneal mesotheliomas that frequently invaded the diaphragm and other organs; their incidence varied in different strains: mesotheliomas were induced in 12/31 (39%) mice of the C3H strain, and 9/32 (28%) mice of the BALB/c strain; low incidences of mesothelioma were obtained in C57BL/6 mice (1/31) and DBA/2 mice (1/26), and none in Swiss mice (0/30) and AKR mice (0/32). Most of the induced mesotheliomas were of the mixed type and mainly fibrous. In addition, lesions consisting of severe mesothelial hyperplasia associated with tissue necrosis and inflammation were considered as possible early stages of mesothelioma development (91). This study shows marked susceptibility differences among different mouse strains to mesothelioma induction by a polynuclear aromatic hydrocarbon. This model, which has not been used so far for mechanism

studies, could provide clues to the underlying genetic susceptibility factors.

Other organic chemicals were linked to mesothelioma induction, as reviewed by Peterson et al (92), Ilgren and Wagner (10) and Ilgren (11). Among them, sterigmatocystin given by repeated intraperitoneal injections, which induced mesotheliomas in 50% of Wistar rats (93); possibly 1-nitroso-5,6-dihydrouracyl given in three intraperitoneal injections to MRC-Wistar rats (2/40 with mesothelioma) (94); *N*-methyl-*N*-nitrosourea given repeatedly intraperitoneally to guinea pigs (only one mesothelioma) (95); and diethylstilbestrol, which, after subcutaneous implantation in squirrel monkeys, resulted in uterine malignant mesotheliomas in 7/10 animals (96).

Ferric saccharate and nitrilotriacetic acid (NTA) were injected intraperitoneally daily for 3 months in male Wistar rats, separately or together: 9/19 rats given ferric saccharate (5 mg Fe/kg body weight/day, 6 days/week for 3 months) developed mesotheliomas from the serosa of the tunica vaginalis or spermatic cord; among 19 rats treated with ferric saccharate and also with NTA (83.5 mg/kg body weight, 6 days/week for 5 months), seven developed mesotheliomas at the same locations and six had widespread peritoneal mesotheliomas; the mesotheliomas showed all three histologic types; none were found in rats given NTA alone or just saline (97). Potassium bromate ($KBrO_3$), an oxidizing agent, given in drinking water to F344 rats, induced mesotheliomas on the surfaces of various abdominal organs in males, in 59% at 500 ppm and 33% at 250 ppm, but in 6% in untreated controls (!), with none in female F344 rats, female B6C3F1 mice, and male Syrian golden hamsters; different durations of treatment in male rats gave mesothelioma incidences near 40% for treatments up to 52 weeks, and 75% for 104 weeks of treatment (98).

The reports of the National Toxicology Program (NTP) (18), National Institute of Environmental Health Sciences, were reviewed for tests resulting in mesotheliomas. The following long-term tests in male F344 rats yielded high incidences of mesotheliomas originating in the peritoneum/tunica vaginalis testis: (a) by inhalation: 1,2-dibromoethane (up to 25/50 rats with mesotheliomas); (b) by feeding: o-nitrotoluene (up to 44/60 and to 54/60 in a stop-exposure test); 2,2-bis(bromomethyl)-1,3-propanediol (up to 26/60 in a stop-exposure test); (c) by gavage: glycidol (up to 39/50); (d) by intraperitoneal injection: cytembena (a cytostatic agent) (up to 37/50). Lower incidences of mesotheliomas were induced by the following compounds: (e) by inhalation: dichloromethane (up to 5/50); (f) by feeding: pentachlorophenol (up to 9/50); ethyl tellurac (up to 8/50); nitrofurazone (up to 7/50); o-toluidine (no incidence given); 3,3'-dimethoxybenzidine dihydrochloride (up to 6/60); 3,3'-dimethylbenzidine dihydrochloride (up to 4/60); pentachlorophenol (up to 9/50 in a stop-exposure study); (g) by gavage: methyleugenol (up to 12/50); trichloroethylene (up to 5/50); (h) by dermal application: 2,3-dibromo-l-propanol (up to 4/50). In addition, a test of acronycine by intraperitoneal injection in Sprague-Dawley rats induced mesotheliomas (in the abdomen or tunica

vaginalis) in both male and female rats, but the incidence was not clear because of high mortality. The historical control incidence of mesotheliomas in male F344 rats was reported from two laboratories as 2.9% and 2.1%. No mesotheliomas were reported in all the studies on female F344 rats, or in any of the tests in mice.

Radioactivity was shown to induce mesotheliomas; in rats exposed to $^{239}PuO_2$ by intraperitoneal injection, 27% developed epithelial mesotheliomas and 38% sarcomatous mesotheliomas; the tumor incidence was dose-dependent and a greater dose was required to induce epithelial than sarcomatous mesotheliomas (99). When chrysotile or 3,4-benzo[*a*]pyrene was injected with the $^{239}PuO_2$, the resultant tumor incidence was additive [Sanders, 1973, quoted by Hahn et al (100)]. $^{239}PuO_2$ also induced pleural mesotheliomas by inhalation or intrapleural injection (101). Four life-span inhalation studies of radionuclides were reviewed by Hahn et al (100) for the induction of mesotheliomas; the rats were exposed briefly (10–40 min) per nasum once or repeatedly (seven times over 1 year); in a total of 3076 rats (approximately equally divided by sex), exposed by inhalation to aerosols of $^{239}PuO_2$, mixed uranium-plutonium oxide, or $^{144}CeO_2$, a total of 28 pleural mesotheliomas were induced (21 epithelial-papillary diffuse, two epithelial-papillary focal, two sarcomatous, and three mixed); four mesotheliomas were found in 1641 controls (0.24%). These studies showed that mesotheliomas can be induced with either alpha- or (less effectively) with beta-emitting radionuclides.

Combined Exposures

Warren et al (14) reported a large study in NEDH rats of both sexes treated with UICC chrysotile by the intratracheal, intrapleural, and intraperitoneal routes, alone or combined with x-radiation or 3-methylcholanthrene administration. The results (limited by incomplete reporting) are indicative of a marked synergism of asbestos with radiation and with methylcholanthrene for peritoneal, but not pleural, mesotheliomas. Combined effects in mesothelioma induction were also reported for intrapleural chrysotile combined with radon 222 inhalation in rats (102).

In a study of the combined effects of asbestos inhalation coupled with the inhalation of a particulate material, namely, quartz or rutile (a titanium dioxide polymorph), unexpected results were obtained (103). Rats of the AF/Han strain (sex not specified) were exposed to inhalation (5 hours/day at 10 μg/m^3) of UICC chrysotile or to a sample of long-fiber amosite. Separate groups were exposed to the same asbestos inhalation schedule followed by a 2 hours/day inhalation of either rutile (at 10 μg/m^3) or quartz (at 2.2 μg/m^3). The inhalation exposures were continued for 1 year, with a 2-year follow-up. Early lung lesions from quartz and asbestos were more diffuse than those from asbestos alone. After 6 months, the asbestos + quartz groups showed more extensive fibrosis and alveolar epithelial hyperplasia, while titanium dioxide did not change the degree of fibrosis seen with asbestos alone. The incidence of pleural mesotheliomas was 6/38 (16%) with chrysotile

+ quartz, 0/37 with chrysotile alone and 2/41 with chrysotile + rutile; and it was 8/39 (21%) with amosite + quartz, 2/40 with amosite alone and 2/40 with amosite + rutile. Peritoneal mesotheliomas were 1/40 with amosite alone, 2/40 with amosite + rutile, 1/39 with amosite + quartz, and none with chrysotile. The induced mesotheliomas were histologically biphasic. The remarkable finding that the induction of pleural mesotheliomas by asbestos inhalation is significantly increased by quartz was paralleled by the increase in pulmonary fibrosis. Quartz also increased the incidence of pulmonary adenocarcinomas in comparison with the two groups treated with asbestos alone. The role of quartz, an ubiquitous dust and a known pulmonary carcinogen, in the potentiation of pleural mesothelial carcinogenesis remains to be further investigated. Quartz shares several molecular mechanisms with asbestos (104), and may also have affected the transport of inhaled fibers to the pleura.

Induction by SV40

High incidences of mesotheliomas were induced in 21-day-old male Syrian golden hamsters following intracardiac, intraperitoneal, and intrapleural injections of wild-type (wt) simian virus 40 (SV40) (see also Chapter 3). Specifically, wt SV40 830 resulted in the induction of 13/21 (62%) mesotheliomas by intracardiac injection, and wt SV40 776 resulted in 2/5 (40%) mesotheliomas by intracardiac and 4/6 (67%) mesotheliomas by intraperitoneal injection. When injected intrapleurally, both wt strains resulted in a 100% incidence of mesotheliomas (6/6 and 5/5, respectively) with no mesotheliomas in controls (105). The tumors developed between 3 and 6 months after injection. After intraperitoneal or intracardiac injection, the tumors formed a continuous layer over pleural and pericardial surfaces, obliterating the cavities; and those induced by intraperitoneal injection spread widely over the serosal surfaces. No distant metastases were observed. The histologic appearance of the tumors was mostly of the mixed type, with spindle cell and epithelioid areas in the same tumor, but some tumors showed only one type of differentiation. The diagnosis of mesothelioma was confirmed by histochemical staining, showing production of hyaluronic acid, and by electron microscopic features. Tumor cell lines were established from these mesotheliomas (105).

Mesotheliomas in hamsters induced by asbestos or by SV40 were compared (106). Intrapleural injection of crocidolite asbestos induced mesotheliomas in hamsters in a dose-dependent manner; the tumors appeared late (>18 months) and were small and well differentiated, with adjacent pleural fibrosis, and rarely caused local invasion and death. In contrast, SV40-induced hamster mesotheliomas showed no adjacent pleural fibrosis, and they were large and multicentric, with both epithelioid and sarcomatoid areas; histologically they were anaplastic and showed invasion of adjacent tissues; and they were uniformly fatal within 3 to 6 months. Such different patterns in the histopathology and clinical course of two animal models of mesothelioma obtained by different etiologic agents should prove valuable for

investigating the respective molecular pathways involved in their pathogenesis.

Induction by Other Viruses

Proliferative lesions of the pleura, pericardium, and peritoneum were reported in Swiss mice inoculated in utero with polyoma virus, but none of the lesions progressed to invasion or metastasis; the lesions showed hypertrophied mesothelial cells over a thickened hyalinized matrix, or stalks attached to the visceral surface and covered by one to five layers of cuboidal mesothelial cells, sometimes forming polypoid masses (107). In a group of 16 Syrian golden hamsters injected intratracheally with polyoma virus (which induced lung carcinomas and liver angiomas), one hamster developed a bilateral pleural mesothelioma (108); given the absence of spontaneous mesotheliomas in hamsters (see above), this is probably a polyoma-induced tumor.

The MC29 avian leukosis virus (an RNA virus) induced mesotheliomas in 35% of chickens injected in the peritoneal, pericardial, and air sac cavities (109). Diffuse mesotheliomas were induced in chickens of the inbred SC line, by inoculation of preparations of v-*src* DNA (the oncogene of Rous sarcoma virus); pRLV-*src* resulted in mesothelioma incidences of 9/10 by intravenous, and 22/37 by intraperitoneal administration; and intraperitoneal pJDΔ 11 construct gave 10/13 mesotheliomas. The mesotheliomas in all these experiments were of the three morphologic types: epithelial, fibrous, and mixed (110).

Mechanisms and Pathways

Mechanisms Specific to Fibrous Minerals: The roles of fiber type, dimensions, durability, surface properties, deposition, translocation, and dissolution have been shown to be of critical importance in many studies (42,54,68,70,111–114). The critical factors are fiber dimensions (≥8 μm in length and ≤0.25 μm in diameter), the number of fibers, their chemical composition, and the durability, or biopersistence, of the fiber type. Suzuki (115) remarked on the need for a long latent period for mesothelioma induction, dependent on the longevity of the species: ≥7 months in mice, ≥1 year in rats, ≥6 years in baboons, and ≥20 years or longer in humans.

In considering the dose of fibrous minerals that was used in most animal experiments, especially in single injection tests, one may become concerned that the induction of mesotheliomas is obtained by high doses, as compared with the amount of fiber inhaled in human exposures over a long time. The animal models are often primarily chosen to obtain the desired end point, i.e., the development of tumors comparable with those of human pathology. Concerning the dose, one has to remember that particulate and fibrous minerals act by mechanisms involving the reactivity of their surfaces, not their bulk, and that therefore their total weight is not representative of the active surface molecular layer, a fundamental difference from the mode of action of soluble carcinogens. It is also important to consider the probability of tumor

occurrence in relation to the number of individuals exposed and the duration of their survival, which are obviously very different between groups of experimental animals and human populations. The fact that the carcinogenic activity of fibrous minerals has been observed under many different test conditions, species, and routes of administration, and its correspondence with human occupational epidemiology, leave no doubt about the carcinogenicity of these materials. More difficult is the evaluation of negative results obtained in certain tests, in terms of risk estimates for human exposure. Here, dose-response relationships are important in the experimental models as well as in corresponding human exposures, and mechanistic considerations can contribute to the understanding of the results. An example is given by a recent report on biosoluble synthetic mineral fibers tested by intraperitoneal injection in rats (57). Much remains to be investigated about the mechanisms by which fibrous minerals induce tumors, especially mesotheliomas. For example, asbestos fibers (chrysotile) were found to be effective in transfecting exogenous plasmid DNA in monkey cells COS-7, suggesting another possible role for the fibers in the mechanism of neoplastic transformation (116). Well-defined biologic models can provide new insights into the mechanisms of fiber carcinogenesis.

An extensive critical discussion was provided by the consensus report of a group of experts convened by the International Agency for Research on Cancer (IARC) (117), who considered fiber characterization, biopersistence, genotoxicity, cell proliferation, animal models in relation to species, routes of exposure and doses, and the relevance of in vivo and in vitro assays and of mechanistic data. The report recognized that fibers can activate macrophages and epithelial cells to release inflammatory mediators, cytokines, and growth factors, which may alter epithelial and mesothelial cell proliferation; that fibers can bind to the plasma membrane and activate cells; and that asbestos fibers can activate multiple intracellular signaling pathways and transcription factors, including oxidative stress-related pathways via redox-sensitive transcription factors such as nuclear factor NFκB and the activator protein AP-1. The report expressed concern about the interpretation of experiments using intratracheal and intracavity injections, because high-dose exposures may result in uneven deposition. It recommended that a multidose chronic inhalation study should be undertaken in rats and hamsters, using a well-characterized amphibole sample, and including relevant short-term end points or biomarkers, to be evaluated in future mechanistic studies.

A workshop report on chronic inhalation testing, sponsored by the U.S. Environmental Protection Agency (118), reviewed test methods and conditions. Concerning mesotheliomas, it noted that the rat inhalation model may not be sensitive enough to the induction of mesotheliomas (except for erionite), and that the hamster appeared more sensitive than the rat with respect to fiber-induced mesothelioma, but less sensitive to the induction of lung tumors and fibrosis. Commenting on histopathologic evaluations, it recommended that a dissecting microscope should be used to examine for mesotheliomas (a practice that has not been widely used).

Muhle and Pott (119) expressed their concern about the adequacy of inhalation models for risk evaluation of asbestos fibers, considering that rat lungs weighing about 1 g at the start of an experiment, with survival of little more than 2 years, are compared with human lungs of 1000 g, surviving several decades, and that the number of cells at risk and the number of cell generations is much higher in humans. They stated, "All German institutions that were involved with the assessment of the cancer risk from fibers concluded that the inhalation model with rats is not sensitive enough to predict cancer risk due to the much longer lifespan of humans." The complexities and pitfalls of human risk evaluations based on tests on different animal species were reviewed by Maxim and McConnell (120). With advances in tracing the molecular pathways of mesothelioma carcinogenesis, one hopes that the biologic significance of different animal models will become better understood.

Molecular Mechanisms in Mesothelioma Pathogenesis, as Studied In Vivo in Animal Models: As reviewed in this chapter, mesotheliomas can be induced in animal models by a variety of agents, not only by fibrous minerals, but also by several organic carcinogens, by radioactive materials, and by viruses. In addition, co-carcinogenic effects have been reported, for example, by inhalation of quartz particles combined with asbestos fibers.

Considerable advances have been made in the past decade in identifying molecular pathways that characterize mesothelioma cells. Most of these studies have used human or animal mesothelial cells or mesothelioma cells in culture and were recently reviewed (121). The study of messenger RNA (mRNA) expression patterns at different stages of asbestos-induced carcinogenesis in rats showed that several genes (c-*myc*, *fra-1*, and *egfr*) were upregulated in pretumorous tissue, in asbestos-induced tumors and in cells treated with asbestos in vitro; and proteins associated with cell adhesion were also upregulated (122).

The role of the tumor suppressor genes was studied mostly in human mesotheliomas and derived cell lines, as recently reviewed (123). Homozygous loss of the *p16* INK4 locus was found in the tumor cells, and alteration of $p16^{INK4\alpha}$ appears to play a critical role in mesothelial cell tumorigenesis. Alterations of the p16(INK4) locus were found in about one third of human malignant mesothelioma specimens (124). It was concluded that the available data suggest that alteration of either product of the *CDKN2A* locus, i.e., $p14^{ARF}$ or $p16^{INK4\alpha}$, contributes to the pathogenesis of mesothelioma (123).

Wild-type *p53* was detected in human mesothelioma-derived cell lines at levels about four times higher than in human fibroblasts (125). In rat mesotheliomas induced by erionite or asbestos and in cell lines derived from spontaneous or induced rat mesotheliomas, *p53* was found to be rarely mutated (126). The role of *p53* was discussed above in relation to mesothelioma induction in transgenic $p53^{+/-}$ mice.

The rat neurofibromatosis 2 (NF2) tumor suppressor gene was found to be mutated in 40% of human mesotheliomas; in contrast, rat

mesotheliomas and derived cell lines showed no DNA sequence alterations, suggesting a different role of this gene between human and rat asbestos-induced mesotheliomas (127).

Cells derived from the appropriate animal models, such as mesotheliomas induced by intraperitoneal injection of asbestos, were used to identify the transcription factor AP-1 as a major target of asbestos-induced signaling pathways, and the induction of *c-fos* and *c-jun* mRNAs in rat pleural mesothelial cells early after exposure to carcinogenic fibers (128). The AP-1–dependent member of the *fos* family of proto-oncogenes, *fra-1*, was recently identified as a factor required for asbestos-induced transformation of mesothelial cells through the extracellular signal-regulated kinase – mitogen-activated protein kinase (ERK-MAPK) cascade (129). The multifunctional cytokine interleukin-6 (IL-6) was shown to be an autocrine growth factor for normal human pleural mesothelial cells and to be expressed together with mRNA for the transmembrane components of the IL-6 receptor complex, i.e., the IL-6 binding molecule, gp80, and the signaling molecule, gpl30 (130). The interconnections of the pathways of the IL-6 complex, the AP-1 and other transcription factors, and the redox-sensitive pathways were pointed out (131). The role of several growth factors, including platelet-derived growth factor (PDGF), has been considered (132). In murine peritoneal mesotheliomas, a profound downregulation of lymphocyte surface markers was found in tumor-infiltrating lymphocytes; significant amounts of transforming growth factor (TGF)-β, IL-6, IL-1, and of tumor necrosis factor (TNF)-α were produced by the mesothelioma cells (133,134).

Two recent studies evaluated the expression of transmembrane adhesion molecules in human mesotheliomas and in other tumor types. Comparing epithelial-type cadherin (E-cadherin), E-selectin and vascular cell adhesion molecule (VCAM) in mesotheliomas and peripheral pulmonary adenocarcinomas, it was found that only E-cadherin showed a significant difference of expression: highly positive in the adenocarcinomas, but negative or weak in the mesotheliomas (135). In a study on 20 human diffuse mesotheliomas (16 epithelioid and four sarcomatous), compared with other tumors, E-cadherin was found positive in 4/20 (20%) and neural-type cadherin in 14/20 (70%), and immunoreactivity was observed mainly in epithelioid-shaped tumor cells (136). Whether this marker for differential diagnosis is applicable to the various animal models of mesothelioma remains to be determined.

Several of these signaling and transcription pathways are related to conditions involving chronic inflammation and fibrosis, and have been studied also for silica-induced pathology (104). Their specificity in relation to mesothelioma induction remains to be further defined.

The concept of "pathway pathology" was recently proposed to refer to different pathways of signaling molecules and transcription factors linked to specific morphologic patterns in the corresponding tumor pathology (137). Such criteria of molecular pathway identification could be applied to the study of tumors of different etiologies; they

would become pointers to the causative agents and mechanisms of different groups of tumors, and could be termed "pathway etiopathogenesis" (104). The critical differences in the pathology of mesotheliomas induced in hamsters by intrapleural injection of either asbestos or SV40, cited above (106), could be a fruitful case study for both of these approaches to molecular pathways.

Conclusion

This review of in vivo animal models of mesothelioma shows that in the 40 years since their first development they have provided solid evidence for the experimental induction of mesothelioma by a variety of carcinogenic agents, most prominently fibrous minerals, and by some viruses, especially SV40, which was first identified by animal tests as a specific etiologic agent of mesothelioma. The close similarity of histopathology in the animal models and in human mesotheliomas has strengthened their usefulness for mechanism studies. Variations in susceptibility to mesothelioma induction by species, strain, sex, route of exposure, and site of origin offer a choice of animal models for mechanism studies. The high susceptibility of hamsters versus rats, male versus female rats, and of peritoneal and especially testicular mesothelium in rats, demonstrated in a number of studies with different agents, could be used to investigate the underlying mechanisms.

The recent expansion of our knowledge of molecular pathways involved in carcinogenesis opens up a remarkable opportunity for a better definition of the relationships of specific etiologic factors and cofactors and induced tumor types. The development of in vitro cellular models, derived from established in vivo models, both human and animal, allows further comparative investigations. One wishes to see a greater development of animal models addressed to specific mechanisms and pathways. The recent study of mesothelioma in *p53* transgenic mice (64) provides a stimulating example. Transgenic and gene-deleted (knockout) animal models offer further opportunities to explore molecular mechanisms and pathways, and to evaluate their relevance for conditions of genetic susceptibility, tumor biology, and potential tumor therapy.

References

1. Mancuso TF, Coulter EJ. Methodology in industrial health studies. Arch Environ Health 1963;6:210–226.
2. Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. JAMA 1964;188:22–26.
3. Selikoff IJ, Churg J, Hammond EC. Relation between asbestos exposure and mesothelioma. N Engl J Med 1965;272:560–565.
4. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North West Cape Province. Br J Ind Med 1960; 17:260–271.

5. Battifora H, McCaughley WTE. Tumors of the serosal membranes. In: Rosai J, Sobin LH, eds. Atlas of Tumor Pathology. Third series, Fascicle 15. Washington, DC: Armed Forces Institute of Pathology, 1995:17–99.
6. Craighead JE, Abraham JL, Churg A. The pathology of asbestos-associated diseases of the lungs and pleural cavities: diagnostic criteria and proposed grading schema. Arch Pathol Lab Med 1982;106:541–596.
7. Wagner JC. Experimental production of mesothelial tumours of the pleura by implantation of dusts in laboratory animals. Nature 1962;196:180–181.
8. Smith WE, Miller L, Elsasser RE, Hubert DD. Tests for carcinogenicity of asbestos. Ann NY Acad Sci 1965;132:456–488.
9. Stanton MF, Wrench C. Mechanisms of mesothelioma induction with asbestos and fibrous glass. J Natl Cancer Inst 1972;48:797–821.
10. Ilgren EB, Wagner JC. Background incidence of mesothelioma: animal and human evidence. Regul Toxicol Pharmacol 1991;13:133–149.
11. Ilgren EB. Mesotheliomas of Animals. A Comprehensive, Tabular Compendium of the World's Literature. Boca Raton, FL: CRC Press, 1993.
12. Morris HP, Wagner BP, Ray FE, Snell KC, Stewart HL. Comparative study of cancer and other lesions of rats fed N,N'-2,7-fluorenylbisacetamide or N-2-fluorenylacetamide. Natl Cancer Inst Monogr 1961;5:1–54.
13. Sass B, Rabstein LS, Madison R, Nims RM, Peters RL, Kelloff GJ. Incidence of spontaneous neoplasms in F344 rats throughout the natural lifespan. J Natl Cancer Inst 1975;54:1449–1456.
14. Warren S, Brown CE, Chute RN, Federman M. Mesothelioma relative to asbestos, radiation and methylcholanthrene. Arch Pathol Lab Med 1981; 105:305–312.
15. Tanigawa H, Onodera H, Maekawa A. Spontaneous mesothelioma in Fischer rats—a histological and electron microscopic study. Toxicol Pathol 1987;15:157–163.
16. Pott F, Ziem U, Reiffer F-J, Ernst H, Mohr U. Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. Exp Pathol 1987; 32:129–152.
17. Minardi F, Maltoni C. Results of recent experimental research on the carcinogenicity of natural and modified asbestos. Ann NY Acad Sci 1988;534:754–761.
18. National Toxicology Program (NTP). NTP Web site: www.ntp.server.nih.gov/search/mesothelioma, 2003.
19. Mohr U, Rittinghausen S, Takenaka S, Dungworth DL, Pylev LN. Tumours of the lower respiratory tract and pleura in the rat. In: Turusov V, Mohr U, eds. Pathology of Tumours in Laboratory Animals, 2nd ed, vol 1. Tumours of the Rat. IARC Scientific Publication No. 99. IARC, 1990: 275–299.
20. Gould DH. Mesotheliomas of tunica vaginalis propria and peritoneum in Fischer rats. Vet Pathol 1977;16:372–379.
21. Burek JD. Pathology of Aging Rats. West Palm Beach, FL: CRC Press, 1978: 135–137.
22. Deerberg F, Rehm S. Spontaneous mesotheliomas in Han-WIST rats. Z Versuchtierkd 1981;23:296–302.
23. Mostofi FK, Sesterhenn LA, Bresler VM. Tumours of the testis. In: Turusov V, Mohr U, eds. Pathology of Tumours in Laboratory Animals. Volume I, Tumours in the Rat. IARC Scientific Publication No. 99. IARC, 1990: 275–299.
24. Mohr U, Emura M, Dungworth DL, Ernst H. Tumours of the lower respiratory tract. In: IARC. Pathology of Tumours in Laboratory Animals,

2nd ed, vol 3. Tumours of the hamster. IARC Scientific Publication No. 126. IARC, 1996:189–222.
25. International Agency for Research in Cancer. Pathology of Tumours in Laboratory Animals, 2nd ed, vol 2. Tumours of the Mouse. IARC Scientific Publication No. 111. IARC, 1994.
26. Johnson NF. The utility of animal inhalation studies to assess the risk of mineral fiber-induced pulmonary cancer. In: D'Amato R, Slaga TJ, Farland WH, Henry C, eds. Relevance of Animal Studies to the Evaluation of Human Cancer Risk. New York: Wiley-Liss, 1992:19–36.
27. Kane AB. Mechanisms of fibre carcinogenesis. In: Kane A, Boffetta P, Saracci R, Wilbourn JD, eds. Mechanisms of fibre carcinogenesis. IARC Scientific Publication No. 140. IARC, 1996:11–34.
28. Kane AB. Animal models of malignant mesothelioma. In: Rom WN, ed. Environmental and Occupational Medicine, 3rd ed. Philadelphia: Lippincott-Raven, 1998:377–386.
29. Pass HI, Hadjiev O, Carbone M. Animal models of mesothelioma. In: Teicher BA, ed. Tumor Models in Cancer Research. Totowa, NJ: Humana Press, 2002:507–520.
30. International Agency for Research on Cancer. Asbestos. In: IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, vol 14. IARC, 1977:1–106.
31. International Agency for Research on Cancer. Erionite. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol 42. IARC, 1987:225–239.
32. International Agency for Research on Cancer. Man-made mineral fibres. In: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, vol 43. IARC, 1988:39–171.
33. International Agency for Research on Cancer. Man-made vitreous fibres. In: IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, vol 81. IARC, 2002:33–377.
34. Wagner JC. The induction of tumours by the intrapleural inoculations of various types of asbestos dust. In: Severi L, ed. Lung Tumours in Animals. Perugia, Italy: Division of Cancer Research, 1966:589–606.
35. Wagner JC, Berry G. Mesotheliomas in rats following inoculation with asbestos. Br J Cancer 1969;23:567–581.
36. Wagner JC, Berry G, Timbrell V. Mesotheliomata in rats after inoculation with asbestos and other materials. Br J Cancer 1973;28:173–187.
37. Wagner JC, Berry G, Skidmore JW. Studies of the carcinogenic effect of fibre glass of different diameters following intrapleural inoculation in experimental animals. In: Le Vee WN, Schulte PA, eds. Occupational Exposure to Fibrous Glass. DHEW Publication No. (NIOSH) 76–151; NTIS Publication No. PB-258869. Cincinnati, OH: DHEW, 1976:193–204.
38. Pylev LN, Shabad LM. Some results of experimental studies in asbestos carcinogenesis. In: Bogovski P, Gilson JC, Timbrell V, Wagner JC, eds. Biological Effects of Asbestos. IARC Scientific Publication No. 8. IARC, 1973:99–105.
39. Monchaux G, Bignon J, Jaurand MC, et al. Mesotheliomas in rats following inoculation with acid-leached chrysotile asbestos and other mineral fibres. Carcinogenesis 1981;2:229–236.
40. Stanton MF, Layard M, Tegeris A, Miller E, May M, Kent E. Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. J Natl Cancer Inst 1977;58:587–603.

41. Stanton MF, Layard MW. Carcinogenicity of natural and man-made fibers. In: Margison PG, ed. Advances in Medical Oncology, Research and Education. Vol I. Carcinogenesis. Oxford: Pergamon Press, 1979:181–187.
42. Stanton MF, Layard M, Tegeris A, et al. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. J Natl Cancer Inst 1981;67:965–975.
43. Maltoni C, Minardi F, Morisi L. The relevance of the experimental approach in the assessment of the oncogenic risks from fibrous and nonfibrous particles. The ongoing project of the Bologna Institute of Oncology. Med Lavoro 1982;73:394–407.
44. Wagner JC, Skidmore JW, Hill RJ, Griffith DM. Erionite exposure and mesotheliomas in rats. Br J Cancer 1985;51:727–730.
45. Pylev LN, Kulagina TF, Vasilieva LA, Chelischev NF, Berenstein BG. Blastomogenic activity of erionite (nidale erionite) (Russ.). Gig Tr Prof Zabol 1986;161:33–37.
46. Hill RJ, Edwards RE, Carthew P. Early changes in the pleural mesothelium following intrapleural inoculation of the mineral fiber erionite and the subsequent development of mesotheliomas. J Exp Pathol 1990;71: 105–118.
47. Smith WE, Hubert DD. The intrapleural route as a means for estimating carcinogenicity. In: Karbe E, Park JF, eds. Experimental Lung Cancer. Carcinogenesis and Bioassays. New York: Springer-Verlag, 1974:92–101.
48. Pott F, Roller M, Ziem U, et al. Carcinogenicity studies on natural and man-made fibres with the intraperitoneal test in rats. In: Bignon J, Peto J, Saracci R, eds. Nonoccupational Exposure to Mineral Fibres. IARC Scientific Publication No. 90. IARC, 1989:173–179.
49. Roller M, Pott F, Kamino K, Althoff GH, Bellmann B. Results of current intraperitoneal carcinogenicity studies with mineral and vitreous fibres. Exp Toxicol Pathol 1996;48:3–12.
50. Roller M, Pott F, Kamino K, Althoff GH, Bellmann B. Dose-response relationships of fibrous dusts in intraperitoneal studies. Environ Health Perspect 1997;105(suppl 5):1253–1256.
51. Rittinghausen S, Ernst H, Muhle H, Fuhst R, Mohr U. Histopathological analysis of tumor types after intraperitoneal injection of mineral fibres in rats. In: Brown RC, Hoskins JA, Johnson NF, eds. Mechanisms in Fibre Carcinogenesis. New York: Plenum Press, 1991:81–89.
52. Miller BG, Searl A, Davis JMG, et al. Influence of fibre length, dissolution and biopersistence on the production of mesothelioma in the rat peritoneal cavity. Ann Occup Hyg 1999;43:155–166.
53. Maltoni C, Minardi F. Recent results of carcinogenicity bioassays of fibres and other particulate materials. In: Bignon J, Peto J, Saracci R, eds. Nonoccupational Exposure to Mineral Fibres. IARC Scientific Publication No. 90. IARC, 1989:46–53.
54. Davis JMG, Bolton RE, Miller BG, Niven K. Mesothelioma dose response following intraperitoneal injection of mineral fibres. Int J Exp Pathol 1991; 72:263–274.
55. Davis JMG, Jones AD. Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. Br J Exp Pathol 1988;69:717–739.
56. Davis JMG, Addison J, McIntosh C, Miller BG, Niven K. Variations in the carcinogenicity of tremolite dust samples of differing morphology. Ann NY Acad Sci 1991;643:437–490.
57. Grimm HG, Bernstein DM, Attia M, Richard J, de Reydellet A. Experience from a long-term carcinogenicity study with intraperitoneal injection of biosoluble synthetic mineral fibers. Inhal Toxicol 2002;14:855–882.

58. Suzuki Y, Kohyama M. Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. Environ Res 1984;35:277–292.
59. Özesmi M, Patiroglu TE, Hillerdal G, Özesmi C. Peritoneal mesothelioma and malignant lymphoma in mice caused by fibrous zeolite. Br J Ind Med 1985;42:746–749.
60. Moalli PA, MacDonald JL, Goodglick LA, Kane AB. Acute injury and regeneration of the mesothelium in response to asbestos fibers. Am J Pathol 1987;128:426–445.
61. Davis MR, Manning LS, Whitaker D, Garlepp MJ, Robinson BW. Establishment of a murine model of malignant mesothelioma. Int J Cancer 1992;52:881–886.
62. Goodglick LA, Vaslet CA, Messier NJ, Kane AB. Growth factor responses and protooncogene expression of murine mesothelial cell lines derived from asbestos-induced mesotheliomas. Toxicol Pathol 1997;25:565–573.
63. Marsella JM, Liu BL, Vaslet CA, Kane AB. Susceptibility of p53-deficient mice to induction of mesothelikoma by crocidolite asbestos fibers. Environ Health Perspect 1997;105(suppl 5):1069–1072.
64. Vaslet CA, Messier NJ, Kane AB. Accelerated progression of asbestos-induced mesotheliomas in heterozygous $p53^{+/-}$ mice. Toxicol Sci 2002;68: 331–338.
65. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of inhalation of asbestos in rats. Br J Cancer 1974;29:252–269.
66. Davis JMG, Beckett ST, Bolton RE, Collings P, Middleton AP. Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. Br J Cancer 1978;37:637–688.
67. Davis JMG, Addison J, Bolton RE, Donaldson K, Jones AD, Smith T. The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. Br J Exp Pathol 1986;67:415–430.
68. Pott F. Neoplastic findings in experimental asbestos studies and conclusions for fiber carcinogenesis in humans. Ann NY Acad Sci 1991;643: 205–218.
69. Johnson NF, Edwards RE, Munday DE, Rowe N, Wagner JC. Pluripotential nature of mesotheliomata induced by inhalation of erionite in rats. Br J Exp Pathol 1984;65:377–388.
70. Davis JMG. Mineral fibre carcinogenesis: experimental data relating to the importance of fibre type, size, deposition, dissolution and migration. In: Bignon J, Peto J, Saracci R, eds. Non-occupational Exposure to Mineral Fibres. IARC Scientific Publication No. 90. IARC, 1989:33–45.
71. McConnell EE, Axten C, Hesterberg TW, et al. Studies on the inhalation toxicology of two fibrglasses and amosite asbestos in the Syrian golden hamster. Part II. Results of chronic exposure. Inhal Toxicol 1999;11: 785–835.
72. Gelzleichter TR, Bermudez E, Mangum JB, et al. Comparison of pulmonary and pleural responses of rats and hamsters to inhaled refractory ceramic fibers. Toxicol Sci 1999;49:93–101.
73. Bunn WB, Bender JR, Hesterberg TW, Chase GR, Konzen JL. Recent studies of man-made vitreous fibers. Chronic animal inhalation studies. J Occup Med 1993;35:101–113.
74. Everitt JI, Bermudez E, Mangum JB, et al. Pleural lesions in Syrian golden hamsters and Fischer-344 rats following intrapleural instillation of man-made ceramic or glass fibers. Toxicol Pathol 1994;22:229–236.
75. Saffiotti U, Williams AO, Daniel LN, Kaighn ME, Mao Y, Shi X. Carcinogenesis by crystalline silica: animal, cellular and molecular studies. In:

Castranova V, Vallyathan V, Wallace WE, eds. Silica and Silica-Induced Lung Diseases. Boca Raton, FL: CRC Press, 1996:345–381.
76. Wagner MMF, Wagner JC, Davies R, Griffiths DM. Silica-induced malignant histiocytic lymphoma: incidence linked with strain of rat and type of silica. Br J Cancer 1980;41:908–917.
77. Shabad LM, Pylev LN, Krivosheeva LV, Kulagina TF, Nemenko BA. Experimental studies on asbestos carcinogenicity. J Natl Cancer Inst 1974; 52:1175–1187.
78. Mohr U, Pott F, Vonnahme F-J. Morphological aspects of mesotheliomas after intratracheal instillation of fibrous dusts in Syrian golden hamsters. Exp Pathol 1984;26:179–183.
79. Adamson IY, Bakowska J, Bowden DH. Mesothelial cell proliferation: a nonspecific response to lung injury associated with fibrosis. Am J Respir Cell Mol Biol 1994;10:253–258.
80. Adamson IY, Prieditis H, Young L. Lung mesothelial cell and fibroblast responses to pleural and alveolar macrophage supernatants and to lavage fluids from crocidolite-exposed rats. Am J Respir Cell Mol Biol 1997;16: 650–656.
81. LaRocca P, Rheinwald JG. Co-expression of simple epithelial keratins and vimentin by human mesothelium and mesothelioma in vivo and in culture. Cancer Res 1984;44:2991–2999.
82. Adamson IY, Bakowska J. KGF and HGF are growth factors for mesothelial cells in pleural lavage fluid after intratracheal asbestos. Exp Lung Res 2001;27:605–616.
83. Adamson IY, Bakowska J, Prieditis H. Proliferation of rat pleural mesothelial cells in response to hepatocyte and keratinocyte growth factors. Am J Respir Cell Mol Biol 2000;23:345–349.
84. Chouroulinkov I. Experimental studies on ingested fibres. In: Bignon J, Peto J, Saracci R, eds. Non-occupational Exposure to Mineral Fibres. IARC Scientific Publication No. 90. IARC, 1989:112–126.
85. Truhaut R, Chouroulinkov I. Effect of long-term ingestion of asbestos fibres in rats. In: Bignon J, Peto J, Saracci R, eds. Non-occupational Exposure to Mineral Fibres. IARC Scientific Publication No. 90. IARC, 1989: 127–133.
86. Greenblatt M, Lijinsky W. Nitrosamine studies: neoplasms of liver and genital mesothelium in nitrosopyrrolidine-treated MRC rats. J Natl Cancer Inst 1972;48:1687–1696.
87. Berman J, Rice JM. Mesotheliomas and proliferative lesions of the testicular mesothelium produced in Fischer, Sprague-Dawley, and Buffalo rats by methyl(acetoxymethyl)nitrosamine (DMN-OAc). Vet Pathol 1979;16: 574–582.
88. Cabral JRP, Neal GE. Testicular mesotheliomas in rats exposed to *N*-2-fluorenylacetamide (FAA). Tumori 1983;69:195–199.
89. Snellings WM, Weil CS, Maronpot RR. A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. Toxicol Appl Pharmacol 1984;75:105–117.
90. Lynch DW, Lewis TR, Moorman WJ, et al. Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. Toxicol Appl Pharmacol 1984;76:69–84.
91. Rice JM, Kovatch RM, Anderson LM. Intraperitoneal mesothelioma induced in mice by a polycyclic aromatic hydrocarbon. J Toxicol Environ Health 1989;27:153–160.
92. Peterson JT Jr, Greenberg SD, Buffler PA. Non-asbestos-related malignant mesothelioma. Cancer 1984;54:951–960.

93. Terao K. Mesotheliomas induced by sterigmatocystin in Wistar rats. Gann 1978;69:237–247.
94. Pelfrene A, Garcia H. Histology of chemically induced mesotheliomas in MRC-Wistar rats. Tumori 1975;61:509–516.
95. Rao MS, Reddy JK. Pathology of tumors developed in guinea pigs given intraperitoneal injections of N-methyl-N-nitrosourea. Neoplasma 1977;24: 57–62.
96. McClure NM, Graham CE. Malignant uterine mesotheliomas in squirrel monkeys following diethylstilbestrol administration. Lab Anim Sci 1973; 23:493–498.
97. Okada S, Hamazaki S, Toyokuni S, Midorikawa O. Induction of mesothelioma by intraperitoneal injections of ferric saccharate in male Wistar rats. Br J Cancer 1989;60:708–711.
98. Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y. Toxicity and carcinogenicity of potassium bromate—a new renal carcinogen. Environ Health Perspect 1990;87:309–335.
99. Sanders CI, Jackson TA. Induction of mesotheliomas and sarcomas from hot spots of $^{239}PuO_2$ activity. Health Phys 1972;22:755–759.
100. Hahn FF, Haley PJ, Hubbs AF, Hoover MD, Lundgren DL. Radiation-induced mesotheliomas in rats. In: Brown RC, Hoskins JA, Johnson NF, eds. Mechanisms in Fibre Carcinogenesis. New York: Plenum Press, 1991: 91–99.
101. Sanders CL. Pleural mesotheliomas in the rat following exposure to PuO_2. Health Phys 1992;63:695–697.
102. Lafuma J, Morin M, Poncy JL, Masse R. Mesothelioma induced by intrapleural injection of different types of fibres in rats; synergistic effect of other carcinogens. In: Wagner JC, ed. Biological Effects of Mineral Fibres. IARC Scientific Publication No. 30. IARC, 1980:311–320.
103. Davis JMG, Jones AD, Miller BG. Experimental studies in rats on the effects of asbestos inhalation coupled with the inhalation of titanium dioxide or quartz. Int J Exp Pathol 1991;72:501–525.
104. Saffiotti U. From human and experimental silica carcinogenesis to molecular mechanisms, transcription factors and signaling pathways. Am J Ind Med Submitted.
105. Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. Am J Pathol 1993;142:1524–1533.
106. McConnell EE, Carbone C. A comparison of pleural mesotheliomas induced by asbestos and SV40 virus in Syrian golden hamsters. Inhal Toxicol 2000;12(suppl 3):173–181.
107. Stanton MF, Stewart SE, Eddy BE, Blackwell RH. Oncogenic effect of tissue-culture preparations of polyoma virus on fetal mice. J Nat Cancer Inst 1959;23:1441–1475.
108. Rabson AS, Branigan WJ, Legallais FY. Lung tumors produced by intratracheal inoculation of polyoma virus in Syrian golden hamsters. J Natl Cancer Inst 1960;25:937–965.
109. Chabot JF, Beard D, Langlois AJ, Beard JW. Mesotheliomas of peritoneum, epicardium, and pericardium induced by strain MC29 avian leukosis virus. Cancer Res 1970;30:1287–1308.
110. England JM, Panella MJ, Ewert DL, Halpern MS. Induction of a diffuse mesothelioma in chickens by intraperitoneal inoculation of v-*src* DNA. Virology 1991;182:423–429.
111. Wagner JC, Griffith DM, Hill RJ. The effect of fibre size on the in vivo activity of UICC crocidolite. Br J Cancer 1984;49:453–458.

112. Jaurand MC. Observations on the carcinogenicity of asbestos fibers. Ann NY Acad Sci 1991;643:258–270.
113. Pott F. Detection of mineral fibre carcinogenicity with the intraperitoneal test—Recent results and their validity. Ann Occup Hyg 1995;39:771–779.
114. Walker C, Everitt J, Barrett JC. Possible cellular and molecular mechanisms for asbestos carcinogenicity. Am J Ind Med 1992;21:253–273.
115. Suzuki Y. Comparability of mesothelioma in humans and in experimental animal studies. Ann NY Acad Sci 1991;643:219–222.
116. Appel JD, Fasy TM, Kohtz DS, Kohtz JD, Johnson EM. Asbestos fibers mediate transformation of monkey cells by exogenous plasmid DNA. Proc Natl Acad Sci USA 1988;85:7670–7674.
117. International Agency for Research on Cancer. Consensus report. In: Kane A, Boffetta P, Saracci R, Wilbourn JD, eds. Mechanisms of fibre carcinogenesis. IARC Scientific Publication No. 140. IARC, 1996:1–9.
118. Vu V, Barrett JC, Roycroft J, et al. Workshop Report. Chronic inhalation toxicity and carcinogenicity testing of respirable fibrous particles. Regul Toxicol Pharmacol 1996;24:202–212.
119. Muhle H, Pott F. Asbestos as reference material for fibre-induced cancer. Int Arch Occup Environ Health 2000;73:S53-S59.
120. Maxim LD, McConnell EE. Interspecies comparisons of the toxicity of asbestos and synthetic vitreous fibers: a weight-of-the-evidence approach. Regul Toxicol Pharmacol 2001;33:319–342.
121. Mossman BT, Gruenert DC. SV40, growth factors, and mesothelioma. Am J Respir Cell Mol Biol 2002;26:167–170.
122. Sandhu H, Dehnen W, Roller M, Abel J, Unfried K. mRNA expression patterns in different stages of asbestos-induced carcinogenesis in rats. Carcinogenesis 2000;21:1023–1029.
123. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002;29:2–17.
124. Hirao T, Bueno R, Chen CJ, Gordon GJ, Heilig E, Kelsey KT. Alterations of the p16(INK4) locus in human malignant mesothelial tumors. Carcinogenesis 2002;23:1127–1130.
125. Bocchetta M, Di Resta I, Powers A, et al. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. Proc Natl Acad Sci USA 2000;97:10214–10219.
126. Kleymenova EV, Horesovsky G, Pylev LN, Everitt J. Mesotheliomas induced in rats by the fibrous mineral erionite are independent from p53 alterations. Cancer Lett 1999;147:55–61.
127. Kleymenova EV, Bianchi AA, Kley N, Pylev LN, Walker CL. Characterization of the rat neurofibromatosis 2 gene and its involvement in asbestos-induced mesothelioma. Mol Carcinog 1997;18:54–60.
128. Heinz NH, Janssen YM, Mossman BT. Persistent induction of c-fos and c-jun expression by asbestos. Proc Natl Acad Sci USA 1993;90:3299–3303.
129. Ramos-Nino ME, Timblin C, Mossman BT. Mesothelial cell transformation requires increased AP-1 binding activity and ERK-dependent Fra-1 expression. Cancer Res 2002;62:6065–6069.
130. Fujino S, Yokohama A, Kohno N, Hiwada K. Interleukin 6 is an autocrine growth factor for normal human pleural mesothelial cells. Am J Respir Cell Mol Biol 1996;14:508–515.
131. Gerwin BI. Cytokine signaling in mesothelial cells: receptor expression closes the autocrine loop. Am J Respir Cell Mol Biol 1996;14:505–507.
132. Pass HI, Mew DJY. In vitro and in vivo studies of mesothelioma. J Cell Biochem Suppl 1996;24:142–151.

133. Bielefeldt-Ohmann H, Fitzpatrick DR, Marzo AL, et al. Patho- and immunobiology of malignant mesothelioma: characterisation of tumour infiltrating leukocytes and cytokine production in a murine model. Cancer Immunol Immunother 1994;39:347–359.
134. Fitzpatrick DR, Bielefeldt-Ohmann H, Himbeck RP, Jarnicki AG, Marz AL, Robinson BW. Transforming growth factor-beta: antisense RNA-mediated inhibition affects anchorage-independent growth, tumorigenicity and tumor-infiltrating T-cells in malignant mesothelioma. Growth Factors 1994;11:29–44.
135. Muller AM, Weichert A, Muller KM. E-cadherin, E-selectin and vascular cell adhesion molecule: immunohistochemical markers for differentiation between mesothelioma and metastatic pulmonary adenocarcinoma? Virchows Arch 2002;441:41–46.
136. Laskin WB, Miettinen M. Epithelial-type and neural-type cadherin expression in malignant noncarcinomatous neoplasms with epithelioid features that involve the soft tissues. Arch Pathol Lab Med 2002;126:425–431.
137. Rosner A, Miyoshi K, Landesman-Bollag E, et al. Pathway pathology. Histological differences between Erb/Ras and Wnt pathway transgenic mammary tumors. Am J Pathol 2002;161:1087–1097.

5

Mesothelial and Mesothelioma Cell Lines

Agnes B. Kane

Primary Mesothelial Cell Lines

Primary cultures of mesothelial cells have been established from rats, rabbits, mice, and humans (Table 5.1). Mesothelial cell lines provide several advantages for experimental studies: they provide a large number of cells isolated from a single donor, cell lines can be isolated from genetically engineered mice, and primary cell lines limit the number of animals required for experiments. However, cell lines have several disadvantages: variability among donors, variability in culture conditions in different laboratories, potential phenotypic and genetic instability, and a limited life span in vitro (reviewed in ref. 1). Some of these disadvantages can be overcome by quality control procedures. For example, cell lines should not be passaged indefinitely; frozen stocks should be maintained and thawed at regular intervals to prevent phenotypic and genetic instability (reviewed in ref. 2). As in all cell culture models, precautions are required to prevent cross-contamination and contamination with bacteria or viruses. DNA profiles could be useful to identify cell lines; for example Manning et al (3) established initial genetic profiles for their panel of human malignant mesothelioma cell lines. All cultures should be screened for *Mycoplasma* and other pathogens (2).

Technical details regarding primary human mesothelial cell cultures have been summarized by Versnel et al (1) and Gerwin (4). Briefly, primary human mesothelial cells require enriched culture media supplemented with 10% to 20% fetal bovine serum, exogenous growth factors [usually epidermal growth factor (EGF)], insulin, transferrin, and hydrocortisone. Rabbit, mouse, and rat primary mesothelial cells require similar growth conditions, with the important exception that growth of rat pleural mesothelial cells is inhibited by EGF. As reviewed by Walker et al (5), there are additional differences in expression of growth factors and their receptors between human and rat mesothelial cells (6). Differences in growth factor responses have been described in primary human mesothelial cell cultures derived from different donors (7).

Table 5.1. Primary mesothelial cell lines

Source	Isolation procedure	Reference
Human pericardial effusion	None	Castor and Naylor (53)
Rat parietal pleura	Mechanical scraping	Thiollet et al (54) Bermudez et al (9)
Rabbit pleura	Mechanical scraping or enzymatic digestion	Antony et al (55)
Mouse parietal peritoneum	Enzymatic digestion	Cistulli et al (14)
Human omentum	Enzymatic digestion	Stylianou et al (56) Yáñez-Mó et al (18)
Human peritoneal effusion	Plating overnight	Wu et al (20) Jonjic et al (57)
Human pleural effusion	Plating overnight	Reviewed in Versnel et al (1) and in Lechner et al (7)
Human peritoneal dialysis effluent	Centrifugation	Yáñez-Mó et al (18)

Mesothelial cell cultures have been characterized by morphology, electron microscopy, immunocytochemistry, and cytogenetics (1,8). Although mesothelial cells can form monolayers with epithelial morphology, this growth pattern can be altered in vitro as described below. At the ultrastructural level, mesothelial cells typically show surface microvilli, abundant mitochondria, extensive rough endoplasmic reticulum, perinuclear intermediate filaments, desmosomes, and tight junctions (9). Immunocytochemistry is useful to confirm expression of markers specific for mesothelial cells, especially coexpression of intermediate filaments, keratin, and vimentin (10) and expression of the Wilms' tumor suppressor gene, *WT1* (11). These markers are also useful for the immunohistochemical diagnosis of human malignant mesotheliomas (12,13). Cytogenetic studies of human mesothelial cell lines reveal a normal karyotype that may acquire abnormalities after several passages (1). One primary murine mesothelial cell line has been reported that spontaneously acquired a point mutation in exon 5 of the *p53* tumor suppressor gene. This mutation increased growth rate in vitro; however, it did not confer tumorigenicity (14).

Primary cell lines provide a valuable model to study the cell biology and differentiation of normal mesothelial cells. Primary cultures have also been used to investigate the toxicologic effects of asbestos and man-made mineral fibers (15). The responses of mesothelial cells to various growth factors and cytokines are discussed in Chapter 7.

The mesothelium is derived embryologically from the mesoderm. At approximately embryonic day 7.5 in the mouse, epithelial cells undergo mesenchymal differentiation to form the mesoderm cell layer. This morphologic differentiation is governed by transcription factors *snail* and *slug* that modulate expression of cadherins and cytoskeletal proteins characteristic of mature mesothelial cells (16,17). In response to mechanical injury, peritoneal dialysis, or chronic inflammation, mesothelial cells also revert from an epithelial to a mesenchymal phe-

notype. This transdifferentiation is termed the epithelial-mesenchymal transition (18) and has been investigated in primary cultures of human mesothelial cells isolated from reactive peritoneal effusions or dialysis effluent (Table 5.1). In these pathologic conditions, human mesothelial cells detach from the mesothelial monolayer and survive in suspension. When these reactive mesothelial cells are placed in monolayer culture, they express epithelial or mesenchymal phenotypes (18,19). Wu et al (20) first characterized the expression of cytoskeletal proteins including actin, vimentin, and several cytokeratins by mesothelial cells isolated from ascitic fluid. Modulation of the epithelial phenotype in vitro depended on culture conditions: serum, EGF, and hydrocortisone induced a mesenchymal phenotype (21), while supplementation with retinoic acid induced an epithelial phenotype (22). The epithelial–mesenchymal transition of reactive human mesothelial cells in vitro is characterized by reduced expression of some cell surface proteoglycans (syndecan-4, glypican-1), the *WT1* tumor suppressor gene (19), and decreased expression of E cadherin in parellel with expression of the transcription factor *snail* (18). Transdifferentiation of omental mesothelial cells in vitro was also induced by mechanical wounding of mesothelial monolayers or by exposure to the inflammatory mediators, transforming growth factor-β_1 (TGF-β_1) or interleukin-1β (IL-1β) (18).

Mesothelial cells are sensitive target for transformation by asbestos fibers. The biologic basis for this increased sensitivity is unknown. Studies conducted with cell culture models have provided evidence that the iron-catalyzed generation of reactive oxygen species is a plausible mechanism for asbestos carcinogenicity (23). Reactive oxygen species have been implicated in asbestos-induced apoptosis (24,25), chromosomal damage (26), oxidative DNA damage (27), and DNA strand breaks (28) in human and rat pleural mesothelial cells. Variations in antioxidant defense mechanisms have been hypothesized to contribute to pulmonary disease induced by fibers and particulates (29). The antioxidant defense pathways of primary rat pleural mesothelial cells have been characterized in detail; these cultures have low catalase activity and depend primarily on the glutathione pathway for protection against oxidant stress (30). These mechanistic studies suggest that mesothelial cells are highly susceptible to DNA and chromosomal damage in response to asbestos exposure. Mesothelial cells with asbestos-induced DNA damage that escape apoptosis may be precursors for the development of malignant mesothelioma (24).

In Vitro Transformation of Mesothelial Cells

In vitro models of cell transformation have been developed to assess the ability of viral, physical, or chemical agents to induce immortalization and transformation of target cells (reviewed in ref. 31). Primary cultures of rat pleural mesothelial cells have been used to investigate the ability of chrysotile asbestos fibers to induce colony formation and tumorigenicity (Table 5.2). Unfortunately, rodent cells, including mesothelial cells, become spontaneously immortalized at late passages.

Table 5.2. In vitro models of mesothelial cell transformation

Model	Treatment	Experimental end points		Reference
		Colony formation	Tumorigenicity	
Rat pleural mesothelial cells	None	+ (late passage)	+ (late passage)	Jaurand et al (32)
	None	+ (late passage)	?	Funaki et al (33)
	Chrysotile asbestos	+ (early or middle passage)	–	Jaurand et al (32)
Human peritoneal mesothelial cells	Activated H-*ras* oncogene	+	–	Tubo and Rheinwald (39)
	SV40 large-T antigen	+	–	O'Connell and Rheinwald (41)
Human pleural mesothelial cells	SV40 large-T antigen	+	–	Ke et al (34)
	SV40 large-T antigen + activated H-*ras* oncogene	+	+	Reddel et al (40)
Human pleural mesothelial cells	hTERT	–	?	Yu et al (42)
	hTERT + SV40 large-T and small-t antigens	+	?	
	hTERT + SV40 small-t antigen + activated H-*ras*	+	?	
Human pleural mesothelial cells	SV40 virus infection	+	?	Bocchetta et al (36)
	Crocidolite asbestos	–	?	
	SV40 large-T antigen	–	?	
	SV40 large-T and small-t antigens	+	?	
	SV40 large-T and small-t antigens + crocidolite asbestos	+	?	
	SV40 large-T antigen + crocidolite asbestos	+	?	

T, t, tumor.

In two rat mesothelial models, these spontaneously immortalized cultures showed disorganized growth, loss of contact inhibition, decreased doubling times, and growth in soft agar. In both models, immortalized cell populations became aneuploid with trisomy of chromosome 1 (32,33). Late passages of spontaneously immortalized rat pleural mesothelial cells were tumorigenic after subcutaneous injection in nude mice (32). Single or repeated exposures of rat pleural mesothelial cells to chrysotile asbestos fibers induced in vitro transformation and tumorigenicity at earlier passages (32). In contrast to rodent mesothelial cells, human mesothelial cells stop dividing after 15 (34) to 55 (35) population doublings and do not immortalize spontaneously. Exposure of human pleural mesothelial cells to crocidolite asbestos fibers is toxic and does not induce transformation in vitro (36). Human peritoneal cells can be induced to proliferate indefinitely (greater than 100 population doublings) by transfection with *hTERT*, the catalytic subunit of telomerase. These immortalized cultures are still dependent on EGF, hydrocortisone, or serum for growth and do not show morphologic characteristics of transformed cells (35).

Cell transformation models are valuable tools to identify specific genes responsible for immortalization and tumorigenicity. Initial studies using rodent fibroblasts identified at least two collaborating oncogenes (e.g., *ras* and *myc*) that were required for in vitro transformation (37). In contrast, human cells cannot be fully transformed by these collaborating oncogenes unless exposed to chemical or physical carcinogenic agents (reviewed in ref. 38). Human peritoneal mesothelial cells transfected with activated H-*ras* oncogene show characteristics of transformed cells; however, they are not tumorigenic when injected subcutaneously in nude mice (39). A human pleural mesothelial line (Met5A) was stably immortalized by simian virus 40 (SV40) early region that encoded large-tumor antigen; these cells formed colonies in vitro and were hypodiploid with multiple chromosomal abnormalities but they are not tumorigenic (34). However, Met5A cells transfected with activated H-*ras* oncogene were tumorigenic in nude mice (40).

With the recent discovery of SV40 viral DNA sequences in human malignant mesotheliomas (see Chapter 3), additional assays have been conducted to investigate specific genes required for in vitro transformation of human pleural mesothelial cells (Table 5.2). Human peritoneal mesothelial cells appear to be more susceptible to transformation by SV40 large-tumor antigen (41) than pleural mesothelial cells (36). Human pleural mesothelial cells immortalized by transfection of *hTERT* show in vitro transformation by SV40 large-T and small-t antigens or by SV40 small-t antigen plus activated H-*ras* oncogene (42). Crocidolite asbestos in combination with SV40 large-tumor or large-tumor and small-tumor antigens induced in vitro transformation and clonal chromosomal aberrations (36).

Most tumor cells, including human malignant mesotheliomas, show a common set of characteristics: autonomous cell growth, resistance to growth-inhibitory signals, evasion of apoptosis, indefinite proliferation potential, angiogenesis, and invasion and metastasis (reviewed in ref.

43). Most tumor cell lines, including rodent and human mesothelioma cell lines (reviewed below and in Chapter 6) show multiple genetic alterations and genetic instability that may have enabled tumor cells to acquire these characteristics. Numerous in vitro studies using rodent or human mesothelial cell lines exposed to asbestos fibers have demonstrated their genotoxic and clastogenic activities (reviewed in ref. 44). In these in vitro cell transformation models, rat and human mesothelial cells exposed to asbestos fibers also show chromosomal damage (32,34,36). It is hypothesized that reactive oxygen species generated in response to asbestos fibers induce chromosomal or genetic instability that enables subsequent immortalization and transformation by re-expression of *hTERT*, SV40 large-tumor and small-tumor antigens, and activation of intracellular signaling pathways (reviewed in Chapter 2). Most of these genetic changes that have been defined as the minimal requirements for transformation of human cells (38) are also found in human malignant mesotheliomas. For example, telomerase activity has been detected in human pleural mesotheliomas (45,46). Inactivation of the pRB growth inhibitory pathway by SV40 large-tumor antigen or deletion of the *p16* tumor suppressor gene has been described in human and murine mesothelioma cell lines. The *p53* tumor suppressor gene that controls cell cycle checkpoints and apoptosis is inactivated by SV40 large-tumor antigen. Silencing and deletion of the *p14* or $p19^{ARF}$ tumor suppressor genes have been found in both human and murine mesothelioma cell lines (reviewed in Chapter 6). Finally, exposure of mesothelial cells to asbestos fibers in vitro activates multiple cell signaling pathways leading to sustained cell proliferation (as described in Chapter 2).

Malignant Mesothelioma Cell Lines

Cell lines have been derived from rodent (Table 5.3) or human malignant mesotheliomas (Table 5.4). Most of the rodent cell lines were derived after direct intrapleural or intraperitoneal injection of asbestos fibers; no cell lines have been isolated from rodents exposed to fibers by inhalation. Despite the unnatural route of delivery, these rodent malignant mesotheliomas produced by direct injection of fibers closely resemble human malignant mesotheliomas with respect to their morphology, pattern of growth, and natural history (reviewed in ref. 47). A limited number of molecular studies have been carried out with

Table 5.3. Rodent malignant mesothelioma cell lines

Source	Exposure	Reference
Rat peritoneum	Crocidolite asbestos	Gormley et al (58)
Rat pleura	Crocidolite asbestos	Whitaker et al (59)
Rat peritoneum	Chrysotile or crocidolite asbestos	Craighead et al (8)
Rat peritoneum	Spontaneous	Funaki et al (33)
Mouse peritoneum	Crocidolite asbestos	Davis et al (60)
Mouse peritoneum	Crocidolite asbestos	Goodglick et al (51)

Table 5.4. Human malignant mesothelioma cell lines

Source	Reference
Pleural effusions or biopsies	Gerwin et al (6)
Pleural effusions, biopsies, or tumors	Versnel et al (61) Demetri et al (62)
Pleural effusions or biopsies	Pelin-Enlund et al (63)
Pleural effusions	Manning et al (3)
Pleural effusions or biopsies	Schmitter et al (64)
Pleural biopsies	Taguchi et al (65)
Pleural effusions or biopsies	Zeng et al (66) Pass et al (68)

rodent malignant mesothelioma cell lines; in general, the murine cell lines resemble human mesothelioma cell lines with respect to common alterations in tumor suppressor genes, especially deletions of *p16* and $p19^{ARF}$ (reviewed in ref. 48). Rodent and human malignant mesothelioma cell lines are important for testing of novel therapeutic strategies.

There are several caveats in using mesothelioma cell lines to investigate the pathogenesis of diffuse malignant mesothelioma. First, most of the available rodent and human malignant cell lines have been derived from malignant tumors. These malignant cell lines represent the end point in the neoplastic process and provide limited information about the molecular events involved in earlier stages of tumor development and progression (47). Second, rodent cells are more easily immortalized than human cells. Immortalized human cell lines and most malignant cell lines have acquired expression of telomerase. In contrast, in most adult mouse tissues, telomerase is expressed constitutively. Third, fewer genetic changes are required to induce cancer in murine models in comparison to human cancers (reviewed in 38). Newer genetically engineered mice may be developed to replicate more closely the molecular alterations found in human malignant mesotheliomas. Finally, cell lines propagated as monolayers in vitro do not represent the complex tumor microenvironment in vivo (49,50). Growth of mesothelioma cell lines as spheroids or cocultured with stromal cells would more accurately model tumors in situ. We have developed an in-vivo, ex-vivo approach to study the molecular pathogenesis of malignant mesothelioma induced by direct intraperitoneal injection of crocidolite asbestos fibers in mice. Mesothelial cell lines were derived from mice at various intervals in the development of these tumors representing reactive, preneoplastic, or neoplastic mesothelial cells (51). We used complementary DNA (cDNA) microarrays (52) to develop gene expression profiles of these murine mesothelial cell lines. Upregulation of genes involved in signal transduction and cell proliferation was found in preneoplastic mesothelial cell lines. Neoplastic mesothelial cell overexpressed genes involved in cell proliferation, altered cell cycle regulation, and resistance to apoptosis. It is hypothesized that additional genes are upregulated at later stages in the development of malignant mesothelioma that allow these tumors to induce angiogenesis and invade (Fig. 5.1). The importance of these genetic alterations in the pathogenesis of mesotheliomas can be assessed using genetically engineered mice as described in Chapter 4.

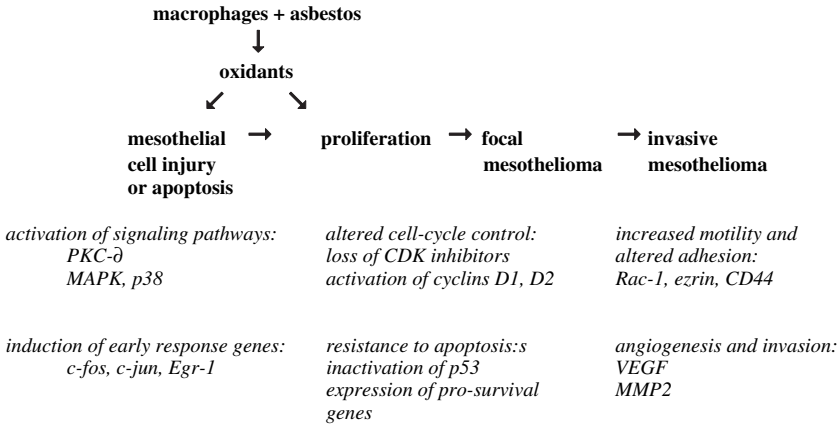


Figure 5.1. Molecular pathogenesis of malignant mesothelioma. CDK, cyclin-dependent kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PKC, protein kinase C; VEGF, vascular endothelial growth factor.

Acknowledgment

The research conducted in the author's laboratory was supported by National Institutes of Health (NIH) grant R01 ES03721 from the National Institute of Environmental Health Sciences.

References

1. Versnel MA, van der Kwast TH, Hoogsteden HC, et al. Establishment and characteristics of human normal and malignant mesothelial cell lines. In: Jaurand M-C, Bignon J, eds. The Mesothelial Cell and Mesothelioma. New York: Marcel Dekker, 1994:169–186.
2. Masters JRW. Human cancer cell lines: fact and fantasy. Nature Rev Mol Cell Biol 2000;1:233–236.
3. Manning LS, Whitaker D, Murch AR, et al. Establishment and characterization of five human malignant mesothelioma cell lines derived from pleural effusions. Int J Cancer 1991;47:285–290.
4. Gerwin BI. Mesothelial carcinogenesis: possible avenues of growth promotion. In: Jaurand M-C, Bignon J, eds. The Mesothelial Cell and Mesothelioma. New York: Marcel Dekker, 1994:223–244.
5. Walker C, Bermudez E, Evertt J. Growth factor and receptor expression by mesothelial cells: a comparison between rodents and humans. In: Harris CC, Lechner JF, Brinkley BR, eds. Cellular and Molecular Aspects of Fiber Carcinogenesis. New York: Cold Spring Harbor Laboratory Press, 1991: 149–158.
6. Gerwin BI, Lechner JF, Reddel RR, et al. Comparison of production of transforming growth factor-β and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. Cancer Res 1987; 47:6180–6184.
7. Lechner JF, LaVeck MA, Gerwin BI, et al. Differential responses to growth factors by normal human mesothelial cultures from individual donors. J Cell Physiol 1989;139:295–300.

8. Craighead JE, Akley NJ, Gould LB, et al. Characteristics of tumors and tumor cells cultured from experimental asbestos-induced mesotheliomas in rats. Am J Pathol 1987;129:448–462.
9. Bermudez E, Everitt J, Walker C. Expression of growth factor receptor RNA in rat pleural mesothelial cells in culture. Exp Cell Res 1990;190:91–98.
10. Mackay AM, Tracy RP, Craighead JE. Intermediate filament proteins in asbestos-induced mesotheliomas of the rat. Cancer Res 1987;47:5461–5468.
11. Walker C, Rutten F, Yuan X, et al. Wilms' tumor suppressor gene expression in rat and human mesothelioma. Cancer Res 1994;54:3101–3106.
12. Zeng L, Fleury-Feith J, Monnet I, et al. Immunocytochemical characterization of cell lines from human malignant mesothelioma: characterization of human mesothelioma cell lines by immunocytochemistry with a panel of monoclonal antibodies. Hum Pathol 1994;25:227–234.
13. Ordóñez NG. Immunohistochemical diagnosis of epithelioid mesotheliomas: a critical review of old markers, new markers. Hum Pathol 2002;33: 953–967.
14. Cistulli CA, Sorger T, Marsella JM, et al. Spontaneous *p53* mutation in murine mesothelial cells: increased sensitivity to DNA damage induced by asbestos and ionizing radiation. Toxicol Appl Pharmacol 1996;141:264–271.
15. Lechner JF, Gerwin BI, Reddel RR, et al. Studies on human mesothelial cells: effects of growth factors and asbesti-form fibers. In: Harris CC, Lechner JF, Brinkley BR, eds. Cellular and Molecular Aspects of Fiber Carcinogenesis. New York: Cold Spring Harbor Laboratory Press, 1991: 115–130.
16. Cano A, Pérez-Moreno MA, Rodrigo I, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2000;2:76–83.
17. Carver EA, Jiang R, Lan Y, et al. The mouse snail gene encodes a key regulator of the epithelial-mesenchymal transition. Mol Cell Biol 2001;21: 8184–8188.
18. Yáñez-Mó M, Lara-Pezzi E, Selgas R, et al. Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells. N Engl J Med 2003;348: 403–413.
19. Gulyás M, Dobra K, Hjerpe A. Expression of genes coding for proteoglycans and Wilms tumor susceptibility gene 1(WT1) by variously differentiated benign human mesothelial cells. Differentiation 1999;65:89–96.
20. Wu Y-J, Parker LM, Binder NE, et al. The mesothelial keratins: a new family of cytoskeletal proteins identified in cultured mesothelial cells and nonkeratinizing epithelia. Cell 1982;31:693–703.
21. O'Connell ND, Rheinwald JG. Regulation of the cytoskeleton in mesothelial cells: reversible loss of keratin and increase in vimentin during rapid growth in culture. Cell 1983;34:245–253.
22. Kim KH, Stellmach V, Javors J, et al. Regulation of human cell differentiation: opposing roles of retinoids and epidermal growth factor in the expression of intermediate filament proteins. J Cell Biol 1987;105:3039–3051.
23. Kamp DW, Graceffa P, Pryor WA, et al. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 1992;12:293–315.
24. Broaddus VC, Yang L, Scavo LM, et al. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. J Clin Invest 1996;98:2050–2059.
25. Bérubé KA, Quinhan TR, Fung H, et al. Apoptosis is observed in mesothelial cells after exposure to crocidolite asbestos. Am J Respir Cell Mol Biol 1996;15:141–147.

26. Lechner JF, Tokimwa T, LaVeck M, et al. Asbestos-associated chromosomal changes in human mesothelial cells. Proc Natl Acad Sci USA 1985;82:3884–3888.
27. Fung H, Kow YW, Van Houten B, et al. Patterns of 8-hydroxydeoxyguanosine formation in DNA and indications of oxidant stress in rat and human pleural mesothelial cells after exposure to crocidolite asbestos. Carcinogenesis 1997;18:825–832.
28. Ollikainen T, Linnainmaa K, Kinnula VL. DNA single strand breaks induced by asbestos fibers in human pleural mesothelial cells in vitro. Environ Mol Mutagenesis 1999;33:153–160.
29. Driscoll KE, Carter JM, Borm PJA. Antioxidant defense mechanisms and the toxicity of fibrous and nonfibrous particles. Inhal Toxicol 2002;14: 101–118.
30. Kinnula VL, Everitt JI, Mangum JB, et al. Antioxidant defense mechanisms in cultured pleural mesothelial cells. Am J Respir Cell Mol Biol 1992;7: 95–103.
31. Jaurand M-C, Barrett JC. Neoplastic transformation of mesothelial cells. In: Jaurand M-C, Bignon J, eds. The Mesothelial Cell and Mesothelioma. New York: Marcel Dekker, 1994:207–222.
32. Jaurand M-C, Saint-Etienne L, Van der Meeren A, et al. Neoplastic transformation of rodent cells. In: Harris CC, Lechner JF, Brinkley BR, eds. Cellular and Molecular Aspects of Fiber Carcinogenesis. New York: Cold Spring Harbor Laboratory Press, 1991:131–148.
33. Funaki K, Everitt J, Bermudez E, et al. Trisomy of rat chromosome 1 associated with mesothelial cell transformation. Cancer Res 1991;51:4059–4066.
34. Ke Y, Reddel RR. Gerwin BI, et al. Establishment of a human in vitro mesothelial cell model system for investigating mechanisms of asbestos-induced mesothelioma. Am J Pathol 1989;134:979–991.
35. Dickson MA, Hahn WC, Ino Y, et al. Human keratinocytes that express hTERT and also bypass a $p16^{INK4a}$-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. Mol Cell Biol 2000;20:1436–1447.
36. Bocchetta M, DiResta I, Powers A, et al. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. Proc Natl Acad Sci USA 2000;97:10214–10219.
37. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 1983;304:596–602.
38. Hahn WC, Weinberg RA. Rules for making human tumor cells. N Engl J Med 2002;347:1593–1603.
39. Tubo RA, Rheinwald JG. Normal human mesothelial cells and fibroblasts transfected with the *EJras* oncogene become EGF-independent, but are not malignantly transformed. Oncogene Res 1987;1:407–421.
40. Reddel RR, Malan-Shibley L, Gerwin BI, et al. Tumorigenicity of human mesothelial cell line transfected with EJ-ras oncogene. J Natl Cancer Inst 1989;81:945–948.
41. O'Connell TM, Rheinwald JG. Biology of normal, malignant, and oncogene-transfected human mesothelial cells in culture. In: Harris CC, Lechner JF, Brinkley BB, eds. Cellular and Molecular Aspects of Fiber Carcinogenesis. New York: Cold Spring Harbor Laboratory Press, 1991:55–66.
42. Yu J, Boyapati A, Rundell K. Critical role for SV40 small-t antigen in human cell transformation. Virology 2001;290:192–198.
43. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.

44. Kane, AB. Mechanisms of mineral fibre carcinogenesis. In: Kane AB, Boffetta P, Saracci R, Wilbourn JD, eds. Mechanisms of Fibre Carcinogenesis. Lyon: IARC Scientific Publications No. 140, 1996:11–34.
45. Dhaene K, Hübner R, Kumar-Singh S, et al. Telomerase activity in human pleural mesothelioma. Thorax 1998;53:915–918.
46. Dhaene K, Wauters J, Weyn B, et al. Expression profile of telomerase subunits in human pleural mesothelioma. J Pathol 2000;190:80–85.
47. Kane AB. Animal models of malignant mesothelioma. In: Rom WN, ed. Environmental and Occupational Medicine, 3rd ed. Philadelphia: Lippincott-Raven, 1998:377–386.
48. Kane AB. Oncogenes and tumor suppressor genes in the carcinogenicity of fibers and particles. Inhal Toxicol 2000;12:133–140.
49. Coussens LM, Werb Z. Inflammatory cells and cancer: think different! J Exp Med 2001;193:F23–F26.
50. Tlsty TD. Stromal cells can contribute oncogenic signals. Semin Cancer Biol 2001;11:97–104.
51. Goodglick LA, Vaslet CA, Messier NJ, Kane AB. Growth factor responses and protooncogene expression of murine mesothelial cell lines derived from asbestos-induced mesotheliomas. Toxicol Pathol 1997;25:565–573.
52. Miller LD, Long PM, Wong L, et al. Optimal gene expression analysis by microarrays. Cancer Cell 2002;2:353–361.
53. Castor CW, Naylor B. Characteristics of normal and malignant human mesothelial cells studied in vitro. Lab Invest 1969;20:437–443.
54. Thiollet J, Jaurand MC, Kaplan H, et al. Culture procedure of mesothelial cells from the rat parietal pleura. Biomedicine 1978;29:69–73.
55. Antony VB, Owen, CL, Hadley KJ. Pleural mesothelial cells stimulated by asbestos release chemotactic activity for neutrophils in vitro. Am Rev Respir Dis 1989;139:199–206.
56. Stylianou E, Jenner LA, Davies M, et al. Isolation, culture and characterization of human peritoneal mesothelial cells. Kidney Int 1990;37:1563–1570.
57. Jonjic N, Peri G, Bernasconi S, et al. Expression of adhesion molecules and chemotactic cytokines in cultured human mesothelial cells. J Exp Med 1992;176:1165–1174.
58. Gormley IP, Bolton RE, Brown G, et al. Studies on the morphological patterns of asbestos induced mesotheliomas "in vivo" and "in vitro." Carcinogenesis 1980;2:219–231.
59. Whitaker D, Shilkin KB, Walters MNI. Cytologic and tissue culture characteristics of asbestos-induced mesothelioma in rats. Acta Cytol 1984;28: 185–189.
60. Davis MR, Manning LS, Whitaker D, et al. Establishment of a murine model of malignant mesothelioma. Int J Cancer 1992;52:881–886.
61. Versnel MA, Bouts MJ, Hoogsteden HC, et al. Establishment of human malignant mesothelioma cell lines. Int J Cancer 1989;44:256–260.
62. Demetri GD, Zenzie BW, Rheinwald JG, et al. Expression of colony-stimulating factor genes by normal human mesothelial cells and human malignant mesothelioma cell lines in vitro. Blood 1989;74:940–946.
63. Pelin-Enlund K, Husgafvel-Pursiainen K, Tammilehto L, et al. Asbestos-related malignant mesothelioma: growth, cytology, tumorigenicity and consistent chromosome findings in cell lines from five patients. Carcinogenesis 1990;11:673–681.
64. Schmitter D, Lauber B, Fagg B, et al. Hematopoietic growth factors secreted by seven human pleural mesothelioma cell lines: interleukin-6 production as a common feature. Int J Cancer 1992;51:296–301.

65. Taguchi T, Jhanwar SC, Siegfried JM, et al. Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma. Cancer Res 1993;53:4349–4355.
66. Zeng L, Buard A, Monnet I, et al. In vitro effects of recombinant human interferon gamma on human mesothelioma cell lines. Int J Cancer 1993; 55:515–520.
67. Xu L, Flynn BJ, Ungar S, et al. Asbestos induction of extended lifespan in normal human mesothelial cells: interindividual susceptibility and SV40 T antigen. Carcinogenesis 1999;20:773–783.
68. Pass H, Stevens E, Oie H, et al. Characteristics of nine newly derived mesothelioma cell lines. Ann Thorac Surg 1995;59(4):835–844.

6

Cytogenetics of Malignant Mesothelioma

Sinoula Apostolou, Binaifer R. Balsara, and Joseph R. Testa

Pleural malignant mesotheliomas (MMs) are aggressive tumors that generally affect individuals older than 50 years of age and occur more frequently in men than in women (1). They are derived from mesothelial cells lining the pleural, pericardial, and peritoneal cavities. Approximately 3000 patients are diagnosed with MM in the United States each year. Its frequency is increasing worldwide, and this trend is expected to continue until the year 2020 (2). The increasing incidence of MM over the past 40 years is a reflection of exposure to asbestos fibers in industrialized countries, particularly in connection with the mining and shipyard industries (2). Epidemiologic studies have established that exposure to asbestos fibers is associated with about 80% of the cases (3); however, recent studies have implicated simian virus 40 (SV40) in the etiology of some MMs (reviewed in refs. 4–6).

Malignant mesothelioma is characterized by a long latency of 20 to 40 years between exposure to asbestos and tumor development, indicating that multiple somatic genetic alterations may be required for tumorigenic conversion of a normal mesothelial cell. Early evidence to support this idea was provided by karyotypic analyses, which revealed multiple cytogenetic alterations in most human MMs (reviewed in ref. 7). Specific chromosomal changes are not shared by all MMs; however, several prominent sites of chromosomal loss have been identified in this malignancy. Tumor suppressor genes (TSGs) located in these deleted chromosomal regions may be responsible for the tumorigenic conversion of mesothelial cells, and recent studies have begun to identify the specific TSGs that contribute to the development and progression of MM. This chapter presents an overview of recurrent chromosomal imbalances and molecular genetic alterations characteristic of this malignancy.

Cytogenetic Assessment of Malignant Mesotheliomas

Chromosome banding techniques have revealed that most MMs have complex karyotypes (reviewed in refs. 7 and 8). Karyotypes of 39 MMs (9,10) have repeatedly exhibited extensive aneuploidy and structural

rearrangements of various chromosomes, particularly the short (p) arms of chromosomes 1, 3, and 9, and the long (q) arm of chromosome 6. Loss of one copy of chromosome 22 is the single most consistent numerical change seen in MMs. Losses or rearrangements of chromosomes 4, 14, and 17 and gain of chromosome 7 also have been commonly observed. Deletions and unbalanced rearrangements accounted for overlapping losses from the chromosome region 1p21-22 in 32 of 39 (82%) cases. Twenty-five of 39 (64%) MMs possessed interstitial deletions or other rearrangements resulting in losses from 3p21. Twenty cases (51%) showed losses from 6q, with the shortest region of overlap (SRO) being 6q15-21. Losses involving 9p were detected in 31 (79%) cases, with the SRO being 9p21-22. Loss or relative deficiency of chromosome 17 was observed in 11 of 39 (28%) cases. Loss of a copy of chromosome 22 was documented in 26 cases (67%). These recurrent losses of 1p, 3p, 6q, 9p, 17p, and 22 frequently occurred in combination in a given tumor. The complexity of the cytogenetic alterations observed suggest the emergence of tumor progression-associated changes. However, since cytogenetic data do not exist for early neoplastic/preneoplastic lesions of the mesothelium, it is not possible to discriminate between alterations associated with initiation and those associated with progression of the disease. However, the accumulated losses of DNA sequences from chromosomes 1p, 3p, 6q, 9p, 17p, and 22 appear to play a significant role in the pathogenesis of MM.

Comparative genomic hybridization (CGH) analysis has also revealed recurrent genomic imbalances in MM. Comparative genomic hybridization to metaphase chromosomes is a DNA-based, molecular cytogenetic technique that facilitates the identification of chromosome imbalances within the entire tumor genome in a single experiment. The CGH analyses were performed on 24 MM cell lines derived from patients from the United States (11); each of these cell lines exhibited numerous (6 to 25) genomic imbalances. Loss of 22q, documented in 14 of 24 (58%) cell lines, was the most prominent alteration. Also in agreement with earlier karyotypic findings, losses of 1p, 3p, 6q, and 9p were common, with each being detected in about 30% to 40% of cell lines. Moreover, the metaphase-CGH analysis uncovered other recurrent chromosome losses not highlighted by previous karyotypic studies. In particular, 13 of 24 MMs (54%) showed losses of part or all of 15q, with the SRO being 15q11.1-21. Additionally, losses of 14q24.2-qter and 13q12-14 were each observed in 42% of the cell lines. The most frequently overrepresented chromosomal arm was 5p (54% of cases), suggesting the involvement of a putative oncogene(s) in this region.

Many of the common genomic imbalances identified in MM cases from the United States were also detected in a series of MM specimens from Finland (12,13). Prominent among the recurrent alterations detected were losses of chromosome arms 4q, 6q, 9p, 13q, 14q, and 22q. However, three prominent imbalances in the series of MMs from the United States, i.e., losses of 15q11-21, 8p21-pter, and 3p21, were each observed in only one of 42 of the Finnish cases. Such variation between the data from Finland and from the United States may reflect dissimilarities in the type of asbestos exposure or genetic differences in the

study populations. Alternatively, such discrepancies may be related to the presence of SV40 in MMs from the United States and the absence of SV40 in MMs from Finland (14).

A recent study reported genomic imbalances in 77 MM tumors (15). Common losses clustered at 1p, 3p, 4p, 4q, 6q, 9p, 13q, 14q, and 22q. Abnormalities observed were similar to the ones reported in our study with the three most common changes being loss of 9p21, 4q31-32 and 22q, each observed in about 30% to 35% of cases. While there were many similarities in the frequencies of various genomic imbalances between epithelioid and sarcomatoid MMs, several chromosomal locations (3p, 7q, 15q, 17p) showed significant variations (15). Deletion at 3p21 was common in epithelioid tumors but rare in sarcomatoid and biphasic tumors. Similarly, loss of 17p was common in epithelioid tumors (25%) but was present in only 4% of sarcomatoid tumors. Loss of 7q, which is associated with poor prognosis in other tumor types, was observed in ~20% of sarcomatoid MMs but was not observed in epithelioid cases (15). Likewise, losses of 15q14-15 were seen in 20% of sarcomatoid tumors but not in epithelioid types. Moreover, the incidence of amplicons was four- to fivefold higher in sarcomatoid than in epithelioid MMs. Figure 6.1 summarizes CGH findings on 166 primary MM specimens and cell lines reported to date in five separate series from the United States and Europe (11–13,15,16).

Deletion Mapping of Recurrent Chromosomal Losses

As an initial approach for the isolation of putative TSGs important in the development or progression of MM, the frequently deleted regions defined by cytogenetic studies, i.e., 1p, 3p, 4p/q, 6q, 9p, 13q, 14q, 15q, 17p, and 22q, have been mapped at the molecular genetic level by loss of heterozygosity (LOH) analysis using polymorphic DNA markers (Table 6.1). Loss of heterozygosity is the most common type of somatic genetic alteration found in solid tumors. It occurs as a consequence of interstitial deletions, aneuploidy, or aberrant mitotic recombinational events (17,18), and implicates the presence of a recessive mutation in the remaining allele of a TSG located within the affected region of the genome (19). The recurrent genomic losses observed in MMs are consistent with the probability of a recessive mechanism of oncogenesis. Results of these investigations have been reviewed in detail elsewhere (7,8) and are briefly summarized below.

Chromosome 1p22

To map the critically deleted segment of 1p, LOH analyses were performed on 50 MMs using an extensive panel of microsatellite markers distributed throughout the short arm of chromosome 1 (20). Allelic losses at 1p21-22 were detected in 36 cases (72%), and the SRO was a 4-cM (centimorgan) segment within 1p22. The involvement of *BCL10* (17), a gene located at 1p22 that encodes a protein containing an N-terminus caspase recruitment domain homologous to the motif found in several regulatory and effector apoptotic molecules, was

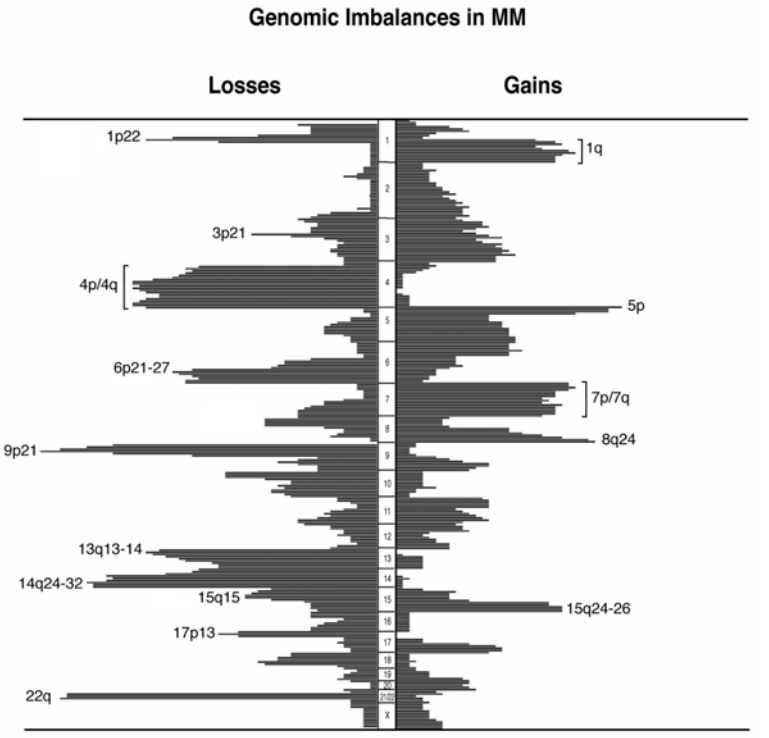


Figure 6.1. Bar plot of genomic imbalances observed in 166 primary malignant mesothelioma (MM) specimens and MM cell lines reported in the literature. Gains and losses are plotted for every chromosome band. Individual chromosomes are designated in the middle of the bar plot. The most frequent sites of chromosome loss (left) and gain (right) are as indicated.

Table 6.1. Summary of allelic losses and tumor suppressor genes associated with multistep tumorigenesis in human malignant mesothelioma

Chromosome region	Incidence of allelic loss	Tumor suppressor genes
1p22	**72%**[1]	**—**
3p21.3	**63%**[1]	***RASSF1A***
4p15	**>50%**[2]	**—**
4q25–34	**60%–80%**[2]	**—**
6q14–25	**60%**[1]	**—**
9p21	**83%**[1,4]	***p16^{INK4a}, p14^{ARF}***
13q13.2–14.2	**67%**[1]	**—**
14q	**43%**[1]	**—**
15q15	**48%**[1]	**—**
17p13	**40%**[3]	***TP53***
22q12	**72%**[1]	***NF2***

[1] Percentages shown reflect incidence of allelic loss observed in MM cell lines examined by the authors. *Note*: Each of these common sites of allelic loss was confirmed in a subset of cases for which corresponding tumor tissue was available.

[2] Allelic loss observed in MM tumor specimens examined by Shivapurkar et al (28).

[3] S.C. Jhanwar, personal communication.

[4] At *CDKN2A/ARF* locus, 85% of cell lines exhibited homozygous losses.

investigated in 50 MM cell lines (21). Mutations of *BCL10* were initially identified in mucosa-associated lymphoid tissue (MALT) lymphomas and other tumors including tumor cell lines derived from several MMs (22). Our reverse-transcription polymerase chain reaction (RT-PCR) analyses demonstrated that all MM cell lines and normal mesothelial cells examined expressed *BCL10* at similar levels. Sequence analyses revealed several nucleotide alterations in MM samples that were also observed in a panel of 50 genomic DNA samples from healthy donors, indicating that the nucleotide differences seen in MM represented polymorphisms and not mutations.

Chromosome 3p21

Chromosome 3p is a common site of allelic loss in MM (23,24). The LOH from 3p was detected in 15 of 24 (63%) MMs we examined (23). The highest frequency of allelic loss was at 3p21.3. Losses from this region have also been reported in other malignancies, particularly lung tumors, suggesting that perturbation of a TSG(s) located at this site may play a role in the development of multiple tumor types. The nature of the TSG(s) located in this region is unknown, although a homozygous deletion of the beta-catenin gene (*CTNNB1*), located at 3p21.3, was reported in one MM cell line (25). The remaining nine MM cell lines and tumor specimens did not display deletions or aberrant expression of *CTNNB1*. Another study has revealed frequent epigenetic inactivation of a RAS association domain family protein from the lung tumor suppressor locus 3p21.3 (26). The RAS effector homologue, *RASSF1*, is located in a 120-kilobase (kb) region of minimal homozygous deletion observed in some lung carcinomas. Three *RASSF1* transcripts have been identified, one of which (transcript A) was missing in all small cell lung cancers examined (26). Loss of expression was correlated with methylation of the CpG-island promoter sequence of *RASSF1A*. The promoter region of this putative TSG is frequently methylated in MM, and its methylation is correlated with loss of *RASSF1A* expression and the presence of SV40 (27).

Chromosome 4

Frequent losses of chromosome 4 in both MMs and lung carcinomas have been reported (28). Three nonoverlapping regions of chromosomal loss were identified—4q33-34, 4q25-26, and 4p15.1-15.3—suggesting that several TSGs localized to chromosome 4 may contribute to the pathogenesis of MM.

Chromosome 6q14-25

The LOH analysis of 6q in MMs revealed a complex pattern of allelic loss (29). The LOH at 6q was demonstrated in 28 of 46 MMs (61%), and deletions fell into several discrete regions including 6q14-21, 6q16.3-21, 6q21-23.2, and 6q25. Multiple nonoverlapping regions of 6q loss have also been described in other types of malignancy, such as non-

Hodgkin's lymphoma, suggesting that several putative TSG(s) in 6q may play a role in the development of multiple tumor types.

Chromosome 9p21

Homozygous deletions of the tumor suppressor gene $p16^{INK4a}$, localized within chromosome 9p21, occurr at high frequencies in cell lines derived from various types of cancer (30). $p16^{INK4a}$ encodes a protein capable of binding to the cyclin-dependent kinase CDK4, which thereby inhibits the catalytic activity of the CDK4/cyclin D enzymes. To assess the possible involvement of $p16^{INK4a}$ in MM, deletion mapping was performed on 40 MM cell lines (31); 34 (85%) of these cell lines possessed homozygous deletions of one or more $p16^{INK4a}$ exons and another had a point mutation in $p16^{INK4a}$. Downregulation of $p16^{INK4a}$ was observed in four of the remaining cell lines. Homozygous deletions of $p16^{INK4a}$ were identified in five of 23 (22%) MM tumor specimens. Moreover, abnormal expression of $p16^{INK4a}$ was also reported in all 12 MM specimens and all 15 MM-derived cell lines examined by immunohistochemistry (32). In xenograft experiments, reexpression of $p16^{INK4a}$ in MM cells resulted in cell-cycle arrest and cell death, as well as inhibition of tumor formation or diminished tumor size (33).

In many cases, homozygous deletions of the *CDKN2A/ARF* locus, which encodes the alternative TSG products $p16^{INK4a}$ and $p14^{ARF}$, also leads to inactivation of $p14^{ARF}$, since $p16^{INK4a}$ and $p14^{ARF}$ share exons 2 and 3, although their reading frames differ. Thirty-six of our 40 MM cell lines showed homozygous deletions of one or more $p14^{ARF}$ exons. $p14^{ARF}$ is essential for the activation of p53 in response to the action of certain oncogene products such as Ras (34). The $p16^{INK4a}$ product, on the other hand, induces cell cycle arrest by inhibiting the phosphorylation of the retinoblastoma protein, pRb. Therefore, homozygous loss of $p14^{ARF}$ and $p16^{INK4a}$ would collectively affect both p53- and pRb-dependent growth regulatory pathways, respectively. Interestingly, in vitro work has demonstrated that adenovirus-mediated transfer of $p14^{ARF}$ in MM cell lines induces G1 arrest and apoptotic cell death (35), supporting the notion that both products of the *CDKN2A/ARF* locus may contribute to the pathogenesis of MM.

Chromosomes 13q13.3-14.2 and 14q

To define the SRO of deletions from these chromosomes, we performed LOH analyses on 30 MMs using 25 microsatellite markers mapped to 13q and 21 markers mapped to 14q (36). Twenty of 30 MMs (67%) displayed allelic loss of at least one marker in 13q. The SRO was delineated as a 7-cM region located at 13q13.3-14.2. Thirteen of 30 MMs (43%) displayed allelic losses from 14q, with at least three distinct regions of LOH located at segments q11.2-13.2, q22.3-24.3, and q32.12. These data highlight a single region of chromosomal loss in 13q in many MMs, implicating the involvement of a TSG that is fundamental to the pathogenesis of this malignancy. To date, two TSGs have been identified in chromosome 13: *RB1*, located at 13q14, and *BRCA2*,

located at 13q12-13. The LOH data suggest that these genes can be excluded as candidates for 13q loss in MM, as they reside outside the SRO. Moreover, loss of expression of *RB1* is rare in MM (37). In comparison, the lower incidence and diffuse pattern of allelic losses in 14q suggest that several TSGs localized to this chromosome arm may contribute to tumorigenic progression in some MMs.

Chromosome 15q15

The CGH analyses demonstrated losses from 15q in 13 of 24 (54%) MM cell lines examined, and LOH analyses revealed allelic losses from one or more 15q loci in 10 of these 13 cell lines (11). The SRO was located at 15q11.1-15. Losses involving this region have also been observed in other types of cancer, such as metastatic tumors of the breast, lung, and colon, suggesting that this region harbors a TSG that may contribute to the progression of a variety of epithelial cancer types. We also performed a high-density LOH analysis of 46 MMs (38). These studies have defined a minimally deleted region of approximately 3 cM, which was established to reside at 15q15 by fluorescence in situ hybridization analysis of probes known to map to this region.

Chromosome 17p13

Preliminary studies have demonstrated abnormalities of 17p in approximately 40% of MM cell lines examined either by cytogenetics alone or in combination with restriction fragment length polymorphism (RFLP) analysis (S.C. Jhanwar, personal communication). The *TP53* gene is located at chromosome 17p13, and occasional mutations of *TP53* have been reported in MM (39,40).

Chromosome 22

As stated earlier, loss of a copy of chromosome 22 is a frequent occurrence in MM, and extensive LOH analysis of chromosome 22 in MM has not been performed since an entire copy of chromosome 22 is lost in most cases. Although the neurofibromatosis type 2 TSG, *NF2*, which encodes *merlin* or *schwannomin*, predisposes affected individuals primarily to tumors of neuroectodermal origin, somatic mutations of *NF2* have occasionally been identified in apparently unrelated malignancies (41). Although the precise function of *merlin* is unknown as yet, it has been shown to play a role in cell adhesion, spreading, and motility. Thus, *NF2* loss-of-function mutations may contribute to tumor invasiveness and metastasis. This notion is supported by recent work demonstrating that merlin is phosphorylated by p21-activated kinase (Pak) (42,43), a common downstream target of Rac/cdc42, and Pak is known to regulate motility in mammalian cells (44).

Our mutational studies of *NF2* in MM revealed nucleotide mutations in 8 of 15 (53%) cell lines (41). The mutations, which included deletions and insertions and one nonsense mutation, predicted truncated forms of the NF2 protein. Similar results were reported by Sekido et al (45),

who detected somatic mutations in one MM specimen and in 7 of 17 (41%) MM cell lines. In our study, the mutations observed in complementary DNAs (cDNAs) from MM cell lines were confirmed in genomic DNA from six matched primary tumor specimens (41). The two cDNA alterations that could not be confirmed by genomic analysis were both splicing related: i.e., deletion of exon 10 in one cell line, and a 43-bp insertion between exons 13 and 14 in the other.

In a follow-up investigation, we detected mutations in the *NF2* coding region in 12 of 23 (52%) additional MM cell lines (46). Western blot analyses revealed loss of *merlin* expression in each of the 12 cell lines exhibiting alterations of the *NF2* gene. In addition, two cell lines from our earlier study, which lacked NF2 expression and possessed *NF2* mutations, were also examined. The LOH analyses were performed on 25 MM cell lines using two polymorphic DNA markers residing at or near the *NF2* locus in chromosome 22q12. Eighteen of the 25 cell lines (72%) showed losses at one or both of these loci. All cases exhibiting mutation and aberrant expression of *NF2* displayed LOH, consistent with biallelic inactivation of *NF2* in MM.

Conclusion

There is now a large body of experimental and epidemiologic data in support of the assertion that asbestos, or at least amphibole asbestos, causes MM. The data also suggest that exposure to asbestos may not be sufficient for MM development. Other factors, such as genetic predisposition and SV40, may render some individuals more susceptible to asbestos carcinogenicity. Cytogenetic and molecular genetic studies indicate that MM results from the accumulation of numerous somatic genetic events, mainly deletions, suggesting a multistep cascade involving the inactivation of multiple TSGs (Table 6.1). To date, several TSGs have been shown to be frequently altered in MMs, and their disruption would be expected to have profound consequences on the growth and behavior of a mesothelial cell. Moreover, the critically deleted regions identified in MM overlap with sites commonly deleted in several other human malignancies. Thus, the identification of TSGs in MM may be helpful in elucidating pathogenetic mechanisms important in other more common cancers, as well. The discovery of all of the critical somatic genetic alterations in MM and understanding how each of them contributes to the pathogenesis of this malignancy may ultimately lead to the design of more effective therapeutic strategies. The identification of these somatic genetic changes should be facilitated by the recent development of array-CGH, a powerful new method for high-resolution profiling of genomic imbalances (47). This methodology uses assembled arrays of several thousand cloned human DNA sequences, at ≤1-megabase intervals, representing segments located throughout the genome. Array-CGH permits fine mapping of genomic imbalances encompassing known genes as opposed to the very limited resolution (10–20 megabases) resolved by metaphase-CGH. Array-CGH allows for rapid and reliable assessment of DNA copy number

changes across the entire genome and could potentially lead to the identification of novel MM genes whose products may serve as targets for therapeutic intervention in this disease.

References

1. Antman KH, Pass HI, Schiff PB. Management of mesothelioma. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Principles and Practice of Oncology. Philadelphia: Lippincott Williams & Wilkins, 2001:1943–1969.
2. Attanoos RL, Gibbs AR. Pathology of malignant mesothelioma. Histopathology 1997;30:403–418.
3. Craighead JE, Mossman BT. The pathogenesis of asbestos-associated diseases. N Engl J Med 1982;306:1446–1455.
4. Butel JS, Lednicky JA. Cell and molecular biology of simian virus 40: implications for human infections and disease. J Natl Cancer Inst 1999;91: 119–134.
5. Carbone M, Fisher S, Powers A, Pass HI, Rizzo P. New molecular and epidemiological issues in mesothelioma: role of SV40. J Cell Physiol 1999; 180:167–172.
6. Carbone M, Rizzo P, Pass HI. Simian virus 40, poliovaccines and human tumors: a review of recent developments (meeting review). Oncogene 1997; 15:1877–1888.
7. Murthy SS, Testa JR. Asbestos, chromosomal deletions, and tumor suppressor gene alterations in human malignant mesothelioma. J Cell Physiol 1999;180:150–157.
8. Testa JR, Pass HI, Carbone M. Molecular biology of mesothelioma. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Principles and Practice of Oncology. Philadelphia: Lippincott Williams & Wilkins, 2001:1937–1943.
9. Testa JR, Jhanwar SC. Molecular genetics of malignant mesothelioma. In: Light RW, Lee G, eds. Pleural Disease: An International Textbook. London: Arnold Publishers, (in press.)
10. Taguchi T, Jhanwar SC, Siegfried JM, Keller SM, Testa JR. Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma. Cancer Res 1993;53:4349–4355.
11. Balsara BR, Bell DW, Sonoda G, et al. Comparative genomic hybridization and loss of heterozygosity analyses identify a common region of deletion at 15q11.1-15 in human malignant mesothelioma. Cancer Res 1999;59: 450–454.
12. Bjorkqvist AM, Tammilehto L, Anttila S, Mattson K, Knuutila S. Recurrent DNA copy number changes in 1q, 4q, 6q, 9p, 13q, 14q and 22q detected by comparative genomic hybridization in malignant mesothelioma. Br J Cancer 1997;75:523–527.
13. Bjorkqvist AM, Tammilehto L, Nordling S, et al. Comparison of DNA copy number changes in malignant mesothelioma, adenocarcinoma and large-cell anaplastic carcinoma of the lung. Br J Cancer 1998;77:260–269.
14. Hirvonen A, Mattson K, Karjalainen A, et al. SV40-like DNA sequences not detectable in Finnish mesothelioma patients not exposed to SV40 contaminated poliovaccines. Mol Carc 1999;26:93–99.
15. Krismann M, Muller KM, Jaworska M, Johnen G. Molecular cytogenetic differences between histological subtypes of malignant mesotheliomas: DNA cytometry and comparative genomic hybridization of 90 cases. J Pathol 2002;197:363–371.

16. Kivipensas P, Bjorkqvist AM, Karhu R, et al. Gains and losses of DNA sequences in malignant mesothelioma by comparative genomic hybridization. Cancer Genet Cytogenet 1996;89:7–13.
17. Seemayer TA, Cavenee WK. Molecular mechanisms of oncogenesis. Lab Invest 1989;60:585–599.
18. Weinberg RA. Tumor suppressor genes. Science 1991;254:1138–1146.
19. Knudson AG Jr. Hereditary cancers disclose a class of cancer genes. Cancer 1989;63:1888–1891.
20. Lee W-C, Balsara B, Liu Z, Jhanwar SC, Testa JR. Loss of heterozygosity analysis defines a critical region in chromosome 1p22 commonly deleted in human malignant mesothelioma. Cancer Res 1996;56:4297–4301.
21. Apostolou S, De Rienzo A, Murthy SS, Jhanwar SC, Testa JR. Absence of *BCL10* mutations in human malignant mesothelioma. Cell 1999;97:684–686.
22. Willis TG, Jadayel DM, Du M-Q, et al. *Bcl10* is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. Cell 1999; 96:35–45.
23. Lu YY, Jhanwar SC, Cheng JQ, Testa JR. Deletion mapping of the short arm of chromosome 3 in human malignant mesothelioma. Genes Chromosomes Cancer 1994;9:76–80.
24. Zeiger MA, Gnarra JR, Zbar B, Linehan WM, Pass HI. Loss of heterozygosity on the short arm of chromosome 3 in mesothelioma cell lines and solid tumors. Genes Chromosomes Cancer 1994;11:15–20.
25. Shigemitsu K, Sekido Y, Usami N, et al. Genetic alteration of the beta-catenin gene (CTNNB1) in human lung cancer and malignant mesothelioma and identification of a new 3p21.3 homozygous deletion. Oncogene 2001;20:4249–4257.
26. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet 2000;25:315–319.
27. Toyooka S, Carbone M, Toyooka K, et al. Progressive aberrant methylation of the RASSF1A gene in simian virus 40 infected human mesothelial cells. Oncogene 2002;21:4340–4344.
28. Shivapurkar N, Virmani AK, Wistuba II, et al. Deletions of chromosome 4 at multiple sites are frequent in malignant mesothelioma and small cell lung carcinoma. Clin Cancer Res 1999;5:17–23.
29. Bell DW, Jhanwar SC, Testa JR. Multiple regions of allelic loss from chromosome arm 6q in malignant mesothelioma. Cancer Res 1997;57: 4057–4062.
30. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. Science 1994;264:436–440.
31. Cheng JQ, Jhanwar SC, Klein WM, et al. *p16* alterations and deletion mapping of 9p21-p22 in malignant mesothelioma. Cancer Res 1994;54: 5547–5551.
32. Kratzke RA, Otterson GA, Lincoln CE, et al. Immunohistochemical analysis of the p16INK4 cyclin-dependent kinase inhibitor in malignant mesothelioma. J Natl Cancer Inst 1995;87:1870–1875.
33. Frizelle SP, Grim J, Zhou J, et al. Re-expression of p16INK4a in mesothelioma cells results in cell cycle arrest, cell death, tumor suppression and tumor regression. Oncogene 1998;16:3087–3095.
34. Palmero I, Pantoja C, Serrano M. p19ARF links the tumour suppressor p53 to Ras. Nature 1998;395:125–126.
35. Yang CT, You L, Yeh CC, et al. Adenovirus-mediated p14(ARF) gene transfer in human mesothelioma cells. J Natl Cancer Inst 2000;92:636–641.

36. De Rienzo A, Jhanwar SC, Testa JR. Loss of heterozygosity analysis of 13q and 14q in human malignant mesothelioma. Genes Chromosomes Cancer 2000;28:337–341.
37. Van Der Meeren A, Seddon MB, Kispert J. Lack of expression of the retinoblastoma gene is not frequently involved in the genesis of human mesothelioma. Eur Respir Rev 1993;3:177–179.
38. De Rienzo A, Balsara BR, Apostolou S, Jhanwar SC, Testa JR. Loss of heterozygosity analysis defines a 3-cM region of 15q commonly deleted in human malignant mesothelioma. Oncogene 2001;20:6245–6249.
39. Cote RJ, Jhanwar SC, Novick S, Pellicer A. Genetic alterations of the p53 gene are a feature of malignant mesothelioma. Cancer Res 1991;51:5410–5416.
40. Metcalf RA, Welsh JA, Bennett WP, et al. p53 and Kirstein-*ras* mutations in human mesothelioma cell lines. Cancer Res 1992;52:2610–2615.
41. Bianchi AB, Hara T, Ramesh V, et al. Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumour types. Nat Genet 1994;6:185–192.
42. Kissil JL, Johnson KC, Eckman MS, Jacks T. Merlin phosphorylation by p21-activated kinase 2 and effects of phosphorylation on merlin localization. J Biol Chem 2002;277:10394–10399.
43. Xiao GH, Beeser A, Chernoff J, Testa JR. p21-activated kinase links Rac/Cdc42 signaling to merlin. J Biol Chem 2002;277:883–886.
44. Sells MA, Boyd JT, Chernoff J. p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. J Cell Biol 1999;145:837–849.
45. Sekido Y, Pass HI, Bader S, et al. Neurofibromatosis type 2 (*NF2*) gene is somatically mutated in mesothelioma but not in lung cancer. Cancer Res 1995;55:1227–1231.
46. Cheng JQ, Lee W-C, Klein MA, Cheng GZ, Jhanwar SC, Testa JR. Frequent alterations of *NF2* and allelic loss from chromosome band 22q12 in malignant mesothelioma: evidence for a two-hit mechanism of *NF2* inactivation. Genes Chromosomes Cancer 1999;24:238–242.
47. Snijders AM, Nowak N, Segraves R, et al. Assembly of microarrays for genome-wide measurement of DNA copy number. Nat Genet 2001;29:263–264.

7

Growth Factors and Malignant Mesothelioma

Paola Cacciotti, Luciano Mutti, and Giovanni Gaudino

Growth factors can act as positive or negative modulators of cell proliferation, differentiation, motility, and angiogenesis. The interaction of these signal molecules with their membrane receptors triggers a number of intracellular signaling pathways, resulting in the activation or repression of various subset of genes. Aberrations in these biochemical signals are linked to developmental abnormalities or to a series of chronic diseases, including cancer. Tumor malignant cells arise as the result of a stepwise progression of genetic events, including deregulated expression of growth factors or of molecules involved in their signaling pathways (1).

The proliferation of normal human and rodent mesothelial cells is regulated by exposure to several growth factors, including epidermal growth factor (EGF) (2,3), tumor necrosis factor-α (TNF-α) (4), platelet-derived growth factor (PDGF) (5), hepatocyte growth factor (HGF) (6), and keratinocyte growth factor (KGF) (7).

This chapter focuses on the several growth factors expressed by mesothelial and malignant mesothelioma cells (MMCs), and discusses how deregulation of their biologic activities is responsible for the onset and progression of this tumor (Table 7.1).

Epidermal Growth Factor and Its Related Molecules

Epidermal growth factor (EGF) has a profound effect on the differentiation of specific cells in vivo and is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin. The EGF precursor exists as a membrane-bound molecule that is proteolytically cleaved to generate the 53-amino acid peptide growth factor that stimulates cells to divide (8).

Epidermal growth factor is a powerful mitogen for human mesothelial cells too. Autotransphosphorylation and activation of the EGF tyrosine kinase receptor (EGFR) occurs after exposure to asbestos triggering the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) cascade. The MAPK activation by asbestos is

Table 7.1. Overview of growth factors expressed by human mesothelial cells (HMCs) and malignant mesothelioma cells (MMCs)

Growth factor	Receptor	Biologic activity in HMC and MMC
EGF	EGF-R	Growth, differentiation, synthesis of glycosaminoglycans and MMP
TGF-α	EGF-R	Growth
$TGF\beta_1$ $TGF\beta_2$	TGFβ-R	Growth, change of morphology, regulation of pleural inflammation, reduced T-lymphocyte infiltration
TNF-α PDGF-AA	TNFα-R	Proliferation, collagen production, acquisition of fibroblastoid morphology, and upregulation of the synthesis of MMP
PDGF-AB PDGF-BB	PDGFR-α	Growth, motility, hyaluronan, and collagen synthesis
PDGF-AB PDGF-BB	PDGFR-β	Growth, motility, hyaluronan, and collagen synthesis
IGF-I	IGFI-R	Proliferation, proteoglycan synthesis
IGF-II	IGFII-R	Proliferation, proteoglycan synthesis
VEGF	KDR/FIt-1	Proliferation, angiogenesis
FGF-1 FGF-2	FGF-R	Angiogenesis, synthesis of hyaluronan, and proteoglycans
HGF	MET	Proliferation, motility, morphology, invasion, and angiogenesis

EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

attenuated by generic inhibitors of growth factor receptor interactions, like suramin, as well as by tyrphostin AG 1478, a specific inhibitor of the EGFR tyrosine kinase activity (3). Although both asbestos-transformed MMCs and spontaneously transformed mesothelial cells express functional EGFRs, only cells transformed by exposure to asbestos fibers express into conditioned medium TGF-α, a growth factor with high affinity for EGFR (9). Interestingly, while TGF-α inhibits the growth of spontaneously transformed mesothelial cells, it stimulates the proliferation of asbestos-transformed cells, as demonstrated by the inhibition of growth observed after incubation with neutralizing antibody raised against TGF-α. Taken together, these data indicate that TGF-α acts as an autocrine growth factor for asbestos-transformed rat mesothelial cells and suggest that differences in mesothelioma etiology may be linked to differences in the molecular alterations present in these tumors (10).

Epidermal growth factor is not only a mitogen but it may also play a role in the process of cell differentiation and the synthesis of glycosaminoglycans in mesothelial cells (11). In addition, it has been recently demonstrated that many different growth factors including EGF, TGF-α, amphiregulin, heparin-binding EGF, beta-cellulin (BTC), stem cell factor, insulin-like growth factors I and II, acidic and basic fibroblast growth factors, and HGF regulate the expression in malignant mesothelioma cells of the extracellular matrix metalloproteinases

(MMPs), molecules playing a key role in tumor cell invasion and metastasis (12).

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) 1 and 2 are dimeric multifunctional polypeptide that control proliferation, differentiation, and other functions in many cell types. Many cells synthesize TGF-β_1 and essentially all of them have specific receptors for this peptide. TGF-β_1 regulates the actions of many other peptide growth factors and determines a positive or negative direction of their effects.

Both TGF-β_1 and -β_2 are secreted by human and murine MMCs through an autocrine mechanism. They may both reduce T-lymphocyte infiltration into tumors and modulate malignant growth of tumor cells, as demonstrated by experiments with antisense oligonucleotides in vitro and in vivo (13). Moreover, TGF-β is responsible of evident morphologic changes in mesothelial cells (14). Both mesothelial cells and cells infiltrating in the pleural space can secrete TGF-α, because high levels of this growth factor were found in pleural effusions and in pleural tissues during disease processes. Also, TGF-β may participate in the regulation of pleural inflammation and enhance both cell proliferation and pleural fluid formation (15), partially due to induction of vascular endothelial growth factor (VEGF) (16). Cell lines derived from MM patients show considerably higher levels of TGF-β messenger RNA (mRNA) expression when compared with normal mesothelial cells. Treatment with exogenous TGF-β has no effects on growth of the MM cells, while the proliferation of the mesothelial cells is slightly induced (17).

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is a homotrimer multifunctional proinflammatory cytokine localized in membrane belonging to the tumor necrosis factor superfamily. It also exists as an extracellular soluble form derived from the membrane form by proteolytic processing. This cytokine is mainly secreted by macrophages that can bind to, and thus functions through, its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is involved in the regulation of a wide spectrum of biologic processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation, and has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.

In human mesothelial cells TNF-α induces the acquisition of fibroblastoid morphology and upregulates the synthesis of matrix metallo-

proteinase-9 (MMP-9) and type I collagen, which may facilitate peritoneal extracellular matrix remodeling and fibrogenesis (18). Also, TNF-α induces a significant increase in cell proliferation and collagen production of rat pleural mesothelial cells in vitro, suggesting a role for this molecule in healing of the pleura after tissue injury (19).

Platelet-Derived Growth Factors AA, AB, and BB

The proteins AA, BB, and AB are members of the platelet-derived growth factor (PDGF) family and are mitogenic factors for cells of mesenchymal origin characterized by a motif of eight cysteines. They can exist either as homodimers (AA and BB) or as a heterodimer (AB) stabilized by disulfide bonds. The PDGF-α receptor binds all three dimeric forms of PDGF, whereas the PDGF-β receptor binds PDGF-BB with high affinity and PDGF-AB with lower affinity, but not PDGF-AA (20) (Fig. 7.1). They are released by platelets upon wounding and play an important role in stimulating adjacent cells to grow and thereby heal the wound.

Expression of the PDGF receptor (PDGFR) has been detected both in normal mesothelial cells and in MMCs. However, several MMC lines, but not normal mesothelial cells, display constitutively enhanced expression of the c-*sis* (PDGF-BB) and PDGF-AA genes. This PDGF-dependent autocrine circuit has been postulated to play a role in

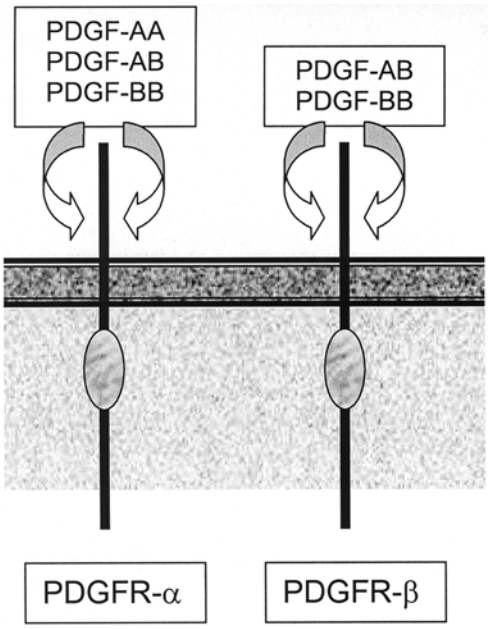


Figure 7.1. Selective binding of platelet-derived growth factor (PDGF) ligands (PDGF-AA, -AB, and -BB) to PDGF receptors.

the etiology of this type of malignancy (21). Several independent studies demonstrated that normal mesothelium is responsive to PDGF predominantly via PDGFR-α and at lesser extent via PDGFR-β receptor, whereas the autocrine stimulation of growth in mesothelioma cells hangs on the PDGF/PDGFR-β interaction (5,22,23). The pattern of PDGF and PDGF receptor expression in mesothelial cells largely corresponds to expression of PDGF and its receptors in vitro (24).

There are two PDGF-AA transcript isoforms differing in the presence or absence of an alternative exon-derived sequence. However, both normal mesothelial cells and MMCs predominantly express the PDGF-AA transcript lacking the exon-6-derived sequence, which encodes a cell-retention signal. This means that the PDGF-AA protein is most likely secreted by both cell types and may be involved in autocrine growth stimulation via PDGF-α receptors in mesothelial cells. As well, it might also have a paracrine function if it is secreted by malignant mesothelial cells that do not express the receptor. Moreover, the enhancement of transcription seems to be the most likely mechanism for the elevated mRNA levels of PDGF-AA gene in human malignant mesothelioma cells (25). In addition, TGF-β_1, secreted in active form by mesothelial cells, may play a role in the regulation of differential PDGF-R expression, by downregulation of a still lower PDGF-α receptor mRNA level in malignant mesothelioma cells (24).

Overexpression of PDGF-AA is responsible for autocrine downregulation of its receptor. Surprisingly, the PDGF-AA/PDGFR autocrine loop is antiproliferative for mesothelioma cells in vitro, whereas proliferation is stimulated by abrogation of PDGF-α expression. This suggests that PDGF-AA does not contribute to tumorigenicity by the autocrine stimulation of growth. On the other hand, in vivo PDGF-AA overexpression is associated with augmented tumorigenicity, and abrogation of PDGF-AA expression decreases tumor incidence and increases latency period to tumor formation. Thus, the tumorigenic effect of PDGF-AA must act through paracrine mechanisms relevant at early stages of tumor initiation (26). The absence of alterations of PDGF expression in rat mesothelioma, in contrast to what occurs in the human disease, suggests that the production of this growth factor by transformed mesothelial cells may be a species-specific mechanism (27).

Platelet-derived growth factor stimulates mesothelial cell proliferation in vitro and in vivo (28) as well as hyaluronan synthesis in patients with mesothelioma, as demonstrated by partial inhibition by an antiserum raised against PDGF (29). Moreover, PDGF stimulates collagen synthesis that, if combined with increased proliferation, may be important in healing the pleura injured during the progression of the disease (2). Finally, migration of mesothelioma cells on fibronectin, laminin, or collagen-type IV in response to PDGF-BB and inhibition of this effect after pretreatment with blocking antibodies to α3β1 integrin were described, suggesting that cooperation between PDGFR-β and integrin α3β1 is necessary for the motile response of MMCs to PDGF-BB (30).

Insulin-Like Growth Factors

Insulin-like growth factors I and II (IGFs) are polypeptides structurally and functionally related to insulin but having a much higher growth and differentiation-promoting activity.

Cell lines derived from normal rat mesothelium as well as cell lines derived only from spontaneous rat mesotheliomas, but not from asbestos-induced rat mesotheliomas, showed expression of RNA transcripts for IGF-II. All these cell lines expressed receptors for IGF-I and IGF-II, as well as insulin receptors. Coexpression of IGF-II and its cognate receptor suggests that IGF-II acts as an autocrine growth factor in the spontaneously immortalized cells and in the cells derived from the spontaneous rat tumors. Growth induced by IGF-II secreted into conditioned medium can be inhibited using an IGF-II–specific antibody in a dose-dependent manner. These data suggest that IGF-II expression may be involved in the spontaneous alteration of rat mesothelial cells and may function as an autocrine or paracrine growth factor to modulate the growth of these cells in vitro and in vivo. Ubiquitous expression of IGF-II by cells that have not been exposed to asbestos and the lack of IGF-II expression by asbestos-transformed cells suggest that the mechanisms of changes in growth factor expression differ in mesothelial cells transformed by different mechanisms (31). Similar results were also observed in vitro with IGF-I in human mesothelial cells (32). It was also shown that the existence of stimulatory effects of IGF-I on matrix proteoglycan synthesis was mediated via receptor-growth factor complexes and the protein tyrosine kinase intracellular pathway (33). The inhibitory effect of IGF-1 receptor antisense transcripts on hamster mesothelioma has been demonstrated by decreased growth and tumorigenicity in vitro and in vivo. These results may suggest interesting implications for a therapy of the human mesothelioma (34).

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a potent angiogenic protein with a selective mitogenic effect on endothelial cells known to be involved in many normal and pathologic processes.

Coexpression of VEGF and its receptors flt-1 and KDR has been reported in samples of mesothelioma, suggesting a potential autocrine loop for malignant pleural mesothelioma cells (35). Malignant mesothelioma cells produce significantly higher VEGF levels than normal mesothelial cells, and this growth factor is found at higher levels in the pleural effusions of MM patients than in the effusions of patients with nonmalignant pleural disease. In addition, VEGF induces increased proliferation of MMCs in a dose-dependent way, via activation of its tyrosine kinase receptor, and can have an impact on patient survival, not only by promoting angiogenesis but also by directly stimulating tumor growth (36).

Simian virus 40 (SV40)–large-tumor antigen (Tag) expression potently increases VEGF protein and mRNA levels in several human

mesothelial cell (HMC) lines and concomitant expression of SV40–small-tumor antigen (tag) enhances Tag function, suggesting that VEGF regulation by SV40 transforming proteins can represent a key event in SV40 signaling relevant for tumor progression (37,38). The closely related molecule, VEGF-C, is also implicated in malignant mesothelioma growth; VEGF-C and its cognate receptor VEGFR-3 are coexpressed in mesothelioma cell lines, and a functional VEGF-C autocrine growth loop was demonstrated in mesothelioma cells (39). Moreover, human MMCs, but not normal mesothelial cells, express a catalytically active lipoxygenases (5-LO), a key regulator of MMC proliferation and survival via a VEGF-related circuit (40).

Angiogenesis is an important part of normal and pathologic processes, including tumor growth, metastasis, inflammation, and wound healing, and VEGF is the best known angiogenic factor, implicated in tumor-associated microvascular hyperpermeability and carcinogenesis. An increased expression of VEGF was found in biphasic and epithelioid mesotheliomas and malignant pleural effusions. Vascular permeability was proportionally increased with VEGF levels in the malignant pleural effusions (41).

Fibroblast Growth Factors 1 and 2

Acidic and basic fibroblast growth factors (FGF-1 and -2) are potent angiogenic cytokines. These proteins are members of the fibroblast growth factor (FGF) family, and FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biologic processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion. These proteins function as modifiers of endothelial cell migration and proliferation, as well as angiogenic factors. They act as mitogens for a variety of mesoderm- and neuroectoderm-derived cells in vitro, and thus are thought to be involved in organogenesis.

Their expression levels correlate significantly with a poor survival of MM patients, supporting the assumption that selective angiogenic cytokines might contribute to the progressive changes of mesothelioma by tumor angiogenesis (42). The expression of angiogenic factors may represent useful markers for diagnosis and prediction of disease outcome. Basic fibroblast growth factor (bFGF) is a potent angiogenic factor that promotes in vitro growth of endothelial cells and in vivo vessel formation. It displays stimulatory effects for the synthesis of hyaluronan and proteoglycans, via protein tyrosine kinase activity elicited by receptor-ligand complexes through an autocrine stimulatory mechanism (11).

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF), also known as scatter factor (SF), is a multifunctional factor involved both in development and tissue

repair, as well as pathologic processes such as cancer and metastasis. It is a dimer of an alpha chain and a beta chain linked by a disulfide bonds and contains four kringle domains. It is a potent mitogen for mature parenchymal hepatocyte cells, seems to be an hepatotrophic factor, and acts as growth factor for a broad spectrum of tissues and cell types. It has no detectable protease activity. It has been identified in vivo in many types of tumors together with its tyrosine kinase receptor, c-Met.

Hepatocyte growth factor and its receptor c-Met are often expressed by normal human mesothelial cells and MMCs. Moreover, coexpression of HGF and its receptor was also observed in many samples of mesothelioma, suggesting that the HGF/c-Met signaling system may play a role in the development of this tumor, by either autocrine or paracrine mechanisms. In addition, c-Met expression was found in cells obtained from pleural fluids of patients with mesothelioma (6). In vitro HGF acts as a strong chemoattractant for human MMCs and stimulates motility in all mesothelioma cell lines tested. Furthermore, HGF can stimulate mesothelioma cell migration that can be blocked in the presence of neutralizing anti-HGF monoclonal antibodies. Addition of HGF to mesothelioma cells cultured on collagen type IV is associated with a change of morphology and induction of bipolar shape and protrusion of prominent pseudopodia. Moreover, HGF is mitogenic for mesothelioma cells, suggesting that expression of HGF/c-Met is involved not only in mesothelioma progression but also in its growth (6). In addition, the ability to secrete HGF/SF seems to be correlated with the fibroblast-like morphology, and in general the biologic activity of this growth factor is dependent on the cell phenotype, because HGF induces both cell-spreading and proliferation in epithelioid cells but only stimulation of cell motility in fibroblastoid cells (43). This growth factor also enhances cell adhesion and invasion, as demonstrated by the HGF-induced synthesis of many matrix metalloproteinases and serine proteases critical for tumor progression (44). On the basis of the significantly higher microvessel density values of malignant mesotheliomas overexpressing HGF/SF, it is absolutely possible that HGF/SF also may be an additional relevant factor in tumor angiogenesis in malignant pleural mesotheliomas (45).

Interestingly, the urokinase-type plasminogen activator receptor (uPAR) expression is induced by exposure to asbestos at the surface of rabbit and human mesothelial cells, suggesting that altered expression of this receptor could be involved in asbestos-induced remodeling of the pleural mesothelium, partially due to the uPAR-dependent HGF activation (46). Finally, other findings suggest that when SV40 infects HMCs, it causes Met activation via an autocrine loop, replicates in HMCs, and infects other adjacent HMCs, inducing an HGF-dependent Met activation, change of morphology, and increase of S-phase entry (Fig. 7.2). This mechanism may explain how a limited number of SV40-positive cells may be sufficient to direct noninfected HMCs toward malignant transformation (47).

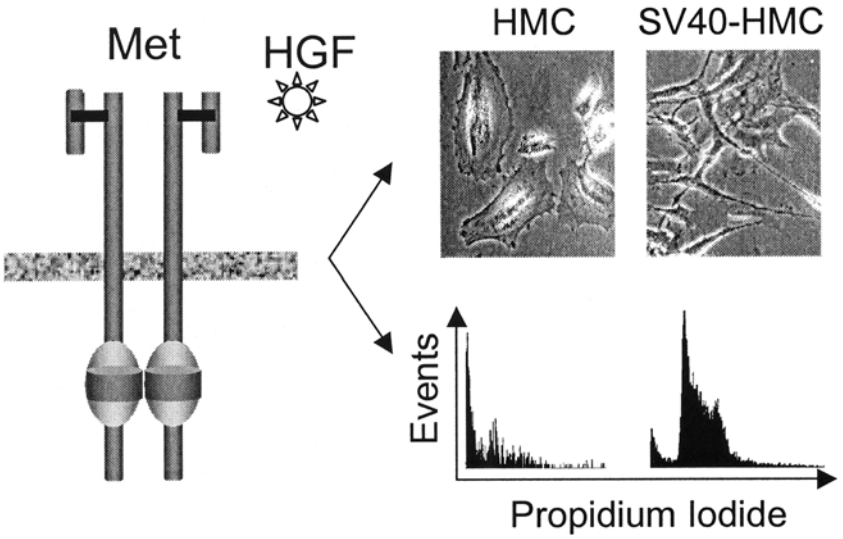


Figure 7.2. Hepatocyte growth factor (HGF)/Met autocrine loop induces change of morphology (upper panel) and S-phase entry (lower panel) in SV40 human mesothelial cells.

References

1. Comoglio PM, Boccaccio C. Scatter factors and invasive growth. Semin Cancer Biol 2001;11:153–165.
2. Owens MW, Milligan SA. Growth factor modulation of rat pleural mesothelial cell mitogenesis and collagen synthesis. Effects of epidermal growth factor and platelet-derived factor. Inflammation 1994;18:77–87.
3. Zanella CL, Posada J, Tritton TR, Mossman BT. Asbestos causes stimulation of the extracellular signal-regulated kinase 1 mitogen-activated protein kinase cascade after phosphorylation of the epidermal growth factor receptor. Cancer Res 1996;56:5334–5338.
4. Goldberg JL, Zanella CL, Janssen YM, et al. Novel cell imaging techniques show induction of apoptosis and proliferation in mesothelial cells by asbestos. Am J Respir Cell Mol Biol 1997;17:265–271.
5. Gerwin BI, Lechner JF, Reddel RR, et al. Comparison of production of transforming growth factor-beta and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. Cancer Res 1987; 47:6180–6184.
6. Klominek J, Baskin B, Liu Z, Hauzenberger D. Hepatocyte growth factor/scatter factor stimulates chemotaxis and growth of malignant mesothelioma cells through c-met receptor. Int J Cancer 1998;76:240–249.
7. Adamson IY, Bakowska J. KGF and HGF are growth factors for mesothelial cells in pleural lavage fluid after intratracheal asbestos. Exp Lung Res 2001;27:605–616.
8. Mroczkowski B, Reich M, Chen K, Bell GI, Cohen S. Recombinant human epidermal growth factor precursor is a glycosylated membrane protein with biological activity. Mol Cell Biol 1989;9:2771–2778.
9. Bermudez E, Everitt J, Walker C. Expression of growth factor and growth factor receptor RNA in rat pleural mesothelial cells in culture. Exp Cell Res 1990;190:91–98.

10. Walker C, Everitt J, Ferriola PC, Stewart W, Mangum J, Bermudez E. Autocrine growth stimulation by transforming growth factor alpha in asbestos-transformed rat mesothelial cells. Cancer Res 1995;55:530–536.
11. Tzanakakis GN, Hjerpe A, Karamanos NK. Proteoglycan synthesis induced by transforming and basic fibroblast growth factors in human malignant mesothelioma is mediated through specific receptors and the tyrosine kinase intracellular pathway. Biochimie 1997;79:323–332.
12. Liu Z, Klominek J. Regulation of matrix metalloprotease activity in malignant mesothelioma cell lines by growth factors. Thorax 2003;58:198–203.
13. Marzo AL, Fitzpatrick DR, Robinson BW, Scott B. Antisense oligonucleotides specific for transforming growth factor beta2 inhibit the growth of malignant mesothelioma both in vitro and in vivo. Cancer Res 1997;57: 3200–3207.
14. Ikubo A, Morisaki T, Katano M, et al. A possible role of TGF-beta in the formation of malignant effusions. Clin Immunol Immunopathol 1995;77: 27–32.
15. Lee YC, Lane KB. The many faces of transforming growth factor-beta in pleural diseases. Curr Opin Pulmon Med 2001;7:173–179.
16. Gary Lee YC, Melkerneker D, Thompson PJ, Light RW, Lane KB. Transforming growth factor beta induces vascular endothelial growth factor elaboration from pleural mesothelial cells in vivo and in vitro. Am J Respir Crit Care Med 2002;165:88–94.
17. Kuwahara M, Takeda M, Takeuchi Y, Harada T, Maita K. Transforming growth factor beta production by spontaneous malignant mesothelioma cell lines derived from Fischer 344 rats. Virchows Arch 2001;438:492–497.
18. Zhu Z, Yao J, Wang F, Xu Q. TNF-alpha and the phenotypic transformation of human peritoneal mesothelial cell. Chin Med J (Engl) 2002;115: 513–517.
19. Owens MW, Grimes SR. Pleural mesothelial cell response to inflammation: tumor necrosis factor-induced mitogenesis and collagen synthesis. Am J Physiol 1993;265:L382–388.
20. Heldin CH, Backstrom G, Ostman A, et al. Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. EMBO J 1988;7:1387–1393.
21. Versnel MA, Hagemeijer A, Bouts MJ, van der Kwast TH, Hoogsteden HC. Expression of c-sis (PDGF B-chain) and PDGF A-chain genes in ten human malignant mesothelioma cell lines derived from primary and metastatic tumors. Oncogene 1988;2:601–605.
22. Ascoli V, Scalzo CC, Facciolo F, Nardi F. Platelet-derived growth factor receptor immunoreactivity in mesothelioma and nonneoplastic mesothelial cells in serous effusions. Acta Cytol 1995;39:613–622.
23. Ramael M, Buysse C, van den Bossche J, Segers K, van Marck E. Immunoreactivity for the beta chain of the platelet-derived growth factor receptor in malignant mesothelioma and non-neoplastic mesothelium. J Pathol 1992; 167:1–4.
24. Langerak AW, van der Linden-van Beurden CA, Versnel MA. Regulation of differential expression of platelet-derived growth factor alpha- and beta-receptor mRNA in normal and malignant human mesothelial cell lines. Biochim Biophys Acta 1996;1305:63–70.
25. Langerak AW, Dirks RP, Versnel MA. Splicing of the platelet-derived-growth-factor A-chain mRNA in human malignant mesothelioma cell lines and regulation of its expression. Eur J Biochem 1992;208:589–596.
26. Metheny-Barlow LJ, Flynn B, van Gijssel HE, Marrogi A, Gerwin BI. Paradoxical effects of platelet-derived growth factor-A overexpression in malig-

nant mesothelioma. Antiproliferative effects in vitro and tumorigenic stimulation in vivo. Am J Respir Cell Mol Biol 2001;24:694–702.
27. Walker C, Bermudez E, Stewart W, Bonner J, Molloy CJ, Everitt J. Characterization of platelet-derived growth factor and platelet-derived growth factor receptor expression in asbestos-induced rat mesothelioma. Cancer Res 1992;52:301–306.
28. Mutsaers SE, McAnulty RJ, Laurent GJ, Versnel MA, Whitaker D, Papadimitriou JM. Cytokine regulation of mesothelial cell proliferation in vitro and in vivo. Eur J Cell Biol 1997;72:24–29.
29. Asplund T, Versnel MA, Laurent TC, Heldin P. Human mesothelioma cells produce factors that stimulate the production of hyaluronan by mesothelial cells and fibroblasts. Cancer Res 1993;53:388–392.
30. Klominek J, Baskin B, Hauzenberger D. Platelet-derived growth factor (PDGF) BB acts as a chemoattractant for human malignant mesothelioma cells via PDGF receptor beta-integrin alpha3beta1 interaction. Clin Exp Metastasis 1998;16:529–539.
31. Rutten AA, Bermudez E, Stewart W, Everitt JI, Walker CL. Expression of insulin-like growth factor II in spontaneously immortalized rat mesothelial and spontaneous mesothelioma cells: a potential autocrine role of insulin-like growth factor II. Cancer Res 1995;55:3634–3639.
32. Lee TC, Zhang Y, Aston C, et al. Normal human mesothelial cells and mesothelioma cell lines express insulin-like growth factor I and associated molecules. Cancer Res 1993;53:2858–2864.
33. Syrokou A, Tzanakakis GN, Hjerpe A, Karamanos NK. Proteoglycans in human malignant mesothelioma. Stimulation of their synthesis induced by epidermal, insulin and platelet-derived growth factors involves receptors with tyrosine kinase activity. Biochimie 1999;81:733–744.
34. Pass HI, Mew DJ, Carbone M, Donington JS, Baserga R, Steinberg SM. The effect of an antisense expression plasmid to the IGF-1 receptor on hamster mesothelioma proliferation. Dev Biol Stand 1998;94:321–328.
35. Konig J, Tolnay E, Wiethege T, Muller K. Co-expression of vascular endothelial growth factor and its receptor flt-1 in malignant pleural mesothelioma. Respiration 2000;67:36–40.
36. Strizzi L, Catalano A, Vianale G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol 2001;193:468–475.
37. Cacciotti P, Strizzi L, Vianale G, et al. The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. Am J Respir Cell Mol Biol 2002; 26:189–193.
38. Catalano A, Romano M, Martinotti S, Procopio A. Enhanced expression of vascular endothelial growth factor (VEGF) plays a critical role in the tumor progression potential induced by simian virus 40 large T antigen. Oncogene 2002;21:2896–2900.
39. Masood R, Kundra A, Zhu S, Xia G, Scalia P, Smith DL, Gill PS. Malignant mesothelioma growth inhibition by agents that target the VEGF and VEGF-C autocrine loops. Int J Cancer 2003;104:603–610.
40. Romano M, Catalano A, Nutini M, et al. 5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. FASEB J 2001;15:2326–2336.
41. Zebrowski BK, Yano S, Liu W, et al. Vascular endothelial growth factor levels and induction of permeability in malignant pleural effusions. Clin Cancer Res 1999;5:3364–3368.

42. Kumar-Singh S, Weyler J, Martin MJ, Vermeulen PB, Van Marck E. Angiogenic cytokines in mesothelioma: a study of VEGF, FGF-1 and -2, and TGF beta expression. J Pathol 1999;189:72–78.
43. Harvey P, Warn A, Dobbin S, et al. Expression of HGF/SF in mesothelioma cell lines and its effects on cell motility, proliferation and morphology. Br J Cancer 1998;77:1052–1059.
44. Harvey P, Clark IM, Jaurand MC, Warn RM, Edwards DR. Hepatocyte growth factor/scatter factor enhances the invasion of mesothelioma cell lines and the expression of matrix metalloproteinases. Br J Cancer 2000;83: 1147–1153.
45. Tolnay E, Kuhnen C, Wiethege T, Konig JE, Voss B, Muller KM. Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma. J Cancer Res Clin Oncol 1998;124:291–296.
46. Perkins RC, Broaddus VC, Shetty S, Hamilton S, Idell S. Asbestos upregulates expression of the urokinase-type plasminogen activator receptor on mesothelial cells. Am J Respir Cell Mol Biol 1999;21:637–646.
47. Cacciotti P, Libener R, Betta P, et al. SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. Proc Natl Acad Sci USA 2001;98:12032–12037.

8

Oncogenes and Tumor Suppressor Genes in Malignant Mesothelioma

Robert A. Kratzke and Adi F. Gazdar

Malignant mesothelioma is a disease strongly associated with carcinogen exposure (1). As has been observed in a wide variety of other carcinogen-associated solid cancers, mesothelioma tumors accumulate a spectrum of acquired genetic lesions during the molecular pathogenesis leading to overt cancer. Perhaps reflecting the unique history of carcinogen exposure routinely seen in mesothelioma, many of the well-characterized mutations found in other cancers such as p53 and *ras* family alterations are not a common feature in malignant mesothelioma (1). Nonetheless, a variety of well-defined molecular abnormalities have been identified in the majority of cases of mesothelioma. As has often been the case in cancer genetics, the first information regarding genetic alterations in mesothelioma came from tumor karyotypic or family studies.

Although generally observed to be a disease strongly associated with asbestos exposure, familial clustering of mesothelioma independent of asbestos exposure has been reported (2–6). Epidemiologic data suggest that there may be a possible familial predisposition to mesothelioma, but the molecular basis for this remains unclear (7). Examples of chromosome 9p or 22 abnormalities have been reported in single cases or families with early-onset mesothelioma, but observations such as these have occurred infrequently and largely have confirmed known genetic loci that are involved in mesothelioma pathogenesis (2,6). The lack of a heritable model of mesothelioma, such as defined in breast cancer or colon cancer, has concentrated studies on the genetics of asbestos-induced mesothelioma. In these more common cases of sporadic mesothelioma with no obvious familial clustering or early onset, both of which are ultimately rare in mesothelioma, the most frequent cytogenetic abnormality in tumors reported has been the loss of chromosome 22 (8). As will be seen, this marks one of the most common somatic genetic targets, NF2, identified to date in mesothelioma. Other frequently observed karyotypic abnormalities include loss of the short arm (p) of chromosomes 1, 3, and 9 (8–10). Again, one of these recurring molecular lesions, loss of the 9p21 locus, has been correlated with corresponding loss of multiple well-characterized tumor suppressor

gene function. In addition, loss of the genetic material from the long arm of chromosomes 6, 13, and 15 has also been commonly identified in mesothelioma tumors (1). Reminiscent of investigations in a spectrum of cancers, gross karyotypic abnormalities have given useful guidance as to the location of somatic mutations in mesothelioma associated with loss of function and malignant transformation. These loss of function genes, or tumor suppressor genes, regulate a variety of molecular phenomena ranging from regulating the cell cycle, to cellular homeostasis and repair, to programmed cell death mechanisms. In a similar way, reduplication, rearrangement, or amplification of specific sequences can likewise give an indication of gain of function genetic abnormalities. However, few examples exist of such gain of function genetic abnormalities phenomenon in mesothelioma. For example, gain of genetic material has been reported for 5p; however, no specific recurrent gene rearrangements that might indicate a gain of function fusion gene product have been identified (1).

This chapter discusses the known acquired genetic alteration and when possible correlates them with the previously documented cytogenetic data. However, unlike general models in other solid tumors, little is known about the relative timing or order of these genetic and epigenetic lesions. Nonetheless, recent data from animal models can give us an indication of which molecular events are directly correlated with potential carcinogenic stimuli such as asbestos or simian virus 40 (SV40) infection.

Loss of Function from Cyclin-Dependent Kinase Inhibitors at the 9p21 Locus in Mesothelioma

Multiple lines of research have revealed that among the most common acquired genetic abnormalities in cancer is loss of G1 to S checkpoint control (Table 8.1) (11). Among the most critical elements in maintaining control at this checkpoint is the presence of hypophosphorylated pRb in maintaining the cell in a quiescent state (12). Processive phosphorylation of the 105-kd *Rb* (retinoblastoma susceptibility gene) gene product (pRb) is mediated by the family of cyclin-dependent kinases (CDKs) in conjunction with their corresponding S phase cyclins consisting primarily of cyclin E and the members of the cyclin D family (11). The D family of cyclins, among the earliest expressed proteins in the cell cycle, can physically interact with both pRb (13) and their associated CDKs, resulting in the critical phosphorylation of pRb.

The beginning of the understanding of the G1 to S molecular checkpoint can be traced to the cloning and characterization of the retinoblastoma susceptibility gene (*Rb*). The identification of the *Rb* gene in 1986 as being the genetic element that is mutated or deleted in the germline of kindreds affected with inherited retinoblastoma was the first proof of the tumor suppressor gene paradigm (14) as put forth by Knutson 15 years earlier. This model, which largely defines the tumor suppressor gene, is currently understood to postulate that loss of both alleles of a normal genetic element, in the case of familial retinoblastoma this

Table 8.1. Molecular alterations commonly present in malignant mesotheliomas (MM)

Gene	Comment	Reference
p16^{INK4a}	Usually downregulated by multiple mechanisms	12
p14^{ARF}	Another product of the *p16^{INK4a}* but seldom methylated or silenced in MM	27
p27	Low expression is a good prognosis marker	34
Rb	Seldom mutated, but inactivated by SV40 tumor antigen (Tag)	86
p53	Seldom mutated, but inactivated by SV40 Tag	86,87
Bcl-x	Downregulated frequently	66
NF2	Mutations common in MM	47
WT1	Frequently upregulated	80
Telomerase	Expressed in almost all MM; induced during SV40 transformation	88
EGFR	Upregulated by asbestos	89
MET oncogene	Upregulated by SV40	90
Notch1 oncogene	Upregulated by SV40	91

being the two alleles of the *Rb* gene, leads to loss of cellular homeostasis and transformation. This "two-hit" model applies to most of the somatic genetic lesions identified in mesothelioma and discussed in this chapter. In fulfillment of this model, the *Rb* gene locus of 13q14 is commonly deleted in a variety of other cancers, most notably lung and bladder cancer, diseases in which consequent loss of the *Rb* gene product, pRb, is found to be quite common (15). The irony of somatic mutations acquired in mesothelioma, however, is that although abnormalities of 13q are also common in mesothelioma, somewhat surprisingly, loss of function of pRb is rarely if ever observed in mesothelioma (12). Therefore, although loss of function at the G1 to S checkpoint occurs in mesothelioma, it is not accounted for by deletion or mutation the *Rb* gene.

As previously mentioned, processive phosphorylation of pRb leads to inactivation of pRb and the consequent release of the cell from the block into S phase. Inhibition of this CDK-mediated phosphorylation occurs via a family of proteins known appropriately as CDK inhibitors (CDKIs) (11). The first described CDKI, p16^{INK4a} (CDKN2, MTS) was first found to be commonly deleted in a form of familial melanoma associated with loss of genetic material at 9p21 (16). It had been known that 9p21 is a frequently targeted region of the genome in a variety of cancers including lung, bladder, and brain tumors. Therefore, it was not surprising to find that p16^{INK4a} expression was also lost in many of these same cancers. What was somewhat unexpected, though, was the tight correlation between loss of p16^{INK4a} function and the presence of wild-type pRb in many cancers, and vice versa (17–19). Since, as previously discussed, pRb was not found to be a common site of loss of function in mesothelioma, it might be anticipated that an alternative but equally critical aspect of the G1 to S checkpoint, such as p16^{INK4a}, would be affected in mesothelioma.

A variety of studies prior to the localization of the $p16^{INK4a}$ gene to the 9p21 locus had identified loss of 9p as a common event in mesothelioma tumors and cell lines (9,10). Both the aforementioned retention of wild-type pRb expression in mesothelioma and the subsequent identification of the 9p locus as being the location of the CDK inhibitor $p16^{INK4a}$, made this gene product a likely candidate tumor suppressor gene deleted in mesothelioma. When mesothelioma cell lines and tumors were examined for presence of the $p16^{INK4a}$ gene product, it was found to be absent in all cases (12,20,21). Although this was not unexpected, since many of the cell lines examined possess disruption of the 9p21 locus, the extremely high frequency of loss of $p16^{INK4a}$ gene product expression was surprising. Virtually all mesothelioma tumors and cell lines examined to date have lost detectable expression of the $p16^{INK4a}$ gene product.

Tightly linked at the 9p21 locus to the $p16^{INK4a}$ gene is the highly homologous $p15^{INK4b}$ gene (11). This 15-kd protein CDKI possesses much of the same biochemical activity as $p16^{INK4a}$, but its mutational spectrum is somewhat different. While $p16^{INK4a}$ appears to be one of the most common genetic lesions acquired in solid tumors, $p15^{INK4b}$ is affected at a lower frequency in many of the common cancers. For example, in lung cancer, where the 9p21 locus is intact in a large number of cases, the inactivation of the two genes via epigenetic mechanisms, such as DNA hypermethylation, is not concordant (22). This pattern of differential inactivation is similar to that observed between $p16^{INK4a}$ and the third gene encoded at this locus, $p14^{ARF}$, yet a third regulatory gene located at the 9p21 locus (see below). In the case of the homologous CDKI, $p15^{INK4b}$, the gene is differentially inactivated in a significant percentage of cases of both leukemia and myelodysplastic syndrome, where expression of the other 9p21 regulatory genes are maintained (23,24). Nonetheless, in mesothelioma many tumors have lost gross genetic material at the 9p21 locus, and have consequently co-deleted all the genes encoded there (20). As will be discussed, nondeletional loss of gene expression via epigenetic mechanisms of the genes at the 9p21 locus is less common in mesothelioma than in other cancers (25–27). With the high rate of deletion of the 9p21 locus, it is not surprising that at least one study has determined that 72% of mesothelioma tumors have co-deleted the genes for both $p15^{INK4b}$ and $p16^{INK4a}$, and presumably for the other regulatory gene encoded at this locus, $p14^{ARF20}$.

In light of the high frequency of loss of $p16^{INK4a}$ product in mesothelioma, it has been suggested that this may be a good target for gene replacement therapy (28,29). Reexpression of $p16^{INK4a}$ protein is associated with cell cycle arrest, apoptosis, and cell death in mesothelioma, and prolonged survival in mesothelioma xenograft models (28,29). Alternative methods of mediating the $p16^{INK4a}$ gene reexpression may also serve as potential novel therapies for mesothelioma. As in other cancers that lack $p16^{INK4a}$ expression but retain normal 9p alleles, DNA hypermethylation of the $p16^{INK4a}$ gene accounts for a small percentage of $p16^{INK4a}$ gene silencing in mesothelioma (25–27). It is interesting to note that a small percentage of mesothelioma tumors have demon-

strated response to methylation inhibiting cytidine analogues in phase II clinical trials.

Loss of genetic material and consequent gene expression at the 9p21 locus results in at least one additional well-characterized molecular defect in mesothelioma. In one of the more surprising findings in cell cycle and cancer molecular genetics, the DNA sequence encoding the *p16*^{INK4a} gene also encodes an alternative cell cycle regulatory gene in a unique reading frame, thus coding for a second protein with an entirely different amino acid sequence and molecular weight (11). This 14-kd protein, known as p14^{ARF} (alternative reading frame), inhibits *mdm2*-mediated degradation of the well-characterized p53 tumor suppressor gene (11). This model predicts that in the absence of p14^{ARF}, wild-type p53 will be highly unstable due to a very short protein half-life. Potentially, then, loss of genetic material at the 9p21 locus leads to loss of cell cycle regulation through both the pRb and p53 pathways. Data from murine knockout models suggest that loss of p14^{ARF} may be a stronger cancer initiating event than loss of p16^{INK4a}, although similar data from human cancer is not as convincing with differential loss of one or the other gene product being observed via methylation in tumors with intact 9p21 locus (30).

Loss of p14^{ARF} gene expression appears to be as equally common in mesothelioma as the loss of p16^{INK4a} protein (1,27). Studies on protein expression have been limited to this point due to the lack of appropriate immunologic reagents, but reexpression of p14^{ARF} appears to mediate many of the same effects in mesothelioma cells and xenografts that p16^{INKa} reexpression does (31). In a correlation that strongly mimics the previously described situation in the p16^{INK4a}/pRb genetic switch in mesothelioma, p53 mutations are rarely found in mesothelioma (1,32). Rather, the prevailing model suggests that loss of p53 checkpoint is mediated through loss of p14^{ARF} expression and consequent enhanced *mdm2*-mediated p53 degradation (11,33). This fascinating picture of dual loss of control at two of the best characterized cell cycle checkpoints by disruption of a single genetic locus has proven to be a surprising twist in the study of cancer biology. Although it may prove that only one of these pathways is the critical step in the development of mesothelioma, at this time the evidence suggests that loss of both of the proteins encoded for at the 9p21 locus is important in the pathogenesis of mesothelioma.

Additional Abnormalities of Cyclin-Dependent Kinase Inhibitors in Mesothelioma

Gross abnormalities in expression or mutations of *p15*^{INK4b} and *p16*^{INK4a} are likely the most common genetic abnormalities of the CDK inhibitor family of proteins not only in mesothelioma but in other solid tumors as well. Nonetheless, differential expression of some of the other well-characterized CDK inhibitors such as p27 (p27^{kip1}) and p21 (WAF1/CIP1) has been documented. In mesothelioma, studies have identified either elevated p27 expression by immunohistochemistry to

be a positive prognostic sign or the converse, low expression of p27, to be a poor prognostic sign (34–36). For example, in one study low expression was associated with a median survival of 4 to 5 months, while normal or elevated expression of p27 in mesothelioma portends a more favorable prognosis with a median survival of 10 to 11 months (34). Similar prognostic significance of low p27 expression has been identified in other solid tumors such as breast cancer (37–40). In a like manner, the p21 CDK inhibitor has been identified as differentially expressed in mesothelioma tumors by immunohistochemistry (41,42). The original identification of p21 was as a factor upregulated by the expression of wild-type p53, and thus it might be anticipated that most mesothelioma tumors express detectable p53 (43). Alternatively, as previously discussed, p53 may be destabilized or inactivated by secondary means, and thus any factor potentially dependent on the presence of wild-type p53 for expression, such as p21, may be relatively absent. Along these lines, in studies only about 35% of mesothelioma tumors possess easily detectable p21 expression (41,42). However, the relatively few studies published have varied in the conclusion of whether there is any clinical significance in the p21 expression pattern in mesothelioma.

Mutations in the NF2 Gene Are a Common Feature of Mesothelioma

Neurofibromatosis type 2 (NF2) is an autosomal-dominant disease characterized by development of brain tumors and schwannomas, particularly involving the eighth cranial nerve (44). Epidemiologic studies have demonstrated a linkage to the long arm of chromosome 22 (22q), and subsequent positional cloning isolated the *NF2* gene as the targeted gene whose loss of function accounts for this clinical syndrome (45,46). The 70-kd *NF2* gene product is a moesin ezrin radixin-like protein that maps to 22q11-q13.1 and has also has been named both *merlin* (moesin ezrin radixin-like protein) and *schwannomin*. As discussed previously, loss of chromosome 22 appears to be the most commonly detected cytogenetic reported in malignant mesothelioma (8). Following the identification of the *NF2* gene on chromosome 22 as the affected gene in familial neurofibromatosis, a variety of investigators examined *NF2* gene expression in mesothelioma (47,48). An initial study of 15 mesothelioma cell lines revealed abnormalities in single-strand conformation polymorphism (SSCP) in 53% of cDNAs from mesothelioma. Subsequent sequencing revealed a high frequency of mutations in the NF2 protein coding sequence, resulting in truncated *merlin* protein in all eight of the samples with abnormal SSCP migration (48). When primary tumors were examined, all but one of the matching primary tumors possessed the identical mutation found in the paired cell line. Similar studies have reported 41% of tumors or cell lines with detectable mutations or deletions in NF2 transcripts in mesothelioma, while no such abnormalities were observed in lung cancer (47). Similar to the situation with the genes at the 9p21 locus,

the *NF2* gene appears to function as a classic tumor suppressor gene in mesothelioma and likely is the second most common somatic mutation in this disease.

Although the high rate of mutation of *NF2* transcripts in mesothelioma cell lines and tumors indicates it is a frequent target of inactivation in the development of mesothelioma, it is interesting to note that mesothelioma is not part of the well-described clinical syndrome of neurofibromatosis 2 (44). However, this is not unlike what has been observed in other familial cancer syndromes, including the rare inherited abnormalities of 9p21 associated with melanoma or pancreatic cancer. The clinical syndrome of neurofibromatosis is exceedingly rare, and presumably the carrier rate of germline mutations of NF2 is as rare as 1 in 40,000 (44). It has been theorized that if germline mutations in NF2 predispose patients to mesothelioma in the face of asbestos exposure, the low frequency of both the incidence of *NF2* germline mutations and asbestos exposure would make the predisposition very difficult to detect. Along these lines, a recent case was reported of an asbestos-exposed NF2 patient who developed mesothelioma within several years of his exposure, rather than the common prolonged latency period of 20 years or more (2). It seems likely that NF2 patients are at increased risk of mesothelioma following additional exposure to asbestos, or perhaps following infection with SV40 or other putative mesothelioma promoting agents. However, the coincident low rate of germline *NF2* mutation carriers and intense asbestos exposure makes detection of this potential heritable predisposition to mesothelioma uncommon.

Alterations of p53 Appear Infrequently in Malignant Mesothelioma

Mutations of the *p53* gene are among the most frequent and best documented acquired genetic abnormalities in solid tumors (49). The *p53* locus is located at 17p13, a common hot spot for karyotypic abnormalities in a wide spectrum of cancers. As its name indicates, the *p53* gene encodes a 53-kd phosphoprotein that appears to play a key role in maintenance of the integrity of genetic information. Mutations of *p53* generally are missense mutations resulting in expression of a full-length inactive gene product that is preferentially stabilized when compared to the relatively short-lived wild-type p53 protein. As such, cancers that stain strongly for p53 protein on immunohistochemistry often, but not invariably, possess mutant p53 protein, whereas tumors that stain weakly are more often than not wild type. However, because of some variability between the correlation of strong immunostaining and mutation of the p53 protein, studies on the frequency of mutation of *p53* in cancers can come to divergent opinions (50).

Abnormalities of 17p have been noted in mesothelioma, although at a lower frequency than in many other tumors (1). When mesothelioma cell lines or tumors have been examined, generally few mutations in *p53* have been found (51–54). One explanation for this divergence from

the mutation patterns seen in other cancers may be the presence of SV40 tumor antigen (Tag) as a putative co-carcinogen. It has been known that SV40 Tag can bind to and inactivate wild-type p53, implicating that infection with SV40 may serve as a method for inactivating *p53* in the absence of overt mutation. In recent years, the detection of SV40 viral DNA in mesothelioma tumors has generated much interest in this hypothesis, and this issue is discussed in detail elsewhere in this text (1). Alternatively, as discussed previously, the absence of $p14^{ARF}$ in mesothelioma would be expected to result in loss of the inhibition of *mdm2*-mediated degradation of *p53*, leading to a shorter protein half-life. Of interest, *p53* mutations have rarely been observed in experimental models of asbestos-derived mesothelioma in rodents (55,56), but *p53* deficient mice are more susceptible to asbestos-induced mesothelioma (57,58). These findings strongly argue that loss of *p53* function plays a direct role in the development of mesothelioma, but more likely through alternative mechanisms than direct mutation or deletion of the *p53* gene product.

Several investigators have reported increased levels of p53 protein in mesothelioma tumors as detected by immunohistochemistry (51,52). Rates of overexpression have been reported to be as high as 35% in resected tumors. In addition, one report identified two of four mesothelioma cell lines with missense mutations by DNA sequencing (59). However, these reports should probably be regarded as the exception rather than the rule. Circulating autoantibodies to p53 are often seen in solid tumors and are thought to represent an immunologic response to the presentation of mutant p53 antigen, but these autoantibodies are rarely detected in patients with mesothelioma, although perhaps only in a small percentage of patients (less than 10%) (60). Moreover, the p53 antisera detected in these patients in this study had relatively low titers that did not vary with treatment, indicating that perhaps they bear little relevance to the p53 status of the corresponding tumor.

Similar to the reports on reexpression of $p16^{INK4a}$ and $p14^{ARF}$, virally mediated reexpression of p53 can result in cell growth inhibition and xenograft inhibition in mesothelioma cells (61). Although this may seem to argue for the presence of directly inactivated p53 in mesothelioma that can then be ameliorated by the addition of exogenous wild-type p53, it is also consistent with a model of secondary inactivation of p53 from the presence of SV40 Tag or from protein destabilization in the absence of $p14^{ARF}$. In the latter two examples it may be predicted that overexpression of exogenous p53 will overcome these mechanisms of p53 inactivation and thus reestablish normal cell homeostasis. Consistent with this interpretation, the replication sufficient ONYX-015 adenovirus, which has a selective cytolytic effect in p53-deficient cells, demonstrated cell killing in a mesothelioma cell line (MS-1) that retained both normal p53 and $p14^{ARF}$, while three cell lines (NCI-H28, NCI-H513, 211H) that possessed wild-type p53 but absent $p14^{ARF}$ were killed by the ONYX-015 virus (62). When $p14^{ARF}$ was reintroduced into these three cell lines, they became resistant to ONYX-015–mediated killing as well, strongly arguing that p53 inactivation in these mesothelioma cell lines is mediated in part by the absence of $p14^{ARF}$ protein.

However, these findings cannot eliminate the probable important role played by the presence of possible SV40 Tag in moderating or inactivating wild-type p53 function in mesothelioma.

Apoptosis-Mediating Gene Defects in Mesothelioma

Both the death receptor and mitochondrial-mediated pathways play significant roles in cell death and cancer progression (63). Although much recent interest has focused on abnormalities of caspase activity in cell immortalization and cancer pathogenesis, little is known regarding this pathway and the pathogenesis of mesothelioma (64,65). In contrast, abnormalities of the Bcl-2 pathway (an inhibitor of the mitochondrial pathway) have been described in mesothelioma, but to a limited degree. In one of the largest studies conducted on this subject, researchers in Finland identified Bcl-2 positivity in seven out of 35 (20%) mesothelioma tumors as analyzed by immunohistochemistry (66). Strong expression of the related antiapoptotic proteins Bcl-X was found in all cases. The absence of Bcl-2 expression in mesothelioma was strongly correlated, in this relatively small series, with a higher apoptotic index and, paradoxically, statistically significant poorer survival. Although a similar paradoxical result has been reported in other cancers, these findings are surprising unless they are indicative of a high tumor burden turnover in aggressive disease. No similar correlations were found in apoptotic index with Bcl-X expression, but given the universally elevated levels detected it is understandable if no variation can be detected.

In the face of elevated expression of the antiapoptotic *Bcl-X* gene product, investigators have designed systems to attempt to downregulate its expression and render the mesothelioma cells more susceptible to apoptosis. Expression of antisense Bcl-X has been reported to engender apoptosis in two mesothelioma cell lines following diminished transcription of *Bcl-X* transcripts (67). In a like manner, treatment of mesothelioma cells with sodium phenylbutyrate has been described as leading to Bcl-X downregulation and cell death in mesothelioma (68). In a somewhat different pharmacologic maneuver, these same investigators introduced the proapoptotic *Bak* gene into mesothelioma cells to counteract the overexpressed Bcl-X protein and found apoptosis occurred in two cell lines (69). It is hard to generalize from these experiments which, if any, of these potential therapeutic modalities could prove of value in treating mesothelioma, but it seems clear that as in many other solid tumors, overexpression of the antiapoptotic Bcl family of proteins plays a significant role in the pathogenesis of mesothelioma, and downregulation of these proteins can mediate cell death in mesothelioma cell lines and tumors.

The death receptor pathway is initiated by death inducing ligands such as *TRAIL* binding to their specific cell surface receptors and triggering a cascade involving caspase 8 and possibly caspase 10. There are four known *TRAIL* receptors: *DR4* and *DR5*, which initiate the apoptotic pathway upon activation, and two decoy receptors, *DcR1*

and *DcR2*, which lack a death domain and are presumed to be anti-apoptotic. However, recent data indicate that the decoy receptors are methylated and silenced in pediatric tumors (70). Our unpublished data indicate that methylation of the decoy receptors is one of the most frequent molecular changes present in virtually all cancer types, with very high frequencies in mesotheliomas. These observations indicate that the role of decoy receptors need to be reevaluated, and that silencing of the decoy receptors may aid cell survival rather than prevent apoptosis.

Epigenetic Inactivation of RASSF1A Occurs in Mesothelioma and After Simian Virus 40 Infection in Mesothelial Cells

Many of the classic studies of the past decade in somatic mutations in solid tumors have revolved around the central tenet that cancer is a disease of acquired genetic damage. However, it is now clear that epigenetic mechanisms, including DNA hypermethylation, play a significant role in gene silencing in cancer. As an example, as has been previously discussed, up to 10% of mesothelioma tumors inactivate $p16^{INK4a}$ gene expression following acquired DNA hypermethylation, although it must be noted that this rate of methylation is much lower than that found in many other common cancers (26,27). Until recently, however, the methylation pattern of other genes in mesothelioma has not been extensively studied. A series profiling 66 mesothelioma and 40 lung adenocarcinomas for methylation has yielded fascinating and compelling results (25). Methylation profiling of seven genes commonly inactivated by epigenetic mechanisms in cancer was carried out in 66 mesothelioma samples. In summary, it was found that the overall rate of gene methylation ("methylation index") was significantly lower than that found in adenocarcinoma of the lung. However, one gene, the candidate tumor suppressor gene *RASSF1A*, was methylated at a remarkably high frequency in this large cohort of mesothelioma tumors (32%). The *RASSF1A* gene is a 39-kd *ras*-associated protein that fulfills the criterion of a classic tumor suppressor gene in lung cancer (71–73). The gene maps to the 3p21.3 locus, a common sight for loss of heterozygosity and cytogenetic abnormalities in a wide variety of cancers, including mesothelioma. An additional intriguing finding arising from this methylation survey is that the mesothelioma tumors analyzed that possessed evidence of SV40 Tag sequences (52% of the tumors examined) were the subset that also possessed, significantly, a higher methylation index (25). If SV40 infection mediates or is associated with epigenetic cellular events, such as DNA hypermethylation, it may give insight into the relatively fewer somatic genetic lesions seen in mesothelioma as compared to other carcinogen associated malignancies.

As has been discussed in this chapter and elsewhere in this text, there appears to be significant evidence that expression of SV40 Tag plays a role in the pathogenesis of mesothelioma (1). Identification of the presence of distinct methylation patterns in SV40 Tag-positive mesothe-

lioma tumors raises the question of cause and effect. Recent investigations on the acquired genetic and epigenetic abnormalities that occur following SV40 infection of mesothelial cells have revealed an interesting pattern of DNA hypermethylation in these cells. In early passage following infection, no methylation of seven genes previously identified as being targets of methylation in mesothelioma was found. However, later passages of SV40-infected mesothelial cells have been found to possess progressive methylation of the *RASSF1A* gene and a consequent decrease in transcript (74). Consistent with methylation at this locus, treatment with the cytosine analogue and methylation inhibitor 5-aza-2′-deoxycytidine led to reexpression of the *RASSF1A* transcripts. These findings provide a true mechanistic link between known tumor suppressor gene inactivation and SV40 infection.

Dominant-Negative WT1 Gene Abnormalities Are Detected in Mesothelioma

Identification of karyotypic abnormalities at 11p13 in pediatric nephroblastoma (Wilms' tumor) led to the subsequent cloning in 1990 of the Wilms' tumor susceptibility gene 1 (*WT1*) (75). The *WT1* locus had already been shown to fulfill one of the main criteria of a classic tumor suppressor gene even prior to the identification of the coding sequence when it was demonstrated in 1987 that reconstitution of 11p to nephroblastoma cells reversed the malignant phenotype (76). Subsequent analyses have demonstrated loss or inactivation of *WT1* in most cases of pediatric nephroblastoma or Wilms' tumor. The *WT1* gene product is a 52- to 54-kd protein with a complex expression pattern involving up to 24 isoforms (77). The protein possesses a series of zinc finger motifs consistent with a DNA-binding protein, and disruption of *WT1* in murine models leads to severe developmental abnormalities in the urogenital system. Similar to the pattern seen with other tumor suppressor genes identified first in germline cancer syndromes, acquired mutations of *WT1* have also been noted in a variety of other malignancies as well (78).

Mutations of the *WT1* gene in mesothelioma were noted after the rather striking finding that WT1 protein is routinely expressed in normal mesothelium just as it is expressed in normal urogenital tissues (79). In addition, mesothelioma tumors were found to generally express elevated levels of WT1 protein as detected by immunohistochemistry (80,81). The presence of nuclear staining for WT1 has been reported in 75% to 100% of mesothelioma tumors and cell lines examined to date. This has led to WT1 expression being developed as a potentially useful diagnostic test in differentiating mesothelioma tumors from lung cancer, since normal WT1 expression is rarely detected in lung or lung cancers (81). Mutational analyses, however, have yielded only sporadic missense mutation identified in mesothelioma samples. Mutations of *WT1* in mesothelioma, as well as in other cancers, generally target the DNA binding motif of exons 7 through 10 containing the zinc finger motifs. By way of interaction with DNA through the zinc finger motifs,

the *WT1* gene product is thought to function as a transcriptional suppressor (82). In an intriguing alteration of the standard loss of function abnormalities of tumor suppressor genes, mutations of *WT1* in the zinc finger motif have been demonstrated not only to abrogate the transcriptional repression activity of wild-type *WT1* but also to result in a dominant-negative transcriptional activator phenotype (82). Such dominant-negative or activating mutations of *WT1* occur in both germline nephroblastoma and acquired mutations in cases such as mesothelioma (79), but probably at a low frequency. Additional reports indicate DNA hypermethylation of the *WT1* may occur with a high frequency in mesothelioma at a CpG island with the 5′ end of the gene, but the correlation of this finding with gene product expression remains unclear (83).

Relationship to Simian Virus 40

While the relationship of the SV40 virus and the pathogenesis of malignant mesothelioma (MM) is discussed elsewhere in this book, the presence of SV40 is associated with certain molecular changes that are discussed briefly here for the sake of completeness. These changes include activation or upregulation of the *Met* and *Notch1* oncogenes and of insulin-like growth factor I (84). In addition, methylation and silencing of the *RASSF1A* gene is significantly higher in virus containing tumors (25,74). The Tag present in SV40-positive MMs has been demonstrated to be capable of binding to and inhibiting cellular p53 and retinoblastoma family proteins (84). In an in vitro model, both SV40 and asbestos acted as co-carcinogens (85), suggesting that both of these etiologic factors contribute to the molecular pathogenesis of MM in their own unique manner. Of notable interest, both the Tag protein of SV40 and deletions of 9p induced by asbestos exposure may contribute to inhibition of the cell cycle and of the p53 protein.

Conclusion

Mesothelioma is a cancer marked by a distinct pattern of mutations unlike most other solid tumors. Direct mutation of genes such as *p53* or *ras* family members may be rare and distinctly different from the well-characterized spectrum of mutations seen in the more common epithelial malignancies such as lung or bladder cancer. This may be partially a result of the unique contributions of asbestos and SV40 infection as co-carcinogens in mesothelioma. Nonetheless, aberrations of the regulatory genes expressed at the 9p21 locus ($p16^{INK4a}$, $p14^{ARF}$) and chromosome 22 (*NF2*) are clearly among the highest frequencies observed in human cancers. In addition, recent investigations into epigenetic inactivation following SV40 infection may yield new gene targets such as *RASSF1A* that are inactivated by pathways that have not been sufficiently explored in the past. The presence of normal DNA within mesothelioma tumors is likely not to be equated with the presence of a normal protein phenotype in the future. This holds the promise

of potentially reversible epigenetic or infectious molecular defects accounting for much of the transformed phenotype in mesothelioma, and the distinct possibility of novel therapeutic modalities that may reverse these acquired defects in gene expression patterns.

References

1. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002;29:2–17.
2. Baser ME, De Rienzo A, Altomare D, et al. Neurofibromatosis 2 and malignant mesothelioma. Neurology 2002;59:290–291.
3. Kane MJ, Chahinian AP, Holland JF. Malignant mesothelioma in young adults. Cancer 1990;65:1449–1455.
4. Risberg B, Nickels J, Wagermark J. Familial clustering of malignant mesothelioma. Cancer 1980;45:2422–2427.
5. Hammar SP, Bockus D, Remington F, Freidman S, LaZerte G. Familial mesothelioma: a report of two families. Hum Pathol 1989;20:107–112.
6. Musti M, Cavone D, Aalto Y, Scattone A, Serio G, Knuutila S. A cluster of familial malignant mesothelioma with del(9p) as the sole chromosomal anomaly. Cancer Genet Cytogenet 2002;138:73–76.
7. Roushdy-Hammady I, Siegel J, Emri S, Testa JR, Carbone M. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 2001;357:444–445.
8. Flejter WL, Li FP, Antman KH, Testa JR. Recurring loss involving chromosomes 1, 3, and 22 in malignant mesothelioma: possible sites of tumor suppressor genes. Genes Chromosomes Cancer 1989;1:148–154.
9. Taguchi T, Jhanwar SC, Siegfried JM, Keller SM, Testa JR. Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma. Cancer Res 1993;53:4349–4355.
10. Cheng JQ, Jhanwar SC, Lu YY, Testa JR. Homozygous deletions within 9p21-p22 identify a small critical region of chromosomal loss in human malignant mesotheliomas. Cancer Res 1993;53:4761–4763.
11. Sherr CJ. Cancer cell cycles. Science 1996;274:1672–1677.
12. Kratzke RA, Otterson GA, Lincoln CE, et al. Immunohistochemical analysis of the p16INK4 cyclin-dependent kinase inhibitor in malignant mesothelioma. J Natl Cancer Inst 1995;87:1870–1875.
13. Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A, Weinberg RA. Physical interaction of the retinoblastoma protein with human D cyclins. Cell 1993;73:499–511.
14. Friend SH, Horowitz JM, Gerber MR, et al. Deletions of a DNA sequence in retinoblastomas and mesenchymal tumors: organization of the sequence and its encdoded protein. Proc Natl Acad Sci USA 1987;84:9059–9063.
15. Kratzke RA, Shimizu E, Kaye FJ. Oncogenes in human lung cancer. Cancer Treat Res 1992;63:61–85.
16. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. Science 1994;264:436–440.
17. Kelley MJ, Nakagawa K, Steinberg SM, Mulshine JL, Kamb A, Johnson BE. Differential inactivation of CDKN2 and Rb protein in non-small-cell and small-cell lung cancer cell lines. J Natl Cancer Inst 1995;87:756–761.
18. Kratzke RA, Greatens TM, Rubins JB, et al. Rb and p16INK4a expression in resected non-small cell lung tumors. Cancer Res 1996;56:3415–3420.

19. Otterson GA, Kratzke RA, Coxon A, Kim YW, Kaye FJ. Absence of p16INK4 protein is restricted to the subset of lung cancer lines that retains wild-type RB. Oncogene 1994;9:3375–3378.
20. Xio S, Li D, Vijg J, Sugarbaker DJ, Corson JM, Fletcher JA. Codeletion of p15 and p16 in primary malignant mesothelioma. Oncogene 1995;11: 511–515.
21. Cheng JQ, Jhanwar SC, Klein WM, et al. p16 alterations and deletion mapping of 9p21-p22 in malignant mesothelioma. Cancer Res 1994;54: 5547–5551.
22. Zhou JX, Niehans GA, Shar A, Rubins JB, Frizelle SP, Kratzke RA. Mechanisms of G1 checkpoint loss in resected early stage non-small cell lung cancer. Lung Cancer 2001;32:27–38.
23. Daskalakis M, Nguyen TT, Nguyen C, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. Blood 2002;100:2957–2964.
24. Quesnel B, Guillerm G, Vereecque R, et al. Methylation of the p15(INK4b) gene in myelodysplastic syndromes is frequent and acquired during disease progression. Blood 1998;91:2985–2990.
25. Toyooka S, Pass HI, Shivapurkar N, et al. Aberrant methylation and simian virus 40 tag sequences in malignant mesothelioma. Cancer Res 2001;61: 5727–5730.
26. Wong L, Zhou J, Anderson D, Kratzke RA. Inactivation of p16(INK4a) expression in malignant mesothelioma by methylation. Lung Cancer 2002; 38:131–136.
27. Hirao T, Bueno R, Chen CJ, Gordon GJ, Heilig E, Kelsey KT. Alterations of the p16(INK4) locus in human malignant mesothelial tumors. Carcinogenesis 2002;23:1127–1130.
28. Frizelle SP, Grim J, Zhou J, et al. Re-expression of p16INK4a in mesothelioma cells results in cell cycle arrest, cell death, tumor suppression and tumor regression. Oncogene 1998;16:3087–3095.
29. Frizelle SP, Rubins JB, Zhou JX, Curiel DT, Kratzke RA. Gene therapy of established mesothelioma xenografts with recombinant p16INK4a adenovirus. Cancer Gene Ther 2000;7:1421–1425.
30. Kamijo T, Zindy F, Roussel MF, et al. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell 1997;91:649–659.
31. Yang CT, You L, Yeh CC, et al. Adenovirus-mediated p14(ARF) gene transfer in human mesothelioma cells. J Natl Cancer Inst 2000;92:636–641.
32. Lee WC, Testa JR. Somatic genetic alterations in human malignant mesothelioma (review). Int J Oncol 1999;14:181–188.
33. Quelle DE, Zindy F, Ashmun RA, Sherr CJ. Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. Cell 1995;83:993–1000.
34. Beer TW, Shepherd P, Pullinger NC. p27 immunostaining is related to prognosis in malignant mesothelioma. Histopathology 2001;38:535–541.
35. Bongiovanni M, Cassoni P, De Giuli P, et al. p27(kip1) immunoreactivity correlates with long-term survival in pleural malignant mesothelioma. Cancer 2001;92:1245–1250.
36. Beer TW. Immunohistochemical MIB-1 and p27 as prognostic factors in pleural mesothelioma. Pathol Res Pract 2001;197:859.
37. Catzavelos C, Tsao MS, DeBoer G, Bhattacharya N, Shepherd FA, Slingerland JM. Reduced expression of the cell cycle inhibitor p27Kip1 in non-small cell lung carcinoma: a prognostic factor independent of Ras. Cancer Res 1999;59:684–688.

38. Yang RM, Naitoh J, Murphy M, et al. Low p27 expression predicts poor disease-free survival in patients with prostate cancer. J Urol 1998;159:941–945.
39. Fredersdorf S, Burns J, Milne AM, et al. High level expression of p27(kip1) and cyclin D1 in some human breast cancer cells: inverse correlation between the expression of p27(kip1) and degree of malignancy in human breast and colorectal cancers. Proc Natl Acad Sci USA 1997;94:6380–6385.
40. Tan P, Cady B, Wanner M, et al. The cell cycle inhibitor p27 is an independent prognostic marker in small (T1a,b) invasive breast carcinomas. Cancer Res 1997;57:1259–1263.
41. Isik R, Metintas M, Gibbs AR, et al. p53, p21 and metallothionein immunoreactivities in patients with malignant pleural mesothelioma: correlations with the epidemiological features and prognosis of mesotheliomas with environmental asbestos exposure. Respir Med 2001;95:588–593.
42. Baldi A, Groeger AM, Esposito V, et al. Expression of p21 in SV40 large T antigen positive human pleural mesothelioma: relationship with survival. Thorax 2002;57:353–356.
43. el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75:817–825.
44. Evans DG, Sainio M, Baser ME. Neurofibromatosis type 2. J Med Genet 2000;37:897–904.
45. Rouleau GA, Merel P, Lutchman M, et al. Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. Nature 1993;363:515–521.
46. Trofatter JA, MacCollin MM, Rutter JL, et al. A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 1993;72:791–800.
47. Sekido Y, Pass HI, Bader S, et al. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. Cancer Res 1995;55:1227–1231.
48. Bianchi AB, Mitsunaga SI, Cheng JQ, et al. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc Natl Acad Sci USA 1995;92:10854–10858.
49. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 1993;329:1318–1327.
50. Greatens TM, Niehans GA, Rubins JB, et al. Do molecular markers predict survival in non-small-cell lung cancer? Am J Respir Crit Care Med 1998; 157:1093–1097.
51. Liu BC, Fu DC, Miao Q, Wang HH, You BR. p53 gene mutations in asbestos associated cancers. Biomed Environ Sci 1998;11:226–232.
52. Mayall FG, Jacobson G, Wilkins R. Mutations of p53 gene and SV40 sequences in asbestos associated and non-asbestos-associated mesotheliomas. J Clin Pathol 1999;52:291–293.
53. Ni Z, Liu Y, Keshava N, Zhou G, Whong W, Ong T. Analysis of K-ras and p53 mutations in mesotheliomas from humans and rats exposed to asbestos. Mutat Res 2000;468:87–92.
54. Pass HI, Stevens EJ, Oie H, et al. Characteristics of nine newly derived mesothelioma cell lines. Ann Thorac Surg 1995;59:835–844.
55. Unfried K, Roller M, Pott F, Friemann J, Dehnen W. Fiber-specific molecular features of tumors induced in rat peritoneum. Environ Health Perspect 1997;105S:1103–1108.
56. Kleymenova EV, Horesovsky G, Pylev LN, Everitt J. Mesotheliomas induced in rats by the fibrous mineral erionite are independent from p53 alterations. Cancer Lett 1999;147:55–61.

57. Vaslet CA, Messier NJ, Kane AB. Accelerated progression of asbestos-induced mesotheliomas in heterozygous p53+/− mice. Toxicol Sci 2002;68: 331–338.
58. Marsella JM, Liu BL, Vaslet CA, Kane AB. Susceptibility of p53-deficient mice to induction of mesothelioma by crocidolite asbestos fibers. Environ Health Perspect 1997;105 (suppl 5):1069–1072.
59. Cote RJ, Jhanwar SC, Novick S, Pellicer A. Genetic alterations of the p53 gene are a feature of malignant mesotheliomas. Cancer Res 1991;51:5410–5416.
60. Creaney J, McLaren BM, Stevenson S, et al. p53 autoantibodies in patients with malignant mesothelioma: stability through disease progression. Br J Cancer 2001;84:52–56.
61. Giuliano M, Catalano A, Strizzi L, Vianale G, Capogrossi M, Procopio A. Adenovirus-mediated wild-type p53 overexpression reverts tumourigenicity of human mesothelioma cells. Int J Mol Med 2000;5:591–596.
62. Yang CT, You L, Uematsu K, Yeh CC, McCormick F, Jablons DM. p14(ARF) modulates the cytolytic effect of ONYX-015 in mesothelioma cells with wild-type p53. Cancer Res 2001;61:5959–5963.
63. Rudin CM, Thompson CB. Apoptosis and disease: regulation and clinical relevance of programmed cell death. Annu Rev Med 1997;48:267–281.
64. Harada K, Toyooka S, Shivapurkar N, et al. Deregulation of caspase 8 and 10 expression in pediatric tumors and cell lines. Cancer Res 2002;62: 5897–5901.
65. Shivapurkar N, Toyooka S, Eby MT, et al. Differential inactivation of caspase-8 in lung cancers. Cancer Biol Ther 2002;1:65–69.
66. Soini Y, Kinnula V, Kaarteenaho-Wiik R, Kurttila E, Linnainmaa K, Paakko P. Apoptosis and expression of apoptosis regulating proteins bcl-2, mcl-1, bcl-X, and bax in malignant mesothelioma. Clin Cancer Res 1999;5:3508–3515.
67. Smythe WR, Mohuiddin I, Ozveran M, Cao XX. Antisense therapy for malignant mesothelioma with oligonucleotides targeting the bcl-xl gene product. J Thorac Cardiovasc Surg 2002;123:1191–1198.
68. Mohiuddin I, Cao X, Fang B, Nishizaki M, Smythe WR. Significant augmentation of pro-apoptotic gene therapy by pharmacologic bcl-xl down-regulation in mesothelioma. Cancer Gene Ther 2001;8:547–554.
69. Pataer A, Smythe WR, Yu R, et al. Adenovirus-mediated Bak gene transfer induces apoptosis in mesothelioma cell lines. J Thorac Cardiovasc Surg 2001;121:61–67.
70. van Noesel MM, van Bezouw S, Salomons GS, et al. Tumor-specific down-regulation of the tumor necrosis factor-related apoptosis-inducing ligand decoy receptors DcR1 and DcR2 is associated with dense promoter hypermethylation. Cancer Res 2002;62:2157–2161.
71. Pfeifer GP, Yoon JH, Liu L, Tommasi S, Wilczynski SP, Dammann R. Methylation of the RASSF1A gene in human cancers. Biol Chem 2002;383:907–914.
72. Dreijerink K, Braga E, Kuzmin I, et al. The candidate tumor suppressor gene, RASSF1A, from human chromosome 3p21.3 is involved in kidney tumorigenesis. Proc Natl Acad Sci USA 2001;98:7504–7509.
73. Dammann R, Takahashi T, Pfeifer GP. The CpG island of the novel tumor suppressor gene RASSF1A is intensely methylated in primary small cell lung carcinomas. Oncogene 2001;20:3563–3567.
74. Toyooka S, Carbone M, Toyooka KO, et al. Progressive aberrant methylation of the RASSF1A gene in simian virus 40 infected human mesothelial cells. Oncogene 2002;21:4340–4344.

75. Call KM, Glaser T, Ito CY, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 1990;60:509–520.
76. Weissman BE, Saxon PJ, Pasquale SR, Jones GR, Geiser AG, Stanbridge EJ. Introduction of a normal human chromosome 11 into a Wilms' tumor cell line controls its tumorigenic expression. Science 1987;236:175–180.
77. Yang Y, Gubler MC, Beaufils H. Dysregulation of podocyte phenotype in idiopathic collapsing glomerulopathy and HIV-associated nephropathy. Nephron 2002;91:416–423.
78. Little M, Wells C. A clinical overview of WT1 gene mutations. Hum Mutat 1997;9:209–225.
79. Park S, Schalling M, Bernard A, et al. The Wilms tumour gene WT1 is expressed in murine mesoderm-derived tissues and mutated in a human mesothelioma. Nat Genet 1993;4:415–420.
80. Amin KM, Litzky LA, Smythe WR, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. Am J Pathol 1995;146:344–356.
81. Foster MR, Johnson JE, Olson SJ, Allred DC. Immunohistochemical analysis of nuclear versus cytoplasmic staining of WT1 in malignant mesotheliomas and primary pulmonary adenocarcinomas. Arch Pathol Lab Med 2001;125:1316–1320.
82. Gubler MC. WT1, a multiform protein. Contribution of genetic models to the understanding of its various functions. J Am Soc Nephrol 2002;13: 2192–2194.
83. Kleymenova EV, Yuan X, LaBate ME, Walker CL. Identification of a tumor-specific methylation site in the Wilms tumor suppressor gene. Oncogene 1998;16:713–720.
84. Gazdar AF, Butel JS, Carbone M. SV40 and human tumours: myth, association or causality? Nat Rev Cancer 2002;2:957–964.
85. Bocchetta M, Di Resta I, Powers A, et al. Human mesothelial cells are unusually susceptible to simian virus 40- mediated transformation and asbestos cocarcinogenicity. Proc Natl Acad Sci USA 2000;97:10214–10219.
86. Pipas JM, Levine AJ. Role of T antigen interactions with p53 in tumorigenesis. Semin Cancer Biol 2001;11:23–30.
87. Carbone M, Rizzo P, Grimley PM, et al. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. Nat Med 1997;3:908–912.
88. Foddis R, De Rienzo A, Broccoli D, et al. SV40 infection induces telomerase activity in human mesothelial cells. Oncogene 2002;21:1434–1442.
89. Zanella CL, Timblin CR, Cummins A, et al. Asbestos-induced phosphorylation of epidermal growth factor receptor is linked to c-fos and apoptosis. Am J Physiol 1999;277:L684–693.
90. Cacciotti P, Strizzi L, Vianale G, et al. The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. Am J Respir Cell Mol Biol 2002;26:189–193.
91. Bocchetta M, Miele L, Pass HI, Carbone M. Notch-1 induction, a novel activity of SV40 required for growth of SV40-transformed human mesothelial cells. Oncogene 2003;22:81–89.

9

Angiogenesis and Mesothelioma

Alfonso Catalano, Luigi Strizzi, and Antonio Procopio

Malignant mesothelioma (MM) is a primary tumor of the pleura and peritoneum. Malignant mesotheliomas that are limited to other organs are extremely rare, though several cases of pericardial MM have been reported (1). A unique feature of MM is its strong relationship with asbestos exposure (2,3), which has recently led to great public concern in view of the ubiquitous presence of that mineral. Insulation, construction, shipyard industries, and automobile brakes are among the many sources of occupational exposure (4). Exposure can be not only occupational but also environmental, or even familial by household contamination (5).

The mechanisms of MM pathogenesis have not been fully elucidated. Asbestos fibers could work their way through the lung tissues to damage pleura and produce adhesions and plaques. Changes observed in target tissues exposed to asbestos include hyperplasia, metaplasia, DNA synthesis, and increased production of oxygen free radicals. Activation of diacylglycerol, protein kinase C, and ornithine decarboxylase also has been reported in a pathway similar to classic tumor promoters, such as phorbol esters (6–8). Moreover, crocidolite fibers, which are the major tumorigenic asbestos fibers, induce angiogenesis in the peritoneal lining of MM animal models (9). Thus, ingrowth of new blood vessels around clusters of asbestos fibers may also facilitate the later emergence of MM at these sites.

Angiogenesis plays an important role in the growth, progression, and metastasis of solid tumors (10). Further, quantitative histologic studies have suggested that angiogenesis, as assessed by intratumoral microvascular density (IMD) and total microvascular area (MVA), correlates with poor prognosis in several human neoplasms (11,12). Malignant mesothelioma demonstrated a higher IMD than colon and breast tumors (13–15). This value was significantly and independently related to survival adjusted for other known prognostic variables in MM, such as histologic type, stage, and age (16). This might call for an IMD profile to be provided as part of the pathologic evaluation of tumor specimens from patients with MM.

Currently, many angiogenic factors have been identified to be released from tumor-associated inflammatory cells, extracellular matrix,

or tumor cells, which support and stimulate angiogenesis (17). Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGFs), transforming growth factor-β (TGF-β), hepatocyte growth factor/scatter factor (HGF/SF), insulin-like growth factor-I (IGF-I), and various cytokines like interleukins (IL)-6 and -8 are strong regulators of angiogenesis with a paracrine loop mechanism (18–20). All these factors were identified in MM (21–23), which may affect tumor angiogenesis by positively regulating endothelial cell proliferation, motility, and vascular permeability (17). However, the primary cause of fatality in MM is local invasion of the primary tumor, unlike other solid tumors where metastasis is most commonly seen. Malignant mesothelioma cells show a high proliferation rate as evidenced by a high mitotic count and by methods to demonstrate proliferating cell nuclear antigens and silver-stained nucleolar organizing regions. In addition, MM presents with minimal central necrosis, despite its huge size (24,25). Thus, it was postulated that several growth factors with angiogenic capacity are also additional effects regarding the process of MM carcinogenesis, growth dependency patterns, and long latency.

Role of Vascular Endothelial Growth Factor in Malignant Mesothelioma Cell Growth and Progression

Various autocrine stimulatory effects of angiogenic growth factors such as IGF-I, PDGFs, and VEGF, via their receptors, have been recently postulated in the pathogenesis of MM (26–28). Among these, VEGF seems to play a key role on MM biology, as VEGF binds with high affinity to the tyrosine kinase receptors VEGFR-1 (also known as flt-1) and R-2 (also known as Flk-1/KDR) expressed by endothelial cells (29). The receptors VEGFR-1 and R-2 are also expressed by the tumor cells of Karposi's sarcoma, ovarian and breast cancers, and in choriocarcinoma, melanoma, and ovarian cancer cell lines, suggesting that the role of the VEGF signaling pathway extends beyond angiogenesis in solid tumors (30). In addition, VEGF overexpression has been demonstrated in MM tissues and cell lines (Fig. 9.1) and its protein production was correlated with poor survival (28). Expression of VEGFR-1 and R-2 by malignant cells within MM tumors also has been reported (28).

Human recombinant (rh)-VEGF was used to treat several MM cells to demonstrate that VEGF can stimulate DNA synthesis and cell proliferation. Additionally, monoclonal antibodies that neutralize VEGF retard the induction of DNA synthesis by rh-VEGF in MM cells, supporting the contention that VEGF directly stimulates MM cell growth (28). In this and other studies, VEGF induction of cell proliferation appears to be mediated by VEGFR-2 (28,31). Also, VEGF induces higher levels of VEGFR-2 autophosphorylation in MM cells and initiates a range of cellular responses, including proliferation (32). Although not yet studied, VEGF binding to VEGFR-1 may mediate other responses in MM cells. In monocytes, which express only VEGFR-1, VEGF induces cell migration and the production of tissue factor,

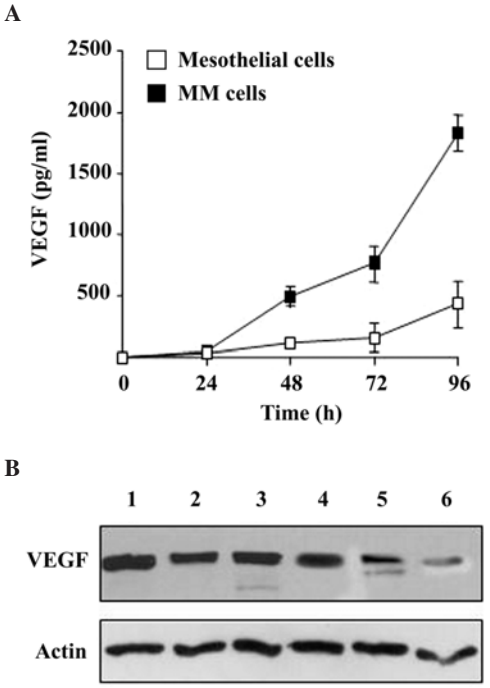


Figure 9.1. *Vascular endothelial growth factor (VEGF) protein expression in mesothelioma and mesothelial cells*; VEGF release was identified by enzyme-linked immunosorbent assay (ELISA) of several cell culture condition (A) and by Western blot (B). A: VEGF production was determined in malignant mesothelioma (MM) and mesothelial supernatants at 24-hour intervals. B: Lane 1, positive control; lane 2–5, several MM cell lines; lane 6, mesothelial cells.

whereas production of matrix metalloproteinases 1, 3, and 9 in smooth muscle cells also may be mediated via the VEGFR-1 receptor (32). Interestingly, VEGFR-1 demonstrates higher affinity ligand binding than R-2 and may act competitively to regulate VEGF-induced mitogenesis (32). Consequently, binding of VEGF to both VEGFR-1 and R-2 expressed by MM cells may have implications for a variety of processes involved in tumor progression, including stimulation of tumor cell proliferation, degradation of extracellular matrix, and tumor cell migration.

Regulation of Vascular Endothelial Growth Factor Expression by Potential Transforming Factors in Malignant Mesothelioma

The mechanism for VEGF upregulation in MM tumors is unknown. Previous studies have shown that p53 represses VEGF transcription by preventing the binding of Sp-1 to the VEGF promoter (33). More recently the involvement of Src kinase activity in p53 inhibition of VEGF transcription has been assessed (34). In addition, p16 and Rb

family members can inhibit VEGF expression (35). Therefore, it is possible that cell cycle regulatory proteins generally acting in the G1 phase of the cell cycle could have similar effects, or that these proteins control distinct pathways with a common end point. We have recently observed that VEGF upregulation is involved in the cell cycle perturbation caused by p53 inactivation in response to simian virus 40 (SV40) infection (36).

Simian virus 40 encodes two transforming proteins, the large-tumor antigen (Tag) and the small-tumor antigen (tag); SV40-Tag, a 90-kd nuclear phosphopolypeptide, is essential for virus growth and sufficient to induce mesothelial cell transformation in the absence of cell lysis (37). Although SV40-Tag displays pleotropic actions on multiple potential mechanisms of cell transformation (38), it has been proposed that it may facilitate cell transformation by binding and inactivating p53 and retinoblastoma (Rb) tumor suppressor proteins (39). The presence of nucleic acids and proteins of SV40 has been observed in most MMs (36). Moreover, SV40 infection represents a negative prognostic cofactor for patients affected by MM (40). This provocative finding is intriguing and its significance is as yet unknown, although it was noted that the early polio vaccines from 1954 until 1960 were contaminated with SV40. More than 50 studies have confirmed that at least 60% of MMs contain and express SV40. These results suggest that SV40 may intervene in the pathogenesis of MM. We found a physiologic relationship between SV40-related proteins and VEGF expression in MM cells. Moreover, since VEGF also acts as a potent autocrine growth factor to MM cells, we antagonized VEGF activity in Tag-expressing MM cells using an adenoviral vector encoding a soluble form of Flt-1 (Ad.sFlt-1). sFlt-1 expression abrogated both Flk-1/KDR phosphorylation and DNA synthesis induced by SV40-Tag in MM cells (Fig. 9.2). These data strongly indicate that VEGF signaling induced by SV40-Tag contributes to cell cycle modulation promoted by SV40.

In addition, the involvement of 5-lipoxygenase (5-LO) activity in induction of VEGF transcription has been also observed; 5-LO is suggested to be involved in the mechanisms of mesothelial cell carcinogenesis (41). Lipoxygenase isoforms are expressed in human mesothelial cells, and a metabolically active 5-LO is selectively upregulated in neoplastic phenotypes of these cells. It also observed that 5-LO inhibition resulted in MM growth arrest and apoptosis. Finally, VEGF release and messenger RNA (mRNA) levels are regulated by 5-LO activity in MM cells, and this regulation is a crucial mechanism of 5-LO actions on proliferation and apoptosis. Since VEGF simultaneously can be induced by both SV40-Tag and 5-LO function to accomplish cellular transformation, its upregulation could represent common molecular strategies for potential transforming factors to regulate proliferation and tumor progression. However, the roles of these molecules in tumorigenesis need to be studied more closely.

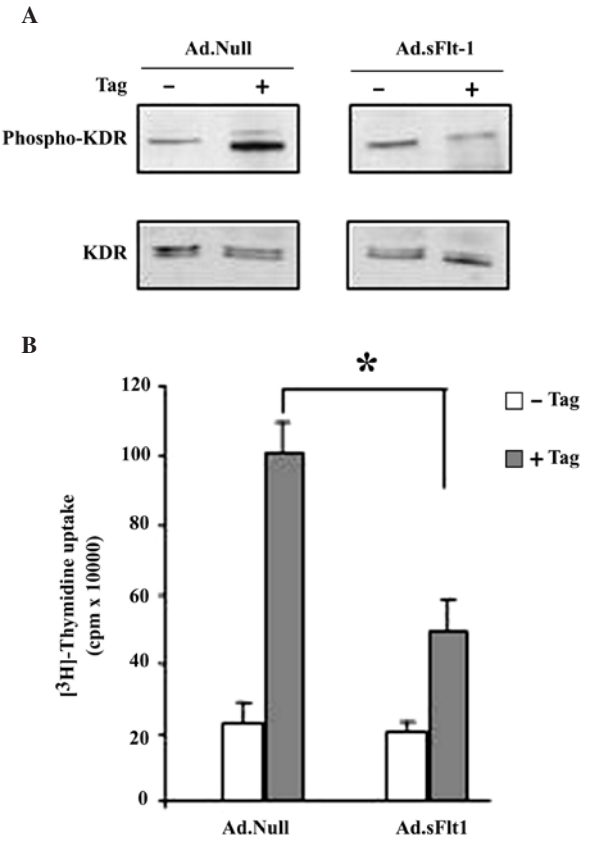


Figure 9.2. *Inhibition of VEGF pathway(s) blocked MM cell growth triggered by simian virus 40 large-tumor antigen (SV40-Tag).* The MM cells were transfected with 4.0 μg of pw2dl (+ Tag) or with pw101 (– Tag), incubated for 24 hours, then infected with Ad.sFlt-1 or Ad.Null (50 p.f.u. × cell) for an additional 48 hours. A: Cell lysates were separated by 7.5% polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. Blots were exposed to antiphosphotyrosine monoclonal antibody for 1 hour at room temperature (upper panels), stripped and reblotted with an anti-flk-1/KDR antibody (lower panels). The bands were then visualized using the ECL Western blotting system. B: The MM cells were seeded into 24-well plates for 12 hours before transfection experiments. At the end of the incubations, [^{3}H]-Thymidine (0.5 μCi/mL) was added for an additional 4 hours. Results are mean ± standard deviation (SD) from $n = 3$ with duplicates (*, $p < .05$ vs. control).

Antiangiogenic Agents as Therapeutic Tools for Malignant Mesothelioma

Given the role of VEGF in MM, fumagillin, endogenous angiostatic substances, such as endostatin and angiostatin, and synthetic angiogenesis inhibitors, such as thalidomide, were used against MM cell growth (42). All these substances inhibit neovascularization and angiogenesis in organ cultures as well as tumor-induced neovascularization in vivo. However, the mechanisms responsible for these effects are dif-

ferent among antiangiogenic drugs. For example, angiostatin induces cell growth inhibition by inhibiting HGF-induced phosphorylation of c-*met*, Akt, and extracellular signal-regulated kinase (ERK)1/2 (43). Only fumagillin seems to inhibit endothelial proliferation through MetAP2 inactivation, which is an enzyme involved in the removal of the N-terminal methionine from proteins and peptides and is an inhibitor of phosphorylation of initiation factor eIF-2-associated 67-kd protein, p67 (44).

We hypothesized a direct inhibitory effect of these molecules on tumor cell proliferation. Our results showed that only the angiostatic agent, fumagillin, at concentrations comparable to those used for endothelial cell inhibition, arrested the growth of MM cells which expressed high levels of MetAP2. In fact, normal mesothelial (NM) cells treated with fumagillin that had poor MetAP2 expression did not show any significant alteration of proliferation (Fig. 9.3). We have also demonstrated that this growth inhibition effect of fumagillin was associated with downregulation of bcl-2 expression and cell death by apoptosis. Interestingly, a decrease of telomerase activity in a time frame required for fumagillin to induce downregulation of bcl-2 expression was observed. Several groups have now reported that overexpression of bcl-2 or bcl-XL confers protection upon mitochondria, making it more difficult for many stimuli to induce pore opening and release of AIF and cytochrome c, inducing apoptosis (45). In our MM cells, MetAP2-positive, stable overexpression of bcl-2 inhibited the reduction of telomerase activity and reverted the induction of apoptosis by fumagillin. Our finding indicates a close relationship among MetAP2, survival factor bcl-2, and telomerase activity in neoplastic cells compared to normal mesothelial cells. It remains to be resolved whether the observed modulation of telomerase activity by fumagillin is mediated via changes in bcl-2 expression, or both bcl-2 expression and telomerase activity are regulated via a fumagillin-responsive common pathway(s). It also remains to be seen in these cells whether the regulation of telomerase activity is a phenomenon restricted to bcl-2, or is a general event associated with other antiapoptotic gene products such as bcl-XL.

Since the activation of telomerase activity and bcl-2 deregulation had been shown to be associated with the development of human cancer (46), our finding of potential involvement of MetAP2 in the deregulation of telomerase activity through a bcl-2–dependent mechanism may provide an important insight into the role of MetAP2 activity during cell growth and also suggest the potentially clinical use of fumagillin, or its derivatives, in therapy for MM. Recently, TNP-470, belonging to the fumagillin family, was shown to inhibit growth factor–induced DNA synthesis of vascular smooth muscle cells and induced apoptosis and senescence in human hepatoma cells (50). These data suggest a broad range of effects of these compounds and involve this parent compound of fumagillin in apoptotic and senescence pathway(s). In our study, fumagillin did not inhibit the production of MM cell growth factors, such as FGF-2 and VEGF. In contrast, it inhibited growth factor-induced DNA synthesis of both MM and NM cells. Recently, growth

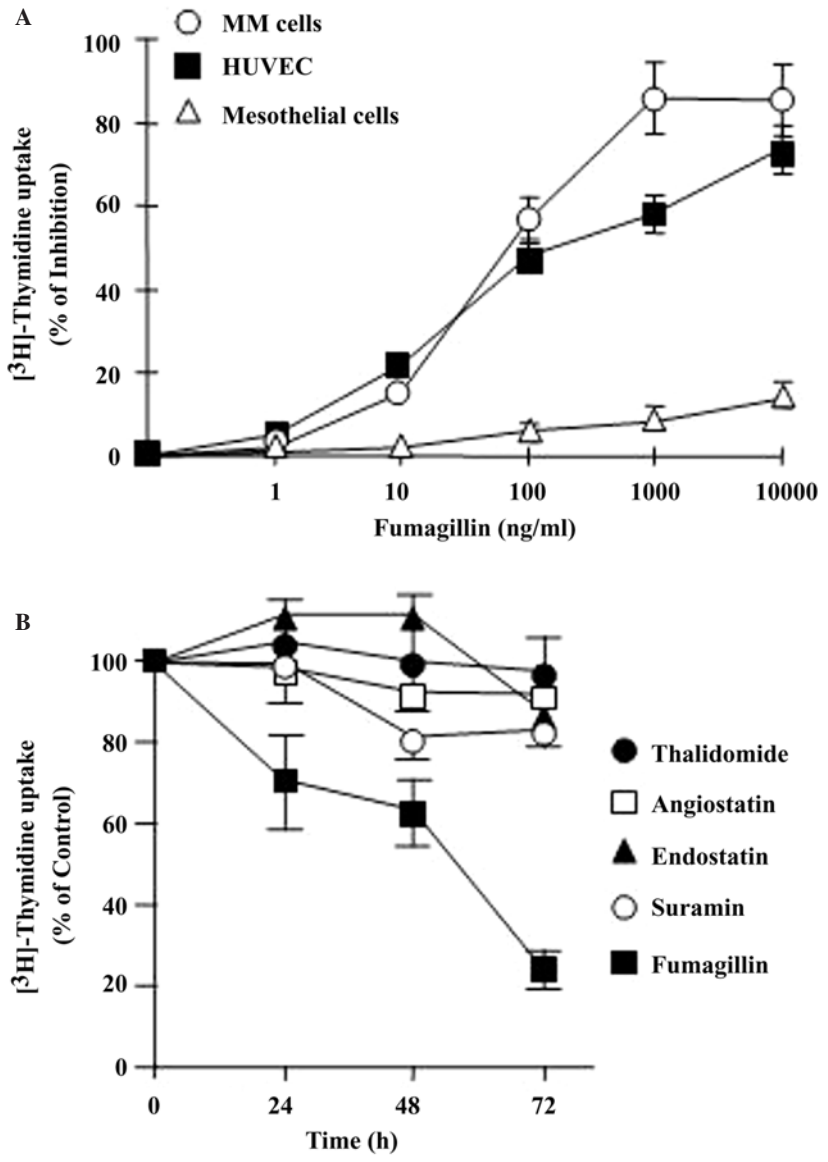


Figure 9.3. *Effect of antiangiogenic agents on cell proliferation in MM and mesothelial cells.* A: MM, HUVEC, or mesothelial cells were treated with varying concentrations of fumagillin for 24 hours. [^{3}H]-thymidine uptake was then determined. Results are means ± SD from $n = 2$ with triplicate measurements. B: MM cells were incubated for different times with fumagillin, thalidomide, angiostatin, endostatin, or suramin. At the end of the incubation, DNA synthesis was assessed by [^{3}H]-thymidine uptake. Data points depict mean values ± SD from two experiments with quadruplicate determinations (*, $p < .05$).

factors, like VEGF, have been shown to increase bcl-2 expression, and it was shown that FGF-2 inhibited cell apoptosis by bcl-2–dependent and –independent mechanisms in endothelial cells (47). In our study, fumagillin antagonized VEGF proliferative effects. Thus, bcl-2 appeared to be one of many potentially downregulated proteins by

fumagillin. In recent years, approaches such as identification of agent(s) that can modulate bcl-2 have become the subject of active investigation to control cancer cell growth. In addition, telomerase has also attracted a great deal of interest as a possible target in cancer biology. The apparent lower levels of measurable telomerase activity in normal mesothelial cells and its detection in human mesothelioma cells have raised the possibility that telomerase may also serve an important target to control the growth of this tumor.

In spite of wide occurrence of deregulation of bcl-2 and telomerase activity in cancer cells, to date, to the best of our knowledge, no close linkage between MetAP2 and these two phenotypes has been reported. Our findings of the modulation of telomerase activity by inhibition of MetAP2 activity via a widely deregulated survival factor, bcl-2, and their implication in an apoptotic pathway, could open new possibilities to develop novel strategies for MM by co-targeting angiogenic and apoptotic pathways.

Acknowledgments

This work was supported by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC) and from the Italian Ministero dell' Università e della Ricerca Scientifica (ex 60%) to A. Procopio. We thank the laboratory staff of the Institute of Experimental Pathology at the University of Ancona. A. Catalano is recipient of a Fondazione Italiana Ricerca sul Cancro (FIRC) fellowship.

References

1. Warren WH. The clinical manifestations and diagnosis of mesothelioma. In: Kittle CF, ed. Mesothelioma: Diagnosis and Management. Chicago: Year Book, 1987:31.
2. Doll R, Peto J. Other asbestos-related neoplasms. In: Antman K, Aisner J, eds. Asbestos-Related Malignancy. Orlando, FA: Grune & Stratton, 1987:81.
3. Elmes PC. The natural history of diffuse mesothelioma. In: Bogovoski P, Gilson JC, Timbrell V, Wagner JC, eds. Biological Effects of Asbestos. Lyon, France: International Agency for Research on Cancer, 1973:267.
4. Nicholson WJ, Perkel G, Selikoff IJ. Occupational exposure to asbestos: population at risk and projected mortality—1980–2030. Am J Indust Med 1982;3:259.
5. Whitwell F, Scott J, Grimshaw M. Relationship between occupations and asbestos-fibre content of the lungs in patients with pleural mesothelioma, lung cancer, and other diseases. Thorax 1977;32:377.
6. Barrett JC, Lamb PW, Wiseman RW. Multiple mechanisms for the carcinogenic effects of asbestos and other mineral fibers. Environ Health Perspect 1989;81:81.
7. Marsh JP, Mossman BT. Mechanisms of induction of ornithine decarboxylase activity in tracheal epithelial cells by asbestiform minerals. Cancer Res 1988;48:709.
8. Mossman BT, Bignon J, Corn M, et al. Asbestos: scientific developments and implications for public policy. Science 1990;247:294.

9. Branchaud RM, MacDonald JL, Kane AB. Induction of angiogenesis by intraperitoneal injection of asbestos fibers. FASEB J 1989;3:1747–1752.
10. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990;82:4–6.
11. Gasparini G, Harris AL. Clinical importance of the determination of tumour angiogenesis in breast carcinoma: much more than a new prognostic tool. J Clin Oncol 1995;13:765–782.
12. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N Engl J Med 1991; 324:1–8. 1996;2:167–168.
13. Gasparini G, Harris AL. Clinical importance of the determination of tumour angiogenesis in breast carcinoma: much more than a new prognostic tool. J Clin Oncol 1995;13:765–782.
14. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N Engl J Med 1991; 324:1–8.
15. Vermeulen PB, Verhoeven D, Fierens H, et al. Microvessel quantification in primary colorectal carcinoma: an immunohistochemical study. Br J Cancer 1995;71:340–343.
16. Kumar-Singh S, Vermeulen PB, Weyler J, et al. Evaluation of tumour angiogenesis as a prognostic marker in malignant mesothelioma. J Pathol 1997;182:211–216.
17. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991;64: 327–336.
18. Friesel RE, Maciag T. Molecular mechanism of angiogenesis: fibroblast growth factor signal transduction. FASEB J 1995;9:919–925.
19. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146:1029–1039.
20. Folkman J. Tumor angiogenesis and tissue factor (comment). Nat Med 1996;2:167–168.
21. Kumar-Singh S, Weyler J, Martin MJ, Vermeulen PB, Van Marck E. Angiogenic cytokines in mesothelioma: a study of VEGF, FGF-1 and -2, and TGF beta expression. J Pathol 1999;189:72–78.
22. Tolnay E, Kuhnen C, Wiethege T, Konig JE, Voss B, Muller KM. Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma. J Cancer Res Clin Oncol 1998;124:291–296.
23. Antony VB, Hott JW, Godbey SW, Holm K. Angiogenesis in mesotheliomas. Role of mesothelial cell derived IL-8. Chest 1996;109:21S–22S.
24. Ramael M, Jacobs W, Weyler J, et al. Proliferation in malignant mesothelioma as determined by mitosis counts and immunoreactivity for proliferating cell nuclear antigen (PCNA). J Pathol 1994;172:247–253.
25. Bethwaite PB, Delahunt B, Holloway LJ, Thornton A. Comparison of silver-staining nucleolar organizer region (AgNOR) counts and proliferating cell nuclear antigen (PCNA) expression in reactive mesothelial hyperplasia and malignant mesothelioma. Pathology 1995;27:1–4.
26. Lee TC, Zhang Y, Aston C, et al. Normal human mesothelial cells and mesothelioma cell lines express insulin-like growth factor I and associated molecules. Cancer Res 1993;53:2858–2864.
27. Langerak AW, De Laat PAJM, Van Der Linden-Van Beurden CAJ, et al. Expression of platelet derived growth factor and receptors in human malignant mesothelioma in vitro and in vivo. J Pathol 1996;178:151–160.

28. Strizzi L, Catalano A, Vianale G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol 2001;193:468–475.
29. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353–364.
30. de Jong JS, van Diest PJ, van der Valk P, Baak JP. Expression of growth factors, growth inhibiting factors, and their receptors in invasive breast cancer. I. An inventory in search of autocrine and paracrine loops. J Pathol 1998;184:44–52.
31. Ferrara N. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol 2001;280:1358–1366.
32. Zhang L, Yu D, Hu M, et al. Wild-type p53 suppresses angiogenesis in human leiomyo sarcoma and synovial sarcoma by transcriptional suppression of vascular endothelial growth factor expression. Cancer Res 2000;60:3655–3661.
33. Pal S, Datta K, Mukhopadhyay D. Central role of p53 on regulation of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) expression in mamary carcinoma. Cancer Res 2001;61:6952–6957.
34. Claudio PP, Stiegler P, Howard CM, et al. RB2/p130 gene-enhanced expression down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in vivo. Cancer Res 2001;61:462–468.
35. Catalano A, Romano M, Martinotti S, Procopio A. Enhanced expression of vascular endothelial growth factor (VEGF) plays a critical role in the tumor progression potential induced by simian virus 40 large T antigen. Oncogene 2002;21:2896–2900.
36. Carbone M, Rizzo P, Grimley PM, et al. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. Nat Med 1997;3:908–912.
37. Bocchetta M, Di Resta I, Powers A, Fresco R, Tosolini A, Testa JR. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. Proc Natl Acad Sci USA 2000;97:10214–10219.
38. DeCaprio JA. Biologicals 1999;27:23–28.
39. Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994;9:1781–1790.
40. Procopio A, Strizzi L, Vianale G, et al. Genes Chromosomes Cancer 2000; 29:173–179.
41. Romano M, Catalano A, Nutini M, et al. 5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. FASEB J 2001;15:2326–2336.
42. Catalano A, Romano M, Robuffo I, Strizzi L, Procopio A. Methionine aminopeptidase-2 regulates human mesothelioma cell survival: role of Bcl-2 expression and telomerase activity. Am J Pathol 2001;159:721–731.
43. Wajih N, Sane DC. Angiostatin selectively inhibits signaling by hepatocyte growth factor in endothelial and smooth muscle cells. Blood (in press).
44. Zamzami N, Brenner C, Marzo I, Susin SA, Kroemer G. Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. Oncogene 1998;16:2265–2282.
45. Liu JP. Studies of the molecular mechanisms in the regulation of telomerase activity. FASEB J 1999;13:2091–2104.
46. Zhang Y, Griffith EC, Sage J, Jacks T, Liu JO. Cell cycle inhibition by the anti-angiogenic agent TNP-470 is mediated by p53 and p21/WAF1/CIP1. Proc Natl Acad Sci USA 2000;97:6427–6431.
47. Dias S, Shmelkov SV, Lam G, Rafii S. VEGF(165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. Blood 2002;99:2532–2540.

10

Immune Status and Mesothelioma

Elliott Kagan

Although there is a considerable body of published literature concerning the putative role of immune status in the pathogenesis and progression of common malignancies such as lung and breast cancers, this area of research previously had been relatively neglected with respect to malignant mesothelioma, a comparatively uncommon tumor. Over the past decade, however, the development of animal mesothelioma models and the widespread availability of mesothelioma cell lines to researchers has focused increasing interest in this area. Furthermore, occupational and environmental asbestos exposure hitherto had been regarded as the most important global causes of mesothelioma and, since inhaled asbestos fibers have been shown to suppress innate cellular immunity, studies of asbestos-exposed individuals and of experimental asbestos exposure have provided valuable insight into how altered immune status may allow mesothelial tumors to escape immune surveillance. It is also conceivable that variability in host immune status, coupled with individual differences in genetic susceptibility to mesothelioma among similarly exposed subjects (1,2), may account for the considerable variation in incidence of mesothelioma in different exposure settings, which can span two orders of magnitude (3–5). Given the now well-recognized association of simian virus 40 (SV40) with malignant mesothelioma (6), opportunities now exist to study the immune status and to develop vaccination protocols of seropositive subjects at risk.

Innate Immunity Against Mesothelioma Cells

Non–major histocompatibility complex (MHC)-restricted cytotoxic lymphocytes have the capacity to lyse tumor cell targets of various origins and comprise natural killer (NK) cells, NK T cells, and γδ T cells (7). All are derived from a common lymphoid precursor but differentiate along separate pathways. Whereas NK cells are $CD56^+$ but lack the CD3 and T-cell receptor markers, NK T cells and γδ T cells coexpress CD3 as well as differing forms of the T-cell receptor. Unlike conventional T cells,

γδ T cells do not express CD4 or CD8. It is of interest that established γδ T-cell clones have been shown to demonstrate varying degrees of cytotoxicity against individual mesothelioma cell lines (8). The NK cells can be stimulated to proliferate ex vivo by the addition of interleukin-2 (IL-2) to produce lymphokine-activated killer (LAK) cells (9), which have been shown to exhibit a broader spectrum of tumoricidal activity than do NK cells (10). Similarly, NK T cells can be greatly expanded ex vivo by the timed addition of interferon-γ (IFN-γ), IL-2, and anti-CD3 to generate cytokine-induced killer (CIK) cells (7). All these cell types are involved in innate immune responses to tumors, but they lack the capacity for immunologic memory.

In cancer-bearing hosts, NK cells have been considered to be the major component of antitumor immunity responsible for rapid elimination of malignant cells, and there is evidence that adoptively transferred LAK cells can selectively localize in solid tumors tissue and eliminate established tumors (11). However, there have been few clinical studies of NK or LAK cell function in mesothelioma patients. In one study, 70% of patients demonstrated significantly depressed NK cell activity, a finding that was improved by the addition of IFN-γ to the patients' peripheral blood lymphocytes in vitro (12). In this regard, it should be noted that NK cells have inhibitory receptors whose ligands are MHC class I molecules (7,13). This could provide one explanation as to why mesothelioma patients have impaired NK cell killing, since mesothelioma cell lines are known to express human leukocyte antigen (HLA) class I molecules constitutively (14,15). In another study, patients demonstrated significant reduction of LAK cell activity against mesothelioma cell targets, a phenomenon that appeared to be due to prostaglandin-induced immunosuppression, since LAK functional activity was restored by the addition of 10 μg/mL of indomethacin to the patients' lymphocytes (16). Nevertheless, LAK cell killing of both freshly explanted mesothelioma cells and established mesothelioma cell lines has been shown to be significantly greater than that exhibited by NK cells (17).

Asbestos Exposure and Innate Immunity

Since asbestos workers are at risk of developing mesothelioma and other cancers, clinical studies of such individuals have provided insight into how tumors might escape immune surveillance in susceptible individuals with disordered immunoregulation. There is substantial evidence that systemic T-cell function is depressed in asbestotic subjects. Several studies have shown that such individuals manifest impaired peripheral blood T cell responsiveness to mitogens, findings that were independent of age or smoking status (18–21). Defective lymphokine generation also has been described in association with asbestosis (22). When delayed-type hypersensitivity responses were assessed in asbestos workers and in unexposed subjects, a disproportionate number of individuals with radiologically detectable asbestosis demonstrated cutaneous anergy to either de novo (2,4-dinitrochlorobenzene) or recall

(tuberculin, streptokinase-streptodornase, or *Candida albicans*) antigens (18,23,24). Moreover, there was a good correlation between the presence of cutaneous anergy and the duration of asbestos exposure. Likely explanations for these findings include the presence of circulating "inhibitors" of lymphoid cell activation (18,25) and the selective loss of circulating T-suppressor (T_S) cells that has been demonstrated in patients with asbestosis (20). The fact that chrysotile asbestos fibers can induce the temporary downregulation of cell surface CD4 and CD45RA expression in cultured human peripheral blood mononuclear cells (26) provides additional support for the latter notion, since $CD4^+$ $CD45RA^+$ T cells have been shown to induce the activation of $CD8^+$ T_S cells (27). Whatever mechanisms are involved, the impairment of innate immunity that has been detected in asbestos workers may be a consequence of the translocation of inhaled asbestos fibers to lymph nodes and to the spleen (28–30).

The use of flow cytometry has demonstrated notable differences in the proportions of T-cell subsets in the blood and bronchoalveolar lavage (BAL) of asbestos-exposed subjects when compared with those in unexposed individuals. Peripheral lymphopenia has been well described in association with asbestosis (21,31–33), and in one study the intensity of the lymphopenia correlated with the radiographic severity of asbestosis (33). The number of circulating T cells (as defined by CD3 and other pan–T-cell markers) also has been shown to be significantly reduced in asbestotic subjects compared with that in unexposed controls (21,31–35). Analysis of T-cell subsets in the peripheral blood generally has demonstrated a reduction in the proportions of T cells expressing either the helper/inducer ($CD4^+$) or suppressor/cytotoxic ($CD8^+$) phenotypes. A correlation also has been shown between the intensity of asbestos exposure and a decreased blood $CD8^+/CD4^+$ ratio when corrections were made for the confounding effects of smoking (33,34).

Several studies have analyzed the composition of BAL lymphoid cell populations in asbestos-exposed individuals (32,35–37). A notable finding in most of these studies has been a lymphocytic alveolitis, characterized by increased percentages and numbers of BAL lymphocytes and $CD4^+$ cells with reductions in the proportions of $CD8^+$ cells. These changes were most likely to occur in asbestos-exposed subjects with radiographic stigmata of asbestosis or asbestos-related pleural injury.

Asbestos exposure also is known to influence NK function. Asbestos fibers were shown to impair peripheral blood and BAL NK cell activity in vitro, an effect that was induced by all commercial types of asbestos and that was prevented by pretreatment of the lymphoid cells with recombinant interleukin-2 (rIL-2) (38). The same group subsequently showed that chrysotile (but not amphibole) fibers also significantly suppressed the in vitro activity of LAK cells from normal subjects (39). Although one study has demonstrated normal NK functional activity in asbestos workers (38), others have shown that NK function declines with increasing intensity or duration of asbestos exposure (40–42). Immune abnormalities that are detectable in asbestos workers are summarized in Table 10.1.

Table 10.1. Effects of asbestos on T-cell, natural killer (NK)-cell, and lymphokine-activated killer (LAK)-cell function

Cell type	Functional effect	References
T cells	Peripheral lymphopenia	21,31–33
	Decreased circulating T-cell numbers	21,31–35
	Loss of suppressor T cells	20
	Downregulated $CD4^+$ and $CD45RA^+$ expression	26
	Impaired mitogen responsiveness	18–21
	Impaired lymphokine production	22
	Cutaneous anergy to recall and de novo antigens	18,23,24
	Inhibitors of lymphoid activation	18,25
	Decreased blood $CD8^+/CD4^+$ ratio	33,34
	Bronchoalveolar lavage (BAL) lymphocytic alveolitis	32,35–37
	Decreased BAL $CD8^+/CD4^+$ ratio	32,36,37
NK cells	Impaired blood and/or BAL NK activity	38,40–42
LAK cells	Impaired LAK cell activity	39

Tumor-Infiltrating Lymphocytes (TIL) in Mesothelioma

Lymphocytic infiltrates are not usually prominent within the stroma of mesothelioma biopsies and there are few reports of their possible significance. An early South African study of 58 cases noted that 94% of those having a minimal or absent lymphocytic reaction had a mean survival of 9 months after diagnosis, whereas two thirds of those having a significant lymphocytic response survived longer than 18 months after diagnosis (43). The authors concluded that the presence of a significant lymphocytic infiltration was indicative of a better prognosis. A recent British study of only 15 mesothelioma cases has, however, disputed this assertion (44). Nevertheless, the earlier authors' conclusions are supported by an anecdotal report of transient, spontaneous regression of a pleural mesothelioma characterized by a prominent lymphocytic infiltrate in association with serologic reactivity against autologous mesothelioma antigens, findings that essentially disappeared when the patient eventually succumbed to her tumor (45).

Adaptive Immunity Against Mesothelioma

There is evidence that mesotheliomas sometimes can be immunogenic and that affected patients are capable of mounting specific serologic responses to their tumors. In one study of 29 mesothelioma patients, 28% manifested high-titer immunoglobulin G (IgG) antibodies detectable by Western blot analysis against a variety of different antigens, some of them nuclear, expressed by a range of tumor cells of differing lineage (46). Moreover, antibody titer also increased in tandem with disease progression. In contrast, normal sera displayed no such reactivity. In a follow-up study, the same group identified six patient-specific nuclear antigens using the technique of serologic identification by recombinant expression cloning (SEREX) and pooled patients' sera as the probe against an expressed complementary DNA (cDNA) library

derived from a cloned mesothelioma cell line (47). However, none of the antigens detected were uniquely expressed in mesothelioma cells, and it is not clear whether the patients' serologic responses necessarily conferred immune protection against their tumors.

Of central importance is the ability of cancer patients to mount an effective cell-mediated immune response against their tumors by generating effector $CD8^+$ cytotoxic T lymphocytes (CTL) against tumor-associated antigens. Efficient activation of CTL requires tumor-associated peptides to be presented in the context of membrane-bound MHC class I complexes, the presence of membrane co-stimulatory molecules such as CD80 and CD86 on antigen-presenting cells, the induction of IL-2 and other cytokines by $CD4^+$ helper T cells, and the differentiation of recruited $CD8^+$ T cells into CTL (48). As is the case with many other cancers, however, the milieu is not conducive for efficient activation of CTL in patients with mesothelioma, allowing the tumor to evade the host's adaptive response. There are a number of possible explanations for this state. These include altered MHC class I phenotypes on tumor cells (49), inadequate expression of co-stimulatory molecules (50,51), insufficient numbers of tumor-specific $CD4^+$ helper T cells that may be needed to synergize with CTL (52), secretion of transforming growth factor-β (TGF-β), generation of reactive nitrogen species (RNS), upregulated tumor cyclooxygenase-2 (COX-2) expression and prostaglandin E_2 secretion (53,54), and the generalized immunosuppression associated with advanced cancer (48).

A daunting challenge to circumvent the possibility of tumor escape in individuals at risk of developing mesothelioma (e.g., asbestos workers or SV40-exposed subjects) would be to vaccinate them against putative mesothelioma-associated antigens in order to generate CTL in immunologically uncompromised hosts. One study took a step in that direction by identifying seven candidate peptides within the Tag protein domain required for SV40 transformation that could bind and stabilize HLA-A*0201 molecules, the most widely expressed human MHC allelic product (55). Two of those peptides were shown to be immunogenic because they induced SV40 large-tumor antigen (Tag)-specific CTL from healthy peripheral blood lymphocytes and one was found to be endogenously processed by an SV40-transformed human mesothelial cell line. Recently, other investigators have generated CTL from the peripheral blood of one of three $HLA\text{-}A2^+$ mesothelioma patients using one of the same candidate peptides used in the earlier study (56). Activity of CTL was shown to be directed against an $HLA\text{-}A2.1^+$ mesothelioma cell line transfected with SV40 Tag cDNA in an MHC class I–restricted manner.

Oxidant-Mediated Immunosuppression and Mesothelioma

A number of studies have shown that inhaled asbestos fibers can induce the formation of reactive oxygen and nitrogen species in the lungs and pleura. Iron-rich crocidolite asbestos fibers have been shown to gener-

ate hydroxyl radical (·OH) formation via superoxide ($\cdot O_2^-$)-driven, iron-catalyzed Haber-Weiss (Fenton) reactions, which have been implicated in asbestos-induced injury, as evidenced by catalase-mediated inhibition of asbestos-induced mesothelial cell apoptosis (57) and lung inflammation and fibrogenesis (58). The use of a rat asbestos inhalation model also has demonstrated that both crocidolite and chrysotile asbestos fibers stimulate the formation of reactive nitrogen species in vivo as a consequence of persistent pleuropulmonary inflammation, macrophage recruitment, and cytokine secretion (59–61). Notably, inhaled asbestos fibers induced upregulated inducible nitric oxide synthase (iNOS) expression and nitric oxide radical (·NO) production by pleural macrophages (59,60) as well as nitrotyrosine formation in visceral and parietal pleural mesothelial cells (60). Nitrotyrosine is a surrogate marker for peroxynitrite ($ONOO^-$), a highly reactive oxidizing and nitrating species that has been shown to activate the extracellular signal-regulated kinase (ERK) signaling pathway by targeting the epidermal growth factor (EGF) receptor, Raf-1 and MEK independently (62). In this regard, it is significant that crocidolite and chrysotile exposure recently were shown to induce protracted in vivo ERK activation in association with tyrosine nitration in the rat lung (61). Since ERK activation can induce phosphorylation and stimulate the DNA-binding activity of c-Fos, Fra-1, and other activator protein-1 (AP-1) transcription factors, it is conceivable that prolonged induction of targeted AP-1 family members may lead to deregulation of cell proliferation and differentiation that may play a role in asbestos-mediated oncogenesis (63). Support for this notion is provided by the recent demonstration that inhibition of MEK1 in the ERK signaling pathway by the inhibitor PD98059 resulted in reversion of the transformed phenotype in rat mesothelioma cell lines (64).

Several studies indicate that the generation of RNS can induce or potentiate an immunosuppressive state by promoting apoptosis in T cells. Murine models of trypanosomiasis (65) and histoplasmosis (66) are associated with immunosuppression of antigen-specific lymphoproliferative responses and elevated levels of apoptosis in splenocytes, findings that are significantly reversible by treatment of infected mice with N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of ·NO production. Moreover, the in vitro addition of L-NMMA to peripheral blood mononuclear cells from AIDS patients reduced lymphocyte apoptosis and facilitated recovery of lymphoproliferative responses, whereas co-incubation of the patients' lymphocytes in vitro with ·NO donors significantly increased the severity of apoptosis (67). The addition of $ONOO^-$ to normal human peripheral blood T cells also has been shown to suppress mitogen- and CD3-mediated lymphocyte activation and proliferation by promoting impaired tyrosine phosphorylation and apoptosis (68). It is noteworthy that overexpression of iNOS has been detected in 74% to 100% of mesothelioma biopsies but rarely or not at all in nonmalignant or nonreactive pleural tissues (54,69). Moreover, culture supernatants of cytokine-treated human colon carcinoma cells that contained high levels of ·NO significantly suppressed human lymphocyte mitogen-induced proliferation (70).

Transforming Growth Factor-β–Mediated Immunosuppression and Mesothelioma

Transforming growth factor-β (TGF-β) is a pleiotropic cytokine that exists in three isoforms, TGF-β_1, TGF-β_2, and TGF-β_3, and, when secreted, exists predominantly in a latent form bound to a latency-binding peptide. When activated by protease action, TGF-β mediates its effector functions via its cognate receptors (TβR-I and TβR-II) and the Smad protein signaling pathway (71). Tumor-specific mutations of TβR-I and especially of TβR-II have been described in several different types of malignancies (72) as have mutations of smad2 (73) and smad4 (74). Transforming growth factor-β appears to have a biphasic role in tumorigenesis: in the early phases, it acts as a tumor suppressor, whereas in the later stages, when tumor cells have escaped its antiproliferative effects and start to secrete high amounts of TGF-β, this cytokine may act to promote tumor invasion and metastasis (75). Noteworthy in this respect is the fact that both human and murine mesothelioma cells, as well as cell lines derived from human, murine, and rat mesotheliomas, have been shown to express and secrete TGF-β (76–80), while antisense oligonucleotides targeting TGF-β messenger RNA (mRNA) inhibited tumor cell proliferation (78) and anchorage-independent growth (77). The importance of secreted TGF-β within the local milieu of mesothelioma patients is underscored by the finding of TGF-β_1 and TGF-β_2 levels in pleural effusions caused by mesotheliomas that were three to six times as high as those detected in effusions caused by primary lung cancers (81). Genetic factors can influence the effects of TGF-β–mediated signaling. Thus, in one study, *TβR-I*(*6A*), a polymorphic allele of *TβR-I*, was identified as a tumor susceptibility allele in cancer patients (82), whereas another study demonstrated that the 129J mouse strain was uniquely resistant to the fibrogenic effects of asbestos and manifested a delayed response to the fibroproliferative effects of TGF-β_1 (83).

Immunosuppressive effects of TGF-β on innate and adaptive immunity have been noted in a number of model systems that have shown that this cytokine can suppress lymphocyte activation, proliferation, and function both in vitro and in vivo (84). Also, TGF-β has been shown to block LAK activity of human lymphocytes in a dose-dependent fashion (85). Conversely, splenic LAK cell activity from mice injected with a human glioma cell line was greatly enhanced when the glioma cells were transfected with antisense TGF-β_1 prior to being injected (86). In another study, cloned NK T cells established from TIL in a B16 melanoma that were shown to secrete TGF-β were able to inhibit in vivo antitumor responses (87). Furthermore, TGF-β–transduced dendritic cells in C57BL10 ($H2b^+$) mice showed marked impairment in allostimulatory activity of C3H/HeJ T cell ($H2k^+$) T cells in vitro and were able to prolong heart allografts' survival in vivo (88). Importantly, TGF-β also can inhibit the generation of $CD8^+$ CTL both in vitro (89) and in vivo (90), and T-cell–specific blockade of TβR-II–mediated signaling has allowed mice to effectively overcome live tumor challenge via generation of a tumor-specific immune response (91).

Asbestos-exposed individuals may especially be prone to the immunomodulatory effects of TGF-β in the pleural microenvironment. All three TGF-β isoforms have been detected in both lung parenchymal and pleural fibrotic lesions in lung sections from Canadian asbestos miners and millers (92) and in the evolving lung lesions of rats exposed to chrysotile asbestos by inhalation (93). Since pleural plaques can coexist with mesothelioma (94), these could provide a ready source of TGF-β production in some patients that might facilitate the progression of their tumors. It is also possible that protracted asbestos-induced TGF-β secretion in the lungs may diffuse across to the pleural space in a manner analogous to that seen when rats transduced intratracheally with an adenovector overexpressing the active form of TGF-β_1 were shown to develop significant pleural fibrosis (95).

Conclusion

Although malignant mesothelioma may not represent a classic immunogenic tumor, there is abundant evidence suggesting that immune status may play an ancillary role in determining host susceptibility to the development and progression of mesothelioma. As illustrated in Figure 10.1, it is conceivable that cytokine-driven, persistent pleural inflammation and macrophage recruitment, in association with locally generated reactive oxygen and nitrogen species and TGF-β secretion, may provide a favorable milieu for the development and progression of mesothelioma in genetically susceptible subjects who were occupationally or environmentally exposed to asbestos. Similar considerations possibly may pertain to erionite-exposed persons who developed mesothelioma in the Cappadocian region of Turkey (2,96).

Attempts to boost the antitumor immune response in mesothelioma patients have served as the basis for several immunotherapeutic clinical trials, which generally have had disappointing results (97). These have included intrapleural or intratumoral instillation of IL-2, IFN-γ, or granulocyte macrophage colony-stimulating factor (98–102), intrapleural infusion of activated macrophages and IFN-γ (103), and intrapleural gene therapy approaches using replication-deficient adenovirus-mediated delivery of herpes simplex virus–thymidine kinase (Ad.HSV-tk) to transduce the patients' mesothelioma cells (104,105). Some of the limitations in these approaches have been in the selection of patients with advanced tumor stage or in whom there was inadequate debulking of tumor prior to commencing immunotherapy.

Acknowledgments

This work was supported by grant HL-54196 from the National Institutes of Health and grant MDA905-01-1-0001 from the U.S. Department of Defense.

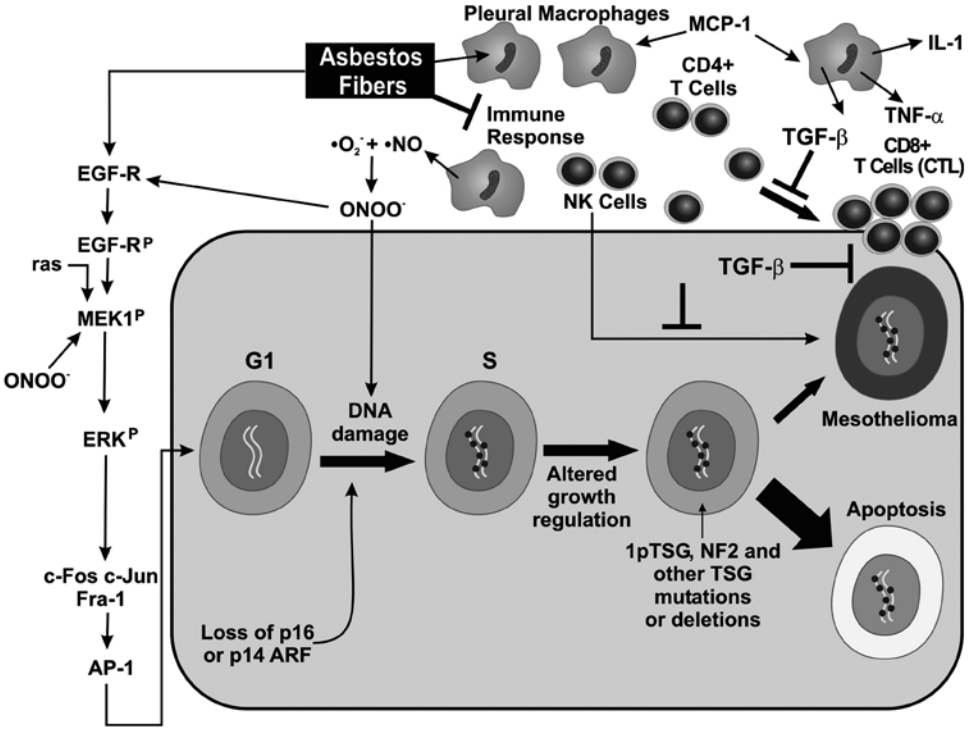


Figure 10.1. Hypothetical schema illustrating the role of cytokine-mediated pleural inflammation, reactive nitrogen species and transforming growth factor-β (TGF-β) in the pathogenesis of asbestos-induced malignant mesothelioma. Rapid accumulation of successive mutations or deletions of targeted tumor suppressor genes (TSG) may favor a pathway of mesothelial cellular proliferation and transformation rather than apoptosis. Crossed bars indicate an inhibitory effect. [Adapted from Rizzo et al (106).]

References

1. Musti M, Cavone D, Aalto Y, Scattone A, Serio G, Knuutila S. A cluster of familial malignant mesothelioma with del(9p) as the sole chromosomal anomaly. Cancer Genet Cytogenet 2002;138:73–76.
2. Roushdy-Hammady I, Siegel J, Emri S, Testa JR, Carbone M. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 2001;357:444–445.
3. Robinson CF, Petersen M, Palu S. Mortality patterns among electrical workers employed in the U.S. construction industry, 1982–1987. Am J Ind Med 1999;36:630–637.
4. Sluis-Cremer GK, Liddell FD, Logan WP, Bezuidenhout BN. The mortality of amphibole miners in South Africa, 1946–80. Br J Ind Med 1992;49: 566–575.
5. Selikoff IJ, Seidman H. Asbestos-associated deaths among insulation workers in the United States and Canada, 1967–1987. Ann NY Acad Sci 1991;643:1–14.
6. Gazdar AF, Butel JS, Carbone M. SV40 and human tumors: myth, association or causality? Nature Cancer Rev (in press).
7. Lowdell MW, Lamb L, Hoyle C, Velardi A, Prentice HG. Non-MHC-restricted cytotoxic cells: their roles in the control and treatment of leukaemias. Br J Haematol 2001;114:11–24.

8. Mavaddat N, Robinson BW, Rose AH, Manning LS, Garlepp MJ. An analysis of the relationship between gd T cell receptor V gene usage and non-major histocompatibility complex-restricted cytotoxicity. Immunol Cell Biol 1993;71(part 1):27–37.
9. Sussman JJ, Shu S, Sondak VK, Chang AE. Activation of T lymphocytes for the adoptive immunotherapy of cancer. Ann Surg Oncol 1994;1:296–306.
10. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med 1982;155:1823–1841.
11. Basse PH, Whiteside TL, Herberman RB. Cancer immunotherapy with interleukin-2-activated natural killer cells. Mol Biotechnol 2002;21: 161–170.
12. Lew F, Tsang P, Holland JF, Warner N, Selikoff IJ, Bekesi JG. High frequency of immune dysfunctions in asbestos workers and in patients with malignant mesothelioma. J Clin Immunol 1986;6:225–233.
13. Lanier LL. NK cell receptors. Annu Rev Immunol 1998;16:359–393.
14. Christmas TI, Manning LS, Davis MR, Robinson BW, Garlepp MJ. HLA antigen expression and malignant mesothelioma. Am J Respir Cell Mol Biol 1991;5:213–220.
15. Orengo AM, Spoletini L, Procopio A, et al. Establishment of four new mesothelioma cell lines: characterization by ultrastructural and immunophenotypic analysis. Eur Respir J 1999;13:527–534.
16. Manning LS, Bowman RV, Davis MR, Musk AW, Robinson BW. Indomethacin augments lymphokine-activated killer cell generation by patients with malignant mesothelioma. Clin Immunol Immunopathol 1989;53:68–77.
17. Manning LS, Bowman RV, Darby SB, Robinson BW. Lysis of human malignant mesothelioma cells by natural killer (NK) and lymphokine-activated killer (LAK) cells. Am Rev Respir Dis 1989;139:1369–1374.
18. Kagan E, Solomon A, Cochrane JC, et al. Immunological studies of patients with asbestosis. I. Studies of cell-mediated immunity. Clin Exp Immunol 1977;28:261–267.
19. Haslam PL, Lukoszek A, Merchant JA, Turner-Warwick M. Lymphocyte responses to phytohaemagglutinin in patients with asbestosis and pleural mesothelioma. Clin Exp Immunol 1978;31:178–188.
20. Gaumer HR, Doll NJ, Kaimal J, Schuyler M, Salvaggio JE. Diminished suppressor cell function in patients with asbestosis. Clin Exp Immunol 1981;44:108–116.
21. deShazo RD, Nordberg J, Baser Y, Bozelka B, Weill H, Salvaggio J. Analysis of depressed cell-mediated immunity in asbestos workers. J Allergy Clin Immunol 1983;71:418–424.
22. Lange A, Smolik R, Chmielarczyk W, Garncarek D, Gielgier Z. Cellular immunity in asbestosis. Arch Immunol Ther Exp (Warsz) 1978;26:899–903.
23. Pierce R, Turner-Warwick M. Skin tests with tuberculin (PPD) *Candida albicans* and *Trichophyton spp.* in cryptogenic fibrosing alveolitis and asbestos related lung disease. Clin Allergy 1980;10:229–237.
24. Lange A, Garncarek D, Tomeczko J, Ciechanowski G, Bisikiewicz R. Outcome of asbestos exposure (lung fibrosis and antinuclear antibodies) with respect to skin reactivity: an 8-year longitudinal study. Environ Res 1986; 41:1–13.
25. Rola-Pleszczynski M, Lemaire I, Sirois P, Masse S, Begin R. Asbestos related changes in pulmonary and systemic immune responses—early enhancement followed by inhibition. Clin Exp Immunol 1982;49:426–432.

26. Kinugawa K, Ueki A, Yamaguchi M, et al. Activation of human CD4+CD45RA+ T cells by chrysotile asbestos in vitro. Cancer Lett 1992;66:99–106.
27. Yamashita N, Clement LT. Phenotypic characterization of the post-thymic differentiation of human alloantigen-specific CD8+ cytotoxic T lymphocytes. J Immunol 1989;143:1518–1523.
28. Auerbach O, Conston AS, Garfinkel L, Parks VR, Kaslow HD, Hammond EC. Presence of asbestos bodies in organs other than the lung. Chest 1980;77:133–137.
29. Dodson RF, Williams MG Jr, Corn CJ, Brollo A, Bianchi C. Asbestos content of lung tissue, lymph nodes, and pleural plaques from former shipyard workers. Am Rev Respir Dis 1990;142:843–847.
30. Roggli VL, Benning TL. Asbestos bodies in pulmonary hilar lymph nodes. Mod Pathol 1990;3:513–517.
31. Kagan E, Solomon A, Cochrane JC, Kuba P, Rocks PH, Webster I. Immunological studies of patients with asbestosis. II. Studies of circulating lymphoid cell numbers and humoral immunity. Clin Exp Immunol 1977;28: 268–275.
32. Costabel U, Bross KJ, Huck E, Guzman J, Matthys H. Lung and blood lymphocyte subsets in asbestosis and in mixed dust pneumoconiosis. Chest 1987;91:110–112.
33. Peng L, Wang X. Lymphocyte B and T cell subsets in peripheral blood from patients with asbestosis. Br J Ind Med 1993;50:183–184.
34. Miller LG, Sparrow D, Ginns LC. Asbestos exposure correlates with alterations in circulating T cell subsets. Clin Exp Immunol 1983;51:110–116.
35. Sprince NL, Oliver LC, McLoud TC, Eisen EA, Christiani DC, Ginns LC. Asbestos exposure and asbestos-related pleural and parenchymal disease. Associations with immune imbalance. Am Rev Respir Dis 1991;143:822–828.
36. Wallace JM, Oishi JS, Barbers RG, Batra P, Aberle DR. Bronchoalveolar lavage cell and lymphocyte phenotype profiles in healthy asbestos-exposed shipyard workers. Am Rev Respir Dis 1989;139:33–38.
37. Rom WN, Travis WD. Lymphocyte-macrophage alveolitis in nonsmoking individuals occupationally exposed to asbestos. Chest 1992;101:779–786.
38. Robinson BW. Asbestos and cancer: human natural killer cell activity is suppressed by asbestos fibers but can be restored by recombinant interleukin-2. Am Rev Respir Dis 1989;139:897–901.
39. Manning LS, Davis MR, Robinson BW. Asbestos fibres inhibit the in vitro activity of lymphokine-activated killer (LAK) cells from healthy individuals and patients with malignant mesothelioma. Clin Exp Immunol 1991; 83:85–91.
40. Yoneda T, Kitamura H, Narita N, Mikami R, Yokoyama K. NK cell activity in asbestosis. Eur J Respir Dis 1986;68:64–67.
41. deShazo RD, Morgan J, Bozelka B, Chapman Y. Natural killer cell activity in asbestos workers. Interactive effects of smoking and asbestos exposure. Chest 1988;94:482–485.
42. Froom P, Lahat N, Kristal-Boneh E, Cohen C, Lerman Y, Ribak J. Circulating natural killer cells in retired asbestos cement workers. J Occup Environ Med 2000;42:19–24.
43. Leigh RA, Webster I. Lymphocytic infiltration of pleural mesothelioma and its significance for survival. S Afr Med J 1982;61:1007–1009.
44. Mudhar HS, Fisher PM, Wallace WA. No relationship between tumour infiltrating lymphocytes and overall survival is seen in malignant mesothelioma of the pleura. Eur J Surg Oncol 2002;28:564–565.

45. Robinson BW, Robinson C, Lake RA. Localised spontaneous regression in mesothelioma—possible immunological mechanism. Lung Cancer 2001; 32:197–201.
46. Robinson C, Robinson BW, Lake RA. Sera from patients with malignant mesothelioma can contain autoantibodies. Lung Cancer 1998;20:175–184.
47. Robinson C, Callow M, Stevenson S, Scott B, Robinson BW, Lake RA. Serologic responses in patients with malignant mesothelioma: evidence for both public and private specificities. Am J Respir Cell Mol Biol 2000;22:550–556.
48. Foss FM. Immunologic mechanisms of antitumor activity. Semin Oncol 2002;29:5–11.
49. Ruiz-Cabello F, Cabrera T, Lopez-Nevot MA, Garrido F. Impaired surface antigen presentation in tumors: implications for T cell-based immunotherapy. Semin Cancer Biol 2002;12:15–24.
50. Leong C, Marley J, Loh S, Robinson B, Garlepp M. Induction and maintenance of T-cell response to a nonimmunogenic murine mesothelioma cell line requires expression of B7-1 and the capacity to upregulate class II major histocompatibility complex expression. Cancer Gene Ther 1996;3:321–330.
51. Leong CC, Marley JV, Loh S, Milech N, Robinson BW, Garlepp MJ. Transfection of the gene for B7-1 but not B7-2 can induce immunity to murine malignant mesothelioma. Int J Cancer 1997;71:476–482.
52. Marzo AL, Lake RA, Robinson BW, Scott B. T-cell receptor transgenic analysis of tumor-specific CD8 and CD4 responses in the eradication of solid tumors. Cancer Res 1999;59:1071–1079.
53. Edwards JG, Faux SP, Plummer SM, et al. Cyclooxygenase-2 expression is a novel prognostic factor in malignant mesothelioma. Clin Cancer Res 2002;8:1857–1862.
54. Marrogi A, Pass HI, Khan M, Metheny-Barlow LJ, Harris CC, Gerwin BI. Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2): in vitro antiproliferative effects of a COX-2 inhibitor. Cancer Res 2000;60:3696–3700.
55. Velders MP, Macedo MF, Provenzano M, et al. Human T cell responses to endogenously presented HLA-A*0201 restricted peptides of simian virus 40 large T antigen. J Cell Biochem 2001;82:155–162.
56. Bright RK, Kimchi ET, Shearer MH, Kennedy RC, Pass HI. SV40 Tag-specific cytotoxic T lymphocytes generated from the peripheral blood of malignant pleural mesothelioma patients. Cancer Immunol Immunother 2002;50:682–690.
57. Broaddus VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. J Clin Invest 1996;98:2050–2059.
58. Mossman BT, Marsh JP, Sesko A, et al. Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene glycol-conjugated catalase in a rapid inhalation model of asbestosis. Am Rev Respir Dis 1990;141:1266–1271.
59. Choe N, Tanaka S, Xia W, Hemenway DR, Roggli VL, Kagan E. Pleural macrophage recruitment and activation in asbestos-induced pleural injury. Environ Health Perspect 1997;105(suppl 5):1257–1260.
60. Tanaka S, Choe N, Hemenway DR, Zhu S, Matalon S, Kagan E. Asbestos inhalation induces reactive nitrogen species and nitrotyrosine formation in the lungs and pleura of the rat. J Clin Invest 1998;102:445–454.
61. Iwagaki A, Choe N, Li Y, Hemenway DR, Kagan E. Asbestos inhalation induces tyrosine nitration associated with extracellular signal-regulated

kinase 1/2 activation in the rat lung. Am J Respir Cell Mol Biol 2003; 28:51–60.
62. Zhang P, Wang YZ, Kagan E, Bonner JC. Peroxynitrite targets the epidermal growth factor receptor, Raf-1, and MEK independently to activate MAPK. J Biol Chem 2000;275:22479–22486.
63. Reddy SP, Mossman BT. Role and regulation of activator protein-1 in toxicant-induced responses of the lung. Am J Physiol 2002;283:L1161–L1178.
64. Ramos-Nino ME, Timblin CR, Mossman BT. Mesothelial cell transformation requires increased AP-1 binding activity and ERK-dependent Fra-1 expression. Cancer Res 2002;62:6065–6069.
65. Martins GA, Cardoso MA, Aliberti JC, Silva JS. Nitric oxide-induced apoptotic cell death in the acute phase of *Trypanosoma cruzi* infection in mice. Immunol Lett 1998;63:113–120.
66. Wu-Hsieh BA, Chen W, Lee HJ. Nitric oxide synthase expression in macrophages of *Histoplasma capsulatum*-infected mice is associated with splenocyte apoptosis and unresponsiveness. Infect Immun 1998;66:5520–5526.
67. Mossalayi MD, Becherel PA, Debre P. Critical role of nitric oxide during the apoptosis of peripheral blood leukocytes from patients with AIDS. Mol Med 1999;5:812–819.
68. Brito C, Naviliat M, Tiscornia AC, et al. Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxynitrite-driven apoptotic death. J Immunol 1999;162:3356–3366.
69. Soini Y, Kahlos K, Puhakka A, et al. Expression of inducible nitric oxide synthase in healthy pleura and in malignant mesothelioma. Br J Cancer 2000;83:880–886.
70. Kojima M, Morisaki T, Tsukahara Y, et al. Nitric oxide synthase expression and nitric oxide production in human colon carcinoma tissue. J Surg Oncol 1999;70:222–229.
71. Dennler S, Goumans MJ, ten Dijke P. Transforming growth factor b signal transduction. J Leukoc Biol 2002;71:731–740.
72. Pasche B. Role of transforming growth factor beta in cancer. J Cell Physiol 2001;186:153–168.
73. Eppert K, Scherer SW, Ozcelik H, et al. MADR2 maps to 18q21 and encodes a TGFb-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 1996;86:543–552.
74. Hahn SA, Schutte M, Hoque AT, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 1996;271:350–353.
75. de Caestecker MP, Piek E, Roberts AB. Role of transforming growth factor-b signaling in cancer. J Natl Cancer Inst 2000;92:1388–1402.
76. Bielefeldt-Ohmann H, Fitzpatrick DR, Marzo AL, et al. Patho- and immunobiology of malignant mesothelioma: characterisation of tumour infiltrating leucocytes and cytokine production in a murine model. Cancer Immunol Immunother 1994;39:347–359.
77. Fitzpatrick DR, Bielefeldt-Ohmann H, Himbeck RP, Jarnicki AG, Marzo AL, Robinson BW. Transforming growth factor-b: antisense RNA-mediated inhibition affects anchorage-independent growth, tumorigenicity and tumor-infiltrating T-cells in malignant mesothelioma. Growth Factors 1994;11:29–44.
78. Marzo AL, Fitzpatrick DR, Robinson BW, Scott B. Antisense oligonucleotides specific for transforming growth factor b2 inhibit the growth of

malignant mesothelioma both in vitro and in vivo. Cancer Res 1997;57: 3200–3207.
79. Kumar-Singh S, Weyler J, Martin MJ, Vermeulen PB, Van Marck E. Angiogenic cytokines in mesothelioma: a study of VEGF, FGF-1 and -2, and TGF b expression. J Pathol 1999;189:72–78.
80. Kuwahara M, Takeda M, Takeuchi Y, Harada T, Maita K. Transforming growth factor b production by spontaneous malignant mesothelioma cell lines derived from Fischer 344 rats. Virchows Arch 2001;438:492–497.
81. Maeda J, Ueki N, Ohkawa T, et al. Transforming growth factor-b 1 (TGF-b 1)- and b 2-like activities in malignant pleural effusions caused by malignant mesothelioma or primary lung cancer. Clin Exp Immunol 1994;98:319–322.
82. Pasche B, Kolachana P, Nafa K, et al. *TbR-I(6A)* is a candidate tumor susceptibility allele. Cancer Res 1999;59:5678–5682.
83. Warshamana GS, Pociask DA, Sime P, Schwartz DA, Brody AR. Susceptibility to asbestos-induced and transforming growth factor-b_1-induced fibroproliferative lung disease in two strains of mice. Am J Respir Cell Mol Biol 2002;27:705–713.
84. Beck C, Schreiber H, Rowley D. Role of TGF-b in immune-evasion of cancer. Microsc Res Tech 2001;52:387–395.
85. Geller RL, Smyth MJ, Strobl SL, et al. Generation of lymphokine-activated killer activity in T cells. Possible regulatory circuits. J Immunol 1991;146: 3280–3288.
86. Yamanaka R, Tanaka R, Yoshida S, Saitoh T, Fujita K, Naganuma H. Suppression of TGF-b1 in human gliomas by retroviral gene transfection enhances susceptibility to LAK cells. J Neurooncol 1999;43:27–34.
87. Tamada K, Harada M, Abe K, et al. Immunosuppressive activity of cloned natural killer (NK1.1+) T cells established from murine tumor-infiltrating lymphocytes. J Immunol 1997;158:4846–4854.
88. Takayama T, Kaneko K, Morelli AE, Li W, Tahara H, Thomson AW. Retroviral delivery of transforming growth factor-b1 to myeloid dendritic cells: inhibition of T-cell priming ability and influence on allograft survival. Transplantation 2002;74:112–119.
89. Ranges GE, Figari IS, Espevik T, Palladino MA. Inhibition of cytotoxic T cell development by transforming growth factor b and reversal by recombinant tumor necrosis factor a. J Exp Med 1987;166:991–998.
90. Fontana A, Frei K, Bodmer S, et al. Transforming growth factor-b inhibits the generation of cytotoxic T cells in virus-infected mice. J Immunol 1989; 143:3230–3234.
91. Gorelik L, Flavell RA. Immune-mediated eradication of tumors through the blockade of transforming growth factor-b signaling in T cells. Nat Med 2001;7:1118–1122.
92. Jagirdar J, Lee TC, Reibman J, et al. Immunohistochemical localization of transforming growth factor beta isoforms in asbestos-related diseases. Environ Health Perspect 1997;105(suppl 5):1197–1203.
93. Perdue TD, Brody AR. Distribution of transforming growth factor-b_1, fibronectin, and smooth muscle actin in asbestos-induced pulmonary fibrosis in rats. J Histochem Cytochem 1994;42:1061–1070.
94. Bianchi C, Brollo A, Ramani L, Zuch C. Pleural plaques as risk indicators for malignant pleural mesothelioma: a necropsy-based study. Am J Ind Med 1997;32:445–449.
95. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-b1 induces prolonged severe fibrosis in rat lung. J Clin Invest 1997;100:768–776.

96. Baris YI, Sahin AA, Ozesmi M, et al. An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Urgup in Anatolia. Thorax 1978;33:181–192.
97. Nowak AK, Lake RA, Kindler HL, Robinson BW. New approaches for mesothelioma: biologics, vaccines, gene therapy, and other novel agents. Semin Oncol 2002;29:82–96.
98. Astoul P, Viallat JR, Laurent JC, Brandely M, Boutin C. Intrapleural recombinant IL-2 in passive immunotherapy for malignant pleural effusion. Chest 1993;103:209–213.
99. Boutin C, Nussbaum E, Monnet I, et al. Intrapleural treatment with recombinant g-interferon in early stage malignant pleural mesothelioma. Cancer 1994;74:2460–2467.
100. Castagneto B, Zai S, Mutti L, et al. Palliative and therapeutic activity of IL-2 immunotherapy in unresectable malignant pleural mesothelioma with pleural effusion: results of a phase II study on 31 consecutive patients. Lung Cancer 2001;31:303–310.
101. Davidson JA, Musk AW, Wood BR, et al. Intralesional cytokine therapy in cancer: a pilot study of GM-CSF infusion in mesothelioma. J Immunother 1998;21:389–398.
102. Robinson BW, Mukherjee SA, Davidson A, et al. Cytokine gene therapy or infusion as treatment for solid human cancer. J Immunother 1998;21: 211–217.
103. Monnet I, Breau JL, Moro D, et al. Intrapleural infusion of activated macrophages and g-interferon in malignant pleural mesothelioma: a phase II study. Chest 2002;121:1921–1927.
104. Sterman DH, Molnar-Kimber K, Iyengar T, et al. A pilot study of systemic corticosteroid administration in conjunction with intrapleural adenoviral vector administration in patients with malignant pleural mesothelioma. Cancer Gene Ther 2000;7:1511–1518.
105. Albelda SM, Wiewrodt R, Sterman DH. Gene therapy for lung neoplasms. Clin Chest Med 2002;23:265–277.
106. Rizzo P, Bocchetta M, Powers A, et al. SV40 and the pathogenesis of mesothelioma. Semin Cancer Biol 2001;11:63–71.

11

Extracellular Matrix and Mesothelioma: Some Clues to the Invasive Behavior of Mesothelioma

Julius Klominek and Dan Hauzenberger

Malignant mesotheliomas are highly aggressive diffuse tumors arising from mesothelial-lined surfaces. Mesotheliomas spread along mesothelial-lined surfaces to involve the pericardium, contralateral hemithorax, and peritoneal cavity by invasion through the diaphragm. The resulting tumor often forms diffuse thickening of involved surfaces rather than solitary rounded lesions as seen in other neoplasms (1,2). Malignant mesotheliomas also invade the underlying basement membrane and produce metastases in up to 80% of patients (3–5). Invasion through needle biopsy tracts and incision in the thoracic wall is a common feature in malignant mesotheliomas (6,7). During this process mesothelioma cells must interact with extracellular matrix proteins, growth factors embedded in it, and stromal cells, which participate in synthesis and modifications of this microenvironment. Today, the central theme of research about tumor etiology, progression, and metastasis focuses more on the crosstalk between tumor cells, extracellular matrix, and a variety of host cells rather than the behavior of individual tumor cells taken out of their microenvironmental context (8).

This chapter summarizes evidence describing various aspects of mesothelioma interactions with different components of the extracellular matrix, and the possible role of these interactions in the invasive behavior of this tumor.

Extracellular Matrix

Extracellular matrix (ECM) can be defined as a complex mixture of proteins, proteoglycans, and adhesive glycoproteins that provides structural and mechanical support to cells and tissues (9,10). Moreover, it is a reservoir of active and latent growth factors (11,12). The components of the ECM act in concert with growth factors and other cells and molecules present in it, to regulate a wide variety of cellular processes both in health and disease.

The ECM appears during evolution with the onset of multicellular life (13). Depending on the tissue environment, the cells interacting with the ECM respond through appropriate matrix receptors by changing gene expression and differentiation (14). Other cellular events affected by cell–matrix interactions include cell growth, cell death, adhesion, migration, and invasion. In turn, these cell–matrix interactions regulate physiologic processes such as embryonic development, tissue morphogenesis, and angiogenesis (14,15).

Under normal physiologic conditions, ECM units called basement membrane (16) and underlying tissue stroma maintain highly ordered and complex tissue architecture (17). Thus, these matrix boundaries delineate tissues and suppress inappropriate mixing of cells. During dynamic phases such as morphogenesis and wound healing, maintenance of tissue architecture is governed through signaling by soluble and solid-phase molecules of the ECM as well as by cell–cell communication (18,19). In malignancy there is an imbalance in these signals leading to violation of normal tissue boundaries (20). With acquisition of malignant phenotype, sooner or later, altered cell–ECM and cell–cell signals lead to release of normal constraints, enabling some malignant cells to migrate out of their original site and invade adjacent tissues (21,22). Today we have abundant evidence showing that ECM should no longer be considered as a reactive component without major biologic significance but rather an active player in tumor development, invasion, and metastasis.

Basement Membrane and Stroma Composition

Basement membrane is a dense matrix of collagen and glycoproteins such as fibronectin, laminin, and proteoglycans (23). Its primary role is physical cell support, control of cell polarity, and differentiation. Basement membranes, with few exceptions, do not contain any pores large enough to allow cell transmigration. It follows, then that invasion of the basement membrane is an active process.

Basement membrane is normally produced and deposited by epithelial, endothelial, or mesothelial cells, which then remain in close contact with its components (17). These contacts are mediated by integrin and nonintegrin receptors that recognize glycoproteins such as fibronectin, laminin, and collagen type IV (24–28).

Interstitial stroma is composed of various components of the ECM and stromal cells. Solid-phase and soluble components of stroma include glycoproteins, collagens, glycosaminoglycans, and elastin (17,29,30). The predominant cell of the stroma is fibroblast, which is responsible for the production of different collagens, proteolytic enzymes and their inhibitors, as well as growth factors. Other cells represented in the stromal compartment include myofibroblasts, immune cells such as lymphocytes and dendritic cells, and inflammatory cells such as monocytes, granulocytes, and vascular cells (31).

Extracellular matrix composition varies somewhat between different organs. Depending on the specific tissues and organs, appropriate cells

deposit matrix proteins that support specialized physiologic functions. Under normal conditions ECM is not permeable for cell movement. However, during wound healing, angiogenesis, inflammation, or tumor cell invasion, ECM undergoes enzymatic degradation and remodeling, allowing appropriate cells to transmigrate (32). Migration occurs along different components of the ECM in concert with growth factors or motility factors (33–36).

Basement Membrane and Stroma in Normal Mesothelium and Mesotheliomas

Composition and function of the pleura and peritoneum have been studied in different animals and humans. Key features such as presence of single cell layer basement membrane and underlying stroma are a constant finding between species (37–42). Normal mesothelium of pleura or the abdominal cavity is a single layer of distinctly polarized mesothelial cells that rests on a continuous basement membrane (Fig. 11.1). Besides providing mechanical support and controlling architectural arrangement of these cells, basement membrane is also involved in modulation of permeability characteristics of the pleura and peritoneum (43–45).

In early quantitative studies of pleural ECM, collagens as a group and particularly, collagen type I, were described to compose a major portion of the pleural connective tissue (46–48). Subsequent studies allowed more precise identification of individual collagen types and other ECM components of the basement membrane.

Collagens

The collagens are a family of at least 20 different molecules characterized by common structural features (49). These include a triple helical region, nonhelical regions, and globular domains. Collagens provide mechanical stability and strength, interacting with one another or with other ECM components. Fibrillar collagen types I, II, III, V, and XI are predominant components of stroma. Collagen types IV, VI, VIII, and

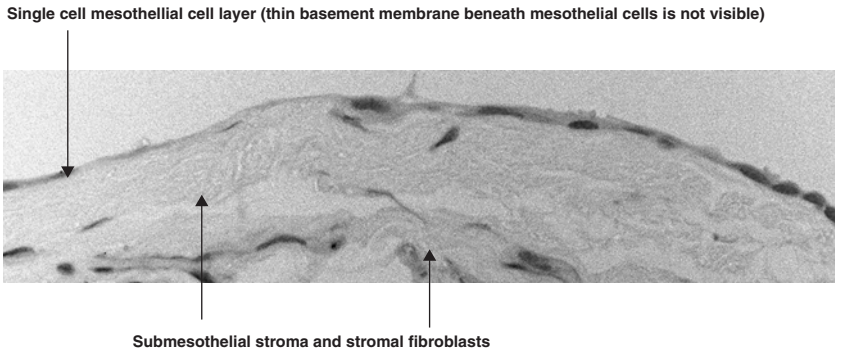


Figure 11.1. Normal human mesothelial cells and underlying submesothelial stroma.

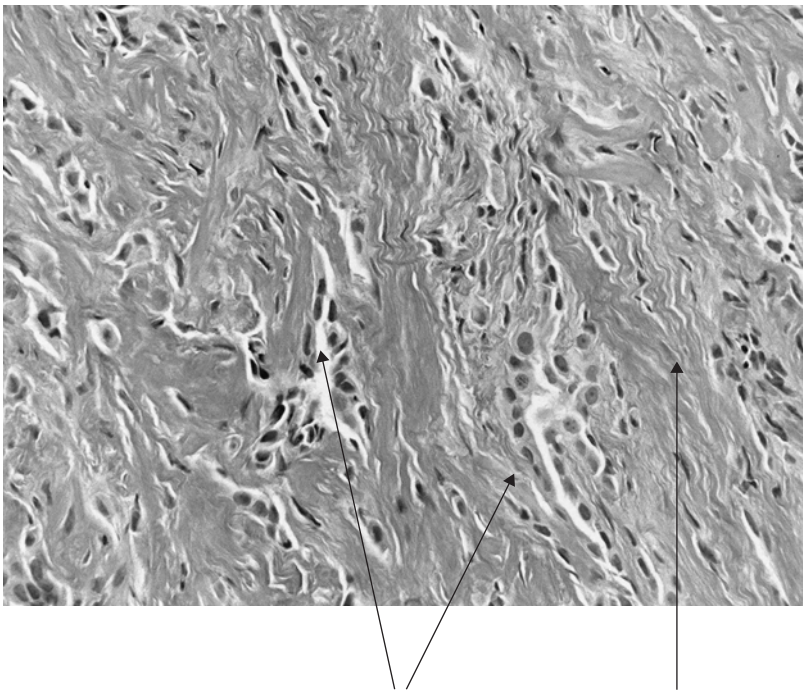


Figure 11.2. Malignant mesothelioma cells embedded in extracellular matrix.

X do not form fibrils but are cross-linked into a three-dimensional network (50).

Normal mesothelium rests on a basement membrane that contains collagen types I, III, and IV (40,51). Studies of paraffin-embedded tissues from normal adults and adults with active pleuritis showed that areas of mesothelial cell injury were associated with loss of the submesothelial basement membrane and that mesothelial cells play an active role in production of ECMs during healing of pleural injury (42).

The presence of collagen in malignant mesotheliomas has been noted by light (1,52,53), and electron microscopy (54,55). Large amounts of collagenous matrix were particularly prominent in the desmoplastic variant of malignant mesothelioma (56,57). Besides demonstration of tumor cells and tumor cell invasion, the presence of collagenous matrix is a key diagnostic feature of desmoplastic tumors (58,59), including malignant mesothelioma (60). Collagenous matrix is also present in the sarcomatous and epithelial types of mesothelioma (Fig. 11.2) (60).

These early light and electron microscopic findings have later been corroborated using immunohistochemical methods. Using polyclonal anticollagen type I and type IV antibodies, both types of collagen have been demonstrated in clinical specimens of malignant mesothelioma (61,62). The data from these studies indicated that malignant mesotheliomas have the ability to synthesize basement membrane components such as collagen type IV (3,62). Using monoclonal antibodies to colla-

gen type IV, Di Muzio et al (63) demonstrated the presence of this molecule around individual mesothelioma cells of epithelial subtype. This feature distinguished epithelial mesotheliomas from peripheral lung adenocarcinomas. Additional data confirming collagen synthesis were obtained from studies of malignant mesotheliomas in culture. In these studies, light microscopic, chemical, and immunohistochemical methods were used to demonstrate production of collagens in malignant mesotheliomas (64–67).

Collagens and other components of the ECM act in concert with growth factors and motility factors during tumor cell invasion and metastasis (32,33,36). Growth factors with motility-inducing properties such as hepatocyte growth factor (HGF), have been shown to stimulate chemotaxis, chemokinesis, and invasiveness in malignant mesothelioma cell lines in the presence of matrix proteins (66,68). Malignant mesotheliomas synthesize and secrete ECM proteins and growth factors such as HGF (66,69,70). The same cells also express appropriate receptors for these molecules (70,71) and migrate to these self-secreted matrix proteins and growth factors. Taken together, the findings indicate a possible autocrine motility-stimulating loop in malignant mesotheliomas. Accordingly, these in vitro properties of malignant mesothelioma cells may provide some clues to the highly motile, invasive characteristics of this tumor in vivo (66,70).

Fibronectin

Fibronectin is a multifunctional glycoprotein that is found in two different forms: soluble, found in body fluids, and solid, found in loose connective tissue and most basement membranes. In vivo, physiologic sources of fibronectin are hepatocytes that produce soluble forms and fibroblasts, and endothelial cells (and many others) that synthesize solid forms of this molecule (72–74). Fibronectin acts through several distinct domains that promote cell adhesion, cell migration, and matrix assembly. In addition, fibronectin is an essential component mediating cell–collagen interactions. The interactions among collagen, proteoglycans, and fibronectin are important in matrix assembly and in development of the structural organization of the ECM (75,76). In tumors, fibronectin often accumulates in the stroma (77,78). Interestingly, at the invasive edge of mammary carcinomas, fibronectin is lost in a majority of cases (79).

Cultured normal mesothelial cells and their malignant counterparts synthesize and secrete fibronectin in vitro (66,67,80,81). In addition, normal and malignant mesothelial cells have the ability to assemble fibronectin into homogeneous fibrillar arrays of organized matrix, located primarily between the cells (66,82). Evidence from other tumors, such as synovial sarcoma, indicates that these tumors have the ability to produce and secrete fibronectin in vivo (58). However, in patients with mesothelioma, fibronectin has been identified only in pleural fluids (83).

Experiments with malignant mesothelioma cell lines indicate that these cells adhere, spread, and migrate in response to both soluble

and solid forms of fibronectin (66,67,71). In analogy to collagens and laminin, the presence of fibronectin is an absolute requirement for growth factor–induced migration of both normal and malignant mesothelial cells (84,85).

Laminin

Laminins are major components of basement membranes mediating a variety of functions such as cell adhesion, cell migration, neurite outgrowth, cellular proliferation, and basement membrane assembly (86). Laminin was first isolated from the matrix of mouse EHS tumor (87) and is now known to consist of a family of proteins that is composed of variably expressed chains that generate several different isoforms that vary in size, composition, and structure (88). Laminins are produced by a large variety of cells (9). Together with collagen type IV, laminin forms the structural framework of basement membrane, where it is responsible for induction of epithelial cell differentiation and establishment of cell polarity (89). In malignancy, laminin has been suggested to mediate adhesion of tumor cells to the basement membrane prior to invasion (90). In experimental models, adhesion to laminin plays a role in cell attachment, cell migration, and hematogenous metastasis (21,91,92).

Normal and malignant mesothelial cells synthesize and secrete laminins in vitro and in vivo (62,66,67,80). In histologic sections of malignant mesotheliomas, laminin staining was primarily located in the cytoplasm, but in some areas laminin was also demonstrated extracellularly. However, demonstration of laminin immunoreactivity in mesotheliomas has no diagnostic or prognostic value (62). Laminin production by primary breast carcinomas was also investigated using immunohistochemistry on archival specimens from a retrospective series with long-term follow-up. Laminin production was found to be independent of the clinical and pathologic variables analyzed (93).

In malignant mesotheliomas in cell culture, laminin induced cell adhesion, spreading, and chemotactic and haptotactic migration, indicating its role in tumor cell invasion (66,67,71).

Thrombospondin

Thrombospondins (TSPs) are a family of secreted, modular glycoproteins whose functions are not well understood. Unlike the various structural proteins of the ECM, TSPs do not appear to contribute directly to the integrity of a physical entity, such as a fiber or a basement membrane (94). Rather, it seems that these proteins act contextually to influence cell function by modulating cell–matrix interactions (95).

The role of thrombospondin-1 was investigated in relation to prognosis in surgically resected malignant pleural mesothelioma. Expression of thrombospondin messenger RNA (mRNA) has been demonstrated both in normal pleural tissue and normal lung tissue. Significantly higher expression was found in a majority of resected malignant mesotheliomas (96). These results were consistent with pre-

vious data gathered from investigations of other tumors (97). Although thrombospondin-1 was overexpressed in the majority of mesotheliomas, the level of expression had little prognostic value (96).

Elastin

Elastin, a cross-linked protein of the ECM, is the major component of several elastic tissues such as lung, blood vessels, and skin (98). Elastin as part of ECM was shown to mediate cell adhesion of monocytes, fibroblasts, and tumor cells (99). Elastin-derived peptides, especially a hexapeptide, VGVAPG, is chemotactic for several types of cells such as monocytes and fibroblasts and for certain tumor cells (100). Elastin production has been demonstrated in normal mesothelial cells in culture (80) but not in malignant mesotheliomas. However, a 67-kd elastin/laminin receptor has been identified in human malignant mesotheliomas by immunohistochemistry (62). Interestingly, experimental evidence from other tumors links a 67-kd elastin/laminin receptor to invasion and metastasis formation and poor prognosis in breast cancer patients (27,32,101).

Glycosaminoglycans and Proteoglycans

Glycosaminoglycans (GAGs) are linear polysaccharides consisting of repeating disaccharide units. They include heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronic acid (HA) (17,102,103).

Among GAGs, hyaluronic acid is the molecule that has been most extensively studied. It has been proposed to have functional importance in processes such as embryogenesis, angiogenesis, cell growth and migration, wound healing, and the formation of high molecular mass aggregates with various proteoglycans (103,104). Its role in malignancy is at least threefold: it functions as a template for assembly of other pericellular macromolecules, it interacts directly with cell surface receptors that transduce intracellular signals, and it promotes anchorage-independent growth and invasiveness (105).

Mayer and Chaffee (106) had observed the association between hyaluronic acid and malignant mesothelioma as early as 1939. Since then a large number of studies have investigated the functional and diagnostic importance of HA in this tumor (107–112). These and other studies not mentioned in this chapter, frequently identify elevated HA levels in pleural effusions or serum of patients with malignant mesothelioma. High levels of HA in pleural effusions appear to be related to the epithelial differentiation of malignant mesothelioma, whereas low or "normal" levels of HA are found more frequently in fibrous mesotheliomas (113). Elevated HA can also be found in pleural effusions of patients with rheumatoid arthritis and other inflammatory conditions, adding to the controversy surrounding the clinical usefulness of this test in mesothelioma and limiting its wide acceptance (114). The source of HA in patients with malignant mesothelioma has also been subject to controversy. Microscopic examination of mesothelioma biopsies often reveals the presence of noncollagenous matrix sur-

rounding mesothelioma cells (60,109). Hyaluronic acid was also demonstrated on human malignant mesothelioma cells growing in nude mice xenografts (54). However, in 1993 Asplund et al (115) proposed that normal human mesothelial cells rather than their malignant counterparts were the source of HA in malignant mesothelioma, and that secretion of HA into pleural fluids was induced by growth factors.

Hyaluronic acid synthases (HAS) are enzymes responsible for synthesis of HA in plasma membrane. Using reverse-transcription polymerase chain reaction (RT-PCR), HAS1 was identified in malignant mesothelioma of the epithelial subtype (115). In addition, increasing concentrations of newly synthesized HA, chondroitin sulfate, and heparan sulfate was demonstrated in cultured malignant mesothelioma cells (117,118). Addition of growth factors such as platelet-derived growth factor BB (PDGF-BB) to malignant mesothelioma culture medium led to a 10-fold increase of HA synthesis (118). Taken together, these data show that at least some malignant mesotheliomas synthesize HA and other glycosaminoglycans. Recent data indicate that HA contributes also to malignant phenotype in malignant mesothelioma, as it has the capacity to stimulate proliferation and migration through interaction with HA receptor CD44 (119).

The proteoglycan family of ECM and cell surface molecules contains more than 30 molecules that perform a variety of different functions in the ECM. Proteoglycans act as tissue organizers, influence cell growth and the maturation of specialized tissues, play a role as biologic filters, modulate growth factor activities, regulate collagen fibrillogenesis and skin tensile strength, affect tumor cell growth and invasion, and influence corneal transparency and neurite outgrowth (120).

The term *proteoglycan* refers to its molecular structure, consisting of a protein core to which glycosaminoglycan side chains are attached. Functional characterization of proteoglycans reflects the location of the molecule, whether mainly found in association with the cell surface or the ECM (120).

Syndecans are a cell-associated proteoglycans that interact with growth factors, ECM components, enzymes, protease inhibitors, and chemokines. In malignant mesotheliomas, syndecan-1 expression was demonstrated in epithelial subtype and epithelial components in biphasic form. In the sarcomatous type of mesothelioma, expression of syndecan-1 was weak or absent, indicating that this molecule is related to differentiation of mesotheliomas (116,121).

Integrins

The integrins are a large family of membrane glycoproteins consisting of an α and β subunit, where a single β subunit is noncovalently associated with one of several possible α subunits (122). Integrins mediate cell–matrix and cell–cell adhesion, a function that has been implicated in processes like development, the immune response, hemostasis, and maintenance of tissue architecture (26,123). Integrins also participate in a number of pathologic conditions, such as inflammation, tumor cell invasion, and metastasis (25). At present there are

at least 24 αβ heterodimers formed of nine different β subunits and 18 α subunits.

Integrin ECM ligands include a variety of molecules such as collagen type I and IV, laminin, fibronectin, vitronectin, von Willebrand's factor, and thrombospondin (26). Integrins also have a signaling function where signals can be transduced in both directions, so-called inside-out and outside-in signaling (124). Malignant cells often have abnormal integrin function (124). Tumor cells generally show decreased integrin function, which occurs either because the cells have fewer integrins as a result of dedifferentiation, or because integrin function is suppressed through oncogenic transformation (125). In carcinomas, there is a shift in integrin expression from those that favor the ECM present in normal epithelium to other integrins (e.g., $\alpha_3\beta_1$ and $\alpha_v\beta_3$) that preferentially bind the degraded stromal components produced by matrix proteases (126,127).

Normal human mesothelial cells and malignant mesothelioma cells express a rather homogeneous pattern of α and β integrin subunits (Table 11.1) (71). Notably, high levels of $\alpha_3\beta_1$ integrin are consistently expressed in both normal and malignant mesothelial cells (71,128,129), whereas expression of classic lymphocyte fibronectin receptor is generally absent or very low (71,128,130). Vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are also a constant feature of both normal and malignant mesothelial cells. Vitronectin is a multifunctional adhesive protein present in large concentrations in serum. In addition, vitronectin diffuses into ECM, where it may bind and become concentrated as compared with other serum proteins (131), and may also be found in pleural and bronchoalveolar lavage fluid (132). Experimental evidence indicates that normal mesothelial cells recognize and internalize vitronectin-coated asbestos fibers via $\alpha_v\beta_5$ integrin (132). Thus, this finding provides clues to how integrins participate in asbestos-induced biologic effects.

In malignant mesotheliomas, integrins appear to have two distinct functions: they mediate cell attachment to ECM, and cell migration (71,130). Experiments with antiintegrin antibodies revealed that preincubation of mesothelioma cells with antibodies to β_1, α_2, α_5, and α_6 subunits inhibited both cell attachment and cell migration to fibronectin, laminin, and collagen type IV. Interestingly, in some mesothelioma cell lines preincubation of cells with α_3 antibodies inhibited cell migration, without any effect on cell attachment (71). These observations confirm an important role of integrins in attachment and migration of tumor cells and may contribute to our understanding of the highly motile, invasive behavior of malignant mesotheliomas.

Crosstalk between integrins and growth factor receptors is an important mechanism during normal development and pathologic processes (133). In malignant mesothelioma cells crosstalk between α_3 integrin and PDGF receptor β is a prerequisite for PDGF-BB–induced chemotaxis (34). In vivo, PDGF is synthesized and released by several stromal cell types during wound healing (134). The PDGF produced around damaged tissue could attract certain mesothelioma cells to invade biopsy tracts and incisions as often seen in patients with malignant

Table 11.1. Patterns of integrins expressed in mesothelial and mesothelioma cells

Cell line	β1	β2	β3	β4	αL	α1	α2	α3	α4	α5	α6	αv	αvβ3	αvβ5
STAV AB	++	ND	−	+	ND	−	+	++	−	−	+	+	ND	ND
STAV FCS	++	ND	−	+	ND	−	+	+++	−	−	+	+	ND	ND
SPC111	++	ND	−	+	ND	+	+	++	−	−	+	+	ND	ND
SPC212	+++	ND	+	+	ND	+	+	++	−	−	+	++	+	++
ZL5	+++	−	−	+	−	+	+	+++	+	+	+	+	−	+
ZL34	++	ND	+	+	ND	+	+	++	−	−	+	+	ND	ND
M14K	+++	−	−	−	−	−	+	+++	−	−	+	++	ND	++
Mg38K	+++	−	−	+	−	−	++	+++	+	+	+	++	−	++

Expression of integrins in normal mesothelial cells *in vitro*

Cell line	β1	β2	β3	β4	αL	α1	α2	α3	α4	α5	α6	αv
NHM	+++	ND	+	+	ND	−	+	++	−	−	+	++
CuNHM	++	ND	+	−	ND	−	+	++	−	−	−	++

Expression of integrins in malignant mesothelioma cells *in vivo*

Case	β1	β3	β4	α1	α2	α3	α4	α5	α6	αv
Pleura	+	−	−/+	−	−/+	+	−/+	+	+	+
Node	+	−	+	+	−	+	−	+	+	+
Metastasis	+	−	+	−	−	+	−	+	+	+

ND, not done; NHM, normal human mesothelial cells; CuNHM, cultured normal human mesothelial cells.

mesotheliomas (135). We hypothesize that inhibition of PDGF-induced cell migration using novel small molecular drugs could contribute to the reduction of mesothelioma cell invasion into needle biopsy tracts and incisions.

Matrix Metalloproteases

Degradation of and migration through ECM barriers, such as basement membrane and stroma, is a complex process that requires the production, release, and activation of extracellular degrading enzymes (20). Inappropriate overexpression of one or more of these enzymes has been shown to occur in almost all cells of the tumor–host microenvironment, for example, tumor cells, fibroblasts, and recruited macrophages (136). Remodeling of the ECM is confined to the immediate pericellular environment of the cell and is not solely dependent on the amount of proteolytic enzymes present but on the balance of activated proteases and their naturally occurring inhibitors (137).

Matrix metalloproteases (MMPs) are a family of zinc atom-dependent endopeptidases with specific and selective activities against many components of ECM. This family currently consists of at least 20 members, and most of them are secreted as zymogens that must be activated extracellularly (20).

The expression of MMPs in human tumors is the result of a complex interaction between tumor cells and stromal host cells (e.g., fibroblasts, endothelial cells, and inflammatory cells), which all actively participate in the production of these enzymes (138). The proteolytic remodeling of the ECM by MMPs does more than allow tumor cells to locally invade and form metastases. Another major consequence of matrix degradation by MMPs is the release of ECM-sequestered growth factors, several of which play an important role in tumor cell survival and proliferation, as well as angiogenesis (139). Importantly, similar mechanisms are shared by physiologic and tumorigenic invasion. The difference between them is that physiologic invasion is regulated, whereas tumorigenic invasion appears to be perpetual (140).

Normal human pleural and peritoneal mesothelial cells secrete MMP-2 and MMP-9 and the counterregulatory tissue inhibitors of metalloproteases (TIMP). Secretion of these enzymes is probably involved in ECM turnover following serosal injury (141).

Malignant mesotheliomas in culture produce several proteases belonging to the MMP family of enzymes. These include MMPs 1, 2, 3, 7, 9, 10, membrane-bound MT1-MMP, and TIMP 1, 2, and 3 (68,142,143). Production of these enzymes was regulated by growth factors such as HGF, epidermal growth factor (EGF), transforming growth factor-α, β-cellulin, heparin-binding EGF, amphiregulin, insulin-like growth factor I, and acidic and basic fibroblast growth factors (68,142–144). Interestingly, several of these growth factors also stimulate migration of malignant mesothelioma cells (Fig. 11.3) (70,144,145).

In addition to the known MMPs, malignant mesothelioma cells secrete not yet characterized enzymes that specifically degrade fi-

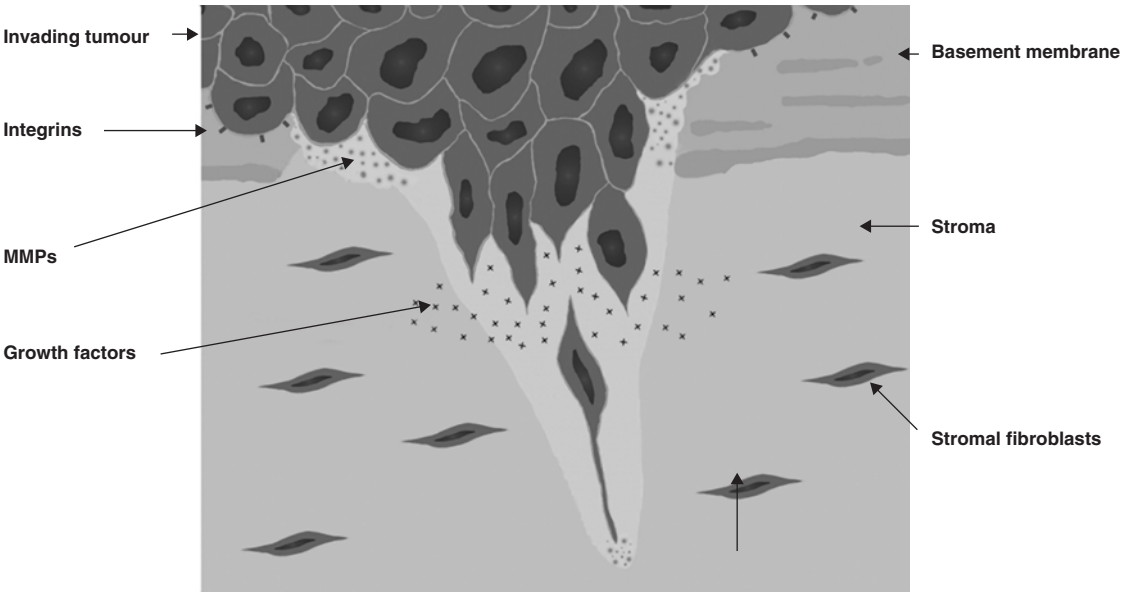


Figure 11.3. Proposed schematic model of mesothelioma cell invasion. Tumor cells attach to the extracellular matrix via integrins. Matrix metalloproteases (MMPs) degrade and alter matrix composition. Interaction with matrix components or their degradation products induce cell migration. Growth factors are either released from the matrix upon degradation, secreted by stromal cells, or secreted by tumor cells in an autocrine fashion. Released or secreted growth factors act in concert with integrins to additionally propel tumor cell migration. Release of MMPs by tumor or stromal cells is modulated by secretion of tissue inhibitors of proteases. Growth factors may also regulate MMP release.

bronectin, vitronectin, and laminin but not collagen (142). While these results do not provide specific answers to how these enzymes participate in matrix remodeling in vivo, it is apparent that mesotheliomas produce a wide array of matrix-degrading proteases that may contribute to highly invasive behavior of this tumor.

Conclusion

Malignant mesotheliomas synthesize, secrete, and assemble a wide array of matrix proteins. They also express receptors that bind these matrices and secrete enzymes that have the capacity to degrade ECM components alone or in collaboration with host cells. New knowledge of interactions between mesothelioma cells and their microenvironment creates new possibilities, which will allow us to better understand the etiology, progression, and spreading of this tumor. These new insights that are emerging within the field of tumor biology and mesothelioma research will help us to generate new questions and ideas that in the near future will translate into diagnostic and therapeutic modalities. Here are some examples: one of the longstanding questions in mesothelioma research is the cellular origin of this tumor. Are mesothelial or submesothelial cells responsible? Perhaps we should rephrase the question and ask how mesothelial cells interact with submesothelial cell

populations and its surrounding matrix to give rise to mesothelioma. Erlotinib (Tarceva), Gefitinib (Iressa), and Imatinib (Gleevec) are new small-molecule drugs that target receptor tyrosine kinases (EGF receptor, c-kit, and PDGF receptor) expressed often on mesothelioma cells. Interestingly, these drugs not only are cytostatic but also act in the mesothelioma microenvironment by inhibiting cell migration and MMP production (146). In the near future, results of clinical trials with these drugs will be available. Other drugs that act in the mesothelioma microenvironment are MMP inhibitors. Despite initial disappointments in clinical trials, MMP inhibitors may have a role in treatment and prevention of invasion and metastases (147). Finally, cDNA microarray studies of malignant mesotheliomas reveal distinct genes that may support development of new diagnostic tools for mesothelioma or become potential drug targets for treatment of this tumor.

Acknowledgments

This work was supported by research grants from the Swedish Heart and Lung Foundation. We also thank Drs. Anders Hjerpe and Arrigo Capitanio for providing photographs of normal mesothelium and malignant mesothelioma.

References

1. Klemperer P, Rabin CB. Primary neoplasms of the pleura. Arch Pathol 1931;11:385–412.
2. Semb G. Diffuse malignant pleural mesothelioma: a clinicopathological study of 10 fatal cases. Acta Chir Scand 1963;126:78–91.
3. Wilson GE, Hasleton PS, Chatterjee AK. Desmoplastic malignant mesothelioma: a review of 17 cases. J Clin Pathol 1992;45:295–298.
4. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. J Clin Oncol 1989;7:1157–1168.
5. Moskal TL, Urschel JD, Anderson T, Antkowiak JG, Takita H. Malignant pleural mesothelioma: a problematic review. Surg Oncol 1999;7:5–12.
6. Chahinian AP, Pajak TF, Holland JF, Norton L, Ambinder RM, Mandel EM. Diffuse malignant mesothelioma. Prospective evaluation of 69 patients. Ann Intern Med 1982;96:746–755.
7. Hillerdal G. Malignant mesothelioma 1982: review of 4710 published cases. Br J Dis Chest 1983;77:321–343.
8. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature 2001;411:375–379.
9. Timpl R, Dziadek M. Structure, development, and molecular pathology of basement membranes. Int Rev Exp Pathol 1986;29:1–112.
10. Akiyama Y, Jung S, Salhia B, et al. Hyaluronate receptors mediating glioma cell migration and proliferation. J Neurooncol 2001;53:115–127.
11. Klagsburn M, Baird A. A dual receptor system is required for basic fibroblast growth factor activity. Cell 1991;67:229–231.
12. Roberts AB, McCune BK, Sporn MB. TGF-beta regulation of extracellular matrix. Kidney Int 1992;41:557–559.

13. Damsky C, Sutherland A, Fisher S. Extracellular matrix 5: adhesive interactions in early mammalian embryogenesis, implantation and placentation. FASEB J 1993;7:1320–1329.
14. Damsky CH, Werb Z. Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. Curr Opin Cell Biol 1992;4:772–781.
15. Ingber DE, Folkman J. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. J Cell Biol 1989;109:317–330.
16. Carter WG, Kaur P, Gil SG, Gahr PJ, Wayner EA. Distinct functions for integrins alpha 3 beta 1 in focal adhesions and alpha 6 beta 4/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. J Cell Biol 1990;111:3141–3154.
17. Timpl R. Structure and biological activity of basement membrane proteins. Eur J Biochem 1989;180:487–502.
18. Bissell MJ, Hall GH, Parry G. How does the extracellular matrix direct gene expression? J Theor Biol 1982;99:31–68.
19. Lin CQ, Bissell MJ. Multi-faceted regulation of cell differentiation by extracellular matrix. FASEB J 1993;7:737–743.
20. Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Biol 1993;9:541–573.
21. Liotta LA. Tumor invasion and metastases—role of the extracellular matrix: Rhodes memorial award lecture. Cancer Res 1986;46:1–7.
22. Liotta LA, Rao CN, Barsky SH. Tumor invasion and the extracellular matrix. Lab Invest 1983;49:636–649.
23. Yurchenco PD, Schittny JC. Molecular architecture of basement membranes. FASEB J 1990;4:1577–1590.
24. Humphries MJ, Olden K, Yamada KM. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. Science 1986;233:467–470.
25. Hynes RO. Integrins: a family of cell surface receptors. Cell 1987;48: 549–554.
26. Ruoslahti E. Integrins. J Clin Invest 1991;87:1–5.
27. Rao C, Castronovo V, Schmott MC, et al. Evidence for a precursor of the high-affinity metastasis associated murine laminin receptor. Biochemistry 1989;28:7476–7486.
28. Aznavoorian S, Stracke ML, Krutzsch H, Schiffmann E, Liotta LA. Signal transduction for chemotaxis and haptotaxis by matrix molecules in tumor cells. J Cell Biol 1990;110:1427–1438.
29. Kadler K. Extracellular matrix I. Fibril-forming collagens. Protein Profile 1994;1:519–638.
30. Yurchenco PD. Assembly of laminin and collagen type IV into basement membrane networks. In: Yurchenco PD, Birk DE, Mecham RP, eds. Extracellular Matrix Assembly and Structure. San Diego: Academic Press, 1994.
31. Tlsty TD. Stromal cells can contribute oncogenic signals. Semin Cancer Biol 2001;11:97–104.
32. Aznavoorian S, Murphy AN, Stetler-Stevenson WG, Liotta LA. Molecular aspects of tumor cell invasion and metastasis. Cancer 1993;71:1368–1383.
33. McCarthy J, Turely EA. Effects of extracellular matrix components on cell locomotion. Crit Rev Oral Biol Med 1993;4:619–637.
34. Klominek J, Baskin B, Hauzenberger D. Platelet-derived growth factor BB is a chemoattractant for human malignant mesothelioma cells and its

action is mediated through platelet derived growth factor receptor β interactions. Clin Exp Metastasis 1998;16:529–539.
35. Doerr ME, Jones JI. The roles of integrins and extracellular matrix proteins in the insulin-like growth factor I-stimulated chemotaxis of human breast cancer cells. J Biol Chem 1996;271:2443–2447.
36. Wells A, Kassis J, Solava J, Turner T, Lauffenburger DA. Growth factor-induced cell motility in tumor invasion. Acta Oncol 2002;41:124–130.
37. Wang NS. Ultrastructure of rabbit pleura. Am Rev Respir Dis 1974;110: 623–633.
38. Legrand M, Pariénte R, André J, Chrétien J, Brouet G. Ultrastructure de la plèvre pariétale humaine. Presse Med 1971;79:2515–2520.
39. Whitaker D, Papadimitriou J. Mesothelial healing: morphological and kinetic investigations. J Pathol 1985;145:159–175.
40. Stylianou E, Jenner LA, Davies M, Coles GA, Williams JD. Isolation, culture and characterization of human peritoneal mesothelial cells. Kidney Int 1990;37:1563–1570.
41. Bermudez E, Everitt J, Walker C. Expression of growth factor and growth factor receptor RNA in rat pleural mesothelial cells in culture. Exp Cell Res 1990;190:91–98.
42. Davila RM, Crouch EC. Role of mesothelial and submesothelial stromal cells in matrix remodeling following pleural injury. Am J Pathol 1993;142: 547–555.
43. Pinchon MC, Ebert RV. Pleural permeability in rat I. Ultrastructural basis. Cell Biol 1980;37:269–272.
44. Agostoni E, Zocchi L. Mechanical coupling and liquid exchanges in the pleural space. Clin Chest Med 1998;19:241–260.
45. Hirsch A, Bernaudin JF, Nebut M, Soler P. The pleural mesothelium, structure and function. Bull Eur Physiopathol Respir 1976;12:367–406.
46. Borenstein P, Sage H. Structurally distinct collagen types. Annu Rev Biochem 1980;49:957–1003.
47. Gay RE, Rhodes RK. Immunolocalisation of the Genetically Distinct Collagen Types in the Pathology of Connective Tissue. New York: Marcel Dekker, 1986.
48. Isoda K, Maeda T, Hamamoto Y. Collagen-producing mesothelial cells in Adriamycin-induced pleuritis in rat. Acta Pathol Jpn 1987;37:1305–1317.
49. Bork P. The modula structure of vertebrate collagens. FEBS Lett 1992;100: 49–54.
50. Van Der Rest M, Garrone R. Collagen family of proteins. FASEB J 1991;5: 2814–2823.
51. Harvey W, Amlot PL. Collagen production by human mesothelial cells in vitro. J Pathol 1983;139:337–347.
52. Godwin MC. Diffuse mesotheliomas: with comment on their relation to localised fibrous mesotheliomas. Cancer Metastasis Rev 1957;10:298–319.
53. McCoughey WTE. Primary tumours of the pleura. Ann NY Acad Sci 1958; 132:603–613.
54. Suzuki Y, Chahinian P, Ohnuma T. Comperative studies of human malignant mesothelioma in vivo, in xenografts in nude mice, and in vitro. Cancer 1987:334–344.
55. Suzuki Y, Churg J, Kannerstein M. Ultrastructure of human malignant diffuse mesothelioma. Am J Pathol 1976;85:241–262.
56. Churg J, Rosen SH, Moolten S. Histological characteristics of mesothelioma associated with asbestos. Ann NY Acad Sci 1965;132:614–622.
57. Cantin R, Al-Jabi M, McCoughey WTE. Desmoplastic diffuse mesothelioma. Am J Surg Pathol 1982;6:215–222.

58. Guarino M, Christensen L. Immunohistochemical analysis of extracellular matrix components in synovial sarcoma. J Pathol 1994;172:279–286.
59. Valensi QJ. Desmoplastic malignant melanoma: a light and electron microscopic study of two cases. Cancer 1979;43:1148–1155.
60. Adams VI, Unni KK. Diffuse malignant mesothelioma of pleura: diagnostic criteria based on an autopsy study. Am J Clin Pathol 1984;82:15–23.
61. Kataoka H, Wickström B, Klominek J, Gay RE, Gay S, Hjerpe A. Immunocytochemical demonstration of collagen types I and IV in cells isolated from malignant mesothelioma and in lung cancer cell lines. Lung Cancer 1990;6:16–27.
62. Kallianpur AR, Carstens PH, Liotta LA, Frey KP, Siegal GP. Immunoreactivity in malignant mesotheliomas with antibodies to basement membrane components and their receptors. Modern Pathol 1990;3:11–18.
63. Di Muzio M, Spoletini L, Strizzi L, et al. Basal lamina reduplication in malignant epithelioid pleural mesothelioma. Ultrastruct Pathol 1998;22: 467–475.
64. Alvarez-Fernandez E, Diez-Nau MD. Malignant fibrosarcomatous mesothelioma and benign pleural fibroma (localized fibrous mesothelioma) in tissue culture. Cancer 1979;43:1658–1663.
65. Behbehani AM, Hunter WJ, Chapman AL, Lin F. Studies of human mesothelioma. Hum Pathol 1982;13:862–866.
66. Klominek J, Robért K-H, Sundqvist K-G. Chemotaxis and haptotaxis of human malignant mesothelioma cells. Effects of fibronectin, laminin, type IV collagen and an autocrine motility factor-like substance. Cancer Res 1993;53:4376–4382.
67. Scarpa S, Giuffrida A, Fazi M, et al. Migration of mesothelioma cells correlates with histotype-specific synthesis of extracellular matrix. Int J Mol Med 1999;4:67–71.
68. Harvey P, Clark IM, Jaurand M-C, Edwards DR. Hepatocyte growth factor/scatter factor enhance the invasion of mesothelioma cell lines and the expression of matrix metalloproteases. Br J Cancer 2000;83:1147–1153.
69. Harvey P, Warn A, Newman P, Perry LJ, Ball RY, Warn RM. Immunoreactivity for hepatocyte growth factor/scatter factor and its receptor, met, in human lung carcinomas and malignant mesotheliomas. J Pathol 1996; 180:389–394.
70. Klominek J, Liu Z, Baskin B, Hauzenberger D. Human hepatocyte growth factor/scatter factor stimulats chemotaxis and growth of malignant mesothelioma cells through c-met receptor. Int J Cancer 1998;76:240–249.
71. Klominek J, Sumitran Karuppan S, Hauzenberger D. Differential motile response of human malignant mesothelioma cells to fibronectin, laminin and collagen type IV: the role of β1 integrins. Int J Cancer 1997;72:1034–1044.
72. Ruoslahti E, Vaheri A. Novel human serum protein from fibroblast plasma membrane. Nature 1974;248:789–791.
73. Hynes RO, Yamada KM. Fibronectins: multifunctional modular glycoproteins. J Cell Biol 1982;95:369–377.
74. Hynes RO. Fibronectins. New York: Springer-Verlag, 1990.
75. Ruoslahti E. Fibronectin and its receptors. Annu Rev Biochem 1988;57: 375–413.
76. Woods A, Couchman JR. Adhesion and cytoskeletal organisation of fibroblasts in response to fibronectin fragments. EMBO J 1986;5:665–670.
77. Christensen L. The distribution of fibronectin, laminin and tetranectin in human breast cancer with special attention to the extracellular matrix. APMIS 1992;suppl 26.

78. Loridon-Rosa B, Vielh P, Cuadrado C, Buriton P. Comperative distribution of fibronectin and vitronection in human breast and colon carcinomas. An immunofluorescence study. Am J Clin Pathol 1988;90:7–16.
79. Christiansen L, Nielsen M. Stromal fibronectin staining pattern and metastasizing ability of human breast carcinoma. Cancer Research 1988: 6227–6233.
80. Rennard SI, Jaurand M-C, Bignon J, et al. Role of pleural mesothelial cells in the production of the submesothelial connective tissue matrix of lung. Am Rev Respir Dis 1984;130:267–274.
81. Kinnula VL, Linnala A, Viitala E, Linnainmaa K, Virtanen I. Tenascin and fibronectin expression in human mesothelial cells and pleural mesothelioma cell-line cells. Am J Respir Cell Mol Biol 1998;19:445–452.
82. Ferriola PC, Stewart W. Fibronectin expression and organization in mesothelial and mesothelioma cells. Am J Physiol 1996;271:L804–L812.
83. Siri A, Carnemolla B, Raffanti S, Castellani P, Balzano E, Zardi L. Fibronectin concentrations in pleural effusions of patients with malignant and non-malignant diseases. Cancer Lett 1984;22:1–9.
84. Klominek J, Hauzenberger D, Sundqvist K-G. Platelet derived growth factor (PDGF) is a chemoattractant for human malignant mesothelioma cells. Keystone Symposia Abstract 1994.
85. Palmer U, Liu Z, Broomé U, Klominek J. Epidermal growth factor receptor ligands are chemoattractants for normal human mesothelial cells. Eur Respir J 1999;14:405–411.
86. Beck K, Hunter I, Engel J. Structure and function of laminin: anatomy of a multidomain glycoprotein. FASEB J 1990;4:148–160.
87. Timpl R, Rodhe H, Gehron Robey P, Rennard SI, Foidart JM, Martin GR. Laminin—a glucoprotein from basement membranes. J Biol Chem 1979; 254:97–120.
88. Timpl R, Brown JC. The laminins. Matrix Biol 1994;14:275–281.
89. Adams JC, Watt FM. Regulation of development and differentiation by extracellular matrix. Dev Biol 1993;117:1183–1198.
90. Rao CN, Marguiles IMK, Tralka TS, Terranova VP, Madri JA, Liotta LA. Isolation of a subunit of laminin and its role in molecular structure of tumour cell attachment. J Biol Chem 1982;257:9740–9744.
91. McCarthy JB, Palm SL, Furcht LT. Migration by haptotaxis of a Schwann cell tumor line to a basement glycoprotein laminin. J Cell Biol 1983;97: 772–777.
92. McCarthy JB, Furcht LT. Laminin and fibronectin promote the haptotactic migration of B16 mouse melanoma cells in vitro. J Cell Biol 1984;98: 1474–1480.
93. Pellegrini R, Martignone S, Tagliabue E, et al. Prognostic significance of laminin production in relation with its receptor expression in human breast carcinomas. Breast Cancer Res Treat 1995;35:195–199.
94. Bornstein P. Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. J Cell Biol 1995;130:503–506.
95. Bornstein P. Thrombospondins as matricellular modulators of cell function. J Clin Invest 2001;107:929–934.
96. Ohta Y, Shridhar V, Kalemkerian GP, Bright RK, Watanabe Y, Pass HI. Thrombospondin-1 expression and clinical implications in malignant pleural mesothelioma. Cancer 1999;85:2570–2576.
97. Varani J, Rister BL, Hughes LA, Carey TE, Fligiel SEG, Dixit VM. Characterisation of thrombospondin synthesis, secretion and cell surface expression by human tumour cells. Clin Exp Metastasis 1989;7:265–276.

98. Starcher BC. Lung elastin and matrix. Chest 2000;117(suppl):229S–234S.
99. Bornstein P. Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. J Cell Biol 1995;130:503–506.
99. Fülöp T, Larbi A. Putative role of 67 kDa elastin-laminin receptor in tumour invasion. Semin Cancer Biol 2002;12:219–229.
100. Blood CH, Sasse J, Brodt P, Zetter BR. Identification of a tumour cell receptor for VGVAPG, an elastin-derived chemotactic peptide. J Cell Biol 1988:107.
101. Martignone S, Menard S, Bufalino R, et al. Prognostic significance of the 67-kilodalton laminin receptor expression in human breast carcinomas. J Nat Cancer Inst 1993;85:398–402.
102. Farquhar MG. The glomerular basement membrane: a selective macromolecular filter. In: Hay ED, ed. Cell Biology of Extracellular Matrix. New York: Plenum, 1981:335–378.
103. Kjellén L, Lindahl U. Structure and functions of proteoglycans. Annu Rev Biochem 1991;60:853–856.
104. Laurent TC, Fraser JRE. Hyaluronan. FASEB J 1992;6:2397–2404.
105. Toole BP. Hyaluronan promotes the malignant phenotype. Glycobiology 2002;12:37R–42R.
106. Meyer K, Chaffee E. Hyaluronic acid in pleural fluid associated with malignant tumour involving pleura and peritoneum. Proc Soc Exp Biol Med 1939;42:797–800.
107. Arai H, Endo M, Sasai Y, et al. Histochemical demonstration of hyaluronic acid in a case of pleural mesothelioma. Am Rev Respir Dis 1975;111: 699–702.
108. Arai H, Kang K, Sato H, et al. Significance of the quantification and demonstration of hyaluronic acid in tissue speciments from the diagnosis of pleural mesothelioma. Am Rev Respir Dis 1979;120:529–532.
109. Wagner JC, Munday DE, Harington JS. Histochemical demonstration of hyaluronic acid in pleural mesotheliomas. J Pathol Bacteriol 1962;84: 73–78.
110. Roboz J, Greaves J, Silides D, Chachinian P, Holland JF. Hyaluronic acid content of effusions as a diagnostic aid for malignant mesothelioma. Cancer Res 1985;45:1850–1854.
111. Frebourg T, Lerebours G, Delpech B, et al. Serum hyaluronate in malignant pleural mesothelioma. Cancer 1987;59:2104–2107.
112. Pettersson T, Fröseth B, Riska H, Klockars M. Concentration of hyaluronic acid in pleural fluid as a diagnostic aid for malignant mesothelioma. Chest 1988;94:1037–1039.
113. Thylen A, Levin-Jacobsen AM, Hjerpe A, Martensson G. Immunohistochemical differences between hyaluronan- and non-hyaluronan-producing malignant mesothelioma. Eur Respir J 1997;10:404–408.
114. Söderblom T, Pettersson T, Nyberg P, Teppo AM, Linko L, Riska H. High pleural fluid hyaluronan concentrations in rheumatoid arthritis. Eur Respir J 1999;13:519–522.
115. Asplund T, Versnel MA, Laurent TC, Heldin P. Human mesothelioma cells produce factors that stimulate the production of hyaluronan by mesothelial cells and fibroblasts. Cancer Res 1993;53:388–392.
116. Dobra K, Andang M, Syrokou A, Karamanos NK, Hjerpe A. Differentiation of mesothelioma cells is influenced by the expression of proteoglycans. Exp Cell Res 2000;258:12–22.
117. Klominek J, Robért K-H, Hjerpe A, Wickström B, Gahrton G. Serum-dependent growth patterns of two, newly established human mesothelioma cell lines. Cancer Res 1989;49:6118–6122.

118. Tzanakakis GN, Karamanos NK, Klominek J, Hjerpe A. Glycosaminoglycans from two human malignant mesothelioma cell lines: determination, distribution, and effect of platelet-derived growth factor on their synthesis. Biochem Cell Biol 1995;73:59–66.
119. Nasreen N, Mohammed KA, Hardwick J, et al. Low molecular weight hyaluronan induces malignant mesothelioma cell (MMC) proliferation and haptotaxis: role of CD44 receptor in MMC proliferation and haptotaxis: Immunohistochemical localization of transforming growth factor beta isoforms in asbestos-related diseases. Oncol Res 2002;13:71–78.
120. Iozzo RV. Matrix proteoglycans: from molecular design to cellular function. Annu Rev Biochem 1998:609–652.
121. Kumar-Singh S, Jacobs W, Dhaene K, et al. Syndecan-1 expression in malignant mesothelioma: correlation with cell differentiation, WT1 expression, and clinical outcome. J Pathol 1998;186:300–305.
122. Hemler ME. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. Annu Rev Immunol 1990;8:365–400.
123. Ruoslahti E, Giancotti FG. Integrins and tumor cell dissemination. Cancer Cells 1989;1:119–126.
124. Jones JL, Walker RA. Integrins: a role as signalling molecules. Mol Pathol 1999;52:208–213.
125. Friedrichs K, Ruiz P, Franke F, Gille I, Terpe HJ, Imhof BA. High expression level of alpha 6 integrin in human breast carcinoma is correlated with reduced survival. Cancer Res 1995;55:901–906.
126. Varner JA, Cheresh DA. Integrins and cancer. Curr Opin Cell Biol 1996; 8:724–730.
127. Lukashev ME, Werb Z. ECM signalling: orchestrating cell behaviour and missbehaviour. Trends Cell Biol 1988;8:437–441.
128. Giuffrida A, Vianale G, Di Muzio M, et al. Modulation of integrin expression on mesotheliomas: the role of different histotypes in invasiveness. Int J Oncol 1999;15:437–442.
129. Rihn BH, Mohr S, McDowell SA, et al. Differential gene expression in mesothelioma. FEBS Lett 2000;480:95–100.
130. Barth TF, Bruderlein S, Rinaldi N, Mechtersheimer G, Moller P. Pleural mesothelioma mimics the integrin profile of activated, sessile rather than detached mesothelial cells. Int J Cancer 1997;72:77–86.
131. Hayman EG, Pierschbacher MD, Ohgren Y, Ruoslahti E. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. Proc Natl Acad Sci USA 1983;80:4003–4007.
132. Boylan AM, Sanan DA, Sheppard D, Broaddus VC. Vitronectin enhances internalization of crocidolite asbestos by rabbit pleural mesothelial cells via the integrin alpha v beta 5. J Clin Invest 1995;96:1987–2001.
133. Eliceiri BP. Integrin and growth factor receptor crosstalk. Circ Res 2001;89: 1104–1110.
134. Raines EW, Ross R. Platelet-derived growth factor in vivo. In: Westermark B, Sorg C, eds. Biology of Platelet-Derived Growth Factor. Basel: Karger, 1993:74–114.
135. Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. Chest 1995;108:754–758.
136. Gottesman M. The role of proteases in cancer. Semin Cancer Biol 1990; 1:97–100.
137. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991;64: 327–336.

138. McCawley LJ, Matrisian LM. Matrix metalloproteinases: multifunctional contributors to tumor progression. Mol Med Today 2000;6:149–156.
139. Stamenkovic I. Matrix metalloproteinases in tumor invasion and metastasis. Semin Cancer Biol 2000;10:415–433.
140. Chang C, Werb Z. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. Trends Cell Biol 2001;11:S37–43.
141. Marshall BC, Santana A, Xu QP, et al. Metalloproteinases and tissue inhibitor of metalloproteinases in mesothelial cells. Cellular differentiation influences expression. J Clin Invest 1993;91:1792–1799.
142. Liu Z, Ivanoff A, Klominek J. Expression and activity of matrix metalloproteases in human malignant mesothelioma cell lines. Int J Cancer 2001; 91:638–643.
143. Hirano H, Tsuji M, Kizaki T, et al. Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinase, collagens, and Ki67 antigen in pleural malignant mesothelioma: an immunohistochemical and electron microscopic study. Med Electron Microsc 2002;35:16–23.
144. Liu Z, Klominek J. Regulation of matrix metalloproteases activity in malignant mesothelioma cell line by multiple growth factors. Thorax 2003.
145. Harvey P, Warn A, Dobbin S, et al. Expression of HGF/SF in mesothelioma cell lines and its effects on cell motility, proliferation and morphology. Br J Cancer 1998;77:1052–1059.
146. Liu Z, Klominek J. Inhibition of proliferation, migration and matrix metalloproteases production in malignant mesothelioma cell lines by tyrosine kinase inhibitor ZD1839 (Iressa). Proceedings of the 6th Meeting of the International Mesothelioma Intrest Group Abstract 32, 2002.
147. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295:2387–2392.

12

Genomics and Proteomics in Mesothelioma

Anil Wali and Harvey I. Pass

Advances in molecular biology over the past decade have improved our understanding of genetic, transcriptional, and translational alterations in human cancers. The sequencing of the human genome has resulted in the identification of many known and novel genes. Several groups are engaged in determining the interactions and regulation of all these genes to ascertain their function in early detection and prevention of cancer. Recent advances in functional genomic technology have begun to investigate interactive pathways to elucidate what, where, when, and how these genes are expressed in an orchestrated fashion. Other groups have concentrated on proteomics, or the study of proteins, including their relative amount, distribution, posttranslational modifications, functions, and interactions to address fundamental biologic questions in the progression of a disease from a normal to a cancerous state. This chapter discusses the functional genomics and expression proteomics approaches employed to date in general and their relevance to mesothelioma in particular. It is our attempt to provide both novice and experienced investigators in this field with novel methodologies used in other types of cancers that might ultimately lead to the early detection and treatment of mesothelioma.

Genomics in Cancer Research

The Human Genome Project analysis has described 30,000 to 50,000 genes after DNA sequencing analyses. In spite of 20% to 30% differences observed between the predicted transcriptomes by International Human Genome Sequencing Consortium (HGSC) and Celera Genomics, these data have provided a tremendous stimulus for systematic analysis of various types of cancer. High-resolution analysis of chromosomal aberrations, genome-wide mutation screens, and expression profiling have given investigators a comprehensive view of genetic alterations in many cancers. These high-throughput technologies are being vigorously pursued to gain a complete list of the molecular and genetic causes that drive malignant transformation and

the possible therapeutic options that may be exploited for clinical benefit.

Comparative Genomic Hybridization (CGH) Analysis

Comparative genomic hybridization (CGH) analysis is a DNA-based molecular cytogenetic technique that allows the identification of chromosomal imbalances (gains, losses, and amplification of DNA sequences) in an entire tumor genome in a single experiment. An equal proportion of biotin-labeled tumor DNA and digoxigenin-labeled normal reference DNA is hybridized to normal metaphase chromosomes. The variance in signal intensities of the two fluorochromes [fluorescein isothiocyanate (FITC) for tumor DNA and the tetrarhodamine isothiocyanate (TRITC) for the normal DNA] are detected in a fluorescence microscope, and captured images are then evaluated with a special image analysis program. The over- and under-represented DNA segments are quantitated by FITC/TRITC ratios for every single chromosome. The accumulation of various clonal chromosomal deletions in most malignant mesotheliomas is indicative of a multistep progression of tumorigenesis, such as gene point mutations, partial deletion, epigenetic silencing, gene amplification, gene rearrangements, and/or complete gene loss. All these mechanisms could be involved in the genesis of a mesothelioma from an occult to advanced stage. Cytogenetic and loss of heterozygosity (LOH) analyses of malignant mesothelioma have demonstrated frequent deletions of specific sites within chromosome arms 1p, 3p, 6q, 9p, 13q, 15q, and 22q and trisomies and polysomies of chromosomes 1, 5, 7, 11, 12, and 20. Furthermore, current information has confirmed the involvement of these five tumor suppressor genes: p16, p15, p53, NF2, and WT1.

Restriction Landmark Genome Scanning (RLGS)

Restriction landmark genome scanning (RLGS) is a highly resolving gel-based technique in which several thousand fragments in genomic digests are visualized simultaneously and quantitatively analyzed. The genomic DNA is radioactively labeled at cleavage sites specific for a rare cleaving restriction enzyme, Not1, followed by first-dimension electrophoresis. By subjecting separated DNA fragments to in situ digestion with a frequent cutter prior to a second-dimension electrophoresis, several thousand fragments from the genome can be resolved and visualized (1). The digestion of genomic DNA by NotI prior to labeling generates landmarks that allow visualization of DNA fragments that occur preferentially in CpG islands (2). Because of the localization of CpG islands in proximity to transcribed sequences (3), there is a strong possibility that NotI fragments detected in RLGS scans occur in the vicinity of coding sequences.

There are many applications of RLGS that stem from its quantitative reproducibility. For instance, RLGS could be useful for studies of restriction fragment length polymorphisms; for identifying genomic insertions, deletions, or amplifications; and for identifying somatic methylation changes (4,5). The widespread use of RLGS has been

hampered by difficulty in deriving sequence information for displayed fragments and a lack of whole-genome sequence-based framework for interpreting RLGS patterns. Recently, a collaborative effort among several laboratories has resulted in the development of bioinformatic tools for comparisons of sample-derived RLGS patterns with patterns predicted from the human genome sequence and displayed as virtual genome scans (VGS). The tools developed allow sequence prediction of fragments in RLGS patterns obtained with different restriction enzyme combinations. The utility of VGS is demonstrated by the identification of restriction fragment length polymorphisms, and of amplifications, deletions, and methylation changes in tumor-derived CpG islands and the characterization of an amplified region in a breast tumor that spanned <230 kilobase (kb) on 17q23 (6). Recently, using RLGS in combination with promoter methylation studies, a novel lung cancer–related gene, bone morphogenetic protein 3B (BMP3B) was identified on chromosome 10q11 (7).

Only one study utilizing RLGS in mesothelioma has been described: RLGS analysis using Not1-EcoRV-Hinf1 restriction enzyme digestion on five malignant pleural mesothelioma was carried out that showed losses in chromosomal locations 22q13.1, 17q23.2, 4p16.2, 12p13.2 and 1p34.1 (unpublished data).

Serial Analysis of Gene Expression (SAGE)

Serial analysis of gene expression (SAGE) is a comprehensive method for analysis of gene expression patterns (8). There are three main underlying principles that define SAGE methodology: (1) A short sequence tag [10–14 base pair (bp)] contains sufficient information to uniquely identify a transcript provided that the tag is obtained from a unique position within each transcript. (2) Sequence tags can be linked together to form long serial molecules that can be cloned and sequenced. (3) Quantitation of the number of times a particular tag is observed provides the expression level of the corresponding transcript. Recent technologic advances have made large-scale gene expression measurements routine. Serial analysis of gene expression counts polyadenylated transcripts by sequencing a short 14-bp tag at the gene's 3′ end, adjacent to the last restriction site, normally NlaIII. All expressed transcripts with a NlaIII site can be "tagged" and counted efficiently in large numbers (typically <50,000 per RNA sample) by using automated sequencing. The tag counts are then archived electronically for future analysis and digital comparisons. To provide quantitative expression levels on a genome-wide scale, the Cancer Genome Anatomy Project (CGAP) uses the SAGE project as one of the largest suppliers of a public gene expression database (9,10). These data are posted at the National Center for Biotechnology Information's SAGEmap Web site (http://www.ncbi.nlm.nih.gov/SAGE), where SAGE tags are assigned to UniGene clusters, differentially expressed tags can be identified, and the expression level of a particular tag can be displayed (11,12). SAGEmap has been quite a powerful tool, but recently an improvised version has been reported, known as SAGE

Genie (http://cgap.nci.nih.gov/SAGE). It consists of SAGE Anatomic Viewer, which allows nearly any gene's transcript levels to be easily viewed in normal and malignant tissues. The anatomic view is based on a growing set of over 5.2 million SAGE tags assembled from 114 cell types, plus new Web tools to view these data. These informatics allow SAGE Genie to automatically identify SAGE tags from a gene's primary or alternatively polyadenylated transcript while screening for experimental artifacts. A large archive of SAGE data is now more accurately and easily viewed by using SAGE Genie, including a means to see anatomically based gene expression (13). To date, SAGE analysis has compared the gene epression from a surgically resected malignant pleural mesothelioma (MPM) to the patient's autologous normal mesothelium and the results have been posted on the Web: (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM727).

SELEX-SAGE

To determine the location and relative strength of all transcription-factor binding sites within a genome is important not only for a comprehensive understanding of gene regulation but also for effective promoter applications. A bioinformatically driven experimental method has been devised to accurately define the DNA-binding sequence specificity of transcription factors. Computer simulations showed that several thousand low- to medium-affinity sequences are required to generate a profile of desired accuracy. To produce data on this scale, a method combining systematic evolution of ligands by exponential enrichment (SELEX) and serial analysis of gene expression (SAGE) protocols was coupled to an automated quality-controlled sequence extraction procedure. This allowed the sequencing of a database of more than 10,000 potential DNA ligands for the CTF/NFI transcription factor. The database is publicly available at http://www.isrec.isb-sib.ch/selex_nf1/. The resulting binding-site model defines the sequence specificity of this protein with a high degree of accuracy not achieved earlier, and thereby makes it possible to identify previously unknown regulatory sequences in genomic DNA (14).

Gene Expression Profile Using DNA Microarrays

Global gene expression profiles for several types of tumors, using DNA microarrays, have been published recently that delineate distinct patterns of gene expression among subsets of related tumors (15). These studies have examined pathologically homogeneous as well as heterogeneous set of tumors to identify clinically relevant subtypes, high-stage versus low-stage tumors of the same lineage to identify molecular correlates, and tumors of different lineages for specific molecular signatures. The landmark study that caught much attention in this area was the uncovering of novel tumor subtypes of diffuse large B-cell lymphoma (16). Two molecularly distinct forms of this tumor were uncovered that were reflective of different stages of B-cell differentiation. One type expressed genes characteristic of germinal center B cells

with significantly better overall survival than the second type, which expressed genes normally induced during in vitro activation of peripheral blood B cells. This study marked the beginning of identifying previously undetected and clinically significant subtypes of lymphoma. Recently, two groups have been able to show a clear distinction among acute lymphoblastic leukemia (ALL) subtypes and acute myelogenous leukemia (AML) and the mixed-lineage leukemia (MLL) (17).

Differential Gene Expression Patterns in Mesotheliomas

The differential display technology works by systematic amplification of the 3′ terminal portions of messenger RNAs (mRNAs) by using anchored primers that bind 5′ boundary of the poly-A tails by reverse transcription, followed by polymerase chain reaction (PCR) amplification with additional upstream primers of arbitrary sequences and resolution of those fragments on a DNA sequencing gel. This methodology helps visualize all the expressed genes using side-by-side comparisons between or among related cells (18). To better understand malignant mesothelioma pathobiology, researchers have used the technique of differential display to compare gene expression patterns in mesothelioma, normal pleura, and normal lung. The human inhibitor of apoptosis protein-1 gene (*IAP-1*/*MIHC*/*cIAP2*) was discovered to be highly expressed in malignant pleural mesothelioma (MPM) tumors and cell lines (19). The overexpression of IAP-1 mRNA and protein was validated by multiple methods, including real-time quantitative reverse-transcription PCR and Western blot analysis. The main drawback of the above technique has been low specificity, which results in false positives due to the low annealing temperature of PCR. To circumvent these shortcoming, modifications with the longer arbitrary primer design (25-mer) and lock-docking oligo (dT) primers (29-mer) for RNA fingerprinting have yielded high-stringency PCR products relative to differential display. This modified differential display method, called *RNA fingerprinting*, has been used to identify mRNAs that were differentially expressed during human mesothelial cancer progression. Five different clones were identified using this procedure. Two clones were expressed in the metastatic mesothelioma cell line M1A and the malignant mesothelioma cell line M1, one clone was expressed uniquely in the metastatic cell line M1A, and one clone was solely expressed in the normal mesothelial cells. Three clones had no homology to known genes, whereas the other two clones had a striking sequence homology to the M130 antigen and rab 12 mRNA, respectively. These sequence tags may be of interest as a specific mesothelioma tumor marker (20). A mesothelioma cell line with retained ability to differentiate into either epithelial or fibroblast-like phenotype has been studied to identify the genes related to tumor cell differentiation using subtractive hybridization. Nine genes were found to be selectively overexpressed in the epithelial sub-line, compared to only two genes in the fibroblast-like phenotype. One of the genes that was differentially expressed by the epithelial cells was thioredoxin, a small redox-active protein associated with cell growth and differentiation (21).

Subtractive complementary DNA (cDNA) hybridization has been shown to identify and isolate cDNAs from differentially expressed genes. In general, it involves hybridization of two populations of cDNAs (test and control) and then separation of the unhybridized fraction (target) from hybridized common sequences that would represent the unique differentially expressed genes among the two populations. An improvisation of this methodology was achieved by a PCR-based cDNA subtraction method called suppression subtractive hybridization (SSH) (22), which is used to selectively amplify target cDNA fragments (differentially expressed) and simultaneously suppress nontarget DNA amplification by attaching long inverted terminal repeats to the DNA fragments. The mRNA expression patterns at different stages of asbestos-induced carcinogenesis in rats have been monitored by SSH and array assay. Several genes were found to be upregulated in pretumorous tissues from asbestos-treated rats, in asbestos-induced tumors, and in cells treated with asbestos in vitro. The upregulation of the proto-oncogenes c-*myc*, *fra-1*, and *egfr* in fiber-induced carcinogenesis was demonstrated at different stages of carcinogenesis. The upregulation of osteopontin, zyxin, and integrin-linked kinase in this study was indicative of a possible link between fibers, integrin-linked signal transduction, and extracellular matrix proteins (23). Recently, these studies were further substantiated by another group showing induction of CD44 and c-*met* were linked to *fra-1* expression using microarrays (24).

One of the first mesothelioma cDNA array experiments was carried out on four malignant mesothelioma (MM) cell lines and two primary mesothelial cell cultures established from pleural fluid of noncancer patients. Human cancer gene filters including 588 genes were used for the cDNA array experiments. The study revealed 26 overexpressed genes that play a role in the regulation of cell cycle, cell growth, and DNA damage repair, and 13 underexpressed genes encoding growth factors, receptors, and proteins involved in cell adhesion, motility, and invasion and that are common to three or four MM cell lines. The study presented gene expression profiles in MM cell lines and showed the involvement of several genes, such as *JAGGED1*, *ser/thr*, protein kinase *NIK*, *Ku80*, and cyclin *D2*, to be novel in MM (25). Similar conclusions were drawn using high-density filter arrays of more than 6500 genes to compare constitutive gene expression of mesothelioma cells to that of pleural cells. Most of the highly expressed transcripts were common to both cell lines and included genes associated with stress response and DNA repair. Interestingly, fewer than 300 genes that differed between cell lines were involved in macromolecule stability, cell adhesion and recognition, cell migration (invasiveness), and extended cell division (25–27).

To make a pathologic distinction between MPM and adenocarcinoma (ADCA) of the lung has been quite challenging given the existing set of markers for this purpose. Recently, a simple technique, based on the expression levels of a small number of genes, was designed to accurately distinguish between genetically disparate tissues using gene expression ratios and rationally chosen thresholds. The study tested the

fidelity of ratio-based diagnosis in differentiating between MPM and lung cancer in 181 tissue samples (31 MPM and 150 ADCA). Validation of this microarray data and ratio-based diagnosis was performed using calretinin/claudin-7 and VAC-β/TACSTD1 ratios; the two ratios correctly identified 23 of 24 samples. Using two and three expression ratios, it was found that the differential diagnoses of MPM and lung ADCA were 95% and 99% accurate, respectively (29,30).

Recently, cDNA microarray filters with 4132 clones were used to identify differential gene expression profile among four control pleural tissue samples and 16 mesothelioma patient tumor specimens (31). Use of various normalization and analysis approaches showed significant induction of 166 genes and downregulation of 26 genes among these two groups. Expression profiling showed marked upregulation of genes involved in glucose metabolism, protein translation, and cytoskeletal remodeling pathways. Prominent upregulated genes included *gp96*, lung resistance-related protein, galectin-3-binding protein, laminin receptor, and voltage-dependent anion channels.

Pass et al (32) have very recently shown that gene expression profiles in malignant pleural mesothelioma can predict time to progression and survival patterns among two separate series of patients who underwent cytoreduction at two different centers in the United States. The study involved gene expression profile analysis on 21 MPM patient tumor samples that had identical postoperative adjuvant therapy (Fig. 12.1). Analysis of Affymetrix gene chips U95, representing 12,000 gene probe sets, was performed using dChip, significance of analysis (SAM), and GeneSight software packages. A neural network was constructed using a common set of 27 gene classifiers that could segregate patient populations based on short or long survival after debulking of their tumors. The 27 gene classifiers were able to predict actual time to progression and survival with 95.2% accuracy in one test set, whereas 76% accuracy was achieved in the separate validation set of MPMs. These data are indicative of pretherapy gene expression analysis that can be beneficial in predicting clinical outcomes of patients undergoing surgical treatments. Considering all the published literature so far on gene expression profiling on MPM cases, it is plausible to conceive of having a precise "genetic signature" among patient populations that can predict the patient's stage, histology, response to therapeutic options, and clinical outcome.

Proteomics in Cancer Research

An important step in the postgenomics era has been decoding the functions of some 30,000 genes that are scattered among 3.2 billion nucleotides. Research in proteomics involves study of the structure, function, and expression analysis of all proteins in the normal as well as pathophysiologic conditions during various stages of development. Cancer proteomics can be defined as the implementation of proteomic platforms to identify and quantitate differentially expressed proteins relative to normal tissue from preneoplasia to neoplasia. Recent

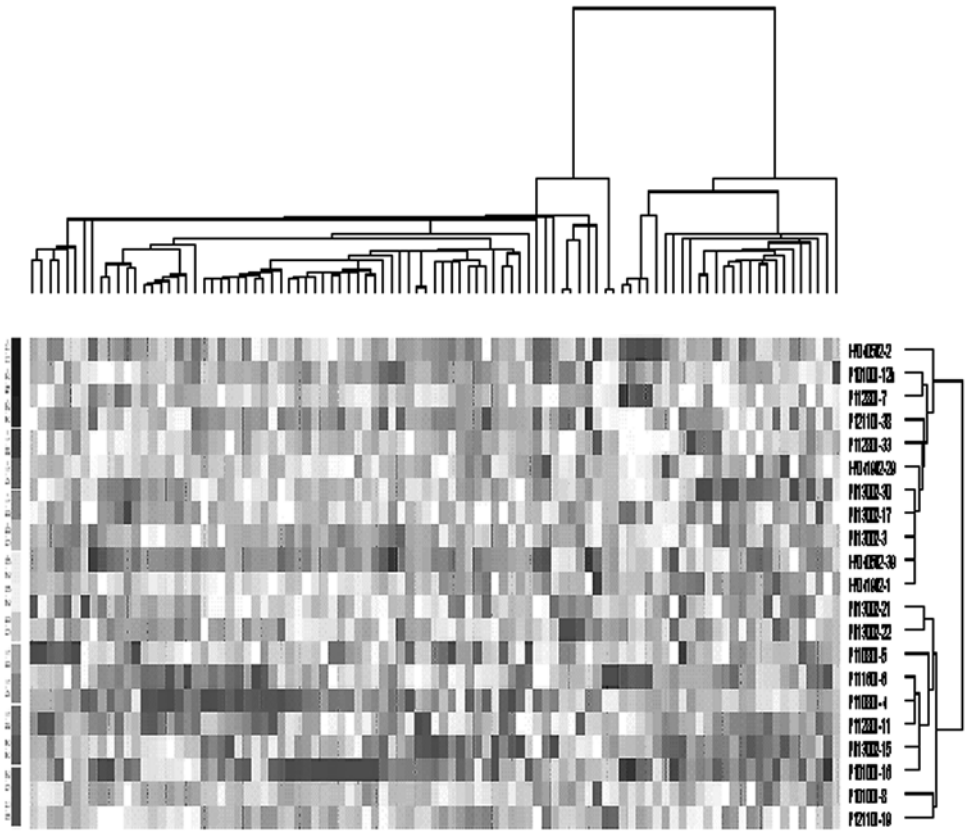


Figure 12.1. Gene expression arrays were performed in 21 patients having surgery for malignant pleural mesothelioma. Twenty-seven significant genes common to both dChip and SAM analyses from the original set of 95 genes depicted here were able to define two groups of patients whose time to progression and survival were significantly different (25).

methodologies in proteomics also can be used to identify early detection biomarkers for cancer, prognostication, and identification of potential therapeutic targets. Valuable discoveries of biomarkers have been possible because of the proteome research efforts that entailed both the interaction between the functional pathways of a cell and its impact on environmental milieu. Both posttranscriptional and translational modifications are necessary in the proper expression and function of proteins. Differential splicing can yield several RNA transcripts from one gene. Furthermore, many posttranslation modifications have been shown to alter function, stability, protein–protein and DNA–protein interactions, and targeting (33), all of which ultimately yield a potentially large number of protein products from one gene. Prominent changes have been observed during the conversion of a healthy cell into a neoplastic cell that could result in altered expression, detrimental protein modifications, and cell localization, which ultimately result in aberrant cellular function. Identification and in-depth understanding of all these processes are the main thrust in cancer proteomics.

Technologies Involved in Expression Proteomics

The most widely used techniques for the characterization of proteins are two-dimensional gel electrophoresis (2DGE), mass spectrometry (MS), amino acid composition analysis, and peptide sequence tagging. Some approaches include recombinant proteins obtained using cDNA expression libraries and phage-display libraries. Some approaches use high-throughput antibody arrays. Matrix-assisted laser desorption ionization (MALDI), surface-enhanced laser desorption ionization (SELDI), laser capture microdissection (LCM), and capillary electrophoresis (CE) have been added to the proteomics tool set. Other techniques include isotope-coded affinity tags (ICAT) and 2D differential fluorescence gel electrophoresis (DIGE) in order to quantify relative protein expressions. We summarize these technologies here in relation to the advances made in various types of cancers that could be implemented in the field of mesothelioma carcinogenesis.

Two-dimensional gel electrophoresis (2DGE) has been the method of choice in quantitative proteomics. In brief, protein samples are denatured and separated on the basis of their charge through isoelectric focusing. Almost complete resolution of both basic and acidic proteins have been achieved by the introduction of immobilized pH gradients into this system (34). The proteins are further separated by migration in a polyacrylamide gel on the basis of their molecular weights. Silver-staining protocols have enhanced resolution and visualization of 3000 proteins on a single gel. Fluorescent dyes have been developed to make the protein samples accessible to mass spectrometry (35). Laser densitometers with high resolutions have been used in spot detection of stained gels that can be further analyzed with software packages such as PDQUEST (36) and Phoretix (37) for all quantitation purposes. Ratio analysis is the most common form of detecting quantitative changes among different protein samples. Two-dimensional gel electrophoresis is currently being modified to the point where it can be used in high-throughput platforms (38).

Mass spectrometry (MS)-based methodologies have proved to be a powerful means for obtaining peptide mass fingerprints for proteins resolved in 2DGE gels. Protein databases and reference maps have been generated to document changes in protein expression resolved by 2DGE from various cell types and different stages of tumor development. Specialized software packages available on the World Wide Web servers such as ExPaSy and the World 2DGE page (39), have been utilized by biomarker research investigators to compare 2D gel patterns with one another and with reference maps on the Internet. Using this techonology, the proteome of 150 bladder tumors was observed to decline in the expression of specific cytokeratins, psoriasin, galectin 7, and stratifin in tumors with a low degree of differentiation (40). A map of healthy and renal cell carcinoma (RCC) proteins has been constructed through 2DGE analysis of healthy and RCC kidney tissue, which led to the identification of ubiquinol cytochrome C reductase as a potential biomarker (41). A comprehensive analysis of the proteome in lung cancer has already been undertaken (42). In a recent study

involving a series of 93 lung adenocarcinomas (64 stage I and 29 stage III) and 10 uninvolved lung samples, nine candidate proteins such as antioxidant enzyme AOE372, adenosine triphosphate (ATP) synthase subunit d (ATP5D), β1,4-galactosyltransferase, cytosolic inorganic pyrophosphatase, glucose-regulated Mr 58,000 protein, glutathione-S-transferase M4, prolyl 4-hydroxylase β subunit, triosephosphate isomerase, and ubiquitin thiolesterase (UCHL1) were identified as being significantly overexpressed in lung adenocarcinomas. The expression of these proteins was increased from 1.4- to 10.6-fold as compared with uninvolved lung tissue (43).

To classify human lung cancer tumors, matrix-assisted laser desorption/ionization mass spectrometry was recently performed on frozen tissue sections. Proteomic spectra were aligned from 79 lung tumors and 14 normal lung tissues, and a class-prediction model was built with the proteomic patterns in a training cohort of 42 lung tumors and eight normal lung samples. To assess statistical significance, a blinded test cohort of 37 lung tumors and six normal lung samples was used. A class-prediction model was based on 1600 differentially expressed peaks that perfectly classified lung cancer histologies, and distinguished primary tumors from metastases with 85% accuracy in the training cohort. This model nearly perfectly classified samples in the independent blinded test cohort. Furthermore, a proteomic pattern composed of 15 distinct mass spectrometry peaks could distinguish between good and poor prognosis among non–small-cell lung cancer (NSCLC) patients (44). To date, our laboratory is the only one that has performed 2DGE on four mesothelioma tumors and their respective cell lines (Fig. 12.2).

Several ionization techniques, such as electrospray ionization and MALDI, have facilitated the characterization of proteins by MS (45). For sequence-based determinations by MS, proteins are cleaved with trypsin or cyanogen bromide into peptide fragments and separated by high-pressure liquid chromatography (HPLC) followed by tandem MS. The peptides are then subjected to short pulses of ultraviolet radiation under reduced pressure. Some of the peptides are ionized and accelerated in an electric field and subsequently turned back through an energy correction device (46). Peptide mass is derived through a time-of-flight (TOF) measurement of the elapsed time from acceleration-to-field free drift or through a quadrupole detector. A peptide mass map is generated with the sensitivity to detect molecules at a few parts per million. Hence, a spectrum is generated with the molecular mass of individual peptides, which are used to search databases to find matching proteins. This approach is termed "peptide sequence tagging." The short (2–4 amino acids) sequence can be derived by fragmentation of the parent ion into three complementary segments: the mass of an N-terminal fragment, the mass of a C-terminal fragment, and the partial amino acid sequence between them.

The alternative process of ionization, through the electrospray ionization, involves dispersion of the sample through a capillary electrophoresis (CE TOF MS) (47). The charged peptides pass through a mass spectrometer under reduced pressure and are separated accord-

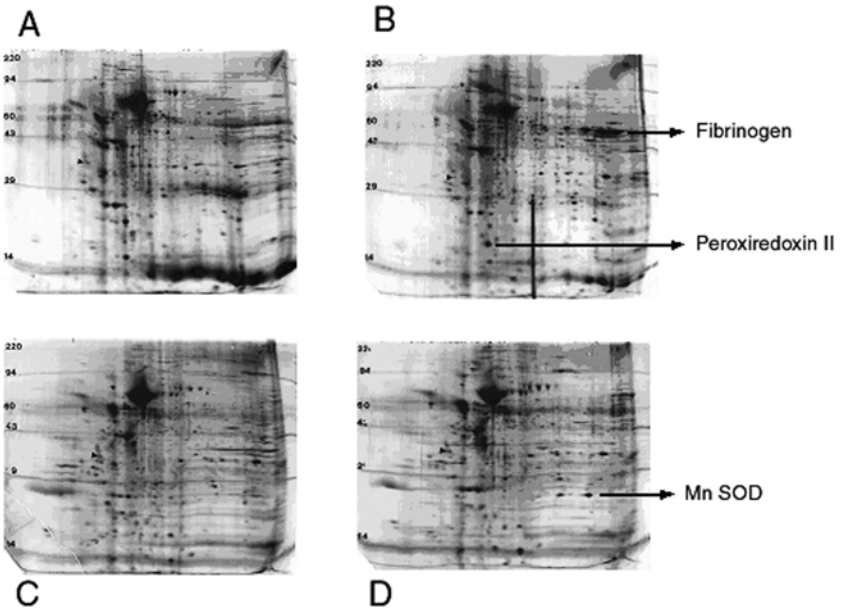


Figure 12.2. A: Two-dimensional gel electrophoresis proteomic analysis of normal human peritoneum. B: Malignant pleural mesothelioma (MPM) tumor. C: Simian virus 40 large-tumor antigen (SV40 Tag) immortalized human mesothelial cell line (MeT5A). D: Corresponding MPM patient–derived cell line using (2DGE). These 2DGE images were analyzed by the Phoretix 2D software package to identify differentially expressed proteins in mesothelial cells and mesotheliomas. Three prominent spots (arrows) were chosen for further analysis using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and identified as fibrinogen, peroxiredoxin II, and manganese (Mn) superoxide desmutase (SOD). All three proteins have been implicated in the pathogenesis of malignant pleural mesothelioma by various laboratories (75–77).

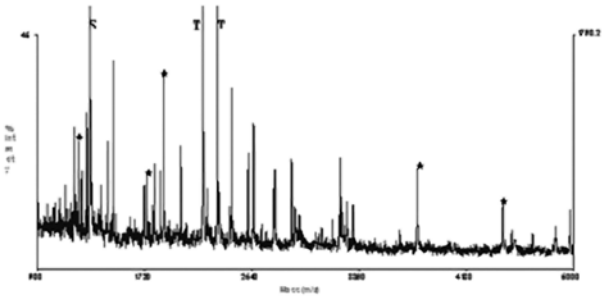
ing to their mass-to-charge ratios through electric fields. After separation through 2DGE, digested peptide samples can be delivered to the mass spectrometer through a "nanoelectrospray" or directly from a liquid chromatography column (liquid chromatography-MS), allowing for real-time sequencing and identification of proteins. Some modifications have led to the MALDI quadrupole TOF instrument, which combines peptide mapping with the peptide sequencing approach (48,49). Accurate identification of posttranslational modifications, such as phosphorylation and glycosylation, have been possible by the assessment of mass shifts. The MALDI-TOF-MS of 2DGE-separated proteins has been pivotal in the identification of multimeric isoforms of manganese superoxide dismutase found to be expressed exclusively in RCCs (47). The MALDI-MS system has also helped to detect increases in the expression of nuclear matrix, redox, and cytoskeletal proteins in breast carcinoma compared to benign tumors (36) and identification of peroxiredoxin II (Prx II) by peptide mass fingerprinting in MPM (Fig. 12.3).

Amino acid composition analysis is an efficient method for protein identification (50). The method is complementary to MS and represents a useful analytical tool for the mapping of proteins of interest.

Direct mapping and imaging of biomolecules present in frozen sections has been recently achieved by an innovative technology termed "imaging MS." For this analysis, frozen tissue sections mounted on a metal plate, coated with ultraviolet-absorbing matrix are placed in the MS, and the specimens are processed as described previously for liquid samples (51). Imaging MS has shown promise in several applications such as discovery of biomarker tissue localization, molecular interactions of tumor cells, and assessment of surgical complexities of tumors (52).

Identification of Prx II by MALDI-MS, tryptic peptide map and aa sequence analysis

A



B

Apparent mass	Theoretical mass	Position	Peptide sequence
1212.52	1212.39	140 - 150	QITVNDLPVGR
1735.69	1736.02	93 - 109	EGGLGPLNIPLLADVTR
1864.15	1864.19	92 -109	KEGGLGPLNIPLLADVTR
3804.88	3805.16	158 - 191	LVQAFQYTDEHGEVCPAAWKPGS DTIKPNVDDSK
4460.54	4459.89	158 -196	LVQAFQYTDEHGEVCPAAWKPGS DTIKPNVDDSKEYFSK

C

```
  1  masgnarigk papdfkatav vdgafkevkl sdykgkyvvl ffypldftfv cpteiiaftt
 61  vkrtsaklgc evlgvsvdsq fthlawintp RKEGGLGPLN IPLLADVTRr lsedygvlkn
121  degiayrglf iidgkgvlrQ ITVNDLPVGr svdealrLVQ AFQYTDEHGE VCPAAWKPGR
181  DTIKPNVDDS KEYFSKhn
```

Figure 12.3. Identification of peroxiredoxin II (Prx II) by peptide mass fingerprint. A: One protein spot (marked in Fig. 12.4) was excised from the gel, and digested with trypsin and analyzed by MALDI-MS. Peptide mass spectrum was obtained by MALDI–time-of-flight (TOF) mass spectrometry. B: Masses of five tryptic peptides were matched with Prx II within the 10-ppm range by database searching. The corresponding mass peaks are marked with asterisks (A). C: The sequence of Prx II is represented by a single-letter code for amino acids. Sequence coverage by five peptides is indicated by capital letters.

Most of the problems associated with sample preparations with MALDI-MS have been overcome with the advent of surface-enhanced laser desorption-ionization (SELDI) (53). The main principle in SELDI is surface-enhanced affinity capture through the use of specific probe surfaces or chips. This protein biochip is the counterpart of the array technology in the genomic arena that forms the basis for Ciphergen's ProteinChip array SELDI MS technology (49). A 2DGE analysis separation is not required for SELDI analysis as it can bind protein molecules based on its defined chip surfaces. Chips with broad binding properties, including immobilized metal affinity capture, and with biochemically characterized surfaces, such as antibodies and receptors, form the core of SELDI (49). The advantage of this MS technology involves no preprocessing of the samples before analysis, and that has expedited both biomarker discovery and protein profiling in small sample volumes in a short period of time. After being captured on the SELDI protein biochip array, proteins are detected through the ionization-desorption, TOF-MS process. A retentate (proteins retained on the chip) map is generated in which the individual proteins are displayed as separate peaks on the basis of their mass and charge (m/z). The SELDI technology has been used to provide protein fingerprints (54), which may provide insights into changing protein expression from healthy to benign, and subsequently from premalignant to malignant lesions. Recently, distinct SELDI protein profiles and patterns for individual cells and cancer type have been evaluated, including prostate, lung, and ovarian cancer (55). The versatile platform of the ProteinChip SELDI-MS technology has given a major impetus to the proteomics field, not only for the discovery and protein profiling applications, but also as a potential multiplex immunoassay tool. For this application, antibody rather than a chemical matrix is bound to the chip array to capture the protein antigen. This format has been successfully used to develop both single and multiplex versions of the SELDI immunoassay for detection and measuring prostate-specific antigen (PSA) and prostate-specific membrane antigen in body fluids (56,57).

Pass et al investigated SELDI-TOF to analyze protein expression profiles in pleural effusions from patients with cytologically documented benign inflammatory fluid collections, non-MM malignant effusions, and MM effusions. The 58 discovery samples were randomized in a 96-well format for fractionation, and a validation set of 50 blinded samples was then randomized and processed in a similar fashion followed by analysis with classification algorithms defined with data from the discovery samples.

Spectra from the discovery sample set were analyzed using the Biomarker Wizard in ProteinChip Software version 3.0 to detect peak clusters, which were subjected to univariate statistical analysis using the Mann-Whitney test of means. All peak clusters were also subjected to multivariate statistical analysis using Ciphergen's Biomarker Patterns Software (BPS, 1) and Salford Systems' TreeNet. The 58 discovery samples consisted of 30 patients with MM, 22 noncancer control patients, and six control patients with non-MM cancers. Data from these 58 samples were utilized for the discovery of biomarker candi-

Table 12.1. Results for predictive algorithms for malignant mesothelioma (MM) vs. control pleural effusions

	Three nonneutrophil peaks	Six peaks	All peaks
Sensitivity (%)	**82**	**88**	**100**
Specificity (%)	**91**	**88**	**88**
Positive predictive value (%)	**92**	**88**	**89**
Negative predictive value (%)	**81**	**88**	**100**

dates and for the determination of classification algorithms to be tested with data from the validation set of 50 blinded samples.

From the biomarker discovery phase of the project, the six best candidates as determined by univariate analysis and BPS were chosen for purification and identification. They had approximate molecular masses of 11.6, 10.8, 3.4, 5.0, 6.6, and 4.6 kd and were all decreased in amount for the MM patients relative to the control discovery samples.

Three different predictive algorithms were defined utilizing the TreeNet software with sets of markers selected from the discovery samples. With the predictive algorithms, three classification predictions were established for the 50 validation samples using the different marker combinations. The unblinding of individual patient diagnosis enabled calculation of the accuracy of the three predictions and this is seen in Table 12.1.

Isotope-coded affinity tags (ICAT) has been the latest addition to the arsenal of new technologies developed in the field of proteomics (60). This technique utilizes a thiol-specific reactive group (iodoacetamide) to react with free cysteine residues in the denatured protein samples via two ICAT reagents, such as d8-ICAT (X = deuterium) heavy; and d0-ICAT (X = hydrogen) light reagents. A nonreactive linker incorporates heavy or light ICAT reagent with the biotin affinity tag group in that sample. The control and experimental samples are combined, proteolyzed, fractionated, and avidin affinity enriched before LC/MS is performed to quantify ratios of all peptides having a mass difference of 8 atomic mass unit (amu). The most impressive usage of ICAT technology has resulted in quantifying the relative level of expression of 524 proteins that could be potential serum markers of neoplastic prostate disease (61). Recent progress with ICAT technology has incorporated ^{13}C rather than 8 deuteriums into the heavy reagent. An alternative approach to quantitative protein profiling has been developed by using Cy-3 and Cy-5 in vitro labeling of control and experimental proteins, which has increased the magnitude of the linear response range significantly, compared to existing staining protocols. This differential 2D DIGE has been used in the proteomic expression analysis of esophageal and breast cancer cell systems (62,63).

Protein and Antibody Microarrays

Biochip-based microarrays containing spotted antigens or antibodies have been developed to study protein–protein interactions, biomarkers,

and humoral response to cancer (64,65). Arrays of clones from phage-display libraries have been probed with antigen-coated filters for high-throughput antibody screening (66). Protein microarrays have been prepared by printing the proteins on coated microscope slides using purified proteins, recombinant proteins, and crude mixtures or antibodies with a robotic arrayer. Protein solutions to be measured are labeled by covalent linkage of a fluorescent dye to the amino groups on the proteins (67). Protein arrays have been used to identify and track cancer progression by immobilization of proteins from pure populations of microdissected cells from various tissues (68). Synthesis of protein and antibody arrays has proven to be more costly and labor-intensive compared to DNA arrays. It is expected, however, that the availability of large antibody arrays will enhance the discovery of differential biomarkers in normal and diseased tissue. For excellent reviews on current and innovative technologies used in protein-antibody array applications for medical research, see Cahill (69) and others (64,65).

Tissue Microarrays

Tissue microarrays have become an integral part of high-throughput molecular profiling of tumor specimens, and are used for rapid validation of genomic and proteomic arrays (70). Arrays are generated by robotic punching out of small cylinders (0.6 mm × 3–4 mm high) of tissue from thousands of individual tumor specimens embedded in paraffin and then arrayed in a large paraffin block. Tissue from as many as 600 specimens can be represented in a single "master" paraffin block. Subsequent serial sections of the same tissue array helps analysis of multiple tumor samples in parallel by immunohistochemistry, fluorescence in situ hybridization, and RNA–RNA in situ hybridization. Tissue arrays have thus expedited the simultaneous analysis of tumors from many different patients at different stages of disease. Disadvantages of this technique are that a single core is not representative because of tumor heterogeneity and uncertainty of antigen stability on long-term storage of the array. Large-scale validation efforts are currently ongoing in breast and prostate cancers that are expected to strengthen protein expression profiling (71,72). The biggest advantage of this technology has been the rapid turnaround time to assess expression profiles and patterns with clinical outcomes from large cohorts. Our laboratory has produced mesothelioma tumor arrays consisting of 60 MPM tumors per slide that are being used on a routine basis to validate other ongoing genomic and proteomic initiatives (Fig. 12.4).

Computational Methods and Bioinformatics

Computation and bioinformatic tools have become an essential component of biologic research. The amount and diversity of the data being generated by different technologies are challenging, and the data are impossible to organize or analyze without computational assistance. In functional genomics, a great deal of effort has been devoted in generating gene expression profiles using either Web-based (dchip) or commercially available (GeneSight; Gene Spring) software packages.

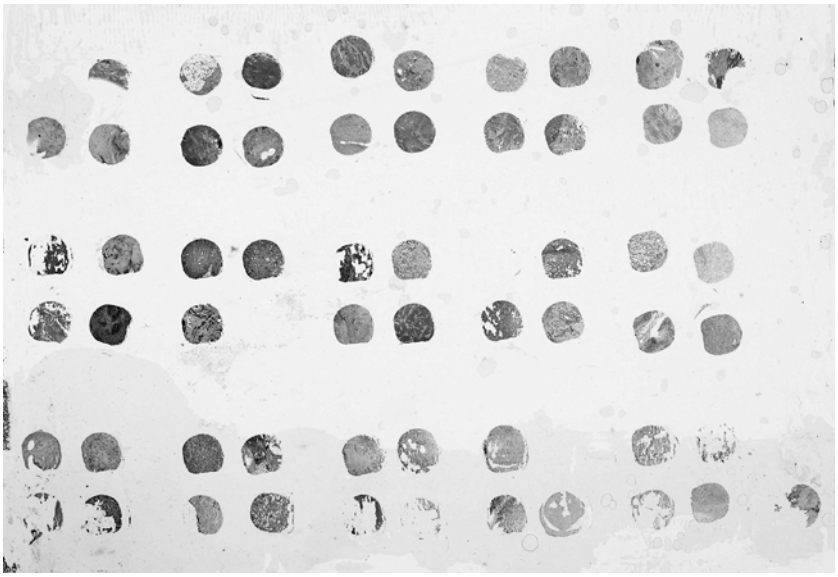


Figure 12.4. Analysis of intelectin protein expression in MPM tissue microarray containing 53 samples. The localization of the primary antibody using color development by an avidin-biotin detection kit illustrates the structural pattern of the array with cylindrical tissue samples.

The same holds true for proteomics to validate across the different technologies. The main databases serving as the targets for MS data searches are the expressed sequence tag and the protein sequence databases, which contain protein sequence information translated from DNA sequence data (46). The 2DGE-related protein databases are helping map proteins from specific cells and from different stages of tumor development (73). Many of these are public domain, available through the World Wide Web on servers such as ExPaSy and the World 2DGE page (39). Annotated protein databases, such as SWISS-PROT and TrEMBL, are fast becoming critical proteome resources (74). Such tools facilitate the analysis of posttranslational modifications and three-dimensional structure and physicochemical properties of identified proteins (33). These databases are becoming invaluable resources of protein maps from such tissues as breast and bladder transitional cell and squamous cell carcinomas (33).

Future of Genomics and Proteomics in Mesothelioma

The global analysis of gene expression patterns in mesothelioma is likely to contribute to early detection, better prognostication, and acceleration in the discovery of novel therapeutic options. Moreover, the handling and analysis of the types of data to be collected in proteomic investigations of mesothelioma represents an emerging field. New techniques and new collaborations among surgeons, oncologists, basic research and computer scientists, and biostatisticians are warranted.

There is a need to develop and integrate database repositories from various sources, and to develop efficient and valid methods of data analysis. Proteomics will complement genomic-based approaches in the study of mesothelioma. As new protein biomarkers will be discovered through proteomic approaches, exciting information is expected to emerge from various collaborative efforts that can ultimately pave the way for the early detection, diagnosis, and possible treatment of this insidious disease.

References

1. Hatada I, Hayashizaki Y, Hirotsune S, Komatsubara H, Mukai T. A genomic scanning method for higher organisms using restriction sites as landmarks. Proc Natl Acad Sci USA 1991;88:9523–9527.
2. Lindsay S, Bird AP. Use of restriction enzymes to detect potential gene sequences in mammalian DNA. Nature 1987;327:336–338.
3. Larsen F, Gundersen G, Lopez R, Prydz H. CpG islands as gene markers in the human genome. Genomics 1992;13:1095–1107.
4. Kim D, LaQuaglia MP, Yang SY. A cDNA encoding a putative 37 kDa leucine-rich repeat (LRR) protein, p37NB, isolated from S-type neuroblastoma cell has a differential tissue distribution. Biochim Biophys Acta 1996; 1309:183–188.
5. Eng C, Herman JG, Baylin SB. A bird's eye view of global methylation. Nat Genet 2000;24:101–102.
6. Rouillard J, Erson AE, Kuick R, et al. Virtual genome scan: a tool for restriction landmark-based scanning of the human genome. Genome Res 2001; 11:1453–1459.
7. Dai Z, Zhu WG, Popkie AP, et al. Promoter methylation and silencing of bone morphogenetic protein 3B (BMP3B) in non-small cell lung cancer identifies a novel lung cancer gene on 10q11 [abstract]. Proc Am Assoc Cancer Res 2002;43:2333.
8. Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. Science 1995;270:484–487.
9. Weeraratna AT, Becker D, Carr KM, et al. Generation and analysis of melanoma SAGE libraries: SAGE advice on the melanoma transcriptome. Oncogene 2004;1–11.
10. Lal A, Lash AE, Altschul SF, et al. A public database for gene expression in human cancers. Cancer Res 1999;59:5403–5407.
11. Strausberg RL. The Cancer Genome Anatomy Project: new resources for reading the molecular signatures of cancer. J Pathol 2001;195:31–40.
12. Lash AE, Tolstoshev CM, Wagner L, et al. SAGEmap: a public gene expression resource. Genome Res 2000;10:1051–1060.
13. Brentani H, Caballero OL, Camargo AA, et al. Proc Natl Acad Sci USA 2003; 100:13418–13423.
14. Roulet E, Busso S, Camargo AA, Simpson AJ, Mermod N, Bucher P. High-throughput SELEX SAGE method for quantitative modeling of transcription-factor binding sites. Nat Biotechnol 2002;20:831–835.
15. Friedrich MJ. Genomics and proteomics may help clinicians individualize cancer treatment. JAMA 2002;287:1931–2932.
16. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403: 503–511.

17. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002;30(1):41–47.
18. Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 1992;257:967–971.
19. Gordon GJ, Appasani K, Parcells JP, et al. Inhibitor of apoptosis protein-1 promotes tumor cell survival in mesothelioma. Carcinogenesis 2002;23: 1017–1024.
20. Frank S, von Specht BU, Farthmann EH, Hirsch T. Identification of genes involved in human mesothelial cancer progression using a modified differential display technique. Cancer Lett 1998;123:7–14.
21. Sun X, Dobra K, Bjornstedt M, Hjerpe A. Upregulation of 9 genes, including that for thioredoxin, during epithelial differentiation of mesothelioma cells. Differentiation 2000;66:181–188.
22. Diatchenko L, Lukyanov S, Lau YF, Siebert PD. Suppression subtractive hybridization: a versatile method for identifying differentially expressed genes. Methods Enzymol 1999;303:349–380.
23. Sandhu H, Dehnen W, Roller M, Abel J, Unfried K. mRNA expression patterns in different stages of asbestos-induced carcinogenesis in rats. Carcinogenesis 2000;21:1023–1029.
24. Ramos-Nino ME, Scapoli L, Martinelli M, Land S, Mossman BT. Microarray analysis and RNA silencing link fra-1 to cd44 and c-met expression in mesothelioma. Cancer Res 2003;63:3539–3545.
25. Kettunen E, Nissen AM, Ollikainen T, et al. Gene expression profiling of malignant mesothelioma cell lines: cDNA array study. Int J Cancer 2001; 91:492–496.
26. Rihn BH, Mohr S, McDowell SA, et al. Differential gene expression in mesothelioma. FEBS Lett 2000;480:95–100.
27. Mohr S, Keith G, Galateau-Salle F, Icard P, Rihn BH. Cell protection, resistance and invasiveness of two malignant mesotheliomas as assessed by 10K-microarray. Biochim Biophys Acta 2004;1688:43–60.
28. Siddiq F, Wali A, Lonardo F, Carbone M, Pass HI. Downregulation of TIMP-1 and osteonectin gene expression alters cellular properties of MPM-derived cell line (Abstract). Proc Am Assoc Cancer Res 2004;45:420.
29. Gordon GJ, Jensen RV, Hsiao LL, et al. Translation of microarray data into clinically relevant cancer diagnostic tests using gene expression ratios in lung cancer and mesothelioma. Cancer Res 2002;62:4963–4967.
30. Gordon GJ, Jensen RV, Hsiao LL, et al. Using gene expression ratios to predict outcome among patients with mesothelioma. J Natl Cancer Inst 2003; 95:598–605.
31. Singhal S, Wiewrodt R, Malden LD, et al. Gene expression profiling of malignant mesothelioma. Clin Cancer Res 2003;9:3080–3097.
32. Pass HI, Liu Z, Wali A, et al. Gene expression profiles predict survival and progression of pleural mesothelioma. Clin Cancer Res 2004;10:849–859.
33. Banks RE, Dunn MJ, Hochstrasser DF, Sanchez J-C, Blackstock W, Pappin DJ. Proteomics: new perspectives, new biomedical opportunities. Lancet 2000;356:1749–1756.
34. Hanash SM. Biomedical applications of two-dimensional electrophoresis using immobilized pH gradients: current status. Electrophoresis 2000;21: 102–109.
35. Steinberg T, Jones LJ, Haugland RP, Singer VL. SYPRO ruby and SYPRO red protein gel stains. Anal Biochem 1996;239:223–237.
36. Bergman AC, Benjamin T, Alaiya A, et al. Identification of gel-separated tumor marker proteins by mass spectrometry. Electrophoresis 2000;21: 679–686.

37. www.nonlinear.com.
38. Lopez MF, Kristal BS, Chernokalskaya E, et al. High-throughput profiling of the mitochondrial proteome using affinity fractionation and automation. Electrophoresis 2000;21:3427–3440.
39. Hochstrasser DF, Appel RD, Golaz O, Pasquali C, Sanchez JC, Bairoch A. Sharing of World Wide Web spread knowledge using hypermedia facilities and fast communication protocols (Mosaic and World Wide Web): the example of ExPaSy. Methods Inf Med 1995;34:75–78.
40. Ostergaard M, Wolf H, Orntoft TF, Celis JE. Psoriasin (S100A7): a putative urinary marker for the follow-up of patients with bladder squamous cell carcinomas. Electrophoresis 1999;20:349–354.
41. Sarto C, Marocchi A, Sanchez JC, et al. Renal cell carcinoma and normal kidney protein expression. Electrophoresis 1997;18:599–604.
42. Hanash SM. Global profiling of gene expression in cancer using genomics and proteomics. Curr Opin Mol Ther 2001;3:538–545.
43. Chen G, Gharib TG, Huang CC, et al. Proteomic analysis of lung adenocarcinoma: identification of a highly expressed set of proteins in tumors. Clin Cancer Res 2002;8:2298–2305.
44. Yanagisawa K, Shyr Y, Xu BJ, et al. Proteomic patterns of tumour subsets in non-small-cell lung cancer. Lancet 2003;362:433–439.
45. Hillenkamp F, Karas M, Beavis RC, Chait BT. Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. Anal Chem 1991;63:1193A–1203A.
46. Andersen JS, Mann M. Functional genomics by mass spectrometry. FEBS Lett 2000;480:25–31.
47. Krutchinsky AN, Zhang W, Chait BT. Rapidly switchable matrix-assisted laser desorption/ionization and electrospray quadrupole-time-of-flight mass spectrometry for protein identification. J Am Soc Mass Spectrom 2000; 11:493–504.
48. Schevchenko A, Loboda A, Shevchenko A, Ens W, Standing KG. MALDI quadrupole time-of-flight mass spectrometry: a powerful tool for proteomic research. Anal Chem 2000;72:2132–2141.
49. Merchant M, Weinberger SR. Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry. Electrophoresis 2000;21:1164–1167.
50. Humphery-Smith I, Cordwell SJ, Blackstock WP. Proteome research: complementarity and limitations with respect to the RNA and DNA worlds. Electrophoresis 1997;18:1217–1242.
51. Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM. Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues. Nat Med 2001;7:493–496.
52. Chaurand P, Stoeckli M, Caprioli RM. Direct profiling of proteins in biological tissue sections by MALDI mass spectrometry. Anal Chem 1999;71:5263–5270.
53. Hutchens TW, Yip T-T. Rapid Commun Mass Spectrom 1993;7:576–580.
54. Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 2002;359:572–577.
55. Kozak KR, Amneus MW, Pusey SM, et al. Identification of biomarkers for ovarian cancer using strong anion-exchange ProteinChips: potential use in diagnosis and prognosis. Proc Natl Acal Sci USA 2003;100:12343–12348.
56. Xiao Z, Cazares LH, Wright GL. A novel biochip SELDI mass spectrometry immunoassay for quantitation of prostate specific membrane antigen (PSMA) in body fluids [abstract]. Proc Am Assoc Cancer Res 2000;41: 316.

57. Xiao Z, Jiang X, Beckett ML, Wright GL Jr. Generation of a baculovirus recombinant prostate-specific membrane antigen and its use in the development of a novel protein biochip quantitative immunoassay. Protein Exp Purification 2000;19:12–21.
58. Adam B-L, Davis JW, Cazares LH, Schellhammer PF, Lynch DF, Wright GL Jr. Identifying the signature proteins of prostate cancer in seminal plasma by SELDI affinity mass spectrometry [abstract]. Proc Am Assoc Cancer Res 2000;41:564.
59. Moody TW, Walters J, Zakowicz H, et al. Surface enhanced laser desorption/ionization analysis of human lung cancer specimens [abstract]. Proc Am Assoc Cancer Res 2001;42:59.
60. Aebersold R. Quantitative proteome analysis: methods and applications. J Infect Dis 2003;187 (suppl 2):S315–S320.
61. Martin DB, Gifford DR, Wright ME, et al. Quantitative proteomic analysis of proteins released by neoplastic prostate epithelium. Cancer Res 2004; 64:347–55.
62. Zhou G, Li H, DeCamp D, et al. 2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. Mol Cell Proteomics 2002;1(2):117–124.
63. Gharbi S, Gaffney P, Yang A, et al. Evaluation of two-dimensional differential gel electrophoresis for proteomic expression analysis of a model breast cancer cell system. Mol Cell Proteomics 2002;1(2):91–98.
64. James P. Chips for proteomics: a new tool or just hype? BioTechniques 2002; 33:S4–S13.
65. Kusnezow W, Hoheisel JD. Antibody microarrays: promises and problems. BioTechniques 2002;33:S14–S23.
66. De Wildt RMT, Mundy CR, Gorick BD, Tomlinson IM. Antibody arrays for high throughput screening of antibody-antigen interactions. Nat Biotech 2000;18:989–994.
67. Haab BB, Dunham MJ, Brown PO. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Biol 2001;2:1–13.
68. Paweletz CP, Charboneau L, Bichsel VE, et al. Protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Proc Am Assoc Cancer Res 2001;42:55.
69. Cahill DJ. Protein and antibody arrays and their medical applications. J Immunol Methods 2001;250:81–91.
70. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4: 844–847.
71. Camp RL, Carette LA, Rimm DL. Validation of tissue microarray technology in breast cancer. Lab Invest 2000;80:1943–1949.
72. Mucci NR, Akdas G, Manely S, Rubin MA. Neuroendocrine expression in metastatic prostate cancer: evaluation of high throughput tissue microarrays to detect heterogeneous protein expression. Hum Pathol 2000;31: 406–414.
73. Oh JMC, Hanash SM, Teichroew D. Mining protein data from two-dimensional gels: tools for systematic post-planned analyses. Electrophoresis 1999;20:766–774.
74. Bairoch A, Apweiler R. The SWISS-PROT protein sequence data bank and its supplement TrEMBL in 1998. Nucleic Acids Res 1998;26:38–42.
75. Shetty S, Kumar A, Pueblitz S, et al. Fibrinogen promotes adhesion of monocytic to human mesothelioma cells. Thromb Haemost 1996;75: 782–790.

76. Kinnula VL, Lehtonen S, Sormunen R, et al. Overexpression of peroxiredoxins I, II, III, V, and VI in malignant mesothelioma. J Pathol 2002;196:316–323.
77. Kahlos K, Paakko P, Kurttila E, Soini Y, Kinnula VL. Manganese superoxide dismutase as a diagnostic marker for malignant pleural mesothelioma. Br J Cancer 2000;82:1022–1029.

13

Asbestos Mineralogy and Health Effects

Meral Dogan and A. Umran Dogan

Fibers and fibrous minerals, for example, the asbestos minerals, erionite (one of the many natural and synthetic zeolite species) (1), fiberglass, or other silica forms (diatoms) have been shown to be extremely hazardous. Their airborne character is paramount, and the specific gravity of the species, the size, and an appropriate morphology that permits suspension are of primary consideration. Asbestos as a ubiquitous natural resource refers to several types of fibrous minerals formed by earth processes and made up of microscopic bundles of fibers. The dangers associated with inhalation of asbestos fibers have been known for more than 30 years. Asbestos is known as a group A human carcinogen. The potential hazards of exposure to asbestos materials are of concern worldwide. There are several modes of exposure to airborne fibers including occupational exposure and the erosion of natural deposits in asbestos-bearing rocks. Asbestos may also be dispersed in water from a number of sources, including erosion of natural deposits, corrosion, and disintegration of asbestos materials.

Governments and industries have introduced regulatory measures requiring safety controls throughout the product life cycle to limit asbestos exposure to the general public and workers. Although asbestos materials have been well documented as to their physical and chemical characteristics, they remain under investigation both by mineralogists studying geologic aspects and by pathologists/epidemiologists studying medical aspects. The term *asbestos* may be well known, but the precise definition, safe level of exposure, duration of exposure, and asbestos types of these fibrous materials still raise questions and often lead to differences of opinions and arguments as well as legal disputes (2).

Mineralogy of Asbestos Group Minerals

The six different types of asbestos fibers are divided into two mineral groups based upon the crystalline structures: serpentine and amphibole asbestos. Asbestiform minerals are not always found with a

fibrous habit. Tremolite, for example, occurs naturally in three distinct morphologic forms or mineral habits. It may occur as asbestos, splintery fibers, or in massive crystalline deposits. Any mechanical manipulation of asbestos rocks rapidly produces many long, thin fibers/fibrils, since for the most part, asbestos fibrils are easily separable because of translocation along a twin plane, which produces a much-reduced cohesion. A lot of data have been accumulated that suggest that amphibole asbestos and its nonasbestos analogues possess very different biologic potential. Davis et al (3) demonstrated that although asbestiform tremolite was extremely carcinogenic when injected into the peritoneal cavities of rats, nonasbestiform tremolite samples had little or no carcinogenic potential. These observations suggest that the tremolite contamination of any material may present a concern only if thin asbestiform fibers are present.

Serpentine Group Asbestos Minerals

Serpentine group asbestos mineral is chrysotile. Chrysotile fibers are found as veins in serpentines, in serpentinized ultramafic rocks, and in serpentinized dolomitic rocks. Chrysotile is one of the three polymorphs of serpentine group minerals that have sheet or layer structure. It is a hydrated magnesium silicate and its stoichiometric chemical composition may be given as $Mg_3Si_2O_5\ (OH)_4$. Most of the industrial chrysotile fibers are extracted from deposits where fiber length can reach several centimeters. Fiber growth may occur in massive serpentinite at a right angle to the walls of cracks, which are referred to as cross-vein chrysotile or cross-fibers, or inclined or parallel to the vein axes called slip fibers, or as dispersed aggregates of short fibers with no preferential orientation, which are called mass-fiber deposits.

Among three principal serpentine minerals, the distinction between asbestos and nonasbestiform varieties is that the nonasbestiform antigorite and lizardite are arranged to form a sheet structure, and the crystals are platy, that is, they have one short dimension and two longer dimensions, like a saucer. The crystal structure of chrysotile, always in fibrous form, is discretely distinct from that of the amphiboles. Chrysotile has an octahedral brucite layer $(Mg_6O_4(OH)_8)^{-4}$ intercalated between each silicate tetrahedral sheet. In the asbestiform variety of serpentine, chrysotile sheets $(Si_4O_{10})^{-4}$ are rolled up tightly to form fibers.

The serpentine's theoretical formula is

$$(Mg_{3\text{-}x\text{-}y}\ R^{2+}_x\ R^{3+}_y)\ (Si_{2-y}\ R^{3+}_y)\ O_5\ (OH)_4$$

where R^{2+} is Fe^{2+}, Mn^{2+}, or Ni^{2+}; and R^{3+} is Al^{3+} or Fe^{3+}.

In the octahedral brucite layer, magnesium can be substituted by several divalent ions, such as Fe^{2+}, Mn^{2+}, or Ni^{2+}. In the tetrahedral layer, silicon may be replaced by Al^{3+} or Fe^{3+}. The other two polymorphs are known as antigorite and lizardite. Their composition is $Mg_3Si_2O_5\ (OH)_4$. The essential difference of the three minerals is 1:1 layers in the structure. The silicate and brucite layers share oxygen atoms. The distance is 0.305 nm in the silicate layer and 0.342 nm in the brucite layer (4).

This mismatch of O—O distances induces curvature and 1:1 layers are concentrically coiled, producing hollow tubes parallel to the a-axis in chrysotile. The concentric sheets forming fibers have a curvature radius from 2.5 to 3.0 nm for the internal layers up to approximately 25 nm for the external layers (5). Electron microscopy studies indicate that the unit fiber (fibril) cross section appears in a concentric or spiral arrangement. In the lizardite, these layers are planar, which is characteristic of sheet silicates. Antigorite structure is corrugated.

Stacking of the tetrahedral and octahedral sheets in the chrysotile structure has been shown to yield three types of chrysotile fibers:

1. Clino-chrysotile: Monoclinic stacking, x-axis is parallel to the fiber axis
2. Ortho-chrysotile: Orthorhombic stacking, x-axis is parallel to the fiber axis
3. Para-chrysotile: 180-degree rotation of two-layer structure, y-axis is parallel to the fiber axis

Amphibole Group Asbestos Minerals

The chemical composition of amphibole group asbestos minerals can vary widely and reflects the complexity of the environment in which they formed. The commercially used asbestiform amphiboles are actinolite, tremolite, anthophyllite, amosite, and crocidolite. They are all hydrated silicates, which have double tetrahedral chains with Si_8O_{22} composition that extend along the c-axis. Amphiboles are distinguished from one another by the number of the cations Ca, Fe, Mg, and Na that they contain.

The amphibole group has the general chemical formula of

$$A_{0-1}B_2C_5T_8O_{22}(OH, F, CI, O)_2$$

where A represents zero to one Na^+ or K^+ in the A site, two ions of Mg^{2+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Na^+, or Li^+ enter the M4 sites, five ions of Mg^{2+}, Fe^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Cr^{3+}, or Ti^{4+} enter M1, M2, and M3 sites, and eight ions of Si^{4+} or Al^{3+} enter the T site. The remaining entry in the formula (OH, F, Cl, O) indicates anions that occupy another site. Complete ionic substitution may take place between Fe^{3+} and Al, and between Ti and other c-type cations, and there is partial substitution of Al for Si in the tetrahedral chains. Thus, from their respective composition, amphibole fibers can be viewed as a series of minerals. For example, the magnesium in tremolite is partly replaced by divalent iron in the c-position to yield actinolite.

The theoretical formulas for the amphibole group of asbestos minerals are as follows:

Actinolite: $Ca_2\,(Mg, Fe^{2+})_5\,Si_8\,O_{22}\,(OH)_2$
Tremolite: $Ca_2\,Mg_5\,Si_8\,O_{22}\,(OH)_2$
Anthophyllite: $Mg_7\,Si_8\,O_{22}\,(OH)_2$
Amosite: $(Fe^{2+})_2\,(Fe^{2+}Fe^{3+}, Mg)_5\,Si_8\,O_{22}\,(OH)_2$
(Cummingtonite-Grunerite series)
Crocidolite: $Na_2\,(Fe^{2+}, Mg)_3\,Fe^{3+}{}_2\,Si_8\,O_{22}\,(OH)_2$ (Riebeckite)

The term *amosite* is applied to brown industrial asbestos; it is an acronym for "asbestos minerals of South Africa" with the addition of the usual "-ite" suffix to designate a mineral. The term *crocidolite* is applied to blue amphibole asbestos. Asbestiform varieties of several other amphiboles have been identified. Other minerals are similar to asbestos in their particle shape, but they do not possess the characteristics of asbestos.

The amphibole group of silicates is composed of very common minerals in igneous and metamorphic rocks. The minerals show long prismatic or needle-like (acicular) crystal habits or morphologies. Because of their peculiar structure, the amphiboles have a distinctive cleavage that results in acicular or needle-like morphology when the minerals are crushed. A tiny oblong form often appears naturally in sedimentary deposits if the primary rocks are eroded, or when mined and milled as part of the extraction of other minerals. However, only when amphiboles form fibers or adopt an asbestiform habit should they be classified as asbestos (6).

Unlike chrysotile fiber, the atomic structure of amphibole does not inherently lead to fiber formation; instead it results from multiple nucleation and specific growth conditions. Asbestiform and nonasbestiform amphiboles are similar in their crystalline structure, but different in the macroscopic scale. The asbestiform amphiboles tend to have a larger number of crystal defects such as twinning, Wadsley defects, and chain width disorder than nonasbestiform varieties.

Identification of Asbestos

Over the years much data have been accumulated about asbestos, which suggests that amphibole asbestos and its nonasbestos analogues possess very different biologic potential. Davis et al (3) demonstrated that although asbestiform tremolite was extremely carcinogenic when injected into peritoneal cavities of rats, nonasbestiform tremolite samples had little or no carcinogenic potential. Therefore, it is important to distinguish between asbestiform and nonasbestiform amphiboles and types of fibers in bulk, air, and tissue samples. There are some problems related to the mineralogic techniques necessary to prepare and characterize samples. The designation of the shape and size of fibrous materials can be relatively easily revealed by optical examination. Optics became the technique of choice to investigate the occurrence of inorganic fibrous airborne particulates at occupational sites, in schools, or any buildings, and even outdoors where filters could be set up to obtain a representative aliquot of the air. However, the light (optical) microscope does not have enough spatial resolution and so is not sufficient on its own for positive identification of minerals. It is difficult to identify some fibers such as chrysotile in the tissue samples under the optical microscope because of the small fiber sizes.

Since the small fiber size of chrysotile in the tissue samples preclude the use of optical microscopes, morphologic, chemical, and structural identifications are done by combinations of methods in order to make

unambiguous mineral identifications. The crystal chemical range of potentially hazardous inorganic and mineral species should be accurately identified. Morphologic identifications can be performed by using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Chemical information is most commonly obtained by energy dispersive spectroscopy (EDS) or wavelength dispersive spectroscopy (WDS), which is an integral part of SEM or TEM. A relative error percentage for EDS is about 10% and for WDS is about 1%. Therefore, EDS provides only semiquantitative information, but WDS provides more quantitative information on chemical composition of the sample. Crystal structures can be determined by electron diffraction (ED) on samples. Powder x-ray diffraction (XRD) is a powerful technique providing that enough material is available, but not for a mineral present at low percentage in tissue and air samples. Certain regulations may require specific species of amphiboles; thus, quantitative chemical data may be necessary. For example, substitution solid solution series of amphiboles, such as a tremolite and an actinolite, must be identified. The SEM studies combined with EDS may not be conclusive because of the lack of information on the mineral structure.

It is also very difficult to observe chrysotile through the electron microscope because of its beam sensitivity. Analysts tend to measure fibers that are more stable under beam conditions. Lung burden studies indicate that chrysotile is often inhaled as a shorter fiber than amphiboles. Therefore, in a tissue with both amphibole and chrysotile, it is possible to make a misjudgment unless the fibers are identified individually.

The levels of sensitivity using the high-resolution techniques now available mandate that we follow up the reactions delineated as interference of inorganic materials in the biologic environment. The information on the inorganic fibrous particulates can be matched with the equally high-resolution techniques applied to analyses of tissues, with data gathered at the cellular and molecular levels. The advances in techniques increase the possibilities that we can test hypotheses and, it is hoped, gain greater understanding from the anatomic to the genetic of the reactions that lead to induction of disease. Coordinating ultramicroscopic levels with the health and mineralogic investigations for a particular geographic area should enable us to refine the possibilities. The exchange of information among the several disciplines is needed to advance our knowledge.

Asbestos Materials and Their Properties

An environment-friendly product is defined as one that is made from simple starting materials, produced by low-energy–consuming technology, has a long useful service life and presents a low risk during its manufacture, transportation, storage, use, and disposal. Asbestos fits the definition with the exception of risk factors. The asbestos bundles have splaying ends and are extremely flexible and can be woven. It possesses high tensile strength, resistance to chemical and thermal degra-

dation, and high electrical resistance. Fibers are not volatile or soluble. Asbestos has been mined for its useful properties for years.

Asbestos is commonly used in heat, thermal, and acoustic insulation; fire proofing; and in other building materials, including floor and ceiling tiles, wallboards, siding, pipes, adhesives, roofing shingles and felt, base flashing, fire doors, electrical panel partitions, electrical cloth, textured paintings/coatings, taping compounds, table tops, laboratory hoods, laboratory gloves, fire blankets and curtains, joint compounds, spackling compounds, packing materials, thermal paper products, chalkboards, elevator brake shoes, HVAC duct insulation, boiler insulation, breaching insulation, electric wiring insulation, ductwork flexible fabric connections, cooling towers, pipe insulation, heating and electrical ducts, vinyl wall coverings, and high-temperature gaskets.

Today, only the chrysotile type of asbestos is used. The industry now markets dense and nonfriable materials including chrysotile-cement building materials, friction materials, gaskets, and certain plastics. Chrysotile and its nonfriable products, such as chrysotile cement, are claimed to be used in complete safety if properly controlled throughout the product life cycle. However, there is debate about the hazards of chrysotile.

Alternative Products

There are alternative products on the market. The manufacturing cost is 20% to 30% higher, and many of the products have been demonstrated to be less resistant to heat, humidity, and temperature contrasts (freeze-thaw), and they are not as durable as chrysotile-reinforced products. These fibers, including cellulose, are quite biopersistent, and thus require care during manufacture, handling, and use. In addition, many of these asbestos-free materials have poor technical performance or durability.

Replacement products contain natural or synthetic fibers that can be hazardous as well. However, unlike chrysotile, few countries have introduced appropriate regulations for these substitute materials. Thus, scientists have begun to raise concerns over the health effects of some of the fibers used to replace chrysotile.

Asbestos Exposures

Occupations involving a risk of exposure to asbestos include mining and milling of minerals containing asbestos; manufacturing, stripping, repair, or maintenance of materials or products containing asbestos; demolition or repair of plants or structures containing asbestos; transportation, storage, and handling of asbestos or materials containing asbestos; and other occupations involving a risk of exposure to airborne asbestos fibers.

Removal of asbestos insulation should be considered a last resort and it should be undertaken only when the materials are beyond repair or at the time of major renovation work or building demolition. Asbestos

removal is a very costly operation, which must be conducted by highly specialized contractors. Hasty elimination of asbestos insulation considerably increases the probability that controls will not be adequately enforced, thus presenting a source of risk not only for the workers, but for building occupants as well.

Chrysotile, the most common serpentine fiber, is known to be considerably less hazardous than the amphibole varieties. It has curly fibers that are unlikely to remain suspended in the air, and it does not stay in the lungs very long. Thus, in general, chrysotile is a less dusty material and is more easily eliminated from the human body than amphiboles. The controlled use of chrysotile allows its continued use in high-density products, provided permissible exposure limits of 1.0 F/cc or below (F is the degree of fineness of abrasive particles) are recommend by a group of experts from the World Health Organization (WHO). However, increasing evidence about the hazards of occupational and environmental exposure to chrysotile was presented at a 3-day conference on Parliament Hill, Ottawa, on September 12, 2003. The chrysotile producers of Zimbabwe, Canada, Brazil, and Swaziland, which together account for 75% of world exports of chrysotile fibers, have signed a Memorandum of Understanding (MOU), the objective of which is to supply chrysotile fibers only to those companies that demonstrate compliance with international rules and standards.

We can now ask more precise questions based on data accumulated over the many years of scientific research. Potentially hazardous inorganic and mineral species have been accurately identified (7). The health responses are well documented (8). The crossover of information among the several disciplines will be needed to advance our knowledge.

Occupational and Environmental Health Hazards Related to Asbestos Exposure

Three different types of diseases that are associated with the inhalation of the various types of asbestos fibers have been identified: asbestosis (form of fibrosis), lung cancer, and mesothelioma of the pleura (lining of the lung and chest cavity) or peritoneum (lining of the abdomen). Asbestosis is characterized by shortness of breath and cough. It may lead to severe impairment of respiratory function and ultimately death because presently there is no cure for the disease.

Cancers of the larynx, pancreas, esophagus, colon, and kidney have also been linked to asbestos exposure, but the increased risk is not as great as with the respiratory system. It is possible to test for the presence of fibers in urine, feces, or mucus.

At least from the diagnostic perspective, asbestos has another effect—it can cause pleural lesions, visible some decades after exposure. Calcified pleural plaques (CPPs) and pleural thickening (PT) are the lesions. Epidemiologic studies indicate that high frequency of malignant pleural mesothelioma (MPM), CPP, and PT constitute important pulmonary health problems in some countries, including

Italy (i.e., Corsica) (9,10), Greece (11), Turkey (12–15), Cyprus (16), and the United Kingdom (17). It has been predicted that within a few years in the United Kingdom, for example, mesothelioma will be the cause of death in 1 in 150 males born between 1945 and 1950 (17). Dogan (18,19) suggested that although MPM cases were not observed where low doses (0.054–0.1 fiber/mL in the air samples) of long, but thick, splintery fibers, and short and thin mixed asbestos fiber exposures are present, there is a high incidence of CPP and PT in Central Anatolia in Turkey.

Asbestos and Mesothelioma

A layer of specialized cells called mesothelial cells lines the chest and abdominal cavities, and the cavity around the heart. These cells also cover the outer surface of most internal organs. The tissue formed by these cells is called the mesothelium. The mesothelium helps protect the organs by producing special lubricating fluid that makes the organs move around. Tumors of the mesothelium can be benign (noncancerous) or malignant (cancerous). Malignant mesothelioma is a cancerous tumor of the pleura or peritoneum. About three fourths of malignant mesothelioma occurrences start in the chest cavity and are known as pleural mesothelioma. Another 10% to 20% begin in the abdomen and are called peritoneal mesothelioma. The mesothelium of the pericardial cavity is called the pericardium. Cancer cells can invade and damage nearby tissues and organs. They can also metastasize from their original site to other parts of the body. The covering layer of the testicles is actually an outpouching of peritoneum into the scrotum. It is subject to a rare form of cancer. The risk of developing a mesothelioma is related to how much asbestos a person was exposed to and how long this exposure lasted. People exposed at an early age, for a long period of time, and at higher levels are most likely to develop this cancer. Although the risk of developing mesothelioma rises with the amount of asbestos exposure, it is clear that genetic factors also play a role in determining who develops the disease (20,21). This explains why not all persons exposed to high levels of asbestos dust develop mesothelioma.

Despite standard “dust” levels that have been in existence since the 1970s, in some countries the number of cases of lung cancer and mesothelioma grows and the controversy still persists.

The discovery that exposure to asbestos is linked to mesothelioma was first made in 1960 (22) and again in 1965 (23); these studies documented the high incidence of the disease among people working at or living near crocidolite asbestos mines, as well as in household members of workers at these mines. From the 1940s through the 1970s, crocidolite and another amphibole, amosite, were used extensively, either alone or in conjunction with chrysotile, in friable insulation applications in the shipbuilding and construction industries, primarily in North America and Europe. These sprayed-on applications have been discontinued since the 1970s. To a lesser extent, amphiboles were also

used in the manufacture of asbestos-cement pipe. The amphibole hypothesis, officially introduced in 1990 (24), states that chrysotile asbestos is not a potent cause of malignant mesothelioma, supporting the findings of Doll and Peto (25) and the U.S. Environmental Protection Agency (26). The amphibole hypothesis has raised many crucial issues and served to focus research on still partially unanswered questions of why different exposed populations have experienced such different rates of major asbestos diseases. McDonald et al (27), Stayner et al (28), and Cullen (29) also suggested that chrysotile may be less potent than some amphibole asbestos minerals in causing mesothelioma. Some publications, however, suggested that chrysotile asbestos is the main cause of pleural mesothelioma in humans (30–33).

Whether chrysotile fibers on their own can ever cause mesotheliomas is still debated. Other evidence of the connection between chrysotile exposure and mesothelioma has been provided by the cohort study of Quebec chrysotile miners (27), as reported at the September 12, 2003, Conference of Canadian Asbestos.

Epidemiologic studies have shown an excess of developing mesothelioma among residents in Biancavilla, Sicily, Italy, and a new asbestos amphibole, fluoro-edenite, appears responsible for the high incidence of malignant pleural mesothelioma (34).

Mesothelioma is more common in people who have had serious lung diseases such as tuberculosis. The median survival varies from 4 to 18 months in different studies. However, the prognosis depends on the stage of the tumor.

Mesothelioma does not generally cause symptoms in the early stages. Symptoms of mesothelioma in the lining of the lung can include shortness of breath, pain in the lower back or the side of the chest, persistent cough and hoarseness, difficulty in swallowing, unexplained weight loss, and sweating. Symptoms of mesothelioma in the abdominal lining may include abdominal pain, swelling of the abdomen, nausea and vomiting, loss of appetite, unexplained weight loss, and change in bowel habits. These signs and symptoms usually indicate problems other than cancer. However, people who have been exposed to asbestos who notice any symptoms should see their doctor.

Differentiation of the tumor from other conditions of the pleura and other types of cancer can be difficult. Since mesothelioma can affect the lungs and abdomen, doctors may also carry out some of the tests commonly used to detect lung or stomach cancer, such as chest x-ray, to show tumor and possibly pleural effusion, thoracic computed tomography (CT), cytology from pleural fluid, and open lung biopsy.

Patients with mesothelioma usually had a rapid demise (within a year in many cases), in spite of the fact that the exposure to asbestos may have been relatively mild and taken place over 30 years (25). Malignant mesothelioma affects men more frequently than women (35). Sustained exposure to asbestos or erionite is the main risk factor. It can take 15 to 40 years following these fiber exposure for mesothelioma to develop. However, smoking dramatically increases the risk among the toxic fiber exposed. Mesothelioma may also develop follow-

ing exposure to radiation from a substance called thorium dioxide, which was used to create x-rays of blood vessels until 1955. Other suspected causes include biogenic silica fibers, chronic irritation stemming from tuberculosis and other factors.

Simian virus 40 (SV40) has been detected in human tumors in over 40 laboratories, and many of these reports linked SV40 to mesotheliomas. The presence of SV40 in mesothelioma and other human tumor types has been reported by negative findings (36). However, three independent panels established a positive link between SV40 in human mesothelioma and brain tumors (37,38).

Mechanisms of the Potentially Hazardous Minerals

It is generally accepted that to be pathogenic to the lung or pleura, fibers must be long, thin, and durable. Researchers still debate the safe level of exposure, but it is known that the greater and the longer the exposure, the greater the risk of contracting an asbestos-related disease. Investigators suggested mechanisms of disease induction that went beyond physical trauma. Fiber chemistry may also be significant. The body has multifunctional chemical cascades that are only partially understood. Some investigators suggested that health causation mechanisms could be small differences in the morphology of the particulates or in the chemical character of the particulates, and particularly the surfaces of the asbestos materials. One of the hypotheses came with the investigations on crocidolite, and in the series of tremolite-actinolite-ferroactinolite (39). The presence of Fe, both Fe^{2+} and Fe^{3+}, states in the amphibole could initiate a cascade of cell responses leading to an activated oxygen ligand thought to be a carcinogenic agent. For example, asbestiform species within the tremolite-ferroactinolite series is present at Libby, Montana, where the mining of vermiculate has an accompanying fibrous Fe-containing amphibole species in the gangue. Libby's population has a high incidence of mesothelioma.

Some individuals appear to be more susceptible while others develop cancer after only limited exposure. There is evidence of a genetic disposition among the affected population (20,40).

Research into the potential causative effects of these diseases, including exposure to fiberglass, suggests that the inorganic materials were foreign bodies in the biologic environment. The longer a foreign substance persists in the body, the more likely it is to cause cellular damage and lead to accelerated cell reproduction and chromosomal damage, which are associated with tumor growth. Many studies using intrapleural or intraperitoneal injection have demonstrated that long and thin fibers were the most effective in producing mesotheliomas, once they were within the body cavities (41–44). Fibers longer than 20 μm are more potent than fibers shorter than 10 μm with respect to the induction of pulmonary tumors and fibrosis by inhalation (45,46). Dodson et al (47) confirmed that the most dangerous fibers were more than 8 μm in length and less than 0.25 μm in diameter. However, both

the chrysotile and amphibole fibers in the pleura/plaques of all of these studies have been reported as consistently shorter than those in the parenchyma itself. Repeated episodes of cell tissue injury followed by proliferation and genetic damage may give rise to a tumor that proliferates autonomously (48).

The identification of a particular hazardous species from areas where disease, such as mesothelioma, is endemic showed that minerals other than those originally designated could be present (49,50). Both amphibole- and chrysotile-type mineral fibers in lung tissue from several mesothelioma cases and some controls were studied by Jones et al (51), McDonald et al (52), Mowe et al (53), Gaudichet et al (54), McDonald et al (55), Rogers et al (56), and Rodelsperger et al (57).

It has been accepted that amphiboles are more toxic than chrysotiles. The greater toxicity of amphiboles is linked to durability in the lung. Chrysotile fibers dissolve relatively quickly, but amphiboles persist at sites of tumor development and serve as the stimulus for euplastic (new tissue) growth (58,59). The kinetics for amphiboles and chrysotile fibers are different in human lung tissue (60–62). Amphibole fiber concentrations increase with the duration of exposure, whereas chrysotile concentrations do not. In addition to the biopersistence of amphiboles, the Fe content of particles appears to trigger an oxidative stress process—the generation of active oxygen species (AOS), which some researchers believe can cause membrane damage, induce the release of inflammatory compounds, which can lead to fibrosis and lung cancer, and even cause DNA strand breaks. Fe-containing particles can produce AOS by an oxidation mechanism.

Wagner et al (63) and Davis et al (64) also have amplified the importance of the numbers of very fine fibers for determination of chrysotile pathogenesis. Brooke Mossman, of the University of Vermont College of Medicine, suggests that the lower amounts and bioavailability of Fe in chrysotile fibers may render them less biologically active over time. It may be that asbestos causes cancer by physically irritating cells rather than by chemical effects. Other studies have confirmed the importance of fiber length and geometry in the generation of AOS by alveolar macrophages. Longer fibers and particles are generally relatively inactive (65). When fibers are inhaled, most are cleared from the nose, throat, trachea, or bronchi. Fibers are cleared by sticking to mucus inside air passages and being coughed up or swallowed. The long, thin fibers are less readily cleared, and they may reach the ends of the small airways and penetrate into the pleural lining of the lung and chest wall. These fibers may then directly injure mesothelial cells of the pleura, and eventually cause mesothelioma.

Prognostic factors in oncology assist in the selection of patients who are more likely to benefit from clinical treatment. These factors in mesothelioma were studied in the past two decades by Chahinian et al (66), Samson et al (67), Antman et al (68), Calavrezos et al (69), Spirtas et al (70), Tammilehto (71), Boutin et al (72), De Paugher Manzini et al (73), Ruffie et al (74), Currau et al (75), Herndon et al (76), and Edwards et al (77). It is hoped that these studies will provide insight into the biology of cancer. Recently, gene therapy studies including suicide gene

therapy, genetic immunopotentiation, and suicide gene plus allogenic vaccine were reported (78–81).

The particles initiated cellular responses to an unexpected trauma, and a normal repair mechanism was the deposition of a fibrous protein, collagen, in excessive concentrations at the site of trauma. This reaction is also encountered with other trauma such as the invasion of bacteria, for example, in the lung environment or when cuts are healing in the skin. The physical rejection of the particles can be envisioned, but the local reactions that lead to scarring depend not only on the fiber reaching the delicate tissues of the alveoli deep within the lung but also on local cell responses.

Conclusion

Asbestos, characterized as a group A human carcinogen, is a generic name given to the fibrous variety of six naturally occurring minerals: chrysotile, actinolite, tremolite, anthophyllite, amosite, and crocidolite. The permissible exposure limits recommended by WHO is 1.0 F/cc or below. The identification of asbestos fibers can be performed through morphologic, crystal structural, and compositional analyses. It is widely accepted that asbestos fibers can be associated with asbestosis, lung cancer, and mesothelioma. Despite extensive cancer studies in humans, certain controversies remain about asbestos exposure and cancer. Today, only chrysotile is used as an asbestos material because it is considered to be less potent. The key questions concern whether or not, and to what extent, exposure to chrysotile asbestos, including its natural contaminant tremolite, causes mesothelioma. Many companies ceased production of asbestos-containing insulations, plasters, ceiling tiles, and cement products because of liability issues. However, there is a continued demand for inexpensive and durable construction materials.

Acknowledgment

We acknowledge Prof. Robert L. Brenner of the Department of Geoscience, the University of Iowa, for critically reading an earlier version of the manuscript.

References

1. Dogan AU. Mesothelioma in Cappadocian Villages. Indoor Built and Environment 2003;12(6):367–375.
2. Carbone M, Setlak P, Bocchetta M, et al. Genetic susceptibility to mesothelioma. In: Peters GA, Peter BJ, eds. The Asbestos Legacy—The Sourcebook on Asbestos Diseases, vol 23. LexisNexis 2001;151–168.
3. Davis JMG, Bolton RE, Miller BG, Nieven K. Mesothelioma dose-response following intrapertoneal injection of mineral fibers. Int Exp Pathol 1991; 72:263–274.

4. Skinner HCW, Ross M, Frondel C. Asbestos and Other Fibrous Materials. NewYork: Oxford University Press, 1988.
5. Yada K. Study of microstructure of chrysotile asbestos by high resolution electron microscopy. Acta Crystallography 1971;27:659–664.
6. United States Environmental Protection Agency. Master Testing List. Washington, DC: Office of Prevention, Pesticides and Toxics, USEPA, 1992.
7. Wiley AG, Verkouteren JR. Amphibole asbestos, Libby, Montana. Am Mineralogist 2000;85:1540–1542.
8. Finkelmen R, Skinner HCW, Plumlee GS, Bunnell JE. Medical geology. Geotimes 2001;46:20–23.
9. Boutin C, Viallat JR, Steinbauer J, Massey DG, Charpin D, Mouries JC. Bilateral pleural plaques in Corsica: a non-occupational asbestos exposure marker. Eur J Respir Dis 1986;69:4–9.
10. Rey F, Boutin C, Steinbauer J, Viallat JR, et al. Environmental pleural plaques in an asbestos exposed population of northeast Corsica. Eur Respir J 1993;6:978–982.
11. Constantopoulos SH, Goudevenos JA, Saratsiz N, Langer AM, Selikoff IJ, Moutsopoulos HM. Metsova lung: pleural calcification and restrictive lung function in northwestern Greece. Environmental exposure to mineral fiber as etiology. Environ Res 1985;38:319–331.
12. Barıs YI. Asbestos and erionite related chest diseases. Ankara, Turkey: Semih Ofset Matbaacılık Ltd., 1987.
13. Coplu L, Dumortier P, Demir AU, et al. An epidemiological study in an Anatolian Village in Turkey environmentally exposed to tremolite asbestos. J Environ Pathol Toxicol Oncol 1996;13(I):1–7.
14. Karakoca Y, Emri S, Cangir AK, Baris YI. Enviromental pleural plaques due to asbestos and fibrous zeolite exposure in Turkey. Indoor Built Enviroment 1997;6:100–105.
15. Yazicioglu S, Ilcayto R, Balci K, Sayli BS, Yorulmaz B. Pleural calcification, pleural mesotheliomas and bronchial cancers caused by tremolite dust. Thorax 1980;35:564–569.
16. McConnochie K, Simonato L, Mavrides P, Chritofides P, Pooley FD, Wagner JC. Mesothelioma in Cyprus: the role of tremolite. Thorax 1987;42:342–347.
17. Peto J, Decarli A, La Vecchia C, Levi F, Negri E. The European mesothelioma epidemic. Br J Cancer 1999;79:566–572.
18. Dogan M. Environmental health problems related to mineral dusts: examples from Central Anatolia, Turkey. Environ Geol 2002;41(5):571–579.
19. Dogan M. Sources and types of mineral dust in regions of Turkey with endemic malignant mesothelioma. Indoor and Built Environment 2003;12: 377–383.
20. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002;29(1):2–17.
21. Powers A, Carbone M. The role of environmental carcinogens, viruses, and genetic predisposition in the pathogenesis of mesothelioma. Cancer Biol Ther 2002;1(4):350–355.
22. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the north western Cape Province. Br J Ind Med 1960; 17:260–271.
23. Newhouse ML, Thompson H. Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. Br J Ind Med 1965; 22(4):261–269.
24. Mossman BT, Bignon J, Corn M, Seaton A, Gee JB. Asbestos scientific development and implications for public policy. Science 1990;247:294–301.

25. Doll R, Peto J. Asbestos-effects on health exposures and mortality among chrysotile asbestos workers. Part 1: exposure estimates. Am J Med 1985; 26:31–447.
26. United States Environmental Protection Agency. Airbone asbestos-health assessment update. Document No: EPA-600/8-84003F. Washington, DC: Environmental Protection Agency, Office of Health and Environment Assessment, 1986.
27. McDonald JC, Liddell FD, Dufresne A, McDonald AD. The 1891–1920 birth cohort of Quebec chrysotile miners and millers: mortality 1976–88. Br J Ind Med 1993;50(12):1073–1081.
28. Stayner LT, Dankovic DA, Lemen RA. Occupational exposure to chrysotile asbestos and cancer risk: a review of the amphibole hypothesis. Am J Public Health 1996;86:179–186.
29. Cullen MR. The amphibole hypothesis of asbestos related cancer. Am J Public Health 1996;86:179–186.
30. Kohyama N, Suzuki Y. Asbestos in insulation workers. Ann NY Acad Sci 1991;27–52.
31. Huncharek M. Asbestos and cancer. Epidemiological and public health controversies. Cancer Invest 1994;12:214–222.
32. Nicholson WJ, Landrigan PJ. The carcinogencity of chrysotile asbestos. Adv Modern Environ Toxicol 1994;22:407–423.
33. Smith AH, Wright CC. Chrysotile asbestos is the main cause of pleural mesothelioma. Am J Ind Med 1996;30:252–266.
34. Cardile V, Renis M, Scifo C, et al. Behaviour of the new asbestos amphibole fluoro-edenite in different lung cell systems. Int J Biochem Cell Biol 2004;1–12.
35. Testa JR, Carbone M. Mesothelioma. In: Schwab M, ed. Encyclopedic Reference of Cancer. New York: Springer-Verlag, 2002;545–550.
36. Strickler HD. The International SV40 Working Group. Cancer Epidemiol Biomed Prevent 2001;10:523–532.
37. Rizzo P, Bocchetta M, Powers A. SV40 and the pathogenesis of mesothelioma. Semin Cancer Biol 2001;11:63–71.
38. Carbone M, Pass HI, Miele L, Bocchetta M. New developments about the association of SV40 with human mesothelioma. Oncogene 2003;22:5173–5180.
39. Verkouteren JR, Wylie AG. The tremolite-actinolite-ferroactinolite series: systematic relationships between cell parameters, optical properties, and habit, and evidence of discontinuities. Am Mineralogist 2000;85:1239–1254.
40. Roushdy-Hammady I, Siegel I, Emri S, Testa JR, Carbone M. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. 2001;357(9254):444–445.
41. Pott F, Huth F, Friedrichs HK. Tumors in rats after intraperitoneal injection of asbestos dusts. Naturwissenschaften 1972;59:318–332.
42. Pott F, Huth F, Fredrichs KH. Tumorigenic effect of fibrous dusts in experimental animals. Environ Health Perspect 1974;9:313–315.
43. Stanton MF, Wreinch C. Mechanism of mesothelioma induction with asbestos and fibrous glass. J Natl Cancer Inst 1972;48:797–821.
44. Stanton MF, Layard M, Tegeris A, Miller MM, Kent E. Carcinogenecity of fibrous glass: pleural response in the rat in relation to fiber dimension. J Natl Cancer Inst 1977;58:587–603.
45. Davis JMG. Mineral Fibre Carcinogenesis: Experimental Data Relating to the Importance of Fiber Type, Size, Deposition, Dissolution, and Migration. International Agency for Research on Cancer 1989;33–35.
46. Davis JMG, Addison J, Bolton RE, Donaldson K, Jones AD, Smith T. The pathogenicity of long versus short fiber samples of amosite asbestos

administered to rats by inhalation and intraperitoneal injection. Br J Exp Pathol 1986;67:415–430.
47. Dodson RF, O'Sullivan M, Corn CJ, McLarty JW, Hammar SM. Analysis of asbestos fibers in lung tissue from mesothelioma patients. Ultrastruct Pathol 1997;21:321–336.
48. Antoniades HN. Linking cellular injury to gene expression and human proliferative disorders: examples with the PDGF genes. Mol Carcinogen 1992;6:175–180.
49. Barıs YI, Artvinli M, Sahin AA. Environmental mesothelioma in Turkey. Ann Acad Sci 1979;330:423–432.
50. Wiley AG, Huggins CW. Characterization of winchite-asbestos from Allamore Talc District, Texas. Canad Mineralogist 1980;18:101–107.
51. Jones JSP, Roberts GH, Pooley FD, et al. The pathology and mineral content of lung in cases of mesothelioma in the United Kingdom in 1976. In: Wagner JC, ed. Biological Effects of Mineral Fibers. Lyon, France: IARC, 1980;187–199.
52. McDonald AD, McDonald JC, Pooley FD. Mineral fiber content of lung in mesothelial tumors in North America. Ann Occup Hyg 1982;26:417–422.
53. Mowe G, Gylseth B, Hartveit F, et al. Fiber concentration in lung tissue of patients with malignant mesothelioma: a case-control study. Cancer 1985; 56:1089–1093.
54. Gaudichet A, Janson X, Manchaux G, et al. Assessment by analytical microscopy of the total lung fiber burden in mesothelioma patients matched with four other pathological series. Ann Occup Hyg 1988;32: 213–223.
55. McDonald JC, Armstrong B, Case BW, et al. Mesothelioma and asbestos fiber type: evidence from lung tissue analyses. Cancer 1989;63:1544–1547.
56. Rogers AJ, Leigh J, Berry G, et al. Relationship between lung asbestos fiber type and concentration and relative risk of mesothelioma. A case-control study. Cancer 1991;67:1912–1920.
57. Rodelsperger K, Woitowitz HJ, Bruckel B, et al. Dose-response relationship between amphibole fiber lung burden and mesothelioma. Cancer Detect Prev 1999;23:183–193.
58. Jaurand MC, Bignon J, Sebastian P, Goni J. Leaching of chrysotile asbestos in human lungs. Environ Res 1979;14:245–254.
59. Jaurand MC, Gaudichet A, Halpern S, Bignon J. In vitro biogradation of chrysotile fibres by alveolar macrophages and mesothelial cells in culture—comparison with a pH effect. Br J Ind Med 1984;41:389–395.
60. Wagner JC, Chamberlain M, Brown RC, et al. Biological effects of tremolite. Br J Cancer 1982;45:352–360.
61. Wagner JC, Skidmore JW, Hill RJ, Griffiths DM. Mesotheliomas in rats. Br J Cancer 1986;51:727–730.
62. Sebastien P, McDonald JC, McDonald AD, Case B, Harley R. Respiratory cancer in chrysotile textile and mining industries. Br J Ind Med 1989; 46:180–187.
63. Wagner JC, Berry G, Skidmore JW, Pooley FD. The comparative effects of three chrysotile by injection and inhalation in rats. In: Wagner JC, ed. Biological Effects of Mineral Fibers. IARC Publication 30. Lyon: International Agency Research on Cancer 1980;363–373.
64. Davis JMG, Addison J, Bolton RE, Donaldson K, Jones AD. Inhalation an injection studies in rats using dust samples from chrysotile asbestos prepared by a wet dispersion process. Br J Exp Pathol 1986;67:113–129.
65. Hansen K, Mossman BT. Generation of superoxide (O^{-}_{2}) from alveolar macrophages exposed to asbestiform and nonfibrous particles. Cancer Res 1987;47:1681–1686.

66. Chahinian AP, Pajak TF, Holland JF. Diffuse malignant mesothelioma. Ann Intern Med 1982;96:746–755.
67. Samson MK, Wasser LP, Borden EC, et al. Randomized comparison of cyclophosphamide, imidazole carboxamide, and Adriamycin versus cyclophosphamide and Adriamycin in patients with advanced stage malignant mesothelioma. A Sarcome Intergroup Study. J Clin Oncol 1987;5:86–91.
68. Antman KH, Shemin R, Ryan L. Malignant mesothelioma: prognostic variables in a registry of ISO patients, The Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. J Clin Oncol 1988;6:147–153.
69. Calavrezos A, Koschel G, Huselman H, et al. Malignant mesothelioma of the pleura. Klin Wochenschr 1988;66:607–613.
70. Spirtas R, Connelly RR, Tucker MA. Survival patterns for malignant mesothelioma: The SEER experience. Int J Cancer 1988;41:525–530.
71. Tammilehto L. Malignant mesothelioma: prognostic factors in a prospective study of 9 patients. Lung Cancer 1992;8:175–184.
72. Boutin C, Rey F, Gouvernet J, et al. Thoroscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Cancer 1993;72:394–404.
73. De Pangher Manzini V, Brollo A, et al. Prognostic factors of malignant mesothelioma of the pleura. Cancer 1993;72:410–417.
74. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 188 consecutive patients. Cancer 1993;72:410–417.
75. Currau D, Sahmoud T, Therasse P, et al. Prognostic factors in patients with pleural mesothelioma: the European Organization for Research and Treatment of Cancer experience. J Clin Oncol 1998;16:145–152.
76. Herndon JE, Green MR, Chahinian AP, et al. Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the Cancer and Leukemia Group B. Chest 1998;113:723–731.
77. Edwards JG, Abrams KR, Leverment JN, et al. Prognostic factors for malignant mesothelioma in 142 patients: validation of CALGB and EORTC prognostic scoring systems. Thorax 2000;55:731–735.
78. Schwarzenberger P, Harrison L, Freeman S, et al. Treatment of three patients with malignant mesothelioma with the gene modified cell line PA1-STK. Proc Am Soc Clin Oncol 1998;17:447a.
79. Sterman DH, Kaiser LR, Albelda SM. Advances in the treatment of malignant pleural mesothelioma. Chest 1999;116:504–520.
80. Mukherjee S, Haenel T, Himbeck R, et al. Replication-restricted vaccinia as a cytokine gene therapy vector in cancer: persistent transgene expression despite antibody generation. Cancer Gene Ther 2000;7:663–670.
81. Nowak AK, Lake RA, Kindler HL, Robinson BWS. New approaches for mesothelioma: biologic, vaccines, gene therapy, and other novel agents. Semin Oncol 2002;29(1):82–96.

14

Molecular Epidemiology of Mesothelioma

Riccardo Puntoni, Paolo G. Cerrano, and Rosangela Filiberti

Over the past 50 years epidemiology has been involved in the field of cancer research. By studying the association between risk factors and cancer occurrence, epidemiologists have contributed to the identification of the most important determinants of cancer in humans. In recent years, epidemiologists have concentrated on the link between genetic and environment in carcinogenesis, by focusing interests on low-level exposures.

Indeed, traditional epidemiology, called "black box epidemiology," is unable to study the mechanistic aspects of a disease (1). Therefore, the design of epidemiologic studies has been enriched by introducing biologic markers (Fig. 14.1), and step-by-step molecular epidemiology has been created. This new research, Perera (2) states, "seeks to combine the precision of laboratory methods to quantify carcinogenic dose or preclinical response in humans, with the relevance and rigor of analytic epidemiology." This new research philosophy is based on properly designed epidemiologic studies that take into account the control of confounding factors, the selection of appropriate control groups, the power of the studies, and the extent to which a biologic marker can predict cancer occurrence (3,4).

The aim of molecular epidemiology is to assess individual exposures to carcinogens and to quantify genetic damages linked to individual susceptibility, in order to estimate cancer risk at the individual level. Studies of genetically susceptible subgroups can detect high-risk subjects and can implement new methodologies to prevent cancer at the primary (chemoprevention) or secondary (screening programs) prevention level. Moreover, a better understanding of the natural history of cancer may also improve cancer treatment, by selecting the patients who will be able to benefit from specific therapies. In addition, a multidisciplinary approach between molecular biologists and epidemiologists, as well as physicians and biostatisticians, is needed.

The outcome of malignant mesothelioma (MM) is poor, and the therapy of the disease has not progressed in the past decade (5). But MM is largely preventable because the causative factors are mostly of

environmental origin. Nevertheless, genetic factors appear to be important by affecting individual susceptibility to carcinogens.

Many years ago traditional epidemiology highlighted the carcinogenetic effect of asbestos (6–8). Today, the molecular epidemiology approach has a great capability to study MM, by assessing susceptibility factors that might predispose to cancer and by detecting markers to monitor cancer risk in individuals exposed to carcinogens and to improve patient management.

The molecular events that underlie the development of this neoplasm have not yet been completely elucidated, such as the possible relationship between asbestos and simian virus 40 (SV40), and the intrinsic predisposition of mesothelial cells to accumulate genetic damages. It is clear that multiple genetic alterations are required for malignant transformation of mesothelium, as suggested from the long latency period between exposure and the onset of disease (9). Different mechanisms are involved in the etiology of MM, such as modified gene expression, gene silencing, gene amplification or rearrangement, complete gene loss, modified expression of their protein products, or combinations of multiple mechanisms. Other mechanisms include genomic instability, reduced DNA repair capacity and individual susceptibility (10).

In Figure 14.2 a multistep process of risk is hypothesized for MM. Environmental exposure, which may be monitored with specific exposure markers, such as asbestos bodies in the lung or SV40 antibodies, increases the risk of damaging DNA at the individual level. This event may be monitored through different markers of DNA damage and of susceptibility to DNA damage. Therefore, the presence of damage itself increases the risk of gene impairment. It is supposed that asbestos fibers interact with mesothelial cells, generating reactive free radicals (11), which may influence the activation of some oncogenes or the inhibition of suppressor genes by interfering with mechanisms of cell growth and with the expression of cytokines and growth factors or mutated proteins (11,12). The biologic features of the involved genes affect the aggressiveness and, consequently, the prognosis of disease.

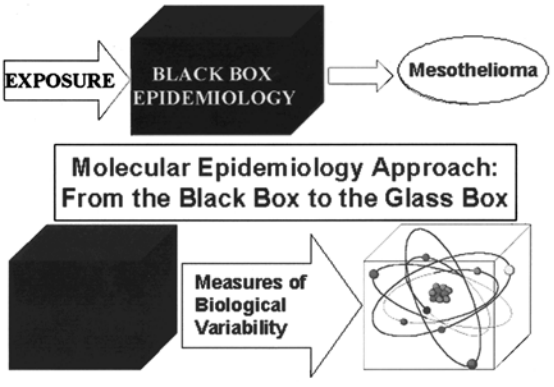


Figure 14.1. Paradigm of molecular epidemiology.

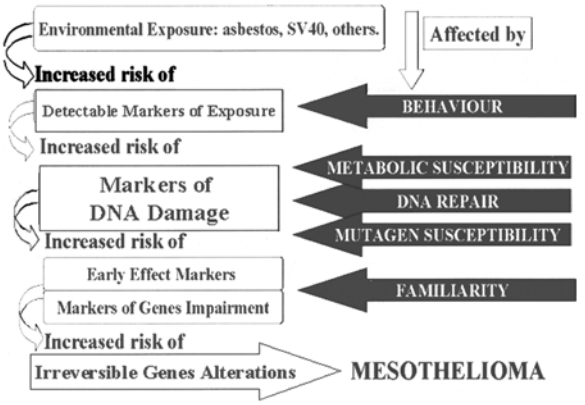


Figure 14.2. The multistep process of risk.

This chapter summarizes the principal ideas and methodologies used in the field of molecular epidemiology that can be applied to increase our understanding of the prevention and surveillance of malignant mesothelioma.

Biomarkers

Biomarkers are measurable biologic indicators of exposure, effect, susceptibility, or disease state that are used to understand the mechanisms of cancer progression (13). Even organs or tissues that are not considered directly involved in the carcinogenetic process can show a response proportional to the effective biologic dose. These tissues are commonly known as "surrogate cell populations." Chromosomal aberrations (CA) (14) or micronuclei (MN) in lymphocytes can be considered indirect indicators of response that takes place in target organs (e.g., pleura) and can therefore be considered as "surrogate" markers. Overall, this aspect is relevant in biomonitoring studies performed in living patients, since target tissues are often obtained by invasive procedures.

Studies with biomarkers may contribute to clarifying the etiology of cancer and improving risk estimates, leading to better preventive strategies.

Biomarkers of Susceptibility

Biomarkers indicate whether an individual is particularly sensitive in relation to the events produced by exposure. They highlight the differences among individuals or populations that may affect the response to asbestos, SV40, or other environmental agents. These differences depend on genetic or other individual features influencing the response of the target tissue. We have divided these markers into two main groups: metabolic susceptibility markers and DNA repair markers.

Metabolic Susceptibility Markers

The activity of the enzymes involved in the metabolism of carcinogens has great variability among individuals, due to the existence of a polymorphism in the genes coding for these enzymes. These differences may be inherited and can lead to conspicuous differences in individual sensitivity to the effects of chemical exposure, modifying the ability of a chemical to interact with proteins, RNA, or DNA.

Metabolic susceptibility markers can assess interindividual variations in the activities of metabolizing enzymes responsible for activation (phase I reactions) or deactivation (phase II reactions) of environmental or endogenous toxicants (16,17). Some metabolic susceptibility genes have been considered in recent years as risk factors for lung cancer and for human MM.

The most important polymorphic genes involved in respiratory cancer risk, as reported in the literature, are:

1. the genes of the cytochrome P450 (CYP) family, which mediate the phase I reactions of metabolic activation;
2. the phase II genes glutathione S-transferases (GSTs) and *N*-acetyltransferases (NATs);
3. the microsomal epoxide hydrolase (mEH) gene, which plays a dual role in bioactivation and detoxification of procarcinogens.

Many P450 genes are polymorphic, including *CYP1A1*, whose product metabolizes polycyclic aromatic hydrocarbons (PAHs) such as benzopyrene (BP). The higher lung cancer risk from the "susceptible" *CYP1A1* genotype was seen in light smokers, whereas heavy smokers with this genotype had less than twice the risk of heavy smokers without the genotype (18,19). The gene *GST* has a basic role in phase II reactions to deactivate carcinogens. About 40% to 50% of Caucasians possess a *GSTM1* null genotype (20). The state of acetylation is controlled by two autosomal alleles in a single locus; rapid acetylation is dominating, whereas slow acetylation is recessive. Approximately half of the Western population is slow acetylating (NAT).

Microsomal epoxide hydrolase (mEPHX) is a critical metabolic enzyme involved in the activation and subsequent detoxification of procarcinogens and plays a role in the metabolic activation of the PAHs.

Metabolic genes encoding for enzymes involved in conjugation and detoxification are likely to be implicated in the MM carcinogenetic pathway, due to the presence of free radicals generated from asbestos exposure.

One study showed that nonmalignant asbestos-related diseases develop more frequently in occupationally asbestos-exposed subjects carrying a homozygous deletion (null genotype) of *GSTM1* gene (21). A second study reported that "individuals with homozygous deletion of the *GSTM1* gene and a *NAT2* slow-acetylator genotype who are exposed to high levels of asbestos appear to have enhanced susceptibility to asbestos-related pulmonary disorders" (22). On the contrary, the *GSTM1* genotype did not prove to interact with asbestos exposure in the risk of lung cancer (23).

A paper on MM was published in 1995 (24) that reported that individuals with combined *GSTM1* and *NAT2* defects had about a fourfold risk of developing MM compared to those with the *GSTM1* gene and *NAT2* fast acetylator genotype [odds ratio (OR) = 3.6; 95% confidence interval (CI) = 1.3–9.6]. Moreover, the risk among subjects highly exposed to asbestos with the double at-risk genotype was more than sevenfold greater than those with the more beneficial genotypes of both *GSTM1* and *NAT2* genes (OR = 7.4; 95% CI = 1.6–34.0).

We have carried out a preliminary study by analyzing the distribution of *CYP1A1*, *mEH*, *GSTM1*, *GSTT1*, and *NAT2* genotypes in an Italian study population consisting of 55 MM patients and 200 population controls. The combination of the *NAT2* fast acetylator and the *GSTM1* null genotype posed a significantly increased risk of MM compared to the combination of the *NAT2* slow acetylator and the functional *GSTM1* genotype. A combined effect was also observed for the *NAT2* fast acetylator and the *mEH* low-activity genotypes compared with the *NAT2* slow acetylator and the high *mEH* activity genotype combination.

When the MM patients were stratified according to the degree of asbestos exposure, the putative *mEH* high-activity genotypes appeared to be totally absent among the patients with low or unlikely exposure to asbestos. The association reached the statistical significance when cases with high activity were compared with cases of intermediate or low activity, or intermediate plus low activity cases combined.

The most remarkable combined effect of borderline significance was observed for the concurrent presence of the *NAT2* fast acetylator genotype and the *mEH* low-activity genotype, compared to the combination of the *NAT2* slow acetylator genotype and the high *mEH* activity genotype.

Our preliminary results strengthen the hypothesis that metabolic gene polymorphisms involved in oxidation processes have a role in modulating individual susceptibility to MM in subjects with different degrees of asbestos exposure.

The lack of any association between *CYP1A1* genotypes and MM corroborates the hypothesis that PAHs, the main metabolic substrates of this enzyme, have no direct effect in the development of malignant mesothelioma.

In an immunohistochemistry experiment, expression of the GST subclasses alpha, mu, and pi in 20 patients with nonneoplastic mesothelium and in 57 patients with malignant mesothelioma was studied. The expression of GST pi was reported to be positively correlated with increased survival in MM. Therefore, the authors concluded that GST and P-170 glycoprotein may contribute to the resistance to cytotoxic drugs frequently observed in these tumors, but no correlation was demonstrated between GST and P-170 expression (25).

DNA Repair Markers

The repair of DNA damage, i.e., base excision repair and nucleotide excision repair, protects the cell from the injuries of mutagens and it is necessary for the maintenance of genomic stability. Failure of this

system, originally demonstrated in individuals with xeroderma pigmentosum, can lead to cancer. It has been estimated that inherited defects in the DNA repair system can account for 15% to 25% cases—and even more—of sporadic cancers of different organs (26).

The assays of DNA repair capacity have been recently grouped into four categories on the basis of the evaluation of DNA damage induced with chemicals or physical agents. The evaluation tests are the mutagen sensitivity assay, induced micronuclei, and the comet assay. Moreover, there are more accurate measures of repair kinetics, such as the host cell reactivation assay, measures of genetic variation associated with DNA repair, and indirect tests of DNA repair, such as unscheduled DNA synthesis (27).

There is no direct information available on DNA damage and MM. Here some data related to asbestos and SV40, the two major determinants of MM are reported. Asbestos does not significantly induce gene mutations in bacterial and mammalian systems but causes structural and numerical chromosome aberrations in cultured mammalian cells. It has been found that asbestos fibers produce a cell transformation and genotoxicity characterized by the formation of aneuploid cells, abnormal anaphases, chromosomal aberrations, DNA single-strand breaks, and DNA repair in human mesothelial cells (28–35).

In SV40 immortalized cell lines an interference of tumor (T) antigen with DNA repair has been reported (28). Simian Virus 40 large-T antigen (SVLTAg) has been widely used to immortalize cells. It was hypothesized that DNA mismatch repair (MMR) activity is important during SVLTAg-induced immortalization and that the immortalized cells are deficient in repairing G:T, A:C, and G:G mispairs in bacteriophage M13mp2 (29). In addition, the *p53* tumor suppressor gene is able to activate excision repair that is ultraviolet (UV) induced in human cells. The SVLTAg binds p53 protein and can interfere with its function. Simian virus 40 transformation was shown to reduce the levels of DNA repair, most likely because of the inhibition of normal p53 function by LTAg (30). Tag expression in mesothelial cells might have both adverse and beneficial effects by impairing the control of DNA integrity and enhancing apoptosis, respectively (31). As SV40 appears to play a possible role in the impairment of DNA repair mechanisms in mesothelial cells, an additive effect with asbestos fibers may be hypothesized.

In consideration of the DNA damages generated by asbestos or SV40 exposure, all markers reported in the above-mentioned four categories could be useful to estimate DNA repair capability at the individual level. The DNA repair capability in MM patients is still overlooked in the literature.

Micronucleus Test as an Index of Susceptibility to Malignant Mesothelioma

The decrease in DNA repair leads to increased genetic damage as measured by cytogenetic damage, including formation of micronuclei.

The micronucleus assay is the method we have chosen for assessing chromosome damage because it enables both chromosome loss and chromosome breakage to be measured reliably (36). The micronucleus test (MT) in peripheral blood lymphocytes (PBLs) seems to be a useful method for monitoring individuals with genetic instability (37), and recent evidence suggests the usefulness of MT as a screening test for carriers of specific mutations in evaluating cancer susceptibility (38). Micronuclei (MN) are small amounts of DNA that arise in the cytoplasm when chromatid/chromosomal fragments are not incorporated into daughter nuclei during mitosis, often because these fragments do not possess a centromere. Acentric fragments remain behind at anaphase, whereas chromosomal elements with centromeres are drawn toward the spindle poles (39). Therefore, the formation of micronuclei requires a dividing cell population. Micronuclei are about 1/20 to 1/5 the size of the main nucleus. Usually there is only one micronucleus formed per cell (40). The frequency of micronuclei is usually reported as the number of cells containing micronuclei per total cells counted.

A study was carried out to evaluate, by the modified cytokinesis-blocked method of Fenech and Morley (41), the MN frequency in PBLs of patients with pleural MM compared to lung cancer (LC) patients and two control groups [patients with BRDs, such as chronic obstructive pulmonary disease, asbestosis and silicosis, and healthy controls (HCs)] in order to ascertain the relevance of this biomarker to express the susceptibility of individuals to develop pleuropulmonary tumors. Analysis was performed blindly of the subjects' status only on binucleated (BN) lymphocytes with preserved cytoplasm. An average of 2000 cells were analyzed for each subject, and all reported MN counts were the mean of duplicate determinations. Means, medians, and standard deviations were calculated in terms of BN cells with MN: (BN – MN)/1000 BN cells. Nonparametric tests were used to check the differences among the groups. A significant increased MN frequency in PBLs was observed only in patients with MM in comparison with all other groups. No difference was observed between LC patients and HCs, or among the different types of BRD subjects (Fig. 14.3).

Asbestos exposure has never been associated with a high frequency of MN and DNA alterations. Moreover, numerical and structural chromosomal aberrations and an increase in MN frequency were also reported in human cells in a number of studies (42–44). Since PBLs are not the direct target for asbestos fibers, an increase in the cumulative genetic damage in this surrogate tissue supplies an index of the cumulative genetic damage occurring during the PBLs' life span (45).

A study on breast cancer has indicated a close relationship between the presence of a *BRCA1* mutation and sensitivity for the induction of micronuclei. The authors, in contrast to the results with the micronucleus assay, found no significant difference between women with and without a *BRCA1* mutation with respect to the induction and repair of DNA damage in the comet assay. The results suggested a normal rate of damage removal and points to a disturbed fidelity of DNA repair (46). The increase of micronucleated PBLs in MM, patients together

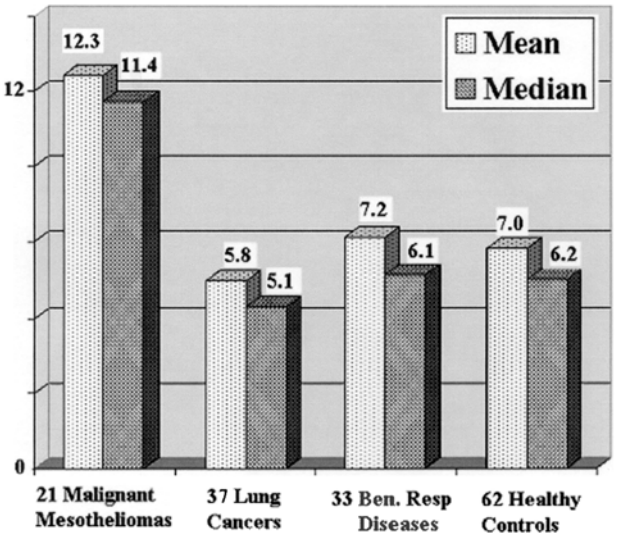


Figure 14.3. Binucleated cells with micronuclei in study subjects.

with the report of an association of this event with genetic characteristics in other neoplasia, is consistent with the existence of genetic factors predisposing to the development of MM related to defects in DNA repair systems.

Biomarkers of Exposure, Diagnosis, and Prognosis

The alterations observed in tumor suppressor genes and dominant oncogenes may be found in applications to molecular epidemiology and to clinical work. The impairment of the genes is persistent, so it may reflect etiologic exposure. It may provide a useful tool for diagnostic tests, and even for early detection, since the expression of certain markers may occur prior to the development of some overt malignancies (47). Moreover, the impairment may act as a prognostic marker, providing a good estimate of tumor aggressiveness and, eventually, it may be able to influence therapy decisions, because therapies can be addressed to the oncogenes or their protein products (48).

Oncoproteins have access to the extracellular environment and are detectable in peripheral fluids such as serum or plasma, so the measure of their circulating level provides an interesting opportunity to assess the diagnostic and prognostic value of a marker overexpression in patients with tumors that are not easily accessible to biopsy (49).

We briefly describe some markers of genes impairment, according to their possible utility in the molecular epidemiology of MM.

Tumor Suppressor Genes

A critical role in the development and progression of MM is played by the loss or inactivation of tumor suppressor (50). $p16^{INK4}$ is a protein product of the *CDKN2* tumor suppressor gene and is being studied as one of the most interesting markers in MM, from a diagnostic and a therapeutic point of view (51,52). It has been reported that deletion of

$p16^{INK4a}$ occurs in from 22% to 70% of primary mesotheliomas (10,53). Since the prevalence of asbestos fibers was found to be lower in MM patients with any p16 alteration than in those with no p16 alteration, it is possible that deletion of p16 occurs in a relatively susceptible subset of MM (54). Homozygous *CDKN2A* deletion may be a diagnostic marker to characterize malignant mesothelial cells from benign reactive cells (55). Loss of $p16^{INK4}$ protein expression can result also from epigenetic mechanisms, such as an abnormal DNA hypermethylation. The inhibition of methylation in mesothelioma may provide a potential treatment target in some MM (56,57).

The commonest genetic alterations observed in human cancer are linked to mutations in the *p53* tumor suppressor gene. p53 Antibodies (p53-Abs) have been indicated as possible biomarkers for early diagnosis and prognosis in various neoplasms (49,58), and sometimes have been associated with a poor prognosis and poor survival (59). In addition, serum p53-Abs could be useful in identifying individuals at high cancer risk. This has been suggested by studies on a group of workers with occupational exposure to vinyl chloride who were found to be seropositive even more than 11 years before the clinical detection of angiosarcoma of the liver (60), or on patients with chronic obstructive pulmonary disease (COPD) who had elevated serum p53-Abs about 7 months before the diagnosis of lung cancer (61). The *p53* gene is rarely mutated in MM (52,62–64), but p53 immunoreactivity in tumor tissue was shown in a proportion ranging from 25% to 86% (65,66). There are few data in the literature on p53-Abs in MM, and the results are not encouraging concerning their value as diagnostic or prognostic indicators (67,68).

We analyzed the anti-p53 autoantibody level in 30 MM patients, 48 LC patients, 55 subjects with benign lung diseases (BLDs), and in 51 HCs. In our investigation 7.4% of the MMs, 17% of the LCs, 3.6% of BLDs, and none of the HCs had elevated serum levels of the anti-p53 autoantibodies (69). According to these data, the presence of detectable p53-Abs in serum of patients with MM appears to be occasional and does not seem to serve as either a diagnostic or a prognostic indicator. Nevertheless, the presence of two positives among patients at high risk of developing pleuropulmonary malignancies underlines the need for further investigation with prolonged follow-up and an increased number of subjects.

Some genes have been recognized to be hallmarks of MM and suitable for differentiating MM from other tumors. Among these, the Wilms' tumor 1 susceptibility (WT1) is selectively expressed in MM (70). The detection of WT1 messenger RNA (mRNA) and of the WT1 protein is particularly useful in differentiating MM from adenocarcinoma in tissue sections of pleural tumors (71).

Oncogenes

The role of *ras* in the development of MM is uncertain. No mutation of H-*ras* has been found in MM tissues by Cristaudo et al (72), and results from other studies indicate that the K-*ras* proto-oncogene cannot play a critical role in the induction of mesothelioma by asbestos, either in

humans or in rats (73). On the contrary, positivity for p21 was found in 35% of MM tissues examined by Isik et al (68). In this study, a relationship between asbestos exposure and p21 was found, since immunopositivity for p21 was higher for patients with environmental asbestos exposure and was correlated to exposure times (68). Interesting results come from the determination of p21 expression in serum. A cohort study on serum samples of 46 pneumoconiosis patients revealed two MM patients, both positive for p21. In this case, the protein seemed to support the role of an early diagnostic marker, since the positivity of the MM patients preceded by 11 and 26 months the clinical evidence of the tumor (74). The role of p21 as a prognostic marker is controversial (68,75).

Growth Factors

In recent years, attention has been focused on oncogenes' causing the production of growth factors or their receptors that have intrinsic tyrosine kinase activity. Receptor tyrosine kinases have become therapeutic targets for molecularly aimed therapies, and it is possible that treatment of MM patients may benefit from this research (31–33).

In a pilot study on 62 MMs, 35 LCs, 51 nonneoplastic subjects exposed to asbestos and assumed to be at-risk controls (RCs), and 25 HCs, we investigated the role of *platelet-derived growth factor* (PDGF), which is involved in the pathogenesis of MM (76–78). Only a few studies have investigated PDGF levels in the blood of neoplastic patients (74,79,80). Brandt-Rauf et al (74) reported a higher serum level of PDGF in advanced pneumoconiosis cases than in patients with disease at an earlier stage. According to this study, patients with higher PDGF levels had an increased probability of having disease progression, suggesting that serum PDGF levels may be a marker for the development of severe and progressive asbestos-related diseases. We found positive values in 42% of MMs, 8% of RCs, 3% of LCs, and in 4% of HCs (preliminary data, unpublished). These results indicate that high serum PDGF-AB levels could be used as a suggestive indicator of MM. This hypothesis, and the fact that PDGF is thought to be an autocrine growth factor for mesothelioma, support the trials that are testing a highly selective inhibitor of the PDGF receptor tyrosine kinase as a therapeutic agent (81).

The epidermal growth factor receptor (EGFR) family is a group of four structurally similar tyrosine kinases, among which there are the EGFR and HER2/neu protein, encoded by the HER-2/*neu* gene. Immunoreactivity for HER2/neu was found in 97% of MM patients (82). The protein has been detected at increased serologic levels in subjects at risk of cancer, such as in asbestosis patients, who later developed lung cancer (83). Thus, it may constitute a marker of cancer risk (83), and possibly of exposure. In fact, an association between occupational exposures, mainly asbestos, and enhanced secretion of the protein was found among healthy asbestos workers without asbestosis or cancer (84,85).

Suggestions for the utility of circulating HER-2/neu protein as an independent biologic prognostic factor come from a study on patients

with advanced non–small-cell lung cancer (NSCLC) who submitted to standard combination chemotherapy. In this study elevated serum level of HER-2/neu protein were associated with a poor survival outcome, even if no significant differences were observed in HER-2/neu serum concentration in lung cancer patients and a group of matched healthy controls (86,87).

Several studies have demonstrated that both EGF and its receptor (EGFR) are involved in the development and progression of MM (88,89), and are correlated with poor prognosis in some types of tumors. Autophosphorylation of EGFR occurs in mesothelial cells after exposure to asbestos, and may initiate cell-signaling cascades that are important in asbestos-induced carcinogenesis (90). Modification of phosphorylation provides a rationale for the preventive and therapeutic approaches to lung cancers and mesothelioma (91). Other studies in vitro or on animal models have shown that an agent that significantly inhibits EGFR may be an effective therapeutic option for patients with MM (81,92).

The data in the literature support the diagnostic importance of the hepatocyte growth factor/scatter factor (HGF/SF) and its receptor in MM (82,93,94). Moreover, studies on lung cancer showed that when c-*met* is mutated or overexpressed in malignant cells it serves as an important therapeutic indication (95), while elevated HGF is associated with a poor prognosis and may be useful as a marker of risk in early-stage tumors (96).

In our experience, the concentration of both HGF and EGF markers in MM was double the concentration in HC. In this case, positivity was found in 60% of MM patients and in none of the HCs for HGF, and in 50% of MMs and 18% of HCs for EGF. In addition, a significantly correlation existed between the two markers. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are potent angiogenic factors that promote vessel formation. An effective therapeutic approach for MM (97,99) may derive from VEGF. The concentration of this factor was significantly higher in pleural effusions of MM patients than in those from patients with nonmalignant pleural disease, and an inverse correlation between serum VEGF levels and MM outcome was found (100).

The survival of MM patients seems to be affected also by bFGF, even if it is expressed more in nonmalignant than in malignant pleural effusions (101).

References

1. Skrabanek P. The emptiness of the black box. Epidemiology 1994;5:553–555.
2. Perera FP. Molecular cancer epidemiology: a new tool in cancer prevention. J Natl Cancer Inst 1987;78:887–898.
3. Hulka BS, Wilcosky T. Biological markers in epidemiologic research. Arch Environ Health 1988;43:83–89.

4. Wogan GN. Molecular epidemiology in cancer risk. Assessment and prevention: recent progress and avenues for future research. Environ Health Perspect 1992;98:167–178.
5. Waller DA. The role of the surgery in diagnosis and treatment of malignant pleural mesothelioma. Curr Opin Oncol 2003;15:139–143.
6. Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. JAMA 1964;188:22–26.
7. Wagner JC, Gilson JC, Berry G, Timbrell V. Epidemiology of asbestos cancer. B Med Bull 1971;27:71–76.
8. Puntoni R, Vercelli M, Merlo F, Valerio F, Santi L. Mortality among shipyard workers in Genoa, Italy. Ann NY Acad Sci 1979;330:353–377.
9. Huncharek M. Genetic factors in the aetiology of malignant mesothelioma. Eur J Cancer 1995;31A:1741–1747.
10. Lechner JF, Tesfaiqzi J, Gerwin BI. Oncogenes and tumor-suppressor genes in mesothelioma—a synopsis. Environ Health Perspect 1997;105: 1061–1067.
11. Kamp DW, Weitzman SA. The molecular basis of asbestos induced lung injury. Thorax 1999;54:638–652.
12. Heintz NH, Janssen YM, Mossmann BT. Persistent induction of c-*fos* and c-*jun* expression by asbestos. Proc Natl Acad Sci USA 1993;90:3299–3303.
13. Committee on Biological Markers of the National Research Council: biological markers in environmental health research. Environ Health Perspect 1987;74:3–9.
14. Bonassi S, Hagmar L, Stromberg U, et al, for the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. Cancer Res 2000;60:1619–1625.
15. Bartsch H, Aitio A, Camus AM, et al. Carcinogen-metabolizing enzymes and susceptibility to chemical carcinogenesis. IARC Sci Publ 1982;39:337–350.
16. Hanke JZ. Genetic susceptibility to toxic substances and its relationship to carcinogenesis. IARC Sci Publ 1984;59:99–106.
17. Dejmek A, Brockstedt U, Hjerpe A. Immunoreactivity of pleural malignant mesotheliomas to glutathione S-transferases. APMIS 1998;06:489–494.
18. Segers K, Kumar-Singh S, Weyler J, et al. Glutathione S-transferase expression in malignant mesothelioma and non-neoplastic mesothelium: an immunohistochemical study. Cancer Res Clin Oncol 1996;122:619–624.
19. Seidegard PG, Vorachek VR, Pero RW, Pearson WR. Hereditary differences in the expression of human glutathione trasferase active in *trans*-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA 1988; 85:7293–7297.
20. Zhao H, Spitz MR, Gwyn KM, Wu X. Microsomal epoxide hydrolase polymorphisms and lung cancer risk in non-Hispanic whites. Mol Carcinog 2002;33,2:99–101.
21. Smith CM, Kelsey KT, Wiencke JK, Leyden K, Stephen L, Christiani DC. Inherited glutathione S-transferase deficiency is a risk factor for pulmonary asbestosis. Cancer Epidemiol Biomarkers Prev 1994;3:471–477.
22. Hirvonen A, Saarikoski ST, Linnainmaa K, et al. Glutathione S-transferase and N-acetyltransferase genotypes and asbestos-associated pulmonary disorders. J Natl Cancer Inst 1996;88:1853–1856.
23. Stucker I, Boffetta P, Antilla S, et al. Lack of interaction between asbestos exposure and glutathione S-transferase M1 and T1 genotypes in lung carcinogenesis. Cancer Epidemiol Biomarkers Prev 2001;10:1253–1258.

24. Hirvonen A, Pelin K, Tammilehto L, Karjalainen A, Mattson K, Linnainmaa K. Inherited GSTM1 and NAT2 defects as concurrent risk modifiers in asbestos-related human malignant mesothelioma. Cancer Res 1995;55:2981–2983.
25. Segers K, Kumar-Singh S, Weyler J, et al. Glutathione S-transferase expression in malignant mesothelioma and non-neoplastic mesothelium: an immunohistochemical study. J Cancer Res Clin Oncol 1996;122:619–624.
26. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol 2003;21:1174–1179.
27. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 2000;92:874–897.
28. Renier A, Yegles M, Buard A, et al. Use of mesothelial cell cultures to assess the carcinogenic potency of mineral or man made fibers. Cell Biol Toxicol 1992;8:133–139.
29. Ollikainen T, Linnainmaa K, Kinnula VL. DNA single strand breaks induced by asbestos fibers in human pleural mesothelial cells in vitro. Environ Mol Mutagen 1999;33:153–160.
30. Okayasu R, Takahash S, Yamada S, Hei TK, Ullrich RL. Asbestos and DNA double strand breaks. Cancer Res 1999;59:298–300.
31. Liu W, Ernst JD, Courtney Broaddus V. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage, and apoptosis in mesothelial cells. Am J Respir Cell Mol Biol 2000;23:371–378.
32. Digweed M, Demuth I, Rothe S, et al. SV40 large T-antigen disturbs the formation of nuclear DNA-repair foci containing MRE11. Oncogene 2002; 21:4873–4878.
33. Yeh CC, Lee C, Huang MC, Dahiya R. Loss of mismatch repair activity in simian virus 40 large T antigen-immortalized BPH-1 human prostatic epithelial cell line. Mol Carcinog 2001;31:145–151.
34. Bowman KK, Sicard DM, Ford JM, Hanawalt PC. Reduced global genomic repair of ultraviolet light-induced cyclobutane pyrimidine dimers in simian virus 40-transformed human cells. Mol Carcinog 2000;29:17–24.
35. Levresse V, Moritz S, Renier A, et al. Effect of simian virus large T antigen expression on cell cycle control and apoptosis in rat pleural mesothelial cells exposed to DNA damaging agents. Oncogene 1998;16:1041–1053.
36. Fenech M. The in vitro micronucleus technique. Mutat Res 2000;455:81–95.
37. Maluf SW, Erdtmann B. Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis in single-cell gelelectrophoresis. Cancer Genet Cytogenet 2001;124:71–75.
38. Trenz K, Rothfuss A, Schutz P, Speit G. Mutagen sensitivity of peripheral blood from women carrying a BRCA1 or BRCA2 mutation. Mutat Res 2002;500:89–96.
39. Schmid W. The micronucleous test. Mutat Res 1975;31:9–15.
40. Schlegel R, MacGregor JT, Everson RB. Assessment of cytogenetic damage by quantification of micronuclei in human peripheral blood erythrocytes. Cancer Res 1986;46:3717–3721.
41. Fenech M, Morley AA. Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation. Mutat Res 1986;161:193–198.
42. Lechner JF, Tokiwa T, La Veck M, et al. Asbestos-associated chromosomal changes in human mesothelial cells. Proc Natl Acad Sci USA 1985;82: 3884–3888.
43. Dopp E, Schuler M, Schiffmann D, Eastmond DA. Induction of micronuclei, hyperploidy, and chromosomal breakage affecting the centric/

pericentric regions of chromosomes 1 and 9 in human amniotic fluid cells after treatment with asbestos. Mutat Res 1977;377:77–87.
44. Keane MJ, Stephens JW, Zhong BZ, Miller WE, Ong TM, Wallace WA. A study of the effect of chrysotile fiber surface composition on genotoxicity *in vitro*. J Toxicol Environ Health 1999;57:529–541.
45. Bolognesi C, Filiberti R, Neri M, et al. High frequency of micronuclei in peripheral blood lymphocytes as index of susceptibility to pleural mesothelioma. Cancer Res 2002;62:5418–5419.
46. Rothfuss A, Schutz P, Bochum S, et al. Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. Cancer Res 2000;60:390–394.
47. Partanen R, Koskinen H, Oksa P, et al. Serum oncoproteins in asbestosis patients. Clin Chem 1995;41:1844–1847.
48. Taylor JA. Oncogenes and their applications in epidemiologic studies. Am J Epidemiol 1989;130:6–13.
49. Brandt-Rauf PW. Biomarkers of gene expression: growth factors and oncoproteins. Environ Health Perspect 1997;105S4:807–816.
50. Lee WC, Testa JR. Somatic genetic alterations in human malignant mesothelioma (review). Int J Oncol 1999;14:181–188.
51. Frizelle SP, Grim J, Zhou J, et al. Re-expression of p16INK4a in mesothelioma cells results in cell cycle arrest, cell death, tumor suppression and tumor regression. Oncogene 1998;16:3087–3095.
52. Papp T, Schipper H, Pemsel H, et al. Mutational analysis of N-ras, p53, p16INK4a, p14ARF and CDK4 genes in primary human malignant mesotheliomas. Int J Oncol 200;18:425–433.
53. Xio S, Li D, Vijg J, et al. Codeletion of p15 and p16 in primary malignant mesothelioma. Oncogene 1995;11(3):511–515.
54. Hirao T, Bueno R, Chen CJ, et al. Alterations of the p16(INK4) locus in human malignant mesothelial tumors. Carcinogenesis 2002;23:1127–1130.
55. Illei PB, Ladanyi M, Rusch VW, et al. The use of CDKN2A deletion as a diagnostic marker for alignant mesothelioma in body cavity effusions. Cancer 2003;99:51–56.
56. Kratzke RA, Otterson GA, Lincoln CE, et al. Immunohistochemical analysis of the p16INK4 cyclin-dependent kinase inhibitor in malignant mesothelioma. J Natl Cancer Inst 1995;87:1870–1875.
57. Wong L, Zhou J, Anderson D, et al. Inactivation of p16(INK4a) expression in malignant mesothelioma by methylation. Lung Cancer 2002;38:131–136.
58. Lubin R, Zalcman G, Bouchet, et al. Serum p53 antibodies as early markers of lung cancer. Nature Med 1995;1:701–702.
59. Zalcman G, Tredaniel J, Schlichtholz B, et al. Prognostic significance of serum p53 antibodies in patients with limited-stage small cell lung cancer. Int J Cancer (Pred Oncol) 2000;89:81–86.
60. Trivers GE, Cawley HL, DeBenedetti VM, et al. Anti-p53 antibodies in sera of workers occupationally exposed to vinyl chloride. J Natl Cancer Inst 1995;87:1400–1407.
61. Trivers GE, De Benedetti VMG, Cawley H, et al. Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. Clin Cancer Res 1996;2:1767–1775.
62. Metcalf RA, Welsh JA, Bennett WP, et al. p53 and Kirsten-ras mutations in human mesothelioma cell lines. Cancer Res 1992;52:2610–2615.
63. Mor O, Yaron P, Huszar M, et al. Absence of p53 mutations in malignant mesotheliomas. Am J Respir Cell Mol Biol 1997;16:9–13.

64. Kitamura F, Araki S, Suzuki Y, et al. Assessment of the mutations of p53 suppressor gene and Ha- and Ki-ras oncogenes in malignant mesothelioma in relation to asbestos exposure: a study of 12 American patients. Ind Health 2002;40:175–181.
65. Ramael M, Lemmens G, Eerdekens C, et al. Immunoreactivity for p53 protein in malignant mesothelioma and non-neoplastic mesothelium. J Pathol 1992;168: 371–375.
66. Esposito V, Baldi A, De LA, et al. p53 immunostaining in differential diagnosis of pleural mesothelial proliferations. Anticancer Res 1997;17:733–736.
67. Creaney J, McLaren BM, Stevenson S, et al. p53 autoantibodies in patients with malignant mesothelioma: stability through disease progression. Br J Cancer 2001;84:52–56.
68. Isik R, Metintas M, Gibbs AR, et al. p53, p21 and metallothionein immunoreactivities in patients with malignant pleural mesothelioma: correlations with the epidemiological features and prognosis of mesotheliomas with environmental asbestos exposure. Respir Med 2001;95: 588–593.
69. Neri M, Betta P, Marroni P, et al. Serum anti-p53 autoantibodies in pleural malignant mesothelioma, lung cancer and non-neoplastic lung diseases. Lung Cancer 2003;39:165–172.
70. Amin KM, Litzky LA, Smythe WR, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. Am J Pathol 1995;146:344–356.
71. Hecht JL, Lee BH, Pinkus JL, et al. The value of Wilms tumor susceptibility gene 1 in cytologic preparations as a marker for malignant mesothelioma. Cancer 2002;96:105–109.
72. Cristaudo A, Vivaldi A, Sensales G, et al. Molecular biology studies on mesothelioma tumor samples: preliminary data on H-ras, p21, and SV40. J Environ Pathol Toxicol Oncol 1995;14:29–34.
73. Ni Z, Liu Y, Keshava N, et al. Analysis of K-ras and p53 mutations in mesotheliomas from humans and rats exposed to asbestos. Mutat Res 2000;468:87–92.
74. Brandt-Rauf PW, Smith S, Hemminki K, et al. Serum oncoproteins and growth factors in asbestosis and silicosis patients. Int J Cancer 1992;50: 881–885.
75. Baldi A, Groeger AM, Esposito V, et al. Expression of p21 in SV40 large T antigen positive human pleural mesothelioma: relationship with survival. Thorax 2002;57:353–356.
76. Langerak AW, De Laat PA, Van der Linden-Van Beurden CA, et al. Expression of platelet-derived growth factor (PDGF) and PDGF receptors in human malignant mesothelioma in vitro and in vivo. J Pathol 1996;178: 151–160.
77. Ascoli V, Scalzo CC, Facciolo F, Nardi F. Platelet-derived growth factor receptor immunoreactivity in mesothelioma and nonneoplastic mesothelial cells in serous effusions. Acta Cytol 1995;39:613–622.
78. Metheny-Barlow LJ, Flynn B, van Gijssel HE, et al. Paradoxical effects of platelet-derived growth factor-A overexpression in malignant mesothelioma. Antiproliferative effects in vitro and tumorigenic stimulation in vivo. Am J Respir Cell Mol Biol 2001;24:694–702.
79. Mossman BT, Gruenert DC. SV40, growth factors, and mesothelioma. Another piece of the puzzle. Am J Respir Cell Mol Biol 2002;26:167–170.

80. Ariad S, Seymour L, Bezwoda WR. Platelet-derived growth factor (PDGF) in plasma of breast cancer patients: correlation with stage and rate of progression. Breast Cancer Res Treat 1991;20:11–17.
81. Nowak AK, Lake RA, Kindler HL, et al. New approaches for mesothelioma: biologics, vaccines, gene therapy, and other novel agents. Semin Oncol 2002;29:82–96.
82. Thirkettle I, Harvey P, Hasleton PS, et al. Immunoreactivity for cadherins, HGF/SF, met, and erbB-2 in pleural malignant mesotheliomas. Histopathology 2000;36:522–528.
83. Brandt-Rauf PW, Luo JC, Carney WP, et al. Detection of increased amounts of the extracellular domain of the c-erbB-2 oncoprotein in serum during pulmonary carcinogenesis in humans. Int J Cancer 1994;56:383–386.
84. Lahat N, Froom P, Kristal-Boneh E, et al. Increased serum concentration of growth factor receptors and Neu in workers previously exposed to asbestos. Occup Environ Med 1999;56:114–117.
85. Krajewska B, Lutz W, Pilacik B. Determination of blood serum oncoprotein NEU and antioncoprotein p-53—molecular biomarkers in various types of occupational exposure. Int J Occup Med Environ Health 1998;11: 343–348.
86. Filiberti R, Marroni P, Paganuzzi M, et al. c-erbB-2 protein in serum of primary lung cancer patients. Cancer Detect Prev 2002;26:64–68.
87. Ardizzoni A, Cafferata MA, Paganuzzi M, et al. Study of pretreatment serum levels of HER-2/neu oncoprotein as a prognostic and predictive factor in patients with advanced nonsmall cell lung carcinoma. Cancer 2001;82:1896–1904.
88. Morocz IA, Schmitter D, Lauber B, et al. Autocrine stimulation of a human lung mesothelioma cell line is mediated through the transforming growth factor alpha/epidermal growth factor receptor mitogenic pathway. Br J Cancer 1994;70:850–856.
89. Vogelzang NJ. Emerging insights into the biology and therapy of malignant mesothelioma. Semin Oncol 2002;29:35–42.
90. Pache JC, Janssen YM, Walsh ES, et al. Increased epidermal growth factor-receptor protein in a human mesothelial cell line in response to long asbestos fibers. Am J Pathol 1998;152:333–340.
91. Manning CB, Cummins AB, Jung MW, et al. A mutant epidermal growth factor receptor targeted to lung epithelium inhibits asbestos-induced proliferation and proto-oncogene expression. Cancer Res 2002;62:4169–4175.
92. Janne PA, Taffaro ML, Salgia R, et al. Inhibition of epidermal growth factor receptor signaling in malignant pleural mesothelioma. Cancer Res 15:5242–5247.
93. Tolnay E, Kuhnen C, Wiethege T, et al. Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma. J Cancer Res Clin Oncol 1998;124:291–296.
94. Harvey P, Warn A, Newman P, et al. Immunoreactivity for hepatocyte growth factor/scatter factor and its receptor, met, in human lung carcinomas and malignant mesotheliomas. J Pathol 1996;180:389–394.
95. Maulik G, Shrikhande A, Kijima T, et al. Role of the hepatocyte growth factor receptor, c-Met, in oncogenesis and potential for therapeutic inhibition. Cytokine Growth Factor Rev 2002;13:41–59.
96. Siegfried JM, Weissfeld LA, Luketich JD, et al. The clinical significance of hepatocyte growth factor for non-small cell lung cancer. Ann Thorac Surg 1998;66:1915–1918.

97. Cacciotti P, Strizzi L, Vianale G, et al. The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. Am J Respir Cell Mol Biol 2002;26:189–193.
98. Catalano A, Romano M, Martinotti S, et al. Enhanced expression of vascular endothelial growth factor (VEGF) plays a critical role in the tumor progression potential induced by simian virus 40 large T antigen. Oncogene 2002;25:2896–2900.
99. Masood R, Kundra A, Zhu S, et al. Malignant mesothelioma growth inhibition by agents that target the VEGF and VEGF-C autocrine loops. Int J Cancer 2003;104:603–610.
100. Strizzi L, Catalano A, Vianale G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol 2001;193:468–475.
101. Strizzi L, Vianale G, Catalano A, et al. Basic fibroblast growth factor in mesothelioma pleural effusions: correlation with patient survival and angiogenesis. Int J Oncol 2001;18:1093–1098.

15

Malignant Mesothelioma and Erionite

A. Umran Dogan

Cancer has been known for millennia, but the understanding we have of its origins and causes are comparatively recent. Ancient Egyptians first recorded cancer as a disease some 4500 years ago. However, it wasn't until the 18th century that observations on environmental cancers were made, as people started to look for a connection between certain environments, including working practices and human cancer incidence patterns. The idea emerged that the causes of cancer may be divided roughly into two broad categories: exogenous, which is environmental and occupational, and endogenous, which is something inherent in the person. While this has been a useful distinction, advances in genetics now seem to be blurring the boundary. The result is that cancer research has concentrated on the identification of environmental and occupational causes of human cancer. By the late 19th century the study of cancer tissues had revealed that cancer cells were markedly different in biology and cell structures when compared with the normal cells in the surrounding tissue. During the 20th century, the research in cancer increased in an almost exponential fashion. Advances in genetics, biochemistry, and molecular biology have begun to allow some insight into what was happening when a normal cell was changed into a cancerous one and often why it happened. Gene therapy approaches for inherited and acquired lung diseases are reviewed elsewhere (1). Modification of erionite and its effects on in vitro activity is discussed in Brown et al (2). The genetic susceptibility to mesothelioma has been introduced and discussed in the literature (3–6).

Cancer can take many forms and is usually named after the cell type from which it is transformed. Once a cancer cell has arisen, clonal expansion without regard for the surrounding tissue, accounts for the clinical symptoms of the disease. As the tumor grows, continuous dedifferentiation occurs and cells break away to form new cancers at other sites in the body. It is this metastatic growth that accounts for most of the mortality from this disease. A few tumor types are so aggressive in their development that they kill the host before metastasis even begins. One such cancer is mesothelioma, which is a cancer of the lining of the body cavity and named for its development from

the mesothelium. Although these cancers had been known for a long time, only in the past 40 to 50 years have they been accepted as real mesothelial neoplasms, and not secondary tumors. In 1960, Wagner et al (7) described 33 cases of malignant pleural mesothelioma that they believed had developed through environmental exposure to crocidolite, which is blue asbestos. Up to this time, human tumors for which an external cause had been suggested were believed to arise through heavy occupational exposure. That tumors could arise through specific environmental exposure meant that a very much larger population could be at risk.

Then, about 30 years ago, the fear that this discovery engendered was underlined when Professor Izzetin Baris identified large numbers of human mesotheliomas in several villages in central Turkey, in the region known as Central Anatolia. This time, crocidolite, or indeed any other amphibole mineral, was not to blame. Such minerals were not found in this part of the country, although it did seem probable that a fibrous mineral was responsible for the unprecedented level of disease. Examination of the volcanic rock formations that dominate this part of the country revealed the presence of a fibrous zeolite known as erionite, which contained a high percentage of respirable fibers. Animal experiments have now shown that this may be the most potent natural mineral carcinogen in nature. It was far more carcinogenic than crocidolite. With this knowledge, Baris and his colleagues have demonstrated that a major cause of the mesotheliomas (in some cases five out of six family members have presented with mesothelioma) is the erionite. Any role it may have in other cancers is unclear, but in some Cappadocian villages in Central Anatolia cancer is the predominant cause of death, maybe as high as 80% of total deaths, and some 50% of these deaths result from mesothelioma. Since the villagers have constructed their houses from tuffs carved from the often erionite-rich volcanic rock, and because they till the ground containing respirable fibers of erionite, they are exposed to the fibers continuously, indoors and out, throughout their lives. Current attempts at cancer prevention in these villages (now a unique human laboratory) has led to research efforts involving molecular and cellular changes in mineral fiber carcinogenesis, carcinogen avoidance techniques, human lifestyle analysis, nutritional consumption patterns, and chemical/drug prevention concepts. The Turkish Ministry of Health has begun to support efforts aimed at reducing this preventable human cancer problem by establishing hospitals in central Turkey and by sponsoring international meetings in attempts to understand better environment and cancer interrelationships and to identify possible means of prevention.

The problems of natural environmental pollution by mineral fibers are not unique to Turkey, although Turkey has suffered more than any other country. Also, considering the concept of nutritional prevention of cancers, understanding and ensuring good nutrition is a global concern. However, Turkey is a geographically vast country with a large heterogeneous rural community. Most Turkish people survive through subsistence farming, and have a variety of foods, which is a luxury few can experience.

Brief History of the Region

The Cappadocia, "Katpatuka" in Old Persian, means land of beautiful horses. Archaeologic records indicate that Hittites, Phrygians, Persians, and Romans populated the region. After Christianity was accepted as a religion, a monastic life in the region started about 350 A.D. Subsequently, Cappadocia was occupied by Arabs during the 7th and 8th centuries, and Byzantium succeeded the Arabs toward the mid-9th century. In 1071, the Anatolian Seljuks diminished the Eastern Byzantium Empire in the Central and Eastern Anatolia provinces. From the 14th century onward, the Ottomans replaced the Seljuks in ruling the region. All empires left their cultural influences as a precious heritage: more than 400 rock-hewn churches, the remains of Early Christian and Byzantine art, and the Ottomans' hans, caravan serais, medreses, turbes, and mosques.

It is believed that zeolitic tuff was first used during the Roman empire to build houses, construct roads, sewage channels, and milestones (8,9). Therefore, it is logical to assume that exposure to erionite has been continuous and widespread. Thus the diseases associated with erionite have occurred over many centuries in these regions.

Zeolite-Group Minerals

In general, zeolite-group minerals have excellent physical and chemical properties and they are used widely in industry. However, a fibrous form of zeolite, called erionite, has been proven to be the most toxic mineral in humans.

The word *zeolite* comes from the Greek word meaning boiling stone, because of the loss of water when it is heated. Cronsted discovered stilbite, a zeolite-type mineral, in 1756. Currently, a zeolite mineral is defined as a crystalline substance with a structure characterized by a framework of several linked tetrahedra, each consisting of four O atoms surrounding a cation. In the hydrated phases, dehydration occurs at temperatures mostly below about 400°C and is largely reversible. The framework may be interrupted by (OH, F) groups; these occupy a tetrahedron apex that is not shared with adjacent tetrahedra. The tetrahedral arrangement forms lattice structures with relatively large cavities connected by channels. These cavities contain H_2O molecules, and monovalent and divalent cations that balance the charge resulting from a trivalent aluminum ion replacing a quadrivalent silicon ion in the tetrahedra. The cations in the cavities can be exchanged with other cations including mainly sodium, potassium, calcium, magnesium, and also less often barium, strontium, copper, zinc, lead, silver, rubidium, cesium, and ammonium.

The zeolite group of minerals included 32 naturally occurring minerals before 1997. The number of minerals almost tripled when the zeolite-group minerals were reclassified and 13 of them rose to a series status (10,11).

Mineralogy of Erionite

Eakle defined erionite from Durkee, Oregon, in 1898. The mineral occurred as white woolly fibers associated with opal in cavities in rhyolitic welded ash flow tuff. Eakle proposed the name erionite, from the Greek word for wool, because of its woolly aspect.

Earlier erionite studies included Deffeyes (12), Staples and Gard (13), Ames (14), Eberly (15), and Kawahara and Curien (16). Deffeyes (12) improved the crystallographic data of erionite and described erionite from the northern Jersey Valley, Sonoma Range Quadrangle, Nevada; Shoshone Range and valley of Reese River, Nevada; Pine Valley, Nevada; east of Sand Draw, Wyoming; and White River formation, South Dakota.

Erionite, in different parts of the world, has been studied: in the United States by Deffeyes (12), in Italy by Passaglia et al (17) and Passaglia and Tagliavini (18), in Germany by Rinaldi (19), in Crimea by Suprychev and Prokhorov (20), in Antarctica by Vezzalini et al (21), in Mexico by Garcia-Sosa and Rios-Solache (22), and in Japan by Kawahara et al (23), Harada et al (24), Shimazu and Yoshida (25), and Shimazu and Mizoda (26).

The morphology of erionite is hexagonal prisms terminated with the basal pinacoid. Erionite usually occurs as thin fibers, often forming a compact felt, sometimes with a delicate woolly appearance. The occurrence of intergrowth with offretite is common, because both minerals have similar structures. Sometimes a single erionite crystal contains some stacking faults of the offretite, as shown by the transmission electron microscopy (TEM) technique (27). Macro intergrowths have been described by Rinaldi (19). Fibrous erionite-offretite intergrowths over levyne lamellae have been observed by Gottardi and Galli (28).

Definition of Erionite Series

Three types of erionite are described as erionite-Na, the type specimen by Sheppard and Gude (29), erionite-K, the type specimen by Passaglia et al (30), and erionite-Ca, the type specimen by Harada et al (24). If all reliable chemical analyses of erionite and offretites available in the literature are plotted in a discriminatory diagram based on the above chemical parameters, it is evident that none of the proposed criteria satisfactorily defines appropriate compositional fields apt to describe the literature information (30). Chemical analyses are considered to be reliable if (Si + Al) = 36, on the basis of 72 atoms, and balance error (E) < 10%. E% (31) is

$$100 \times [(A1 + Fe)_{ob} - Al_{th}]/Al_{th}$$

where $Al_{th} = Na + K + 2 \times (Ca + Mg + Sr + Ba)$.

Studies of the crystal structure and crystal chemistry of erionite in general, but not Turkish erionite, include those of Alberti et al (32), Gualteri et al (33), and Passaglia and Sheppard (34).

Geologic and Medical Studies of the Region

Previous geologic studies of the area include Sassano (35), Beekman (36), Pasquare (37), Batum (38), Aydin (39), Atabey et al (40,41), Ercan et al (42), Schumacher et al (43), and Le Pennec et al (44). After endemic mesothelioma in the Cappadocia region was reported by Baris (45), Ataman (46,47), Mumpton (48), Forster (49), Bish and Chipera (50), and Temel and Gundogdu (51) surveyed the region and found zeolite-group minerals including erionite.

Previous medical studies of the area include those of Elmes (52,53), Pooley (54,55), Sebastien et al (56,57), Rohl et al (58), Suzuki (59), and Ozesmi et al (60,61).

Mesothelioma and Asbestos

Malignant pleural mesothelioma (MPM) is a relatively rare form of a lung cancer in which thick layers of malignant cancer develop on the outer lining of the lung. Regardless of the source of exposure (occupational or environmental) MPM is a highly lethal disease, with the majority of patients dying within 6 to 18 months. Current therapy is unsatisfactory.

Malignant mesothelioma and exposure to different asbestos group minerals have been studied by many, including McDonald and McDonald (62,63), Hillerdal and Ozesmi (64), Kohyama (65), Leigh et al (66), De Klerk et al (67), Dogan and Emri (68), and Gibbons (69). Between 1959 and 1977, approximately 4500 cases of mesothelioma were diagnosed in the world by McDonald and McDonald (62). The exposure to these carcinogenic materials could either be occupational or environmental. Clinical, epidemiologic, and pathologic surveys and in vivo and in vitro experimental work demonstrate that asbestos is responsible for the etiology of mesothelioma.

Mesothelioma and Erionite

The interest in erionite, a fibrous form of a zeolite-group mineral, has grown after the initial reports of a high incidence of malignant mesothelioma in the villages of Karain and Tuzkoy in Cappadocian region of Turkey by Baris (45), and later a village of Sarihidir by Baris et al (70). Baris et al (70), Ataman (48), Artvinli and Baris (71), Ataman (47), Lilis (72), and Ozesmi et al (60,61) studied the region and attempted to find a relationship between MPM and erionite.

Mumpton (48) reported erionite in the villages where pleural mesothelioma occurs. He also reported that erionite in other villages, such as Sarihidir, reported no cases of mesothelioma. Therefore, he suggested that some other agent might be responsible for the high incidence of mesothelioma in this region. Baris et al (73) and Simonata et al (74) have shown that, contrary to Mumpton (48), mesothelioma also occurred at unusually high rates in Sarihidir village, Turkey.

Rohl et al (58) examined lung tissues and rock samples from this area. They reported significant amounts of tremolite and chrysotile, in addition to erionite. They concluded that their findings were consistent with the published data, which showed a relationship between asbestos (chrysotile or amphibole) exposure and pleural disease. Then they speculated on the existence of an enhanced tumorigenic effect, which was probably produced by a combination of asbestos and erionite. Sebastien et al (75) reported that the high frequency of mesothelioma in the central Turkish villages was related to airborne exposure from the natural mineral fibers. Wagner et al (76) examined the relationship between erionite exposure and mesothelioma, using experimental studies on rats, and found that samples of erionite from Turkey and Oregon produced a very high incidence of mesothelioma.

Health effects of these mineral studies include those of Baris et al (73,77), Casey et al (78,79), Sebastien et al (56,57), Artvinli and Baris (80), Maltoni et al (81), Suzuki (59), Hillerdal and Baris (82), Sebastien et al (75), Kruglikov et al (83), and Tatrai et al (84,85). Casey et al (78,79) reported that fibrosis of the lung and pleura among workers was related to erionite but not to asbestos. Several studies have been conducted on the inhabitants of "mesothelioma" villages in Turkey (with environmental exposure to erionite) and on the inhabitants of control villages. Ferruginous bodies were found in a higher proportion in the sputa of inhabitants of the contaminated villages than in the control villages by Sebastien et al (75). Similarly, although not statistically significant, differences were found for pleural tissue changes by Baris et al (77) and Artvinli and Baris (80) or pleural plaques by Baris et al (73). Hillerdal and Baris (82) reported that pleural calcifications were more frequent in inhabitants of erionite-exposed villages (78/549, 14.2%) and of asbestos-exposed villages (104/446, 23.3%) than of control villages (3/382, 0.8%).

Carcinogeneity studies include those of Baris et al (70,73), Artvinli and Baris (71), McDonald and McDonald (86), Boman et al (87), Artvinli and Baris (88), Simonato et al (74), and Ozesmi et al (60). Most of the data on the carcinogenicity of erionite in humans come from the experience of the inhabitants of the erionite contaminated villages in Central Cappadocia, Turkey. Baris et al (70) reported 25 cases of MPM in a population of 575 inhabitants of Karain between 1970 and 1974; Baris et al (77) reported 28 MPMs in Karain between 1975 and 1979; and Artvinli and Baris (88) examined over 25 years of 312 inhabitants of Tuzkoy between 1978 and 1980 and reported 15 MPMs, 12 malignant peritoneal mesothelioma (MPeMs), and eight lung cancers. The incidence or mortality from mesothelioma was above 1%/year, a rate that is 10,000 times higher than observed among populations nonoccupationally exposed to asbestos from Western Europe or North America.

Baris et al (73) conducted an environmental and epidemiologic study in three contaminated villages (Karain, Sarihidir, and Tuzkoy) and in one control village (Karlik) in the period of 1979 to 1983. They reported that fibers taken from street samples were 2–10, 5–25, 1–29, respectively for Karain, Sarihidir-Tuzkoy, and Karlik; erionite amounts among fibers (>5μm) were 80%, 85%, 60%; numbers of MPeM cases were

(males/females) 12/9, 0/5, 2/1; numbers of MPeM cases were (males/females) 0/0, 0/4, 0/0; numbers of lung cancer cases were (males/females) 2/0, 9/0, 5/1; numbers of other cancers cases were (males/females) 20/11, 5/5, 13/4; and numbers of other causes of death were (males/females) 15/17, 12/6, 13/17; Baris et al (73) confirmed the high mortality from MPM and MPeM, and showed an excess of lung cancer mortality in the contaminated villages. The young age of the patients at the appearance of this respiratory neoplasm was particularly noteworthy.

Boman et al (87) and Ozesmi et al (89) reported seven cases of mesothelioma among about 100 men from one of the Cappadocian villages (Karain) who had immigrated to Sweden. In this group, mesothelioma was the most common cause of death, with an incidence of nearly 1%/year. Metintas et al (90) reported 14 deaths due to MPM among 162 Turkish emigrants from Karain who resided in Sweden. In addition, there were five patients with mesothelioma (four MPM and one MPeM) who were still alive. Thus it is calculated that the risk of mesothelioma for men is 135 times and for the women it is 1336 times greater than for the same sex and age groups in Sweden. The risk increased with time of residing in the village. As in the studies from Turkey, mesotheliomas occurred at a young average age. In subsequent analyses, a cumulative dose of 1 fiber/mL-year was estimated to induce a pleural mesothelioma rate of 996 per 100,000 person-years in the exposed population by Simonato et al (73).

Zeolite Toxicity Experiments Using Animals

Animal experimental studies include those of Suzuki and Kohyama (91), Wagner et al (76), Pylev et al (92,93), Maltoni and Minardi (94), Davis et al (95), Tatrai et al (84,85), and Carthew et al (96). Wagner et al (76) tested natural erionite, synthetic nonfibrous zeolite with the composition of erionite and crocidolite at concentrations of 10 mg/m^3 inhalation in rats. Pleural mesotheliomas were found in 27/28 rats exposed to erionite; one pulmonary and one pleural tumor were found in the 28 rats exposed to synthetic zeolite, and one lung carcinoma was reported in rats exposed to crocidolite. A number of experiments have been conducted on the intrapleural and intraperitoneal administration of various types of erionite in mice and rats. These experiments have all been positive, and showed a very high mesothelioma yield (90% or above) for amounts of erionite above 0.5 or 1 mg. For higher doses, the time of appearance of the tumors was decreased (95,96). Other solid tumors, at the site of inoculation, as well as lymphomas have been occasionally described. Carthew et al (96) compared the relative carcinogenic potency of erionite and asbestos fibers. In experiments based on intrapleural inoculation, erionite was 300 to 800 times more active than chrysotile, and 100 to 500 times more active than crocidolite. In intraperitoneal experiments, erionite was 20 to 40 times more active than chrysotile and 7 to 20 times more active than crocidolite.

Davis (97) showed that intrapleural injection of asbestos produced more tumors than following intrapleural injection. Stanton et al (98) reported that the tumorigenity of asbestos in relation to mesothelioma is attributable to fibers longer than 8 μm and less than 1.5 μm in diameter. Maltoni et al (81) tested erionite and crocidolite fibers for carcinogenicity. They reported pleural mesothelioma after intrapleural injection with erionite fibers, but no pleural tumors among the rats treated at the same time and in the same way with crocidolite. Johnson et al (99) showed that tumors induced by asbestos and erionite are morphologically similar; however, the biologic activity of the two mineral types was different. Suzuki and Kohyama (91) studied the effects of intraperitoneal administration of mordenite and two natural erionites in mice. They found that both erionites produced malignant peritoneal tumors at a high rate, but mordenite did not produce any cancer. Wagner et al (76) showed that the inhalation of erionite in comparison with asbestos produced tumors more rapidly and more frequently.

Palekar et al (100) and Coffin et al (101,102), using both in vitro and in vivo methods, demonstrated that erionite was much more tumorigenic than crocidolite or chrysotile and induced chromosomal abnormalities. Coffin et al studied mechanisms of tumorigenesis and tried to explain why erionite was more tumorigenic than either crocidolite or chrysotile, in spite of the fact that asbestos minerals typically have a far greater percentage of fibers in the length-to-width class considered to be dangerous. They invoked the high internal surface area of erionite (200 m^2/g) when compared with the total surface areas for chrysotile (24 m^2/g) and crocidolite (8–10 m^2/g) as a possible reason for the observed differences in tumorigenesis.

Mortality Studies

Clinical, epidemiologic, and pathologic surveys and in vivo and in vitro experimental studies demonstrate that asbestos is responsible for the etiology of mesothelioma. Epidemiologic and pathologic studies were carried out in South Africa by Wagner et al (7); in the United Kingdom by Newhouse and Thomson (103); in Germany by Bohlig et al (104); in Canada by McDonald et al (105); in France by De Lajarte et al (106); in Australia by Milne (107); and in the United States by Selikoff et al (108), Enterline (109), and Selikoff (110). These studies have emphasized that approximately 70% to 85% of mesothelioma patients have been exposed to asbestos through occupational, environmental, or other means.

Three villages in Central Anatolia, Turkey, namely Tuzkoy, Karain, and Sarihidir, comprise an extremely important field area and are informally referred to as "the death triangle." Since 1975, Baris has been investigating this malignant mesothelioma in these three villages in Nevsehir, Turkey, and he has maintained all patient records of this disease including chest x-rays and personal health statistics. He also gathered the data on the death records of patients who had died of

mesothelioma and other cancers in these villages in Turkey or abroad (i.e., Karain colony villagers in Sweden).

Epidemiologic records for these eight villages between the years 1994 and 1997 have been studied. An extremely high rate of cancer in the young-to-middle-age group was observed in the study area. In vitro and in vivo studies performed by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) also indicate that there is enough evidence to conclude that these fibers are carcinogenic and that the cancer rate in this region is about 1000 times more than the normal rate.

Genetic Studies Suggest Predisposition to Erionite Carcinogenesis

Since erionite was elevated to the group status in 1997 there have been no studies performed that quantitatively characterized erionite-Na, erionite-K, and erionite-Ca in the various erionite villages. Thus we do not know to what types of erionite these villagers are exposed. Family pedigree analyses conducted in the village of Tuzkoy suggested that the carcinogenicity of erionite was more pronounced in certain families. Families with a high incidence of erionite were identified, while in other families living in the same village the incidence of mesothelioma was low. It did not appear that these differences could be explained by different exposures to erionite since all villagers should be exposed to similar amounts of erionite dust. Previous studies suggested that erionite was carcinogenic at very low doses compared to asbestos (77). Thus, the hypothesis of genetic predisposition to erionite carcinogenicity was formulated (4,6). However, this hypothesis must be verified in the other two mesothelioma villages of Karain and "Old" Sarihidir. Moreover, the hypothesis that mineralogic differences among houses within the same village is not responsible for the different incidence of mesothelioma among families in the same village should also be verified by quantitative characterization of the erionite found in these houses. It remains to be demonstrated that there are no chemical differences among different houses in the same villages, or among nearby villages, that could account for the different incidence of mesothelioma. Our research team is investigating these possibilities.

Erionite in Turkey

Previous studies reported that erionite was found only in the three villages of Karain, Sarihidir, Tuzkoy, and that the neighboring villages of Karacaoren (also called Karacaviran) and Yesiloz (also called Tahar) were reported as nonmesothelioma villages by Temel and Gundogdu (51). In contrast, our detailed geologic and mineralogic study of the region showed that erionite is not confined just to these three villages (111,112). In fact, the Karacaoren village is also contaminated with erionite both in the bedrock and the wall rock. Subsequent epidemiologic studies showed that the previously reported nonmesothelioma villages such as

Karacaoren had a high rate of mesothelioma. Therefore, it was established that there was a direct relationship between erionite and nonoccupational malignant mesothelioma in all of these villages in the region.

In Central Anatolia, Turkey, eruptions of volcanoes, mainly Erciyes (3917 m) and Hasandag (3268 m), caused the region to be covered with a thick stratum of lava, volcanic ashes, and a dense tuff layer that formed on the earth's surface. In the Cappadocian region, tuffs accumulated in topographically low areas through both direct airfall contributions and the reworking of larger widespread ash mantles. In time, natural factors such as rain and wind created extraordinary shapes, deep valleys, and natural sculptures of fairy chimneys in the tuff formations of this Cappadocian area. Single-tuff deposits consist of successive accumulations of ash from more than one eruption event. Following deposition, tuffs have undergone a series of geochemical changes involving an early dissolution of glass surfaces and precipitation of grain coating smectite, followed by erionite growth in the pore spaces. A chemical environment of increasing alkalinity is suggested to explain the observed mineralogic changes. Activity diagrams of zeolites by Birsoy (113) also support this theory.

In the United States there are deposits of fibrous zeolites specifically in the western portion of the country. There are homes made of zeolite (erionite) in Oregon and weigh stations made of the same materials in Nevada. Very large amounts of zeolites were also used in pozzolanic cements such as those used in the construction of the Los Angeles aqueduct in California. Recently, a few cases of zeolite-related pulmonary diseases have been reported in the U.S. Therefore, the possibility of increased exposure to zeolites in the western U.S. is anticipated and potential carcinogenic dangers must be evaluated.

Erionite is the only zeolite whose evidence of carcinogeneity has been evaluated. It is classified as a human carcinogen by the IARC (114). In Turkey, erionite-contaminated villages in the Cappadocia region provide a natural laboratory to study the health effect of these carcinogenic minerals, and the villagers who immigrated from Karain to Sweden also form a unique community to study the follow-up effects of zeolite exposure. The cancer rate in these regions is about 1000 times higher than the normal rate. The local saying, "I don't know my father and my father doesn't know his father," indicates that the cancer has been there for centuries.

This problem requires worldwide immediate attention. Although both the exposures and biologic mechanisms are complex, we hope that the multidisciplinary medical geology studies, which combine high-resolution mineralogy and human genetics, will help us understand and control this very malignant human disease.

Conclusion

Mesothelioma, malignant pleural mesothelioma (MPM), or malignant peritoneal mesothelioma (MPeM), is a very lethal disease. Clinical and experimental studies have confirmed a carcinogenic linkage to

erionite, a zeolite-group mineral. In 1975 an extremely high rate of MPM incidence was observed in some villages in the Cappadocian region of Turkey. Further studies showed that the erionite type of zeolite minerals, not asbestos, was the major cause of the epidemic in this area. The high potential of erionite to induce MPM has been confirmed by both epidemiologic and experimental studies.

The World Health Organization (WHO) classified erionite, a zeolite group mineral, as a group I carcinogen (114). The classification is based on evidence in humans, specific diseases from occupational exposures, and health effects noted in animal and cell experiments. Three types of erionite have been described: erionite-Na, erionite-K, and erionite-Ca (10,11). Erionite is observed in the previously reported villages and also in several new villages in the Central Anatolian region of Turkey (111,112).

References

1. Curiel et al. 1996.
2. Brown et al. 1989.
3. Carbone M, Setlak P, Bocchetta M, et al. Genetic susceptibility to mesothelioma. In: The Asbestos Legacy: The Sourcebook on Asbestos Diseases. 2001;23:151–168.
4. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002;29(1):2–17.
5. Dogan AU, Baris YI, Emri S, Testa JR, Carbone M. Familial malignant mesothelioma. Lancet 2001;358:1813–1814.
6. Roushdy-Hammady I, Siegel J, Emri S, Testa JR, Carbone M. Genetic susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 2001;357:444–445.
7. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the north western Cape Province. Br J Ind Med 1960;17:260–271.
8. Mumpton FA. Occurrence and utilization of natural zeolites; a review. International Clay Conference 1975:215–218.
9. Mumpton FA. Discovery and commercial interest in zeolite deposits examined during Zeo-trip '83; an excursion to selected zeolite deposits in eastern Oregon, southwestern Idaho, and northwestern Nevada, and to the Tahoe-Truckee water reclamation plant, Truckee, California, 1983: 66–72.
10. Coombs DS, Alberti A, Armbruster T, et al. Recommended nomenclature for zeolite minerals: report of the Subcommittee on Zeolites of the International Mineralogical Association, Commission on New Minerals and Mineral Names. Canadian Mineralogist 1997;33:1571–1606.
11. Coombs DS, Alberti A, Armbruster T, et al. Recommended nomenclature for zeolite minerals: report of the Subcommittee on Zeolites of the International Mineralogical Association, Commission on New Minerals and Mineral Names. Mineralogical Magazine 1998;62(4):533–571.
12. Deffeyes KS. Erionite from Cenozoic Tuffaceous Sediments, Central Nevada. American Mineralogist 1959;44:501–509.
13. Staples LW, Gard JA. The fibrous zeolite erionite; its occurrence, unit cell, and structure. Mineralogical Magazine 1959;32(247):261–281.

14. Ames LL. Cation sieve properties of the open zeolites, chabasites, mordenite, erionite, and clinoptilolite. Am Mineralogist 1961;46:1120–1131.
15. Eberly PE. Adsorption properties of naturally occurring erionite and its cationic-exchanged forms. Am Mineralogist 1964;49:30–40.
16. Kawahara A, Curien H. La structure cristalline de l'erionite (Crystal structure of erionite). Bull Soc Francaise Mineralogie Cristallographie 1969; 92(3):250–256.
17. Passaglia E, Galli E, Rinaldi R. Levynes and erionites from Sardinia, Italy. Contrib Mineralogy Petrology 1974;43(4):253–259.
18. Passaglia E, Tagliavini A. Erionite from Faedo, Colli Euganei, Italy. Nues Jahrbuch Mineralogie 1995;4:185–191.
19. Rinaldi R. Crystal chemistry and structural epitaxy of offretite-erionite from Sasbach, Kaiserstuhl. Neues Jahrbuch Mineralogie 1976;4:145–156.
20. Suprychev VA, Prokhorov IG. Erionit iz keratofirovykh vulkanitov Karadagskogo zapovednika v Krymu. [Erionite from keratophyre volcanites of the Karadag Reserve in the Crimea.] Mineralogicheskiy Sbornik (L'vov) 1986;40(1):85–88.
21. Vezzalini G, Quartieri S, Rossi A, Alberti A. Occurrence of zeolites from northern Victoria Land (Antarctica). Terra Antarctica 1994;1(1):96–99.
22. Garcia-Sosa I, Rios-Solache M. Sorption of cobalt and cadmium by Mexican erionite. J Radioanalytical Nucl Chem 1997;218(1):77–80.
23. Kawahara A, Takano Y, Takabatake M, Uratani Y. The composition and the crystal structure of erionite from Maze, Niigata prefecture, Japan. Tokyo: Scientific Papers of the College of General Education, University of Tokyo, 1967;17(2):237–248.
24. Harada K, Iwamoto S, Kihara K. Erionite, phillipsite, and gonnardite in the amygdales of altered basalt from Maze, Niigata Prefecture, Japan. Am Mineralogist 1967;52:1785–1794.
25. Shimazu M, Yoshida S. Occurrence of erionite from Iwayaguchi District, Osada area, Niigata prefecture. J Geological Soc Jpn 1969;75(7):389–390.
26. Shimazu M, Mizoda T. Levyne and erionite from Chojabaru, Iki Island, Nagasaki prefecture, Japan. J Japanese Assoc Mineralogists Petrologists Economic Geologists 1972;67(12):418–424.
27. Kokotailo GT, Sawruk S, Lawton SL. Direct observation of stacking faults in the zeolite erionite. Am Mineralogist 1972;57:439–444.
28. Gottardi G, Galli E. Erionites. In: Natural Zeolites. 1985;200–214.
29. Sheppard RA, Gude AJ. Chemical composition and physical properties of the related zeolites offretite and erionite. Am Mineralogist 1969;54(5–6): 875–886.
30. Passaglia E, Artioli G, Gualtieri A. Crystal chemistry of the zeolites erionite and offretite. Am Mineralogist 1998;83:577–589.
31. Passaglia E. The crystal chemistry of chabazites. Am Mineralogist 1970;55: 1278–1301.
32. Alberti A, Martucci A, Galli E, Vezzalini G. A reexamination of the crystal structure of erionite. Zeolites 1997;19(5–6):349–352.
33. Gualtieri A, Artioli G, Passaglia E, Bigi S, Viani A, Hanson JC. Crystal structure-crystal chemistry relationships in the zeolites erionite and offretite. Am Mineralogist 1998;83(5–6):590–606.
34. Passaglia E, Sheppard RA. The crystal chemistry of zeolites. Rev Mineralogy Geochemistry 2001;45:69–116.
35. Sassano G. Acigol bolgesinde Neojen ve Kuvaterner volkanizmasi. [Neogene and Quaternary volcanisms of Acigol region.] MTA report No. 6841. 1964.

36. Beekman PH. Hasandagi-Melendizdagi bolgesinde Pliyosen ve Kuvaterner volkanizma faaliyetleri. [Pliocene and quaternary volcanic activities of Hasan-Melendiz mountains.] MTA journal No. 66. 1966.
37. Pasquare G. Geology of the Cenozoic volcanic area of Central Anatolia. Atti Accac Naz Lincei 1968;9:53–204.
38. Batum I. Nevsehir guney batisindaki Gollu Dag ve Acigol volkanitlerinin jeokimyasi ve petrolojisi. [Geochemistry and petrology of Gollu Mountain and Acigol Volcanics of southwest Nevsehir.] J Earth Sci 1978;4(1–2):70–88.
39. Aydin N. Orta Anadolu masifinin Gumuskent (Nevsehir) dolayinda jeolojik-petrografik incelemeler. [Geological and petrographical study of Central Anatolian massive at Gumuskent (Nevsehir) region.] MTA report No. 206. 1984.
40. Atabey E, Papak I, Tarhan N, Akarsu B, Taskiran MA. Ortakoy (Nigde)-Tuzkoy (Nevsehir)-Kesikkopru (Kirsehir) yoresinin jeolojisi. [Geology of Ortakoy (Nigde)-Tuzkoy (Nevsehir)-Kesikkopru (Kirsehir) regions.] MTA report No. 8156. 1987.
41. Atabey E, Tarhan N, Yusufoglu H, Canpolat M. Hacibektas, Gulsehir, Kalaba (Nevsehir)-Himmetdede (Kayseri) arasinin jeolojisi. [Geology of the region between Hacibektas, Gulsehir, Kalaba (Nevsehir)-Himmetdede (Kayseri).] MTA report No. 8523. 1988.
42. Ercan T, Yildirim T, Akbasli A. Gelveri (Nigde)-Kizilcin (Nevsehir) arasindaki volkanizmanin ozellikleri. [Characteristics of the volcanism between Gelveri (Nigde)-Kizilcin (Nevsehir).] 7th Petroleum Congress of Turkey, 1987;449–460.
43. Schumacher R, Keller J, Bayhan H. Depositional characteristics of ignimbrites in Central Anatolia, Turkey. In: Savascin, Eronat, eds. Proceedings of the International Earth Sciences Congress on Aegean Regions, ed. 1990;2:435–449.
44. Le Pennec JL, Bourdier JL, Froger JL, Temel A, Camus G, Gourgaud A. Neogene ignimbrites of the Nevsehir plateau (central Turkey): stratigraphy, distribution and source constraints. J Volcanology Geothermal Res 1994;63:59–87.
45. Baris YI. Pleural mesotheliomas and asbestos pleurisies due to environmental asbestos exposure in Turkey: an analysis of 120 cases. Hacettepe Bull Med Surg 1975;8:167–185.
46. Ataman G. Les tufs zeolitises de Cappadoce et leur liaison probable avec certain types de cancer du poumon et de mesothelioma pleural. [The zeolitic tuffs of Cappadocia and their probable association with certain types of lung cancer and pleural mesothelioma.] Comptes Rendus Acad Sci Ser D 1978;287:207–210.
47. Ataman G. Mise en evidence du role de l'erionite (zeolite) dans le mesothelioma pulmonaire. [Role of erionite (zeolite) in pulmonary mesothelioma.] Comptes Rendus Hebdomadaires Seances Acad Sci Ser D Sci Naturelles 1980;291(2):167–169.
48. Mumpton FA. Report of reconnaissance study of the association of zeolites with mesothelioma cancer occurrences in central Turkey. Department of Earth Sciences, State University Collage, Brockport, New York, 1979.
49. Forster H. Eine mineralogisch-petrographische untersuchung uber mogliche ursachen von mesotheliomen in Kappadokien/Turkei. [Mineralogy and petrography of rocks causing mesothelioma in Cappadocia, Turkey.] Zbl Arbeitsmed 1982;32:18–27.
50. Bish DL, Chipera SJ. Detection of trace amounts of erionite using x-ray powder diffraction: erionite in tuffs of Yucca Mountain, Nevada, and Central Turkey. Clays and Clay Minerals 1991;39(4):437–445.

51. Temel A, Gundogdu MN. Zeolite occurrences and the erionite-mesothelioma relationship in Cappadocia, Central Anatolia, Turkey. Mineralum Deposita 1996;31:539–547.
52. Elmes PC. Report on visit to Turkey, October 11–15, 1977. Reference PCE/MEW/307A/CF, Medical Research Council Pneumoconiosis Unit, Llandaugh Hospital, Penarth, Glamorgan, Wales, 1977, unpublished report.
53. Elmes PC. Fibrous minerals and health. J Geological Soc Lond 1980; 137(5):525–535.
54. Pooley PD. Report of the examination of Turkish samples: letter report. University College, Cardiff, Wales, 1978, unpublished report.
55. Pooley PD. Evaluation of fiber samples taken from the vicinity of two villages in Turkey. In: Demen R, Dement JH, eds. Dust and Disease. Park Forest South, IL: Pathotox Publication, 1979:41.
56. Sebastien P, Gaudichet A, Bignon J, Baris YI. Zeolite bodies in human lungs from Turkey. Lab Invest 1981;44 (5):420–425.
57. Sebastien P, Gaudichet A, Bignon J, Baris YI. Ferruginous bodies in sputum as an indication of exposure to airborne mineral fibres in the mesothelioma villages of Cappadocia. Arch Environ Health 1981;39:18–23.
58. Rohl AN, Langer AM, Moncure G, Selikoff IJ, Fischbein A. Endemic pleural disease associated with exposure to mixed fibrous dust in Turkey. Science 1982;216(4545):518–520.
59. Suzuki Y. Carcinogenic and fibrogenic effects of zeolites: preliminary observations. Environ Res 1982;27:433–445.
60. Ozesmi M, Hillerdal G, Krause F. Zur Kenntnis kanzerogener und immunologischer Befunde durch den Faserzeolith Erionit. Pneumologie 1990; 44:335–336.
61. Ozesmi M, Karlsson-Parra A, Hillerdal G, Forsum U. Phenotypic characterisation of peripheral blood lymphoid cells in people exposed to fibrous zeolite. Br J Ind Med 1986;43:830–833.
62. McDonald JC, McDonald AD. Epidemiology of mesothelioma from estimated incidence. Prev Med 1977;6:426–446.
63. McDonald JC, McDonald AD. Chrysotile, tremolite, and mesothelioma. Science 1995;267(5199):776–777.
64. Hillerdal G, Ozesmi M. Benign asbestos pleural effusion: 73 exudates in 60 patients. Eur J Respir Dis 1987;71:113–121.
65. Kohyama N. Biological effects of mineral fibers from occupational exposure to non-occupational exposure. J Mineralogical Soc Jpn 1987;18(3): 191–209.
66. Leigh J, Corvalan C, Copland P. Malignant mesothelioma incidence in Australia 1982–1992. 1993;28–30.
67. De Klerk NH, Musk AW, Eccles JL, Hobbs MST. Malignant mesothelioma and exposure to crocidolite at Wittenoom. 1993;25–27.
68. Dogan M, Emri S. Environmental health problems related to mineral dusts in Ankara and Eskisehir, Turkey. Yerbilimleri 2000;22:149–161.
69. Gibbons W. Amphibole asbestos in Africa and Australia: geology, health hazard and mining legacy. J Geological Soc Lond 2000;157(4):851–858.
70. Baris YI, Sahin AA, Ozesmi M, et al. An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Urgup in Anatolia. Thorax 1978;33:181–192.
71. Artvinli M, Baris YI. Malignant mesotheliomas in a small village in the Anatolian region of Turkey: an epidemiologic study. J Natl Cancer Inst 1979;63(1):17–22.

72. Lilis R. Fibrous zeolites and endemic mesothelioma in Cappadocia, Turkey. J Occup Med 1981;23(8):548–550.
73. Baris YI, Simonato L, Artvinli M, et al. Epidemiological and environmental evidence of the health effects of exposure to erionite fibres: a four-year study in the Cappodocian region of Turkey. Int J Cancer 1987;39:10–17.
74. Simonato L, Baris YI, Saracci R, Skidmore J, Winkelmann R. Relation of environmental exposure to erionite fibres to risk of respiratory cancer. In: Bignon J, Peto J, Saracci R, eds. Non-Occupational Exposure to Mineral Fibres. World Health Organization International Agency for Research on Cancer (IARC) Scientific Publications No. 90, 1989;398–405.
75. Sebastien P, Bignon J, Baris YI, Awad L, Petit G. Ferruginous bodies in sputum as an indication of exposure to airborne mineral fibers in the mesothelioma villages of Cappadocia. Arch Environ Health 1984;39(1): 18–23.
76. Wagner JC, Skidmore JW, Hill RJ, Griffiths DM. Erionite exposure and mesotheliomas in rats. Br J Cancer 1985;51:727–730.
77. Baris YI, Saracci R, Simonato L, Skidmore JW, Artvinli M. Malignant mesothelioma and radiological chest abnormalities in two villages in central Turkey—an epidemiological and environmental investigation. Lancet 1981;984–987.
78. Casey KR, Moatamed F, Shigeoka J, Rom WN. Demonstration of fibrous zeolite in pulmonary tissue. Am Rev Respir Dis 1981;123:98.
79. Casey KR, Shigeoka JW, Rom WN, Moatamed F. Zeolite exposure and associated pneumoconiosis. Chest 1985;87:837–840.
80. Artvinli M, Baris YI. Environmental fiber-induced pleuro-pulmonary diseases in an Anatolian village: an epidemiological study. Arch Environ Health 1982;37(3):177–181.
81. Maltoni C, Minardi F, Morisi L. Pleural mesotheliomas in Sprague-Dawley rats by erionite: first experimental evidence. Environ Res 1982;29:238–244.
82. Hillerdal G, Baris YI. Radiological study of pleural changes in relation to mesothelioma in Turkey. Thorax 1983;38(6):443–448.
83. Kruglikov GG, Velichkovskii BT, Garmash TC. Morphology of pneumoconiosis induced by natural zeolite. Gig Tr Prof Zabol 1990;5:14–15.
84. Tatrai E, Bacsy E, Karpati J, Ungary G. On the examination of the pulmonary toxicity of mordenite in rats. Polish J Occup Med Environ Health 1992;5:237–243.
85. Tatrai E, Wojnarovits I, Ungary G. Non-fibrous zeolite induced experimental pneumoconiosis in rats. Exp Pathol 1991;43:41–46.
86. McDonald AD, McDonald JC. Malignant mesothelioma in North America. Cancer 1980;46:1650–1656.
87. Boman G, Schubert V, Svane B, et al. Malignant mesothelioma in Turkish immigrants residing in Sweden. Scand J Work Environ Health 1982;8: 108–112.
88. Artvinli M, Baris YI. Erionite-related diseases in Turkey. In: Beck EG, Bignon J, eds. In Vitro Effects of Mineral Dusts. NATO ASI series G3. 1985:515–519.
89. Ozesmi M, Hillerdal G, Svane B, Widstrom O. Prospective clinical and radiologic study of zeolite-exposed Turkish immigrants in Sweden. Respiration 1990;57(5):325–328.
90. Metintas M, Hillerdal G, Metintas S. Malignant mesothelioma due to environmental exposure to erionite: follow-up of a Turkish emigrant cohort. 1998;
91. Suzuki Y, Kohyama N. Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. Environ Res 1984;35:277–292.

92. Pylev LN, Kulagina TF, Grankina EP, Chelishchev NF, Berenshtein BG. Carcinogenicity of zeolite. Gig Sanit 1989;8:7–10.
93. Pylev LN, Kulagina TF, Vasilyeva LA, Chelischev NF, Berenstein BG. Blostomogenic activity of erionite (nidale erionite). Gig Tr Prof Zabol 1986; 161:33–37.
94. Maltoni C, Minardi F. First available results of long-term carcinogenicity bioassay on detergency zeolites (MS 4A and MS 5A). Ann NY Acad Sci 1988;534:937–985.
95. Davis JMG, Jones AD, Miller BG. Experimental studies in rats on the effects of asbestos inhalation coupled with the inhalation of titanium dioxide or quartz. Int J Exp Pathol 1991;72:501–525.
96. Carthew P, Hill RJ, Edwards RE, Lee PN. Intrapleural administration of fibres induced mesothelioma in rats in the same relative order of hazard as occurs in man after exposure. Hum Exp Toxicol 1992;11:530–534.
97. Davis JMG. The histopathology and ultrastructure of pleural mesotheliomas produced in the rat by injections of crocidolite asbestos. Br J Exp Pathol 1979;60:642–652.
98. Stanton MF, Layard M, Tegeris A, et al. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. J Nat Cancer Inst 1981;67(5):965–975.
99. Johnson NF, Edwards RE, Munday DE, Rowe N, Wagner JC. Pluripotential nature of mesotheliomata induced by inhalation of erionite in rats. Br J Exp Pathol 1984;65:377–388.
100. Palekar LD, Eyre JF, Most BM, Coffin DL. Metaphase and anaphase analysis of V79 cells exposed to erionite, UICC chrysotile and UICC crocidolite. Carcinogenesis 1987;8:553–560.
101. Coffin DL, Palekar LD, Cook PM, Creason JP. Comparison of mesothelioma induction in rats by asbestos and nonasbestos mineral fibers: possible correlation with human exposure data. In: Biological Interaction of Inhaled Mineral Fibers and Cigarette Smoke. Proceedings of an International Symposium/Workshop held at the Battelle Seattle Conference Center, April 10–14, 1989, Seattle, Washington, pp. 347–354.
102. Coffin DL, Peters SE, Palekar LD, Stahel EP. A study of the biological activity of erionite in relation to its chemical and structural characteristics. In: Biological Interaction of Inhaled Mineral Fibers and Cigarette Smoke. Proceedings of an International Symposium/Workshop held at the Battelle Seattle Conference Center, April 10–14, 1988, Seattle, Washington, pp. 313–324.
103. Newhouse M, Thomson H. Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. Br J Ind Med 1965;22: 261–269.
104. Bohlig H, Dabbert AF, Dalquen P, Hain E, Hinz I. Epidemiology of malignant mesothelioma in Hamburg: a preliminary report. Environ Respir 1970;3:365.
105. McDonald AD, Magner D, Eyssen G. Primary malignant mesothelial tumors in Canada, 1960–1968. A pathological review by the mesothelioma panel of the Canadian Tumor Reference Centre. Cancer 1973;31:869–876.
106. De Lajarte M, Cornet E, Corroller J. Etude clinique et professionelle de 54 mesotheliomes pleuraux diffus. Rev Franc Mal Respir 1976;4(2):63.
107. Milne JEH. Thirty-two cases of mesothelioma in Victoria, Australia: a retrospective survey related to occupational asbestos exposure. Br J Ind Med 1976;33:115–122.
108. Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. JAMA 1964;188:22–26.

109. Enterline PE. Mortality among asbestos products workers in the United States. Ann NY Acad Sci 1965;132:156.
110. Selikoff IJ. Asbestos disease in the United States. Rev Fr Mal Respir 1976;4:7–24.
111. Dogan AU. Cappadocian mesothelioma villages. Indoor Built and Environment 2003; in press.
112. Dogan AU. Zeolite mineralogy and Cappadocian erionite. Indoor Built and Environment 2003; in press.
113. Birsoy R. Activity diagrams of zeolites: implications for the occurrences of zeolites in Turkey and of erionite worldwide. Clays and Clay Minerals 2002;50(1):136–144.
114. IARC. Erionite. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Silica and Some Silicates 1997;42:225–239.
115. Baris YI. Environmental asbestos related diseases in Turkey. Hacettepe University, School of Medicine, 1977, unpublished report.
116. Sheppard RA, Gude AJ, Munson EL. Chemical composition of diagenetic zeolites from tuffaceous rocks of the Mojave Desert and vicinity, California. Am Mineralogist, 1965;50:244–249.

16

Determination of Asbestos Exposure by Pathology and Clinical History

Allen R. Gibbs

The determination of whether an abnormal asbestos exposure took place is important in mesothelioma cases because of the potential for financial compensation and for the assessment of the likelihood of further cases occurring from similar occupational, paraoccupational, or environmental circumstances. One should be aware that not all mesotheliomas are associated with asbestos exposure. Spirtas et al (1) found after careful systematic inquiry that 88% of pleural and 54% of peritoneal mesotheliomas could be attributed to asbestos exposure in men in the United States but only 23% of pleural and peritoneal mesotheliomas could be attributed to asbestos in women in the United States. An earlier study of mesotheliomas in North America showed lower figures—50% in men and 5% in women (2).

There are several ways whereby a reasonable determination can be made of whether abnormal asbestos exposure has occurred in an individual. These include (1) a detailed and reliable occupational history; (2) identification of clinical markers of exposure such as pleural plaques, diffuse pleural fibrosis, rounded atelectasis, and asbestosis; (3) histopathologic features, such as pleural plaques and asbestos bodies; and (4) mineral analyses of digested lung tissues.

In most, if not all, parts of the world, there are background exposures to asbestos both inside and outside of buildings. These have arisen from natural outcrops and from industrial activity. These are at very low levels, usually less than 0.001 F/mL (F stands for the degree of fineness of abrasive particles) but in some countries there are higher environmental exposures, for example, Turkey, Corsica, Cyprus, Russia, Czechoslovakia, Austria, Bulgaria, Greece, and New Caledonia, which have given rise to asbestos-related diseases such as pleural plaques and mesotheliomas. Asbestos fibers have been found in the air and water supplies. Airborne levels of asbetos fibers are generally higher in urban than in rural areas but this has not been accompanied by a detectable increase in nonoccupational mesotheliomas (3). Interestingly, a study of airborne asbestos levels in 12 buildings where friable amosite was used as fireproofing material and generally was in poor condition, found indoor concentrations indistinguishable from outdoor levels,

and no evidence of episodic asbestos release was found (4). However, if the fireproofing was knocked out of the ceiling and allowed to fall to the ground, airborne asbestos fiber levels increased for a brief period of time but did not exceed the United States Occupational Safety and Health Administration (OSHA) occupational exposure level for asbestos.

There is a continuum from background exposure to industrially derived exposures to asbestos, and there is no sharp boundary between them. This can give rise to difficulties in determining the background ranges of asbestos for various populations. Indeed, much debate centers on what constitutes a realistic set of controls. Another important point is that when mineral fiber concentrations are determined in the lungs of subjects with mesothelioma in order to determine the likelihood of it being asbestos related, it is important to be aware that background levels of asbestos fibers do exist in the lungs of the general population not occupationally or paraoccupationally exposed to asbestos. These background levels should be determined for the laboratory carrying out the analysis in the individual case because there are technical differences in the way the analyses are carried out by different laboratories, and therefore one cannot use the background range for one laboratory and extrapolate it to another (5).

One should be aware also that asbestos is not a homogeneous entity. There are two main families of asbestos fibers: serpentine and amphibole. These have important physical, chemical, and pathobiologic differences. The sole constituent fiber of the serpentine asbestos group is chrysotile (white), while the amphibole group includes amosite (brown), crocidolite (blue), tremolite, actinolite, and anthophyllite. When assessing a prior asbestos exposure it is useful to determine the fiber type(s) involved because there is a much lower potential for causing mesothelioma from chrysotile exposure than there is from the amphiboles; a study by Hodgson and Darnton (6) estimated a risk ratio for mesothelioma of chrysotile/amosite/crocidolite of 1:100:500.

Clinical History

It continues to disappoint that inquiries into possible exposures to mineral dust, particularly asbestos, are poorly carried out in hospitals that deal frequently with pulmonary diseases. A reliable occupational history is crucial to assessing the risks of occupational disease in a worker and in attribution of a particular disease to an occupational exposure. With respect to mesothelioma, an appropriate latency period from first exposure to asbestos to onset or death from the tumor is necessary for attribution. A review by Lanphear and Buncher (7) of 1690 cases of mesothelioma found that 99% had a latency period of more than 15 years; 96% had a latency period of at least 20 years, and the median latency period was 32 years. In fact, in the series of cases where there was a well-defined period of asbestos exposure, the latency period was almost always in excess of 20 years and averaged 30 to 40 years.

In any individual case a careful inquiry should be made commencing with the individual's first employment and working completely through chronologically until the current or final employment, noting for each the dates of commencement and termination. Careful details of the nature of the various employments should be made because it may not be immediately apparent that there was a potential for asbestos exposure. The reliability of the history varies since in some situations, for example, work as an insulator or in shipbuilding, exposure to asbestos is clear-cut, whereas in other situations, such as the construction industry, the amount and frequency of exposure is variable and depends on the precise work carried out. Direct regular exposure to asbestos is easier to evaluate than indirect intermittent exposures. Sometimes exposures are exaggerated because there is a tendency to assume all visible dust was asbestos, whereas it might have contained other types of mineral dust, particularly where a disease, such as mesothelioma, which is strongly associated with asbestos exposure, is the subject of the inquiry (so-called recall bias) or where there are pending medicolegal proceedings. The recollections of relatives who provide the occupational history of a deceased patient are generally less accurate than if the occupational history had been obtained directly from the patient.

Sometimes exposures to asbestos, particularly tremolite or anthophyllite, have occurred environmentally from birth, for example, in Turkey, Greece, Corsica, New Caledonia, Russia, Czechoslovakia, Austria, Bulgaria, and Finland.

Mesotheliomas have also resulted from exposures to asbestos brought home on the clothes of other family members who worked in a facility using asbestos. Exposures to asbestos in females are more commonly through the paraoccupational than the direct occupational route and these can be equivalent to occupational exposures, which has been confirmed by lung fiber burden analyses in some cases (8). Therefore, it is necessary to make inquiries as to the occupational activities of other family members and whether, if they were occupationally exposed, they wore their dirty workplace clothes home for laundering during the period appropriate for the latency of the tumor.

Accurate, comprehensive, and detailed histories of exposure to agents such as asbestos can be facilitated by the use of questionnaires.

Clinical and Radiologic Markers of Exposure

The clinical and radiologic markers of exposure include pleural plaques, diffuse pleural fibrosis, rounded atelectasis, and asbestosis.

Pleural Plaques

Plaques are pearl gray, smooth, raised nodules, often calcified, which are situated on the parietal pleura, most commonly on the posterolateral and basal parts of the chest wall and diaphragm (Fig. 16.1). They are frequently associated with asbestos exposure especially when large,

Figure 16.1. Pleural plaques appear as pearl gray, smooth, reused nodules.

numerous, and bilateral, but there are other causes such as trauma, old tuberculosis, exposures to talc or mica, and idiopathic causes.

Pleural plaques are benign, and the great majority of individuals with plaques alone have no symptoms or changes detectable by lung function studies. They appear to be related more to amphibole than to chrysotile asbestos exposure. The study by Gibbs (9) of the Quebec chrysotile miners and millers showed that the incidence correlated with tremolite better than with chrysotile concentrations. Pleural plaques can occur with brief, intermittent, low-level exposure, and they have been found in individuals exposed indirectly to asbestos (paraoccupational, neighborhood, environmental). Plaques related to environmental exposure have been associated with the tremolite or anthophyllite types of fiber.

Less than 10% of pleural plaques found at postmortem have been detected in life. This proportion may alter with the increasing use of computed tomography (CT) scanning. Identification of pleural plaques by chest radiographs has a significant error rate, particularly in obese individuals where fat pads can be mistaken for pleural plaques.

Pleural plaques do not begin to show themselves until 15 to 20 years after the first exposure and they may take 30 years for calcification. Their incidence in an asbestos-exposed population increases with time since first exposure. Pleural plaques are a marker of asbestos exposure only and do *not* indicate an increased risk of malignancy (10). For instance, a shipyard worker with plaques is no more likely to develop mesothelioma or lung cancer than a shipyard worker without plaques.

Knowledge of their presence is less informative than an accurate occupational history.

Diffuse Pleural Fibrosis

Diffuse pleural fibrosis predominantly affects the visceral pleura and it can surround the lung completely (11,12). When bilateral and extensive it can be associated with a decrease in vital capacity. It can be associated with quite low exposures to asbestos. The changes are not specific to asbestos and require evidence of an elevated asbestos fiber burden in the lungs to attribute it to asbestos (vide infra).

Rounded Atelectasis

Rounded atelectasis refers to an asymptomatic, peripheral, rounded pulmonary mass 2 to 7 cm in diameter that is attached to the pleura. It can mimic lung cancer on radiologic investigations, but a typical "comet's tail" of vessels and bronchi may be evident linking into the lateral aspect of the mass, which distinguishes it from neoplasia (13,14). Pathologically it consists of dense pleural fibrosis, which is drawn into atelectatic lung parenchyma. Although most closely associated with exposures to asbestos, because of the latter's tendency to induce pleural fibrosis, it has also been described in association with trauma, infection, and other agents such as silica, which can result in pleural thickening (15).

Asbestosis

Asbestosis is defined as diffuse interstitial fibrosis of the lung that has been caused by inhalation of asbestos fibers. Clinically evident and radiologic changes of asbestosis are usually associated with prolonged heavy exposures to asbestos, which are far higher than necessary to produce mesothelioma. Changes of asbestosis are frequently absent in cases of mesothelioma. If they are present, then there is usually a strong and convincing history of asbestos exposure.

Histopathologic Evaluation of Cases

Examination of the pleura at autopsy may reveal the presence of parietal pleural plaques that were not detected during life and it is important that the examiner note their presence, number, and location.

Asbestos Bodies

The main histopathologic evidence for asbestos exposure is dependent on the finding of asbestos bodies in light microscopic sections of lung tissue either by conventional or iron stains. Asbestos bodies are golden, brown, club-shaped, often beaded structures that contain a clear pale transparent straight needle-like core. They are formed by the coating of the asbestos fiber with ferritin and protein and take months or years

to develop after deposition of the fiber in the lung. If the morphologic criteria are carefully adhered to the majority (greater then 95%) of the asbestos bodies are found on examination by electron microscopy with energy dispersive x-ray spectrometry to contain commercial amphibole (crocidolite or amosite) cores. In some areas of the world with environmental exposures to asbestos they contain tremolite or anthophyllite. Asbestos bodies formed from chrysotile are rare. The finding of one convincing asbestos body by light microscopy in a standard histologic section nearly always signifies an above-background exposure. However, ferruginous bodies that are not formed on asbestos fibers can occur, for example, on talc, mica, kaolin, coal, carbon, rutile, and iron (16). These are distinguished by having cores that are yellow or black or platy rather than fibrous. Particular care has to be exercised by the histopathologist when evaluating cases with mixed dust exposures where substantial amounts of sheet silicates (talc, mica, kaolin, etc.) are present; these silicates can be coated to form ferruginous bodies and although these are platy, they can be cut at such an angle as to appear to be fibrous and can be incorrectly identified as asbestos. If the histopathologist finds clusters of asbestos bodies, this usually signifies very high levels of commercial asbestos fibers.

Tissue Digests and Bronchoalveolar Lavage Examinations

When conventional light microscopic examination of tissue sections fails to demonstrate the presence of asbestos bodies, other quantitative approaches can be utilized to demonstrate an elevated fiber burden such as counting asbestos bodies or fibers on lung tissue digests or bronchoalveolar lavage (BAL) samples (5,17–19). The former can be done using light microscopy and the latter necessitates phase-contrast microscopy or electron microscopy. For both approaches the standard reference ranges for the normal population should be determined by the laboratory carrying out the analysis since numerous studies have been published from many countries that have demonstrated the presence of asbestos bodies and fibers in digestates of lung from individuals without occupational exposure to asbestos. Asbestos bodies constitute only about 0.01% to 1% of fibers visible by electron microscopy. Further the proportion of asbestos fibers that become coated to form asbestos bodies varies with a number of factors including fiber type, fiber length, fiber number, and the amount of iron in the tissue, and therefore one cannot calculate a precise fiber load by quantifying the number of asbestos bodies. Analyses using electron microscopic techniques are more time-consuming and costly but are much more sensitive and can provide a precise breakdown of the different fiber types present (20). They should certainly be employed where the light microscopic techniques fail to demonstrate an elevated fiber burden.

Samples of sputum can also be evaluated for the presence of asbestos bodies. Their detection indicates heavy occupational exposure to asbestos even years after cessation of exposure (21). However, the examinations are of little practical use in subjects exposed to relatively light or moderate amounts of asbestos.

References

1. Spirtas R, Heineman EF, Bernstein L. Malignant mesothelioma: attributable risk of asbestos exposure. Occup Environ Med 1994;51:804–811.
2. McDonald AD, McDonald JC. Malignant mesothelioma in North America. Cancer 1980;46:1650–1656.
3. Browne K, Wagner JC. Environmental exposure to amphibole-asbestos and mesothelioma. In: Noland RP, Langer AM, Ross M, Wicks FJ, Martin RF, eds. The Health Effects of Chrysotile Asbestos. The Canadian Mineralogist Special Publication, vol 5. Ottawa, Canada: Mineralogical Association of Canada, 2001:21–28.
4. Nolan RP, Langer AM. Concentration and type of asbestos fibres in air inside buildings. In: Nolan RP, Langer AM, Ross M, Wicks FJ, Martin RF, eds. The Health Effects of Chrysotile. The Canadian Mineralogist Special Publication vol 5. Ottawa, Canada: Mineralogical Association of Canada, 2001:39–51.
5. De Vuyst P, Karjalainen A, Dumortier P, et al. Guidelines for mineral fibre analyses in biological samples: report of the ERS working group. Eur Respir J 1998;11:1416–1426.
6. Hodgson J, Darnton A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann Occup Hyg 2000;44:565–601.
7. Lanphear BP, Buncher CR. Latent period for malignant mesothelioma of occupational origin. J Occup Med 1992;34:718–721.
8. Gibbs AR, Griffiths DM, Pooley FD, Jones JSP. Comparison of fibre types and size distributions in lung tissues of paraoccupational and occupational cases of malignant mesothelioma. Br J Ind Med 1990;47:621–626.
9. Gibbs GW. Etiology of pleural calcification; a study of Quebec chrysotile asbestos miners and millers. Arch Environ Health 1979;34:76–83.
10. Weiss W. Asbestos-related pleural plaques and lung cancer. Chest 1993;103:1854–1859.
11. Stephens M, Gibbs AR, Pooley FD, Wagner JC. Asbestos induced pleural fibrosis. Thorax 1987;42:583–588.
12. Gibbs AR, Stephens M, Griffiths DM, Blight BJN, Pooley FD. Fibre distribution in the lungs and pleura of subjects with asbestos related diffuse pleural fibrosis. Br J Ind Med 1991;48:762–770.
13. Doyle TC, Lawler GA. CT features of rounded atelectasis of the lung. AJR 1984;143:225–228.
14. Gevenois PA, de Maertelaer V, Madani A, et al. Asbestosis, pleural plaques and diffuse pleural thickening: three distinct benign responses to asbestos exposure. Eur Respir J 1998;11:1021–1027.
15. De Vuyst P, Pfitzenmeyer P, Camus PH. Asbestos, ergot drugs and the pleura. Eur Respir J 1997;10:2695–2698.
16. Churg A, Warnock ML. Asbestos and other ferruginous bodies. Am J Pathol 1981;102:447–456.
17. Churg A, Warnock ML. Asbestos fibres in the general population. Am Rev Respir Dis 1980;122:669–678.
18. Whitwell F, Scott J, Grimshaw M. Relationship between occupations and asbestos fibre content of the lungs in patients with pleural mesothelioma, lung cancer and other diseases. Thorax 1977;32:377–386.
19. Ashcroft T, Heppleston AG. The optical and electron microscope determination of pulmonary asbestos fibre concentrations and its relation to the human pathological reaction. J Clin Pathol 1973;26:224–234.

20. Gibbs AR, Pooley FD. Analysis and interpretation of inorganic mineral particles in "lung tissues." Thorax 1996;51:327–334.
21. Paris C, Galateau-Salle F, Creveuil C, et al. Asbestos bodies in the sputum of asbestos workers: correlation with occupational exposure. Eur Respir J 2002;20:1167–1173.

17

Mesothelioma and Asbestos Exposure

J. Corbett McDonald and Alison McDonald

Historical Background

It was the Conference on the Biological Effects of Asbestos at the New York Academy of Sciences, organized by Irving Selikoff in November 1964 (1), that put both mesothelioma and asbestos on the map. Before that meeting, few people in the scientific or general community had much knowledge of either subject. There they learned the nature and numerous essential industrial uses of a group of naturally occurring mineral fibers, collectively known as asbestos, although in fact comprising at least five distinct materials, chemically, physically, and geologically. Of these, chrysotile, a serpentine mineral mined mainly in Quebec and the Ural mountains of Russia, made up over 90%. Of the remainder the two most important were crocidolite and amosite, produced mainly in South Africa and Australia, both amphibole minerals with distinctive qualities valuable for heat insulation, naval marine use, and the production of large-bore cement pipes. Two other amphibole mineral fibers were anthophyllite, of limited production in Finland, and tremolite, little used, though by far the most widespread geologically. Presenters at the conference stated that within some 20 years of the first industrial exploitation of asbestos in the 1880s, workers heavily exposed to airborne fiber and dust developed a distinctive, seriously disabling and sometimes fatal diffuse pulmonary fibrosis, later termed asbestosis. Little was done to limit exposure until the late 1930s, when after a well-conducted survey of four asbestos textile plants in North Carolina, Dreessen et al (2) and others of the U.S. Public Health Service recommended in 1938 that a workplace dust concentration of 5 million particles per cubic foot (about 15 fibers/mL) should not be exceeded. Mainly because of the Second World War, this recommendation was not implemented; and probably for the same reason it went unnoticed that there were case reports by some German pathologists (3) of malignant tumors of the pleura and peritoneum in men with asbestosis. Thus it was only in the 1950s that the causal association of asbestos exposure with lung cancer in the United Kingdom (4), and later with mesothelioma in South Africa (5), was recognized.

Until that time even the very existence of primary malignancies of the mesotheleum was questioned by reputable pathologists. Looking back, however, a review by Saccone and Coblenz (6) in 1943 had included the identification of over 40 cases in autopsies published since 1870, and referred to two cases of "endothelioma" reported in 1767 by Lieutaud in France among 3000 autopsies. That mesothelial cancers in low frequency probably occurred well before the industrial use of asbestos is discussed more fully later. Indeed, a low background incidence of unknown etiology has almost certainly continued, affecting both children and adults.

The Link with Asbestos

Two key events were undoubtedly responsible for organizing the New York Conference. The first was the report in 1960 by Wagner et al (5) of 33 cases of pleural mesothelioma in the northwest Cape Province of South Africa, 28 of which occurred in persons who had either worked in the crocidolite mines or lived close to them, even as children. The second was a study by Hammond et al (7) of 307 deaths in a small cohort of 632 New York insulation workers, including four from pleural and six from peritoneal mesothelioma. At the end of the conference, an expert working group of the International Union Against Cancer (UICC), after review of the evidence presented, made a number of recommendations for epidemiologic studies, in particular into the importance of asbestos fiber type, especially in mining industries rather than in manufacturing, to avoid the complication of exposure to mixtures (8). At the request of the Canadian Federal Government, and with support from the Research Institute of the Quebec Asbestos Mining Association, a comprehensive university-based scientific program was established almost at once (9). Elsewhere, only in the chrysotile mine of Belangero in northern Italy was anything done to implement these recommendations until over 20 years later. As a result, the lack of information on amphibole exposure, comparable to that for chrysotile, greatly delayed our understanding of mineral fiber carcinogenicity.

Early Case-Control Studies

Data from two important case-control studies in the United Kingdom were presented at the New York Conference and published more fully the following year. The first of these was by Elmes et al (10), who studied 42 male cases of pleural mesothelioma and 42 closely matched controls in Belfast, Northern Ireland, with detailed work histories taken from those still living or from relatives of those who had died. Thirty-seven cases had been exposed to asbestos, mainly in plumbing, insulation, and shipyard work, not all heavily and without mention of fiber type, compared with nine controls. The second study, by Newhouse and Thompson (11), was of 83 cases, half in men and half in women, who had died between 1917 and 1950, 56 from pleural and 27 from peritoneal mesothelial tumors, close to the large Cape Asbestos

Table 17.1. Early case-control studies of mesothelioma giving definite or probable occupational exposure to asbestos

First author (reference)	Year	Place	Years diagnosed	Cases/controls	Male (%)	Occupationally exposed (%)	Relative risk
Elmes (10)	1965	Belfast	1950–64	42/42	95	76	3.6
Newhouse (11)	1965	London	1917–64	76/76	49	41	3.9
McEwen (12)	1970	Scotland	1950–67	80/80	91	58	4.2
McDonald (13)	1970	Canada	1960–68	165/165	65	21	7.0
Rubino (14)	1972	Piedmont	1960–70	50/50	64	12	6.0
Ashcroft (15)	1973	Tyneside	1948–67	27/56	88	93	2.3
Hain (16)	1974	Hamburg	1958–68	150/150	71	58	6.3
Zielhuis (17)	1975	Netherlands	1969–71	67/67	94	72	4.0

Source: Based on Table 1 in McDonald and McDonald (3).

Company's factory that opened in 1913 in London's East End. Their control series comprised patients admitted later to the hospital with other diseases, matched for sex and age. The authors acknowledged that neither these control measures nor the interview methods were ideal, but concluded that the case-control comparisons of occupational and residential histories were probably valid. Of 76 pairs, 18 cases (24%) had been employed at the Cape Asbestos factory, five (7%) at other asbestos plants, and eight (11%) as insulators or laggers, compared with one (1%) and four (5%) controls respectively. A further nine cases (12%) were in persons who had lived in the same house as an asbestos worker and were indirectly exposed, compared with one control (1%). Only crocidolite from South Africa was used in the Cape Asbestos factory until 1926, when small quantities of chrysotile and amosite were introduced.

During the next few years, six more case-control studies were published in addition to the two studies cited above, all with a substantially increased relative risk associated with occupational asbestos exposure (Table 17.1). Little attention was given to fiber type explicitly, but it can be seen that five of the eight studies were in shipyard areas where amphibole use was common. Rather different from the other seven, and perhaps more generally informative, was the study across Canada by McDonald et al (13). This entailed an approach to all 423 of the country's pathologists, whereby 165 known deaths from mesothelioma in 1959 to 1968, diagnosed by autopsy or biopsy, were registered (i.e., 1 per million population per annum). Of this total, 65% were in men; 70% were pleural, 27% peritoneal, and 3% pericardial. Detailed occupational and residential histories of exposure to asbestos and six other materials used industrially were obtained "blind" from relatives and friends in 90% of the cases, and from two matched case-control series, one of primary and one of secondary lung cancer, selected from the same autopsy records. An association with definite or probable occupational exposure to asbestos was clearly demonstrated—indeed with the highest relative risk (7.0) of all eight case-control studies—but only 20% of men and one woman had any such contact. Almost all the excess was in the manufacturing and industrial application of asbestos, rather than in mining or milling. No association was found with lesser

degrees of occupational exposure or residence in asbestos-mining areas, but there was a small excess of possible domestic exposures. The smoking histories in the mesothelial tumor and male control groups were almost identical, and considerably lower than those in cases of primary lung cancer, implying that unlike asbestos-related lung cancer, smoking did not contribute to this disease.

In 1972 the survey was repeated, with extension to all pathologists in North America (some 7000 of them), almost all of whom agreed to contribute cases identified at autopsy or biopsy for the one year only (18). A control subject with death from metastatic lung disease from a primary tumor outside the chest, matched for date, sex, and age, was selected from the same pathology file as the case. Relatives were interviewed usually by a public health nurse who was not informed about the case-control status, and detailed residential and occupational histories were recorded. Jobs were also coded blind, using a list compiled by four expert international groups, according to the probability of asbestos exposure. Of 344 male cases of mesothelioma, 188 (55%), compared with 78 (23%) controls, fell into one of the five exposed categories: insulation work, an infrequent occupation in controls, had the highest relative risk (46.1); asbestos production and manufacture were next in line (6.1), followed by employment in heating trades (excluding insulation work), shipyards, and construction (3.4). Subjects from this survey were subsequently used as the base in a case-control analysis of lung burden with the fibers identified and concentrations estimated by electron microscopy. The results of this study are summarized later, together with those from other lung burden analyses.

In the Canadian surveys, 1960 to 1966, the average annual incidence was one case per million persons—about 1.5 for males and 0.8 for females; however, there may have been underreporting during these early years. In 1966 to 1972, the incidence in Canada was 2.9 per million males and 1.4 per million females, and in the United States in 1972 the corresponding rates were 2.7 and 0.8 per million. These estimates were used in 1975 in a geographical analysis of all known cases of mesothelioma worldwide in areas where reported cases could be linked to population estimates. By applying age- and sex-specific rates found in Canada, the number of mesotheliomas expected on this basis was compared with the numbers observed (19). High observed-to-expected ratios were found in many European shipyard cities, notably Walcheren, the Netherlands (23.3), Wilhelmshaven, Germany (21.5), and Plymouth, UK (14.3). In two locations with large asbestos product manufacturing industries, there were also high ratios: Dresden, Germany (16.8) and the Manville-Somerville area of New Jersey (26.5).

In the early 1970s, mesothelioma mortality in North America was already two or three times higher in males than females. This pattern soon became apparent in most industrialized countries and was followed by a steady upward trend in male mortality, which still continues. The steep rise in males, which probably began in the 1940s, reflects a parallel increase in the industrial use of asbestos, from about 1910,

having taken account of a 30- to 40-year latency. As a result of this steady increase, mesothelioma is now responsible for some 20 deaths per million male population in Western Europe and North America compared with an estimated 1 to 2 per million 30 to 40 years ago. In early studies, only a minority of male cases were attributable to occupational exposure, whereas now up to 90% are.

Occupational Risk

Apart from the early case-control studies just described, the main body of information on risks from exposure to airborne asbestos fibers in the workplace is contained in over 40 historical cohort mortality studies reported during the past 30 years. These studies have varied in quality and in the extent to which exposure has been assessed in duration, intensity, or asbestos fiber type. In fewer than 10 cohorts had there been any attempt to estimate exposure intensity for each cohort member, and in very few cohorts indeed was there exposure to only one type of asbestos. These serious deficiencies, given the potentially great difference in carcinogenicity between chrysotile and the amphiboles, render almost uninterpretable the results of the many cohorts exposed to mixtures where one type, usually chrysotile, was said to predominate.

Despite this lack of specificity, cohort surveys, by being prospective, have provided more useful information on occupational risk than was usually obtained from retrospective case-referent studies. This is more true, however, of lung cancer and nonmalignant respiratory disease (NMRD) than of mesothelioma. Standardized mortality ratios (SMRs) can usually be calculated by age, sex, and exposure variables for the former but not the latter, as even now mortality rates for mesothelioma in the general population are seldom available, let alone reliable. Investigators have had to fall back instead on measures of proportional mortality, though these depend enormously on age composition of the cohort and length of follow-up. For example, in the large cohort of Quebec chrysotile miners and millers, described more fully below, the proportion of all deaths ascribed to mesothelioma rose steadily from 3 of 2413 (0.12%) in 1966 to 38 of 8009 (0.47%) in 1992 (20). Even so, in the absence of any better index, proportional rates interpreted with care can be useful. Thus, in Table 17.2, where the salient findings from the 43 main cohort mortality studies published before 1999 are summarized, the differences in proportional mortality for mesothelioma are large and systematic. This is especially so when differences between chrysotile and amphibole exposure within the same industrial sector are considered in more detail.

The largest and most complete of the mining cohorts was of all 10,918 men born in 1891 to 1920 who, in 1966, had served for 1 month or more in the Quebec chrysotile production industry, either as miners or millers in the town of Asbestos or region of Thetford Mines, or in a small asbestos products factory (23). Excluding losses, almost all before 1935 and in men with very short employment, 8009 (82%) of 9780

Table 17.2. Cohort mortality studies of asbestos-exposed workers

First author (reference)	Year	Country	No. of Subjects	Deaths: All causes *n*	All causes %	Lung cancer *n*	Lung cancer SMR	Lung cancer Excess	Lung cancer PMR/1000	Mesothelioma *n*	Mesothelioma PMR/1000	Relevant exposure
Mining and milling												
Piolatto (22)	1990	Italy	952	427	45	22	1.11	2	20.7	2	4.4	Chrysotile
Liddell (23)	1997	Canada	9072	7456	82	597	1.37	161		33		
Meurman (24)	1974	Finland	1092	248	23	21	1.61	8	44.2	0	25.7	Various amphiboles
Brown (25)	1979	U.S.	398	74	19	10	2.90	7		1		
McDonald (26)	1986	U.S.	406	165	41	21	2.45	12		4		
Armstrong (27)	1988	Australia	6505	820	13	91	2.64	57		32		
Sluis-Cremer (28)	1992	South Africa	3212	648	20	26	1.38	7		4		
Sluis-Cremer (28)	1992	South Africa	3430	423	12	27	2.03	14		20		
Manufacturing												
Cement products												
Weiss (29)	1977	U.S.	264	66	25	8	0.93	0	6.0	0	3.3	Chrysotile
Thomas (30)	1982	U.K.	1592	351	22	24	0.93	0		2		
Ohlson (31)	1985	Sweden	1176	220	19	11	1.23	2		0		
Gardner (32)	1986	U.K.	1510	384	25	35	0.92	0		1		
Hughes (33)	1987	U.S. (plant 1)	2565	477	19	48	1.17	7		2		
Finkelstein (34)	1984	Canada	535	108	20	26	4.80	21	55.1	19	20.7	Chrysotile and crocidolite
Alies-Patin (35)	1985	France	1506	206	14	9	1.63	3		4		
Hughes (33)	1987	U.S. (plant 2)	4366	874	20	107	1.44	33		8		
Magnani (36)	1987	Italy	2608	728	28	110	2.68	69		28		
Raffn (37)	1989	Denmark	7996	1305	16	162	1.80	72		13		
Albin (38)	1990	Sweden	1929	592	31	35	2.50	21		13		
Neuberger (39)	1990	Austria	2816	540	19	50	1.72	21		5		
Textiles												
McDonald (40)	1983	U.S.	2543	570	22	59	2.00	29	58.6	1	2.5	Chrysotile
Dement (41)	1994	U.S.	1247	607	49	72	2.25	40		2		
McDonald (42)	1983	U.S.	4137	895	22	53	1.05	3	19.1	14	14.8	Chrysotile and crocidolite
Peto (43)	1985	U.K.	3211	727	23	93	1.44	28		10		

Friction products													
McDonald (44)	1984	U.S.	3641	803	22	73	1.49	24	10.8	0	3.9	Chrysotile
Newhouse (45)	1989	U.K.	9104	2055	23	229	1.03	7		11		
Insulation products													
Seidman (46)	1979	U.S.	Unclear	528	—	76	5.78	63	86.8	14	23.1	Amosite
Acheson (47)	1984	U.K.	4280	333	8	38	1.31	9		5		
Levin (48)	1998	U.S.	753	222	30	35	2.77	22		6		
Filter assembly													
McDonald (49)	1978	Canada	199	56	28	8	2.0	4	57.6	9	102.3	Crocidolite
Jones (50)	1980	U.K.	1088	166	15	12	2.14	6		29		
Talcott (51)	1989	U.S.	33	28	85	8	15.71	8		5		
Acheson (52)	1982	U.K. (a)	757	219	29	15	2.41	9		5		
Acheson (52)	1982	U.K. (b)	570	177	31	7	1.45	2	11.3	1	5.6	Chrysotile
Various products													
Newhouse (53)	1985	U.K.	3000	818	27	158	3.20	109	73.9	60	51.4	Chrysotile and crocidolite
Enterline (54)	1987	U.S.	1074	944	88	77	2.71	49		8		
Liddell (23)	1997	Canada	708	553	78	49	1.34	13		5		
Product application													
Insulation work													
Elmes (55)	1977	U.K.	162	1122	75	24	6.00	20	98.5	24	60.2	Chrysotile, crocidolite, and amosite
Selikoff (56)	1979	U.S. and Canada	17,800	2271	13	397	4.24	303		175		
Newhouse (53)	1985	U.K.	1400	157	11	38	3.55	27		13		
Järvholm (57)	1998	Sweden	248	86	36	11	4.4	8		7		
Dockyard work													
Rossiter (58)	1980	U.K.	6292	1043	17	84	0.84	0	5.3	31	22.8	Chrysotile, crocidolite, and amosite
Kolonel (59)	1985	U.S.	5191	668	12	122	1.08	9		8		

PMR, proportional mortality ratio; SMR, standardized mortality ratio.
Source: Based on Table 5.1 in McDonald (21).

traced had died before 1993; 38 (0.47%) probably from mesothelioma, distributed as follows:

Location	Traced	Deaths	Average exposure (f/mil.y)	Mesothelioma	
				n	PMR (%)
Thetford Mines	**5041**	**4125**	**570**	**25**	***0.61***
Asbestos	**4031**	**3331**	**390**	**8**	***0.22***
Factory (in Asbestos)	**708**	**553**	**201**	**5**	***0.90***

f/mil.y, fibers/million per year; PMR, proportional mortality ratio.

At both the town of Asbestos and the region of Thetford Mines, the average duration of employment for mine, mill, and factory workers was about 10 years, and of those traced, 76% had worked for more than 1 year. No case occurred among some 4400 men employed less than 2 years; eight were in men employed 2 to 5 years, and the remaining 30 in men employed 20 to 49 years. All employees in the factory were also potentially exposed to crocidolite and amosite, mainly used in cement and friction product manufacture. Among these were two cases in men who in 1939 to 1942 worked on the carding of pure crocidolite for military gas mask filters. The exposures, shown in f/mil.y, are approximate and were obtained by conversion from cumulative exposures expressed in million dust particles per cubic foot.y (mpcf.y), accumulated by workers to age 55.

The cohort of 7317 white South African amphibole miners was established in 1981 from records of men employed since 1925 (28). Only some 45% of the cohort were employed for more than 1 year, and 8% for more than 10 years. Excluding 167 lost to follow-up, 1225 of the remaining 7150 had died; 30 (2.4%) probably from mesothelioma, distributed as follows by type of exposure:

Exposure	Traced	Deaths	Average exposure (f/mil.y)	Mesothelioma	
				n	PMR (%)
Crocidolite only	**2893**	**423**	**9.6**	**20**	***4.73***
Amosite only	**2356**	**648**	**15.2**	**4**	***0.62***
Mixed	**509**	**154**	**nk**	**6**	***3.90***

nk, not known.

The cohort of Australian crocidolite miners and millers comprised 6505 men first employed between 1943 and 1967, with 4653 (72%) traced to the end of 1980; by then 820 were known to have died, 32 (3.9%) from mesothelioma (27). Their median cumulative exposure was estimated at 6 fibers per cc/year; their median duration of employment was only 4 months, but their exposure was described as intense. Despite the far lower cumulative exposure experienced by South African and Australian workers than the men in Quebec, their proportional mortality from mesothelioma was about 10 times higher.

Most of the other industry-specific comparisons present much the same pattern. In friction product manufacture, for example, although 11 mesothelioma deaths were observed by Newhouse and Sullivan (45) in a large cohort of over 9000, virtually all of whom were exposed only to chrysotile, 10 of the 11 were definitely, and one possibly, members of a small group who worked for a short time on a special crocidolite contract. In the textile industry, the two cohorts studied by McDonald et al (40,42) were in plants owned by the same company and similar in every way, except that in the one with 14 deaths from mesothelioma, crocidolite was also used. Even more dramatic results were obtained from the two cohorts employed briefly during the early years of the Second World War in Canada (49) and in England (50) on the manufacture and assembly of filter pads made with pure crocidolite for military gas masks. Cases of mesothelioma began to appear in both cohorts 18 years later, with PMRs reaching 16% and 17%, respectively. A disaster of similar severity affected a small group of employees in the United States engaged in the manufacture of cigarette filters, of all things, from crocidolite. In contrast, a study of 570 British workers employed in manufacturing civilian gas masks using chrysotile filters produced only one case, an employee previously exposed to crocidolite (52).

In summary, of 11,538 deaths in the chrysotile cohorts, 44 were from mesothelioma (PMR per thousand 3.8), whereas in the amphibole-related cohorts of 19,622 deaths, 590 were from mesothelioma (PMR per thousand 30.1). While the carcinogenic potency of crocidolite thus seems clear, that of amosite, particularly in mining, is less so. High rates of mesothelioma were observed nevertheless in the manufacturing of insulation materials and among insulation workers, both groups heavily exposed to amosite. In none of the reports on cohorts in Table 17.2, however, is there any reliable indication of risk in relation to estimated intensity of exposure to any specified fiber type; the interpretation of the PMRs, therefore, entails several assumptions.

A further point of interest concerns the general parallel exhibited in Table 17.2 between levels of mesothelioma and lung cancer excess mortality in all industries except textile manufacture. In the four cohorts shown, two of which were in the same plant, there were only two mesothelioma deaths in all, whereas for lung cancer the general pattern was reversed (40,41). In the other two textile plants in which crocidolite was also used, there were 24 deaths from mesothelioma, but with only modest SMRs for lung cancer (42,43). This anomaly, which is confined to the use of chrysotile in this particular plant, has never been satisfactorily explained (60), and is all the more important in that it did not apply to mesothelioma.

Other Causes

Mention has been made of the occurrence of possible cases of mesothelioma in autopsy series at the end of the 19th century. Given the long latency of this disease (20 to 50 years or longer), it is unlikely that they

could have resulted from the industrial exploitation of asbestos, which began in the 1880s and then only on a small scale. This evidence in itself is not conclusive, but there are stronger grounds for both the existence of a low background incidence of mesothelioma of unknown etiology, and for causation by at least one environmental mineral other than asbestos.

Fibrous Erionite

The evidence against fibrous erionite is virtually confined to the disastrous occurrence of unequaled mortality from these tumors in a localized area of central Turkey. The population of three villages in Cappadocia had been exposed since birth to airborne fibers of erionite, a zeolite mineral quite unrelated to asbestos, though with fibers having some physical similarities to crocidolite. The fibers are derived from volcanic rock, or tuff, which is used in the area for construction of houses and other buildings. In the three affected villages, 29 of 125 deaths during a defined period were from pleural mesothelioma, and four others from peritoneal disease (61). Investigation showed that airborne fiber concentrations were higher in the villages affected than in one that was not; also sputum samples from residents in the former contained ferruginous bodies with an erionite core. In most cases exposure was from birth, with death occurring 27 to 40 years later. In common with amphibole fibers, electron microscopic studies of lung tissue showed erionite to be highly biopersistent. Deposits of fibrous erionite occur in volcanic areas elsewhere, for example in some Rocky Mountain states; there has also been some synthetic production for industrial catalytic purposes. No clear link with mesothelioma mortality has been shown with either of these possible sources; perhaps because nowhere other than in Cappadocia has volcanic tuff been used for domestic buildings, resulting in exposure of young children. It is worth adding that deaths from mesothelioma have also been recorded in Scandinavia and other parts of Turkey among former residents of the affected villages.

Apart from asbestos and erionite, no other environmental agent has been incriminated with any certainty. Some suspicion has rested on the long, thin silica fibers created by the burning of sugar cane in India (62) and in the southern states of the United States (63). A similar process might also arise in forest fires, but none of these suggestions has been supported by experimental or lung burden evidence.

Mesothelioma in Children

Despite diagnostic uncertainties, greater even than in adults, it is evident that mesothelioma does occur in children. In a review of 80 cases, reported from 1969 to 1986 (64), the ratio of males to females, aged 1 to 19 years (mean 9.7 years) was 1.4:1 and only two had a possible exposure to asbestos. Of these, 68% were pleural, 25% peritoneal, and 8% pericardial. Given that the latency of mesothelioma is very seldom less than 20 years, and in the Turkish cases at least 27 years, it is most unlikely that childhood cases could be due to asbestos expo-

sure. The incidence of mesothelioma in persons younger than 20 years of age can be roughly estimated from three studies (65). In the Canadian survey from 1960 to 1968, four fatal cases were ascertained by systemic inquiry from all pathologists—a rate of about 0.7 per 10 million per annum. A very similar figure can be derived from 13 cases identified among death certificates in the United States from 1965 to 1968. Finally, data from the Surveillance, Epidemiology, and End Result (SEER) program in the United States, estimated the case incidence from 1973 to 1984, at 0.5 per 10 million. As mesothelioma in children may well be underdiagnosed, a conservative estimate for the annual case incidence in North America may well reach 1 per 10 million, but even so, appreciably lower than any comparable estimate for adults.

Statistical Extrapolation

In Canada, annual incidence rates based on cases of mesothelioma ascertained through pathologists, when extrapolated backward, suggest that male and female rates were similar at or before 1950, at a level of about 1 per million population. This pattern is similar to trends found in the SEER cancer surveillance program in the United States, and for mortality observed in Britain, Finland, Norway, and Denmark, where an increasing male excess appears due to the more frequent history of occupational asbestos exposure in men than in women. In the SEER program, regions with higher age-adjusted incidence rates, presumably attributable to work-related asbestos exposure, had higher ratios of male to female cases than regions with lower rates, and linear extrapolation would suggest that, at the point where the sex ratio is equal, the incidence might be as high as 5 per million, though with wide confidence limits. In Los Angeles County, equal numbers of cases in men and women were without history of exposure to asbestos, suggesting a background incidence of about 2 per million. In France, careful inquiry failed to identify any opportunity for asbestos exposure in younger subjects with mesothelioma, with equal numbers of males and females. In all these various studies, efforts to detect a cause other than asbestos have been largely unsuccessful (65).

Lung Burden Analyses

Valuable though cohort mortality surveys have been in assessing the health effects of asbestos in selected industries, they have not contributed much to knowledge concerning risk in relation to intensity of exposure to specific types of fiber. In diseases such as mesothelioma, the relevant exposures took place many years before adequate measurements of respirable dust particles, let alone fibers, had been made. Any such estimates remain at best a rough surrogate for what an individual worker inhaled and retained. Thus the development in the 1970s of electron microscopy with energy-dispersive analysis, to identify, count, and size mineral fibers in lung tissue, held great potential, though also with limitations. Apart from selective biases resulting from the availability and nature of lung samples obtained for analysis, fibers

seen at autopsy or biopsy may not reflect what were present years earlier; much depends on the ability of fibers to penetrate the airways, and on their subsequent durability and biopersistence. As it is precisely in these latter qualities that chrysotile and the amphiboles differ, any epidemiologic study of lung burden must be carefully controlled and the results interpreted with considerable caution.

Despite these difficulties, there have been several well-designed case-control studies of reasonable quality in Europe, Australia, and America that, though not conclusive, have provided consistent evidence implicating amphibole fibers rather than chrysotile in most cases of mesothelioma (Table 17.3). The two most recent studies from Germany (72) and the United Kingdom (73) are particularly informative in that they addressed risk in relation to fiber concentration in lung tissue, i.e., retained dose. In both these studies, a highly significant linear relationship was observed between odds ratios and concentrations of amphibole fiber, but not with chrysotile. In the German study, risk was greatest with fibers longer than 15 μm, and in the British, short, medium, and long fibers were all associated with risk, but most closely with those in the longest category (≥10 μm). In the latter study, the strong linear trend shown by crocidolite, amosite, and tremolite when combined suggested that their effects were probably additive (Table 17.4). Overall, these analyses indicated that some 80% of cases studied were attributable to amosite or crocidolite, and 7% to tremolite. The contribution of chrysotile could not be reliably assessed because of its low biopersistence, but as over 90% of all asbestos used is chrysotile, for which tremolite is a valid marker, it must be small.

The British study just described was based on a larger number of cases reported by chest physicians in a national surveillance scheme in men younger than 50 years of age at time of diagnosis. It was thought that most, if not all, of the occupational exposures would have been since 1970 when the importation of crocidolite to the UK was virtually eliminated. In fact, it was found that almost all the cases were in men who had started work several years before that date. Of 37 occupations analyzed, odds ratios against expected values obtained from the census were significantly raised in only eight, of which five were in the construction industry: carpenters, plumbers, electricians, insulators, and unskilled workers. The remaining three categories at increased risk were workers in shipbuilding, cement, and mineral product manufacturing, all less important in this than in earlier surveys (74).

The Tremolite Factor

When we began in 1965, at the behest of the UICC Working Group, an extensive program of epidemiologic research in the Quebec asbestos mining industry, it was in the belief that we were dealing with exposure to chrysotile only. Clear evidence was found of a systematic relationship between quantitative estimates of airborne dust particle exposure and all measures of morbidity and mortality of primary interest, including lung cancer, radiographic change, lung function, and

Table 17.3. Analyses of mineral fibers in lung tissue from mesothelioma cases and controls

First author (reference)	Year	Country	Cases	Controls	Odds ratio for amphibole fibers*	Evidence on chrysotile
Jones (66)	1980	U.K.	86 cases notified by coroners and pathologists, 1976	56 cases (lung cancer 27, cerebrovascular disease 29) matched for age, sex, and place	7.4	Chrysotile present in two of four cases without amphiboles
McDonald (67)	1982	Canada and U.S.	99 cases from survey of pathologists	Secondary lung cancer; matched for age, sex, date, and hospital	3.8	In pairs where amphibole content was $<10^6$ F/g closely similar distributions of chrysotile
Mowe (68)	1985	Norway	14 cases, county cancer registry, 1970–9	28 cases excluding malignant and chronic pulmonary disease matched for age, sex, year, and residence	8.5 (based on all types of amphibole fiber)	Fiber type not identified
Gaudichet (69)	1988	France	20 cases from Nantes district, 1980–2	20 each of adenocarcinoma and squamous carcinoma, secondary lung cancer and cardiovascular disease, matched for age, sex, and hospital	Amphibole fiber concentration 2–3 times higher than in controls	Similar concentration in cases and controls
McDonald (70)	1989	Canada	78 cases from survey of pathologists, 1980–4	Nonmalignant nonrespiratory disease, matched for age, sex, date, hospital, and type of sample	6.6 for fibers ≥8 μm in length	Low-level risk in univariate analysis and none in multivariate analysis
Rogers (71)	1991	Australia	221 cases from national surveillance, 1980–5	359 tissue samples from a hospital in Sydney excluding nonmalignant respiratory disease and abdominal cancer; unmatched	16.6 for fibers ≥10 μm in length	7 of 25 cases and 3 of 31 controls without amphibole fibers had $\geq 10^5$ F/g chrysotile
Rödelsperger (72)	1999	Germany	66 cases from five German cities	66 cases undergoing lung resection mainly for lung cancer, matched for age, sex, and region	4.5 for fibers ≥15 μm in clear linear dose response	No increase in odds ratio
McDonald (73)	2001	U.K.	69 male cases aged ≤50 years at diagnosis	57 cases of accidental or sudden death of similar age, sex, and region	Related linearly to concentrations: 0.1–0.9 μg—8.8 (1.8–43.5) 1.0–9.9 μg—59.9 (9.0–40.0)	No significant increase in odds ratio

* Based on Table 5.5 in McDonald (21).

Table 17.4. Distribution of lung fiber concentrations with grouped and continuous odds ratios (OR)

Fiber type	Concentration (*per μg*)	Cases	Controls	Crude OR	Adjusted OR[a]
Crocidolite	0	28	48	1.0	1.0
	0.1–0.9	27	8	5.3 (2.0–14.3)	4.6 (1.3–15.5)
	1.0–9.9	11	1	17.5 (2.0–155)	3.9 (0.3–40.4)
	≥10.0	3	0	∞	∞
	Linear model[b]			13.2 (3.3–44.5)	40.0 (2.6–388)
Amosite	0	13	34	1.0	1.0
	0.1–0.9	23	18	5.6 (1.6–18.8)	5.1 (1.4–18.6)
	1.0–9.9	26	5	24.9 (5.7–108)	17.9 (3.5–91.4)
	≥10.0	7	0	∞	∞
	Linear model[b]			11.4 (2.8–49.2)	14.3 (2.2–113)
Tremolite	0	55	51	1.0	1.0
	0.1–0.9	13	6	2.2 (0.9–6.6)	2.3 (0.7–8.0)
	1.0–9.9	1	0	—	—
	≥10.0	0	0	—	—
	Linear model[b]			6.9 (0.2–30.9)	29.6 (<0–340)
All amphiboles	0	6	28	1.0	1.0
	0.1–0.9	26	24	9.2 (1.9–44.5)	8.8 (1.8–43.5)
	1.0–9.9	28	4	64.7 (9.8–425)	59.9 (9.0–400)
	≥10.0	9	1	55.8 (3.9–792)	—
	Linear model[b]			19.4 (4.2–137)	47.6 (6.0–>999)
Chrysotile	0	14	19	1.0	1.0
	0.1–0.9	28	21	1.5 (0.6–3.9)	1.9 (0.5–6.7)
	1.0–9.9	26	16	2.2 (0.8–6.2)	2.2 (0.6–8.4)
	≥10.0	1	1	—	—
	Linear model[b]			0.1 (<0–1.2)	2.2 (<0–>999)

[a] Crocidolite, amosite, and tremolite are adjusted for each other. Total amphiboles and chrysotile are adjusted for each other.

[b] Average increment in odds ratio per fiber/μg.

Source: From McDonald et al (73).

respiratory symptoms (75). Except at very high exposure levels, these adverse health effects were not severe, and even among 2413 deaths in a cohort of some 10,000 men, only three (0.12%) were ascribed to mesothelioma. This seemed in marked contrast to the findings of Selikoff et al (56) of 22 deaths (5.8%) from this cause among 380 deaths in a small cohort of 632 American insulation workers (76).

It was observed at the outset, and by local physicians for many years, that pleural thickening and calcification were much more frequent among workers in the Thetford Mines region of Quebec than in the town of Asbestos, some 60 miles away. In a detailed study of pleural calcification Gibbs (77), who was responsible for the environmental aspects of our research program, noted in 1972 that these radiographic changes were considerably more prevalent in some mines than in others, suggesting to him that minerals other than chrysotile might be responsible. Over the next few years, a series of studies was published with results based on electron microscopic analyses of lung tissue at autopsy, which, taken together, indicated that the exposure experi-

enced by workers with Quebec chrysotile was much more complicated than had previously been supposed.

First came the observations of Pooley (78), and then of Rowlands et al (79), who found that, in the lungs of former Quebec miners at autopsy, chrysotile and tremolite fibers were present in surprisingly similar concentrations (Fig. 17.1). Later, further analysis of data from these studies suggested that tremolite concentrations were perhaps two to three times higher in the region of Thetford Mines than in the town of Asbestos (80). There followed a much larger investigation by Sébastien et al (81) that, though undertaken for an entirely different purpose, added considerably to several aspects of the tremolite question. The primary objective of this study was to explain the much greater risk of lung cancer, though not of mesothelioma, in asbestos textile workers in Charleston, South Carolina, than in Quebec miners

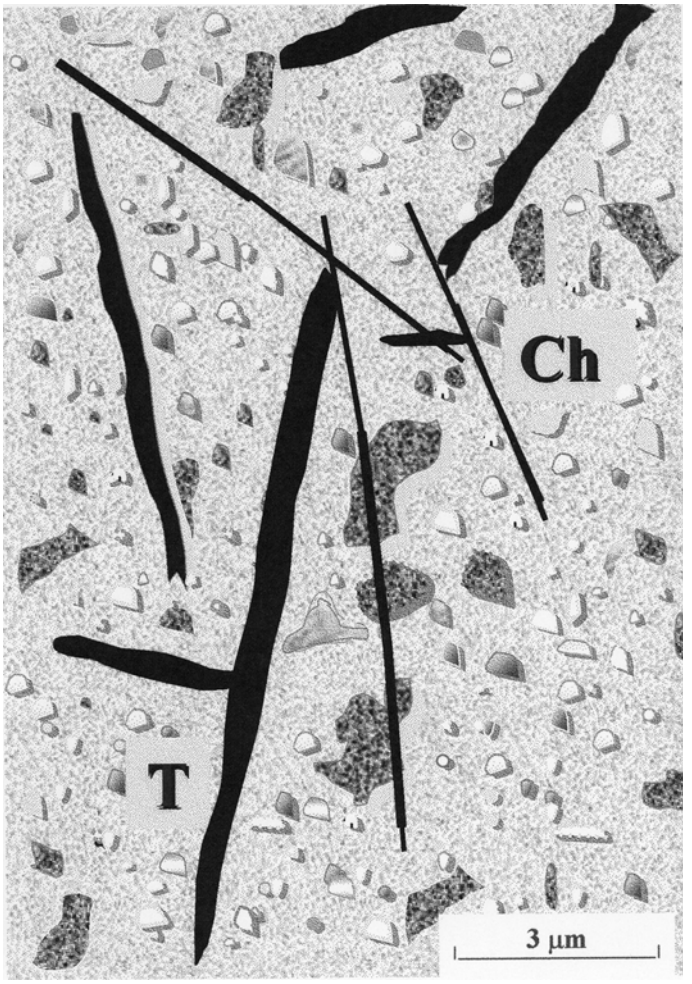


Figure 17.1. Lung of Quebec chrysotile miner at autopsy. Ch, chrysotile fibers; T, tremolite fibers. (*Source*: Copy of photomicrograph kindly provided by Dr. Patrick Sébastien.)

and millers both exposed to chrysotile from the same source. One hundred sixty-one lung tissue samples from deceased cohort members (72 from Charleston and 89 from Thetford Mines) were collected for analysis by transmission electron microscopy. Altogether 1828 chrysotile and 3270 tremolite fibers were identified; in both cohorts tremolite predominated and fiber dimensions were closely similar. Analyses that took account of duration of employment, exposure intensity, and time from last employment to death concluded that none of these variables could explain the higher lung cancer risks observed in textile workers. The possible co-carcinogenic role of mineral oil used to control dust in textile plants was an alternative explanation, which has yet to be adequately tested. However, the findings from this large survey made it possible to address several other questions.

The first of these studies sought to explain the remarkable predominance of tremolite fibers in the lungs of men overwhelmingly exposed at work to chrysotile, although in the city of Thetford Mines tremolite represented only 1.5% of asbestos fibers in the ambient air (82). That this enormous difference was probably due to the far greater durability and biopersistence of tremolite was demonstrated by examining mean lung fiber concentrations in deaths at varying times after the date of last employment. The lungs of even men who died while still employed contained twice as many tremolite fibers as chrysotile; at more than 10 years after leaving work, the ratio rose eightfold (83). In another analysis, the results of which are shown in Figure 17.2, it can be seen that in relation to cumulative exposure, the lung concentration of tremolite in Quebec miners increased linearly, whereas that of chrysotile did not (84). This pattern was virtually identical to that

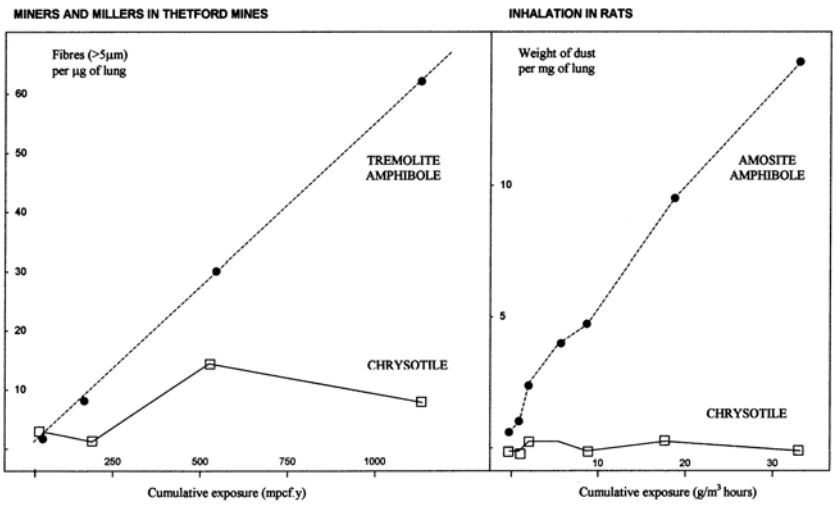


Figure 17.2. Human and experimental data on the relationships between cumulative exposure to asbestos dust and lung retention. [*Source*: Based on Sébastien et al (84).]

reported by Wagner et al (85) in laboratory rats after inhalation of amosite and chrysotile.

In his earlier study of pleural calcification in the region of Thetford Mines, Gibbs (77) had noted that these changes were more common among miners than millers, and particularly in men who had worked in a localized group of mines near the center of the town rather than in other mines located peripherally. He concluded that the cause might be related to some mineral closely associated with chrysotile, possibly mica, talc, or brünnerite, but he did not include tremolite, which even Riordan (86) had rarely mentioned in his comprehensive description of the geology of the region in 1957. Much later, when, by 1992, 38 probable cases of mesothelioma had been identified in the Quebec cohort among over 8000 deaths from all causes, it became clear that even at Thetford Mines they too were unevenly distributed. As mentioned in the previous section, among 4125 deaths in miners and millers at Thetford Mines, there were 25 (0.61%) from mesothelioma; at the town of Asbestos among 3331 deaths, the corresponding figure was 8 (0.24%). At Thetford, however, the cases were more common in miners, whereas at Asbestos the few cases were all in millers. A further detailed examination of work histories of the cases at Thetford showed that man-years of employment were concentrated in a localized area of five mines centrally located (area A), compared with 10 mines located peripherally (area B) (20). These were much the same as those observed by Gibbs for pleural classification. A more detailed analysis was then made of the data for the 83 subjects in Thetford Mines from the study of Sébastien et al (84), using available records of the specific mines in which each man had worked. This showed that the concentration of tremolite fibers, but not of chrysotile, were some four times higher among 58 men in area A (32/μg) than among 25 men in area B (7/μg) ($p = .0002$). A strictly controlled study of deaths from mesothelioma and other cancers, with analysis by logistic regression, was therefore undertaken (87). This showed that the odds ratios (OR) for work in the central mines (area A) were raised substantially and significantly for mesothelioma [OR = 2.55; 90% confidence interval (CI) 1.52–4.27] and lung cancer (OR = 1.98; 90% CI 1.53–2.57), but not in area B or for cancer at other sites in either area. Reanalysis by Sébastien of fibers from his earlier study (81) also confirmed that there was no important difference in their dimensions or composition between the two areas.

None of these findings would necessarily have incriminated tremolite, as opposed to some other mineral with similar geographic distribution, in the absence of independent evidence of the carcinogenicity of fibrous tremolite. The strongest indication of this has been the experience of vermiculite miners and millers in Libby, Montana, exposed to contaminating amphibole fibers in the tremolite series, but to no other form of asbestos. In the early 1980s, parallel but independent studies of mortality and morbidity among the employees of the Libby plant were undertaken by us and the National Institute for Occupational Safety and Health (NIOSH). The results obtained by the two groups provided very similar evidence of high excess mortality from nonmalignant respiratory disease, lung cancer, and mesothelioma (88),

Table 17.5. Mortality in Libby cohort of vermiculite miners exposed to fibrous tremolite (n = 406) (reference: US white males) (90)

		Deaths to July 1983		Deaths since July 1983[a]		Total	
	ICD-9	Observed	SMR	Observed	SMR	Observed	SMR
Respiratory cancers	160–165	23	2.45	21	2.35	44	2.40
All other cancers	140–159, 165–208, 230–239	20	1.09	19	1.29	39	1.18
NMRD	010–018, 460–519	21	2.55	30	3.63	51	3.09
Circulatory disease	390–459	65	0.87	39	1.11	104	0.95
External	800–998	23	1.87	3	1.03	26	1.71
All causes		165	1.17	120	1.43	285	1.27
(incl. mesothelioma)		4	(PMR = 2.4%)	8	(PMR = 6.7%)	12	(PMR = 4.2%)

ICD, International Classification of Diseases; NMRD, nonmalignant respiratory disease.
[a] To January 1, 1999.

and an increased prevalence of small radiographic opacities of between 6% and 10% per 100 F/mL years (89).

As these findings on mortality were based on very small cohorts, a further follow-up to the end of 1998 has recently been completed, allowing a more reliable assessment of risk in relation to estimated exposure. Total deaths to the end of 1998 were lung cancer 44 (SMR 2.40), NMRD 51 (SMR 3.09), all causes 285 (SMR 1.27); included among the total were 12 deaths attributed to mesothelioma (PMR 4.21%) (90) (Table 17.5). Adjusted linear relative risks (per 100 F/mL.y), estimated by Poisson regression, were lung cancer (0.36, 95% CI 0.03–1.20), NMRD (0.38, 95% CI 0.12–0.96), and all causes (0.14, 95% CI 0.05–0.26). The 12 deaths from mesothelioma, though with a typical latency range of 22 to 47 years (median 35.5 years) showed only a limited relationship to estimated exposure. The all-cause linear model would imply a 14% increase in mortality for mine workers exposed occupationally to 100 F/mL.y or 3.2% for a general population exposed for 50 years to an ambient concentration of 0.1 F/mL (90).

Synthesis

Over 40 years have passed since 1960, when 33 cases of pleural mesothelioma were described by Wagner et al (5), almost all from the crocidolite mining region in the northwest Cape Province of South Africa. In the same year, 10 cases of peritoneal mesothelioma were reported by Keal (91) among textile employees of the Cape Asbestos Company in London, exposed to crocidolite from the same source. Recognizing therefore the importance of asbestos fiber type, the UICC Expert Group in 1964 had put priority on epidemiologic studies of miners and millers engaged in the production of the three main types of asbestos rather than on employees in manufacture and industrial application, usually entailing chrysotile-amphibole mixtures. During the next 20 years, unfortunately, most research focused on the latter

and, until the late 1980s, the only production workers studied were miners and millers in Quebec and Italy, both with similar and reassuring results for chrysotile. The first amphibole mine workers for whom there were comparable data were, in fact, the Libby vermiculite employees, exposed incidentally to contamination by fibrous tremolite (26,90). Only later were mortality data published in 1988 on crocidolite miners in Australia (27), and in 1992 on crocidolite and amosite miners in South Africa (28), but in the meantime a considerable number of cohort study results based on workers in the manufacturing industries or in asbestos product use were published. Exposure in all of these cohorts was mainly to chrysotile, but in most of them also to varying proportions of crocidolite or amosite.

Investigators familiar with the disastrous experience of insulation workers in North America, where exposure had also been to chrysotile, and possibly amosite, found it difficult to believe that all types of asbestos were not equally harmful. This view was supported by experimental evidence, which showed that all fiber types were equally carcinogenic, without appreciating that biopersistence and durability would be far more important in humans, with a much longer life span, than in laboratory animals. Against a background of much suspicion and recrimination, the results of the several important cohort studies published in the 1980s failed to have much effect on entrenched and conflicting views. For those who saw chrysotile as a mineral fiber of low carcinogenicity, the findings summarized in Table 17.2 confirmed this opinion. Others, with legitimate concern for control rather than scientific niceties, found little difficulty in maintaining their disbelief. Uncertainties associated with mixed exposures, lack of information on exposure intensity, and statistical chance were often cited; other reasons were less flattering (3).

Some resolution of this unpleasant and unhelpful controversy came with the use of lung tissue analyses in epidemiologic research. Despite difficulties in interpretation of results and the absolute need for properly selected controls (92), these studies demonstrated two things and revealed a third. First, was the clear evidence of an overwhelming predominance, with dose-response, of amphibole fibers in mesothelioma cases; second, that amphibole fibers persist in lung tissue, whereas chrysotile does not. The short life span of laboratory animals could not deal adequately with tumors in humans of long latency. Third, it has only been by analyzing lung tissue that the varying presence of fibrous tremolite has been demonstrated in commercial chrysotile.

Although the recent update on mortality in the Libby vermiculite cohort has indicated that the ability of fibrous tremolite to cause mesothelioma is on a par with crocidolite, it remains almost impossible to estimate the contribution it makes to the carcinogenicity of commercial chrysotile, which greatly varies in level of tremolite content, both geographically and in time. There are two main reasons for this. First is our ignorance of how best to assess exposure to a carcinogenic agent that is biopersistent. Cumulative exposure clearly underestimates the potential effect of a retained carcinogen. The expo-

sure index that appeared to do best in the Libby cohort was one in which the estimated fiber concentration at any year was weighted by residence time. Although conceptually reasonable, such an index was largely determined by estimated airborne fiber concentrations 30 to 50 years before death, for which only the most crude approximations can be guessed in this or any other mortality study. With only 12 mesothelioma deaths in a cohort of 406 men, a statistically significant discrimination between risk and any type of exposure index was not possible. Second, there is the analogous problem that results from a total ignorance of the tremolite concentrations to which Quebec miners and millers were exposed, as far back as 1918, when men who later developed mesothelioma were first employed. These cases were miners rather than millers, and in the relevant period there were almost 30 different mining companies, in few of which were any dust measurements made. Exposure to tremolite would certainly have been intermittent, as evidenced by the fact that mesothelioma risk in the Quebec cohort was related to duration of employment but not to intensity of dust exposure.

Thus, to obtain any kind of answer to the question, we must take account of other types of evidence. For example, there was no case of mesothelioma in the Quebec cohort in men employed less than 2 years, and in the 21 of 38 men with mesothelioma whose lungs were examined at autopsy, amphibole fibers—mostly tremolite and in high concentration—were present in them all (20). In the eight studies of lung fiber burden in mesothelioma cases and controls (Table 17.3), little or no evidence of risk was observed with chrysotile only; indeed, amphibole fibers were present in most cases. Finally, the recent case-referent study in the United Kingdom of young adults with mesothelioma showed that crocidolite and amosite, singly or additively, could account for about 80% of cases, and tremolite for about 7%, leaving very few for chrysotile alone.

Compared with amphibole fibers, pure chrysotile is removed much more rapidly from human tissue, but it is not without some biopersistence. It would be unreasonable, therefore, to conclude that when inhaled in sufficient quantity it carries no mesothelioma risk. It should be remembered, nevertheless, that the epidemiologic evidence reviewed in this chapter reflects exposure levels some 40 or more years ago, orders of magnitude higher than those that prevail today or that should be readily achievable. Unfortunately, past failure to discriminate between the carcinogenicity of chrysotile and the amphiboles allowed the latter to be inadequately controlled too long.

Conclusion

In the discussion session on mesothelioma that followed the presentations by Selikoff, Wagner, Newhouse, Elmes, and others at the New York Conference in 1964, Scheepers (93), a principal discussant, raised two prophetic questions that it has taken 40 years to answer. First, with regard to 11 cases of lung cancer with which he was familiar and whose

predominant exposure had been to chrysotile, he questioned the logic of attributing them to chrysotile when all had "at one time or another also been exposed to other forms of asbestos, mainly amosite or crocidolite." His next paragraph then began with the words "What about tremolite?"! This chapter has been devoted almost entirely to these two questions. As far as mesothelioma is concerned, the number of cases in which exposure has been to commercial chrysotile only, let alone to pure chrysotile, is few; almost all were also exposed to crocidolite, amosite, or chrysotile-amphibole mixtures. The potential importance of amphibole fibers in the tremolite series is only now being appreciated. Its carcinogenicity appears similar to that of crocidolite and, either as a frequent contaminant of chrysotile or as a general environmental pollutant in certain localities, its effects, though yet to be fully assessed, could be very large.

References

1. Selikoff IJ, Churg J. Co-chairmen, Conference on the Biological Effects of Asbestos. Ann NY Acad Sci 1965;132:1–766.
2. Dreessen WC, Dallavalle JM, Edwards TI, Miller JW, Sayers RR. A study of asbestosis in the asbestos textile industry. Public Health Bulletin No. 241. Washington, DC: United States Government Printing Office, 1938.
3. McDonald JC, McDonald AD. The epidemiology of mesothelioma in historical context. Eur Respir J 1996;9:1932–1942.
4. Doll R. Mortality from lung cancer in asbestos workers. Br J Ind Med 1955; 12:81–86.
5. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960; 17:260–271.
6. Saccone A, Coblenz A. Endothelioma of the pleura. Am J Clin Pathol 1943; 13:188–207.
7. Hammond E, Selikoff IJ, Churg J. Neoplasia among insulation workers in the United States with special reference to intra-abdominal neoplasia. Ann NY Acad Sci 1965;132:519–525.
8. Working Group on Asbestos and Cancer. Report and recommendations of the Working Group convened under the auspices of the Geographical Pathology Committee of the International Union Against Cancer. McDonald JC, Working Group Member. Arch Environ Health 1965;11:221–229.
9. McDonald JC. Research on asbestos and health: a progress report to employers and employees of the Quebec Asbestos Mining Industry. McGill Reporter 1970;2:18–19.
10. Elmes PC, McCaughey WTE, Wade OL. Diffuse mesothelioma of the pleura and asbestos. Br Med J 1965;1:350–353.
11. Newhouse ML, Thompson H. Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. Br J Ind Med 1965;22: 261–269.
12. McEwen J, Finlayson A, Mair A, Gibson AAM. Mesothelioma in Scotland. Br Med J 1970;4:575–578.
13. McDonald AD, Harper A, El Attar OA, McDonald JC. Epidemiology of primary malignant mesothelial tumours in Canada. Cancer 1970;26:914–919.

14. Rubino GF, Scansetti G, Donna A, Palestro G. Epidemiology of pleural mesothelioma in north-western Italy (Piedmont). Br J Ind Med 1972;29: 436.
15. Ashcroft T. Epidemiological and quantitative relationships between mesothelioma and asbestos on Tyneside. J Clin Pathol 1973;26:832–840.
16. Hain E, Dalquen P, Bohlig H, Dabbert A, Hinz I. Retrospective study of 150 cases of mesothelioma in Hamburg area. Int Arch Arbeitsmed 1974;33:15–37.
17. Zielhuis RL, Versteeg JPJ, Planteydt HT. Pleural mesothelioma and exposure to asbestos. A retrospective case-control study in the Netherlands. Int Arch Occup Environ Health 1975;36:1–18.
18. McDonald AD, McDonald JC. Malignant mesothelioma in North America. Cancer 1980;46:1650–1656.
19. McDonald JC, McDonald AD. Epidemiology of mesothelioma from estimated incidence. Prev Med 1977;6:426–446.
20. McDonald AD, Case BW, Churg A, et al. Mesothelioma in Quebec chrysotile miners and millers: epidemiology and etiology. Ann Occup Hyg 1997;41:707–719.
21. McDonald JC. Asbestos. In: McDonald JC, ed. Epidemiology of Work Related Diseases, 2nd ed. London: BMJ Books, 2000.
22. Piolatto G, Negri E, La Vecchia C, Pira E, Decarli A, Peto J. An update of cancer mortality among chrysotile asbestos miners in Balangero, Northern Italy. Br J Ind Med 1990;47:810–814.
23. Liddell FDK, McDonald AD, McDonald JC. The 1891–1920 birth cohort of Quebec chrysotile miners and millers: development from 1904 and mortality to 1992. Ann Occup Hyg 1997;41:13–36.
24. Meurman LO, Kiviluoto R, Hakama M. Mortality and morbidity among the working population of anthophyllite asbestos miners in Finland. Br J Ind Med 1974;31:105–112.
25. Brown DP, Dement JM, John M, Wagoner JK. Mortality patterns among miners and millers occupationally exposed to asbestiform talc. In: Lemen R, Dement JM, eds. Dusts and Disease (Occupational and Environmental Exposures to Selected Fibrous and Particulate Dusts). Park forest South, Illinois: Pathotox Publishers, 1979:317–324.
26. McDonald JC, McDonald AD, Armstrong B, Sébastien P. Cohort study of mortality of vermiculite miners exposed to tremolite. Br J Ind Med 1986; 43:436–444.
27. Armstrong BK, De Klerk NH, Musk AM, Hobbs MST. Mortality in miners and millers of crocidolite in Western Australia. Br J Ind Med 1988;45:5–13.
28. Sluis-Cremer GK, Liddell FDK, Logan WPD, Bezuidenhout BN. The mortality of amphibole miners in South Africa, 1946–80. Br J Ind Med 1992;49: 566–575.
29. Weiss W. Mortality of cohort exposed to chrysotile asbestos. J Occup Med 1977;19:737–740.
30. Thomas HF, Benjamin IT, Elwood PC, Sweetnam PM. Further follow-up study of workers from an asbestos cement factory. Br J Ind Med 1982; 39:273–276.
31. Ohlson C-G, Hogstedt C. Lung cancer among asbestos cement workers. A Swedish cohort study and a review. Br J Ind Med 1985;42:397–402.
32. Gardner MJ, Winter PD, Pannett B, Powell CA. Follow-up study of workers manufacturing chrysotile asbestos cement products. Br J Ind Med 1986; 43:726–732.
33. Hughes JM, Weill H, Hammad YY. Mortality of workers employed in two asbestos cement manufacturing plants. Br J Ind Med 1987;44:161–174.

34. Finkelstein M. Mortality among employees of an Ontario asbestos-cement factory. Amer Rev Respir Dis 1984;129:754–761.
35. Alies-Patin AM, Valleron AJ. Mortality of workers in a French asbestos cement factory 1940–1982. Br J Ind Med 1985;42:219–225.
36. Magnani C, Terracini G, Bertolone GP, et al. Mortalita per tumori e altre malattie del sistema respiratorio tra i lavoratori del cemento-amianto a casale Monferrato. Una studia di coorte storico. Med Lav 1987;6:441–453.
37. Raffn E, Lynge E, Juel K, Korsgaard B. Incidence of cancer and mortality among employees in the asbestos cement industry in Denmark. Br J Ind Med 1989;46:90–96.
38. Albin M, Jakobsson K, Attewell R, Johansson L, Welinder H. Mortality and cancer morbidity in cohorts of asbestos cement workers and referents. Br J Ind Med 1990;47:602–610.
39. Neuberger M, Kundi M. Individual asbestos exposure: smoking and mortality—a cohort study in the asbestos cement industry. Br J Ind Med 1990; 47:615–620.
40. McDonald AD, Fry JS, Woolley AJ, McDonald JC. Dust exposure in an American chrysotile textile plant. Br J Ind Med 1983;40:361–367.
41. Dement JM, Brown DP, Okun A. Follow-up study of chrysotile textile workers: cohort mortality and case-control analyses. Am J Ind Med 1994;26: 431–447.
42. McDonald AD, Fry JS, Woolley AJ, McDonald JC. Dust exposure and mortality in an American factory using chrysotile, amosite, and crocidolite in mainly textile manufacture. Br J Ind Med 1983;40:368–374.
43. Peto J, Doll R, Hermon C, Binns W, Clayton R, Goffe T. Relationship of mortality to measures of environmental asbestos pollution in an asbestos textile factory. Ann Occup Hyg 1985;29:305–355.
44. McDonald AD, Fry JS, Woolley AJ, McDonald JC. Dust exposure and mortality in an American chrysotile asbestos friction products plant. Br J Ind Med 1984;41:151–157.
45. Newhouse ML, Sullivan KR. A mortality study of workers manufacturing friction materials: 1941–86. Br J Ind Med 1989;46:176–179.
46. Seidman H, Selikoff IJ, Hammond EC. Short-term asbestos work exposure and long-term observation. Ann NY Acad Sci 1979;330:61–89.
47. Acheson ED, Gardner MJ, Winter PD, Bennett C. Cancer in a factory using amosite asbestos. Int J Epidemiol 1984;13:3–10.
48. Levin JL, McLarty JW, Hurst GA, Smith AN, Frank AL. Tyler asbestos workers: mortality exposure in a cohort exposed to amosite. Occup Environ Med 1998;55:155–160.
49. McDonald AD, McDonald JC. Mesothelioma after crocidolite exposure during gas mask manufacture. Environ Res 1978;17:340–346.
50. Jones JSP, Smith PG, Pooley FD, et al. The consequences of exposure to asbestos dust in a wartime gas-mask factory. In: Wagner JC, ed. Biological Effects of Mineral Fibres 2. IARC Scientific Publications No 30. Lyon, France: IARC, 1980:637–653.
51. Talcott JA, Thurber RN, Kantor AF, et al. Asbestos-associated diseases in a cohort of cigarette-filter workers. N Engl J Med 1989;321:1221–1223.
52. Acheson ED, Gardner MJ, Pippard EC, Grime LP. Mortality of two groups of women who manufactured gas masks from chrysotile and crocidolite asbestos: a 40-year follow-up. Br J Ind Med 1982;39:344–348.
53. Newhouse ML, Berry G, Wagner JC. Mortality of factory workers in East London 1933–80. Br J Ind Med 1985;42:4–11.

54. Enterline PE, Hartley J, Henderson V. Asbestos and cancer: a cohort followed up to death. Br J Ind Med 1987;44:396–401.
55. Elmes PC, Simpson MJC. Insulation workers in Belfast (1940–1975). A further study of mortality due to asbestos exposure. Br J Ind Med 1977; 34:174–180.
56. Selikoff IJ, Hammond EC, Seidman H. Mortality experience of insulation workers in the United States and Canada 1943–1976. Ann NY Acad Sci 1979;330:91–116.
57. Järvholm B, Sandén Å. Lung cancer and mesothelioma in the pleura and peritoneum among Swedish insulation workers. Occup Environ Med 1998; 55:766–770.
58. Rossiter CE, Coles RM. HM Dockyard, Devonport: 1947 mortality study. In: Wagner JC, ed. Biological Effects of Mineral Fibres 2. IARC Scientific Publications No. 30. Lyon, France: IARC, 1980:713–721.
59. Kolonel LN, Yoshizawa CN, Hirohata T, Myers BC. Cancer occurrence in shipyard workers exposed to asbestos in Hawaii. Cancer Res 1985;45: 3924–3928.
60. McDonald JC. Unfinished business: the asbestos textiles mystery. Ann Occup Hyg 1998;42:3–5.
61. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol 42. Silica and Some Silicates. Lyon, France: IARC, 1987:225–239.
62. Das PB, Fletcher AG, Deodhare SG. Mesothelioma in an agricultural community of India: a clinicopathological study. Aust NZ J Surg 1976;46:218–226.
63. Rothschild H, Mulvey JJ. An increased risk for lung cancer mortality associated with sugar cane farming. J Natl Cancer Inst 1982;68:755–760.
64. Fraire AE, Cooper S, Greenberg SD, Buffler P, Langston C. Mesothelioma in childhood. Cancer 1988;62:838–847.
65. McDonald JC, McDonald AD. Mesothelioma: is there a background? Eur Respir Rev 1993;3(11):71–73.
66. Jones JSP, Roberts GH, Pooley FD, et al. The pathology and mineral content of lungs in cases of mesothelioma in the United Kingdom in 1976. In: Wagner JC, ed. Biological Effects of Mineral Fibers. Proceedings of a Symposium held at Lyons, 25–27 September, 1979, vol 1. IARC Scientific Publications No. 30. Lyon, France: IARC, 1980:187–199.
67. McDonald AD, McDonald JC, Pooley FD. Mineral fiber content of lung in mesothelial tumours in North America. Ann Occup Hyg 1982;26:417–422.
68. Mowe G, Gylseth B, Hartveit F, Skaug V. Fibre concentration in lung tissue of patients with malignant mesothelioma. A case-control study. Cancer 1985;56:1089–1093.
69. Gaudichet A, Janson X, Monchaux G, et al. Assessment by analytical microscopy of the total lung fiber burden in mesothelioma patients matched with four other pathological series. Ann Occup Hyg 1988;32(suppl 1):213–223.
70. McDonald JC, Armstrong B, Case BW, et al. Mesothelioma and asbestos fiber type: evidence from lung tissue analyses. Cancer 1989;63:1544–1547.
71. Rogers AJ, Leigh J, Berry G, Ferguson DA, Mulder HB, Ackad M. Relationship between lung asbestos fiber type and concentration and relative risk of mesothelioma—a case-control study. Cancer 1991;67:1912–1920.
72. Rödelsperger K, Woitowitz H-J, Brückel B, Arhelger R, Pohlabeln H, Jöckel K-H. Dose-response relationship between amphibole fiber lung burden and mesothelioma. Cancer Detect Prev 1999;23(3):183–193.

73. McDonald JC, Armstrong BG, Edwards CW, et al. Case-referent survey of young adults with mesothelioma: I. Lung fiber analyses. Ann Occup Hyg 2001;45(7):513–518.
74. McDonald JC, Edwards CW, Gibbs AR, et al. Case-referent survey of young adults with mesothelioma: II. Occupational analyses. Ann Occup Hyg 2001;45(7):519–523.
75. McDonald JC, Becklake MR, Gibbs GW, McDonald AD, Rossiter CE. The health of chrysotile mine and mill workers of Quebec. Arch Environ Health 1974;28:61–68.
76. McDonald JC, McDonald AD, Gibbs GW, Siemiatycki J, Rossiter CE. Mortality in the chrysotile asbestos mines and mills of Quebec. Arch Environ Health 1971;22:677–686.
77. Gibbs GW. Etiology of pleural calcification: a study of Quebec asbestos miners and millers. Arch Environ Health 1979;34:76–82.
78. Pooley FD. An examination of the fibrous mineral content of asbestos in lung tissue from the Canadian chrysotile mining industry. Environ Res 1976;12:281–298.
79. Rowlands N, Gibbs GW, McDonald AD. Asbestos fibers in the lungs of chrysotile miners and millers—a preliminary report. Ann Occup Hyg 1982; 26:411–415.
80. McDonald JC. Epidemiological significance of mineral fiber persistence in human lung tissue. Environ Health Perspect 1994;102(suppl 5):221–224.
81. Sébastien P, McDonald JC, McDonald AD, Case B, Harley R. Respiratory cancer in chrysotile textile and mining industries: exposure inferences from lung analysis. Br J Ind Med 1989;46:180–187.
82. Sébastien P, Plourde M, Robb R, Ross M, Nadon B, Wypruk T. Ambient air asbestos survey in Quebec mining towns: Part 2. Main study. Report EPS 5/AP/RQ-2E. 1986.
83. McDonald JC, McDonald AD. Epidemiology of mesothelioma. In: Liddell FDK, Miller K, eds. Mineral Fibers and Health. Boca Raton, FL: CRC Press, 1991:143–164.
84. Sébastien P, Bégin R, Case BW, McDonald JC. Inhalation of chrysotile dust. In: Wagner JC, ed. The Biological Effects of Chrysotile. Philadelphia: JB Lippincott, 1987:19–29.
85. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of the inhalation of asbestos in rats. Br J Cancer 1974;29:252–269.
86. Riordon PH. The Asbestos belt of South Eastern Quebec: the asbestos deposits of Thetford Mines, Quebec: the British Canadian Mine: Normandie and Vimy Ridge Mines. In: The Geology of Canadian Industrial Mineral Deposits: 6th Commonwealth Mining and Metallurgical Congress. 1957:1–30.
87. McDonald JC, McDonald AD. Chrysotile, tremolite and carcinogenicity. Ann Occup Hyg 1997;41:699–705.
88. Amandus HE, Armstrong BG, McDonald AD, McDonald JC, Sébastien P, Wheeler R. Mortality of vermiculite miners exposed to tremolite. Ann Occup Hyg 1988;32:459–465.
89. Armstrong BG, McDonald JC, Sébastien P, Althouse R, Amandus HE, Wheeler R. Radiological changes in vermiculite workers exposed to tremolite. Ann Occup Hyg 1988;32:469–473.
90. McDonald JC, Harris J, Armstrong B. Mortality in a cohort of vermiculite miners exposed to fibrous amphibole in Libby, Montana. Occ Environ Med 2004;61(4):363–366.

91. Keal EL. Asbestosis and abdominal neoplasms. Lancet 1960;2:1211–1216.
92. McDonald JC. Tremolite, other amphiboles and mesothelioma. Am J Ind Med 1988;14:247–249.
93. Scheepers GWH. Section VIII, Discussant, Conference on the Biological Effects of Asbestos. Ann NY Acad Sci 1965;132:596.

18

From Monkey to Man: The Epidemiologic Evidence of an Association Between Simian Virus 40 and Malignancy

Susan Gross Fisher

Early in the 20th century endemic poliomyelitis gradually evolved into the most devastating epidemic in the Western world. Striking improvements in public health, ironically, were accompanied by more frequent outbreaks of crippling poliomyelitis. By 1950, each new day brought more polio victims, an increasing sense of crisis, and a greater need for an effective therapy. In the United States mass vaccination for paralytic polio began in 1955 with licensing of the Salk inactivated vaccine. It is estimated that by 1960 90% of all persons under 20 years of age had received at least one inoculation; a total of 98 million Americans had been immunized (1). A sharp decline in disease incidence occurred and the spread of this crippling infection was abated.

Amid this chronicle of success was the knowledge that numerous, presumed harmless viruses had been recovered from the primary monkey kidney cell cultures used for the efficient growth of polio virus needed for mass vaccine production. However, in 1961 Eddy and colleagues (2) conducted the first investigations demonstrating the development of tumors in 71% of newborn hamsters injected with polio vaccine culture extracts. The tumors were identified as mesotheliomas, ependymomas, osteogenic sarcomas, and lymphomas. Multiple other investigators confirmed these initial findings (3–6). The oncogenic property of the cell extract was later attributed to a double-stranded DNA virus designated simian virus 40 (SV40), an indigenous pathogen in the African green monkey (7). Diamandopoulos (3) provided compelling evidence of the role of the virus by demonstrating that animals inoculated with anti-SV40 serum demonstrated no tumor growth. Important independent studies by Koprowski et al (8) showed that, in fact, cultured human cells underwent transformation with SV40, raising concerns about the possible consequences of human exposure to this virus.

Simian virus 40 and the closely related human polyomaviruses BK and Jamestown Canyon (JC) produce subclinical infection in immuno-

competent natural hosts. The viruses typically reside in renal epithelial cells, but can spread to other tissues and produce pathologic effects in either immunocompromised hosts or, more importantly, in nonhost species (9). Large-scale vaccine production in the United States necessitated holding large numbers of caged juvenile monkeys for tissue access, amplifying the probability of transmission of SV40 from infected to nonimmune animals. The practice of pooling kidney tissue from multiple animals during vaccine production increased the likelihood of viral contamination of vaccine cultures. It is now well accepted that *at least* 30% and perhaps as much as 70% of inactivated live vaccine produced between 1955 and 1961 was contaminated with SV40 (10). Although the U.S. government established SV40-free vaccine manufacturing requirements in 1961, contaminated vaccine continued to be distributed through 1963.

Epidemiologic Investigations

Early United States Studies

No immediate, unexpected short-term consequences to polio vaccination were reported in a series of field trials involving live poliovirus. Specifically, newborn and infant postvaccination studies were unremarkable, and there were no reports of adverse fetal outcomes following immunization of pregnant women (11). It appeared that exposure to SV40 might be innocuous to humans; however, the tumorigenic effect of the virus in animal models was worrisome. Concerns escalated with additional clinical studies in humans that demonstrated the ability of the virus to replicate, generate subclinical infection, and spread through oral and respiratory routes, suggesting that transmission from polio vaccines to innumerable nonvaccinated, nonimmune human contacts may be possible (12,13). Increasing questions spurred some early epidemiologic investigations to more carefully examine this potential threat to public health.

In 1963 Fraumeni and colleagues (14) conducted a study to identify changes in cancer mortality within 4 years of the initiation of the mass-immunization program. Annual mortality rates among persons less than 25 years of age from 1950 to 1959 were examined. Using published data from the Office of Vital Statistics, the trends over the decade in mortality due to leukemia, selected sites of cancer (brain, kidney, and connective tissue) and all cancers combined were examined. Only minor fluctuations were detected in the age-specific mortality rates from all cancers combined among persons less than 25 years old from 1955 to 1959; however, the leukemia mortality rates increased from 3.5 to 3.8 per 100,000 children ages 5 to 9, and from 2.2 to 2.5 per 100,000 in children ages 10 to 14. No significant changes in mortality for brain, kidney, or connective tissue tumors were noted. Given the very short latency period since vaccination (<5 years), it was unlikely that differences in patterns of mortality would occur.

In a second analysis these investigators included only children who were 6 to 8 years old in 1955, since it was this birth cohort that was eli-

gible for vaccination at the start of the program. In May and June of that year only a small number of lots of vaccine were distributed to each state, and specific records regarding the distribution and contamination of those lots were available. Accessing that data, Fraumeni et al classified the states according to three levels of estimated per capita dose of contaminated vaccine. A cohort analysis was conducted to compare cancer-specific mortality over the 10-year period. Cause-specific mortality data were available from death certificates filed with National Vital Statistics Division of the U.S. Public Health Service (USPHS); the population at risk was defined from age- and state-specific census data available for 1950 and 1960. A comparison of cancer mortality rates among states grouped according to the distribution of SV40 contaminated vaccine showed no change in patterns. Rates of mortality were, in fact, higher among states receiving contaminated vaccine as compared to those not receiving contaminated vaccine; however, this increase was noted before as well as after the introduction of the vaccination program. The investigators concluded that there was no evidence to suggest an increase in cancer mortality related to SV40-contaminated vaccine distribution. In addition, the authors reported that an increase in leukemia rates among 6- to 8-year-olds receiving vaccine free of SV40 in 1956 occurred, suggesting that the reported increase in mortality rate due to leukemia among those less than 25 years of age was independent of contaminated vaccine distribution. To check for the presence of another leukemia-associated agent in the vaccine, vaccine samples were sent to the Laboratory of Viral Oncology, National Cancer Institute, and used for inoculation of mice. After 10 months of observation, no laboratory animals displayed any problems. The authors admit that their negative results are not surprising. They point out that the SV40 exposure in previous animal studies was of a far higher viral titer than that related to the vaccine; that the laboratory animals were exposed as newborns, whereas in the initial vaccination program only school-age children were exposed; and that there was very short follow-up in this study. The investigators emphasized the desirability of long-term studies with attention to those vaccinated in infancy. Continued surveillance was recommended. In addition, it is important to note that this was an ecologic study, i.e., no person-specific observations were included in the analysis, thus no information is available regarding the vaccination status of children who actually died of cancer.

In an attempt to more specifically examine the risk of cancer among individuals known to have received contaminated vaccine as newborns, Fraumeni et al (15) conducted an analysis of 1077 newborns who were vaccinated between 1960 and 1962 at a single medical facility for the purposes of assessing the induction of active immunity to polio with the Sabin vaccine in the presence of maternal antibodies. Within a few days of birth 925 infants in five treatment groups received attenuated oral polio vaccine; the sixth group was administered intramuscular injections of inactivated poliovirus vaccine. Independent of the study purpose, the vaccines administered to each group had differing titers of SV40. Later in infancy, booster injections were given to all chil-

dren, which presumably also contained SV40. Beginning in 1964 Fraumeni et al attempted to follow up this mostly black, highly mobile 1960 birth cohort. With 86% follow-up at 8 years, the investigators documented 11 deaths. This mortality rate was similar to that expected in this population, and no difference in risk of death among vaccine groups was observed. Of note, no deaths due to cancer were recorded. The authors concluded that there was no effect on mortality noted among newborns ingesting SV40 at a dose, which is carcinogenic in hamsters when administered parenterally. This study used the Sabin vaccine, whereas reported tumor growth in animals had been observed with administration of the Salk vaccine. Follow-up was limited to 8 years, although surveillance of the study sample was to be maintained.

Mortimer and coworkers (16) followed the original Fraumeni cohort of 1073 children born in the United States between 1960 and 1962 who received either oral or inactivated vaccine within 3 days of birth. In 1977 to 1979 15 children had died, but no deaths were due to cancer; one cancer death was expected. One girl developed a salivary tumor of "low degree of malignancy." No recurrence occurred after surgical excision. With 87% follow-up for 19 years and the occurrence of only one cancer, the study concluded no carcinogenic effect of SV40 in humans. Given the difficulties of continuing follow-up, termination of surveillance in this cohort was planned. However, due to the identification of SV40 DNA fragments in some tumors from other studies, this cohort follow-up was reactivated. An update of mortality with more than 35 years of cohort follow-up was reported in 2001 by Carroll-Pankhurst et al (17). Forty-four deaths were identified and for 41 of these, death certificates were obtained. Four of the deaths were due to cancer, two due to testicular tumors, and two to leukemia. All four of the deceased subjects had received live, attenuated vaccine as newborns. The investigators noted that the increase in testicular cancer [relative risk (RR) = 37.9; $p = .002$] was particularly interesting since SV40 antigens have been previously detected in seminal fluid (18). The occurrence of death due to leukemia resulted in an RR of 2.62 ($p = .16$); it is notable that hematologic malignancies developed in some of the original laboratory animals injected with SV40. None of the other suspect cancers, i.e., brain, osteogenic sarcoma, or mesotheliomas, were documented to have occurred in this cohort. Given that death rates in this cohort were similar to those expected in similar age, sex, and ethnic groups, there was no suggestion that mortality was underreported. While the follow-up of these subjects exceeded 35 years' duration, the number of expected malignancies for this small cohort was only 3.16, resulting in inadequate power. In fact, the results of this study demonstrate that a threefold increase in cancer risk among persons exposed to SV40-contaminated vaccine as newborns is *not* incompatible with the reported findings of this study.

In a review by Shah and Nathanson (19) published in 1976, the authors add to the evidence related to the carcinogenic effect of SV40 by summarizing an unpublished presentation by Hammond (20) in 1966, who reported on 700,000 subjects, ages 32 to 91, who participated in an American Cancer Society study in which they were questioned

about their history of polio vaccination. In the next 2.5 years 24,000 of these individuals were documented as dead (1962–64). About 25% of the group reported having received one or more doses of vaccine, and these subjects were compared to those who reportedly had received no vaccine. No difference in incidence or type of cancer was detected. Shah and Nathanson question the sensitivity of this investigation given the age of the sample. However, they conclude, "The large numbers do provide a fair degree of assurance that SV40 injections into adults produces no major untoward effect within 5–10 years." Related to the question of cancer risk associated with administration of SV40-contaminated vaccine, it would be unlikely to expect that a carcinogenic effect would be clinically apparent within such a short follow-up. In addition, the rate of polio vaccination among older persons, i.e., those at greatest risk of the occurrence of cancer or death, was likely to be low. Therefore, the detection of a difference in these rates associated with vaccination would be unexpected.

Heinonen and colleagues (21) conducted a study to examine the risk of malignancy among offspring of women immunized against poliomyelitis and influenza or experiencing viral infections during pregnancy. These data were drawn from a prospective, collaborative study of etiologic factors in neurologic and sensory disorders in infancy and childhood. There were 58,807 eligible pregnancies included in the original study sample drawn from 12 U.S. hospitals from 1959 to 1965. After exclusion criteria for this study were considered, 50,897 pregnant women remained in the sample. During the prenatal period, monthly assessments of immunizations, medications, viral infections, and x-ray exposure were made. After delivery, infants were examined three times in the first year of life. Cancer incidence was available during the first year; only mortality and autopsy information was available in years 2 through 4. Prior to their fourth birthday, 24 children developed malignancy; eight were diagnosed with neural tumors, eight developed leukemia, six had renal tumors, one infant had a granulosa cell tumor, and one infant had a hepatoblastoma. In 14 of the 24 malignancies (seven of eight neural tumors), the mothers had received the polio vaccine (7.6 versus 3.1 per 10,000; $p < .05$), yielding an RR of 2.4. The difference in rates of neural tumors among infants whose mothers were vaccinated as compared to those whose mothers did not receive the polio vaccine during pregnancy was statistically significant ($p = .01$). Risk of cancer appeared to be higher when vaccination occurred in the early months of pregnancy. No malignancies were documented among the 3056 infants whose mothers received oral polio vaccine during pregnancy. Race, birth order, mean maternal age, frequency of maternal exposure to abdominal or pelvic radiation, and drug exposures were similar between the two groups. These data suggest that injections of killed polio vaccine in pregnant mothers were associated with malignancies and tumors of neural origin, in particular, in offspring born between 1959 and 1966. This study provides no information regarding vaccination of children after birth. The authors correctly emphasized that the reported findings may have occurred by chance or may have been due to confounding. While the authors concluded

that the present data "suggest" an association between killed polio vaccine in pregnant women and malignancies in their offspring, they almost dismiss the findings, stating that since no other evidence of increased childhood cancer mortality rates in the latter part of the 1950s has been revealed, it is "unlikely that killed polio vaccine had a discernible impact from a public health point of view on the risk of cancer in early childhood." The investigators are quick to point out that "polio immunization virtually eradicated a crippling and frequently lethal disease." This statement is correct but somewhat irrelevant to the scientific question being investigated in this and other similar studies.

International Studies

Although the U.S. immunization program had the greatest scope, many other countries had launched polio vaccination programs; several of these were initiated using U.S.-produced, i.e., contaminated, vaccine. Between 1958 and 1967, Innis (22) studied 816 Australian hospitalized children with malignancy and the same number of hospital controls matched for age and gender. Although the rates of other routine childhood immunizations were similar between the two groups, a significantly greater number of cases over 1 year of age ($n = 618$; 87.5%) than controls ($n = 569$; 80.6%) had been vaccinated for poliomyelitis, suggesting an association between malignancy and polio vaccine administration (odds ratio = 1.69, $p < .001$). The investigator raised questions regarding the comparability of the controls to the cases, given that there was a higher proportion of city dwellers among the controls (but this should bias in favor of the cases). The authors suggest continued surveillance based on these positive findings; however, no additional findings have appeared in the literature.

Olin and Giesecke (23) examined cancer incidence rates in Sweden where vaccination of preschool and school-age children began in 1957 using U.S.-produced vaccine. Approximately 70% of children born between 1946 and 1949 and 59% of those born between 1950 and 1953 were vaccinated with potentially contaminated vaccine. Age-adjusted incidence rates of cancer were reported for 5-year intervals from 1960 through 1990. Although age-standardized incidence rates among boys had increased for brain cancers, but not ependymomas, and mesotheliomas, no association with polio vaccination in children ages 4 to 11 was detected.

Using the National Cancer Registry of the German Democratic Republic, Geissler (24) conducted a large study with significantly longer follow-up than those previously described. The author compared cancer rates among the 885,783 children born between 1959 and 1961, 86% of whom received presumably contaminated Sabin live vaccine beginning in 1960, and 891,321 persons born between 1962 and 1964, most of whom were inoculated with vaccine free of SV40. With 22 years of follow-up, these researchers reported a cancer incidence of 28.7/10,000 among those receiving contaminated vaccine as compared to 30.1/10,000 among those receiving SV40-free vaccine. These inves-

tigators did report detection of SV40-like DNA in several astrocytomas and meningiomas, and the frequency of some intracranial tumors was greater in those exposed to contaminated vaccine. Several questions remain in the interpretation of these data, since for many analyses site-specific rates of malignancy are not presented, only frequencies of incident cancer events.

Recent U.S. Epidemiologic Investigations

With new molecular technologies, the early 1990s brought a growing body of laboratory studies reporting the detection of SV40 DNA in mesotheliomas as well as other types of tumors. Although not all studies supported the association between SV40 and cancer, mounting evidence stimulated additional epidemiologic investigations. With the passage of 40 years since the polio vaccination program was initiated in the United States, and the maturation of a population-based cancer registry in the United States, the time was ripe to reassess the available data using an epidemiologic approach.

The Surveillance, Epidemiology, and End Results (SEER) Program provides population-based, tumor-specific data on all histologically proven cancers occurring in selected geographic sites in the United States. The sample includes approximately 12% of the entire U.S. population and reflects the general characteristics of U.S. residents. All reportable diagnoses of invasive cancer occurring each year since 1973 among residents of the coverage area are included in this database. The consistency, scope, and quality of the SEER system provide an excellent tool for comparing cancer incidence in the United States from 1973. Overinclusion of some minorities and exclusion of many geographic areas, however, may affect type-specific cancer rates depending on the genetic, personal, and environmental risk factors specific to each type.

Strickler and colleagues (25) conducted an ecologic study to examine trends in cancer incidence and mortality related to distribution of the polio vaccine in the United States. These investigators accessed incidence data from SEER and the Connecticut Cancer Registry as well as U.S. mortality rates. Strickler et al compared age-specific incidence rates of ependymoma, osteogenic sarcoma, and mesothelioma in two birth cohorts likely to have received contaminated vaccine and an unexposed birth cohort. Persons born in 1947 through 1952 composed the cohort of persons likely to have been exposed to SV40-containing polio vaccine as children, while a second cohort born between 1956 and 1962 was considered likely to be exposed during infancy. The unexposed group was defined as persons born in 1964 through 1969. Poisson regression was employed to assess whether the age-specific incidence rates varied according to birth cohort. These investigators reported no statistically significant increase in cancer incidence rates among children likely to have received SV40-contaminated polio vaccine more than 35 years ago. There was also no association reported between brain cancer mortality and polio vaccination. The authors, therefore, concluded, “After millions of Americans were parenterally exposed as infants or children, the absence of a discernible effect in our

study adds to the evidence that no relation exists between exposure to SV40-contaminated vaccine and the development of cancer."

As shown in Table 18.1, the SEER database only completely captures tumors occurring during ages 26 to 41 years, 17 to 31 years, and 9 to 24 years in the childhood-exposed, infant-exposed, and unexposed cohorts, respectively, as defined in the study by Strickler et al (25). In fact, for the critical comparison of the childhood-exposed and unexposed cohorts, there is literally not one year of age in which both cohorts are completely represented in the SEER data. The accuracy of statistical conclusions drawn from mathematical models generated from data in which the age distribution of subjects within comparison groups is not overlapping is of serious concern, particularly when the three cancers of interest are highly correlated with age. The data upon which Strickler and colleagues' analysis is based provides inadequate power, and the statistical techniques employed may not represent an optimal assessment of risk in these populations. Hypothesis testing should be conducted only in situations in which the study design allows for comparisons of two comparable samples in which bias is minimized and power is adequate to appropriately answer the scientific question.

Fisher and colleagues (26) also examined trends in overall cancer incidence and the occurrence of the specific tumors linked to SV40 from 1973 to 1993 using the data from SEER. Increases in age-adjusted incidence rates across the 20 years were observed for all sites combined (11.5%) even after exclusion of breast and prostate cancers, which have increased in part due to significant increases in screening. The incidence also increased over time for ependymomas/choroids plexus tumors (25%), other brain tumors (23%), other bone tumors (22.9%), and mesotheliomas (90%). Rates of osteosarcoma over the 20 years remained relatively stable with an increase of only 2.4%. A multitude of host and environmental factors may account for these increases, as well as period-specific changes in cancer diagnosis, disease classification, and cancer reporting policies.

A more specific comparison of birth cohorts selected as likely to have received contaminated polio vaccine (1955–1959) and having very low probability of SV40 exposure by polio vaccination (1963–1967) was also conducted. These years were selected in order to maximize the similarities of age between the two cohorts while avoiding misclassification related to exposure to contaminated vaccine. As shown in Table 18.2, the only ages for which both cohorts are reflected in totality in SEER are ages 18 to 26 (shaded areas). Given that these tumors are less common and that the overlap of age-specific incidence rates in SEER for the two cohorts is limited to these age groups, statistical modeling was not considered to be an appropriate approach for analysis. Since the data reflect only a 9-year age span with similar distribution between cohorts, age adjustment was less of a concern. Table 18.3 provides the average annual incidence rates of specific cancers for 18- to 26-year-olds for each birth cohort. The cancer incidence rate in the exposed cohort is 11% lower than that in the unexposed cohort. This is likely to be due in part to improved reporting over time. In contrast, despite the

Table 18.1. Age of persons from specific birth cohorts by the Surveillance, Epidemiology, and End Results (SEER) program year as defined by Strickler et al (25)

Year of birth	SEER year																				
	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
1947	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
1948	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
1949	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
1950	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
1951	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1952	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
1953	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1954	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1955	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1956	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1957	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1958	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1959	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1960	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1961	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1962	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1963	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1964	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1965	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1966	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1967	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1968	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1969	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1970	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Table 18.2. Age of persons from specific birth cohorts by SEER year as defined by Fisher et al (26)

Year of birth	SEER year																				
	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
1955	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1956	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1957	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1958	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1959	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1960	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1961	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1962	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1963	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1964	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1965	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1966	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1967	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1968	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1969	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1970	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Table 18.3. Average annual cancer incidence rates by tumor type for birth cohorts, ages 18 to 26

	Birth cohorts					
	Exposed (1955–59) n = 2,013,344		Unexposed (1963–67) n = 1,872,998			
	Number of cases	Incidence rate per 100,000	Number of cases	Incidence rate per 100,000	Relative risk (CI) in exposed vs. unexposed	Adjusted RR,* exposed vs. unexposed
All cancer	5512	30.42	5764	34.1	0.89 (0.84, 0.95)	1.0
Ependymoma and choroid plexus	18	0.10	14	0.08	1.20 (0.71, 1.89)	1.37
Other brain cancers	328	1.81	333	1.98	0.92 (0.82, 1.03)	1.05
Osteogenic sarcoma	53	0.29	45	0.27	1.10 (0.81, 1.45)	1.26
Other bone cancers	89	0.49	71	0.42	1.17 (0.94, 1.45)	1.34
Mesothelioma	6	0.03	2	0.01	2.78 (1.02, 6.06)	3.20

* Adjusted increase in incidence was calculated using O − E/E where O equals the observed rate in the exposed cohort and E equals the expected rate in the unexposed group. The expected rate was calculated as 13% less than the rate in the unexposed group to adjust for the 13% lower age-adjusted cancer rate and cancer incidence in 20- to 24-year-olds in 1979 as compared to 1987.
CI, 95% confidence interval.
Source: Fisher et al (26).

small number of cases for some specific tumor types, the risk ratios reflect a 20% increase in the risk of ependymomas/choroids plexus tumors in the exposed cohort as compared to the unexposed cohort, while the risk of other brain tumors is higher among the unexposed group. The relative risks for osteogenic sarcoma and other bone malignancies are also increased in the exposed as compared to the unexposed cohort. As shown in Table 18.3, the 95% confidence intervals for each of these risk estimates cross 1.0, reflecting no statistically significant difference in risk between groups. Given the age of these cohorts any occurrence of mesothelioma would be unlikely; however, eight cases were documented in this analysis, six from the exposed cohort and two from the unexposed cohort, resulting in a relative risk that differed significantly from unity [RR = 2.78; 95% confidence interval (CI) = 1.02, 6.06].

Although the incidence rates of ependymoma/choroids plexus tumors, osteogenic sarcoma, other bone tumors, and mesotheliomas appear higher among the exposed group, the numbers of cases are very small, resulting in extremely limited power to detect a difference in rates if such a difference does exist. Also of importance, a persistent problem inherent in the use of birth cohorts for cancer epidemiologic studies is the period effect of improved reporting of cancer events, increased screening, and more sensitive diagnosis. In this study the period effect may artificially inflate the incidence rates in the unexposed cohort, thereby biasing the risk ratio toward unity. Table 18.3 presents an adjusted relative risk for each tumor type, which accounts for the 13% increase in overall cancer rates in this specific age group over the 20-year period. These adjusted rates suggest that the risk of ependymoma, osteogenic sarcoma, and mesothelioma in the cohort potentially exposed to contaminated vaccine may be increased as much as 37%, 26%, and 220%, respectively. Similarly, any confirmed increases in incidence within the exposed cohort may be due to innumerable factors other than SV40. Therefore, based on this analysis no definitive conclusions can be drawn. The descriptive data suggest that while the attributable risk of SV40 is not likely to be large, further investigation of this association is warranted. In particular, the increase in mesothelioma, although based on extremely small numbers, warrants careful investigation using methods that ensure adequate power to detect an association if, in fact, one truly exists.

Most recently, a report examining the trends in U.S. pleural mesothelioma incidence rates following SV40 contamination of early vaccines was released (27). The most well-established risk factor for mesothelioma is asbestos exposure; however, in 20% to 50% of cases of the disease asbestos exposure is not documented, particularly among females. This is a particularly interesting analysis because the authors attempt to carefully examine pleural mesothelioma incidence trends among adults in various age groups in relation to the probability of their exposure to potentially contaminated vaccine between 1955 and 1961. These investigators provide estimates of likely exposure by age group from data drawn from the national household sample surveys that were conducted annually by the Bureau of the Census. As part of

these surveys, participation in the nationwide inoculation program was monitored. Since survey data were not available for individuals older than 59 years, the rates for the 60- to 70-year age group were estimated based on the trend in lower age groups. These estimates of inoculation rates have not been previously published and provide a good framework for study of this difficult question. This study reports that the rates of mesothelioma have increased on average 3.25% (95% CI = 2.41, 4.09) per year from 1975 through 1997 in males and, similarly, an increase of 2.99% (95% CI = 1.92, 4.08) among females. The authors point out that mesothelioma overall is a rare cancer and that the public health impact of such increases is small. This increase, however, is disappointing given that asbestos exposure has clearly been known for many years to be a significant factor in the development of mesothelioma, and much has been done to decrease this exposure. It is possible that it is too early to see decreasing trends in this disease, but one would anticipate that rates should be decreasing in the very near future. Price (28) points out that there was significant growth in the use of asbestos in the 1930s, and peaked in 1950 where it remained until 1970 when it declined precipitously. Workers born after 1929 have experienced fewer years of exposure at peak asbestos consumption levels. In addition, for those born after 1929, the Occupational Safety and Health Administration reduced its permissible exposure limit four times since 1971, and the Environmental Protection Agency (EPA) restricted use of asbestos in building construction and imposed work practices for building demolitions. In fact, the potential for asbestos exposure is relatively low compared to historical worker exposures.

Strickler and colleagues (27) point out that while mesothelioma incidence rates in age groups most heavily exposed to SV40-contaminated polio vaccine remained stable or decreased from 1975 through 1997, increases in mesothelioma occurred in the older age groups that had only a small likelihood of receiving contaminated vaccine. These age group trends are likely to reflect the potential high exposure to asbestos, which peaked in 1950 when these individuals would have been in the midst of occupational exposure. One may ask, however, if there is no effect of SV40 on mesothelioma rates, then why have the rates among the younger cohorts not dropped more dramatically; perhaps these rates should have decreased by approximately 70% given the authors, estimate of a 60% to 80% disease rate attributable to asbestos. Interestingly, although numbers are small and imprecise, no decreases are noted in younger women, 45 to 65 years of age, in whom the issue of asbestos exposure is likely to be moot. Given the lack of actual descriptive data, including number of cases analyzed by age and gender in this study, it is difficult to interpret. One consideration is very important: given the relatively low prevalence of mesothelioma in men age >85 in the United States, even an extremely large increase in the relative risk of mesothelioma due to SV40 exposure would result in only a small absolute change in rate of disease. The change in incidence of 3.5/100,000 in 1974 (from Fig. 2B) to that of 14/100,000 in 1996–1999 represents a fourfold increase in risk among men over the age of 85. Based on Table 18.1, Strickler et al estimate that a man who is 85 in

1996, and thus would have been 50 years of age in 1961, had more than a 25% chance of being exposed to SV40 vaccine. Therefore, for every 100,000 men over the age of 85 in 1996 we can expect that 25,000 of them were exposed. If all 10/100,000 additional cases of mesothelioma occurring in 1996 are attributable to SV40 exposure, the incidence among SV40-exposed males would be 40/100,000, representing a greater than 10-fold increase in risk. Given that the data as presented in this study are potentially consistent with this magnitude of risk attributable to SV40, this study provides evidence that may be considered to support the possibility of an important carcinogenic effect associated with SV40, as has been suggested by laboratory findings to date.

Summary

In a report released by the Institute of Medicine in October 2002, it was concluded that emerging biologic evidence suggests that SV40 exposure *could* lead to cancer in humans under natural conditions (29). "The principal lines of evidence are based on in vitro and animal studies that demonstrate that SV40 acts in ways consistent with tumorigenesis and that DNA sequences consistent with SV40 have been detected in several types of human tumors." The institute emphasized, however, that *the detection of SV40 in tumors does not, by itself, demonstrate a causal relationship*. Simian virus 40 could merely be a passenger virus. The institute emphasized that, to date, the epidemiologic investigations were sufficiently flawed such that the evidence was inadequate to draw conclusions regarding the role of SV40 in the development of human cancer.

Future epidemiologic investigations of the association between SV40 and malignancy will require access to cancer incidence data from larger, age-matched samples in order to achieve adequate power for drawing conclusions. Analytic approaches, either case-control or cohort designs, require feasible methods of exposure classification in order to overcome the inherent limitations of the ecologic designs that have been used to date. Recall bias, inaccessibility of medical records, inadequate personal health documentation, lack of information regarding viral contamination of vaccine lots, and limited ease of antibody testing are just some of the obstacles to be overcome in the conduct of these studies. Studies specific to mesothelioma are greatly disadvantaged due to the rarity of the tumors and the resulting imprecision of risk estimates. In addition, the fact that the disease is extremely rare before the age of 60 suggests that it remains too early to assess the ultimate impact of SV40 exposure on this disease entity.

Although the public health impact of a role of SV40 in the development of mesothelioma would be relatively small, a similar association in more common cancers such as non-Hodgkin's lymphoma would represent a significant risk to public health. Regardless of incidence, however, the greatest progress in the field of research on SV40 and malignancy has occurred with mesothelioma; studies examining the

potential of SV40 as a therapeutic target in this tumor are already under way. As progress continues in the laboratory, it is critical that further epidemiologic research be undertaken. Creative strategies are critical to provide a better understanding of the role of SV40 in malignancy.

References

1. Communicable Disease Center. Poliomyelitis Surveillance Report No. 248. Atlanta, GA: 1962.
2. Eddy BE, Borman GS, Berkley WH, Young RD. Tumors induced in hamsters by injection of rhesus monkey kidney cell extracts. Proc Soc Exp Biol Med 1961;107:191–197.
3. Diamandopoulos GT. Induction of lymphocyte leukemia, lymphosarcoma, reticulum cell sarcoma and osteogenetic sarcoma in the Syrian golden hamster by ontogenetic DNA simian virus 40. J Natl Cancer Inst 1973;50: 1347–1365.
4. Kirschstein RL, Gerber P. Ependymomas produced after intracerebral inoculation of SV40 into newborn hamsters. Nature 1962;195:298–300.
5. Rabson AS, O'Conor GT, Kirschstein RL, Branigan WJ. Papillary ependymomas produced in Rattus (mastomys) natalensis inoculated with vacuolating virus (SV40). J Natl Cancer Inst 1962;29:765–787.
6. Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. Am J Pathol 1993;142:1524–1533.
7. Eddy BE, Borman GS, Grubbs G, Young RD. Identification of the oncogenic substance in rhesus monkey kidney cell cultures as simian virus 40. Virology 1962;17:65–75.
8. Koprowski H, et al. Transformation of cultures of human tissue infected with simian virus 40. J Cell Comp Physiol 1962;59:281–292.
9. Gerber P, Hottle GA, Grubbs G. Inactivation of vacuolating virus (SV40) by formaldehyde. Proc Soc Exp Biol Med 1961;108:205–209.
10. Shah KV, Willard S, Myers RE, et al. Experimental infections of rhesus with simian virus 40 (SV40). Proc Soc Exp Biol Med 1968;130:196–203.
11. Melnick JL, Stinbaugh S. Excretion of vacuolating SV40 virus (papovirus group) after ingestion as a contaminant of oral poliovaccine. Proc Soc Exp Biol Med 1962;109:965–968.
12. Morris JA, Johnson KM, Aulisio CG, Chanock RM, Knight V. Clinical and serologic responses in volunteers given vacuolating virus (SV40) by respiratory route. Proc Soc Exp Biol Med 1961;108:56–59.
13. Pan American Health Organization and World Health Organization. Field evidence in safety, topic II (B). In: Second International Conference on Live Poliovirus Vaccines. Washington, DC: 1960:113–227.
14. Fraumeni JF, Ederer F, Miller RW. An evaluation of the carcinogenecity of simian virus 40 in man. JAMA 1963;185:713–718.
15. Fraumeni JF, Stark CR, Gold E, Lepow MC. Simian virus 40 in polio vaccine: follow-up of newborn recipients. Science 1970;167:59–60.
16. Mortimer EA Jr, Lepow MC, Gold E, Robbins FC, Burton GJ, Fraumeni JF Jr. Long-term follow-up of persons inadvertently inoculated with SV40 as neonates. N Engl J Med 1981;305:1517–1518.
17. Carroll-Pankhurst C, Engels EA, Strickler HD, Goedert JJ, Wagner J, Mortimer EA. Thirty-five year mortality following receipt of SV40-contaminated polio vaccine during neonatal period. Br J Cancer 2001;85: 1295–1297.

18. Martini F, Iaccheri L, et al. SV40 early region and large T antigen in human brain tumors, peripheral blood cells, and sperm fluids from health individuals. Cancer Res 1996;56(20):4820–4825.
19. Shah K, Nathanson N. Human exposure to SV40: reviews and comments. Am J Epidemiol 1976;103:1–12.
20. Hammond EC. Cancer mortality in relation to SV40 in polio vaccine. Presented at the American Cancer Society Sciences Writers' Seminar, 1966.
21. Heinonen OP, Shapiro S, Monson RR, Hartz SC, Rosenberg L, Slone D. Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. Int J Epidemiol 1973;2:229–234.
22. Innis MD. Oncogenesis and poliomyelitis vaccine. Nature 1968;219:972–973.
23. Olin P, Giesecke J. Potential exposure to SV40 in polio vaccines used in Sweden during 1957: no impact in cancer incidence rates 1960–63. Dev Biol Stand 1998;4:227–233.
24. Geiseler E. SV40 and human brain tumors. Prog Med Virol 1990;37:211–222.
25. Strickler HD, Rosenberg PS, Devesa GG, Hertel J, Fraumeni JF, Goedert JJ. Contamination of poliovirus vaccines with simian virus 40 (1955–1963) and subsequent cancer rates. JAMA 1998;279:292–295.
26. Fisher SG, Weber L, Carbone M. Cancer risk associated with simian virus 40 contaminated polio vaccine. Anticancer Res 1999;19:2173–2180.
27. Strickler HD, Goedart JJ, Devesa SS, Lancy J, Fraumeni JF, Rosenberg PS. Trends in US pleural mesothelioma incidence rates following simian virus 40 contamination of early poliovirus vaccines. J Natl Cancer Inst 2003;95: 38–45.
28. Price B. Analysis of current trends in United States mesothelioma incidence. Am J Epidemiol 1997;145:211–218.
29. Stratton K, Almario DA, McCormick MC, eds. Immunization Safety Review: SV40 Contamination of Polio Vaccine and Cancer. Washington, DC. Institute of Medicine, National Academies Press, 2002.

19

Causes and Prevention of Technical Artifacts When Studying Simian Virus 40 (SV40) in Human Mesotheliomas

Marc Ramael

Introduction to the SV40 Genome, SV40 Early Proteins Large Tumor (T)-Antigen and Small Tumor (t)-Antigen

Simian virus (SV40) was discovered as one of the viruses capable of infecting Macacus rhesus as well as Macacus cynomolgus monkey kidney cells (1). It also had the possibility to infect and to transform human cells grown in vitro. Simian virus 40 is a DNA tumor virus that not only induces tumors in rodents but also is capable of immortalizing human mesothelial cells in vitro. Except for a report demonstrating SV40 in one metastatic melanoma, SV40 was not considered to be oncogenic in humans (2). Simian virus 40 DNA has been found in several human tumors such as choroid plexus tumors, osteosarcomas, malignant mesotheliomas, and lymphoproliferative diseases such as non-Hodgkin's lymphomas (3–5). The role of the SV40 virus in human tumors has been extensively discussed in several excellent reviews (4,6,7).

Simian virus 40 is a double-stranded DNA virus whose genome encodes two tumor (T)-antigens known as large T-antigen and small t-antigen. Replication of the double-stranded DNA genome occurs in the nucleus of the host cell. Transcription of the genome is carried out by host cell RNA polymerase II, and large T-antigen plays a major role in regulating transcription of the viral genome by binding to the origin region of the viral genome. Protein–protein interactions between T-antigen and DNA polymerase alpha directly stimulate replication of the viral genome. Small t-antigen is not essential for virus replication but allows viral DNA to accumulate in the nucleus. Both proteins contain nuclear localization signals, which results in their accumulation in the nucleus, where they migrate after being synthesized in the cytoplasm. After infection, early messenger RNAs (mRNAs) are expressed from the early promoter, which contains a strong transcrip-

tion enhancer element consisting of 72 base pair (bp) sequence repeats. The early proteins synthesized are the two T-antigens, large T- and small t-antigen. As the concentration of large T-antigen builds up in the nucleus, transcription of the early genes is repressed by direct binding of the protein to the origin region of the virus genome. After DNA replication has occurred, transcription of late genes occurs from the late promoter and results in the production of the structural proteins VP1, VP2, and VP3.

The early region of SV40 codes for the 94-kd nuclear large T-antigen (Tag) and the 21-kd small t-antigen (tag), which are responsible for the transforming and oncogenic properties of the virus. The mechanisms by which both proteins induce these events have been studied extensively. The key event is inactivation of the gene products of several tumor suppressor genes such as *p53* and *RB*, which normally inhibit cellular growth (8). Tag can bind and inactivate p53 protein and p107 retinoblastoma protein in malignant mesothelioma (9,10). Tag also exhibits adenosine triphosphatase (ATPase) and helicase activity, the latter of which may contribute to chromosomal breakage and recombination (11). Small t-antigen will enhance the stimulatory effect of Tag on cell proliferation by inhibiting cellular phosphatase 2A, thus inducing the mitogen-activated protein (MAP) kinase cascade and cell proliferation (12). This leads to a powerful combination of loss of cell cycle regulation and marked genomic instability responsible for SV40-induced cell transformation and immortalization (13).

Small t-antigen might play a key role in the development of mesothelioma as tag SV40 DNA mutant viruses are not able to induce mesothelioma when injected intrapleurally in hamsters in contrast to wild-type SV40 (14,15). Virtually all animals injected intrapleurally with the wild-type SV40 died of mesothelioma at 4 to 6 months.

Detection of SV40 DNA in Tissues and Cells by Polymerase Chain Reaction

Methodology for Detection of SV40 DNA

Experimental work suggested that SV40 could cause mesotheliomas. Syrian hamsters injected with SV40 wild-type virus developed malignant tumors such as true histiocytic lymphomas, sarcomas, and mesotheliomas according to the site of injection (15). The SV40 mutant virus that lacked the gene sequence for tag was not able to induce malignant mesothelioma but initiated only the growth of true histiocytic lymphomas or sarcomas (14). In vitro experiments suggest that human mesothelial cells are unusually susceptible to SV40-mediated transformation and asbestos co-carcinogenicity (16). The experimental findings in hamsters prompted Carbone et al (17) to investigate human mesothelial tumor tissue for the presence of SV40. The presence of SV40 DNA in 29 of 48 mesotheliomas was found using polymerase chain reaction (PCR) with several primer sets PYV.for/PYV.rev and SV.for3/SV.rev. These findings were confirmed by sequencing and blotting

experiments. Carbone et al were able to prove that the viral DNA was actively transcribed and translated to viral Tag. Immunohistochemistry carried out on frozen sections as well as immunoprecipitation assays with the mouse monoclonal antibody Pab419 confirmed the presence of viral Tag.

Most studies dealing with the detection of SV40 DNA now describe the use of PCR or a PCR-based assay as the gold standard. The PCR technique described by Bergsagel and coworkers (3) for detection of the SV40 DNA in ependymomas and choroid plexus has become the method used by most research groups. The DNA of the tumor sample is extracted, purified, and amplified with specific beta-globin primers AG1 and AG-2 for checking the integrity and amplifiability of the extracted DNA. All specimens from which adequate DNA is extracted are examined for viral sequences with the primers PYV.for and PYV.rev. These primers amplify a conserved region of the Tag that is common to the papovaviruses SV40 virus, BK, and Jamestown Canyon (JC) virus resulting in an amplicon of 172 bp. This region codes for the Rb, p107, and the Rb2/p130 binding domain of Tag. When using this primer set one only detects papovavirus, but since this primer set does not distinguish among BK, JC, and SV40, additional techniques such as Southern blotting have to be performed for demonstrating with certainty the SV40 origin of the amplified PCR.

Probes specific for BK, JC, SV40 were used in a Southern blot technique to determine whether SV40 was present or whether the amplicon was derived from BK or JC virus. However, under some conditions BK- and JC-specific probes were found to cross-react with SV40. To resolve ambiguities regarding the cross-reactivities of specimens with BK and JC DNA probes, one has to sequence the PCR amplicons. The sequences of BK and JC virus contain an additional 9-bp insert in contrast to SV40, which lacks this 9-bp insert. If fresh tissue or frozen tissue is available for analysis, one can use the primer set SV.for2 and SV.rev amplifying a 574-bp region of the SV40 Tag gene.

The primers SV.for3 and SV.rev can be used to amplify a 105-bp fragment of the SV40 that only partially overlaps the fragment amplified with PYV.for and PYV.rev. The former set can be used easily for analysis of paraffin-embedded, formalin-fixed tissues where the DNA is partially degraded due to formalin fixation and subsequent processing. Other primer sets such as SV5-SV6 result in amplicons of 169 bp, while TA1-TA2 amplify a segment of 441 bp (Table 19.1).

Different positivity rates for SV40 DNA have been found in malignant mesothelioma when using different primer sets. The wide variability in the detection rates may be partly explained by the relatively small numbers of cases analyzed in each study. However, one must take into account the differences in tissue quality, formalin-fixed tissue versus fresh frozen samples, the differences in DNA extraction method, the variability in PCR amplification and detection methods, as well as the geographic differences.

The SV3.for and SV.rev primers amplified SV40 DNA in 90% of the mesothelioma samples (18). The PYV.for and PYV.rev primers amplified SV40 in 70% of the DNAs, and the SV2.for-SV.rev primers ampli-

Table 19.1. Frequently used primer sets for SV40 DNA detection by polymerase chain reaction

Primer pair	Amplicon (base pair)	Position
PYV.for PYV.ref	172	4402–4425
R1 R2	315	266–5195
RA1 RA2	242	266–5195
RA3 RA4	413	358–5119
SV1 SV2	574	Not specified
SV.for2 SV.rev	574	4945–4372
SV.for3 SV.rev	105	4372–4476
SV5 SV6	169	4402–4570
SV8 SV9	289	2548–2821
T3 T4	338	Not specified
TA1 TA2	441	2630–3070
C-terminus	329	2573–2902

fied SV40 DNA in 25% of the cases. The 7/8 primers for the carboxy terminus of Tag amplified SV40 in 38% of the DNAs, important for tissue specific replication of SV40 virus, and 52% showed amplification of the regulatory region. In four cases analyzed this was found to be similar to the situation in the hamster mesothelioma where two 72-bp enhancer elements are present. Duplication of this 72 bp seems to confer a growth advantage to SV40-infected cells. The RA1 and RA2 primers specific for the regulatory region of SV40 amplified SV40 in 50% of the cases. Overall, 24 of 42 patients showed amplification with all sets of primers. The identity of the PCR product was confirmed by restriction enzyme digestion, Southern blot hybridization, and DNA sequencing.

It remains unclear why different primer sets give different results. Mutations and deletions occur in most viruses and also in SV40 virus. Mutations at the 3′ site where the primer has to bind can lead to mis-priming with no resulting Taq polymerase activity and no production of specific SV40 amplicon. However, mutations are unlikely to occur in Rb-pocket binding domain as this site is highly relevant for Tag-

mediated cell transformation. According to this hypothesis, the primer set specific for the Rb-pocket, SV3.for/SV.rev would be expected to give the highest positive results (18,19).

There is little known about the state and nature of the SV40 genome in tumoral cells. The infective viral state of the SV40 genome has so far been isolated from only one choroid plexus tumor (20). In the majority of investigated tumors in this series the SV40 DNA was predominantly present in its full-length episomal state. The same was true of the viral DNA associated with osteosarcomas (21). In the brain tumor study, each tumor appeared to be associated with a single homogeneous viral DNA species defined by its unique sequence related to the variable domain (the last 87 amino acids, 622 to 708) of the C-terminus of Tag. The possible integrated state of the virus has been suggested in three of 69 human papillary thyroid carcinomas and in five of 10 osteosarcomas (22,23). Integration of the SV40 genome into the DNA of the host results in opening of the circular SV40 genome and leads to a linearized SV40 genome. This can lead to disruption of certain genes not only from the host but also of viral genes. It may end in the total loss of viral genes. This phenomenon has also been described for cervical carcinoma–associated oncogenic human papillomavirus (HPV) 16 where integration leads to changes or even loss of the L1 region, resulting in negative PCR results for primers detecting sequences in the L1 region. In contrast, the E6 and E7 regions coding for the oncogenic proteins E6 and E7 remain highly conserved throughout the complete multistep carcinogenesis from dysplasia to frankly malignant and invasive cervical carcinoma (24). It is also noteworthy that different HPV primer sets also give different rates of positivity for a given sample (25). Those primers that amplify smaller segments are found to give a higher positive yield in contrast to those amplifying larger segments. Primer sets detecting sequences in highly conserved regions of HPV such as the E6 and E7 genes also give higher yields than those situated in other regions such as the L1 region known to be less conserved during the carcinogenesis proces. It is very remarkable and maybe also similar to SV40 that in very early lesions the HPV genome is present in the episomal form and that when integration of the genome occurs the lesion starts further to evolve into high-grade dysplasia and invasive frankly malignancy. When applying these findings on the SV40 situation, one can readily appreciate why some SV40 primer sets give different positivity rates as some regions, especially the Rb-binding pocket, remain highly conserved while others may be lost during the multistep carcinogenesis process. It is also understandable that primer sets that amplify smaller segments will yield a higher positivity rate than those that amplify larger segments. One has to bear in mind that some primers can amplify also Tag sequences from other papovaviruses, and even Southern blot techniques with specific probes may not always be sufficient to discern the SV40 Rb-binding pocket from other papova Tags. A likely candidate would be the Tag of the BK virus as this virus has been found to be very ubiquitous in the human population.

The SV40 and BK tags are very similar, but a 9-bp insert facilitates distinction. For this reason, sequencing is the preferred method when

working with PYV.for-PYV.rev and SV3.for-SV.rev primer sets. One cannot rule out with absolute certainty that the sequences detected do not belong to a recombinant SV40-BK virus or another unknown virus.

In one study the presence of SV40 DNA was confirmed in 14 of 25 malignant mesotheliomas using the novel Primed in situ (PRINS) method on paraffin-embedded formalin-fixed tissue specimens (26). The PRINS labeling method is based on a primer-mediated DNA synthesis starting with the annealing of an oligonucleotide DNA primer adjacent to the DNA region of interest. This oligonucleotide serves as a primer for the DNA polymerase Taq polymerase incorporating the four nucleotides dATP, dGTP, dUTP, and dCTP, of which the dUTP is labeled with digoxigenin. The label can be visualized immunohistochemically using alkaline phosphatase-bound antibody or peroxidase-bound antibody. The application of the PRINS methodology has been focused primarily on cytogenetics (27). This method can be applied on paraffin-embedded formalin-fixed tissue sections (28).

The PRINS reactions were carried out with three separate primers, PYV.rev, SV2.for and SV.rev, detecting a DNA sequence coding for the viral tag of SV40. The latter is detecting a region (4425–4402) coding for Tag, which is common to BK, JC, and SV40. The former two primers are SV40 specific. The SV.rev primer is homologous to the 4399 to 4372 region and the SV2.for primer is identical to the region 4920 to 4945. Both are located in the DNA sequence coding for the Tag but outside the Rb-p107-Rb2/p130 binding domain.

Nuclear staining was found in 14 of the 25 investigated mesothelioma tissue specimens using the PYV.rev primer in the PRINS method, indicating the presence of SV40 or SV40-like DNA, as this primer also recognizes a sequence identical to SV40, JC, and BK virus. A separate PRINS reaction with the Sv2.for primer and Sv.rev primer, considered to be specific for the SV40 virus, revealed a nuclear signal in all those cases that tested positive with the PYV.rev primer. The percentage of positive cells varied considerably among different tumors. The percentage of positive neoplastic cells ranged from 2% in an epithelial mesothelioma to nearly 100% in a mesenchymal mesothelioma (Figs. 19.1 and 19.2). Stromal elements such as blood vessels, adipocytes, and inflammatory cells were found not to be positive. In all the cases that tested positive for SV40 or SV40-like DNA, cytoplasmic and nuclear immunoreactivity was found for tag with the monoclonal antibody pAb280. The amount of immunoreactive cells was comparable to those with nuclear signal for SV40-like DNA in a given sample. No nuclear signal indicative for the presence of SV40 or SV40-like DNA or immunoreactivity for tag with the monoclonal antibody Pab 280 was found in those cases with nonneoplastic mesothelium or pleural carcinoma metastasis.

Simian virus 40 DNA and expression of one of the viral proteins (tag) was found in approximately 60% of the investigated mesothelioma cases, in contrast to nonneoplastic mesothelium and carcinoma metastasis, which were negative for both SV40 DNA and SV40 viral antigens.

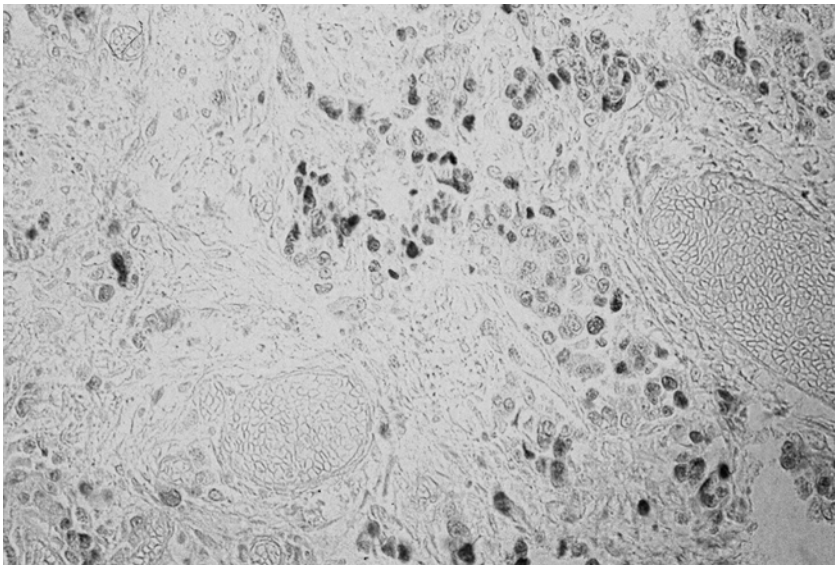


Figure 19.1. Most nuclei of neoplastic mesothelial cells display nuclear staining for simian vacuolating virus (SV40) deoxyribonucleic acid (DNA) with the primed in situ (PRINS) method in an epithelial mesothelioma (SV2 for primer) using dextran polymer-peroxidase visualization.

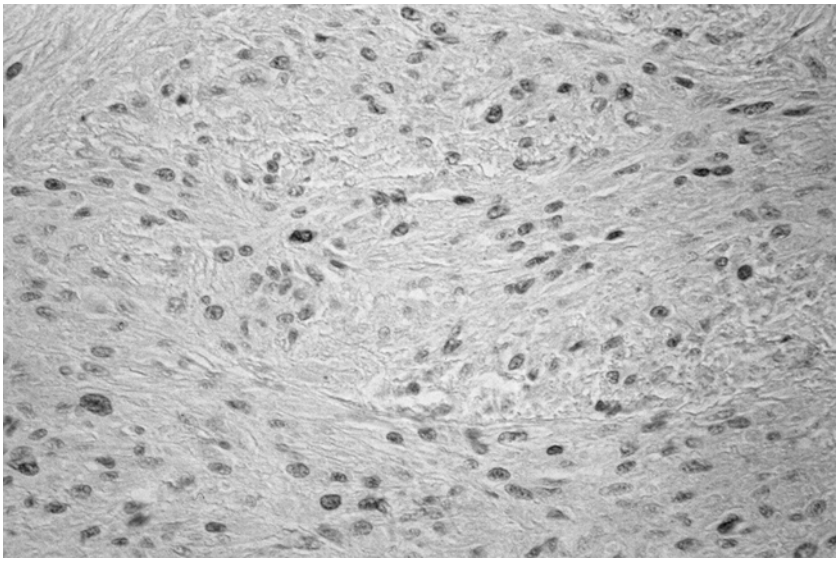


Figure 19.2. Most nuclei of neoplastic mesothelial cells display nuclear staining for simian vacuolating virus (SV40) deoxyribonucleic acid (DNA) with the primed in situ (PRINS) method in a mesenchymal mesothelioma (SV2 for primer) using dextran polymer-peroxidase visualization.

These results suggest that SV40 DNA may be biologically active as there was also immunoreactivity for SV40 antigen in those cases positive for SV40 DNA with the PRINS reaction.

Technical Concerns When Using PCR for SV40 Detection

Polymerase chain reaction contamination could result from the SV40 DNA itself or from plasmids containing various segments of the SV40 genome. It is very unlikely to assume that all reported positive results would be the result of contamination by one or another source. Some laboratories reporting positive results had never before worked with SV40 or known laboratory SV40 strains and some cases displayed several different mutations or deletions. One would not expect such data when dealing with contamination, where always the same bands or band would be found. Tag protein and mRNA were found in mesothelioma, thereby making the possibility of PCR contamination by exogenous SV40 DNA very unlikely.

In some cases, the sequences are from SV40 itself, as the virus could be rescued or extensive sequencing confirmed the SV40 origin. For the majority of cases, where extensive sequencing has not been carried out, it is better to use "SV40 like" instead of SV40. The possibility of a recombinant SV40 cannot be ruled out with certainty, nor can the possibility of a human virus related to SV40 or even a cellular homologue of Tag.

Specificity of PCR for SV40 Detection

The question of specificity of SV40 detection has been challenged on two grounds. The failure of Strickler et al (29) to detect SV40 DNA in their paraffin-embedded samples has been used as an argument to consider SV40 positivity in mesothelioma specimens as a result of intra- or interlaboratory contamination. The possibility of cross-contamination by exogenous SV40 to be high due to the long and widely distributed usage of SV40 early region base vectors and gene constructs was hypothesized (19). This criticism has been addressed by the experiments of Testa and coworkers (30). They reported the conclusions of a multilaboratory study they had directed following the recommendation of the 1997 National Institutes of Health (NIH) conference and upon the request of the International Mesothelioma Interest Group (IMIG). This study included the laboratory of Michele Carbone, who had reported positive findings for SV40 in mesotheliomas in the United States, and that of Kaija Linnainmaa, who had reported negative findings in mesotheliomas in Finland. The laboratory of Kalily, which specialized in JC virus, was also involved in this study to confirm that the isolated DNAs did not belong to other papovaviruses such as BK or JC virus. The mesothelioma material was distributed to four independent laboratories, three of which never had worked with SV40. The DNA was extracted in one single independent center not previously exposed to SV40. This study confirmed the presence of SV40 DNA and proteins in 10 of 12 (83%) mesotheliomas in the United States. The positive and negative controls yielded consistent results in all centers (30). High reliability for SV40 detection was demonstrated by revealing SV40 positivity in

U.S. mesothelioma specimens and absence of SV40 DNA in all investigated Finnish specimens using a double-blind analysis approach (31). To verify these results, Testa et al organized a second study where blinded DNAs of 11 mesothelioma samples in the U.S. together with nine mesothelioma specimens in Turkey were investigated. They found four of 11 U.S. mesotheliomas to be positive for SV40 in contrast to Turkish mesotheliomas, which were all negative for SV40. This discrepancy in results between U.S. mesotheliomas on one side and Turkish as well as Finnish mesotheliomas on the other side, suggested that SV40 prevalence can display regional differences. It is noteworthy that in contrast to the United States, Turkey started to administer polio vaccines in the 1970s that are considered to be cleared of SV40. Whether these differences can be solely attributed to SV40-contaminated polio vaccines or other hitherto unknown factors remains to be determined.

The validity of the contamination theory was further investigated by screening coded DNA aliquots of 32 matched blood samples and prostate biopsies for SV40 DNA by PCR (32). The SV40 DNA was found in nine of 64 specimens and in 31 of 32 patients the prostate and blood samples were completely concordant. The low level of SV40 DNA and the nearly completely concordance is inconsistent with the possibility of contamination. The found positivity was attributed to circulating mononuclear cells in the blood rather than to malignant cells. This underscores the importance of confirming positive PCR results by other techniques, such as immunohistochemistry, in situ hybridization, or microdissection before attributing SV40 to a certain tumor type. Investigation of microdissected mesothelioma cells and nearby stromal cells from the same paraffin section using PCR followed by sequencing displayed SV40 DNA in 57% of the mesothelioma samples. The SV40 sequences were present in both the preinvasive and invasive component of the tumor cells, which indicated that SV40 is present in the early stages of the process. Microdissected cells from matched adjacent lung tissue were negative for SV40 sequences (33).

One research group conducted its expriments with new mesothelioma specimens in a separate laboratory in a new building where experiments with viruses or plasmids had never been carried out (34). The group was able to confirm its former results by demonstrating SV40 regulatory sequences in 10 of 18 mesothelioma samples and SV40 Tag sequences in eight of 10 mesothelioma samples (34,35).

Another study confirmed the presence of SV40 DNA in eight of 12 malignant mesothelioma specimens but detected SV40 DNA only in 11 of 49 lymphoproliferative disorders (5). DNA extractions and PCR reactions were carried out at the same time by the same investigators, thereby ruling out the possibilities that either the technical procedure used was not sufficiently sensitive to detect SV40 or the high percentage of SV40 DNA–positive mesotheliomas might be related to PCR contamination (5).

Reproducibility of the PCR Reaction

The reproducibility of SV40 DNA detection by the PCR technique has been challenged by Strickler and Mulatero. Studies conducted by Shah

upon the request of Strickler and Goedert, failed to detect SV40 DNA in any of the investigated mesothelioma specimens. However, this study was completely based on paraffin-embedded material (Strickler et al, 1997). A number of possibilities were formulated to account for the possible negative data such as limited sensitivity and different technical approach. But it remains unclear why Shah's analyses appear to be at odds with those published by other groups. The author did not use Southern blotting or filter hybridization techniques as confirmatory technique after PCR amplification. In the study of Strickler, only four cases were found positive after ethidium bromide staining of agarose gels compared to 26 of 26 after filter hybridization in Carbone et al's study (17). In a new nine-laboratory multicenter investigation, none of the selected normal human lung tissues and none of the 25 pleural mesothelioma samples obtained from archival samples were reproducibly positive for SV40 DNA (36). Eight laboratories used a PCR assay and one laboratory used Southern blotting without prior PCR amplification. Since several negative control samples gave positive results for SV40 DNA in eight of nine laboratories, one might seriously question the global outcome of this study. The negative results reported by Shah's team may have been caused by the limited sensitivity of the used methodology. Apparently, Shah acknowledged under oath sensitivity problems that raise concerns about the validity of the reported findings (37).

Different methods used for DNA extraction may account for the variable detection rate since the spooling technique used for high-quality DNA extraction from fresh tissue is not applicable to paraffin-embedded, formalin-fixed tissue material. Pass and co-workers stressed the advantage of DNA centrifugation at high speed after phenol-chloroform extraction for efficient isolation of small molecular weight DNA including episomal SV40 DNA. One must take into account that some extraction techniques other than phenol-chloroform extraction may result in less efficient recovery of viral DNA as described for adenovirus DNA and cytomegalovirus DNA (38,39). Additional differences, such as shorter protease digestion time for cultured cells in contrast to cells in tissue blocks, intrinsic differences between cells in culture and cells in tissue, may influence very strongly the performance of the PCR assay for detecting SV40 DNA.

It is interesting to note that Strickler had used minute amounts of DNA extracted from individual paraffin wax sections scraped off glass slides. Estimating an average of 6 pg of genomic DNA/diploid cell and isolating 5000 cells out of one 5-μm-thick paraffin section, one can assume that one can collect 30 ng DNA. This means that approximately 20 to 25 sections are needed to have an optimal amount of DNA.

In contrast, Mulatero et al (40) had relied on antemortem diagnostic biopsy material from which 200 ng of extracted DNA was used per PCR reaction instead of the more optimal amounts of 500 to 1000 pg. The findings of Strickler and Mulatero are in contradiction with the results reported by Carbone and coworkers, who found SV40 DNA in 29 of 48 (60%) mesothelioma tumor samples. However, Carbone and co-workers found their PCR assay was able to detect 1 to 10 genome

copies per PCR reaction in contrast to Strickler, who found a lower sensitivity level of only 10 to 100 SV40 copies with his technique. Carbone et al used the same primer sets as Bergsagel, amplifying the Rb pocket binding domain of Tag, which is the region that binds pRb, p107, and p130/Rb2. Tag expression was detected by immunohistochemistry and Western blot using the anti-Tag pAb 419, which is specific for SV40 Tag and does not recognize Tags from BK and JC virus. Patients' sera were found to contain Tag antibodies. The finding of Tag-protein expression in some human mesotheliomas further decreased the possibility of PCR contamination by commonly used vectors containing SV40 sequences. More than 20 independent research teams have confirmed worldwide the presence of SV40 or SV40-like DNA in pleural malignant mseothelioma (41).

Inadequate sampling can also be a source of negative results. The tumoral component in biopsies can vary strongly in different biopsies and among different patients resulting in different amounts of SV40 DNA. The amount of DNA recovered from open thoracotomy specimens is much greater than the material obtained from fine-needle aspiration biopsy or true-cut biopsy (42). When working on paraffin-embedded, formalin-fixed tissue biopsies, one must take into account the influence of formalin fixation on the DNA, and the subsequent processing. Formalin fixation induces strand breaks in the DNA, resulting in fragmentation and DNA degradation and thus less amplifiable SV40 DNA. The degree of DNA degradation is dependent on the type of fixative used and the duration of fixation (43). Generally speaking, one might assume that amplifiability in these processed tissues is guaranteed up to 200 to 250 bp. It is safe to use the SV.for3/SV.rev primer set when analyzing paraffin-embedded formalin-fixed tissues for the presence of SV40 DNA. A major point of interest is that the integrity of the DNA should always be confirmed by amplifying a genomic sequence, e.g., beta-globin, thereby choosing the size of the amplicon so that it is in the range of amplicon sizes obtained by the SV40-specific primers. It may be possible to amplify larger segments by PCR using paraffin-embedded, formalin-fixed tissues but results may be variable. When comparing studies of different laboratories, one must bear in mind that every pathology laboratory has its own fixatives and protocols for fixation, and that the tissue processing can be quite different from one laboratory to another, and the PCR protocols can display huge differences in sensitivity for detecting SV40 DNA. This makes comparisons between various laboratories as well as interlaboratory studies very difficult especially when the work has been carried out on archival patient material.

Immunohistochemical Demonstration of SV40

Viral Large T-antigen and Small t-Antigen

Tag is the gene product of the early gene of SV40. It is a protein of 94 kd. Tag binds DNA and complexes with the 53-kd protein p53, the gene product of the p53 suppressor gene, which is also required for

initiation of viral DNA replication during lytic growth. In addition, Tag binds DNA polymerase and the transcription factor AP-2 and forms a specific complex with the p105 product of the retinoblastoma suppressor gene.

The small T-antigen is a 19-kd protein found predominantly on the cytoplasm of infected or transformed cells (44). This protein shares 82 amino acids at its amino terminus with Tag; the remaining 92 amino acids are unique. The small T-antigen enhances the transforming capacity of Tag by increasing the production of Tag, by contributing to the inactivation of cellular p53, and by induction of AP-1, leading to enhanced mitosis (12,13,45). Both antigens are encoded by the early region of the SV40 genome.

Widely Used Antibodies to Large T- and Small t-Antigen

Pab419 (Ab-1) is a mouse monoclonal antibody with specificity for an antigen localized to the amino terminal domain of the 94-kd SV40 Tag. The sequences recognized by this reagent are also present on 21-kd SV40 tag. Therefore, the antibody detects Tag as well as tag and will stain SV40-infected cells. The use of the antibody is validated for immunoprecipitation and immunofluorescence (46).

Pab416 (Ab-2) is a mouse monoclonal antibody with specificity for antigenic determinants unique to the SV40 Tag and nonreactive with SV40 tag. Clone Pab416 is derived by immunization with purified Tag and fusion of mouse spleen cells with NS-1 mouse myeloma cells. This antibody is reactive to an epitope situated in the N-terminal region. The antibody is reactive by immunoprecipitation with the 94-kd SV40 Tag and stains SV40-infected cells (46). Cross-reactivity has been noted with BK virus Tag. The use of the antibody has been described for Western blotting, immunoprecipitation, and immunofluorecence. Diffuse nuclear staining is a characteristic staining pattern when using this antibody in an immunohistochemical or immunofluorescent technique on frozen tissue sections.

Pab101 is a mouse monoclonal antibody that is recognizing an epitope situated on the SV40 Tag but does not cross-react with the 21-kd SV40 tag. The antibody reacts with a denaturation-sensitive determinant on Tag. It reacts with higher affinity than Pab100 but does recognize a subclass of Tag that is also recognized by sera from mice bearing SV40-induced tumors. The antibody precipitates Tag but not tag (nor the smaller SV80 tag) from extracts of SV80 cells and SV40-infected TC-7 cells (47,48). Punctuate nuclear staining is observed when immunostaining SV40-infected cells by immunofluorescence but not with BK-infected human cells.

Pab280 (Ab-3) is a mouse monoclonal antibody with specificity for antigenic determinants unique to the SV40 tag and nonreactive with SV40 Tag. Its binding site within the unique region of tag was localized by studying its reaction within SV40 mutants, other papovaviruses, and bacterial expression vectors coding for fragments of tag. The antibody was used to define the cellular localization of tag by immunohistochemistry and by immunoprecipitation of subcellular extracts of

infected cells (49). Pab280 reacts strongly with a cytoplasmic form of tag that appears to be associated with the cytoskeleton and is not detected by antibodies directed to the common N-terminus of tag and Tag (50). Clone Pab280 is derived by immunization of BALB/c mice with tag and fusion of splenocytes with SP20/AG14 mouse myeloma cells. Pab280 is reactive by immunoprecipitation and Western blotting with the 21-kd SV40 tag. The SV40-infected cells are stained by immunohistochemistry by the same antibody applied to frozen or paraffin-embedded, formalin-fixed tissue sections.

Immunohistochemical Studies Detecting the SV40 Proteins

Most investigators have used monoclonal antibodies directed against Tag. Using the mouse monoclonal antibody pAb419 on frozen sections of human pleural malignant mesothelioma biopsies Carbone et al (9,17) demonstrated nuclear expression of the viral Tag in neoplastic mesothelial cells in two separate series. The immunoreactivity was restricted to the nucleus. Cytoplasmic immunoreactivity was not noticed. Immunoreactivity in nonneoplastic elements such as stromal cells or leukocytes was not found. In one of these series the immunohistochemical findings were confirmed by specific mRNA in situ hybridization for Tag. A unique nuclear staining pattern with the monoclonal antibody was found by Orengo et al (51) in two SV40 DNA–positive newly established mesothelioma cell lines. The majority of neoplastic cells (90%) were immunoreactive for Tag without cytoplasmic immunoreactivity. In contrast, Dhaene et al (52) found only cytoplasmic immunoreactivity in their SV40 DNA–positive fresh frozen mesothelioma tissue biopsies and no nuclear immunoreactivity with the Pab419.

Investigations with the Pab101 did not reveal any immunoreactivity in the same mesothelioma cases. These negative results are not so surprising as this antibody is reactive to an antigenic site located at Tag that is very sensitive to denaturation (47,48). Similar results were reported by Galateau-Salle et al (53), working on paraffin-embedded, formalin-fixed mesothelioma tissue biopsies with the same Pab419 monoclonal antibody. One study describes the use of monoclonal antibodies Pab416 and Pab101 for detection of SV40 Tag in paraffin-embedded, formalin-fixed cell block sections from pleural effusions of 32 malignant mesotheliomas. No immunoreactivity was found despite strong staining of positive controls. The authors stated that the small sample size in the cytology block sections, the low viral copy number in infected cells, and the effects of formalin fixation were the reason for their negative immunohistochemical investigation (54). Only cytoplasmic immunoreactivity for SV40 Tag was found in six mesothelioma cell lines with the monoclonal antibodies Pab419 and Pab101 (55). The authors hypothesized the presence of a contaminating 90-kd protein in the commercially available anti-Tag monoclonal antibodies Pab419 and Pab101, resulting in false-positive Western blotting results and immunohistochemistry results. This 90-kd Tag–like protein appeared to be an artifact caused by the incomplete separation of the heavy

(54 kd) and light (25–30 kd) chains of the antibody used in Pilatte's immunoprecipitations (40). When discussing the results in cell lines, one must take into account that a cell line is a very restricted and selected population of tumor cells, thereby raising the question of whether a cell line is still representative, resembling genotypically and fenotypically its tumor of origin. This question is very well illustrated by the enigma of p53 protein overexpression in the thyroid cancer cell line FTC133, which displays strong nuclear immunoreactivity for p53 protein when cultured in vitro, but when the same cultured cells are grown in vivo as tumors in an immunodeficient host p53 protein, immunoreactivity becomes very weak or undetectable (56). The findings of Pilatte et al (55) do not invalidate the data of Carbone and coworkers. One can state with certainty that their findings are not due to a contaminating protein, as PCR and immunoprecipitation data in this study were complemented by the immunodetection of Tag in the nucleus and serologic detection of anti–T-antigen antibodies in the patient sera. Immunoprecipitation experiments conducted by Pilatte et al demonstrated that the contaminating protein did not prevent the detection of Tag in cells that do overexpress this protein. Testa and coworkers (30) observed in some mesothelioma cases cytoplasmic staining with the Pab419 antibody but also nuclear punctuate staining with the monoclonal antibodies Pab419 and Pab101 (Fig. 19.3). Several monoclonal antibodies that react with SV40 Tag also react with proteins found in uninfected and untransformed cells. The proteins were different from each other, e.g., Pab419 reacting with a 35-kd protein. It is

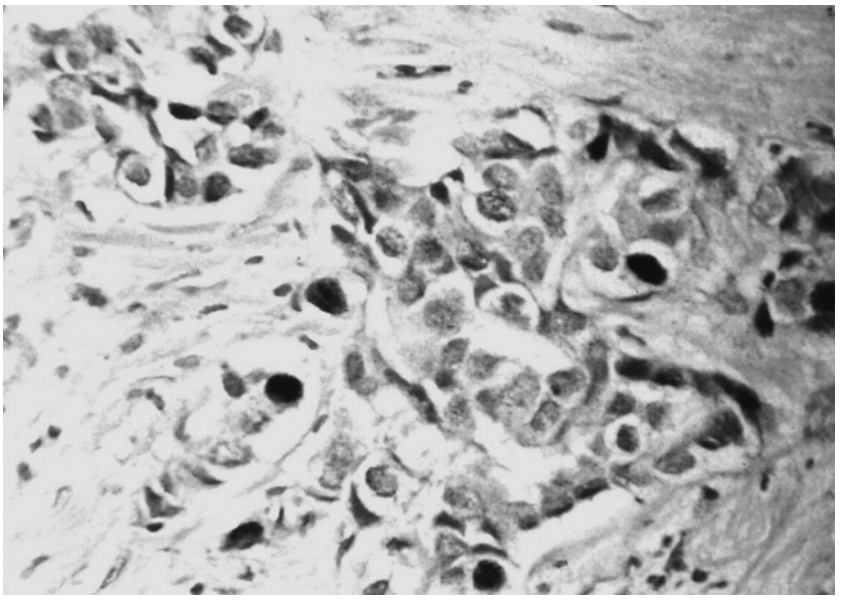


Figure 19.3. Nuclear immunoreactivity for simian vacuolating virus 40 (SV40) in an epithelial mesothelioma (frozen section) with the monoclonal antibody Pab419 visualized with horseradish peroxidase and DAB as substrate.

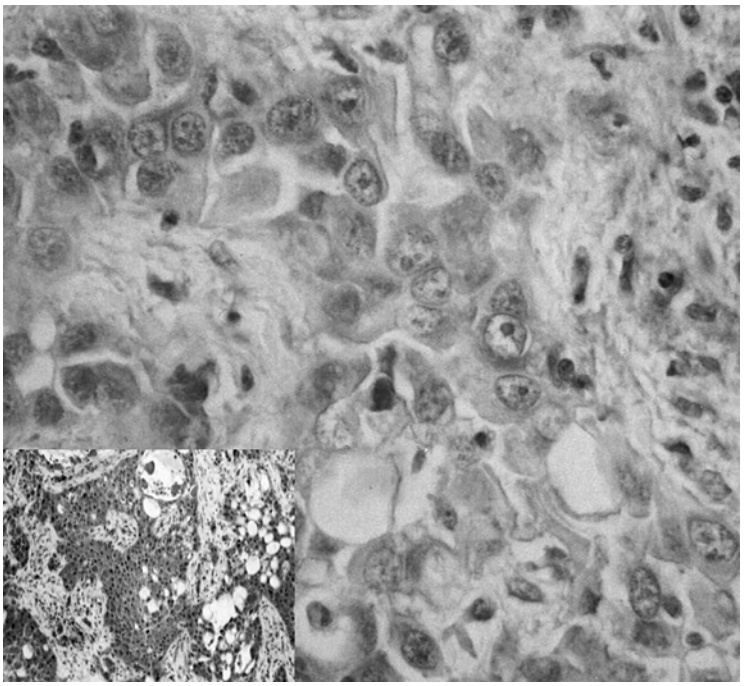


Figure 19.4. Nuclear and cytoplasmic immunoreactivity for simian vacuolating virus 40 (SV40) in an epithelial mesothelioma (paraffin-embedded, formalin-fixed tissue) with the monoclonal antibody Pab280 visualized with peroxidase and DAB as substrate. Not all neoplastic mesothelial cells display both nuclear and cytoplasmic immunoreactivity. The majority of neoplastic cells are immunoreactive (insert).

suggested that although some of these cross-reactions may be fortuitous, they may, as an alternative, reflect similarities of shape and perhaps function between domains of the viral Tag and the relevant host proteins (57).

We found both cytoplasmic and nuclear immunoreactivity with the Pab280 monoclonal antibody (Figs. 19.4 and 19.5). Immunohistochemistry revealed in approximately 60% of paraffin-embedded, formalin-fixed mesothelioma biopsy tissues the presence of tag. These findings were confirmed on the DNA level (26). The antibody is specific for tag and is validated for use in paraffin-embedded, formalin-fixed tissues. Small t-antigen has been found to be present in both the nucleus and the cytoplasm by this antibody. Pab280 reacts strongly with a cytoplasmic form of tag that appears to be associated with the cytoskeleton and is not detected by antibodies directed to the common N-terminus of tag and does not react with Tags. Immunoperoxidase staining of cells infected by the SV40 defective strain SV402 with Pab280 and other T-antibodies demonstrated that this virus produced an N-terminal fragment of Tag as well as tag. In cells infected by the virus, this fragment was located in the cell nucleus but was very unstable.

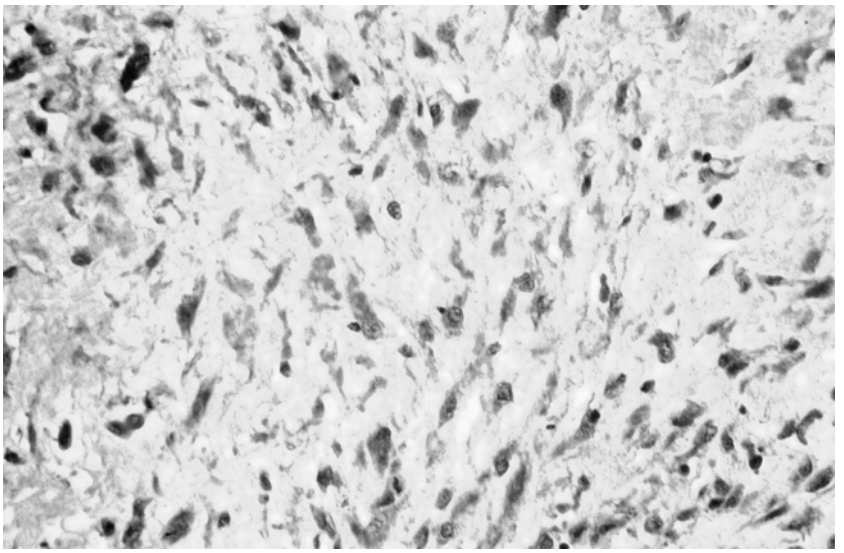


Figure 19.5. Nuclear and cytoplasmic immunoreactivity for simian vacuolating virus 40 (SV40) in a mesenchymal mesothelioma (paraffin-embedded, formalin-fixed tissue) with the monoclonal antibody Pab280 with peroxidase and DAB as substrate.

Specificity of Antibodies and Effects of Fixation on Detection of SV40 Proteins

When discussing the puzzling findings of immunohistochemistry for SV40 antigens in malignant mesothelioma, most authors do not take into account the specificity of the used monoclonal antibody. The widely used Pab419 monoclonal antibody is not only reactive to epitopes situated at Tag but also detects tag. This latter protein is found not only in the nucleus but also in the cytoplasm of infected and transformed cells, thereby explaining the observed cytoplasmic immunoreactivity with this antibody (44,50).

Another point of consideration is that the use of this antibody has been validated for immunohistochemistry only on frozen sections but not for application on paraffin-embedded, formalin-fixed tissues.

Formalin as a cross-linking agent can alter epitopes by rendering them less accessible to the antibody, thereby necessitating the use of antigen retrieval methods. Failure to do this may result in false-negative reports. When addressing the issue of immunoreactivity, one has to take into account that some epitopes are not resistant to formalin fixation, leading to prevention of specific binding to its antibody. Application of the antibody on these fixed tissues can result in false-negative findings. A similar situation has been found when addressing the presence of the nuclear antigen p53 in nonneoplastic mesothelial cells. Formalin fixed mesothelial cells do not display any immunoreactivity for p53 (58). In contrast, the same acetone fixed nonneoplastic mesothelial cells display slight nuclear immunoreactivity with antibodies directed against p53 (59). Application of antigen retrieval

methods, such as pressure cooking in a citrate buffer or a microwave pretreatment in the same buffer, is mandatory when investigating the presence of nuclear antigens such as p53 or hormone receptors for estrogen and progesterone in paraffin-embedded, formalin-fixed tissues. None of the described studies reporting negative findings for SV40 protein in paraffin-embedded mesothelioma tissues addressed this issue properly, thereby invalidating their discussion.

Another effect of prolonged formalin fixation is diffuse cytoplasmic immunoreactivity for nuclear antigens. Cytoplasmic immunoreactivity has also been described for other classic nuclear antigens such as c-myc, the cellular homologue of the v-myc antigen. Fixation times that are longer than 60 to 120 minutes seem to influence the cellular localization of nuclear proteins such as c-myc and result in cytoplasmic immunoreactivity (60). This phenomenon has also been described for c-fos and c-myc in paraffin-embedded, formalin-fixed tissue of pleural malignant mesothelioma (61). It is very remarkable that the studies by Carbone and Orego reporting solely nuclear immunoreactivity in neoplastic mesothelial cells use the same fixation protocol, e.g., short acetone fixation followed by air drying. The studies reporting solely cytoplasmic staining or no immunostaining describe another fixation protocol, e.g., air drying for several hours to overnight followed by acetone fixation. This difference in fixation protocol might be an explanation for the conflicting and at first sight contradictory results reported.

Not only are the antibody and the type of material used critical for obtaining maximal sensitivity and specificity, but also the detection system for revelation of the bound anti–large T-antibody is important. Most research groups describe the use of a classic horseradish peroxidase linked streptavidin-biotin method (ABC method) (17,51,53). However, some groups report the use of more sensitive systems such as dextran polymers coated with alkaline phosphatase, resulting in signal amplification, and the alkaline phosphatase–antialkaline phosphatase (APAAP) method or tyramine amplified immunohistochemistry (26,52,55). These facts also have to be kept in mind when interpreting the results of the various immunohistochemical studies reporting the presence of SV40 antigens in malignant mesothelioma.

Another important point is the use of adequate positive controls when conducting state-of-the-art immunohistochemical surveys. Most studies use SV40-infected or transformed cell lines such as SVEC4-10 or WI-26VA4 or mesothelial cells transfected with SV40. This approach is, in my opinion, scientifically correct, but the positive control cells or tissues have to be processed in the same way as the tissues and cells that are under investigation. In my opinion acetone-fixed, SV40-positive control cells deposited on a glass slide are not adequate controls for an immunohistochemical survey investigating paraffin-embedded, formalin-fixed tissues. Appropriate negative controls have to be included for validating the immunohistochemical procedure. Most studies describe the omission of the first antibody and replacement by buffer solution. It is advisable to include a second negative control per case where the primary antibody has been replaced by another

antibody of the same isotype, at the same concentration but directed against an unrelated antigen normally not present (26,52).

References

1. Sweet B, Hillman R. The vacuolating SV40. Proc Soc Exp Biol Med 1960; 105:420–427.
2. Soriano F, Shelburne E, Gokcen M. Simian virus 40 in a human cancer. Nature 1974;249:421–424.
3. Bergsagel D, Finegold M, Butel J, Kujsky W, Garcea R. DNA sequences similar to those of SV40 in ependymomas and choroid plexus tumours of childhood. N Engl J Med 1992;326:988–993.
4. Carbone M, Rizzo P, Pass HI. Simian virus 40, poliovaccines and human cancer: a review of recent developments. Oncogene 1997;15:1877–1888.
5. Rizzo P, Carbone M, Fisher S, et al. Simian virus 40 is present in most United States human mesotheliomas but is rarely present in non-Hodgkin lymphomas. Chest 1999;116:470S-473S.
6. Ferber D. Monkey virus link to cancer grows stronger. Science 2002;296: 1012–1015.
7. Gazdar A, Butel J, Carbone M. SV40 and human tumours: myth, association or causality? Nature Cancer Rev 2002;2:957–964.
8. Morwood K, Price TNC, Mayne LV. Mutation of p53 is not a prerequisite for immortalization of human fibroblasts by SV40 T antigen. Exp Cell Res 1996;223:308–313.
9. Carbone M, Rizzo P, Grimpley P, et al. Simian virus 40 large T-antigen binds p53 in human mesotheliomas. Nat Med 1997;3:908–912.
10. De Luca A, Baldi A, Esposito V, Howard C, Bagella L, Rizzo P. The retinoblastoma gene family pRb/p105, p1087,pRb2/p130 and simian virus 40 T antigen in human mesotheliomas. Nat Med 1997;8:913–916.
11. Fanning E, Knippers R. Structure and function of simian virus 40 large tumour antigen. Annu Rev Biochem 1992;61:55–85.
12. Bikel I, Montano X, Agha M, et al. SV40 small T-antigen enhances the transformation activity of limiting concentrations of SV40 large T-antigen. Cell 1987;48:321–330.
13. Cicala C, Avantaggiati M, Graessman A, Rundell K, Levine A, Carbone M. Simian virus 40 small t-antigen stimulates viral DNA replication in permissive monkey cells. J Virol 1994;68:3138–3144.
14. Cicala C, Pompetti F, Nguyen P, Dixon K, Levine A, Carbone M. SV40 small t deletion mutants preferentially transform mononuclear phagocytes and B-lymphocytes in vivo. Virology 1992;190:475–479.
15. Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. Am J Pathol 1993;142:1524–1533.
16. Bochetta M, Di Resta I, Powers A, et al. Human mesothelial cells are unusually susceptible to simian virus 40 mediated transformation and asbestos cocarcinogenity. Proc Natl Acad Sci USA 2000;97:10214–10219.
17. Carbone M, Pass H, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994;9:1781–1790.
18. Pass H, Rizzo P, Donington J, Wu P, Carbone M. Further validation of SV40 like DNA in human pleural mesotheliomas. Dev Biol Stand 1998;94:143–145.
19. Griffith D, Nicholson A, Weiss R. Detection of SV40 sequences in human mesotheliomas. Dev Biol Stand 1998;94:127–136.

20. Lednicky J, Garcea R, Bergsagel D, Butel J. Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. Virology 1995;212:710–717.
21. Lednicky J, Stewart A, Jenkins J, Finegold M, Butel J. SV40 DNA in human osteosarcomas shows sequence variation among T-antigen genes. Int J Cancer 1997;72:791–800.
22. Mendoza S, Konishi T, Miller C. Integration of SV40 in human osteosarcoma. Oncogene 1998;17:2457–2462.
23. Pacini F, Vivaldi A, Santoro M, et al. Simian virus 40-like DNA in human papillary thyroid carcinomas. Oncogene 1998;16:665–669.
24. zur Hausen H. Papillomavirus infections—a major cause of human cancers. Biochim Biophys Acta 1996;1288:F55–F78.
25. Kleter B, van Doorn J, ter Schegge J. Novel short fragment PCR assay for highly sensitive broad spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998;53:1731–1739.
26. Ramael M, Nagels J, Heylen H, De Schepper S, Paulussen J, Van Haesendonck C. Detection of SV40 like viral DNA and viral antigens in malignant pleural mesothelioma. Eur Res J 1999;14:1381–1386.
27. Koch J, Kolvraa S, Peetersen P, Gregersen N, Boland L. Oligonucleotide priming methods for the chromosome specific labeling of alpha satellite DNA in situ. Chromosoma (Berlin) 1989;98:259–265.
28. Ramael M, Van Steelandt H, Stuyven G, Van Steenkiste M, Degroote J. Application of the primed in situ labeling (PRINS) method for detection of numerical chromosomal aberrations in paraffin embedded formalin fixed tissue of molar and non-molar pregnancies. Biochemica 1997;2:18–20.
29. Strickler H, Goedert JJ, Fleming M, et al. Simian virus 40 and pleural mesothelioma in humans. Cancer Epidemiol Biomarkers Prev 1996;5:473–475.
30. Testa J, Carbone M, Hirvonen A, et al. A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas. Cancer Res 1998;58:4505–4509.
31. Hirvonen A, Mattson K, Karjalainen A, et al. Simian virus 40 (SV40) like DNA sequences not detectable in Finnish mesothelioma patients not exposed to SV40-contaminated polio vaccines. Mol Carcinogen 1999;26:93–99.
32. Jasani B, Cristaudo A, Emri S, et al. Association of SV40 with human tumours. Semin Cancer Biol 2001;11:49–61.
33. Shivapurkar N, Wiethege T, Wishuba II, et al. Presence of simian virus 40 sequences in malignant mesotheliomas and mesothelial cell proliferations. J Cell Biochem 1999;76:181–188.
34. Cristaudo A, Powers A, et al. SV40 can be reproducibly detected in paraffin embedded mesothelioma samples. Anticancer Res 2000;20:895–898.
35. Cristaudo A, Vivaldi A, Sensales G, et al. Molecular biology studies on mesothelioma tumor samples: preliminary data on H-ras, p21, and SV40. J Environ Pathol Toxicol Oncol 1995;14:29–34.
36. Strickler H, et al. A multicenter evaluation of assays for detection of SV40 DNA and results in masked mesothelioma specimens. Cancer Epidemiol Biomarkers Prev 2001;10:523–532.
37. MacLachlan D. SV40 in human tumours: new documents shed light on the apparent controversy. Anticancer Res 2002;22:957–964.
38. Fahle G, Fisher S. Comparison of six commercial DNA extraction kits for recovery of cytomegalovirus DNA from spiked human specimens. J Clin Microbiol 2000;38:3860–3863.

39. Henderson Y, Liu T, Clayman G. A simple and sensitive method for detecting adenovirus in serum and urine. J Virol Methods 1998;71:51–56.
40. Mulatero C, Surentheran T, Breuer J, Rudd R. Simian virus 40 and human pleural mesotheliomas. Thorax 1999;54:60–61.
41. Klein G, Powers A, Croce C. Association of SV40 with human tumors. Oncogene 2002;21:1141–1149.
42. McLaren B, Haenel T, Stevenson S, Mukerjee S, Robinson B, Lake R. SV40 like sequences in cell lines and tumour biopsies from Australian malignant mesotheliomas. Aust NZ J Med 2000;30:450–456.
43. Greer C, Peterson S, Kiviat N, Manos M. PCR amplification of paraffin embedded tissues: effects of fixative and fixation time. Am J Clin Pathol 1991;95:117–124.
44. Ellman M, Bikel I, Figge J, Roberts I, Schlossman R, Livinston D. Localization of the simian virus 40 small t-antigen in the nucleus and cytoplasm of monkey and mouse cells. J Virol 1984;50:623–627.
45. Tieman F, Zerrahn J, Deppert W. Cooperation of SV40 large and small T-antigens in metabolic stabilization of tumor suppressor p53 during cellular transformation. J Virol 1995;69:6115–6121.
46. Harlow E, Crawford L, Pim D, Williamson N. Monoclonal antibodies specific for simian virus 40 tumour antigens. J Virol 1981;39:861–869.
47. Deppert W. Monoclonal antibodies against SV40 tumour antigens: analysis of antigenic binding sites, using adenovirus 2-simian virus 40 hybrid viruses. J Virol 1981;37:478–482.
48. Gurney E. Monoclonal antibodies against simian virus 40 T-antigens: evidence for distinct subclasses of large T-antigen and for similarities among nonviral T-antigens. J Virol 1980;34:752–763.
49. Montano X, Lane DP. Monoclonal antibody analysis of simian virus 40 small t-antigen. J Virol 1984;51:760–767.
50. Montano X, Lane DP. Monoclonal antibody analysis of simian virus 40 small t-antigen in infected and transformed cells. J Virol 1989;63:3128–3134.
51. Orengo A, Spoletini L, Procopio A, et al. Establishment of four new mesothelioma cell lines: characterization by ultrastructural and immunophenotypic analysis. Eur Respir J 1999;13:527–534.
52. Dhaene K, Verhulst A, Van Marck E. SV40 large T-antigen and human pleural mesothelioma. Screening by tyramine amplified immunohistochemistry. Virchows Arch 1999;435:1–7.
53. Galateau-Salle F, Bidet Ph, Iwatsubo Y, et al. SV40-like DNA sequences in pleural mesothelioma, bronchopulmonary carcinoma and non-malignant pulmonary disease. J Pathol 1998;184:252–257.
54. Simsir A, Fetsch P, Bedrossian C, Ioffe O, Abati A. Absence of SV40 large T-antigen in malignant mesothelioma effusions: an immunocytochemical study. Diagn Cytopathol 2001;25:203–207.
55. Pilatte Y, Vivo C, Renier A, Kheuang L, Greffard A, Jaurand MC. Absence of SV40 large T-antigen expression in human mesothelioma cell lines. Am J Respir Cell Mol Biol 2000;23:788–793.
56. Wynnford-Thomas D. P53 in tumour pathology: can we trust immunohistochemistry? J Pathol 1992;166:329–330.
57. Crawford L, Leppard K, Lane D, Harlow E. Cellular proteins reactive with monoclonal antibodies directed against simian virus 40 T-antigen. J Virol 1982;42:612–620.
58. Ramael M, Eerdekens C, Lemmens G, Deblier I, Jacobs W, Van Marck E. Immunoreactivity for p53 protein in neoplastic and non-neoplastic mesothelial tissues. J Pathol 1992;168:371–375.

59. Dowell S, Derias N, Wilson P, Lane D, Hall P. Expression of p53 in reactive mesothelium. Histopathology 1993;22:96–97.
60. Loke S, Neckers L, Schwab G, Jaffe S. c-Myc protein in normal tissue: effects of fixation on its apparent subcellular distribution. Am J Pathol 1988;131: 29–37.
61. Ramael M, Van den Bossche J, Buysse C, Deblier I, Segers K, Van Marck E. Immunoreactivity for c-fos and c-myc protein with the monoclonal antibodies 14E10 and 6E10 in malignant mesothelioma and non-neoplastic mesothelium. Histol Histopathol 1995;10:639–640.

20

Molecular Detection of Siman Virus 40 in Human Mesothelioma

Bharat Jasani and Katie Ross

Simian virus 40 (SV40) is a polyomavirus of monkey origin. The natural host for SV40 is the Asian macaque, particularly the rhesus (*Macaca mulatta*). Simian virus 40 was discovered as an inadvertent contaminant of some of the early batches (1955–1962) of the inactivated poliovirus vaccine (IPV; Salk vaccine) and the live attenuated oral poliovirus vaccine (OPV; Sabin vaccine) prepared in the United States in rhesus or cynomolgus monkey kidney cells. At about the same time the virus was found to be oncogenic in baby hamsters, causing a select group of tumors (ependymomas, osteosarcomas, lymphomas, and soft tissue sarcomas) depending on the route of viral inoculation. It was also discovered about the same time to be capable of transforming human cells (1).

The recognition of the cross-species oncogenic potential of the virus prompted several epidemiologic investigations stretching over the 1960s and up to the late 1970s. Their aim was to search for any increase in the incidence of tumors in cohorts inadvertently expected to have had received contaminated poliovirus vaccines in the period 1955 to 1962. The results of these and several subsequent investigations have largely proved inconclusive. This has been realized to be due to the difficulty of identifying individuals who were truly exposed to the contaminated vaccines, especially since not all batches were SV40 contaminated and very few batches were tested for the contamination. In addition, the level of contamination of individual batches and the extent of their distribution across the United States were considered to be highly uneven.

The interest in SV40 as a possible human viral carcinogen would have completely waned but for the unexpected molecular findings in the early 1990s of the groups led by Butel in Texas and Carbone in Chicago. Butel's group found SV40 large T-antigen (Tag) oncogene-like DNA presence in human brain tumors (ependymomas and choroid plexus tumors) when searching for equivalent BK virus (BKV) and Jamestown Canyon virus (JCV) gene sequences using a conventional polymerase chain reaction (PCR) technique (2). Similarly, Carbone and colleagues, when studying the capacity of SV40 to cause lymphomas in baby hamsters, by chance discovered SV40's capacity to cause

mesotheliomas in this animal model (3). Carbone et al (4) were prompted by this finding to apply Butel et al's technique to search for SV40 Tag gene presence in human pleural mesothelioma and a set of other tumors. They surprisingly found SV40 Tag DNA sequences in ~70% of human mesothelioma tumor samples and ~30% of osteosarcomas, but none in a variety of other lung and nonlung tumors.

The findings of Butel's group and Carbone's group have been since confirmed by several independent studies (1,5). In addition, more recently the groups led by Butel (6) and Gazdar (7) have produced data to suggest lymphomas to be the fourth major type of tumor showing the presence of episomal SV40 oncogenic sequences. At the same time, Carbone's group (8) has also shown the capacity of the episomal form of SV40 genome to infect human mesothelial cells in a semipermissive mode and facilitate their transformation in the presence of asbestos. The close correspondence observed between the spectrum of SV40 DNA-associated human tumors and the SV40-induced tumors in the hamster model, combined with Carbone et al's in vitro model findings, have led Carbone et al to hypothesize that SV40 may play the role of a co-carcinogen in the causation of human pleural mesothelioma and other SV40-associated human tumors (8,9).

Rationale for SV40 Detection in Human Mesothelioma

The reported rate of detection of SV40 gene sequences in human mesotheliomas has been found to vary from 0% to 90% (5). Demographic differences principally related to the rates of exposure to SV40 contaminant poliovirus vaccines may account for this variation (9). Thus, mesotheliomas from the Turkish and Finnish population unexposed to the contaminated poliovirus vaccines have been recorded to have 0% SV40 DNA association. This is in contrast to the SV40-positive tumor rates of 40% to 90% recorded in parts of the United States and Europe apparently exposed to significant levels of SV40-contaminated IPV and OPV vaccines.

Preliminary reports have suggested morphologic and prognostic differences between SV40-positive and SV40-negative mesotheliomas. The Tag gene- and protein-specific sequences have also been examined as suitable targets for developing SV40 antisense and cytotoxic T-cell therapies, respectively, as well as anti-SV40 prophylactic vaccines.

The above overall findings have generated the need for a reliable method for detection of SV40 in human tissue and tumors on a wide scale for etiopathogenic, diagnostic, prognostic, and therapeutic assessment of mesothelioma cases as well as for effective prophylactic vaccine intervention.

Reliability of SV40 Detection

The reliability of molecular detection of SV40 in the context of human mesotheliomas and other types of human tumors has been constrained by essentially three confounding factors: the low SV40 copy number

presence in the tumor tissue, the episomal status of the tumor-associated SV40 genome, and the potential presence of homologous BKV and JCV human polyomavirus gene sequences. This has necessitated the adoption of methodologic approaches affording the highest assay sensitivity and specificity at the stages of tissue DNA extraction, polymerase chain reaction (PCR) amplification of the target gene sequences, as well as amplicon product analysis.

For the mass screening of tissue material required for demographic causal analysis and prophylactic vaccine testing and application, there is a particular need for a robust, economical, and rapid throughput technique. The latter considerations have led to increasing interest in the adoption of the real-time PCR methodology in preference to the conventional PCR approach.

Reliability of DNA Extraction

The tumor tissue available for analysis is usually a diagnostic biopsy, which may vary in its amount and its composition in terms of the relative proportion of tumor cell content. Therefore, it is essential that the tumor content and quality of the biopsy material included for PCR analysis be assessed histologically in the top and bottom parts of the tissue subjected to the DNA extraction procedure.

The efficiency and quality of DNA extraction depend on the quality of the biopsy material. Thus, a freshly frozen sample usually affords ready extraction of adequate quality and quantity of the target DNA, while paraffin-embedded, formalin-fixed tissue requires a much more vigorous effort.

Since the putative oncogenic form of SV40 genome in human mesothelioma is considered to be episomal, it is essential that the DNA extraction methods used are tailored for efficient extraction of episomal-type small molecular weight DNA fragments of 500 to 5000 base pair length.

Essentially two different types of DNA methods are available: the conventional phenol/chloroform-based organic solvent extraction method, and the modern commercial molecular sieve- or charge separation-based kits. Both these methods have to be preceded by the release of the tissue-bound DNA using adequate proteinase K treatment, because paraffin-embedded, formalin-fixed tissue sections may require as long as 60 to 72 hours (or even longer) of enzyme digestion.

The phenol/chloroform method, though labor intensive, is considered to be the gold standard in that it is designed for total extractable DNA regardless of size. The commercial kit methods, though affording a rapid DNA extraction approach, are not wholly reliable for the extraction of low molecular DNA species. Whichever approach is adopted, it is essential to ensure, by inclusion of appropriate standards, that the desired small viral episomal type DNA of high molecular integrity is efficiently extracted for optimal PCR amplification-based detection.

The reported DNA extraction methods and some of the quality assessment measures are summarized in Table 20.6 and Table 20.7 (pages 341 and 342).

Optimum Polymerase Chain Reaction Conditions

To allow efficient detection of low copy numbers of episomal SV40 associated with human mesotheliomas, it is essential that an adequate amount of the extracted DNA is included in the final PCR mix (e.g., 500 to 1000 ng of DNA per PCR mix is recommended for optimal amplification efficiency).

The choice of the PCR primers and the amplification conditions is based on producing optimally detectable specific target sequence amplification with the minimum number of PCR cycles, to avoid adventitious amplification of any cross-reactive DNA species. Similarly, replicate PCR is conducted on every sample to exclude spurious false-positive results due to chance cross-contamination during tissue sample DNA preparation and subsequent handling. A freshly opened disposable knife should be used for section cutting from each tissue block and discarded after it is used.

The reported conventional and real-time PCR methods used for the detection of SV40 are summarized in Tables 20.1 to 20.9.

Optimum Polymerase Chain Reaction Product Detection

The amplicon product needs to be detected with methods affording the highest objectivity, sensitivity, and specificity. Ethidium bromide–based detection of the gel electrophoresis size-specific PCR product bands has been the most popular approach. However, it requires further confirmation of the specificity of the detected amplicon using either a Southern blot method or DNA sequence analysis.

The reported methods for the detection of SV40-specific PCR amplicon products are summarized in Tables 20.1 to 20.9.

Recommended Method

The PCR method developed by Carbone and colleagues has been recommended as the current gold standard by the multiinstitutional trial (39). The full details of this method have been published by Rizzo et al (40). The essential protocol is listed in the appendix (see below).

Critique of Molecular Detection of SV40

General Considerations

The development of a robust, reliable, and widely applicable molecular method for SV40 detection in human tissue has been necessitated by the inherent incapacity of the current immunologic assays to distinguish SV40 from BKV and JCV. Although immunocytochemical methods are available for in situ identification of SV40, the assay is not

Table 20.1. Reported methodologic details of conventional polymerase chain reaction (PCR)-based studies on human mesothelioma

Primer pair	Amplicon size (base pair, bp)	Position within the genome	Type and amount of tumor sample used for DNA extraction	PCR conditions	Band visualization method	Detection rate	Reference
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	F tissue	40, 52°C	FH	29/48 (60.4%)	10
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	PE tissue	40, 52°C	FH	8/18 (44.4%)	11
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	F and PE tissue, 100 ng DNA/reaction	HS, 45, 63°C	EB	13/28 (46%)	12
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	PE tissue, 300–500 ng DNA/reaction	40, 52°C	FH	26/26 (100%)	13
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	PE tissue	45, 53°C	EB	5/11 (45.5%)	14
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	F tissue 500 ng DNA/reaction	40, 52°C	EB SEQ	9/25 (36%)	15
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	F tissue	45, 52°C	FH	38/42 (90%)	16
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	PE tissue	45, 52°C	EB	4/9 (44.4%)	17
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	F and PE tissue	40, 52°C	EB	57/118 (48%)	18
SV.for3 (4476–4453) SV.rev (4399–4372)	105	4372–4476	PE tissue scraped from slides	40, 52°C	FH	0/50 (0%)	19
SV.for3 (4389–4412) sic SV.rev (4308–4334) sic	105	4308–4412 sic	Cell lines and biopsies	45, 52°C	EB	5/5 (100%) cell lines 7/7 (100%) biopsies	20
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	F tissue	40, 52°C	FH SEQ	29/48 (60.4%)	10
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	F tissue	40, 52°C	FH	10/21 (47.6%)	21
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	PE tissue	40, 52°C	FH SEQ	11/26 (42.3%)	13

PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	PE tissue 200 ng DNA/reaction	40, 52°C	EB	0/12 (0%)	22
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	PE tissue	45, 52°C	EB	4/9 (44.4%)	17
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	PE tissue	HS, 45, 52°C	FH SEQ	32/62 (52%) E MM 17/21 (81%) MM	23
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	PE tissue, pleural effusions	HS, 44, 60°C	FH	9/23 (39.1%) tissue 5/12 (42%) pleural effusions	24
PYV.for (4574–4552) PYV.rev (4425–4402)	172	4402–4574	F tissue	45, 52°C	FH SEQ	29/48 (60.4%)	25
PYV.for (4574–4552) SV.rev (4399–4372)	202	4372–4574	PE tissue scraped from slides	40, 52°C	FH	0/50 (0%)	19
SV.for2 (4945–4970) SV.rev (4399–4372)	574	4945–4372	F tissue 1 μg DNA/ reaction	40, 62°C	EB	0/49 (0%)	26
SV.for2 (4945–4970) SV.rev (4399–4372)	574	4945–4372	F tissue	45, 55°C	FH	10/42 (24%)	16
SV.for2 (4945–4970) SV.rev (4399–4372)	574	4945–4372	PE tissue, pleural effusions	HS, 44, 60°C	FH	9/23 (39.1%) tissue 5/12 (42%) pleural effusions	24
SV.for2 (4945–4970) SV.rev (4399–4372)	574	4945–4372	F tissue	45, 55°C	FH SEQ	29/48 (60.4%)	25
SV.for2 (4857–4881) sic SV.rev (4308–4334) sic	574	4308–4881 sic	Cell lines and biopsies	45, 55°C	EB	0/5 (0%) cell lines 0/7 (0%) biopsies	20
SV8 (2821–2838) SV9 (2548–2565)	289	2548–2821	Cell lines and biopsies	45, 62°C	EB	5/5 (100%) cell lines 7/7 (100%) biopsies	20
SV5 (4551–4570) SV6 (4402–4425)	169	4402–4570	F tissue	45, 59°C	FH SEQ	10/12 (83%)	27
SV1 (not specified) SV2 (not specified)	574	Not specified	PE tissue (Turkey, Italy)	Not specified	EB	0/29 (0%) Turkey 1/1 (100%) Italy	30
RA3 (358–336) RA4 (5119–5142)	413 (485)	358–5119	PE tissue	2 × 35, 52°C	EB SEQ	1/1 (100%)	28

Continued

Table 20.1. *Continued*

Primer pair	Amplicon size (base pair, bp)	Position within the genome	Type and amount of tumor sample used for DNA extraction	PCR conditions	Band visualization method	Detection rate	Reference
RA3 (358–336) **RA4 (5119–5142)**	**482**	**5142–336**	**PE tissue**	**40, 64°C**	**FH**	**10/18 (55.6%)**	**11**
RA1 (266–245) **RA2 (5195–5218)**	**242 (315)**	**266–5195**	**PE tissue**	**2 × 30, 52°C**	**EB** **SEQ**	**1/1 (100%)**	**28**
R1 (266–245) **R2 (5195–5218)**	**315**	**266–5195**	**F tissue**	**40, 60°C**	**EB**	**22/42 (52.4%)**	**16**
Not specified	**739**	**4400–5138**	**Cell lines**	**35, 58°C**	**EB**	**5/5 (100%)**	**29**
TA1 (3070–3049) **TA2 (2630–2652)**	**441**	**2630–3070**	**PE tissue**	**2 × 35, 52°C**	**EB** **SEQ**	**1/1 (100%)**	**28**
T3 (not specified) **T4 (not specified)**	**338**	**Not specified**	**PE tissue (Turkey, Italy)**	**Not specified**	**EB**	**0/29 (0%) Turkey** **1/1 (100%) Italy**	**30**
C-terminus of Tag	**329**	**2573–2902**	**F tissue**	**45, 59°C**	**FH** **SEQ**	**10/12 (83%)**	**27**

EB, ethidium bromide staining; FH, filter DNA hybridization; F, snap-frozen; PE, paraffin-embedded; HS, heparan sulfate; MM, malignant mesothelioma.

Primers' sequences (5′-3′)

Waheed et al	AGTCCTCGAGTCTTTGCAGCTAATGGACCT
	AGTCTCTAGATCCTTTGTGGTGTAAATAGC
SV.for3	TGAGGCTACTGCTGACTCTCAACA
SV.for2	CTTTGGAGGCTTCTGGGATGCAACT
SV.rev	GCATGACTCAAAAAACTTAGCAATTCTG
PYV.for	TAGGTGCCAACCTATGGAACAGA
PYV.rev	GGAAAGTCTTTAGGGTCTTCTACC
T3	ACCACAACTAGAATGCAGTGAAAAAA
T4	GAAGACAGCCAGGAAGAAAATGCTGATAA
R1	AATGTGTGTCAGTTAGGGTGTG
R2	TCCAAAAAAAGCCTCCTCACTACTT
SV1	CTTGGAGGCTTCTGGGATGCAAACT
SV2	GCATGACTCAAAAAACTTAGCAATTCTG
SV5	TAGATTTCCAACCTATGGAACTGAT
SV6	GGAAAGTCCTTGGGGTCTTCTACC
RA1	AATGTGTGTCAGTTAGGGTGTG
RA2	TCCAAAAAAGCCTCCTCACTACTT
RA3	GCGTGACAGCCGGCGCAGCACCA
RA4	GTCCATTAGCTGCAAAGATTCCTC
SV5	TAGATTCCAACCTATGGAACTGAT
SV6	GGAAAGTCCTTGGGGTCTTCTACC
SV8	GCCAGGAAAATGCTGATA
SV9	GATGCTATTGCTTTATTT
TA1	GACCTGTGGCTGAGTTTGCTCA
TA2	GCTTTATTTGTAACCATTATAAG

Source: Adapted from Jasani et al (5).

Table 20.2. Reported real-time PCR methods used for SV40 detection

Detection target	Outline of method	Real-time method	Reference
JCV and BKV	Semi-nested PCR to give qualitative results for differentiation between JCV and BKV Real-time PCR protocol to give quantitative result for assessment of human polyomavirus DNA in urine Overall objective to develop clinical diagnostic assay	Taqman real-time PCR 7700 sequence detection system; Applied Biosystems	31
SV40	Detection of SV40 by real-time PCR using developed primer sets, dependent on the dissociation profile of primer sets; also applies Southern blotting techniques for verification of positives	5700 sequence detection system; Applied Biosciences	32
SV40	Aim to detect SV40 DNA in both mesothelioma and cell line material; reaction kinetics study Further verification of SV40 DNA achieved via Southern blotting	SYBR green binding dye; Applied Biosystems	33
SV40, JCV, and BKV	Detection of SV40, JCV, and BKV in lymphoma samples, from lymph node biopsies and some blood samples; fluorescent Taqman probe approach—direct sequence binding	Taqman real-time PCR 7700 sequence detection system; Applied Biosystems	34
JCV and BKV	Detection of JCV and BKV in renal cell carcinomas and tumors of renal pelvis; fluorescent Taqman probe approach—direct sequence binding Increased number of cycles	Taqman real-time PCR 7700 sequence detection system??; Applied Biosystems	35
SV40	Development of real-time PCR method for quantitative analysis of SV40; subsequent publication aimed at detection of SV40 after purification of pharmaceutical proteins; fluorescent Taqman probe approach—direct sequence binding Standard cycling conditions as recommended, but lowered extension temperature	Taqman real-time PCR 7700 sequence detection system; Applied Biosystems	36
JCV and BKV	The detection and differentiation of JCV and BKV in urine from a diverse sample population—samples from varied clinical sources Fluorescence resonance energy transfer probe method developed	FRET method Roche light cycler	37

Table 20.3. Reported primer pair sets and their targets templates used in real-time PCR studies on SV40 detection

Primer set	Sequence	Primer and probe location	Reference
Semi-nested PCR			31
PV-SNFOR	TCTTTAGTRGTATACACAGCAAA	4307–4329 BKV 4170–4192 JCV	
PV-BACK	GGTGCCAACCTATGGAACAG	4548–4567 BKV 4408–4427 JCV	
BK-FOR	ACAGCAAAGCAGGCAAG	4322–4338 BKV	
JC-FOR	GAGGAATGCATGTGCCCATCT	4229–4248 JCV	
Taqman PCR			
Large T-antigen			
PV-TMFOR	TCTATTACTAAACACAGCTTACT	4342–4365 BKV 4205–4228 JCV	
PV-BACK	GGTGCCAACCTATGGAACAG	4548–4567 BKV 4408–4427 JCV	
PV-PROBE	TGGAAAGTCTTTAGGGTCTTCTACCTT	4387–4413 BKV 4250–4276 JCV	
Large T-antigen			32
SV.for 3	TGAGGCTACTGCTGACTCTCAACA	4476–4453	
SV.rev	GCATGACTCAAAAAACTTAGCAATTCTG	4399–4372	
SV5	TAGATTCCAACCTATGGAACTGAT	4551–4570	33
SV6	GGAAAGTCCTTGGGGTCTTCTACC	4402–4425	
Large T-antigen			34
SV40 5′	TTTGGGCAACAAACAGTGTAGC	3521–3543	
SV40 3′	TGTTTGGTTCTACAGGCTCTGC	3577–3598	
SV40 probe	AAGCAACTCCAGCCATCCATTCTTCTAT	3545–3572	
JCV 5′	AGAGTGTTGGGATCCTGTGTTTT	4298–4320	
JCV 3′	GAGAAGTGGGATGAAGACCTGTTT	4352–4375	
JCV probe	TCATCACTGGCAAACATTTCTTCATGGC	4323–4350	

BKV 5′	**TTGCTTCTTCATCACTGGCAA**	**4455–4475**	
BKV 3′	**AGTCCTGGTGGAGTTCCTTTAATG**	**4515–4538**	
BKV probe	**CATATCTTCATGGCAAAATAAATCTTCATCCCATTT**	**4477–4512**	
BKV capsid			**35**
Taqman 5′	**CCCAGTTAAACTGGACAAAGGC**	**365–386**	
Taqman 3′	**GTTTTACCAACTTTCACWGAAGCTTG**	**437–412**	
Taqman probe	**TGGTTCTGCGCCAGCTGTCACG**	**389–410**	
BKV T-antigen (large and small)			
Taqman 5′	**ATTCATTCTCTTCATTTTATCCTCGTC**	**4986–5012**	
Taqman 3′	**AATCTTCCCTTAATGAGAAAAGCTTATTTA**	**5078–5049**	
Taqman probe	**CCCTTTGTCAGGGTGAAATTCCTTACACTTC**	**5016–5046**	
JCV large T-antigen			
Taqman 5′	**CCACCCCAGCCATATATTGC**	**3493–3512**	
Taqman 3′	**TTGAAAGGTTTAATTTYTTGCTTGATAA**	**3582–3555**	
Taqman probe	**AAACAGCATTGCCATGTGCCCCA**	**3517–3539**	
SV40 large T-antigen			**36**
Taqman 5′ primer	**GTCTTCTACCTTTCTCTTCTTT**	**4416–4438**	
Taqman 3′ primer	**GGAGCAGTGGTGGAA**	**4530–4545**	
Taqman probe	**TGGAGGAGTAGAATGTTGAGAGTC**	**4440–4464**	
VP2			**37**
PoL1s	**CACTTTGGGGGACCTAGT**		
PoL1as	**CTCTACAGTAGCAAGGGATGC**		
PoLP1 probe (fluoresceine)	**TCTGAGGCTGCTGCTGCCACAGGATTT**		
PoLP2 probe (LC red)	**AGTAGCTGAAATTGCTGCTGGAGAGGCTGCT**		

Key: **R** A/G (1:1).
T 5-carboxytetramethylrhodamine (5-TAMRA).

Table 20.4. Reported reaction conditions used in real-time PCR studies on SV40 detection

Reaction Conditions	Cycling Parameters	Reference
Semi-nested PCR: differentiation 30-μL reaction volume 5-μL template 333-nM primer 50 μM each dNTP 1 U *platinum* Taq polymerase 3 μL 10× amplification buffer	*Semi-nested PCR: differentiation* 94°C for 3 min initial denaturation 94°C for 20 s 53°C for 20 s } 30 cycles 72°C for 5 min final extension	31
Taqman round: quantitative for human polyomaviruses 50-μL reaction volume 5-μL template 200 nM each primer 100-nM exonuclease probe 200 μM each dNTP 5 μl 10× amplification buffer 1 μM ROX 2 U *platinum* Taq polymerase	*Taqman round: quantitative for human polyomaviruses* 3-min initial denaturation 94°C for 25 s 65°C for 50 s } 45 cycles	
10-μL extracted DNA template 0.2-μM primer or 0.2-μM SYBR green dye 200 μM each dNTP 10 mM Tris HCl pH 8.0 50 mM KCl 4 mM $MgCl_2$ 5 U Amplitag gold DNA polymerase	50°C for 2 min 95°C for 12 min 95°C for 15 s 55°C for 30 s } 50 cycles	32
25-μL reaction volume 100 ng DNA template 900-nM primers SYBR green PCR mastermix kit used (Applied Biosystems)	Cycling parameters not detailed	33
50-μl reaction volume 100-ng DNA template 50-nM primers SV40 300-nM primers JCV and BKV 200-nM probe Taqman universal Mastermix kit (Applied Biosystems)	50°C for 2 min 95°C 10-min initial denaturation 95°C 15 s 60°C 60 s } 40 cycles	34
50-μL reaction volume 5-μL template 300-nM primer 300-nM probe 200 nM each dNTP 50 mM KCl 10 mM Tris-HCl pH 8.3 5 mM $MgCl_2$ 1.25 U AmpliTaq gold DNA polymerase	95°C for 10 min 95°C for 15 s 60°C for 60 s } 45 cycles	35
50-μL reaction volume 10-μL DNA template 300-nM primer 100-nM probe 5 mM $MgCl_2$ 1.25 U AmpliTaq gold DNA polymerase	95°C for 10-min initial denaturation 95°C for 15 s 55°C for 60 s } 40 cycles	36

Table 20.4. *Continued*

Reaction Conditions	Cycling Parameters	Reference
No details on dNTP concentration or reaction buffer Standard is 1× supplied amplification buffer 200 uM each dNTP		
20-µL reaction volume 5-µL DNA template 4 mM $MgCl_2$ 2 pmol sense primer 8 pmol antisense primer 4 pmol each probe 2 µl 10× FastStart DNA master hybridization probes mix (kit; Roche)	95°C for 10 min 95°C for 10 s 55°C for 10 s 72°C for 10 s } 55 cycles	37

Table 20.5. Reported methods utilized for SV40, JCV, or BKV detection verification in real-time PCR studies on SV40 detection

Method	Reference
Qualitative semi-nested PCR used in conjunction with the Taqman to differentiate the viruses JCV and BKV; Taqman method used to quantify the amount of human polyoma virus in DNA only	31
Further characterized by hybridization; Southern blotting	32
Southern blot analysis	33
No further verification required	34
Utilized cloning from positive samples to achieve duplicate results for quantitation purposes for each patient	35
No further verification required	36
Light cycler melting curve analysis for virus differentiation; probe does not distinguish between viruses but reaction kinetics do	37

Table 20.6. Reported DNA extraction methods used for real-time PCR studies on SV40 detection

Extraction method	Reference
Qiagen DNA Extraction Kit or Tris EDTA buffered proteinase K digestion	31
Proteinase K extraction followed by DNAeasy Cleanup Kit (Qiagen)	32
QIAamp DNA Kit (Qiagen) (both for cell lines and tissue)	33
QIAamp DNA Blood Mini Kit (Qiagen) and proteinase K digestion and organic solvent extraction	34
High pure PCR template preparation kit (Roche Molecular Biolochemicals, NZ) polyA carrier RNA added to elution buffer	35
Needle microdissection of stained tissue from paraffin embedded tissues	36
Laser microdissection (cases of limited tissue availability); both treated with proteinase K in expanded lysis buffer (Boehringer Mannheim)	
DNA prepared with QIAamp blood and tissue kits (Qiagen)	
QIAamp 96 spin Blood Kit (Qiagen) (cell lines used for detection)	37
QIAamp DNA Blood Mini Kit	

Both methods, proteinase K digestion and QIAamp DNA Kits, have previously been evaluated as adequate approaches for the extraction of SV40 DNA from tissues and blood (38,39).

Table 20.7. Reported DNA quality assurance methods used for real-time PCR studies on SV40 detection

Method	Explanation	Reference
Amplification of the β-actin gene	Taqman method to quantify human genomic actin DNA	31
Amplification of the gene glyceraldehyde-6-phosphate dehydrogenase	Amplified on SDS as SV40, used to estimate total cell number as described as single copy gene	32
DNA quantified spectrophotometric analysis Purified SV40 strain 776 used for standard curve and viral loss analysis in extraction	Spiked samples with known copy numbers of SV40; readily accurately quantified Detection of SV40 in spiked cell line extracts showed DNA isolation and purification not compromised.	33
Amplification of human β-globin	Used as an indicator for amplifiable DNA indication in many publications	34
Quantitation of cellular *PDH* gene by Taqman PCR	Evaluate efficiency of microdissection and extraction methods	35
Serial dilution of known quantity of SV40 extracted through kit and quantified on Taqman	Assessed for consistency in extraction through quantification after cycling on Taqman; corresponding DNA quantity analyzed for loss compared to DNA quantity known prior to extraction	36
Amplicon detection through enzyme-linked amplicon hybridization assay (ELAHA) Conventional PCR followed by ELAHA	No method described for extraction quality assurance; this method was used for sensitivity analysis of the developed real-time method, by detecting presence of amplified material through viral specific fluorescent probes; absorbance detection greater than standard denoted a positive result; primers directed at homologous region in JCV and BKV for conventional PCR	37

quantitative and may lack specificity as well as adequate sensitivity for the detection of low levels of Tag expression in neoplastic cells. It also relies on the use of Tag-specific monoclonal antibodies targeted at epitomes based in the variable portion of the Tag, which therefore may be liable to gene mutation–related loss of activity, giving rise to false-negative results.

Although the molecular detection method affords the highest levels of specificity and sensitivity, it is technically demanding in skill and care. It is also labor intensive and time-consuming. The modern real-time PCR method and instrumentation offer a partial solution for wide-scale analysis, but the current methods are still dependent on the verification of SV40-specific sequences as distinct from those of BKV and JCV sequences, with Southern blot or direct sequence analysis. A real-time PCR method capable of directly discriminating the presence of SV40 Lag-specific sequences in a mixture of polymer virus Lag sequences is needed. Recently McKenzie et al (34) have reported a method of this type.

Table 20.8. Comparison of primer sets and locations used in quality assurance methods

Primer set used	Primer location	Reference
huAct se TCACCCACAATGTGCCCATCT huAct as GTGAGGATCTTCATGAGGTAGTCAGTC Taqman huAct F-ATGCCCTCCCCCATGCCATGCCATCCTGCGT-p	Human actin gene 486–506 565–591 516–541	31
Primer sets not given; stated as unpublished data		32
N/A		33
βglobin 5′ primer GGCAACCCTAAGGTGAAGGC βglobin 3′ primer GGTGAGCCAGGCCATCACTA β-globin probe CATGGCAAGAAAGTGCTCGGTTGCCT		34
Taqman 5′ primer TCGATCGGGACTGCTTTCC Taqman 3′ primer CCCACAACCTAGCACCAAAAGA Taqman probe CATCTCCTTTTGCTTGGCAAATCTGATCC	Human *PDH* gene 44–62 123–102 68–96	35
See table below for Taqman primers and probes for real-time assay		36
Primer PoE1s GGAGGAGTAGAAGTTCTAGAA Primer PoE2as TCTGGGTACTTTGTYCTGTA Probe1:PoEBP1 BKV specific Biotinyl-GCTTAACCTTCATGCAGGGTCACA Probe2:PoEJP2 JCV specific Biotinyl-GATGAATGTGCACTCTAATGGTCA	*VP1* gene Location not specified Amplicon size 434	37

Key: T 5-carboxytetramethylrhodamine (5-TAMRA).
F 6-carboxyfluorescin attached to the 5′ terminus (FAM).
p phosphate group attached to the 3′ terminus.

There also remains the need for improving the efficiency of DNA extraction and recovery of episomal copies of SV40 DNA. The currently available commercial kits are not considered to be adequately effective in this respect. There is also a need for developing templates more closely representative of the small episomal form of SV40 viral DNA as standards for determining the adequacy of SV40 DNA extraction from tumor tissue samples and the amplification sensitivity of the PCR method. The use of SV40 DNA derived from semipermissively infected mesothelial cells could be one option.

Special Considerations for PCR Analysis on Paraffin-Embedded Tissue Samples

The extraction method should be standardized to ensure adequate extraction of low molecular weight DNA (at least up to 300-bp size) from paraffin-embedded tissue samples. The resulting DNA extracts

Table 20.9. Reaction conditions and cycling parameters for quality assurance methods

Reaction conditions	Cycling parameters	Reference
50-μL reaction volume 5-μL template 200-nM each primer 100-nM exonuclease probe 200 μM each dNTP 5 μL 10× amplification buffer 1 μM ROX 2 U platinum Taq	3-min initial denaturation 94°C for 25 s 65°C for 50 s 45 cycles Fluorescence read during PCR cycling	31
10-μL extracted DNA template 0.2-μM primer or 0.2 μM SYBR green dye 200-μM each dNTP 10 mM Tris HCl pH 8.0 50 mM KCl 4 mM $MgCl_2$ 5 U AmpliTaq gold DNA polymerase	50°C for 2 min 95°C for 12 min 95°C for 15 s 55°C for 30 s 50 cycles	32
N/A		33
50-μL reaction volume 100-ng DNA template 50-nM primers SV40 300-nM primers JCV and BKV 200-nM probe No concentration given for β-globin primers and probe Taqman Universal Mastermix Kit (Applied Biosystems)	50°C for 2 min 95°C 10-min initial denaturation 95°C 15 s 60°C 60 s 40 cycles	34
50-μL reaction volume 5-μL template 300-nM primer 300-nM probe 200 nM each dNTP 50 mM KCl 10 mM Tris-HCl pH 8.3 5 mM $MgCl_2$ 1.25 U AmpliTaq Gold DNA polymerase	95°C for 10 min 95°C for 15 s 60°C for 60 s 45 cycles	35
50-μL reaction volume 10-μL DNA template 300-nM primer 100-nM probe 5 mM $MgCl_2$ 1.25 U AmpliTaq Gold DNA polymerase No details on dNTP concentration or reaction buffer Standard is 1× supplied amplification buffer 200 uM each dNTP	95°C for 10-min initial denaturation 95°C for 15 s 55°C for 60 s 40 cycles	36
Conventional PCR 50-μL reaction volume 5-μL template 10-pmol primer 200 μM each dNTP 400 μM dUTP 2.5 mM $MgCl_2$ 2 U platinum *Taq* Polymerase 5-μL 10× reaction buffer ELAHA method, detailed in reference	Conventional PCR parameters 94°C for 2 min 94°C for 20 s 55°C for 20 s 45 cycles 72°C for 20 s 72°C for 7-min final extension	37

should also be ensured to be free of inhibitors, which could interfere with the PCR reaction.

The PCR primers should be designed to target regions in the SV40 genome that display the least homology with JCV and BKV sequences and any other cross-reactive DNA sequences. The amplicon size should be designed to be less than 200 bp to avoid false-negative results due to fragmented DNA. Most real-time methods have been designed to compensate for this by having primer design parameters that specify the close proximity of the forward and reverse primer to the probe, and the amplicon region is often recommended to be no greater than 150 bp (for example, as specified in Primer Express software by Perkin Elmer/Applied Biosystems).

Conclusion and Future Work

Ever since the discovery in 1960 of SV40 as a contaminant of poliovirus vaccines and the subsequent realization of its oncogenic properties in the hamster model and its transforming activity in human cells, there has been a steady interest in the analysis of SV40 as a potential human carcinogen. However, the inherent ineffectiveness of cohort-based epidemiologic studies to establish the causal role of SV40 with respect to the suspected forms of human tumors has forced reliance on the analysis of SV40 or the molecular association with human tumors in different demographic settings as an alternative approach. Among the methods available for molecular detection of SV40 in human tissue, the use of the PCR technique has proved the most reliable and versatile. While the majority of the early studies relied on the conventional PCR method, there has been a growing trend toward the use of the more efficient real-time PCR assays for a wide-scale analysis. This chapter presented the principles of the methodologies used thus far and provided an evaluation of their efficacy, particularly with respect to analysis of the large number of tissue samples required for charting the possible causal role of SV40 in human mesothelioma and other tumors. In view of the putative episomal and low copy number SV40 association with human mesothelioma, the need for efficient extraction of this type of DNA linked to high amplification PCR analysis was also discussed.

The putative co-carcinogenic role of SV40 in the formation of certain types of human tumors has raised the need for accurate detection of the viral DNA in human tissue for demographic studies, in diagnostic and prognostic analysis, and in assessing potential therapeutic and prophylactic measures against the virus-associated tumor types. The PCR-based molecular detection of SV40 presently offers the most reliable and versatile approach. However, further refinement and standardization of the current methodology is necessary to allow its widespread acceptance and application. The use of a multiplex real-time PCR method linked to a simultaneous specific detection of SV40, BKV, and JCV DNA offers a possible solution.

Appendix

The protocol for the detection of SV40 in human mesothelioma by PCR is derived from Rizzo et al (40).

DNA Extraction

Fresh/Frozen Tissue

- From 25 to 125 mg frozen/fresh tissue containing a large proportion of well-preserved tumor tissue verified by frozen section histologic analysis
- Oncor (Gaithersburg, MD) commercial kit used for extraction
- Protocol includes lysis step, followed by 12-hour incubation with proteinase K
- DNA recovered by ethanol precipitation and centrifugation (17,400 $\times g$)
- Resuspended in TE buffer
- 1 µg extract used in PCR

Paraffin-Embedded Tissue

- 20 to 50-µm sections from a tissue block containing a large proportion of well-preserved tumor tissue verified by histologic analysis
- Incubation overnight in 1 mL xylene
- Centrifuged, xylene removed, washed twice with 1 mL ethanol, air dried
- Treated with lysis buffer [50 mM Tris-HCl pH 8.5, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.5% Tween 20] with 0.4 mg/mL proteinase K, incubated overnight at 50°C
- Proteinase K added to final concentration 0.8 mg/mL and incubated for a further 2 hours
- Phenol extracted and ultrafiltrated (Centricon 30 tubes, Amicon)
- Final volume in 40 µL water, 10 µL used in PCR

PCR

- Tested for amplification using alu or β-globin specific primers
- Semi-nested method 100 µL reaction (SV40 testing)
- Conditions: 2.5 mM $MgCl_2$, 0.5 µM each primer (rest as stated by manufacturer, Applied Biosystems)
- Cycling parameters: 3 min @ 94°C initial denaturation/hot start, 45 cycles of 1 min @ 94°C, 1 min @ 55–60°C, 1 min @ 72°C
- For paraffin-embedded tissues the annealing stage is set at 2 min @ 55°C
- Primers: Pyv.for and Pyv.rev 173-bp fragment for SV40 JCV and BKV; SV3.for and SV.rev 105-bp fragment of SV40 but also interact with other papovaviruses, used predominantly for paraffin-embedded tissue; SV2.for and SV.rev 576-bp fragment specific for SV40, predominantly used for fresh or frozen tissue

Southern Blotting

- 2% agarose gel used to visualize PCR products/amplicons (20 µL of PCR reaction used)
- Overnight transfer in 0.4 N NaOH to positively charged membrane
- Hybridized with SV40-specific ^{32}P-end-labeled oligonucleotide probe
- 10 pmol probe in 10 µL reaction for labeling, followed by gel filtration for unincorporated ^{32}P removal using Chromaspin columns
- Hybridization overnight in 10 mL hybridization solution: 5× sodium saline phosphate/EDTA (SSPE), 5× Denhardt's solution, 0.5% SDS, and 100 µg/mL of salmon sperm DNA at 52°C
- Filters washed at 52°C with final stringency of 0.5 × SSC 0.1% SDS and exposed to x-ray film at room temperature (≤30 min)

Sequence Analysis

Fragments are separated on 2% agarose gel, removed from gel after Southern blotting determines correct amplicons, and subjected to direct sequence analysis.

References

1. Jasani B, Cristaudo A, Emri SA, et al. Association of SV40 with human tumours. Semin Cancer Biol 2001;11:49–61.
2. Bersagel DJ, Finegold MJ, Butel JS, Kupsky WJ, Garcea RL. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumours of childhood. N Engl J Med 1992;326:988–993.
3. Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. Am J Pathol 1993;142:1524–1533.
4. Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994;9:1781–1790.
5. Jasani B, Jones CJ, Radu C, et al. Simian virus 40 detection in human mesothelioma: reliability and significance of the available molecular evidence. Frontiers Biosci 2001;6:e12–22.
6. Vilzez RA, et al. Association between simian virus 40 and non-Hodgkin lymphoma. Lancet 2002;359:817–823.
7. Shivapurkar N, et al. Presence of simian virus 40 DNA sequences in human lymphomas. Lancet 2002;359:851–852.
8. Gazdar AF, Butel JS, Carbone M. SV40 and human tumours: myth, association or causality. Nature Rev 2002;2:957–964.
9. Carbone M, Pass HI, Miele L, Bocchetta M. New developments about the association of SV40 with human mesothelioma. Oncogene 2003;22:5173–5180.
10. Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1994;9:1781–1790.
11. Cristaudo A, Powers A, Vivaldi A, et al. SV40 can be reproducibly detected in paraffin-embedded mesothelioma samples. Anticancer Res 2000;20:895–898.
12. Dhaene K, Verhulst A, Van Marck E. SV40 large T-antigen and human pleural mesothelioma. Screening by polymerase chain reaction and tyramine-amplified immunohistochemistry. Virchows Arch 1999;435:1–7.

13. Griffiths DJ, Nicholson AG, Weiss RA. Detection of SV40 sequences in human mesothelioma. Dev Biol Stand 1998;94:127–136.
14. Mayall FG, Jacobson G, Wilkins R. Mutations of p53 gene and SV40 sequences in asbestos associated and non-asbestos-associated mesotheliomas. J Clin Pathol 1999;52:291–293.
15. Mutti L, De Luca A, Claudio PP, Convertino G, Carbone M, Giordano A. Simian virus 40-like DNA sequences and large-T antigen-retinoblastoma family protein pRb2/p130 interaction in human mesothelioma. Dev Biol Stand 1998;94:47–53.
16. Pass H, Rizzo P, Donington J, Wu P, Carbone M. Further validation of SV40-like DNA in human pleural mesotheliomas. Dev Biol Stand 1998;94:143–145.
17. Pepper C, Jasani B, Navabi H, Wynford-Thomas D, Gibbs AR. Simian virus 40 large T antigen (SV40LTAg) primer specific DNA amplification in human pleural mesothelioma tissue. Thorax 1996;51:1074–1076.
18. Shivapurkar N, Wiethege T, Wistuba II, et al. Presence of simian virus 40 sequences in malignant mesotheliomas and mesothelial cell proliferations. J Cell Biochem 1999;76:181–188.
19. Strickler HD, Goedert JJ, Fleming M, et al. Simian virus 40 and pleural mesothelioma in humans. Cancer Epidemiol Biomarkers Prev 1996;5:473–475.
20. McLaren BR, Haenel T, Stevenson S, Mukherjee S, Robinson BW, Lake RA. Simian virus (SV) 40 like sequences in cell lines and tumour biopsies from Australian malignant mesotheliomas. Aust NZ J Med 2000;30:450–456.
21. Galateau-Salle F, Bidet P, Iwatsubo Y, et al. Detection of SV40-like DNA sequences in pleural mesothelioma, bronchopulmonary carcinoma and other pulmonary diseases. Dev Biol Stand 1998;94:147–152.
22. Mulatero C, Surentheran T, Breuer J, Rudd RM. Simian virus 40 and human pleural mesothelioma. Thorax 1999;54:60–61.
23. Procopio A, Strizzi L, Vianale G, et al. Simian virus-40 sequences are a negative prognostic cofactor in patients with malignant pleural mesothelioma. Genes Chromosomes Cancer 2000;29:173–179.
24. Strizzi L, Vianale G, Giuliano M, et al. SV40, JC and BK expression in tissue, urine and blood samples from patients with malignant and non-malignant pleural disease. Anticancer Res 2000;20:885–889.
25. Rizzo P, Di Resta I, Powers A, et al. The detection of simian virus 40 in human tumors by polymerase chain reaction. Monaldi Arch Chest Dis 1998;53:202–210.
26. Hirvonen A, Mattson K, Karjalainen A, et al. Simian virus 40 (SV40)-like DNA sequences not detectable in Finnish mesothelioma patients not exposed to SV40-contaminated polio vaccines. Mol Carcinogenesis 1999;26:93–99.
27. Testa JR, Carbone M, Hirvonen A, et al. A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas. Cancer Res 1998;58:4505–4509.
28. Arrington AS, Lednicky JA, Butel JS. Molecular characterisation of SV40 DNA in multiple samples from a mesothelioma. Anticancer Res 2000;20:879–884.
29. Waheed I, Guo ZS, Chen GA, Weiser TS, Nguyen DM, Schrump DS. Antisense to SV40 early gene region induces growth arrest and apoptosis in T-antigen-positive human pleural mesothelioma cells. Cancer Res 1999;59:6068–6073.
30. Emri S, Kocagoz T, Olut A, Gungen Y, Mutti L, Baris YI. Simian virus 40 is not a cofactor in the pathogenesis of environmentally induced malignant pleural mesothelioma in Turkey. Anticancer Res 2000;20:891–894.

31. Biel SS, et al. Rapid quantification and differentiation of human polyomavirus DNA in undiluted urine from patients after bone marrow transplantation. J Clin Microbiol 2000;38:3689–3695.
32. Engels EA, et al. Absence of simian virus 40 in human brain tumours from northern India. Int J Cancer 2002;101:348–352.
33. Gordon GJ, et al. Detection and quantification of SV40 large T-antigen DNA in mesothelioma tissues and cell lines. Oncol Rep 2002;9:631–634.
34. MacKenzie J, et al. Association between simian virus 40 DNA and lymphoma in the United Kingdom. Brief communications. J Natl Cancer Inst 2003;95:1001–1003.
35. Knöll A, et al. Low frequency of human polyomavirus BKV and JCV DNA in urothelial carcinomas of the renal pelvis and renal cell carcinomas. Oncol Rep 2003;10:487–491.
36. Shi L, et al. A real time quantitative PCR-based method for the detection and quantification of simian virus 40. Biologicals 1999;27:241–252.
37. Whiley DM, et al. Detection and differentiation of human polyomaviruses JC and BK by lightcycler PCR. J Clin Microbiol 2001;39:4357–4361.
38. Lednicky et al. Dev Biol Stand 1998;94:155–164.
39. Strickler D, et al. Cancer Epidemiol Biomarkers Prev 2001;10:523–532.
40. Rizzo P, et al. The detection of simian virus 40 in human tumours by polymerase chain reaction. Monaldi Arch Chest Dis 1998;53:202–210.

21

Malignant Mesothelioma Following Radiation

Katherine D. Crew, Alfred I. Neugut, and Karen H. Antman

The strong association between asbestos exposure and malignant mesothelioma has been widely accepted since 1960 (1,2). Although asbestos is the primary etiologic agent for this tumor, a significant number of patients who develop mesothelioma have no known asbestos exposure. Radiation, nonasbestos mineral fibers, organic chemicals, chronic inflammation (3), and simian virus 40 (SV40) exposure (4,5) have also been suggested as risk factors for mesothelioma in humans.

Because asbestos is ubiquitous, past exposures are often difficult to quantitate. Past asbestos exposure may be assessed by a standardized questionnaire that collects information on occupational, paraoccupational, environmental, and domestic contact with asbestos from insulation, mining, milling, heating trades, shipyard work, and construction (6). However, the long latency period of 20 years or longer from the onset of exposure to the development of malignant mesothelioma likely influences the accuracy of the exposure information obtained (1). More objective evidence of asbestos exposure includes radiologic findings such as bibasilar fibrosis and calcified pleural plaques, the presence of asbestos fibers in sputum or bronchoalveolar lavage samples, and evidence of interstitial fibrosis or ferruginous bodies in lung tissue (7). These criteria have been used to try to exclude asbestos as the causal factor in some cases of mesothelioma.

In published case series, the proportion of mesothelioma cases that have an asbestos exposure history ranges from 16% to 77% (8). Of 668 patients who died of malignant mesothelioma in Canada and the United States from 1960 to 1975, only 50% of men and 5% of women had known asbestos exposure (9). Occupational asbestos exposure in women and children is rare; therefore, most asbestos exposure in these individuals is thought to come from a household member who is employed in an asbestos industry. The occurrence of malignant mesothelioma in children may not be related to asbestos at all. In a report of 13 children diagnosed with mesothelioma in the United States, the short latency period from the time of exposure to tumor development and the absence of geographic clustering argued against an environmental

cause for this malignancy in children (10). Therefore, the disease appears to have a "natural" incidence of undetermined origin. Radiation is a possible etiologic agent for mesothelioma that may act independently or may have a synergistic effect with asbestos (11).

Animal Models of Radiation-Induced Mesothelioma

Animal experiments conducted in the 1970s demonstrated that inhaled or implanted plutonium in rodents and dogs could induce epithelial and mesenchymal tumors in the lungs and thorax (12). When plutonium oxide was injected into the peritoneal cavity of rats, 27% of these animals developed epithelial mesotheliomas and 38% developed sarcomas of various types (13). Both the soft tissue sarcomas and mesotheliomas were frequently seen surrounding "hot spots" of plutonium activity in the omentum. The pattern of PuO_2-induced mesothelioma was similar to that observed following intracavitary administration of asbestos fibers.

However, when animals were given inhaled and intrapleurally injected plutonium dioxide, only a small fraction developed pleural mesotheliomas (14). Only five of 2105 rats given aerosolized $^{239}PuO_2$ developed pleural mesotheliomas, and one of 27 rats given intrapleural injection developed these tumors. The reason for the low incidence appeared to be the rapid clearance of $^{239}PuO_2$ to the thoracic, mediastinal, and hepatic lymph nodes, which limited alpha irradiation of the pleural mesothelium and subsequent mesothelioma formation.

Exposure to cerium-144 dioxide led to the development of mesothelioma in four of 566 study animals (15). Evidence supporting a possible synergistic effect of radiation with asbestos comes from experiments showing an increased incidence of mesothelioma in rats after both irradiation and the administration of asbestos compared with animals that received asbestos alone (16).

Carcinogenic Effect of Radiation in Humans

Evidence that radiation is a human carcinogen comes from a number of epidemiologic studies reported in the literature (17,18). Studies on atomic bomb survivors have shown increased rates of cancers of the lung, breast, thyroid, stomach, urinary tract, and colon, as well as leukemia and multiple myeloma (19). Except for radiation-induced leukemias, which develop a few years after exposure, the average latency period for the development of solid tumors after radiation is more than 15 years (20).

Cancer can also occur as a secondary effect of therapeutic radiation (21). Advances in radiation therapy for cancer have led to increased survival for many patients, but a serious late complication of treatment is the development of second primary malignancies. Radiotherapy-related second primary malignancies usually occur about 10 to 20 years after exposure often within the field of irradiation (22–25) or adjacent

to it (26). As the number of long-term cancer survivors increases, second primary malignancies attributable to radiation treatment are becoming more frequent.

Radiation-Induced Sarcomas

Radiation exposure is a risk factor for the development of sarcomas. One study reviewed 344 cases of postirradiation sarcoma (27). The majority of second primary sarcomas were diagnosed in an anatomic site that had been previously exposed to radiotherapy. The mean time interval between the first and second neoplasm was about 11 years. Overall, 67% of the postirradiation sarcomas occurred in women compared to 22% in men (for 11% the gender was not given). This was due to the fact that women have breast and gynecologic tumors that are often treated with radiotherapy and are associated with prolonged survival. Although sarcomas are rare tumors in the general population, their risk is increased in long-term survivors of primary tumors treated with therapeutic irradiation.

In a report of 47 cases of radiation-induced sarcoma, patients exposed at younger ages tended to have a higher risk of developing tumors (28). The Late Effects Study Group assessed the risk of developing second primary neoplasms in patients with a history of childhood cancers, and found that bone and soft tissue sarcomas were the most common second primary malignancies encountered, with 74% of the sarcomas radiation-associated (29).

Malignant mesothelioma represents a type of soft tissue sarcoma (30). Like mixed mesodermal sarcomas of the uterus, synovial cell sarcomas, adenosarcomas, and epitheloid sarcomas, 80% of mesotheliomas have an epithelial component and about half have no sarcomatous elements. Similar to low-grade intraperitoneal and retroperitoneal sarcomas, patients with mesothelioma often die of local complications from the primary lesion rather than distant metastases.

Thorotrast-Related Mesotheliomas

Thorotrast is radioactive thorium dioxide, widely used as a radiographic contrast agent in the 1930s to 1940s, but discontinued after several reports of Thorotrast-induced malignant tumors, including liver cell carcinomas, hemangioendotheliomas, and carcinomas of the bile ducts (31). Dahlgren (32) published a case report of a patient who developed a neurofibrosarcoma of the neck at the site of Thorotrast injection for cerebral angiography. The patient subsequently developed a malignant mesothelioma of the cervical pleura. Maurer and Egloff (33) reported the case of a patient who developed peritoneal mesothelioma 36 years after contamination of the peritoneal cavity with Thorotrast during cholangiography.

In a cohort of 1095 patients who received Thorotrast injections for cerebral arteriography, a total of 368 cancer cases was reported to the Danish Cancer Registry (34). These patients had an increased incidence

of tumors of the liver, gallbladder, and peritoneum, as well as leukemia and multiple myeloma. The increased risk of cancer of the peritoneum (i.e., malignant mesothelioma) seen in this study could be explained by direct alpha radiation from the thorium deposited in the liver or spleen (35).

Case Reports of Radiation-Associated Mesotheliomas

After asbestos exposure, radiation is among the most frequently reported associations with mesothelioma, but the number of well-documented cases is small. Approximately 45 patients with malignant mesothelioma following radiotherapy have been described in the literature (Table 21.1). Pleural and peritoneal mesotheliomas have occurred in patients with prior radiotherapy for Hodgkin's disease (38, 40,53,54,56–58,60), breast cancer (42,51,55,57), testicular cancer (39,42, 43,50,59), cervical cancer (36,44), Wilms' tumor (37,46,48,49), non-Hodgkin's lymphoma (42,47,58), as well as radiation for nonmalignant disease (41,42,52) and Thorotrast exposure (32,33). Many of the primary tumors, including Hodgkin's disease and germ cell tumors, are curable and frequently occur in young individuals.

Among these case reports, the most common first malignancy is Hodgkin's disease. Improvements in the treatment of Hodgkin's disease have allowed many patients to experience long-term survival and cure. In a 15-year follow-up survey of patients treated for Hodgkin's disease, there was a 17.6% cumulative risk (or sixfold excess risk) of second primary cancers compared to a general age-matched population (61). Immunosuppression may contribute to the development of certain second primary tumors after Hodgkin's disease (62). The incidence of second primary solid tumors in patients treated for Hodgkin's disease continues to increase with time (63,64). In addition, all cancers of the stomach, bone, and connective tissue occurred within previously irradiated areas (61). In another cohort of 1329 patients with Hodgkin's disease, the vast majority of second primary solid tumors were observed in patients previously treated with irradiation or alkylating agents, such as cyclophosphamide, nitrogen mustard, procarbazine, and nitrosoureas (63). Sarcomas were the second most common second primary solid tumors after lung cancer. Five patients in this series had soft tissue sarcomas, but there were no reports of mesothelioma. Perhaps this was due to underdiagnosis of mesothelioma or because radiation-related mesothelioma is a rare occurrence.

Sixteen of the 45 published cases of radiation-associated mesotheliomas occurred in patients with prior childhood malignancies. For example, five case reports describe mesothelioma following radiotherapy for Wilms' tumor, which is a relatively rare childhood neoplasm with about a 90% cure rate (65). In the Late Effects Study Group (LESG) report, 1.4% of the 1451 patients with Wilms' tumor developed a second malignant neoplasm, comprising 19% of patients with a second cancer (29). In an update of the LESG, the most common second malignant neoplasms arising from irradiated sites, were bone sarcomas (64). Soft

Table 21.1. Case reports of radiation-induced mesothelioma

Reason for exposure	Sex	Age at exposure (years)	Age at diagnosis (years)	Latent period (years)	Chemotherapy	Histologic type	Site	Outcome	Reference
Thorotrast[a]	F	18	43	25	—	Unknown	Pleura	Unknown	32
Thorotrast[a]	F	23	59	36	—	Mixed	Peritoneum	Death[b]	33
Cervical	F	55	62	7	—	Unknown	Peritoneum	7 mos	36
Wilms' tumor[c]	—	5	21	16	+	Unknown	Unknown	Unknown	37
Hodgkin's	M	29	34	5	Unknown	Sarcomatous	Pleura	Unknown	38
Seminoma	M	50	66	16	—	Epithelial	Peritoneum	24 mos	39
Hodgkin's	M	27	34	7	Unknown	Sarcomatous	Pleura	9 mos	40
Unknown	M	31	57	26	—	Epithelial	Pleura	Unknown	41
Seminoma, lymphoma	M	24	55	31	—	Epithelial	Peritoneum	10 mos	42
Teratocarcinoma	F	6	24	18	+	Epithelial	Peritoneum	5 mos	42
Breast	F	30	40	10	+	Epithelial	Pleura	>48 mos	42
Neck scar[a,c]	F	29	55	26	—	Epithelial	Pleura	Unknown	42
Seminoma	M	33	57	24	+	Epithelial	Pleura	Death[b]	43
Cervical	F	50	59	9	—	Epithelial	Peritoneum	9 mos	44
Hodgkin's	M	23	28	5	+	Unknown	Unknown	1 mo	45
Wilms' tumor	M	3	44	41	—	Epithelial	Pleura	Unknown	46
Wilms' tumor[c]	M	6	22	16	+	Unknown	Pleura	50 mos	46
Non-Hodgkin's lymphoma	M	52	61	9	+	Epithelial	Pleura	14 mos	47
Wilms' tumor	M	2	16	14	+	Epithelial	Pleura	Unknown	48
Wilms' tumor	F	4	28	24	—	Epithelial	Pleura	2 wks	49
Seminoma	M	35	61	26	—	Epithelial	Peritoneum	5 mos	50
Breast	F	34	64	30	—	Unknown	Pleura	13 mos	51
Radiation technologist[a]	M	21	55	34	—	Mixed	Peritoneum	14 mos	52

Hodgkin's	F	4	24	20	—	Epithelial	Pleura	>24 mos	53
Hodgkin's	F	13	22	9	—	Epithelial	Pleura	5 mos	54
Breast	F	65	75	10	—	Epithelial	Pleura	Unknown	55
Breast	F	37	72	35	—	Epithelial	Pleura	Unknown	55
Hodgkin's	F	23	40	17	—	Unknown	Peritoneum	>5 yrs	56
Hodgkin's	M	32	46	14	+	Epithelial	Pleura	12 mos	56
Hodgkin's	M	19	32	13	+	Epithelial	Peritoneum	>30 mos	56
Hodgkin's	M	7	32	25	+	Epithelial	Pleura	Unknown	56
Hodgkin's	M	28	49	21	—	Epithelial	Pleura	Death[b]	57
Hodgkin's	M	21	43	22	—	Mixed	Pleura	Death[b]	57
Hodgkin's	M	20	31	11	—	Epithelial	Pleura	4 mos	57
Hodgkin's	M	7	31	24	+	Epithelial	Pleura	>9 mos	57
Breast	F	55	76	21	+	Sarcomatous	Pleura	Unknown	57
Hodgkin's	M	5	22	17	—	Epithelial	Pleura	3 yrs	57
Hodgkin's	F	1	25	24	—	Epithelial	Pleura	4 yrs	57
Breast	F	49	78	29	—	Epithelial	Pleura	>1 wk	57
Ovarian stromal	F	11	20	9	+	Epithelial	Pleura, Peritoneum	>108 mos	58
Hodgkin's	M	7	18	11	—	Epithelial	Pleura	>7 mos	58
Non-Hodgkin's lymphoma	M	3	16	13	+	Epithelial	Peritoneum	3 mos	58
Teratoma	M	30	65	35	—	Epithelial	Peritoneum	7 mos	59
Seminoma	M	37	54	17	—	Epithelial	Peritoneum	6 mos	59
Hodgkin's	F	25	49	24	—	Unknown	Pericardial	Death[b]	60

[a] Exposure not due to therapeutic radiation for a prior malignancy.
[b] Diagnosed at autopsy.
[c] Mesothelioma did not arise at the site of radiation.

tissue sarcomas were also frequent and approximately 75% arose in previously irradiated tissues. In a retrospective analysis of second primary cancers in patients who survived a childhood cancer, the majority (28 of 36) of second primary cancers was in the field of prior radiotherapy, while 4 of 36 lesions arose outside of the treatment field, including one case of mesothelioma (37).

Of the five cases of mesothelioma following Wilms' tumor, one arose within the irradiated lung field (49), one arose ipsilateral to an irradiated renal fossa (46), and three arose outside the radiation field (37,46,48). A fraction of Wilms' tumor patients carry a genetic predisposition to malignant neoplasms (66). However, malignant mesothelioma as a second primary neoplasm does not appear to be increased in the absence of other predisposing factors. Therefore, genetic predisposition is unlikely to be the sole factor in the development of mesothelioma as a second primary malignancy. Given that the survival of patients with childhood malignancies has improved in recent years (67), an understanding of etiologic factors responsible for second primary neoplasms may come from the study of these children.

The documented case reports also included patients who had radiation exposure for nonmalignant disease. There were two cases of Thorotrast exposure for radiologic studies (32,33), one case of cosmetic irradiation for a neck scar following thyroidectomy for goiter (42), and one case of a patient who received multiple chest x-rays over the course of 26 years to evaluate a radiologic opacity of unclear etiology (41). There was also one case of occupational radiation exposure in a radiation technologist who had an estimated cumulative dose of about 40 to 50 rad, which is a lower dose than that for therapeutic radiation (52).

The literature also includes one case of pericardial mesothelioma in a patient who received mantle field radiotherapy for Hodgkin's lymphoma (60). About 80% of mesotheliomas arise from the pleura and 20% have primarily peritoneal involvement. Primary pericardial mesothelioma is rare. However, in a series of nine patients with pleural mesothelioma examined at autopsy, six had pericardial involvement (30).

Most of the reported patients with radiation-associated mesothelioma did not have documented asbestos exposure, and there appear to be differences between these cases of radiation-associated mesotheliomas and asbestos-induced mesotheliomas (Table 21.2). In a series of

Table 21.2. Clinical features of asbestos-related and radiation-associated mesotheliomas

	Asbestos	Radiation[a]
Median age at diagnosis (years)	**50–70**	**43**
Median latent period (years)	**20–40**	**18**
Male/female ratio	**9:1**	**5:4**
Epithelial/mixed/sarcomatous histology	**5:3:2**	**10:1:1**
Pleural/peritoneal site of involvement	**4:1**	**2:1**
Median months to death after diagnosis	**4–12**	**9**

[a] Based on 45 case reports in the literature.

mesothelioma patients grouped by their level of past asbestos exposure, the patients with asbestos-related mesothelioma were mainly men and had mesotheliomas of varied histologic types, while non–asbestos-related mesotheliomas occurred in more women and were mainly epithelial tumors (41). Among the 45 case reports of radiation-associated mesothelioma, the median age at diagnosis is 43 years (range 16 to 78) compared to the peak occurrence of asbestos-related mesotheliomas in the sixth to seventh decades. The interval between radiation exposure and the appearance of mesothelioma ranged from 5 to 41 years with a median of 18 years, which is consistent with the known latent period of other radiation-induced sarcomas (27). Among the documented cases of radiation-related mesothelioma, 57% of the patients were male and 43% were female, unlike the male predominance seen in mesotheliomas caused by asbestos. As with asbestos-related mesotheliomas, postirradiation mesotheliomas are primarily epithelial in histology (31 epithelial, three mixed, three sarcomatous, eight unknown), pleural in location (29 pleural, 14 peritoneum, one pericardial, one unknown), and rapidly lethal. Patients with radiation-induced mesotheliomas had a median survival of 9 months; 5 cases were diagnosed at autopsy. However, four patients survived more than 3 years (56–58). In general, the median survival of patients with epithelial mesothelioma is 16 months, compared to 12 months for patients with fibrosarcomatous tumors (40).

In at least three cases, mesothelioma arose outside the radiation fields (37,42,46). Tumors found adjacent to radiation ports may represent radiation scatter-induced tumors. The carcinogenic effects of irradiation might be at a site remote from the irradiated organ. Alternatively, these anecdotal reports may represent chance associations.

At least 15 of the 45 patients with radiation-associated mesotheliomas also received chemotherapy. Chemotherapeutic drugs, particularly alkylating agents, are known carcinogens (69–73). Therefore, the pathogenesis of mesothelioma may be multifactorial, with radiation acting synergistically with other known carcinogens.

Mesotheliomas May Be Underdiagnosed and Misdiagnosed

Mesotheliomas were particularly difficult to diagnose in the past due to their nonspecific clinical presentations, the large volume of tissue required for examination, and the lack of experience of most pathologists with the diagnosis. In a series of 123 patients diagnosed with mesothelioma from 1949 to 1980, the median time to diagnosis was 3 months; symptoms appeared from 2 weeks to 2 years prior to diagnosis (40). Thoracotomy or thoracoscopy is generally required to obtain an adequate biopsy. In one case series, the diagnostic yield of pleural fluid for cytology was 4%, for percutaneous needle biopsy 26%, and for open biopsy 70% (74).

Criteria have been established outlining the gross and histologic characteristics and immunohistochemical findings for malignant

mesothelioma (75). In a review of a series of malignant mesotheliomas diagnosed prior to the use of special immunoperoxidase stains, the Canadian Mesothelioma Panel favored the diagnosis in 59% of cases, opposed it in 34%, and was uncertain in 7% (76). The epithelial variant of malignant mesothelioma can easily be mistaken for other neoplasms, especially metastatic adenocarcinomas which are much more common. Unless mesothelioma is considered in the differential diagnosis, special stains that are now used to diagnose these tumors are not routinely performed. Also, electron microscopy may be helpful to confirm the diagnosis. A second primary malignancy may be difficult to differentiate from a recurrence of the first cancer. Therefore, mesotheliomas may be misdiagnosed in patients with a prior malignancy.

Population-Based Studies of Radiation-Associated Mesotheliomas

Population-based surveys have attempted to determine the true incidence of radiation-induced mesotheliomas. Shannon et al (55) published a random retrospective review of 1000 patients without a history of asbestos exposure who developed a unilateral pleural effusion years after having received thoracic radiation for a malignancy. Three patients had radiation-associated malignant pleural mesotheliomas.

Cavazza et al (57) conducted a large, population-based survey of cancer patients who subsequently developed a second primary mesothelioma. The occurrence of mesothelioma as a second primary cancer in the general U.S. population was surveyed using data from the Connecticut Tumor Registry from 1935 to 1972 and the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program from 1973 to 1992. Malignant pleural mesotheliomas occurred as a second primary cancer in 142 of 1,489,643 patients during 6,304,466 person-years of follow-up. The characteristics of this group of patients were somewhat different from those observed in the case reports of radiation-related mesotheliomas. Men comprised 89% of the patients, similar to the male/female ratio for asbestos-related mesothelioma (77). The median latency between the first cancer and the development of malignant mesothelioma was 4.3 years, shorter than generally reported for radiation-induced malignancies. Of the 142 second primary cases of mesothelioma, only 37 received radiation as part of the therapy for their first malignancy. Although second primary cancers may be underreported, malignant mesothelioma as a second primary neoplasm is a very rare event, occurring in approximately 1 in 10,000 cancer patients in this large survey.

Neugut et al (78) published the first controlled study looking at the relationship between thoracic radiation exposure and malignant pleural mesothelioma. They conducted a retrospective cohort analysis of the incidence of malignant pleural mesothelioma after thoracic radiotherapy for breast cancer and Hodgkin's disease using patients registered in the SEER Program from 1973 to 1993. They found that 24.8% of the breast cancer patients and 50.6% of the Hodgkin's disease

patients had received radiotherapy. Six pleural mesotheliomas were diagnosed among the breast cancer patients treated with radiotherapy and four were diagnosed in women not treated with radiation. No mesotheliomas were observed in patients with prior Hodgkin's disease. Therefore, no significant association was found between thoracic radiation for breast carcinoma and Hodgkin's lymphoma and the development of malignant pleural mesothelioma. However, mesotheliomas may have been misdiagnosed as recurrences of breast cancer or Hodgkin's disease, which would lead to underreporting of mesothelioma cases. Another limitation of this study is the lack of reliable mesothelioma diagnosis during the period under study and relatively short follow-up time (maximum of 20 years). In radiation carcinogenesis, not even 30 years of follow-up is adequate to show the total effect of exposure (18).

Conclusion

There is considerable evidence, both in animal models and in humans, of the carcinogenic potential of radiation. Radiotherapy appears to induce a small but significant number of second primary malignancies, both in heavily irradiated tissues and in organs remote from the target area (79). Although second primary malignancies may be a lethal late complication of radiotherapy, the total risk appears to be small compared to its benefits. Various case reports suggest an association between radiation therapy and the subsequent development of malignant mesothelioma. Because of the rarity of radiation-induced mesothelioma, the relatively long latent period, and underdiagnosis, accurate estimates of its incidence are difficult to make. Nevertheless, mesothelioma should be considered in patients with a prior history of radiation who subsequently develop a pleural or peritoneal effusion. As with malignant mesothelioma associated with asbestos exposure, the prognosis is extremely poor. Three possibilities remain with respect to radiation and malignant mesothelioma: (1) radiation acts as an independent carcinogen; (2) radiation potentiates the effects of other carcinogens such as asbestos, chemotherapy, and genetic and environmental factors; and (3) the history of irradiation is incidental in patients who develop mesothelioma.

References

1. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960;17:260–271.
2. Selikoff IJ. Cancer risk of asbestos exposure. In: Hiatt HH, Watson JD, Winsten JA, eds. Origins of Human Cancer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1977:1765–1784.
3. Peterson JT, Greenberg SD, Buffler PA. Non–asbestos-related malignant mesothelioma, a review. Cancer 1984;54:951–960.

4. Carbone M, Fisher S, Powers A, Pass HI, Rizzo P. New molecular and epidemiological issues in mesothelioma: role of SV40. J Cell Physiol 1999;180: 167–172.
5. Mulatero C, Surentheran T, Breuer J, Rudd RM. Simian virus 40 and human pleural mesothelioma. Thorax 1999;54:60–61.
6. Zielhuis RL, Versteeg JP, Planteijdt HT. Pleura mesothelioma and exposure to asbestos. A retrospective case-control study in the Netherlands. Int Arch Occup Environ Health 1975;36:1–18.
7. Gaudichet A, Jaurand MC, Atassi K, et al. Apport du lavage bronchoalveolaire dans l'etude des relations fibres-microenvironnement alveolaire dans l'asbestose pulmonaire. INSERM 1979;84:381–394.
8. Walker AM, Loughlin JE, Friedlander ER, Rothman KJ, Dreyer NA. Projections of asbestos-related disease 1980–2009. J Occup Med 1983;25:409–425.
9. McDonald AD, McDonald JC. Malignant mesothelioma in North America. Cancer 1980;46:1650–1656.
10. Grundy GW, Miller RW. Malignant mesothelioma in childhood: report of 13 cases. Cancer 1972;30:1216–1218.
11. Lafuma J, Hirsch A, Monchaux G, et al. Mesothelioma induced by intrapleural injection of different types of fibres in the rat. Synergistic effect of other carcinogens. In: Wagner JC, ed. Biological Effects of Mineral Fibres. Lyon: IARC Scientific Publication, 1980:311–322.
12. Park JF, Howard EB, Bair WJ. Acute toxicity of inhaled $^{238}PuO_2$ in beagles. BNWL-1050. BNLW Rep 1970;Jan:3.6+.
13. Sanders CL, Jackson TA. Induction of mesotheliomas and sarcomas from "hot spots" of $^{239}PuO_2$ activity. Health Phys 1972;22:755–759.
14. Sanders CL. Pleural mesothelioma in the rat following exposure to $^{239}PuO_2$. Health Phys 1992;63:695–697.
15. Hahn FF, Lundgren DL. Pulmonary neoplasms in rats that inhaled cerium-144 dioxide. Toxicol Pathol 1992;20:169–178.
16. Warren S, Brown CE, Chute RN, Federman M. Mesothelioma relative to asbestos, radiation and methylcholanthrene. Arch Pathol Lab Med 1981; 105:305–312.
17. Mossman KL. Ionizing radiation and cancer. Cancer Invest 1984;2:301–310.
18. Kohn HI, Fry RJM. Radiation carcinogenesis. N Engl J Med 1984;310:504–511.
19. Kato H, Schull WJ. Studies of the mortality of A-bomb survivors. Mortality 1950–1978. Part I. Cancer mortality. Radiat Res 1982;90:395–432.
20. Wakabayashi T, Kato H, Ikeda T. Studies of the mortality of the A-bomb survivors: Report 7: Part III. Incidence of cancer in 1959–1978, based on the tumor registry, Nagasaki. Radiat Res 1983;93:112–146.
21. Harwood AR, Yaffe M. Cancer in man after diagnostic or therapeutic irradiation. In: Penn I, ed. Cancer Surveys, vol 1. Oxford: Oxford University Press, 1982:703–731.
22. Li FP, Cassady JR, Jaffe N. Risk of second tumors in survivors of childhood cancer. Cancer 1975;35:1230–1235.
23. Meadows AT, D'Angio GJ, Evans AE, Harris CC, Miller JC, Mike V. Oncogenesis and other late effects of cancer treatment in children: report of a single hospital study. Radiology 1975;114:175–180.
24. Tefft M, Vawter GF, Mitus A. Second primary neoplasms in children. Am J Roentgenol Radium Ther Nucl Med 1968;103:820–822.
25. Meadows AT, D'Angio GJ, Mike V. Patterns of second malignant neoplasms in children. Cancer 1977;40:1903–1911.
26. Meadows AT, Strong LC, Li FP. Bone sarcoma as a second malignant neoplasm in children: influence of radiation and genetic predisposition. Cancer 1980;46:2603–2606.

27. Robinson E, Neugut AI, Wylie P. Clinical aspects of postirradiation sarcomas. J Natl Cancer Inst 1988;80:233–240.
28. Kim JH, Chu FC, Woodard HQ, Melamed MR, Huvos A, Cantin J. Radiation-induced soft tissue and bone sarcoma. Radiology 1978;129:501–508.
29. Mike V, Meadows AT, D'Angio GJ. Incidence of second malignant neoplasms in children: results of an international study. Lancet 1982;2:1326–1331.
30. Antman KH, Blum RH, Greenberger JS, Flowerdew G, Skarin AT, Canellos GP. Multimodality therapy for malignant mesothelioma based on a study of natural history. Am J Med 1980;68:356–362.
31. Beckenbach H, van Kaick G, Wenz W. Zur diagnostik und therapie von thorotrastinduzierten tumoren. Z Krebsforsch 1970;74:318.
32. Dahlgren S. Effects of locally deposited colloidal thorium dioxide. Ann NY Acad Sci 1967;145:786–790.
33. Maurer RM, Egloff B. Malignant peritoneal mesothelioma after cholangiography with Thorotrast. Cancer 1975;36:1381–1385.
34. Andersson M, Storm HH. Cancer incidence among Danish Thorotrast-exposed patients. J Natl Cancer Inst 1992;84:1318–1325.
35. Van Kaick G, Wesch H, Luhrs H. The German Thorotrast study—report on 20 years follow-up. In: Risks from Radium and Thorotrast. BIR report 21. London: British Institute of Radiology, 1989;98–104.
36. Babcock TL, Powell DH, Bothwell RS. Radiation-induced peritoneal mesothelioma. J Surg Oncol 1976;8:369–372.
37. Li FP. Second malignant tumors after cancer in childhood. Cancer 1977;40:1899–1902.
38. Brody RS, Schottenfeld D, Reid A. Multiple primary cancer risk after therapy for Hodgkin's disease. Cancer 1977;40:1917–1926.
39. Stock RJ, Fu YS, Carter JR. Malignant peritoneal mesothelioma following radiotherapy for seminoma of the testis. Cancer 1979;44:914–919.
40. Brenner J, Sordillo PP, Magill GB, Golbey RB. Malignant mesothelioma of the pleura: review of 123 patients. Cancer 1982;49:2431–2435.
41. Hirsch A, Brochard P, De Cremoux H, et al. Features of asbestos-exposed and unexposed mesothelioma. Am J Ind Med 1982;3:413–422.
42. Antman KH, Corson JM, Li FP, et al. Malignant mesothelioma following radiation exposure. J Clin Oncol 1983;1:695–700.
43. Hoffman RS, Rossof AH, Pazdur R, Slayton R.E. Multiple primary malignancies (letter). J Clin Oncol 1983;2:1308.
44. Beier KM, Gallup DG, Burgess R, Stock RJ. Occurrence of malignant peritoneal mesothelioma after surgery and irradiation for cervical cancer. Gynecol Oncol 1984;17:375–380.
45. Tester WJ, Kinsella TJ, Waller B, et al. Second malignant neoplasms complicating Hodgkin's disease: the National Cancer Institute experience. J Clin Oncol 1984;2:762–769.
46. Antman KH, Ruxer RL, Aisner J, Vawter G. Mesothelioma following Wilms' tumor in childhood. Cancer 1984;54:367–369.
47. Efremidis AP, Waxman JS, Chahinian AP. Association of lymphocytic neoplasia and mesothelioma. Cancer 1985;55:1056–1059.
48. Anderson KA, Hurley WC, Hurley BT, Ohrt DW. Malignant pleural mesothelioma following radiotherapy in a 16-year-old boy. Cancer 1985;56:273–276.
49. Austin MB, Fechner RE, Roggli VL. Pleural malignant mesothelioma following Wilms' tumor. Am J Clin Pathol 1986;86:227–230.
50. Gilks B, Hegedus C, Freeman H, Fratkin L, Churg A. Malignant peritoneal mesothelioma after remote abdominal radiation. Cancer 1988;61:2019–2021.

51. Kawashima A, Libshitz HI, Lukeman JM. Radiation-induced malignant pleural mesothelioma. Can Assoc Radiol J 1990;41:384–386.
52. Horie A, Hiraoka K, Yamamoto O, Haratake J, Tsuchiya T, Sugimoto H. An autopsy case of peritoneal malignant mesothelioma in a radiation technologist. Acta Pathol Jpn 1990;40:57–62.
53. Lerman Y, Learman Y, Schachter P, Herceg E, Lieberman Y, Yellin A. Radiation associated malignant pleural mesothelioma. Thorax 1991;46:463–464.
54. Hofmann J, Mintzer D, Warhol MJ. Malignant mesothelioma following radiation therapy. Am J Med 1994;97:379–382.
55. Shannon VR, Nesbitt JC, Libshitz HI. Malignant pleural mesothelioma after radiation therapy for breast cancer: a report of two additional patients. Cancer 1995;76:437–441.
56. Weissmann LB, Corson JM, Neugut AI, Antman KH. Malignant mesothelioma following treatment for Hodgkin's disease. J Clin Oncol 1996;14: 2098–2100.
57. Cavazza A, Travis LB, Travis WD, et al. Post-irradiation malignant mesothelioma. Cancer 1996;77:1379–1385.
58. Pappo AS, Santana VM, Furman WL, et al. Post-irradiation malignant mesothelioma. Cancer 1997;79:192–193.
59. Amin AMH, Mason C, Rowe P. Diffuse malignant mesothelioma of the peritoneum following abdominal radiotherapy. Eur J Surg Oncol 2001;27: 214–222.
60. Velissaris TJ, Tang ATM, Millward-Sadler GH, Morgan JM, Tsang GM. Pericardial mesothelioma following mantle field radiotherapy. J Cardiovasc Surg 2001;42:425–427.
61. Tucker MA, Coleman CN, Cox RS. Risk of second cancers after treatment for Hodgkin's disease. N Engl J Med 1988;318:76–81.
62. Fisher RI, Devita VT, Bostick F, et al. Persistent immunologic abnormalities in long-term survivors of advanced Hodgkin's disease. Ann Intern Med 1980;92:595–599.
63. Valagussa P, Santoro A, Fossati-Bellani F, Banfi A, Bonadonna G. Second acute leukemia and other malignancies following treatment for Hodgkin's disease. J Clin Oncol 1986;4:830–837.
64. Meadows AT, Baum E, Fossati-Bellani F, et al. Second malignant neoplasms in children: an update from the Late Effects Study Group. J Clin Oncol 1985; 3:532–538.
65. D'Angio GJ, Beckwith JB, Breslow NE. Wilms' tumor: an update. Cancer 1980;45:1791–1798.
66. Knudson AG, Strong LC. Mutation and cancer: a model for Wilms' tumor of the kidney. J Natl Cancer Inst 1972;48:313–324.
67. Myers MH, Heise HW, Li FP, Miller RW. Trends in cancer survival among U.S. white children, 1955–1971. J Pediatr 1975;87:815–818.
68. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. J Clin Oncol 1989;7:1157–1168.
69. Roberts MM, Bell R. Acute leukemia after immunosuppressive therapy. Lancet 1976;2:768–770.
70. Schein PS, Winokur SH. Immunosuppressive and cytotoxic chemotherapy—long-term complications. Ann Intern Med 1975;82:84–95.
71. Silvergleid AJ, Schrier SL. Acute myelogenous leukemia in two patients treated with azathioprine for nonmalignant diseases. Am J Med 1974;57: 885–888.
72. Sotrel G, Jafari K, Lash AF, Stepto RC. Acute leukemia in advanced ovarian carcinoma after treatment with alkylating agents. Obstet Gynecol 1976;47: 67–70.

73. Wall RL, Clausen KP. Carcinoma of the urinary bladder in patients receiving cyclophosphamide. N Engl J Med 1975;293:271–273.
74. Elmes PC, Simpson MJC. The clinical aspects of mesothelioma. Q J Med 1976;45:427–449.
75. Scully RE, Antman KH. Protocol for the examination of specimens from patients with tumors of the peritoneum: a basis for checklists. Arch Pathol Lab Med 2001;125:1174–1176.
76. McDonald AD, McDonald JC. Epidemiologic surveillance of mesothelioma in Canada. Canadian Med Assoc J 1973;109:359–362.
77. Connelly RR, Spirtas R, Myers MH, Percy CL, Fraumeni JF Jr. Demographic patterns for mesothelioma in the United States. J Natl Cancer Inst 1987; 78:1053–1060.
78. Neugut AI, Ahsan H, Antman KH. Incidence of malignant pleural mesothelioma after thoracic radiotherapy. Cancer 1997;80:948–950.
79. Committee on the Biologic Effects of Ionizing Radiations (BEIR V), National Research Council. Health Effects of Exposure to Low Levels of Ionizing Radiation. Washington, DC: National Academy Press, 1990.

22

Genetics and Human Mesothelioma

Michele Carbone and Izzetin Y. Baris

In the villages of Karain (population 600) and Tuzkoy (population 1400) in Cappadocia, a region in Central Anatolia, Turkey, characterized by volcanic tuffs and natural caves, 50% or more of deaths are caused by malignant mesothelioma. These two villages, like most other villages in the region, were built with stones mined from the nearby natural caves. Dr. Y.I. Barish discovered this unique very high incidence of mesothelioma (1). Soon after, scientists looked for asbestos, which in the 1970s was the only known causative factor for mesothelioma. Some asbestos was found (2), but subsequent studies demonstrated that in Cappadocia low asbestos amounts are found almost everywhere because it is a natural component of that volcanic terrain and because asbestos-based stucco (containing tremolite asbestos) has been widely used in building construction (3). It appeared that asbestos could not account for the unique high incidence of mesotheliomas in these two villages (4).

Another type of mineral fiber, erionite, which had been detected in the lungs of several villagers, was suspected as a causative agent (1,4). Erionite is a type of fibrous zeolite commonly found in the stones of the houses of Karain and Tuzkoy. When erionite was injected intrapleurally into animals, it caused mesothelioma, and it was concluded that erionite was the cause of mesothelioma in these villages (5). Erionite, therefore, appeared much more potent than asbestos in causing mesothelioma because more than half of the villagers died of this disease. Erionite was claimed to be the most potent chemical human carcinogen (6). Studies tried to link erionite to other human tumors, but except for mesotheliomas there is no significant difference in the incidence of any other tumor types in these two villages compared with the rest of Turkey (7). Why such a potent carcinogen would specifically cause mesothelioma was unknown. Why about 50% of villagers appeared to suffer no consequence from exposure to such a potent carcinogen was also unknown.

During repeated visits to these villages, we suspected that erionite was not the only cause of mesothelioma. In the villages of Karain and Tuzkoy, mesothelioma developed mostly in certain houses, called the

"houses of death" because all the residents had died of mesothelioma. Residents of nearby houses had not developed mesothelioma. It was stated that the amount of erionite was higher in the "houses of death" but the only proof for this statement was that residents of those houses had died of mesothelioma. If this hypothesis was correct, there had to be an extremely high amount of erionite in the "houses of death," and proportionally a very low amount of erionite in the other houses next to the "houses of death." Yet both groups of houses appeared to have been built at about the same time and with the same type of stones. In Karain and Tuzkoy, families live together for multiple generations; therefore, people living in the same house are often related.

It appeared possible that susceptibility to mesothelioma was genetically transmitted and that the presumed higher amount of erionite in the "houses of death" was a misleading hypothesis. The genetic hypothesis was strengthened by the observation that only one mesothelioma had been observed in Karlik, 1 km south of Karain, with a population of 1500. This mesothelioma had occurred in a woman from Karain who had moved to Karlik when she married a Karlik man. The houses of Karlik appeared identical to those of Karain and had been built with stones mined from the same caves, which are located midway between Karain and Karlik on the same side of the mountain on which these two villages were built. Again the hypothesis was made that there was more erionite in the houses of Karain, but during a local inspection of Karlik and Karain, one of the highest amounts of what appeared to be erionite (erionite has a white color and a soft consistency and looks like an area of decay within the zeolite stones used to build the villages) was found in the stones of the fountain of Karlik, which provides water to the whole village.

The marriage of the woman of Karain with a man of Karlik was unique. Residents of Cappadocia consider people from the two mesothelioma villages "weak" and they are afraid that mesothelioma will spread in their families if they marry with villagers of Karain and Tuzkoy. In fact we observed that people from Karain and Tuzkoy have problems even selling their products to the market because residents of nearby villages are afraid to buy anything that comes from these two villages (7). The consequence of this incredible situation is that most marriages in Karain and Tuzkoy are between villagers, and marriages to people from outside the villages are infrequent.

Since there was reason to suspect that mesothelioma in Karain and Tuzkoy had a genetic cause, we studied this possibility. In an initial study, we constructed preliminary pedigrees from several families, and it was apparent that mesothelioma was genetically transmitted, possibly as an autosomal-dominant disease (8). About 50% of the descendents of affected parents developed mesothelioma, whereas mesothelioma was absent in other families. When members of unaffected families married into affected families, mesothelioma appeared in their descendants (9). Mineralogic analyses revealed no differences in the amount or type of erionite among the "houses of death" and other houses, including those of Karlik (Umran Dogan, University of Ankara, personal communication). These same analyses confirmed the

presence of a very high amount of erionite in the fountain of Karlik. Thus, it appears that in these villages, susceptibility to mesothelioma is genetically transmitted.

The possibility that erionite is a cofactor that causes mesothelioma prevalently in genetically predisposed individuals is presently being investigated in our laboratories in collaboration with Dr. Umran Dogan. Furthermore, we are trying to isolate the putative mesothelioma susceptibility gene that predisposes some families of Karain and Tuzkoy to this malignancy. This should lead to the development of therapeutic approaches for members of these families. It is possible that the same gene that is genetically mutated in Cappadocia may be the target of asbestos and simian virus 40 (SV40) carcinogenesis, and thus it is hoped that the eventual isolation of this putative gene might clarify the molecular pathogenesis of mesothelioma, and also benefit all mesothelioma patients.

Acknowledgments

We are grateful to our colleagues Drs. U. Dogan, M. Dogan, S. Emri, and J.R. Testa for their critical contribution to these studies.

References

1. Baris YI, Sahin A, Ozesmi M, et al. An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Urgup in Anatolia. Thorax 1978;33:181–192.
2. Rohl AN, Langer AM, Moncure G, et al. Endemic pleural disease associated with exposure to mixed fibrous dust in Turkey. Science 1982;216:518–520.
3. Coplu L, Dumortier P, Demir AU, et al. An epidemiological study in an Anatolian village in Turkey environmentally exposed to tremolite asbestos. J Environ Pathol 1982;15:177–182.
4. Baris YI, ed. Asbestos and Erionite Related Chest Diseases. Ankara, Turkey: Semih Offset Matbaacilik, 1987;3–169.
5. Wagner JC, Skidmore JW, Hill RJ, et al. Erionite exposure and mesotheliomas in rats. Br J Cancer 1985;51:727–730.
6. Baris B, Demir AU, Shehu V, et al. Environmental fibrous zeolite (erionite) exposure and malignant tumors other than mesothelioma. J Environ Pathol Toxicol Oncol 1996;15:183–189.
7. Roushdy-Hammady I, Siegel J, Emri S, et al. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 2001;444–445.

23

Clinicians' Approach to Mesothelioma

Philip Harber and J. Bernard .L. Gee

Prior to 1960, malignant mesothelioma (MM) was considered a rare and ill-defined entity of obscure etiology. The recognition of an origin from amphibole asbestos provided a case series of sufficient size both to characterize the tumor and to identify epidemiologically its commonest cause.

Although MM is a relatively rare tumor (1), many clinicians become involved in the care of patients with, or at risk of, MM. Clinicians may play several roles, as summarized in Table 23.1.

The scientific basis guiding approaches of clinicians is discussed throughout this book. In addition, the specific clinical manifestations of the common types of MM are presented in this chapter and Chapters 24 and 25.

Diagnostic Approach

Malignant mesothelioma arise most frequently from the pleura or peritoneum (10–20%), the pericardial site usually being a secondary one. Generally, pleural mesotheliomas are easier to diagnose and recognize than those in the peritoneum.

The common presentations of pleural mesothelioma are unexplained chest pain, dyspnea (often due to the presence of a large pleural effusion), or the incidental detection of a pleural effusion in the course of a routine radiographic examination. Peritoneal mesotheliomas, however, are often detected because of abdominal pain, abdominal mass, or intestinal obstruction.

Malignant mesothelioma is frequently in the differential diagnosis of a patient with a pleural effusion, particularly if there has been exposure to asbestos; however, even among high-risk patients, other etiologies are common. Pleural effusions may be characterized as either a transudation (commonly due to congestive heart failure or fluid overload) or an exudate (often related to infection, trauma, or malignancy including mesothelioma). Diagnostic approaches to patients with

Table 23.1. Roles of clinicians

Counseling asbestos exposed employees who have substantial risk of developing mesothelioma
Counseling members of the general community with very low level exposure
Clinically evaluating patients for whom mesothelioma is in the differential diagnosis (e.g., a patient with a hemorrhagic pleural effusion)
Advising patients about selection of optimal treatment
Providing therapy: surgical, chemotherapeutic, immunotherapeutic, radiotherapeutic
Providing compassionate primary care for patients with this frequently fatal illness
Advising patients about legal aspects
Assessing etiology
Establishing preventive programs

pleural effusions are well summarized elsewhere (2). First, the effusion should be characterized by thoracentesis as either a transudate or an exudate. Generally, transudative effusions do not require further attention to the effusion itself, but rather the underlying etiology such as heart failure should be addressed. For exudative effusions, if the initial thoracentesis does not yield a clear diagnosis such as tuberculosis, further investigation is needed. Cytology studies of the pleural fluid of patients with MM are often inconclusive, but a lung mass may be found by x-ray or computed tomography (CT) scan examination after drainage. Thus, the second step is to perform a biopsy of the pleura or of the lung. Earlier methods of external needle biopsies of the pleura had low sensitivity, whereas open thoracotomy had significant morbidity and mortality. Currently, many consider video-assisted thoracoscopy (VAT) to be an optimal approach since it allows obtaining a sample of reasonable size as well as visual inspection of the pleura to sample the most likely location; associated morbidity is acceptably limited. In addition, where appropriate, lung biopsy may be obtained concomitantly in a VAT. Although hyaluronidase levels were considered useful in the past, they are neither sensitive nor specific for mesothelioma.

Prognosis

The prognosis, unfortunately, is usually dismal. Ominous factors include systemic effects such as fever, weight loss, and lassitude. Nonepithelial forms are more sinister, particularly in men, as are low hemoglobin, elevated platelet counts, and lactate dehydrogenase (LDH). Staging is clearly relevant, with about a mean 30-month survival at stage I and a year or less with more advanced disease. The rare needle-track invasion responds to radiotherapy, but otherwise medical therapy is somewhat experimental, even though thoracocentesis and paracentesis may relieve some symptoms and frequently afford a temporarily acceptable lifestyle.

Clinical Decisions

Table 23.2 summarizes the decisions that patients and clinicians need to make. In some instances, guiding patients to make these

decisions is best accomplished by primary treating physicians, whereas in others, extensive detailed experience with mesothelioma is most relevant.

Extensive surgical approaches are utilized for selected patients with pleural mesothelioma. Therefore, prior to a thoracotomy, patients should be asked if they desire very extensive surgical treatment. Furthermore, the primary physician should confer with the patient about whether a thoracotomy should be conducted in a local facility or if the patient should be referred to one of the centers with extensive experience in the management of MM.

Pathology Considerations for the Clinician

The histopathology of MM is detailed in the Pathology of Mesothelioma section of this book. The following considerations are relevant to the primary clinician.

Confirmation of Diagnosis

Many pathologists have only limited experience with MM, and thus a diagnosis of MM should be confirmed by a pathologist with particular experience in this area. Indeed, many countries have a mesothelioma panel for this purpose.

In addition to routine histologic examination, immunochemical and electron microscopy are useful in establishing the diagnosis. Determination of the histologic types of mesothelioma (epithelial, sarcomatous, and mixed forms) also requires special studies and expertise. The differential diagnosis of mesothelioma includes metastatic carcinoma, bronchogenic carcinoma, and unusual tumors such as sarcomas (3) and small cell desmoplastic carcinoma (4). Occasionally, measurements of simian virus 40 (SV40) components are of particular interest to research scientists and those involved in clinical therapeutic trials for mesothelioma.

Table 23.2. Questions for clinicians to address

Should aggressive therapy be instituted?
Should research approaches be applied to the individual patient?
Should the patient be referred to a distant center or treated locally?
Diagnostic approach: tissue biopsy technique (e.g., pleural biopsy, video-assisted thoracotomy, or open thoracotomy)?
Pathology considerations:
Should the specimen be sent to a pathologist with particular mesothelioma expertise?
Should lung tissue be obtained for asbestos content analysis?
Should SV40 be sought in the tumor tissue?
What exposures have occurred?
Should the patient be encouraged to file a legal action (workers' compensation or tort)?
Are other coworkers or family members also at risk?

Measurement of Asbestos in Lung Tissue

Asbestos, particularly the amphibole varieties closely associated with mesothelioma, is biopersistent and therefore can be measured many years after exposure, thus providing a useful adjunct to a complete occupational and environmental exposure history. Tissue analysis for asbestos fibers (see Chapters 16 and 17) is a specialized technique available in only a limited number of laboratories. Furthermore, this specifically requires biopsy of lung tissue in addition to mesothelioma tumor tissue, since the latter is not a reliable estimate of the lung burden of asbestos fibers. Now that VAT is widely available, the ease of doing such lung biopsy is facilitated.

Although not as precise as analysis of lung tissue, bronchoalveolar lavage fluid may also be evaluated for the presence of asbestos fibers and bodies (5,6). Asbestos fiber levels in MM patients' lungs are typically higher than those of exposed persons without disease, but are lower than those of asbestosis patients (7,8). Asbestos bodies, while less specific, show a similar pattern (9).

Currently, the presence or absence of significantly elevated levels of asbestos in tissue does not affect the diagnostic or therapeutic approach to patients with MM. However, it may be important in obtaining appropriate compensation for an individual by demonstrating definitively that there has been excessive asbestos exposure. While we do not advocate performance of lung biopsy simply for medicolegal reasons, fiber analysis often should be done if a lung biopsy is performed for other reasons. Furthermore, there may be a public health impact of demonstrating that an individual has had significant exposure. A case of MM may be considered a "sentinel event" (10) in a population, indicating a more general exposure has occurred. It may thus help to identify others who are at risk of asbestos-related diseases. For example, tissue analysis demonstrating that a case of pulmonary fibrosis was due to tremolite asbestos contamination of vermiculite helped identify a population of workers that had similar exposures (11).

Determination of Simian Virus 40 Status

Simian virus 40 has been associated with many MMs (see Chapter 3). While it can be reliably detected in paraffin specimens, fresh tissue is preferable. Therefore, if the clinician feels that this should be done, arrangements should be made in advance to process the specimen when it is obtained. For example, it may be either transmitted immediately or quick frozen for subsequent analysis without paraffin embedding. Because its measurement depends upon polymerase chain reaction (PCR) techniques, laboratory contamination must be assiduously avoided.

When is SV40 analysis advisable? At the current time, the presence or absence of SV40 does not materially affect the therapeutic approach. However, there are rapidly evolving developments in this field, and its measurement may become more important in the future. Presence of SV40 is a significant prognostic factor (12), and determination of SV40 is important for research, to improve the understanding of the etiology of this tumor.

Counseling Patients at Risk

Patients frequently ask their physicians about their risk of developing asbestos-related disease such as mesothelioma due to widespread public attention to the health effects of asbestos. Clinicians become involved in the assessment and communication of risk of mesothelioma in several distinct situations: (1) Mesothelioma patients often inquire whether their tumor was exposure related. (2) Relatives or coworkers of an MM patient who also have had exposures to asbestos or SV40 usually have great concern about their personal risk. (3) Currently healthy patients who have had extensive occupational exposure to asbestos need counseling. (4) Patients with limited asbestos exposure also are often concerned. (5) In the future, as information about SV40-contaminated polio vaccine becomes more widespread, patients who received polio vaccine in the 1950s may ask about risk.

The level of anxiety among relatives and coworkers of a mesothelioma patient may be considerably greater than that of an individual with incidental low-level short-term asbestos exposure. However, even in the latter situation, some individuals may have a great deal of concern. Mesothelioma risk is of particular concern for individuals who have been exposed to very low levels of asbestos. Unlike individuals who have had high-level, long-term occupational exposure, for whom mesothelioma is only one of several risks (also asbestosis, pleural plaque, bronchogenic carcinoma, etc.), MM is the only concern with low-level, short-term exposures to amphiboles. Thus, while the absolute risk is much lower than for the occupationally exposed group, the mesothelioma focus is greater.

Physicians should first assess personal risk (integrating estimates of personal exposures with general epidemiologic and toxicologic studies). Then, this information should be communicated effectively in terms the patient can understand (e.g., "1×10^{-6}" is not meaningful to most persons). An individual who nonchalantly accepts the risks of drinking beer, driving without a seatbelt, and skiing may be much more concerned about the much lower risk imposed by inadvertent exposure during asbestos abatement in an office building. The attitude of individuals towards risk is only partially related to the quantitative risk itself. Actual risk may be much lower in community rather than occupational exposures, but several factors common in low-level community-based exposure settings amplify the perception of risk (13). These include (1) the involuntary nature of risk: drinking and driving is a personal choice, but breathing asbestos or receiving a contaminated vaccine is completely out of the control of the individual. (2) Unfamiliarity: automobile accident deaths are familiar to most persons, but MM is not a familiar problem. Hence, the level of concern is greater for this disease than for others, such as bronchogenic carcinoma, with which many individuals have had some personal experience in relatives or coworkers. (3) "Invisible" agents generate more concern than something that can be sensed. (4) "Exotic" risks always generate more concern than more familiar hazards, even if they constitute a lower actual risk; asbestos and SV40 are exotic. (5) Risk to children rather than adults creates great concern.

Table 23.3. Factors affecting individual risk

Asbestos type
Latency
Employment
Job
Tasks
Protective equipment
Family history
Simian Virus 40 exposure (not yet proven)

Assessment of Individual Risk

Several factors determine the likelihood that an individual will develop MM. Risk may vary from very significant in long-term exposed persons to very low in other settings. These factors are summarized in Table 23.3. Furthermore, a similar approach is applicable in determining whether an individual case of MM is likely to have been attributable to a specific exposure (job, agent, etc.). There is a "natural" background risk of MM, although it is relatively low (1). The possible combination of persistent amphibole deposits in the lungs of children and the uncertainty as to the thresholds has raised considerable professional and public anxiety about the risk of these children developing MM later in life. In fact, these risks are extremely small as judged by airborne sampling in such schools (14,15). However, numinous societal anxieties remain, which can only be countered by thoughtful explanation of the real magnitude of the risks (13).

Type of Asbestos

Asbestos is a commercial term referring to a group of minerals with different morphology, surface characteristics, and chemical composition. The two major commercial categories are chrysotile and amphibole. In United States, chrysotile has accounted for most of the commercially applied asbestos, but amphibole asbestos is much more closely associated with mesothelioma risk. Therefore, knowing the amount of chrysotile versus amphibole exposure in an individual can help delineate the actual risk of MM.

While there is a general consensus that the mesothelioma risk posed by amphibole is much greater than that from chrysotile, there is still debate about whether the risk of chrysotile is zero or simply "very low" (16). The commercial categories of amphiboles (crocidolite, amosite, and anthophyllite) and the serpentine (chrysotile) show a descending mesotheliogenic potential, as shown particularly by South African (17,18) and United Kingdom (19) studies. Some of the Canadian mines have been chrysotile contaminated with the noncommercial amphibole tremolite; workers in mines with higher proportions of tremolite have a greater mesothelioma risk.

Epidemiologic studies have not been able to define whether chrysotile per se is associated with mesothelioma because of concomitant exposure to amphibole. Studies of tissue from individuals with

asbestos-related disease and from the general population have been unable to fully resolve this issue because of the frequent contamination of chrysotile with amphibole. In addition, chrysotile tends to fragment or dissolve more quickly than does amphibole. The tissue levels found on biopsy or autopsy may not reflect the actual exposures of individuals many years previously, but rather will progressively underrepresent the amount of chrysotile.

Tremolite, at least in its fibrous habitat, is a powerful mesotheliomagenic agent present in rocks and soils in Asia, Europe, Australia, and the United States, notably California and Montana. It is responsible for MMs in the populations of Cyprus, Christmas Island, and in Libby, Montana. The nonasbestos fibrous zeolite is also an important cause of MMs in Turkey (20). Finnish anthophyllite appears to have a low risk (21). Certain refractory ceramic fibers, a synthetic product, induce mesotheliomas in animals (22).

The potential MM threshold from crocidolite is lower than that required for asbestosis, but the precise value is debated.

Familial Factors

Familial factors have also been invoked with asbestos in MM causation (23). Since families share both genetics and early, particularly childhood, environment, it is difficult to differentiate between these two factors and any potential interaction.

Duration and Era of Work

Certain jobs or tasks have had significant potential for asbestos exposure. The duration of work in these jobs is related to cumulative dose. Some jobs (e.g., insulation worker, pipe fitter, gas mask producer, steam fitter) are well known to entail asbestos exposure, but many other jobs also have had significant exposures.

Knowing the period during which the work was conducted can give insight into the probable extent of exposure. Professional opinion about the quantitative risk exposure and implementation of exposure controls have evolved over the past several decades. For example, it was only in 1970 that the use of asbestos-containing products in consumer settings was outlawed in the United States. The level of workplace exposure that was considered "legally permissible" has changed over time. For example, the U.S. Occupational Safety and Health Administration (OSHA) standards now allow 0.1 fiber per cubic centimeter, whereas in the past, it allowed five fibers per milliliter (24). The use of amphiboles is now rare, but historically it occurred in the 1940s to the late 1960s, thus leaving a long-term legacy of MM, increasingly apparent during the 1990s and 2000s.

Certain tasks and work processes are associated with greater exposure than others. For example, dry transfer of bulk asbestos is associated with a much higher level exposure than is work in a wet state. For some tasks, such as automotive brake-lining work, results are inconsistent (25–27). Work with unencapsulated asbestos leads to much

greater levels of exposure than work with asbestos in a matrix; for example, vinyl asbestos tile includes considerable asbestos, but usually the particle size is so large that the amount reaching the distal lung is low.

Therefore, the clinician should inquire about the specific occupations and locations, dates over which the work was done, tasks performed, details of tasks (e.g., wet or dry), and the proportion of time spent in each task.

The clinician should also look for other signs of asbestos exposure (e.g., pleural plaque) as part of the clinical evaluation if there is concern about mesothelioma risk. Similarly, in an individual with a mesothelioma, it is useful to look for other indicators that provide insight into the asbestos dose. For example, the hemithorax not involved with the tumor should be carefully examined by radiographic imaging techniques (plain radiographs or CT scan) for pleural plaque formation. In addition, it is useful to search for radiographs obtained several years prior to recognition of the MM.

A full occupational and environmental history should be obtained. Often, however, patients may be unaware of some sources of asbestos exposure (28). As discussed earlier, tissue biopsy can facilitate determination of asbestos burden in the lungs.

Latency is the duration of time since the *first* exposure. Generally, MM does not develop for many years (typically 20 to 30) after exposure. Hence, a malignancy developing very soon after an exposure is not likely to be related to that exposure. Several statistical modeling studies suggest that with very long latency the risk may begin to decline.

The presence of other asbestos-related disorders influences the risk of mesothelioma. There are clear data that bronchogenic carcinoma risk is significantly affected by the presence or absence of asbestosis (pulmonary fibrosis). This has not been well demonstrated for MM. Also, while the presence of a pleural plaque is statistically associated with an increased risk of MM, this is likely to be an indirect relationship rather than implying that the plaque itself is a risk factor for mesothelioma (29,30). That is, since pleural plaques occur more frequently in persons with asbestos exposure, it is the exposure, not the plaque per se, that constitutes the risk for an individual with the plaque. A similarly exposed person without a plaque probably has similar mesothelioma risk to someone with a pleural plaque if exposures were the same.

Cigarette smoking is a major risk factor for the development of bronchogenic carcinoma, but not for MM.

Selecting Treatment

Clinicians must guide patients in selecting the optimal treatment. Major questions include:

1. Should treatment be done in a hospital close to home or in a major research center, even if away from home?

2. Should the patient accept randomization into a clinical trial, or should the patient's clinician be allowed to make all decisions on a purely empiric basis?

3. Because mesothelioma, despite therapeutic advances, is often fatal, clear planning for end-of-life care is needed. For example, the clinician should help prepare the patient and family for the need for palliative care. Questions about whether death at home or in a hospice is preferable should be considered.

Counseling Patients about Legal Aspects

Appropriate counseling should alert patients and their families to the possibility of compensation, including workers' compensation and tort-based benefits. However, the decision about instituting a claim should be made by the patient, and the clinician only serves as a general source of information. Needless to say, physicians should not try to be lawyers.

Because of the ubiquitous legal involvement in MM, primary clinicians should advise their patients to clearly identify the role of clinicians with whom they interact. The primary physician of a mesothelioma patient should help identify those clinicians whose interactions with the patient may be affected by the adversarial legal system.

In many states, clinicians have a legal responsibility to report work-related cases to the appropriate public health or regulatory authority. For example, in the state of California, it is a crime to withhold information from the state about a probable work-related condition such as mesothelioma.

Primary and Secondary Prevention Programs

The best "treatment" is prevention. Careful control of exposure to asbestos, based on the current understanding of risk, can help prevent future cases. Nevertheless, because of the population's asbestos burden acquired in prior years, when less attention was paid to asbestos control, asbestos-related mesotheliomas cases are likely to occur for at least 20 more years (1,31–34).

Active screening for case detection of MM is not currently advisable. In the future, studies may possibly demonstrate that recent advances in treatment create a situation in which early detection significantly improves therapeutic outcome; then, appropriate targeted screening programs may be advisable from a public health perspective.

References

1. Price B. Analysis of current trends in United States mesothelioma incidence. Am J Epidemiol 1997;145(3):211–218.
2. Light RW. Clinical practice. Pleural effusion. N Engl J Med 2002;346(25): 1971–1977.

3. Cagle PT, et al. Immunohistochemical differentiation of sarcomatoid mesotheliomas from other spindle cell neoplasms. Am J Clin Pathol 1989; 92(5):566–571.
4. Syed S, et al. Desmoplastic small round cell tumor of the lung. Arch Pathol Lab Med 2002;126(10):1226–1228.
5. Karjalainen A, et al. Asbestos bodies in bronchoalveolar lavage in relation to asbestos bodies and asbestos fibres in lung parenchyma. Eur Respir J 1996;9(5):1000–1005.
6. De Vuyst P, Dumortier P, Gevenois PA. Analysis of asbestos bodies in BAL from subjects with particular exposures. Am J Ind Med 1997;31(6):699–704.
7. Gibbs AR. Role of asbestos and other fibres in the development of diffuse malignant mesothelioma. Thorax 1990;45(9):649–654.
8. Gibbs AR, et al. Comparison of fibre types and size distributions in lung tissues of paraoccupational and occupational cases of malignant mesothelioma. Br J Ind Med 1990;47(9):621–626.
9. Roggli VL, Pratt PC, Brody AR. Asbestos content of lung tissue in asbestos associated diseases: a study of 110 cases. Br J Ind Med 1986;43(1):18–28.
10. Mullan RJ, Murthy LI. Occupational sentinel health events: an up-dated list for physician recognition and public health surveillance. Am J Ind Med 1991;19(6):775–799.
11. Wright RS, et al. Fatal asbestosis 50 years after brief high intensity exposure in a vermiculite expansion plant. Am J Respir Crit Care Med 2002; 165(8):1145–1149.
12. Procopio A, et al. Simian virus-40 sequences are a negative prognostic cofactor in patients with malignant pleural mesothelioma. Genes Chromosomes Cancer 2000;29(2):173–179.
13. Fischhoff B, et al. Acceptable Risk. Cambridge, MA: Cambridge University Press, 1981.
14. Wilson R, et al. Asbestos in New York City public school buildings—public policy: is there a scientific basis? Regul Toxicol Pharmacol 1994;20(2):161–169.
15. Mossman BT, et al. Asbestos: scientific developments and implications for public policy. Science 1990;247(4940):294–301.
16. McDonald JC, et al. Mesothelioma and asbestos fiber type. Evidence from lung tissue analyses. Cancer 1989;63(8):1544–1547.
17. Rees D, et al. Asbestos exposure and mesothelioma in South Africa. S Afr Med J 1999;89(6):627–634.
18. Rees D, et al. Case-control study of mesothelioma in South Africa. Am J Ind Med 1999;35(3):213–222.
19. Hodgson JT, Darnton A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann Occup Hyg 2000;44(8):565–601.
20. Emri S, et al. Lung diseases due to environmental exposures to erionite and asbestos in Turkey. Toxicol Lett 2002;127(1–3):251–257.
21. Meurman LO, Pukkala E, Hakama M. Incidence of cancer among anthophyllite asbestos miners in Finland. Occup Environ Med 1994;51(6):421–425.
22. Hesterberg TW, et al. Chronic inhalation toxicity of size-separated glass fibers in Fischer 344 rats. Fundam Appl Toxicol 1993;20(4):464–476.
23. Dawson A, et al. Familial mesothelioma. Details of 17 cases with histopathologic findings and mineral analysis. Cancer 1992;70(5):1183–1187.
24. Gee B, Bouhuys A. Action on asbestos. N Engl J Med 1971;285(23):1317–1318.
25. Hansen ES. Mortality of auto mechanics. A ten-year follow-up. Scand J Work Environ Health 1989;15(1):43–46.

26. Huncharek M, Muscat J, Capotorto JV. Pleural mesothelioma in a brake mechanic. Br J Ind Med 1989;46(1):69–71.
27. Wong O. Malignant mesothelioma and asbestos exposure among auto mechanics: appraisal of scientific evidence. Regul Toxicol Pharmacol 2001;34(2):170–177.
28. Barbers RG, Abraham JL. Asbestosis occurring after brief inhalational exposure: usefulness of bronchoalveolar lavage in diagnosis. Br J Ind Med 1989;46(2):106–110.
29. Hillerdal G, Lindgren A. Pleural plaques: correlation of autopsy findings to radiographic findings and occupational history. Eur J Respir Dis 1980; 61(6):315–319.
30. Harber P, et al. Pleural plaques and asbestos-associated malignancy. J Occup Med 1987;29(8):641–644.
31. Lilienfeld DE, et al. Projection of asbestos related diseases in the United States, 1985–2009. I. Cancer. Br J Ind Med 1988;45(5):283–291.
32. de Klerk NH, et al. Predictions of future cases of asbestos-related disease among former miners and millers of crocidolite in Western Australia. Med J Aust 1989;151(11–12):616–620.
33. Peto J, et al. Continuing increase in mesothelioma mortality in Britain. Lancet 1995;345(8949):535–539.
34. Peto J, et al. The European mesothelioma epidemic. Br J Cancer 1999; 79(3–4):666–672.

24

Clinical Presentation and Natural History of Mesothelioma: Pleural and Pericardial

A. Philippe Chahinian

This chapter reviews the clinical features of two types of malignant mesothelioma—pleural and pericardial. Although such distinction refers to the cavity of origin of this neoplasm, it is well known that each of these can spread to the other cavity when tumor progression occurs. In a total of 1496 cases of mesotheliomas reviewed pathologically, Suzuki (1) found the primary site to be pleural in 73.1%, peritoneal in 23.7%, and pericardial in 0.3%. The remainder (2.9%) had multicavitary involvement.

Malignant Pleural Mesothelioma

Clinical Presentation

Demographics and General Characteristics

There is uniformly a preponderance of males in all clinical series (Table 24.1) (2–8). This could be related to more common exposure to asbestos, the most important etiologic factor, in males. Men account for 68% to 79% of all cases of pleural mesotheliomas.

Mean and median age at diagnosis are usually between 54 and 59 years, with a very wide range. In fact, pleural mesothelioma can occur at any age, even in children. In a review of 80 cases of malignant mesothelioma in children, mean age was 9.7 years and 59% were boys (9). History of possible asbestos exposure was noted in two children. In addition, one patient had received radiotherapy for Wilms' tumor, and another one had a history of exposure to isoniazid in utero.

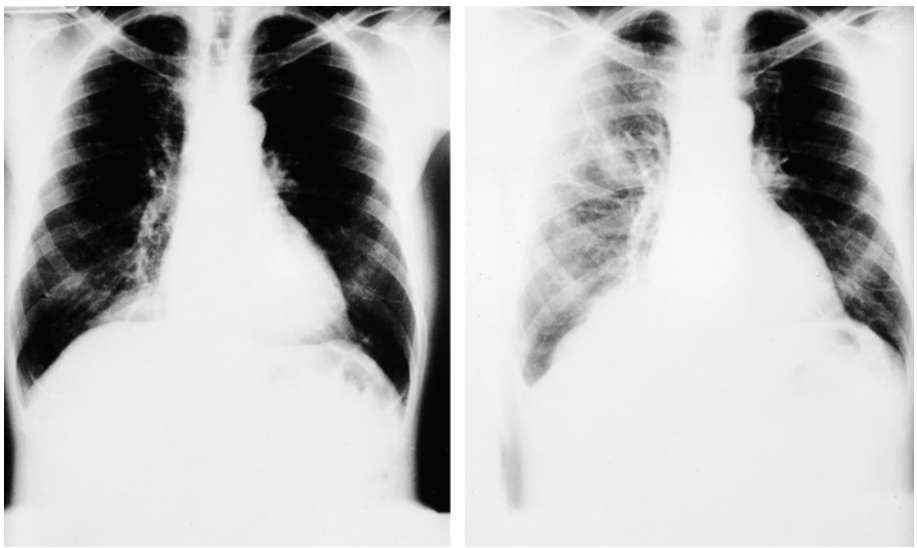
For pleural mesothelioma, the right side is more commonly involved, accounting for about 55% to 65% of cases. This could probably be explained by the preferential inhalation of asbestos fibers in the right lung.

Clinical Symptoms and Diagnosis

Typically the onset of symptoms is gradual and insidious (Fig. 24.1). Since the most common initial manifestation of pleural mesothelioma

Table 24.1 Clinical characteristics of patients with pleural mesothelioma

First Author (Reference) Year No. of Cases	Ratzer (7) 1967 $n = 31$	Chahinian (3) 1982 $n = 57$	Brenner (6) 1982 $n = 123$	Adams (4) 1986 $n = 92$	Ruffie (8) 1989 $n = 332$
Age (years)					
Mean/median	Med. 54	Mean 58	Med. 56	Mean 59	Mean 59
Range	13–70	24–75	5–77	28–80	22–88
Sex M (%)/F (%)	68/32	78/22	68/32	77/23	79/21
Initial symptoms (%)					
Dyspnea	6	37	29	59	29
Chest pain	71	33	37	69	33
Both dyspnea and pain	19	26			28
Cough	13	16	24	27	3
Hemoptysis	6	0			1
Hoarseness		0		3	1
Dysphagia		0			1
Weight loss		14		24	29
Fever		9		33	3
Asymptomatic (%)		4	4		3
Pleural effusion (%)	74	95		79	84
right (%)/left (%)	65/45	66/34	58/42		55/42
Symptoms to diagnosis					
Median		2 mos	3 mos		3.5 mos
Range		0–50 mos	0.5–24 mos		
Delay >6 months (%)		25			28



A B

Figure 24.1. This former asbestos worker was followed by routine periodic chest x-rays. A: A normal x-ray in April 1977. B: Minimal changes on the right side of the diaphragm and blunting of the right costophrenic angle in January 1979. These were the initial signs of pleural mesothelioma.

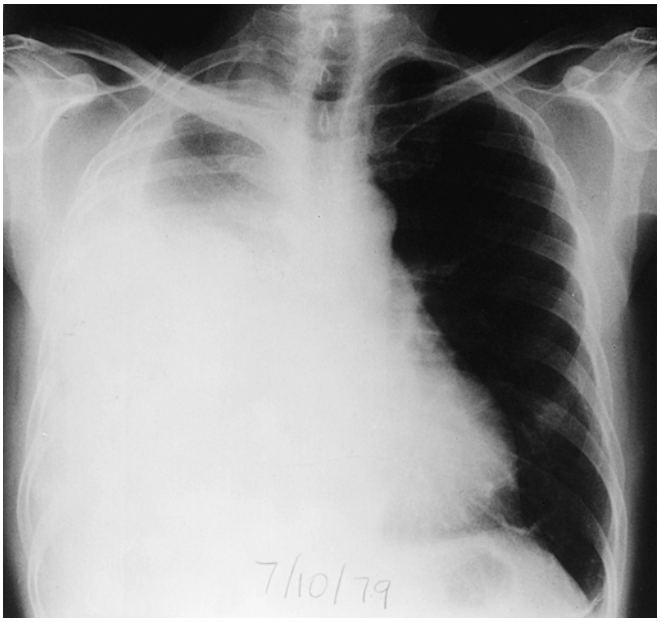


Figure 24.2. Chest x-ray of the same patient as in Figure 24.1 at presentation in July 1979. Massive right pleural effusion. Pleural biopsy showed malignant epithelial mesothelioma.

is a pleural effusion (Fig. 24.2), symptoms are dominated by dyspnea or chest pain. Initial symptoms in representative series are shown in Table 24.1. In our own experience based on 57 patients with pleural mesothelioma, initial symptoms were dyspnea (37%), chest pain (33%), both dyspnea and chest pain (26%), cough (16%), weight loss (14%), and fever without infection (9%) (3). The disease was discovered by routine chest x-ray in only 4% of patients. At this early stage, the degree of dyspnea is often related to the amount of pleural effusion, which occurs in up to 95% of patients (2,3). Chest pain is of the pleuritic type only in 10% of patients (4). More often, it is a steady pain localized to the involved hemithorax. The intensity of the pain is variable, from a dull twinge to a severe ache (7). Fever can be accompanied by night sweats and lead to an erroneous diagnosis of infection, particularly tuberculosis. Other presenting symptoms include, rarely, hemoptysis, dysphagia, Horner's syndrome, and hoarseness (8). Rare acute presentation can occur in less than 10% of patients and are due to spontaneous pneumothorax or acute hemothorax (8).

The presentation of pleural mesothelioma can be particularly challenging in young patients, where the index of suspicion is very low. We previously reported our experience with mesothelioma in young adults (age <40 years). Ten cases were seen at the Mount Sinai Hospital in New York between 1974 and 1987 out of a total of 181 patients with mesothelioma (10); six were pleural and four peritoneal, and age ranged from 24 to 39 years. Seven cases had a history of asbestos exposure, including five by household exposure, usually through the father. The median

latency period between first exposure and diagnosis was 19 years (range 13–34 years). Diagnosis was not suspected in most cases, and the median delay in diagnosis was 5.5 months. The presenting symptoms were diverse and included pain or dyspnea, malaise, cough, and fever. Pain was located at various sites, including any area of the thorax, but also the back or subscapular area. It is therefore important for the clinician to be aware of the possibility of this diagnosis even in young individuals or in children.

Physical findings are almost completely limited to those of a pleural effusion (11). Horner's syndrome is uncommon at this stage. Clubbing is also rare and was reported in about 6% of patients (2). Cardiac abnormalities on initial examination include a pericardial rub (2/57 patients), pericardial knock (1/57 patients), and a murmur of pulmonic stenosis (1/57 patients) (3). Electrocardiographic changes included right bundle branch block (5/57 patients), sinus tachycardia (3/57 patients), nonspecific ST-T changes (3/57 patients), atrial flutter (1/57 patients), and left anterior hemiblock (1/57 patients) (3). The possibility of early pericardial involvement should be considered and evaluated in such cases.

The median interval between first symptom and diagnosis is 2 months, but in our series 25% of patients had symptoms more than 6 months before diagnosis was made (3). Results of radiologic investigations are described elsewhere. Thoracentesis yields a serous to bloody fluid with the characteristics of an exudate (12). Pleural fluid glucose concentration can be low (12), while high levels of hyaluronic acid are highly suggestive of mesothelioma (13). Cytologic diagnosis is difficult. It shows malignant cells in about 35% of cases, but the diagnosis of mesothelioma is made in 10% or less (8,14). Percutaneous pleural needle biopsy can yield the diagnosis in about one third of cases (8). The cytologic and pathologic characteristics of mesothelioma are described elsewhere. Mesothelioma is an important cause of "idiopathic" pleural effusion. In 51 patients with pleural effusion of indeterminate etiology seen at the Mayo Clinic, four were subsequently diagnosed to have malignant mesothelioma (15). When the suspicion of mesothelioma is high enough based on the clinical and radiographic signs, and especially if a history of asbestos exposure is obtained, invasive procedures to obtain a final diagnosis are necessary and include thoracoscopy or thoracotomy.

There is a lack of positive serum markers currently available for the diagnosis of mesothelioma. Serum carcinoembryonic antigen (CEA) is usually within normal limits and is an important marker to distinguish adenocarcinoma from mesothelioma (2). On the other hand, an elevated serum level of hyaluronic acid may prove useful in differentiating mesothelioma from other tumors, or to follow the effect of treatment (2). The levels of CA-125 can also be elevated in mesothelioma. CA-125 is expressed in the nonneoplastic mesothelium and has been detected in 63% of malignant mesothelioma cells by immunohistochemistry, without a clear-cut correlation with serum levels (16). In 32 patients with malignant mesothelioma, we found an elevated serum level of CA-125 (<35 U/mL) in 44% (median 152 U/mL, range 47.6 to 1441 U/mL) (17). Serum levels of CA-125 were more often elevated in

cases of sarcomatous or mixed types (67%) as opposed to epithelial type (35%). Elevated levels were observed both in men (46%) and women (37.5%).

Paraneoplastic Syndromes

The most common paraneoplastic syndrome in pleural mesothelioma is thrombocytosis. We first reported this association in 1982 (3). Thrombocytosis (as defined by a platelet count above 400,000 per microliter) was seen in about 40% of patients at diagnosis and in up to 90% of patients during the course of the disease, a finding that has been confirmed by others (8,16). In addition, thrombocytosis has been linked to a poor prognosis (8,19). It has been suggested in a case of peritoneal mesothelioma that thrombocytosis was secondary to the large amounts of interleukin-6 (IL-6) produced by tumor cells (20), and this was confirmed in 25 patients with pleural mesothelioma (21). We found that serum levels of IL-6, as well as reactive proteins (C-reactive protein, α_1-acid glycoprotein, and fibrinogen) to be significantly higher in mesothelioma patients than in those with adenocarcinoma of the lung (21). There was a correlation between platelet count and serum IL-6 level. Levels of IL-6 in the pleural fluid of mesothelioma patients were even markedly higher than serum levels. In contrast, both serum and pleural fluid levels of tumor necrosis factor-α (TNF-α) were low in mesothelioma patients.

A full leukemoid reaction is much less common. Other hematologic manifestations include clotting abnormalities (venous thrombosis, pulmonary emboli) not necessarily associated with thrombocytosis, as well as disseminated intravascular coagulation and autoimmune hemolytic anemias (2,8). Rare associations with mesothelioma include the syndrome of inappropriate antidiuretic hormone secretion (SIADH), hypoglycemia, and hypercalcemia (2,8). Hyponatremia has been reported in as many as 62% of patients with pleural mesothelioma, but its degree was minimal (mean ± standard deviation = 138 ± 5.4 mmol/L). It was hypothesized that rather than being secondary to ectopic secretion of ADH, it was due to ADH hypersecretion through a vagal reflex, either from involvement of pulmonary baroreceptors or by direct vagal stimulation by tumor (22). Parathyroid hormone–like peptide has been identified in mesothelioma cells, as well as in normal and reactive mesothelial cells (2).

Clinical associations that have been observed in patients with mesothelioma include various immunoproliferative disorders, particularly of B-cell origin (2,23). They include multiple myeloma, plasmacytoma, lymphocytic lymphoma, and chronic lymphocytic leukemia. A case-control study showed an association between occupational exposure to asbestos and large-cell lymphomas of the gastrointestinal tract and oral cavity (24). These observations provide further significance to immunologic abnormalities related to asbestos exposure and mesothelioma. Clinical observations also strongly suggest a genetic susceptibility to mesothelioma. Clusters of cases have been reported in some families, often by household exposure to asbestos, and also in identical twins (2). Similar observations were made after exposure to

erionite in Turkish villages (25). The growing knowledge of the genetic changes associated with mesothelioma will better explain these observations and shed more light on the pathogenesis of the disease.

Differential Diagnosis

Benign mesotheliomas are solitary fibrous tumors of pleura and are usually not related to asbestos exposure. These tumors of the visceral or parietal pleura are often pedunculated, and pleural effusion is exceptional. Most are benign, although a malignant form does rarely occur. Paraneoplastic syndromes that have been observed include clubbing and osteoarthropathy seen in up to 20% to 50% of cases, hyponatremia attributed to SIADH, and hypoglycemia (2).

A very difficult differential is related to the so-called benign asbestos pleurisy, which occurs in about 3% to 5% of asbestos workers (2). Its latency period from first exposure to asbestos is usually less than 20 years, making it the earliest abnormality, compared with other asbestos-related pleural diseases, such as mesothelioma, pleural plaques, and pleural calcifications. Confusion with malignant mesothelioma is common in view of a history of asbestos exposure and a bloody pleural fluid in the majority of cases. In contrast with malignant mesothelioma, however, the pleural effusion resolves spontaneously, but ipsilateral relapses are frequent and contralateral disease may appear. Pleural biopsy shows dense fibrosis with scattered nonmalignant cells. Close follow-up is necessary, since some patients have developed malignant mesothelioma 6 to 12 years after such an episode.

It is also difficult to distinguish malignant mesothelioma from metastatic carcinomas and sarcomas. Confusion with a peripheral adenocarcinoma of the lung metastatic to the pleura is frequent, not only on frozen sections but also on fixed paraffin sections. The pathologic differential diagnosis is discussed elsewhere. Recognizing mesothelioma as the cause of a malignant pleural effusion is important in order to avoid a time-consuming, fruitless, and expensive workup in search of another primary site.

Natural History

The natural history of pleural mesothelioma is of relentless growth in the hemithorax with early involvement of surrounding structures including lung, diaphragm, chest wall, pericardium, mediastinum, and direct spread to the peritoneum and contralateral hemithorax (Fig. 24.3) (2,3). Seeding within the track of needle biopsy or surgical incision is also common (Fig. 24.4). Gradual thickening of the involved visceral and parietal pleura leads to constriction of the hemithorax, and obliteration of the pleural space with decrease or disappearance of pleural effusion at that stage, leading to a "frozen" hemithorax. Characteristic symptoms are increasing pain and dyspnea. Cardiac findings are common at this stage and were reviewed in 64 patients with pleural mesothelioma at our institution (26). The electrocardiogram was abnormal in 89% of patients. Over half (60%) had an arrhythmia, including sinus tachycardia (42%), premature atrial or ventricular contractions (13%), atrial fibrillation

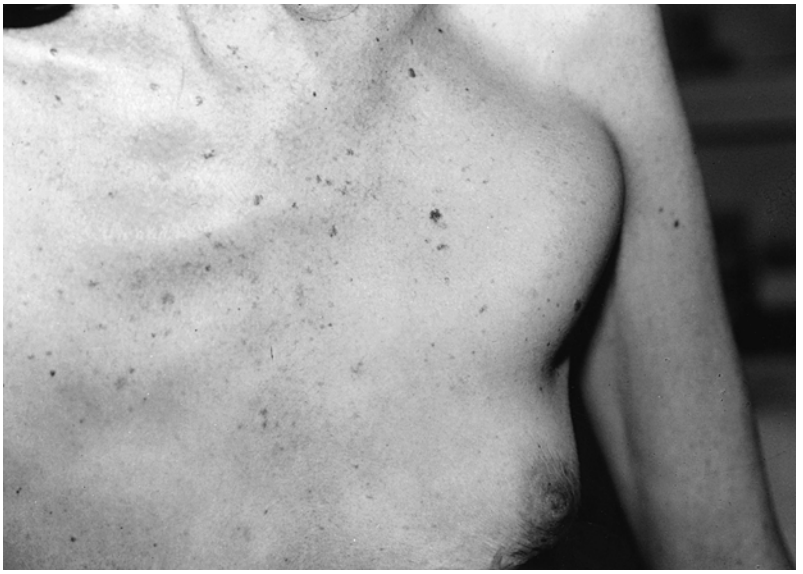


Figure 24.3. Massive chest wall involvement in a patient with pleural mesothelioma.

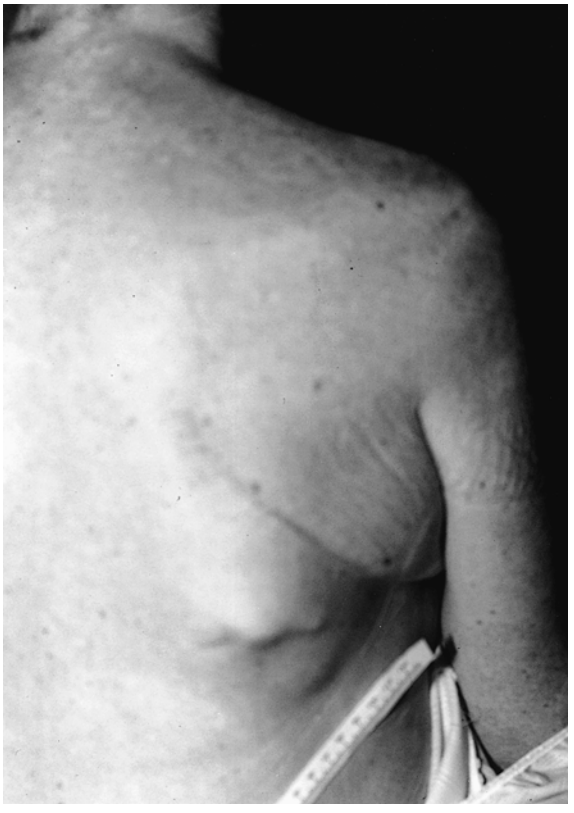


Figure 24.4. Seeding at the surgical scar of prior chest tube insertion in a patient with pleural mesothelioma.

(3%), and atrial flutter (1%). Over one third (37%) had a conduction abnormality, such as complete or incomplete right bundle branch block (27%), or left anterior or posterior hemiblock (8%). Low-voltage QRS was seen in 3% only, and no patient had a left bundle branch block.

Although the clinical picture remains dominated by the local disease, metastases are common and include possible lymphatic spread to mediastinal, cervical, axillary, retroperitoneal, and mesenteric lymph nodes, as well as hematogenous metastases to liver, spleen, adrenals, bone, gastrointestinal tract, pancreas, kidneys, uterus, bone marrow, and even brain (3,27). Such metastases are often found at autopsy, where only 20% of patients with pleural mesothelioma had disease limited to the thorax (2,3), but these metastases rarely contribute to death. It is noteworthy that at autopsy, cardiac invasion to pericardium, epicardium, and even myocardium was found in 74% of patients, most often by direct invasion, and thromboembolic events were noted in 28% (3,27). Two cases of calcified liver metastases have been reported (28,29). These calcifications were attributed to degenerative changes and necrosis of metastases.

In our experience, median survival was 17 months from first symptoms and 13 months from diagnosis, with a survival of 56% at 1 year and 22% at 2 years following diagnosis (3).

Malignant Pericardial Mesothelioma

Whereas pleural mesothelioma commonly spreads to the pericardium, primary pericardial mesothelioma is exceptional but has been well described. It was previously reported under various names including coelothelioma, endothelioma, and endothelial carcinoma (30). Like pleural mesothelioma, histologic types can be epithelial, sarcomatous, or mixed (30,31). Asbestos exposure has been reported, and in prospective studies was found to definite in three of 15 cases (20%) and possible in four of 15 (27%) (32). In further support of this association, asbestos bodies have been occasionally identified within pericardial mesothelioma (33).

Pericardial mesothelioma accounts for about half of all pericardial tumors (34,35). More than 80 cases were reported by 1967. Only a small fraction of patients (less than 20%) had been diagnosed antemortem. Since then progress in imaging and biopsy techniques have allowed definitive diagnosis at presentation. In a more recent review, a total of 28 cases were reported in the English literature from 1972 to 1992. The mean age was 47 years, asbestos exposure was documented in 14% and prognosis remained poor (31). There are over 200 cases reported worldwide (33). In the review by the Armed Forces Institute of Pathology (AFIP), Washington, DC, on 59 patients, the mean age was 46 years, ranging from 2 to 78 years. The male/female ratio was 2:1, somewhat lower than the ratio reported in pleural mesothelioma (33).

A variety of clinical symptoms have been observed, from those of pericardial effusion (often bloody) with dyspnea and pain, to those of constrictive pericarditis or vascular compression (superior vena caval

syndrome, constriction of great vessels) (34). Cardiac tamponade can be the revealing event, or can occur later, often as a terminal manifestation (36). Echocardiography reveals pericardial thickening or effusion, but a mass is detected in only 12% of patients (31). Computed tomography similarly reveals various degrees of pericardial thickening and fluid, and a mass is seen in 44% of cases (31). In addition, search for pleural involvement as well as signs of asbestos exposure (pleural plaques and calcifications) is important. Magnetic resonance imaging is most useful in assessing the disease and evaluating its extent (37). Effusion cytology revealed malignant cells in only 20% of cases (31).

Although pericardial mesothelioma can occasionally mimic tuberculous pericarditis, lupus erythematosus, rheumatic fever, or even cardiac myxoma (33), the major differential diagnosis includes metastatic tumors to the pericardium, by far more common and which can be seen in almost any type of carcinoma, leukemia, and lymphoma. It is often difficult to differentiate mesothelioma from metastatic adenocarcinoma, and special stains as well as electron microscopy are useful. Other primary malignant cardiac tumors, which are usually sarcomas (38), can also be difficult to distinguish from pericardial mesothelioma, especially in its sarcomatous form. Angiosarcoma is the most common primary cardiac malignant tumor and its gross aspect can mimic mesothelioma (33,38). Immunohistochemical stain for factor VIII–related antigen can be helpful, since it is usually positive in angiosarcoma (33). Finally a biphasic aspect (mixed epithelial and sarcomatous) is very characteristic of mesothelioma but two other tumors can present a similar histologic dichotomy, including synovial sarcoma and invasive thymoma (33). The diagnosis of these tumors require detailed gross and microscopic evaluation, which are beyond the scope of this chapter.

Mesothelioma of the atrioventricular node is very rare (about 50 cases reported), and usually is minute or even microscopic (2). Partial or complete nodal heart blocks and sudden death are the major consequences of this tumor. Two thirds occurred in females, and age ranged from an 8-month-old fetus to an 86-year-old woman. The natural history of pericardial mesothelioma, like its pleural counterpart, is of relentless growth. These tumors are usually diffuse, covering most of the heart, often obliterating the pericardial cavity, and may invade the myocardium and invade surrounding tissues (pleura, lung, mediastinal nodes). Distant metastases have also been seen occasionally (34,36).

Treatment is usually purely palliative, and 50% to 60% of patients are dead within 6 months (33,34). The prognosis of pericardial mesothelioma appears clearly worse than that of pleural or peritoneal mesotheliomas (AFIP). Only one patient was reported to be alive at 5 years, following treatment with partial surgical resection and radiation (35). Another patient survived 1 year after similar treatment.

Addendum

Since submission of this manuscript, another marker for mesothelioma has been identified. Mesothelin is a differentiation antigen originating from a precursor protein processed to a 40 kDa cell membrane-bound

protein and a soluble 31 kDa fragment also called megakaryocyte-potentiating factor (39–41). Mesothelin seems to be normally expressed only in mesothelial cells, and its biologic function is unknown, but it may have a role in cell adhesion. Interestingly it can bind to CA-125 (41). It does not seem to affect platelet production in humans. Elevated serum levels of soluble mesothelin have been reported in 37 (84%) of 44 patients with malignant mesothelioma, and in only 3 (2%) of patients with other cancers or inflammatory lung or pleural diseases (39). However elevated serum levels have also been found in other tumors including ovarian, pancreatic, and other carcinomas. The role of mesothelin as a therapeutic target merits further investigations.

References

1. Suzuki Y. Pathology of human malignant mesothelioma. Preliminary analysis of 1,517 mesothelioma cases. Ind Health 2001;39:183–185.
2. Chahinian AP, Pass HI. Malignant mesothelioma. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei E III, eds. Cancer Medicine, 5th ed. Hamilton, Canada: BC Decker, 2000:1293–1312.
3. Chahinian AP, Pajak TF, Holland JF, et al. Diffuse malignant mesothelioma. Prospective evaluation of 69 patients. Ann Intern Med 1982;96:746–755.
4. Adams VI, Unni KK, Muhn JR, et al. Diffuse malignant mesothelioma of pleura. Diagnosis and survival in 92 cases. Cancer 1986;58:1540–1551.
5. Antman K, Shemin R, Ryan L, et al. Malignant mesothelioma. Prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. J Clin Oncol 1988;6:147–153.
6. Brenner J, Sordillo PP, Magill GB, Golbey RB. Malignant mesothelioma of the pleura. Review of 123 patients. Cancer 1982;49:2431–2435.
7. Ratzer ER, Pool JL, Melamed MR. Pleural mesotheliomas. Clinical experiences with thirty-seven patients. AJR 1967;99:863–880.
8. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec. A retrospective study of 332 patients. J Clin Oncol 1989;7:1157–1168.
9. Fraire AE, Cooper S, Greenberg SD, et al. Mesothelioma of childhood. Cancer 1988;62:838–847.
10. Kane JM, Chahinian AP, Holland JF. Malignant mesothelioma in young adults. Cancer 1990;65:1449–1455.
11. Pisani RJ, Colby TV, Williams DE. Malignant Mesothelioma of the pleura. Mayo Clin Proc 1988;63:1234–1244.
12. Taryle DA, Lakshminarayan S, Sahn SA. Pleural mesotheliomas. An analysis of 18 cases and review of the literature. Medicine 1976;55:153–162.
13. Roboz J, Greaves J, Silides D, et al. Hyaluronic acid content of effusions as a diagnostic aid for malignant mesothelioma. Cancer Res 1985;45:1850–1854.
14. Light RW. Pleural effusion. N Engl J Med 2003;346:1971–1977.
15. Ryan Cj, Rodgers RF, Unni KK, Hepper NGG. The outcome of patients with pleural effusion of indeterminate cause at thoracotomy. Mayo Clin Proc 1981;56:145–149.
16. Motoyama T, Watanabe T, Okazaki E, et al. Immunohistochemical properties of malignant mesothelioma cells in histologic and cytologic specimens. Acta Cytol 1995;39:164–170.
17. Chahinian AP, Jogenpally N, Patel R, et al. Serum levels of CA 125 in 32 patients with malignant mesothelioma. Proc Am Soc Clin Oncol 1996;15: 91(abstract).

18. Nakano T, Fujii J, Tamura S, et al. Thrombocytosis in patients with malignant pleural mesothelioma. Cancer 1986;58:1699–1701.
19. Chahinian AP, Pajak TF, Holland JF, Green G. Adverse prognostic significance of thrombocytosis in patients with malignant mesothelioma. Proc Am Assoc Cancer Res 1984;25:161(abstract).
20. Higashihara M, Sunaga S, Tange T, et al. Increased secretion of interleukin-6 in malignant mesothelioma cells from a patient with marked thrombocytosis. Cancer 1992;70:2105–2108.
21. Nakano T, Chahinian AP, Shinjo M, et al. Interleukin 6 and its relationship to clinical parameters in patients with malignant pleural mesothelioma. Br J Cancer 1998;77:907–912.
22. Perks, WH, Stanhope R, Green M. Hyponatraemia and mesothelioma. Br J Dis Chest 1979;73:89–91.
23. Efremidis AP, Waxman JS, Chahinian AP. Association of lymphocytic neoplasia and mesothelioma. Cancer 1985;55:1056–1059.
24. Ross R, Nichols P, Wright W, et al. Asbestos exposure and lymphomas of the gastrointestinal tract and oral cavity. Lancet 1982;2:1118–1119.
25. Carbone M, Kratzke RA, Testa JR. The pathogenesis of mesothelioma. Semin Oncol 2002;29:2–17.
26. Wadler S, Chahinian AP, Slater W, et al. Cardiac abnormalities in patients with diffuse malignant pleural mesothelioma. Cancer 1986;58:2744–2750.
27. Wronsky M, Burt M. Cerebral metastases in pleural mesothelioma. Case report and review of the literature. J Neurooncol 1993;17:21–26.
28. Persaud V, Bateson EM, Bankay CD. Pleural mesothelioma associated with massive hepatic calcification and unusual metastases. Cancer 1970;26:920– 928.
29. Campbell GD, Greenberg SD. Pleural mesothelioma with calcified liver metastases. Chest 1981;79:229–230.
30. Dawe CJ, Wood DA, Mitchell S. Diffuse fibrous mesothelioma of the pericardium. Report of a case and review of the literature. Cancer 1953;6:794– 808.
31. Thomason R, Schlegel W, Lucca M, et al. Primary malignant mesothelioma of the pericardium. Case report and literature review. Tex Heart Inst J 1994; 21:170–174.
32. Beck B, Konetzke G, Ludwig V et al. Malignant pericardial mesothelioma and asbestos exposure. A case report. Am J Ind Med 1982;3:149–159.
33. Burke A, Virmani R. Malignant mesothelioma of the pericardium. In: Tumors of the Heart and Great Vessels. Atlas of Tumor Pathology, third series, fascicle 16. Washington, DC: Armed Forces Institute of Pathology, 1996:181–194.
34. Van de Water JM, Allen WH. Pericardial mesothelioma. Ann Thorac Surg 1967;3:162–165.
35. Steinberg I. Angiocardiography in mesothelioma of the pericardium. 1972;114:817–821.
36. Thomas J, Phythyon JM. Primary mesothelioma of the pericardium. Circulation 1957;15:385–390.
37. Eren NT, Akar AR. Primary pericardial mesothelioma. Curr Treat Options Oncol 2003;3:369–373.
38. Chahinian AP, Gutstein DE, Fuster V. Tumors of the heart and great vessels. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei E III, eds. Cancer Medicine, 5th ed. Hamilton, Canada: BC Decker, 2000:1319– 1321.
39. Robinson BWS, Creany J, Lake R, et al. Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 2003;362:1612–1616.
40. Rump A, Morikawa Y, Tanaka M, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. J Biol Chem 2004;279:9190–9198.
41. Hassan R, Bera T, Pastan I. Mesothelin: A new target for immunotherapy. Clin Cancer Res 2004;10:3937–3942.

25

Clinical Presentation and Natural History of Mesothelioma: Abdominal

Claire F. Verschraegen, Charles R. Key, and Raffit Hassan

Epidemiology and Etiology

The Surveillance, Epidemiology, and End Results (SEER) database showed 5266 cases of mesothelioma (all sites) recorded from 1973 to 1999. The age-adjusted incidence rate of the year 2000 United States standard is 9.7 cases per 1,000,000. The 95% confidence interval rates are 9.4 to 10 cases per 1,000,000. These numbers have not changed much from a previous survey done over 15 years ago (1). Table 25.1 shows the incidence around the country. Seattle and the San Francisco Bay area have higher incidence rates than other regions, especially for males. This increased incidence is related to shipyard work, especially since World War II (2). Usage of asbestos for ship building resulted in long-term exposure of workers for the past 60 years.

Mesothelioma occurs in different anatomic sites that contain mesothelial layers. The most common site is the pleura, followed by the peritoneum, the pericardium, the male genitalia, miscellaneous, and female genitalia. The peritoneal incidence by gender is different from the pleural incidence (Table 25.2). Proportionally, more women develop peritoneal mesothelioma, with an incidence of 24% versus 8% for males, using mesothelioma from any anatomic site as the denominator. In absolute numbers, more males are diagnosed with abdominal mesothelioma, with a frequency of 54.7% versus 45.3% for women (3) (Table 25.3). In the SEER database, the median age at diagnosis was between 65 and 69 years old for males and females. This is slightly higher than the age of the reported case in the literature (Table 25.4). By race, the incidence rates are highest for whites, followed by blacks, and then others.

Asbestos is the best-known environmental agent linked to the development of mesothelioma (see Chapter 2) (4). The literature on asbestos as a cause of pleural/pericardial plaques, asbestosis, and mesothelioma is extensive, and the causal relationship is not discussed in this chapter (5). The number of patients with mesothelioma differs by anatomic site

Table 25.1. Mesothelioma rates per 1,000,000 (age-adjusted to the 2000 U.S. standard)—SEER database

	Rate	SD	Lower CI	Upper CI	Count	Population
Males						
Nine SEER registries	**18**	**0.3**	**17.4**	**18.6**	**4,111**	**299,413,683**
San Francisco–Oakland SMSA	**25.3**	**0.9**	**23.7**	**27.1**	**922**	**46,961,380**
Connecticut	**14.5**	**0.7**	**13.3**	**15.9**	**524**	**41,759,293**
Detroit (Metropolitan)	**13.8**	**0.6**	**12.6**	**15.1**	**551**	**52,102,198**
Hawaii	**13.1**	**1.1**	**11**	**15.5**	**145**	**14,400,110**
Iowa	**11.8**	**0.6**	**10.7**	**13**	**408**	**37,370,945**
New Mexico	**16.8**	**1.1**	**14.7**	**19.2**	**244**	**19,228,865**
Seattle (Puget Sound)	**32.5**	**1.1**	**30.4**	**34.7**	**970**	**40,551,415**
Utah	**15.6**	**1.1**	**13.5**	**18.1**	**202**	**22,128,033**
Atlanta (Metropolitan)	**10.2**	**0.9**	**8.4**	**12.2**	**145**	**24,911,444**
Females						
Nine SEER registries	**3.8**	**0.1**	**3.6**	**4**	**1,155**	**312,419,050**
San Francisco–Oakland SMSA	**4.1**	**0.3**	**3.5**	**4.7**	**203**	**48,509,072**
Connecticut	**3.6**	**0.3**	**3.1**	**4.2**	**177**	**44,547,659**
Detroit (Metropolitan)	**3.4**	**0.2**	**2.9**	**3.9**	**185**	**56,047,986**
Hawaii	**2.4**	**0.5**	**1.6**	**3.5**	**29**	**13,860,703**
Iowa	**3.7**	**0.3**	**3.1**	**4.4**	**163**	**39,570,791**
New Mexico	**4**	**0.5**	**3.1**	**5.1**	**68**	**19,807,655**
Seattle (Puget Sound)	**5.9**	**0.4**	**5.1**	**6.7**	**230**	**41,064,742**
Utah	**2.8**	**0.4**	**2**	**3.7**	**47**	**22,425,156**
Atlanta (Metropolitan)	**2.5**	**0.3**	**1.9**	**3.3**	**53**	**26,585,286**

SD, standard deviation; CI, confidence interval; confidence intervals are 95% for rates; SMSA.
Rates in bold are higher than the mean incidence.

Table 25.2. Incidence by anatomic sites—SEER database

	Male and female	Male	Female
All sites	**5266**	**4111**	**1155**
Digestive system	**621**	**340**	**281**
Abdominal organs	**7**	**4**	**3**
Retroperitoneum	**27**	**14**	**13**
Peritoneum, omentum, and mesentery	**587**	**322**	**265**
Respiratory system	**4532**	**3711**	**821**
Lung and Bronchus	**145**	**113**	**32**
Pleura	**4381**	**3593**	**788**
Respiratory organs	**6**	**5**	**1**
Soft tissue including heart	**34**	**15**	**19**
Female genital system	**17**	**0**	**17**
Corpus and Uterus, NOS	**1**	**0**	**1**
Ovary	**11**	**0**	**11**
Other female genital organs	**5**	**0**	**5**
Male genital system	**29**	**29**	**0**
Testis	**6**	**6**	**0**
Other male genital organs	**23**	**23**	**0**
Miscellaneous	**33**	**16**	**17**

Table 25.3. Relative frequencies of mesothelioma diagnosis per site for males and females

Site	Number of mesothelioma (male)	Number of mesothelioma (female)	Percent (male)	Percent (female)	Percent all sites (male)	Percent all sites (female)
Abdominal	340	281	54.7	45.3	8.3	24.3
Thoracic	3711	821	81.9	18.1	90.2	71.1
Heart	15	19	44	56	0.4	1.6
Female genital	—	17	—	100	—	1.5
Male genital	29	—	100	—	0.7	—
Other	16	17	48	52	0.4	1.5
All sites	4111	1155	78	22	100	100

and by gender (Table 25.3). A higher proportion of women develop the disease in the peritoneal cavity. Although not all mesotheliomas have been linked to asbestos exposure, for patients developing a peritoneal mesothelioma induced by asbestos, exposure to this substance was usually heavier and longer (6,7). The latency between asbestos exposure and onset of disease is shorter for peritoneal than for pleural mesothelioma (20 to 30 versus 30 to 40 years) (8).

Table 25.4. Clinical presentation of diffuse malignant mesothelioma

Characteristic	No. of patients (denominator[a])	Number of publications (8,17–22)	Percent with characteristic
Duration of symptoms			
Under 6 months	53/76	4	70
Over 6 months	23/76		30
Age			
Under 45 years	26/97	6	27
45–60 years	40/97		41
Over 60 years	31/97		32
Symptoms			
Abdominal pain	76/121	7	63
Abdominal mass	31/78	4	40
Increasing abdominal girth	73/105	6	70
Ascites	46/70	5	66
Digestive disturbances[b]	10/30	3	33
Fever	11/55	3	20
Weight loss	27/62	5	44
Thrombocytosis	8/35	2	23
Leukocytosis	4/8	1	50
Pathology			
Epithelial	67/89	4	75
Sarcomatous	8/89		9
Mixed	14/89		16

[a] If a symptom is not stated in a publication, the number of patients cited in the publication is not included in the denominator.

[b] Including anorexia.

Pathology

To establish the diagnosis, a tissue biopsy is essential (see also Pathology of Mesothelioma Section). Without a tissue sample, malignant stromal invasion cannot be detected (9). The biopsy is usually done by a laparoscopic exploratory endoscopy of the peritoneal cavity. The histology of mesothelioma encompasses a spectrum that goes from benign mesothelial hyperplasia (no stromal invasion) to undifferentiated malignant disease. Mesothelial hyperplasia is usually a tissue response to inflammation or trauma, and can be associated with other cancers, presenting a diagnostic challenge. The differential diagnosis includes diffuse malignant mesothelioma, which also can be a diagnostic challenge. Mesothelial hyperplasia may be present for years and it occasionally precedes the diagnosis of diffuse malignant mesothelioma. The different morphologic types of abdominal mesothelial neoplasms that have been described include localized fibrous tumor, adenomatoid tumor, well-differentiated papillary mesothelioma, low-grade cystic mesothelioma, and diffuse malignant mesothelioma (10). The localized fibrous tumor is extremely rare in the abdominal cavity, in comparison to the pleural cavity. The adenomatoid tumor type is encountered most commonly in the genital system, but has been observed on the mesenteric surface or the omentum. Low-grade cystic mesothelioma is also very rare and should not be confused with multilocular peritoneal inclusion cysts, a benign condition. Well-differentiated papillary mesothelioma is the most common variant after diffuse malignant mesothelioma (11,12). It is usually seen in premenopausal women, and could be an incidental finding. When it is symptomatic, it usually presents with ascites, or abdominal pain. Asbestos exposure may not be evident in many well-differentiated cases. Microscopic examination reveals fibrous papillae covered by a mesothelial cell monolayer; the nuclear features are bland and mitoses are rare or absent. A tubulopapillary pattern, branching cords, solid sheets of cells, and deciduoid morphology (13) are sometimes seen. Most well-differentiated papillary mesotheliomas are benign or very low grade and should be observed after diagnostic resection. The differential diagnosis includes a low-grade papillary serous carcinoma of the ovary or peritoneum.

There have been three major variants of diffuse malignant mesothelioma described: the epithelial, the sarcomatous types, and the mixed epithelial/sarcomatous type. Pathology and histologic diagnosis is described in Chapter 31.

Differential Diagnosis

The main differential diagnosis is with adenocarcinoma that invades the peritoneal cavity, such as primary peritoneal tumor, primary ovarian carcinoma especially of the papillary serous type (14), adenocarcinoma of digestive origin, and, rarely, a peritoneal sarcomatosis or a gastrointestinal autonomic nerve tumor (13). Sclerosing mesenteritis sometimes presents a diagnostic problem (15), because of the resulting mesothelial hyperplasia.

Clinical Presentation

Typically, the diagnosis takes a long time to establish. Most of my patients have complained of symptoms for 6 months to more than 2 years prior to diagnosis. In rare instances, patients describe a spontaneous remission with a recurrence more than 5 years later. When the disease becomes symptomatic, patients present with abdominal girth enlargement, ascites, fatigue, anemia, weight loss, night sweats and nocturnal fever, and digestive disturbances. It is fairly common for males to first present with an inguinal hernia, and less often with an umbilical hernia. Women are sometimes diagnosed because of the finding of a pelvic mass. Transmural invasion with involvement of the abdominal musculature is not uncommon, and these patients are usually diagnosed with a sarcoma, despite the epithelial nature of their mesothelioma (Fig. 25.1). Some patients have indolent disease, despite a heavy tumor burden (16). Table 25.4 presents a compilation of peritoneal cases reported in seven medical publications (8,17–22). The most frequent signs are increased abdominal girth with ascites and abdominal pain.

Late complications of mesothelioma are bowel obstruction, and a hypercoagulable state. Care should be taken to prevent deep venous thrombosis and arterial thrombosis, which are relatively frequent in this patient population.

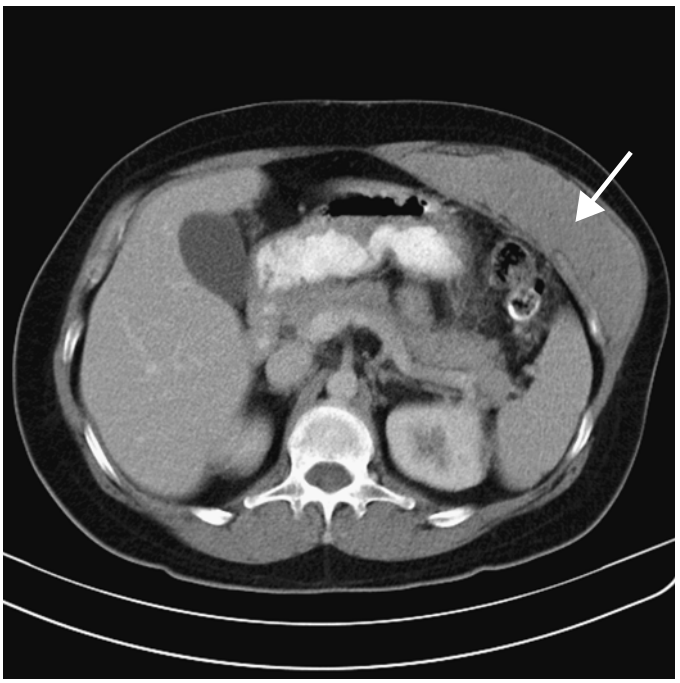


Figure 25.1. Abdominal wall involvement in a patient diagnosed with peritoneal mesothelioma. Thin arrow, parietal involvement.

The laboratory investigations show an increased platelet count in about 50% of patients, and sometime an increase in white blood cells. Low-grade anemia is common. Another feature of peritoneal mesothelioma is that it causes a low serum albumin, in relationship to the peritoneal infiltration. This hypoalbuminemia is usually asymptomatic. The most commonly elevated tumor marker is CA-125, probably a sign of peritoneal irritation by the cancer; it is seen in fewer than half the patients.

The staging of peritoneal mesothelioma has not been established in a reproducible fashion. I distinguish four categories: a localized lesion that can be fully resected [equivalent to stage I of the Butchart et al (23) classification], disease contained into the abdominal cavity on the peritoneal and organ surfaces that can only be debulked at best (equivalent to stage I), disease contained into the abdominal cavity with intraparenchymal invasion of organs such as liver metastases (equivalent to stage IV), and disease extending outside of the peritoneal cavity including lymph node involvement (equivalent to stage III or IV). The last two categories seem to have a worse prognosis. The SEER registry differentiates among localized, regional, distant, or unstaged disease. Of 621 abdominal mesotheliomas, 60 (9.7%) were localized (primary tumor confined to an organ), 72 (11.6%) were regional (tumor involving adjacent structures, or with regional lymph nodes), 402 (65%) were distant (skipped areas between primary tumor and other lesions), and 87 (14%) were unstaged. Because of the uncertainty of these definitions, these numbers have to be taken cautiously. The principal indication from these numbers is that localized peritoneal mesothelioma is very rare.

Pathophysiology

Though the pathophysiology of malignant peritoneal mesotheliomas is not well understood, there have been advances in identifying markers associated with malignant proliferation of mesothelial cells (24). One such molecule is mesothelin, a cell surface glycoprotein present on normal mesothelial cells, which is highly expressed in the majority of patients with epithelial mesotheliomas (25,26). Mesothelin overexpression is also seen in other tumors including serous ovarian carcinomas, pancreatic adenocarcinomas and some squamous cell carcinomas. The exact function of mesothelin is not known but it may be involved in tumor dissemination. Initially, mesothelin is formed as a 69-kd polypeptide with a hydrophobic tail, which is probably removed and replaced by phosphatidylinositol. After glycosylation at one or more of its four putative N-linked glycosylation sites, it is cleaved by a protease to yield the cell-bound 40-kd fragment and a smaller N-terminal fragment, which is shed. This N-terminal fragment was called the megakaryocyte potentiating factor (MPF) since it stimulated the megakaryocyte colony-forming activity of murine interleukin-3 in mouse bone marrow cell culture (27). Also, MPF could play a role in the thrombocytosis seen in the majority of patients with malignant

mesothelioma. Soluble molecules of the mesothelin/MPF family have also been detected in the serum of patients with ovarian cancer, and studies are ongoing to see whether mesothelin levels are elevated in patients with mesothelioma. If this is the case, mesothelin could serve as a tumor marker for this disease (28).

Mesothelin is also an attractive candidate for targeted therapy, given its high expression in patients with mesothelioma and limited expression in normal tissues except normal mesothelial cells. Currently, a phase I study of a recombinant antimesothelin immunotoxin is ongoing in patients with mesotheliomas whose tumors overexpress mesothelin (29).

Treatment

Currently, there is no curative treatment for peritoneal mesothelioma that is appropriate for the majority of patients. Different modalities have been tried, including surgery, chemotherapy, radiation therapy, immunotherapy, and hormonal therapy (30,31). Many patients receive no treatment. The SEER database collected the following information. Of 601 patients for whom treatment records were available, 201 (33%) did not receive any definitive cancer-directed treatment; however, some outpatient chemotherapy treatments may have been missed during data collection. One hundred seven (18%) patients were treated by surgery only, with a total of 195 patients having surgery during the course of therapy. One hundred sixty-two patients (27%) received a medical treatment including chemotherapy with or without hormonal therapy. Forty-two patients were irradiated with (n = 26) or without (n = 16) other therapeutic modalities. Ninety-six patients (16%) received two or more treatment modalities. The treatment was unknown in 17 patients, and two patients received other treatments. Despite these therapeutic efforts, survival remains poor, as described in the next section.

Survival

The 5-year average age-adjusted relative survival rate for all mesothelioma collected in the SEER database over 27 years was 8% overall, 5% for men, and 17% for women. For patients diagnosed with peritoneal mesothelioma, including the "digestive system" category, the 5-year survival rates are 16% overall, 10% for men, and 22% for women. These data indicated that the overall prognosis for patients diagnosed with peritoneal mesothelioma is better than for patients diagnosed with pleural disease. This fact has not well been established in the literature so far (32). Women did better than men, which is also controversial (17,33) (Fig. 25.2).

Survival data were analyzed for patients who received a mesothelioma-specific treatment versus patients who were not specifically treated for mesothelioma. Of 601 patients with an abdominal mesothe-

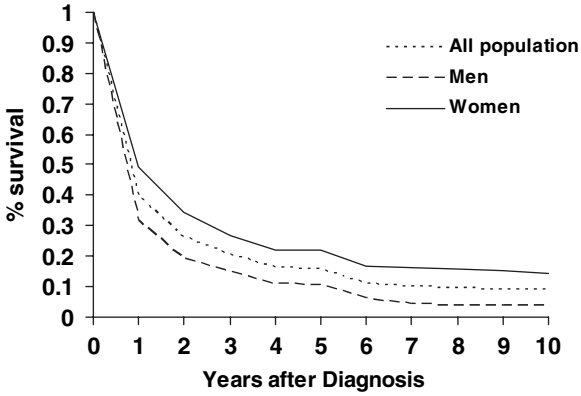


Figure 25.2. Survival of patients diagnosed with peritoneal mesothelioma.

lioma, 87 were excluded from the survival analysis for the following reasons: 68 received a diagnosis of a second primary, seven received a diagnosis at autopsy, six lacked follow-up, and six patients' mesothelioma had other causes. Of 514 analyzable patients, 339 received a mesothelioma-specific treatment, 162 did not, and for 13 patients the treatment was unknown. Median survival was 11 months for treated patients versus 8 months for untreated patients. At 1 year, survival was 47% (±5.5%) for treated patients versus 25% (±7%) for untreated patients (Fig. 25.3). At 5 years, there was no difference, with 16.5% (±4.5%) of treated and 11% (±6.4%) of untreated patients surviving.

Analyzing these data over time shows that there has not been any therapeutic progress within the past 30 years. One-year survival rates

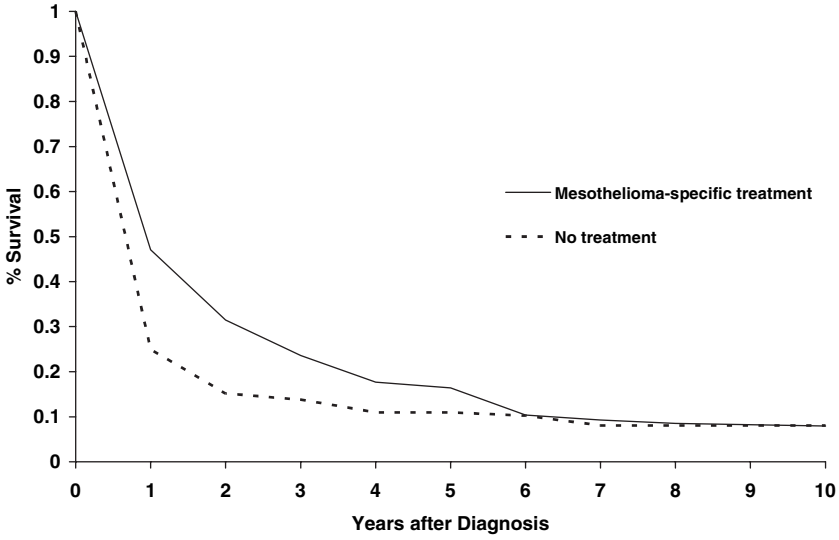


Figure 25.3. Relative survival of untreated versus mesothelioma-specific treated patients.

for treated patients were 47% in the 1970s, 49% in the 1980s, and 44% in the 1990s.

Conclusion

Peritoneal mesothelioma is a disease that can present with a variety of abdominal symptoms, the most common one being increasing girth, or with constitutional symptoms such as fever, night sweats, or weight loss. The prognosis is variable and cannot be predicted accurately despite the symptomatology (16). In absolute numbers, more men than women are affected with this disease; however, the ratio of peritoneal/pleural mesothelioma is greater in women. There are two main types of peritoneal mesothelioma: the diffuse malignant mesothelioma and the well-differentiated mesothelioma seen usually in premenopausal women. The prognosis of patients with well-differentiated disease is better, and these patients should be observed as there is no evidence that treatment improves the prognosis. Patients with diffuse malignant peritoneal disease should be treated on research protocols, as the disease is usually fatal and no treatment has been shown to prolong survival. Multimodality therapy is usually needed in the care of these patients.

Acknowledgments

The authors thank Dr. Harriett Smith for advice with the SEER database, and Dr. Evelyne Loyer for Figure 25.1.

References

1. Connelly RR, Spirtas R, Myers MH, Percy CL, Fraumeni JF Jr. Demographic patterns for mesothelioma in the United States. J Natl Cancer Inst 1987; 78(6):1053–1060.
2. Roggli VL. Malignant mesothelioma and duration of asbestos exposure: correlation with tissue mineral fibre content. Ann Occup Hyg 1995;39(3): 363–374.
3. Roggli VL, Oury TD, Moffatt EJ. Malignant mesothelioma in women. Anat Pathol 1997;2:147–163.
4. Suzuki Y. Pathology of human malignant mesothelioma—preliminary analysis of 1517 mesothelioma cases. Ind Health 2001;39(2):183–185.
5. Roggli VL, Sharma A, Butnor KJ, Sporn T, Vollmer RT. Malignant mesothelioma and occupational exposure to asbestos: a clinicopathological correlation of 1445 cases. Ultrastruct Pathol 2002;26(2):55–65.
6. Britton M. The epidemiology of mesothelioma. Semin Oncol 2002;29(1): 18–25.
7. Neumann V, Gunthe S, Mulle KM, Fischer M. Malignant mesothelioma—German mesothelioma register 1987–1999. Int Arch Occup Environ Health 2001;74(6):383–395.
8. Chahinian AP, Pajak TF, Holland JF, Norton L, Ambinder RM, Mandel EM. Diffuse malignant mesothelioma. Prospective evaluation of 69 patients. Ann Intern Med 1982;96(6 Pt 1):746–755.

9. Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferations. Am J Surg Pathol 2000;24(9):1183–1200.
10. Clement PB. Diseases of the peritoneum. In: Kurman RJ, ed. Pathology of the Female Genital Tract, 4th ed. New York: Springer-Verlag, 1994:647–703.
11. Butnor KJ, Sporn TA, Hammar SP, Roggli VL. Well-differentiated papillary mesothelioma. Am J Surg Pathol 2001;25(10):1304–1309.
12. Daya D, McCaughey WT. Well-differentiated papillary mesothelioma of the peritoneum. A clinicopathologic study of 22 cases. Cancer 1990;65(2): 292–296.
13. Shanks JH, Harris M, Banerjee SS, et al. Mesotheliomas with deciduoid morphology: a morphologic spectrum and a variant not confined to young females. Am J Surg Pathol 2000;24(2):285–294.
14. Bollinger DJ, Wick MR, Dehner LP, Mills SE, Swanson PE, Clarke RE. Peritoneal malignant mesothelioma versus serous papillary adenocarcinoma. A histochemical and immunohistochemical comparison. Am J Surg Pathol 1989;13(8):659–670.
15. Emory TS, Monihan JM, Carr NJ, Sobin LH. Sclerosing mesenteritis, mesenteric panniculitis and mesenteric lipodystrophy: a single entity? Am J Surg Pathol 1997;21(4):392–398.
16. Kerrigan SA, Turnnir RT, Clement PB, Young RH, Churg A. Diffuse malignant epithelial mesotheliomas of the peritoneum in women: a clinicopathologic study of 25 patients. Cancer 2002;94(2):378–385.
17. Antman K, Shemin R, Ryan L, et al. Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. J Clin Oncol 1988;6(1):147–153.
18. Antman KH, Corson JM, Li FP, et al. Malignant mesothelioma following radiation exposure. J Clin Oncol 1983;1(11):695–700.
19. Eltabbakh GH, Piver MS, Hempling RE, Recio FO, Intengen ME. Clinical picture, response to therapy, and survival of women with diffuse malignant peritoneal mesothelioma. J Surg Oncol 1999;70(1):6–12.
20. Jones DE, Silver D. Peritoneal mesotheliomas. Surgery 1979;86(4):556–560.
21. Piccigallo E, Jeffers LJ, Reddy KR, Caldironi MW, Parenti A, Schiff ER. Malignant peritoneal mesothelioma. A clinical and laparoscopic study of ten cases. Dig Dis Sci, 1988;33(5):633–639.
22. van Gelder T, Hoogsteden HC, Versnel MA, de Beer PH, Vandenbroucke JP, Planteydt HT. Malignant peritoneal mesothelioma: a series of 19 cases. Digestion 1989;43(4):222–227.
23. Butchart EG, Ashcroft T, Barnsley WC, Holden MP. Pleuropneumonectomy in the management of diffuse malignant mesothelioma of the pleura. Experience with 29 patients. Thorax 1976;31(1):15–24.
24. Krismann M, Muller KM, Jaworska M, Johnen G. Molecular cytogenetic differences between histological subtypes of malignant mesotheliomas: DNA cytometry and comparative genomic hybridization of 90 cases. J Pathol 2002;197(3):363–371.
25. Chang K, Pai LH, Pass H, et al. Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. Am J Surg Pathol 1992;16(3):259–268.
26. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Nat Acad Sci USA 1996;93(1):136–140.
27. Kojima T, Oh-eda M, Hattori K, et al. Molecular cloning and expression of megakaryocyte potentiating factor cDNA. J Biol Chem 1995;270(37):21984–21990.

28. Scholler N, Fu N, Yang Y, et al. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. Proc Nat Acad Sci USA 1999;96(20): 11531–11536.
29. Hassan R, Kreitman R, Strauss L, et al. SS1(dsFv)PE38 anti-mesothelin immunotoxin in advanced malignancies: phase I and pharmacokinetic study of alternate-day infusion. Proc Am Soc Clin Oncol 2002;21(A29).
30. Sugarbaker PH, Acherman YI, Gonzalez-Moreno S, et al. Diagnosis and treatment of peritoneal mesothelioma: the Washington Cancer Institute experience. Semin Oncol 2002;29(1):51–61.
31. Taub RN, Keohan ML, Chabot JC, Fountain KS, Plitsas M. Peritoneal mesothelioma. Curr Treat Options Oncol 2000;1(4):303–312.
32. Hillerdal G. Malignant mesothelioma 1982: review of 4710 published cases. Br J Dis Chest 1983;77(4):321–343.
33. Sebbag G, Yan H, Shmookler BM, Chang D, Sugarbaker PH, Results of treatment of 33 patients with peritoneal mesothelioma. Br J Surg 2000; 87(11):1587–1593.

26

Staging of Mesothelioma

Raja M. Flores and Valerie W. Rusch

Historically, malignant pleural mesothelioma (MPM) was considered to be a universally fatal disease. Most patients were thought to die within 2 years of diagnosis, and this nihilism led to insufficient attention being given to the staging and treatment of MPM. However, during the past two decades, a few investigators have carefully studied the natural history and treatment of MPM, and we now have a better understanding of the prognosis of early stage disease. Patients with stage Ia tumors can be expected to live up to 2 years without treatment, and patients who undergo surgical resection for stages I and II disease usually live more than 3 years. Effective surgical and multimodality treatments are now available. The surgical mortality of 31% reported by Butchart et al (1) in 1976 for extrapleural pneumonectomy has decreased to 4% and 5%, becoming similar to the mortality of standard pneumonectomy (2). Newer chemotherapeutic regimens, including gemcitabine/cisplatin and pemetrexed/cisplatin, have shown encouraging response rates (3,4). As therapy for MPM improves, accurate staging for the selection of treatment becomes increasingly important.

History of the Staging of Mesothelioma

Prior to this decade, it was difficult to diagnose and to stage MPM accurately. It was frequently misclassified pathologically as metastatic adenocarcinoma, a diagnostic problem that has now been solved by the routine use of a panel of immunohistochemical stains on pleural biopsies. Chest radiography rather than computed tomography (CT) was used as the primary imaging modality, leading to tremendous inaccuracies in clinical stage classification. In addition, there was no widely accepted staging system, making it difficult to assess the natural history and to compare treatment outcomes.

Butchart et al (1) proposed the first staging system in 1976 (Table 26.1), based on their experience with 29 patients who underwent extrapleural pneumonectomy. However, this study was performed before the advent of CT and the extent of disease preoperatively was assessed very

Table 26.1. Staging proposed by Butchart et al

Stage I	**Tumor confined within the "capsule" of the parietal pleura, i.e., involving only ipsilateral pleura, lung, pericardium, and diaphragm**
Stage II	**Tumor invading chest wall or involving mediastinal structures, e.g., esophagus, heart, opposite pleura**
	Lymph node involvement within the chest
Stage III	**Tumor penetrating diaphragm to involve peritoneum; involvement of opposite pleura**
	Lymph node involvement outside the chest
Stage IV	**Distant blood-borne metastases**

Source: Butchart EG, Ashcroft T, Barnsley WC, Holden MP. Pleuropneumonectomy in the management of diffuse malignant mesothelioma of the pleura: experience with 29 patients. *Thorax* 1976;31:15–24, with permission.

crudely. Consequently, this study did not permit accurate correlations of stage and survival. Also, there was a pathologic bias toward identifying patients with the mixed histologic subtype of MPM. It is now well known that the epithelioid subtype is the most common form of MPM, but prior to the routine use of immunohistochemistry and electron microscopy the mixed subtype was the easiest one to identify pathologically. As a result, Butchart's study included 17 patients with mixed histology tumors and only 11 patients with epithelioid MPM (one patient was classified as mesenchymal). Therefore, one may conclude that the epithelioid form of MPM was underdiagnosed at this time.

Several staging systems were proposed over the next 25 years, but each faced limitations similar to the Butchart one, because none was well validated or universally accepted. In 1982 Mattson (5) developed a staging system that was a variation on the Butchart system, and Chahinian et al (6) proposed the first tumor, node, metastasis (TNM)-based staging system. In 1993 the Union Internationale Contre le Cancer [International Union Against Cancer] (UICC) proposed another TNM staging system. In 1999 Sugarbaker et al (7,8) published a revised staging system based solely on patients undergoing extrapleural pneumonectomy. Because of the lack of a universally accepted staging system, the International Mesothelioma Interest Group (IMIG) (9) met to develop an internationally accepted staging system that would be universally accepted and applied to clinical trials. This staging system was accepted by the American Joint Committee on Cancer (AJCC) and UICC and was recently published in the sixth editions of their staging manuals (10,11).

The AJCC Staging System for Malignant Pleural Mesothelioma

T Status

The International Mesothelioma Staging System groups TNM descriptors into a stage I through stage IV classification (Table 26.2) (9). T1 is divided into T1a and T1b, where T1a describes a very early tumor that

Table 26.2. American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) staging system

Primary tumor (T)

- **TX** Primary tumor cannot be assessed
- **T0** No evidence of primary tumor
- **T1** Tumor involves ipsilateral parietal pleura, with or without focal involvement of visceral pleura
- **T1a** Tumor involves ipsilateral parietal (mediastinal, diaphragmatic) pleura; no involvement of the visceral pleura
- **T1b** Tumor involves ipsilateral parietal (mediastinal, diaphragmatic) pleura, with focal involvement of the visceral pleura
- **T2** Tumor involves any of the ipsilateral pleural surfaces with at least one of the following:
  - Confluent visceral pleural tumor (including fissure)
  - Invasion of diaphragmatic muscle
  - Invasion of lung parenchyma
- **T3** Describes locally advanced but *potentially resectable* tumor
  - Tumor involves any of the ipsilateral pleural surfaces with at least one of the following:
  - Invasion of the endothoracic fascia
  - Invasion into mediastinal fat
  - Solitary focus of tumor invading the soft tissues of the chest wall
  - Nontransmural involvement of the pericardium
- **T4** Describes locally advanced *technically unresectable* tumor
  - Tumor involves any of the ipsilateral pleural surfaces with at least one of the following:
  - Diffuse or multifocal invasion of soft tissues of the chest wall
  - Any involvement of rib
  - Invasion through the diaphragm to the peritoneum
  - Direct extension of any mediastinal organs
  - Direct extension to the contralateral pleura
  - Invasion into the spine
  - Extension to the internal surface of the pericardium
  - Pericardial effusion with positive cytology
  - Invasion of the myocardium
  - Invasion of the brachial plexus

Regional lymph nodes (N)

- **NX** Regional lymph nodes cannot be assessed
- **N0** No regional lymph node metastases
- **N1** Metastases in the ipsilateral bronchopulmonary and/or hilar lymph nodes
- **N2** Metastases in the subcarinal lymph nodes and/or the ipsilateral internal mammary or mediastinal lymph nodes
- **N3** Metastases in the contralateral mediastinal, contralateral internal mammary, or hilar lymph nodes and/or the ipsilateral or contralateral supraclavicular or scalene lymph nodes

Distant metastasis (M)

- **MX** Distant metastases cannot be assessed
- **M0** No distant metastasis
- **M1** Distant metastasis present

Stage grouping

Stage I	**T1**	**N0**	**M0**
Stage IA	**T1a**	**N0**	**M0**
Stage IB	**T1b**	**N0**	**M0**
Stage II	**T2**	**N0**	**M0**
Stage III	**T1, T2**	**N1**	**M0**
	T1, T2	**N2**	
	T3	**N0, N1, N2**	
Stage IV	**T4**	**Any N**	**M0**
	Any T	**N3**	**M0**
	Any T	**Any N**	**M1**

Source: American Joint Commission on Cancer. Cancer Staging Manual, 6th ed. New York: Springer-Verlag, 2002: 180–181, with permission.

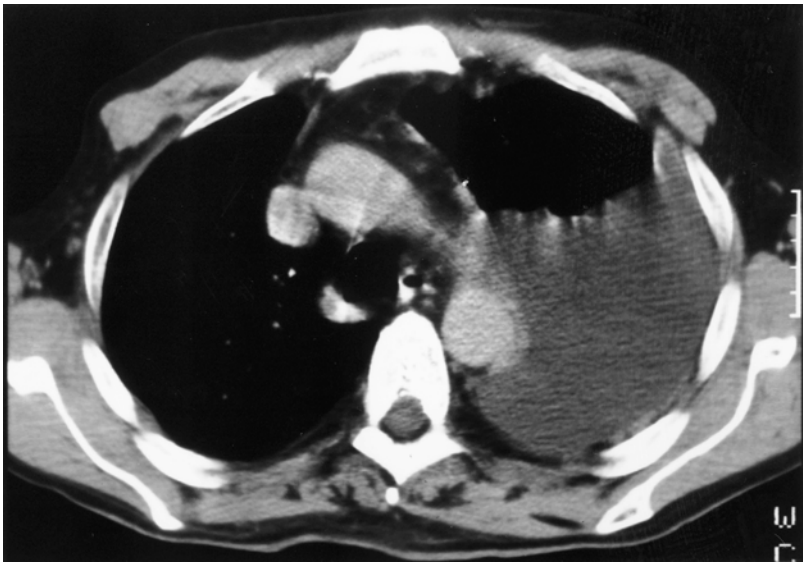


Figure 26.1. Example of stage I malignant pleural mesothelioma (MPM) with a large left pleural effusion and minimal parietal and mediastinal pleural thickening.

involves only the parietal pleura of one hemithorax without mediastinal or diaphragmatic involvement (thus sparing the visceral pleura), and T1b describes an early but slightly more advanced tumor that involves all of the pleural surfaces. T1 tumors are usually associated with a free pleural space and a large effusion (Fig. 26.1).

With tumor growth at the visceral and parietal pleural surfaces, the effusion may resolve or become loculated. This confluence of pleural surfaces designates a tumor as T2 and usually extends to the underlying lung parenchyma. This stage of tumor cannot usually be completely removed without removal of the underlying lung and diaphragmatic muscle. At this stage, only extrapleural pneumonectomy will rid the patient of all gross disease, and pleurectomy/decortication will not.

Stage T3 describes a locally advanced tumor but one that is still amenable to surgical resection by extrapleural pneumonectomy as a method to rid the patient of all gross disease. There is usually involvement of all pleural surfaces (including diaphragm and pericardium) and there may be tumor extension into the endothoracic fascia or mediastinal fat (Fig. 26.2). A solitary, completely respectable focus of tumor extending directly into the chest wall is also included in the T3 category. This usually occurs at a site of previous surgical intervention such as incision or chest tube sites. Although there are no specific size limitations on the extent of chest wall that may be removed, the concept is similar to that for non–small-cell lung cancer: a single focus of tumor invading the chest wall by direct extension is removed en bloc with the entire specimen. This is a very different finding when compared to the locally advanced tumor that is technically unresectable because it

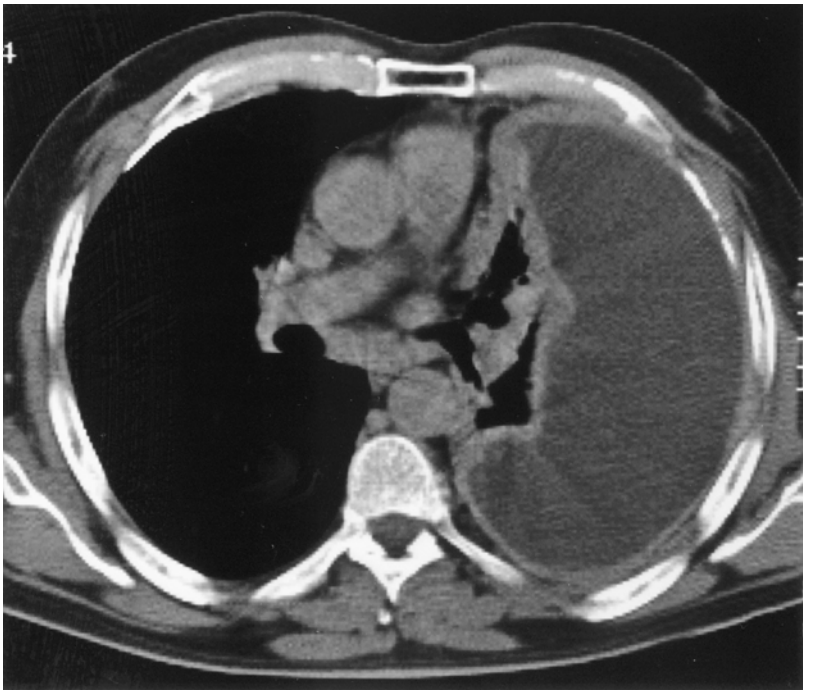


Figure 26.2. Example of more locally advanced MPM, at least T2 and possibly T3 (along anterolateral chest wall) by computed tomography (CT) scan.

diffusely invades the intercostals and chest wall muscles, designated as T4.

Stage T4 indicates a very locally advanced and technically unresectable tumor (Fig. 26.3). It is characterized by diffuse chest wall invasion, direct extension through the diaphragm to the underlying peritoneum, and direct extension to the contralateral pleura, mediastinal organs, spine, myocardium, or internal surface of the pericardium. The differences between T3 and T4 tumors have obvious implications with regard to resectability as well as survival (12,13).

N Status

The N descriptors are identical to those used in the International Lung Cancer Staging System (14). N1 indicates involvement of the ipsilateral lymph nodes from the bronchopulmonary and hilar regions; N2 includes lymph nodes from the ipsilateral mediastinal, internal mammary, and subcarinal regions; and N3 describes supraclavicular, contralateral mediastinal, or contralateral hemithoracic nodal involvement.

Because of current uncertainty about the prognostic difference between N1 and N2 disease, both are grouped into stage III disease. Sugarbaker et al (8) identified a significant difference in survival between patients with negative and positive N2 nodes. Among 176 patients surviving extrapleural pneumonectomy, 136 patients with negative N2 nodes had a significantly better survival than 40 patients with positive nodes. The adverse influence of nodal involvement was not evident in earlier surgical series (15), but analysis of this variable

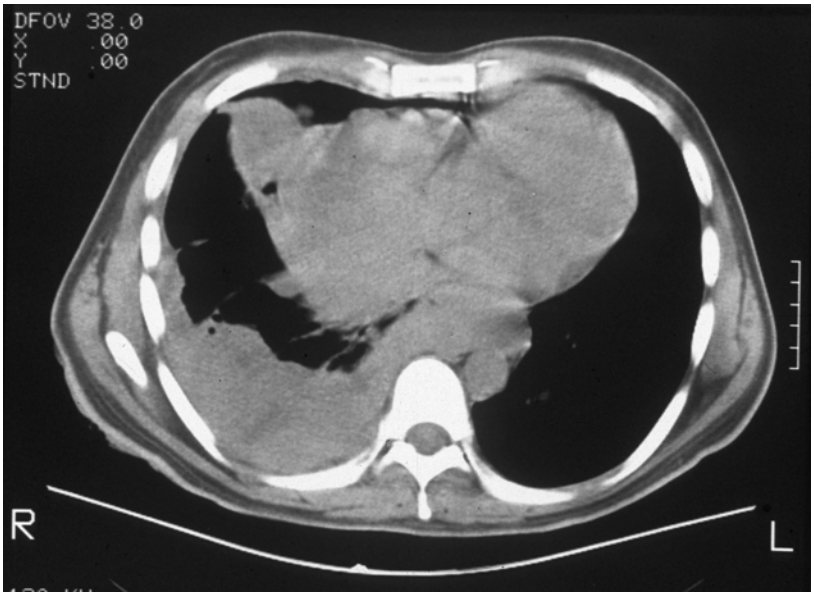


Figure 26.3. Example of very locally advanced MPM, T4 by CT scan. There is massive tumor extending into and shifting the mediastinum.

was confounded by the small numbers of patients in most reports, a lack of routine complete nodal sampling, and the retrospective nature of many analyses. The true incidence of nodal involvement and the routes of lymphatic spread are also poorly understood. It is possible those N2 nodes and the internal mammary nodes may become involved before N1 nodes because of the anatomic extent of MPM and the fact that it apparently arises in the parietal pleura rather than in the parenchyma of the lung. Analysis of our series at Memorial Sloan-Kettering Cancer Center (16) showed that the frequency of nodal metastasis was significantly higher (50%) than in Sugarbaker et al's experience (23%) and emphasizes the importance of systematic nodal dissection for staging. In our experience, both the presence of nodal metastases (N1 and N2) and the number of involved lymph nodes had a prognostic impact on overall survival after surgical resection. As more data become available, new analyses of the patterns of nodal metastasis and of the impact of N1 versus N2 or N3 disease should be performed. Such analyses may lead to future revisions of the AJCC/UICC staging system.

M Status

Although mesothelioma is usually known as a disease that progresses and invades locally, a small but significant number of patients can present with extrathoracic disease. Autopsy series also show that at least half of all patients have widely disseminated disease at the time of death (17). Because of the potential magnitude of surgical procedures, such as extrapleural pneumonectomy, it is important to recognize these patients and spare them an inappropriate operation.

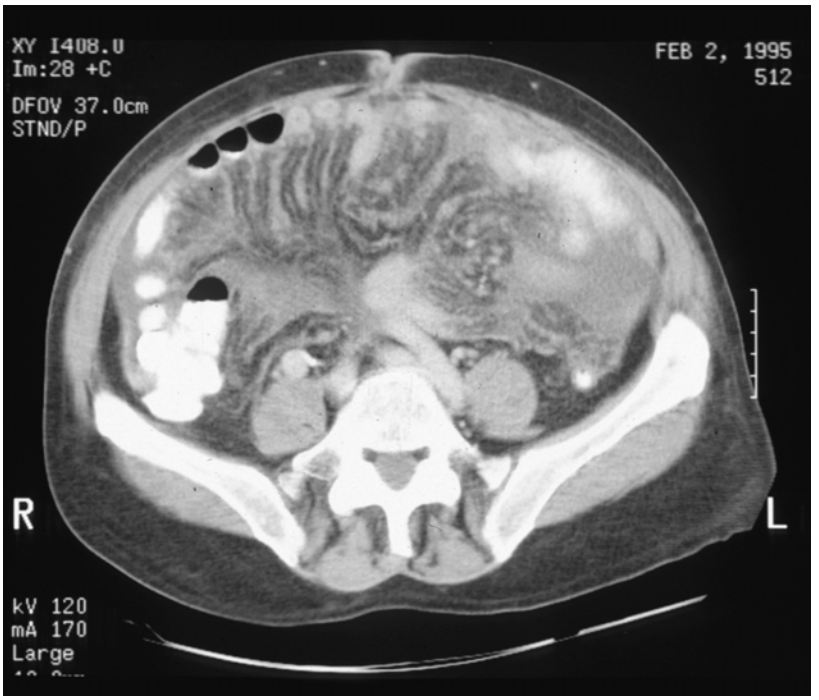


Figure 26.4. Example of MPM metastatic to the omentum and bowel mesentery, M1 by CT scan.

M0 designates no evidence of metastatic disease and M1 describes distant metastasis. In MPM, metastases are often widespread, but the most common sites of disease progression include the peritoneum, contralateral pleura, and contralateral lung (Fig. 26.4). These may develop by direct extension of tumor through the diaphragm (T4 tumor) or as a result of lymphatic or hematogenous dissemination. However, the prognosis is similar no matter what the route of tumor spread.

AJCC Stage Groupings

The TNM descriptors are used to characterize four stages of the disease. Stages I and II include node negative tumors. Stage I is subdivided into 1a and 1b and stage II includes T2N0 tumors. Stage III includes any T3, any N1, or any N2M0 tumors, and stage IV includes any T4, N3, or M1 tumors. Survival at 3 and 5 years for stages I, II, and III is 46% and 28%, 32% and 15%, and 15% and 0%, respectively (Fig. 26.5) (16).

Clinical Staging in Mesothelioma

Computed Tomography

There is some controversy over which imaging study is best and whether magnetic resonance imaging (MRI) adds to CT. At Memorial Sloan-Kettering Cancer Center, we enrolled 95 patients in a prospective

staging protocol. Sixty-five patients underwent CT and MRI followed by surgical resection. The CT and MRI scans were interpreted by independent observers in a blinded fashion, and the imaging findings were then compared to surgical-pathologic staging. Magnetic resonance imaging was slightly more accurate at identifying diaphragmatic invasion, invasion of endothoracic fascia, and solitary resectable foci of chest wall invasion. However, these findings were not significant enough to alter surgical treatment in these patients. Therefore, for cost-effectiveness, we concluded that CT should be considered the standard diagnostic study before therapy (18). However, CT does not reliably identify either N1 or N2 nodal disease and often fails to diagnose chest wall invasion. Consequently, approximately 20% to 25% who undergo surgical exploration are found to have unresectable tumor (19).

Pass et al (20) conducted a study using CT to evaluate the impact of preoperative tumor volume on outcome in patients undergoing resection for pleural mesothelioma. Forty-eight patients had three-dimensional CT reconstructions of preresection and postresection solid tumor volume, and were staged according to the AJCC staging system for mesothelioma, prior to surgical resection with either extrapleural pneumonectomy or pleurectomy/decortication. The median survival for preoperative tumor volume less than 100 cc was 22 months versus 11 months for tumor volume greater than 100 cc ($p = .03$). Progressively higher stage was associated with higher median preoperative volume: stage I, 4 cc; stage II, 94 cc; stage III, 143 cc; stage IV, 505 cc ($p = .007$). Higher tumor volumes were also associated with a greater likelihood of lymph node metastasis. This study showed that preoperative tumor volume assessed by volumetric CT tumor measurement is representative of T status in malignant pleural mesothelioma and can predict survival.

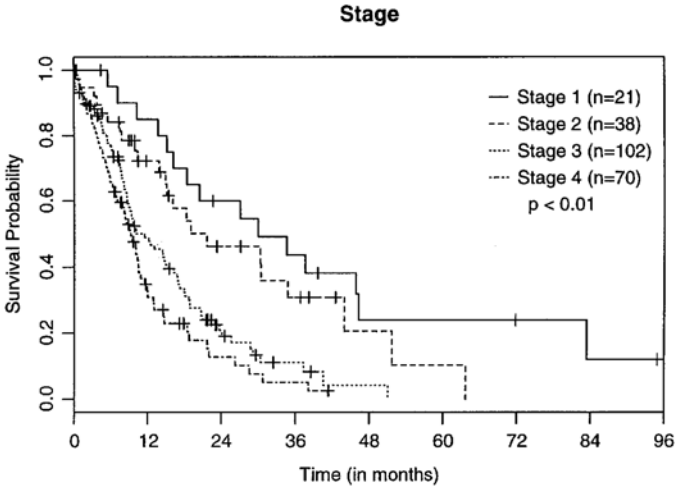


Figure 26.5. Overall survival of 231 patients by stage. When analyzed across all four categories, stage had a highly significant effect on survival ($p < .01$).

Magnetic Resonance Imaging

In 1992, prior to the advent of helical CT scanning, Patz et al (21) from the Brigham and Women's Hospital, reviewed 34 consecutive MPM patients who had a CT scan and MRI prior to surgery. The radiologic review focused on diaphragmatic involvement, chest wall invasion, and mediastinal invasion. The sensitivity was high (>90%) for both CT and MRI in each region. The unresectability rate of patients undergoing thoracotomy was 30%; CT and MRI provided similar information on resectability in most cases. Although the authors state that important anatomic information can be derived from an MRI obtained prior to surgical intervention, this information will rarely preclude patients from surgical exploration. More recent studies have also claimed a slight advantage of MRI over CT. However, there is little evidence that these findings translate into clinically relevant information (22). Therefore, CT scan remains our standard for preoperative locoregional staging.

Positron Emission Tomography

At Memorial Sloan-Kettering Cancer Center, we explored the utility of positron emission tomography (PET) scanning in the preoperative staging of MPM. We reviewed 63 patients who underwent PET scans at our institution during their initial evaluation prior to surgical resection. Fluorodeoxyglucose (FDG) uptake was present in all except one patient with stage Ia disease. We did not find that the PET scan added to the assessment of locoregional disease, especially the determination of T status. Also, PET did not accurately diagnose lymph node involvement. However, a high standard uptake value (SUV) was associated with a greater likelihood of N2 nodal metastases. More importantly, PET was useful in identifying 10% of the patients as having distant disease undetected by CT scan, thereby preventing inappropriate surgical intervention (23).

In addition to identifying patients with stage IV disease, PET scan findings may have prognostic significance. Recently, we evaluated 85 MPM patients who underwent PET scanning at diagnosis and found that there was a linear relationship between increasing SUV and decreasing median survival. The relative risk of death in patients with a SUV of greater than 4 when compared to an SUV of less than 4 was 3.3 ($p = .03$). Figures 26.6 and 26.7 show patients whose tumors had a high and low SUV on PET. In both univariate and multivariable analyses, SUV significantly predicted overall survival. These findings suggest that PET may be used to select patients for treatment (24).

Video-Assisted Thoracic Surgery

Video-assisted thoracic surgery (VATS) is principally used to diagnose MPM. Cytologic yield is low and tissue is frequently necessary to perform immunohistochemistry and electron microscopy, as well as to distinguish MPM from adenocarcinoma. In addition, the identification

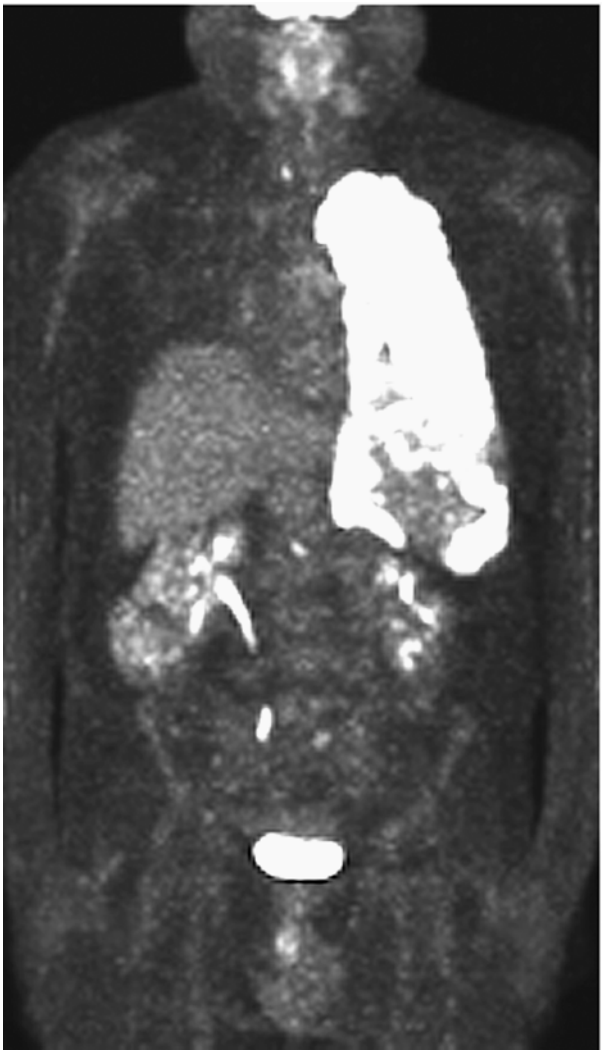


Figure 26.6. Positron emission tomography (PET) demonstrating a high standard uptake value (SUV) in a patient with a left-sided mesothelioma. Tumor involves all pleural and diaphragmatic surfaces.

of histologic subtype is useful in stratifying patients for treatment protocols.

The distinction between T1a and T1b tumors is best made by VATS. In 66 patients undergoing thoracoscopy, Boutin et al (12,25) found subtle differences in the extent of pleural disease that are impossible to identify radiographically and that account for the differences in survival found in patients thought to have similar stage early disease. In this series, 23 patients with stage Ia (parietal pleura only) disease had a median survival of 32.7 months as compared to the 43 patients with stage Ib (parietal and visceral pleura) disease who had a median survival of 7 months.

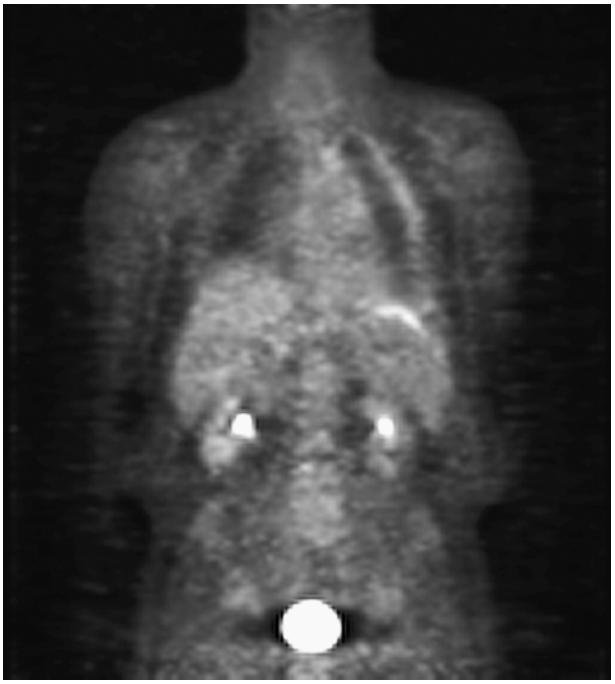


Figure 26.7. Positron emission tomography (PET) demonstrating a low SUV in a patient with a left-sided mesothelioma. Tumor involves the left lower pleural and diaphragmatic areas.

Laparoscopy

At Memorial Sloan-Kettering Cancer Center we conducted a study to determine the utility of laparoscopy in detecting transdiaphragmatic tumor extension when CT findings were equivocal. During a 1-year period, 12 of 36 patients considered for possible thoracotomy and surgical resection had equivocal CT findings of diaphragmatic invasion. All underwent laparoscopy with diaphragmatic and peritoneal biopsies. There were no perioperative complications and the median hospital stay was 1 day. Six patients had biopsy-proven transdiaphragmatic extension or peritoneal studding of tumor. The other six patients subsequently underwent thoracotomy: three had a complete resection, and three had unresectable tumor due to chest wall ($n = 2$) or mediastinal ($n = 1$) invasion. In no case was transdiaphragmatic extension of a tumor seen. This experience demonstrated that laparoscopy is a safe and accurate method for detecting transdiaphragmatic tumor extension when CT fails to do so and should be considered a standard part of prethoracotomy staging in this subset of patients (26).

Mediastinoscopy

The role of mediastinoscopy in the management of malignant pleural mesothelioma is still unclear. Schouwink and colleagues (27) examined

the usefulness of cervical mediastinoscopy in 43 patients. Of the 43 patients, only 24 went on to thoracotomy for pathologic confirmation, and therefore data were not available on the patients with potentially false-negative mediastinoscopy results. Of the 17 patients with enlarged nodes detected by CT scan, only six were confirmed to be positive by cervical mediastinoscopy, emphasizing the fact that lymph nodes that are enlarged on CT are not necessarily malignant. In addition, mediastinoscopy cannot diagnose lymph node metastases that occur frequently in MPM but are in anatomic locations inaccessible to this procedure, including the posterior mediastinal, internal mammary, and peridiaphragmatic regions. Although mediastinoscopy clearly identifies some patients with N2 disease, its role in staging MPM needs further study. Finally, although the presence of N2 disease is generally associated with a worse prognosis, it is not clear that all such patients should be denied surgical resection given the current treatment options.

Summary

Accurate staging of MPM allows us to stratify patients for treatment based on survival and to spare patients with advanced disease the morbidity of nonbeneficial surgical treatment. In MPM, patients with tumors of epithelioid histology and T1 or T2N0 stage have the best prognosis. This select group of patients appears to have a favorable survival with multimodality therapy that includes extrapleural pneumonectomy or pleurectomy/decortication and adjuvant radiation with or without chemotherapy. These findings justify the importance of applying staging systems at diagnosis.

Currently CT and PET scanning provide the most accurate invasive staging and are routinely used at our institution; MRI does not appear to add significantly to CT and PET and should be used selectively; and VATS can provide some additional information about T status, transdiaphragmatic tumor invasion, and peritoneal metastases. Although the current AJCC/UICC staging system and the methods available for clinical staging represent advances made in the management of MPM during the past decade, they are imperfect. Further studies to improve the accuracy of staging in MPM are warranted.

References

1. Butchart EG, Ashcroft T, Barnsley WC, et al. Pleuropneumonectomy in the management of diffuse malignant mesothelioma of the pleura. Experience with 29 patients. Thorax 1976;31:15–24.
2. Rusch VW. Indications for pneumonectomy. Extrapleural pneumonectomy. Chest Surg Clin North Am 1999;9:327–338.
3. Byrne MJ, Davidson JA, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant mesothelioma: a phase II study. J Clin Oncol 1999;17: 25–30.

4. Vogelzang NJ, Rusthoven J, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–2644.
5. Mattson K. Natural history and clinical staging of malignant mesothelioma. Eur J Respir Dis 1982;63(suppl 124):87 (abstract).
6. Chahinian AP, Pajak TF, Holland JF, et al. Diffuse malignant mesothelioma. Prospective evaluation of 69 patients. Ann Intern Med 1982;96:746–755.
7. Sugarbaker DJ, Strauss GM, Lynch TJ, et al. Node status has prognostic significance in the multimodality therapy of diffuse, malignant mesothelioma. J Clin Oncol 1993;11(6):1172–1178.
8. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results of 183 patients. J Thorac Cardiovasc Surg 1999;117:54–65.
9. Rusch VW. The International Mesothelioma Interest Group: a proposed new international TNM staging system for malignant pleural mesothelioma. Chest 1995;108:1122–1128.
10. Union Internationale Contre le Cancer. TNM Classification of Malignant Tumours. New York: Wiley-Liss, 2002.
11. American Joint Committee on Cancer. AJCC Cancer Staging Handbook. New York: Springer-Verlag, 2002.
12. Boutin C, Rey F. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: diagnosis. Cancer 1993; 72:389–393.
13. Tammilehto L. Malignant mesothelioma: prognostic factors in a prospective study of 98 patients. Lung Cancer 1992;8:175–184.
14. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710–1717.
15. Allen KB, Faber LP, Warren WH. Malignant pleural mesothelioma. Extrapleural pneumonectomy and pleurectomy. Chest Surg Clin North Am 1994; 4:113–126.
16. Rusch VW, Venkatraman ES. Important prognostic factors in patients with malignant pleural mesothelioma, managed surgically. Ann Thorac Surg 1999;68:1799–1804.
17. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. J Clin Oncol 1989;7:1157–1168.
18. Heelan RT, Rusch VW, Begg CB, et al. Staging of malignant pleural mesothelioma: comparison of CT and MR imaging. Am J Radiol 1999;172: 1039–1047.
19. Rusch VW, Rosenzweig K, Venkatraman E, et al. A phase II trial of surgical resection and adjuvant high dose hemithoracic radiation for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;122:788–795.
20. Pass HI, Temeck BK, Kranda K, et al. Preoperative tumor volume is associated with outcome in malignant pleural mesothelioma. J Thorac Cardiovasc Surg 1998;115:310–318.
21. Patz EF Jr, Shaffer K, Piwnica-Worms DR, et al. Malignant pleural mesothelioma: value of CT and MR imaging in predicting resectability. AJR 1992; 159:961–966.
22. Knuuttila A, Halme M, Kivisaari L, et al. The clinical importance of magnetic resonance imaging versus computed tomography in malignant pleural mesothelioma. Lung Cancer 1998;22:215–225.
23. Flores R, Akhurst T, Gonen M, Larson S, Rusch V. Positron emission tomography (PET) defines metastatic disease but not locoregional disease in

patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2003;126:11–16.
24. Flores RM, Akhurst T, Gonen M, Larson S, Rusch VW. FDG-PET predicts survival in patients with malignant pleural mesothelioma. Proc Am Soc Clin Oncol 2003;22:620(abstr 2495).
25. Boutin C, Rey F, Gouvernet J, et al. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 2: prognosis and staging. Cancer 1993;72:394–404.
26. Conlon KC, Rusch VW, Gillern S. Laparoscopy: an important tool in the staging of malignant pleural mesothelioma. Ann Surg Oncol 1996;3:489–494.
27. Schouwink JH, Kool LS, Rutgers EJ, et al. The value of chest computer tomography and cervical mediastinoscopy in the preoperative assessment of patients with malignant pleural mesothelioma. Ann Thorac Surg 2003; 75:1715–1719.

27

Prognostic Factors in Mesothelioma

Jeremy P.C. Steele and Dean A. Fennell

Prognostic factors are biologic or physical characteristics of a patient or a cancer that can be used to predict the outcome for the individual. They are of value for the management of mesothelioma because patients need a prognosis in order to be able to make informed decisions about treatment options and to make plans for themselves and their families. Prognostic factors also assist in the selection of patients more likely to benefit from intensive treatments, especially in the context of clinical trials (1). Recently it has become clear that prognostic factors may have an additional benefit: they may give insights into the biology of the cancer being studied, and lead to improved understanding of the molecular pathogenesis (2). This new role for prognostic factors may prove to be the most important of all.

The Cancer and Leukemia Group B and European Organization for Research and Treatment of Cancer Prognostic scoring systems are the two most useful prognostic scoring systems currently available for malignant mesothelioma (3,4). These systems rate performance status, age, histologic subtype, weight loss, and hematologic parameters as the most important prognostic factors for malignant mesothelioma. In the future, molecular biologic markers and DNA expression profiles may be able to give us more insight into mesothelioma and will help in prognostication.

Prognostic factors are especially important in the context of malignant mesothelioma because, until recently, treatment has had relatively little impact on the natural history of the disease. This chapter discusses the known clinical prognostic factors for malignant mesothelioma, focusing on the two best-known current systems, and describes some of the new molecular knowledge that will lead to the development of effective targeted therapies.

Diagnosis of Malignant Mesothelioma

Confirmation of the diagnosis is an essential prerequisite to giving a mesothelioma patient a prognosis. Unfortunately, obtaining a diagnosis of mesothelioma may not be straightforward. The likelihood

of reaching a reliable diagnosis is increased if a multidisciplinary approach is used. Ideally, a thoracic physician, a thoracic surgeon, a medical oncologist, and a specialist pathologist should all be involved. Repeated biopsies may be required to obtain sufficient quantities of high-quality tumor tissue. Most specialists take the view that computed tomography (CT)-guided biopsy or video-assisted thoracoscopic biopsy are the most reliable techniques for obtaining tissue for histology. Blind pleural biopsy has an unacceptably high false-negative rate. Histology must be verified by a pathologist experienced in making the diagnosis of mesothelioma using the relevant immunohistochemistry stains. It is important that the pathologist not only establishes the diagnosis but defines a cell type for the tumor, i.e., epithelioid (also known as epithelial), sarcomatoid (also known as sarcomatous), or mixed histology.

Natural History of Malignant Mesothelioma

Before discussing specific prognostic factors in mesothelioma, it is necessary to give an idea of the typical prognosis for patients with the disease. Most series have shown that the median survival for a patient with mesothelioma is between 4 and 18 months. Three recent phase II chemotherapy trials with response rates greater than 20% reported median overall survivals of 6.0 months (5), 9.5 months (6), and 10.6 months (7). The large international randomized phase III trial of chemotherapy published recently (8), reported a median survival of 12.1 months for the experimental group (treated with pemetrexed and cisplatin) and 9.3 months for the control arm (treated with cisplatin only). This large trial included mainly good performance status patients; patients seen in a mesothelioma clinic will have a wider variety of performance status and some will have a much shorter survival than these data suggest. Despite the suggestion that survival is, at best, about 1 year, it is worth adding that most mesothelioma physicians have patients who survive significantly longer than this, and the occasional patient lives for many years.

Clinical Prognostic Factors for Malignant Mesothelioma

What are the clinical factors that predict a longer survival? There have been many articles published about clinical prognostic factors in mesothelioma in the past 20 years. Table 27.1 summarizes some of the data from these trials. The commonest prognostic factors identified have included histologic cell type, performance status, age, gender, weight loss, chest pain, and clinical stage. Unfortunately, some of these data have conflicted, probably because many of the studies have been of small size and from single centers. Disease staging, treatments given, response assessment methods, and patient eligibility have varied substantially.

Table 27.1. Prognostic factors in malignant mesothelioma—major published series

First author, year (reference)	Median survival	No. of patients	Factor
Samson, 1987 [11]	6	76	S: prior treatment NS: gender, age, PS, histology, asbestos exposure, stage, disease status
Ruffie, 1989 [15]	9	332	S: stage, platelet count, asbestos exposure NS: gender, age, histology, smoking, first symptoms, pleural effusion, weight loss, delay in diagnosis
Antman, 1988 [17]	15	180	S: PS, histology, chest pain, age NS: gender, stage, symptoms to diagnosis time, year of diagnosis, smoking, asbestos exposure
Spirtas, 1988 [19]	7	1475	S: age, gender, treatment, stage, geographic area NS: ethnic group, site, histology, year of diagnosis
Chahinian, 1982 [14]	7	69	S: histology, age, pleural site NS: gender, asbestos exposure, delay in diagnosis
Boutin, 1993 [10]	7–32.7	188	S: absence of weight loss, aspect of visceral pleura, stage, histology NS: age, gender, site of disease, asbestos exposure, symptoms to diagnosis, surgery
De Pangher Manzini, 1993 [16]	13	80	S: age, stage, histologic type NS: gender, PS, period of diagnosis, site of disease, pleural fluid cytology
Calavrezos, 1988 [18]	5–13	132	S: age stage, histology, PS, pain, treatment NS: gender, asbestos exposure, duration of symptoms
Tammilehto, 1992 [12]	9	98	S: PS, gender, histology, diagnostic delay NS: age, stage, treatment, asbestos exposure
Curran, 1998 [4]	12.6	204	S: PS, high WBC count, uncertain histology, gender, sarcomatous, thrombocytosis NS: age
Herndon, 1998 [3]	3.9–9.8	337	S: PS, chest pain, high platelet count, weight loss, high LDH, high WBC count, age, histology NS: gender
Edwards, 2000 [23]	5.9	142	S: gender, weight loss, PS, low Hb, high WBC count, high platelets, histology NS: weight loss

S, significant; NS, nonsignificant; WBC, white blood cell; PS, performance status; LDH, lactate dyhdrogenase.

Source: From Steele JP. Prognostic factors in mesothelioma. Semin Oncol 2002; 29(1):36-40, with permission from Elsevier.

Curran et al (4) made the interesting comment that the statistical analyses commonly used, such as the Cox model (9), are unstable with small series. Because mesothelioma has been a relatively rare form of cancer (at least until recent years), sample sizes have been small, and thus this statistical method may be unreliable. Curran discussed the conflicting data on the importance of patient age in predicting a poor outcome. At least four studies showed that age is not of importance (10–13), whereas several others suggested that it is (14–19). The same is true of gender, performance status, and histologic subtype (4).

Studies have also disagreed on the importance of stage as a prognostic marker. Perhaps surprisingly, stage was not found to be important as a prognostic factor in several studies (4,11,17). The main problem with the existing staging systems is that to obtain full information patients should have had cytoreductive surgery such as extrapleural pneumonectomy. Even at surgical referral centers, most patients are unable to have this operation, thus staging data reported in nonsurgical series are likely to be estimates based on radiology. Recent updates of the two largest surgical series (20,21) have confirmed that, as expected, patients staged surgically as stage I or II survive longer than those with advanced stage mesothelioma. Patients with epithelial cell type did so much better than those with nonepithelial cell type that some surgeons would not consider surgery for this latter group. Since 1998, two important studies have been published that have significantly clarified the important prognostic factors in mesothelioma (3,4). These are discussed below.

The Cancer and Leukemia Group B (CALGB) Prognostic Scoring System

The Cancer and Leukemia Group B (CALGB) examined the individual and joint effect of various pretreatment clinical characteristics on the survival of patients with mesothelioma treated with chemotherapy in a series of sequential phase II trials (3). Over a 10-year period, 337 untreated patients with malignant mesothelioma were registered in phase II studies of 10 different treatment regimens. The median overall survival for patients in these trials ranged from 3.9 to 9.8 months with 1-year survival figures ranging between 14% and 50%. The investigators then used Cox survival models and exponential regression trees to examine the prognostic importance of various pretreatment patient characteristics. The following factors were included:

1. ECOG performance status (PS 0, 1, 2)
2. Epithelial histology (yes/no)
3. Presence of chest pain (yes/no)
4. Presence of dyspnea (yes/no)
5. Duration of symptoms (<3 months, 3–6 months, >6 months)
6. Weight loss in the past 6 months (none or >5%)

7. Asbestos exposure (no/yes/unknown)
8. Smoking history (yes/no)
9. Lactate dehydrogenase (LDH) level >500 IU/L (yes/no)
10. Platelet count >400,000 /μL (yes/no)
11. Hemoglobin (Hb) level /μL (<14.6, ≥14.6)
12. White blood cell count (WBC) /μL (<8.7, ≥8.7)
13. Location of disease involvement (pleural/peritoneal/pericardial)
14. Extent of disease (local or regional/distant)

Survival curves were generated for subgroups defined by these putative prognostic factors, and survival comparisons were made. Patients were split into subgroups using an algorithm that maximized differences in the survival distribution measured by the log rank test. A stepwise analysis generated a regression tree with successive stratification into groups according to prognostic factor with progressively decreasing risk ratio.

Univariate Analysis

Comparison of the subgroups stratified by prognostic factor using the log rank test showed that the following factors were associated with worse outcome:

Poor performance status (PS): Median survival time got worse with increasing PS: for PS 0 survival was 10.9 months, for PS 1 survival was 7.6 months, and for PS 3 survival was only 3.3 months.

Presence of chest pain: Presence of chest pain was associated with a reduced median survival time of 5.4 months compared with 8.8 months for patients without chest pain.

History of dyspnea: A history of dyspnea was associated with reduced median survival time of 6.3 months compared with 8.3 months in the absence of dyspnea.

High platelet count: A platelet count >400,000/μL was associated with a reduced median survival time of 6.2 months compared with 9.4 months for patients with a platelet count <400,000/μL.

Weight loss: The median survival time in patients with significant weight loss was 5.1 months, and 7.9 months for patients not experiencing weight loss.

Elevated LDH level: An elevated LDH level of >500 IU/L was associated with a median survival time of 3.4 months, compared with 7.6 months for patients with an LDH level of <500 IU/L.

Pleural involvement: The presence of pleural involvement was associated with a reduced median survival time of 7.1 months compared with 12.3 months for patients without pleural involvement.

There was a statistically significant linear relationship for white blood cell count and hemoglobin with survival ($p < .001$ for both). An elevated hemoglobin and a low white blood cell count were associated with better prognosis. Age exhibited a statistically significant nonlinear relationship with survival, modeled by a combination of a linear effect of age, and a linear effect for the number of years older than 75 years of age.

Table 27.2. Prognostic groups derived by the Cancer and Leukemia Group B (CALGB) model

Group	Description	No. of patients	Median survival (months)	1-yr survival (%)
1	PS = 0, age < 49 yr PS = 0, age ≥ 49 yr, Hb ≥ 14.6	36	13.9	63
2	PS = 1/2, WBC < 8.7, no chest pain	36	9.5	41
3	PS = 0, age ≥ 49 yr, Hb < 14.6 PS = 1/2, WBC < 15.6, chest pain, no weight loss, Hb ≥ 12.3 PS = 1/2, 9.8 ≤ WBC < 15.6, chest pain, weight loss, Hb ≥ 11.2	146	9.2	30
4	PS = 1/2, 8.7 ≤ WBC < 15.6, no chest pain	33	6.5	25
5	PS = 1/2, WBC < 15.6, chest pain, no weight loss, Hb < 12.3 PS = 1/2, 9.8 ≤ WBC < 15.6, chest pain, weight loss, Hb < 11.2 PS = 1/2, WBC < 9.8. chest pain, weight loss	73	4.4	7
6	PS = 1/2, WBC ≥ 15.6	13	1.4	0

Multivariate Analysis

Multivariate analysis was conducted for all variables on a subset of 195 patients in which all factors were measured. A raised serum LDH >500 IU/L, poor performance status (i.e., PS 1 or 2), the presence of chest pain, an elevated platelet count >400,000/μL, nonepithelial histology, and increasing age >75 years were predictive of a greater risk of dying early. The six prognostic groups determined by using the regression tree and stepwise algorithm are shown in Table 27.2.

In conclusion, the CALGB prognostic scoring system was able to derive various factors strongly linked with a poor outcome for patients with mesothelioma. The most important predictors of a poor prognosis were poor PS, the presence of chest pain, the presence of pleural involvement, breathlessness as a major symptom, high platelet count, significant weight loss, raised LDH, low Hb, high WBC count, age over 75 years, and nonepithelioid histology. On the positive side, the statistical analysis was able to define the best prognostic groups as those containing patients with excellent performance status, age less than 49 years, and normal hemoglobin level.

The European Organization for Research and Treatment of Cancer (EORTC) Prognostic Scoring System

The European Organization for Research and Treatment of Cancer (EORTC) examined data from 204 adult patients with malignant mesothelioma entered into five consecutive EORTC phase II clinical trials from 1984 to 1993 (4). The drugs tested were mitoxantrone, epirubicin, etoposide, and paclitaxel. The Cox model was used to assess 13 factors related to biology and disease history with respect to survival.

The median survival duration was 8.4 months from trial entry and 12.6 months measured from diagnosis.

The putative prognostic factors studied in a total of 204 patients (all with pleural primary tumors) were:

1. Age (≤55 years, >55 years)
2. Interval since first diagnosis (≤50 days, >50 days)
3. Gender (female, male)
4. ECOG performance status (0, 1–2)
5. White blood cell count ($<8.3 \times 10^9$/L, $\geq 8.3 \times 10^9$/L)
6. Platelet count ($\leq 350 \times 10^9$/L, $>350 \times 10^9$/L)
7. Hemoglobin difference (variance of <1 or ≥1 g/dL from stated normal)
8. Modified Butchart staging (I, II, III, IV)
9. Prior treatment (no, yes)
10. Alkaline phosphatase level (normal, abnormal)
11. Lactate dehydrogenase (normal, abnormal)
12. Histologic subtype (epithelial, sarcomatoid, mixed)
13. Certainty of histologic diagnosis (definite, probable/possible)

Continuous variables were divided into two groups with the median as the cutoff point. In the univariate analysis, poor prognosis was associated with five variables. In a multivariate analysis, poor prognosis was associated with:

1. Poor performance status (1 or 2)
2. High white blood cell count ($\geq 8.3 \times 10^9$/L)
3. Low hemoglobin level (<1 g/dL lower than normal)
4. Probable/possible histologic diagnosis of mesothelioma (i.e., uncertain diagnosis)
5. Sarcomatoid histology

Using these five factors the EORTC classified patients into two groups: a good-prognosis group (with a 1-year survival rate of 40%) and a poor-prognosis group (with a 1-year survival rate of 12%).

Multivariate Analysis of the EORTC Prognostic Factors

In a multivariate analysis the Cox multivariate model was based on all of the variables; the model retained the prognostic factors of age, performance status, certainty of histologic diagnosis, histologic subtype, and gender. Based on these five variables, a prognostic score ranging from 0.00 to 2.94 was determined by the following formula:

$$\begin{aligned}\text{Prognostic score} = {} & 0.55 \text{ (if WBC } > 8.3 \times 10^9\text{/L)} \\ & + 0.60 \text{ (if performance status 1 or 2)} \\ & + 0.52 \text{ (diagnosis is probable/possible)} \\ & + 0.67 \text{ (if sarcomatoid subtype)} \\ & + 0.6 \text{ (male gender)}\end{aligned}$$

Based on prognostic score, patients were divided into two groups: a good-prognosis group with a score ≤1.27 (corresponding to having zero, one, or two poor prognostic factors), and a poor-prognosis group

with a score >1.27 (corresponding to having three, four, or five poor prognostic factors. Relative to patients in the low-risk group the high-risk group had a relative risk of 2.9 [95% confidence interval (CI) = 2.0% to 4.1%; $p < .001$]. The median survival times were 10.8 months and 5.5 months for the low- and high-risk groups, respectively. The 1-year survival rates were 40% and 12%, respectively.

It is of concern that patients were randomized into phase II chemotherapy trials with "uncertain" diagnosis. Recent advances in our understanding of the immunohistochemistry of mesothelioma and the more widespread adoption of the multidisciplinary approach to diagnosis and treatment should make this a much rarer occurrence in the future. The EORTC authors made the interesting observation that patients with "uncertain" diagnosis may have appeared to live less long because more time was spent in trying to obtain a diagnosis prior to registration on trial ("lead time bias").

Validation of the CALGB and EORTC Prognostic Models by Other Groups

Fennell et al (22) from the mesothelioma unit of St. Bartholomew's Hospital, London, validated the EORTC model in a group of 145 patients treated in three sequential phase II chemotherapy trials. For the 70 patients treated with single-agent vinorelbine, those predicted as having good prognosis by the EORTC system had a median survival of 19.2 months (95% CI = 14.7–23.7) and those in the poor prognosis group had a median survival of 9.9 months (95% CI = 8.5–11.3).

In 2000 Edwards et al (23) from Leicester, United Kingdom, published a retrospective analysis of a series of 142 mesothelioma patients. Interestingly, some of these patients had had surgical intervention, whereas others were treated with chemotherapy or supportive care. Univariate analysis of prognostic variables was performed using a Cox proportional hazards regression model and statistically significant variables were analyzed further in a forward, stepwise multivariate model. The authors then derived EORTC and CALGB prognostic groups, plotted Kaplan-Meier survival, and calculated survival rates from life tables to see if these prognostic groups predicted outcome for their patients.

Significant poor prognostic factors in univariate analysis included male sex, older age, weight loss, chest pain, poor performance status, low hemoglobin, leukocytosis, thrombocytosis, and nonepithelial cell type. The prognostic significance of cell type, low Hb, high WBC, performance status, and gender was retained in the multivariate model. The overall median survival was 5.9 months. Median 1- and 2-year survival data within prognostic groups from Leicester were comparable to the EORTC and CALGB series. The authors concluded that the EORTC and CALGB prognostic scoring systems should be used both in the assessment of survival data of series in different countries and in the stratification of patients into randomized clinical studies.

Further observational data have been reported from the German Mesothelioma Register by Neumann et al (24). From 1987 to 2000, the German register recorded 4455 patients with malignant mesotheliomas. Survival data were only available for 498 patients of whom 156 survived for more than 2 years. The authors undertook a multivariate analysis using the Cox proportional hazards regression model and showed that the favorable prognostic factors were epithelioid subtype, age less than 60 years, and female gender.

What is interesting about these studies is the importance of systemic biologic measures of disease activity as prognostic factors. Low hemoglobin, high white blood cell count, elevated platelets, and elevated LDH were shown to be important in the CALGB, and high white blood cell count was important in the EORTC system. These parameters are likely to be markers of disease activity and may prove less subjective than some of the prognostic factors described previously such as age and approximate clinical stage. All specialists treating patients with mesothelioma are aware of the importance of systemic symptoms: weight loss, anorexia, lethargy, and night sweats are all frequently seen and refute the common view that mesothelioma is a localized disease that metastasizes only in the end stages. It is likely that these constitutional symptoms reflect the cytokine-rich nature of mesothelioma as described by Fitzpatrick (25). These reports may represent real progress in our understanding of malignant mesothelioma.

Novel Molecular Predictors of Prognosis

There have been a number of publications reporting biologic markers of prognosis in mesothelioma. Many of these markers are overexpressed in malignant mesothelioma and often there are statistical correlations with clinical outcome. The insights provided by such data are exciting and suggest real therapeutic progress is not far away. Some of the most interesting molecular factors are discussed below.

Cyclooxygenase (COX)-2

Cyclooxygenase-2 (COX-2) is an enzyme that catalyzes the initial rate-limiting reaction step in the synthesis of prostaglandins. Cyclooxygenase-2 expression is upregulated in several cancer types, including lung (26), breast (27), and colorectal (28), and is associated with increased tumor cell proliferation and invasiveness (2). Other studies have shown that COX-2 overexpression is a significant poor prognostic factor by univariate analysis in colorectal and gastric cancer, and in stage I adenocarcinoma of the lung. Cyclooxygenase-2 has been implicated in carcinogenesis through the promotion of angiogenesis, formation of carcinogenic metabolites such as maliondialdehyde, and the downregulation of cell-mediated immunity via T-cell anergy (2).

Cyclooxygenase-2 is a target for novel, selective therapeutic intervention and is under investigation for the treatment of solid tumors. Marrogi et al (29) showed that COX-2 is overexpressed in malignant mesothelioma as well as in nonmalignant mesothelial tissues, and

demonstrated in vitro antiproliferative effects of the COX-2 inhibitor NS398. Nonmalignant mesothelial tissues—despite having similar levels of COX-2—were less sensitive to the antiproliferative activity of NS398. Marrogi et al suggested that COX-2 might therefore be a therapeutic target for mesothelioma.

Edwards et al (2) examined the expression of COX-2 and its prognostic significance in snap frozen malignant mesothelioma tissue collected at video-assisted thoracoscopic biopsy or thoracotomy. In 48 cases studied for COX-2 expression by immunohistochemistry, strong cytoplasmic staining was identified in all tissues studied. Expression did not correlate with measured levels of the stable prostaglandin E_2 (PGE_2) derivative bicyclo-PGE_2. Specific COX-2 expression was identified using Western analysis. In univariate statistical analysis, high COX-2 expression on Western blot band densitometry correlated significantly with poor survival ($p = .008$). In multivariate analysis, high COX-2 expression ($p = .0005$), nonepithelioid subtype and chest pain were independent predictors of poor prognosis. The authors concluded that COX-2 expression is a prognostic factor for mesothelioma and is a possible therapeutic target. The studies by Edwards et al and Marrogi et al (29) suggest that COX-2 expression may independently predict survival in malignant mesothelioma and may provide a novel target for therapeutic intervention.

Cyclin-Dependent Kinase Inhibitor p27

The proliferation-associated antigen p27 (kip) is a cell-cycle regulator and cyclin-dependent kinase (CDK) inhibitor. It acts to regulate cell cycle entry into S-phase via direct interaction with cyclin and CDK. The prognostic value of the proliferation-associated antigen p27 has been investigated in malignant pleural mesothelioma (MPM). In a study by Beer et al (30), sections from 36 patients with MPM were immunohistochemically stained for the p27 antigen. Univariate survival analysis was used to determine the effect of p27 on survival. Low p27 expression (<53% of cells positive) was associated with a statistically significant decrease in survival compared with high p27 expression ($p = .04$). Median survival of patients with low p27 was 4 to 6 months, compared with those with high p27 for whom it was 10 to 11 months. The authors conclude that p27 may be an independent prognostic variable in patients with malignant mesothelioma.

Bongiovanni et al (31) also investigated p27 in 63 patients taken from a larger group of 621 pleural mesothelioma patients. Twenty-seven patients were selected with relatively long survival (>24 months), and 36 cases were selected as having a relatively shorter survival (<24 months). The expression of p27 was significantly higher in the long-term surviving group (81%) compared with the short survival group (32%; $p < .0001$). Interestingly, epithelioid histology was associated with higher p27 expression compared with the biphasic type. It was concluded in both studies that p27 may be a useful marker for identifying patients with a more favorable prognosis.

Proliferation Marker MIB-1

MIB-1 is a marker of proliferation. Its expression and prognostic significance was evaluated by Comin et al (32) in a study comparing the immunoreactivity of a series of seven long-term survivors with MPM and a group of control cases with short-term survival. All cases showed MIB-1–positive cells determined by the percentage of nuclear staining. A statistical difference in expression of MIB-1 was observed with significantly greater proliferative activity in the shorter surviving group compared with the controls. A similar finding was also observed by others (31) and suggests that proliferative index may predict outcome for MPM patients.

Angiogenic Cytokines

Angiogenesis is essential for solid tumor proliferation. Malignant mesothelioma is associated with high intratumoral microvascular density, suggesting active angiogenesis. Vascular endothelial growth factor (VEGF), acidic and basic fibroblast growth factors (FGF-1 and -2), and transforming growth factor-β (TGF-β) are all potent angiogenic cytokines. In a study by Kumar-Singh et al (33), increased levels of VEGF, FGF-1, FGF-2, and TGF-β were detected in malignant mesothelioma compared to nonneoplastic mesothelium. When studied together, the levels of these angiogenic cytokines correlated with intratumoral microvascular density and prognosis. Of the cytokines studied individually only FGF-2 correlated with increased tumor invasiveness and worse prognosis.

Ohta et al (34) investigated the prognostic significance of the messenger RNA expression of VEGF, VEGF type C, and their receptors, together with microvessel and microlymphatic density. Fifty-four patients were studied. Vessel density was a negative prognostic indicator that correlated with VEGF expression, indicating an important role for angiogenesis in malignant mesothelioma and the use of vascular density as a prognostic marker.

Glycoprotein 90K

Strizzi et al (35) examined the levels of tumor-associated glycoprotein 90K in the pleural effusions and sera of patients with malignant mesothelioma using enzyme-linked immunosorbent assay (ELISA). This was correlated with immunocytochemistry in malignant mesothelioma sections and compared with benign pleural disease. The average level of glycoprotein 90K was increased in pleural effusions from patients with malignant mesothelioma compared with those of patients with benign pleural disease. Expression of 90K was observed using immunohistochemistry. A positive correlation between 90K and patient survival was reported; using Kaplan-Meier univariate analysis a high serum level of 90K was shown to be associated with a statistically significant increase in survival probability.

Conclusion

Patients with good and poor prognosis can now be determined by well-validated prognostic factors based on the EORTC and CALGB scoring systems. These distinct, but closely related systems, have clarified much contradictory data accrued over the past two decades. The most important poor prognosis predictors are poor performance status, nonepithelial histology, male gender, low hemoglobin, high platelet count, high white blood cell count, and high LDH. In addition to prognostic information these systems have led to insights into the biology of mesothelioma, in particular, the possible role played by cytokine networks in the symptoms experienced by patients with mesothelioma. Prognostic factors may, at last, not simply enable us to predict a worse outcome for some patients compared to others, but help us understand mesothelioma and develop new treatments. Numerous molecular biologic markers of prognosis are under investigation. Overexpression of various cellular proteins has been demonstrated to correlate with the clinical outcome in the source patients. Understanding the importance of these markers in predicting prognosis will lead to better understanding of malignant mesothelioma and will help improve therapy.

References

1. Steele JP, Rudd RM. Malignant mesothelioma: predictors of prognosis and clinical trials. Thorax 2000;55:725–726.
2. Edwards JG, Faux SP, Plummer SM, et al. Cyclooxygenase-2 expression is a novel prognostic factor in malignant mesothelioma. Clin Cancer Res 2002; 8:1857–1862.
3. Herndon JE, Green MR, Chahinian AP, et al. Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the cancer and Leukemia Group B. Chest 1998;113:723–731.
4. Curran D, Sahmoud T, Therasse, et al. Prognostic factors in patients with pleural mesothelioma: the European Organization for Research and Treatment of Cancer experience. J Clin Oncol 1998;16:145–152.
5. Middleton GW, Smith IE, O'Brien ME. Good symptom relief with palliative MVP (mitomycin-C, vinblastine and cisplatin) chemotherapy in malignant mesothelioma. Ann Oncol 1998;9:269–273.
6. Byrne MJ, Davidson JA, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant mesothelioma: a phase II study. J Clin Oncol 1999;17: 25–30.
7. Steele JP, Shamash J, Evans MT, et al. Phase II study of vinorelbine in patients with malignant pleural mesothelioma. J Clin Oncol 2000;18:3912–3917.
8. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–2644.
9. Cox DR. Regression models and life-tables. J R Stat Soc 1972;B34:187–202.
10. Boutin C, Rey F, Gouvernet J, et al. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Cancer 1993;72:394–404.
11. Samson MK, Wasser LP, Borden EC, et al. Randomized comparison of cyclophosphamide, imidazole carboxamide, and Adriamycin versus

cyclophosphamide and Adriamycin in patients with advanced stage malignant mesothelioma: a Sarcoma Intergroup Study. J Clin Oncol 1987;5: 86–91.
12. Tammilehto L. Malignant mesothelioma: prognostic factors in a prospective study of 9 patients. Lung Cancer 1992;8:175–184.
13. Rusch VW, Piantadosi S, Holmes EC. The role of extrapleural pneumonectomy in malignant mesothelioma. J Thorac Cardiovasc Surg 1991;102:1–9.
14. Chahinian AP, Pajak TF, Holland JF. Diffuse malignant mesothelioma. Ann Intern Med 1982;96:746–755.
15. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 188 consecutive patients. Cancer 1993;72:410–417.
16. De Pangher Manzini V, Brollo A, et al. Prognostic factors of malignant mesothelioma of the pleura. Cancer 1993;72:410–417.
17. Antman KH, Shemin R, Ryan L. Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. J Clin Oncol 1988;6:147–153.
18. Calavrezos A, Koschel G, Husselman H, et al. Malignant mesothelioma of the pleura. Klin Wochenschr 1988;66:607–613.
19. Spirtas R, Connelly RR, Tucker MA. Survival patterns for malignant mesothelioma: the SEER experience. Int J Cancer 1988;41:525–530.
20. Rusch VW, Venkatraman ES. Important prognostic factors in patients with malignant pleural mesothelioma, managed surgically. Ann Thorac Surg 1999;68:1799–1804.
21. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117:54–63.
22. Fennell DA, Parmar A, Shamash J, et al. Statistical validation of the EORTC prognostic model for malignant pleural mesothelioma based on three consecutive phase II trials. Lung Cancer 2003;41(suppl 2):S12.
23. Edwards JG, Abrams KR, Leverment JN, et al. Prognostic factors for malignant mesothelioma in 142 patients: validation of CALGB and EORTC prognostic scoring systems. Thorax 2000;55:731–735.
24. Neumann V, Rutten A, Scharmach M, Muller KM, Fischer M. Factors influencing long-term survival in mesothelioma patients—results of the German Mesothelioma Register. Int Arch Occup Environ Health, 2004; Feb 27.
25. Fitzpatrick DR, Peroni DJ, Bielefeldt-Ohmann H. The role of growth factors and cytokines in the tumorigenesis and immunobiology of malignant mesothelioma. Am J Respir Cell Mol Biol 1995;12:455–460.
26. Hida T, Yatabe Y, Achiwa H, et al. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 1998;58:3761–3764.
27. Hwang D, Scollard D, Byrne J, Levine E. Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst 1998;90: 455–460.
28. Sheehan KM, Sheahan K, O'Donoghue DP, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999;282:1254–1257.
29. Marrogi AJ, Travis WD, Welsh JA, et al. Nitric oxide synthase, cyclooxygenase 2, and vascular endothelial growth factor in the angiogenesis of non-small cell lung carcinoma. Clin Cancer Res 2000;6:4739–4744.

30. Beer TW, Shepherd P, Pullinger NC. p27 immunostaining is related to prognosis in malignant mesothelioma. Histopathology 2001;38:535–541.
31. Bongiovanni M, Cassoni P, De Giuli P, et al. p27(kip1) immunoreactivity correlates with long-term survival in pleural malignant mesothelioma. Cancer 2001;92:1245–1250.
32. Comin CE, Anichini C, Boddi V, Novelli L, Dini S. MIB-1 proliferation index correlates with survival in pleural malignant mesothelioma. Histopathology 2000;36:26–31.
33. Kumar-Singh S, Weyler J, Martin MJ, Vermeulen PB, Van Marck E. Angiogenic cytokines in mesothelioma: a study of VEGF, FGF-1 and -2, and TGF beta expression. J Pathol 1999;189:72–78.
34. Ohta Y, Shridar V, Bright RK, et al. VEGF and VEGF type C play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumours. Br J Cancer 1999;81:54–61.
35. Strizzi L, Muraro R, Vianale G, et al. Expression of glycoprotein 90K in human malignant pleural mesothelioma: correlation with patient survival. J Pathol 2002;197:218–223.

28

Radiologic Assessment of Mesothelioma

Samuel G. Armato III, Heber MacMahon, Geoffrey R. Oxnard, Charles L. Croteau, and Nicholas J. Vogelzang

Imaging plays an essential role in the diagnosis, staging, and clinical management of patients with mesothelioma. X-ray imaging techniques [chest radiography and computed tomography (CT)], magnetic resonance imaging (MRI), and positron emission tomography (PET) have all been used to evaluate this disease, although the relative importance of these imaging modalities has changed over time. Our understanding of mesothelioma has been advanced through radiologic examination, and nearly every mesothelioma patient makes numerous trips to the radiology department during the course of treatment. Imaging studies define the morphology and extent of mesothelioma, tumor perfusion, tumor physiology, the presence of mediastinal or chest wall involvement, and the presence of concomitant disease. The image acquisition device (i.e., the hardware) is only one component of the radiologic examination; software tools for the subsequent visualization and postprocessing of the acquired image data are required to extract useful information from the image pixels and to fully exploit the wealth of information contained within the image. This chapter describes the imaging modalities that have been employed for the evaluation of mesothelioma and emphasizes the role of CT in the important task of tumor thickness measurement for the assessment of tumor progression or response to therapy.

Imaging Modalities

Radiography

Chest radiography continues to rank as the most common radiologic procedure performed in the United States. Consequently, initial detection of mesothelioma in a patient is likely to result from a radiographic chest examination. The two-dimensional radiographic projection of mesothelioma with its complex three-dimensional morphology, however, provides neither a sensitive nor a specific diagnosis, and a follow-up study with another imaging modality is almost always indicated. The ability to diagnose mesothelioma on chest radiography

usually occurs at later, more advanced stages of the disease when tumor burden is greater.

Initial radiographic signs of mesothelioma include a unilateral pleural abnormality with an associated ipsilateral pleural effusion, ipsilateral shift of the mediastinum, and unilateral lung volume loss due to encasement of the lung by the tumor (1) (Fig. 28.1). Signs of other asbestos-related disease are usually absent, and the typical finding of diffuse lobulated pleural thickening is indistinguishable from pleural metastases (2,3). At later stages of the disease, radiography may demonstrate thickening of interlobular septa, rib or vertebral body destruction, lymph node metastases, and metastatic pulmonary nodules (4). Contralateral pleural abnormalities, when present, are typically the result of benign asbestos-related disease rather than metastases (5), since mesothelioma generally spreads by contiguous growth; nevertheless, hematogenous spread of mesothelioma may be observed on imaging studies (see Fig. 28.4) and was present in 44 of 66 autopsy cases in one series (6).

Radiography plays a role in the posttherapy follow-up of patients. For example, patients who undergo extrapleural pneumonectomy may be monitored for complications and recurrence with chest radiography once the affected hemithorax has opacified (5). Findings such as mediastinal shift, a new air–fluid level in the affected hemithorax, or nodules in the contralateral lung would indicate that a CT scan is warranted to differentiate between recurrent disease, infection, or a postsurgery complication (5) (Fig. 28.2). More often, however, CT is being used as the sole imaging modality for routine posttherapy follow-up.

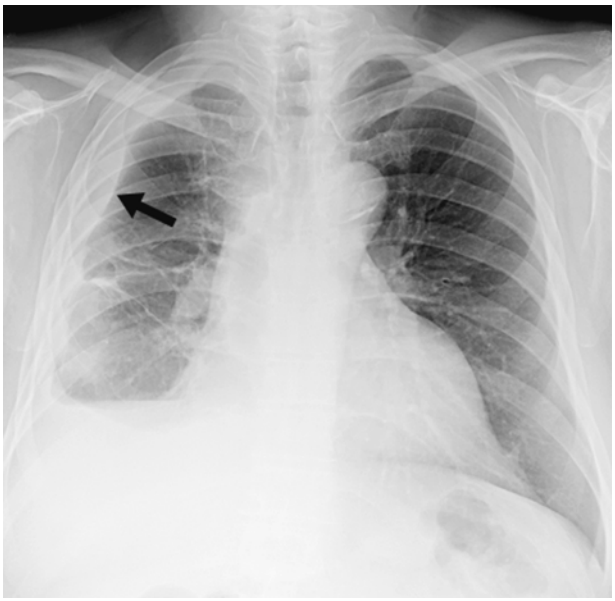


Figure 28.1. Posteroanterior chest radiograph in an 83-year-old man shows mesothelioma diffusely involving the pleura on the right (arrow) accompanied by volume loss of the right hemithorax.

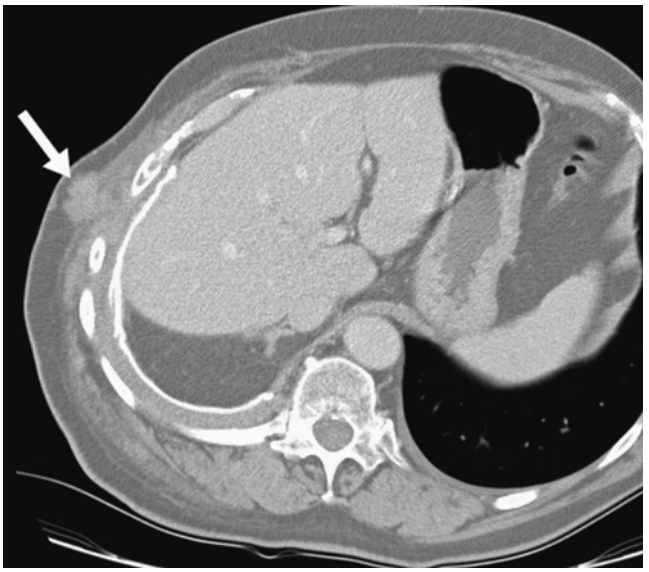


Figure 28.2. A 70-year-old man with prior surgery on the right, which consisted of resection and placement of a synthetic patch (curvilinear bright density just internal to the rib cage). This enhanced computed tomography (CT) scan demonstrates recurrence of disease after surgery as a 22 × 18-mm soft tissue density in the lower right anterior chest wall (arrow).

Computed Tomography

The imaging modality with the greatest impact on the current evaluation of mesothelioma is CT. The transaxial images generated by CT overcome the superposition of anatomic and pathologic structures that limits the two-dimensional projection images acquired by radiography. Accordingly, the spatial extent and radiologic characteristics of mesothelioma tumor may be more clearly appreciated with CT.

The radiologic manifestation of pleural response to a variety of diseases falls into three broad categories: pleural effusion, pleural thickening, and pleural calcification (7). Computed tomography is especially capable of demonstrating such pleural responses. The particular CT findings of mesothelioma, however, are not pathognomonic; a variety of benign and malignant diseases (including metastatic disease, tuberculous pleurisy, empyema, and asbestos-related advanced pleural abnormalities) can have similar characteristics on CT (8,9).

On CT, mesothelioma is characterized by a circumferential, lobulated soft tissue mass that often involves the interlobar fissures and the mediastinal pleura of a hemithorax (2) (Fig. 28.3); bilateral disease is rare (10). Pleural effusions (see Figs. 28.11 and 28.13A below) and nodular pleural thickening, especially in the lower thoracic zone, are typical CT findings in mesothelioma patients (5,10). A tendency for right-sided disease has been observed (10). Intravenous iodinated contrast administered intravenously is typically used to identify mediastinal lymph node enlargement and to determine the relation of lesions to adjacent vascular structures (10); a recognized shortcoming of CT, however, is

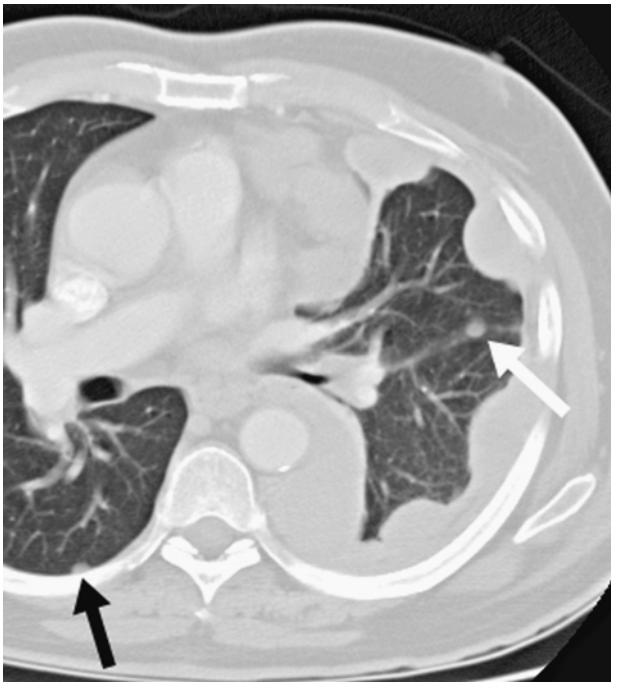


Figure 28.3. Enhanced CT in a 70-year-old man demonstrates left-sided irregular, nodular pleural thickening greater than 1 cm, characteristic of mesothelioma. Focal nodular thickening of the left major fissure is also seen (white arrow). Also of note is a small subpleural nodule (likely metastatic disease) posteriorly on the right (black arrow).

its limited sensitivity for hilar lymph node involvement (10). Although pleural plaques are a common CT finding in mesothelioma, this reflects the role of asbestos exposure in the pathogenesis of both lesions; the possible preneoplastic nature of such plaques has not been proven (11,12).

In a series of 50 patients, Ng et al (13) observed that 76% of the initial CT scans demonstrated pleural effusions, of which the majority were considered "small" (i.e., they occupied less than one third of the hemithorax). Pleural thickening was observed in 94% of cases, of which 72% was nodular, 50% showed a lower zone predominance, and 47% exceeded 1 cm (13). Superior mediastinal pleural thickening was observed in 70% of cases, diaphragmatic crural thickening was demonstrated in 84% of cases, and thickening of the pleural surfaces of the interlobar fissures was present in 84% of cases (13). Kawashima and Libshitz (14) report similar findings. In their series of CT scans from 50 mesothelioma patients, 74% of cases demonstrated pleural effusions (of which approximately half occupied less than one third of the hemithorax), 86% of cases demonstrated thickening of the pleural surfaces of the interlobar fissures, and pleural thickening of various extent, thickness, and nodularity was observed in 92% of cases. Focal pleural masses (ranging from 7 to 18 cm in maximum diameter) were observed

in 8% of cases; half of these cases demonstrated chest wall invasion (14) (Fig. 28.2).

The volumetric extent of disease may be more clearly appreciated with CT than with chest radiography. The CT findings depicting the impact of mesothelioma on the affected hemithorax volume are varied. In response to volume loss of the ipsilateral hemithorax, for example, ipsilateral mediastinal shift may occur (Fig. 28.4). Alternatively, tumor encasement of the ipsilateral lung may result in ipsilateral volume loss *without* mediastinal shift (referred to as the "fixed mediastinum"). Ipsilateral volume loss may also be demonstrated on CT by narrowed intercostal spaces [so-called rib crowding (10)] and ipsilateral hemidiaphragm elevation (14). Substantial pleural effusion or pleural thickening, however, may cause contralateral mediastinal shift with a corresponding increase in ipsilateral hemithorax volume. The CT section in Figure 28.5 represents a hybrid of these mechanisms: ipsilateral volume loss with rib crowding combined with contralateral shift of the mediastinum. Ng et al (13) observed ipsilateral volume loss in 27% of cases, of which 68% demonstrated ipsilateral mediastinal shift; ipsilateral volume increase was observed in 10% of cases, of which 57% demonstrated contralateral mediastinal shift. It is interesting to note that the volume of the affected hemithorax was not substantially altered in 63% of cases at initial CT (13). Kawashima and Libshitz (14) observed ipsilateral volume loss in 42% of cases, of which approxi-

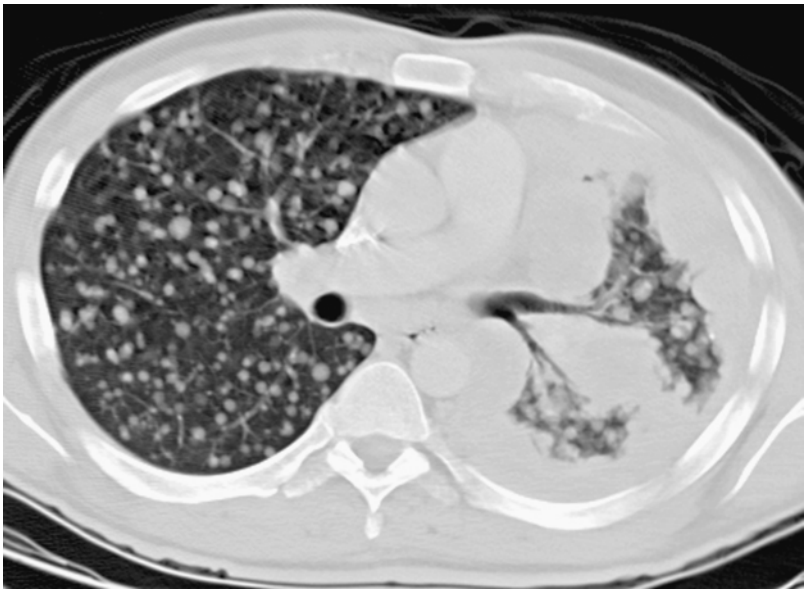


Figure 28.4. Enhanced CT of the chest in a 41-year-old man at the level of the right pulmonary artery shows left-sided pleural thickening with volume loss accompanied by rib crowding and ipsilateral shift of the mediastinum. Numerous sharply circumscribed nodules bilaterally, consistent with hematogenous metastases, are also evident. Pleural thickening involves the left major fissure, which indicates involvement of the visceral pleura.

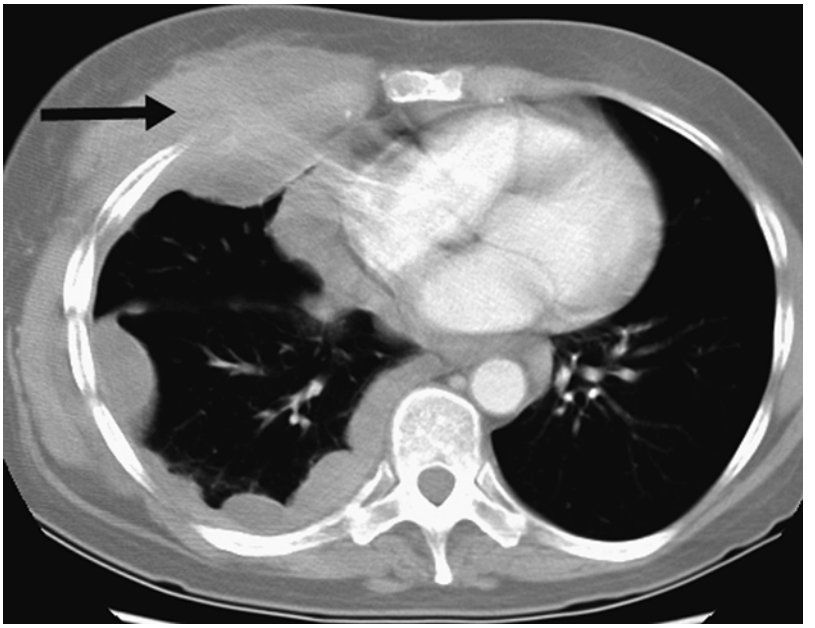


Figure 28.5. Enhanced CT in a 56-year-old woman shows right-sided irregular, nodular thickening of the pleura with rib crowding and contralateral shift of the mediastinum. Involvement of the anterior chest wall and subcutaneous tissue is also seen (arrow).

mately half demonstrated ipsilateral mediastinal shift; contralateral mediastinal shift (due to a large effusion or a combination of effusion and mass) was observed in 14% of cases. Neither change in hemithorax volume nor shift of the mediastinum were observed in 44% of cases (14). Yilmaz et al (10) also noted ipsilateral volume loss with (9% of cases) and without (22% of cases) ipsilateral mediastinal shift, contralateral mediastinal shift due to a large effusion or a combination of effusion and mass (26% of cases), and no change in mediastinal position or affected hemithorax volume (43% of cases).

Although primary pericardial mesothelioma is rare, pericardial invasion of pleural mesothelioma is demonstrated at CT by pericardial thickening with potential concomitant pericardial effusion (5) (Fig. 28.6). It should be noted, however, that distinction between mediastinal pleural disease alone and associated pericardial disease is difficult on CT (14). Some investigators suggest that pericardial involvement should be considered likely when involvement of the mediastinal pleura is bulky or extensive at CT (10).

CT findings are often used in the differential diagnosis of diffuse pleural disease to distinguish between benign pleural disease and mesothelioma (or other malignant pleural disease). The presence of a pleural rind, involvement of the mediastinal pleura, pleural nodularity, and pleural thickening in excess of 1 cm have all been associated specifically with malignant pleural disease (1) and are all well depicted on CT. Moreover, invasion of the chest wall or mediastinum (Figs. 28.2

and 28.7), displacement or destruction of ribs or vertebral bodies (Fig. 28.8), transdiaphragmatic growth (Fig. 28.9), and lymph node metastases (Fig. 28.10) are other CT-based indicators of malignancy (1), although MRI may have advantages over CT with regard to some of these indicators. In a series of 74 patients with diffuse pleural disease, Leung et al (7) observed that among the 71 patients with pleural thickening on CT, four CT findings—presence of a pleural rind, nodular pleural thickening, parietal pleural thickening greater than 1 cm, and mediastinal pleural involvement—were significantly more common in patients with malignant pleural disease than in patients with benign pleural disease. The three patients without pleural thickening demonstrated unilateral pleural effusions, the sole indicator of pleural malignancy in these patients; thus, the authors conclude that absence of pleural thickening does not preclude a malignant diagnosis. The CT findings in mesothelioma patients were the same as the CT findings in patients with metastatic pleural disease, and the CT findings that distinguished mesothelioma from benign pleural disease were essentially the same as those that distinguished malignant pleural disease from benign pleural disease (7). Pleural calcifications were observed to be indicative of a benign process, since none of the 11 mesothelioma patients in this series demonstrated pleural calcifications. Although

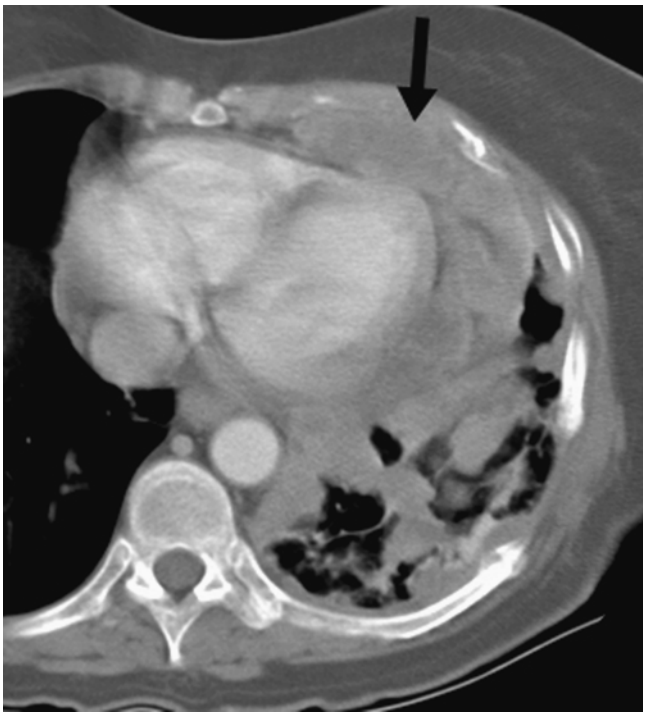


Figure 28.6. Enhanced CT scan at the level of the dome of the right hemidiaphragm in a 62-year-old woman demonstrates malignant mesothelioma involving the pericardium overlying the left ventricle (arrow). Widespread involvement of the pleura and parenchyma on the left is also seen.

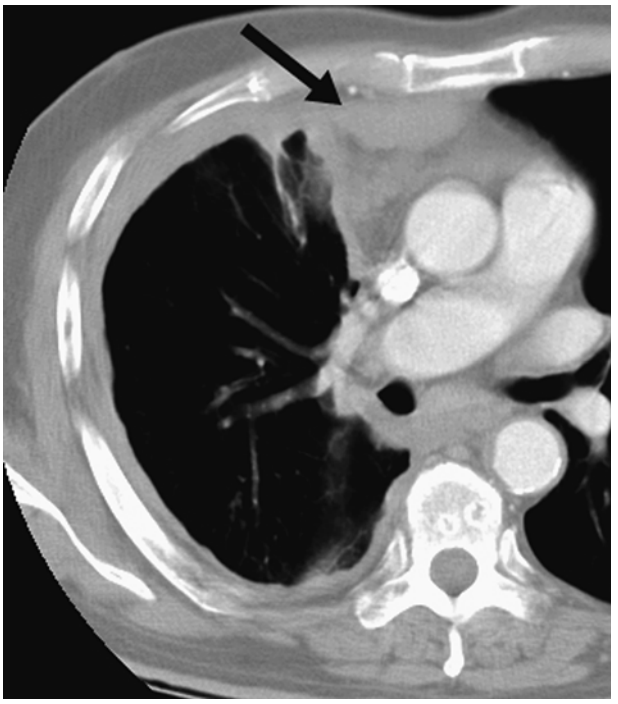


Figure 28.7. Enhanced CT at the level of the right pulmonary artery in a 78-year-old man shows invasion of tumor into the anterior mediastinal fat (arrow). A rind of pleural thickening encircles the entire right lung including the mediastinal pleura anteriorly.

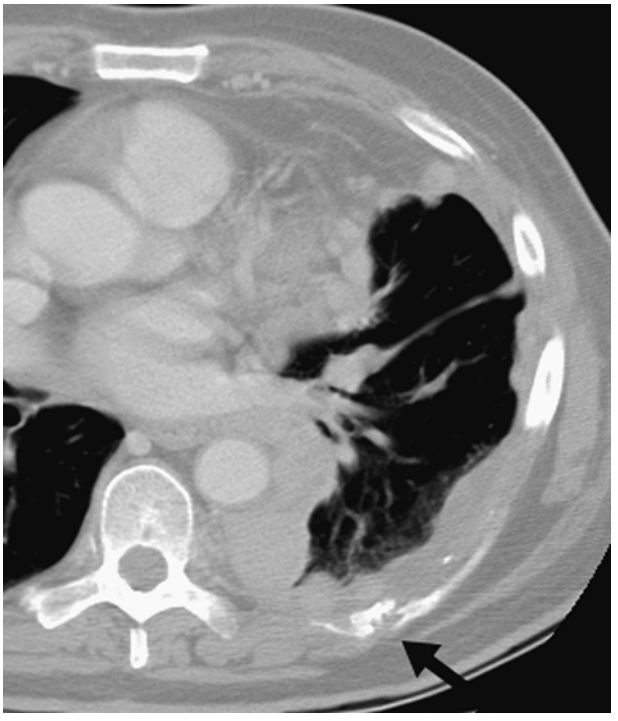


Figure 28.8. Enhanced CT scan at the level of the left atrium in a 70-year-old man reveals extensive pleural thickening on the left with erosive changes in a posterior rib (arrow) due to invasion by tumor.

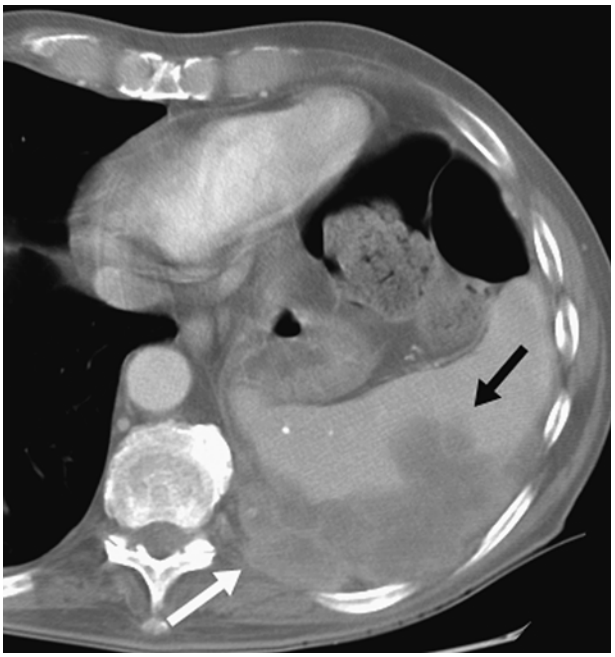


Figure 28.9. Enhanced CT in a 78-year-old man demonstrates evidence of invasion below the diaphragm as indentation of the posterior contour of the spleen (black arrow). Involvement of the posterior chest wall and paraspinal muscles is also seen on the left (white arrow).

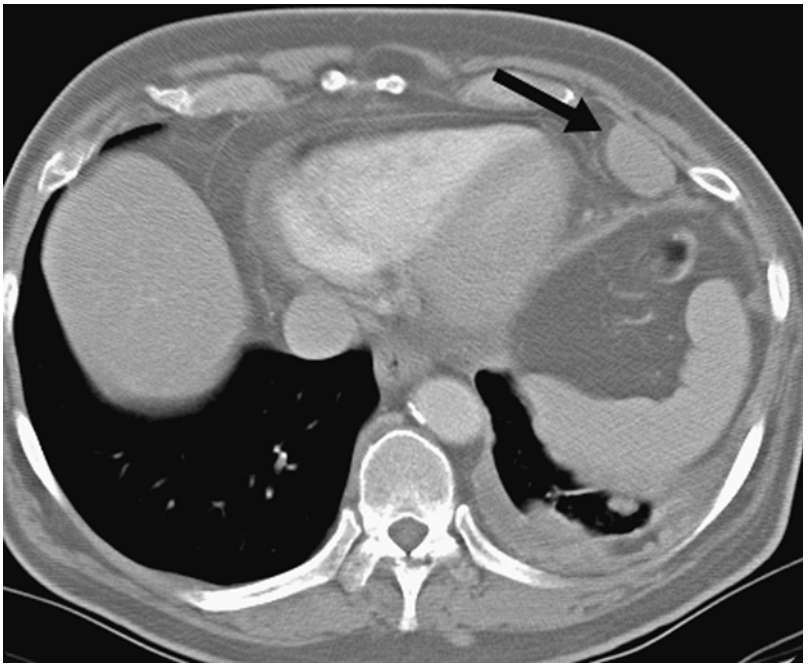


Figure 28.10. Enhanced CT scan through the lung bases at the level of the dome of the liver in a 70-year-old man demonstrates pleural thickening and a 31×20-mm cardiophrenic angle lymph node (arrow) secondary to involvement by mesothelioma.

benign pleural disease in general may present unilaterally, unilateral pleural disease within asbestos-exposed patients was highly specific for malignant disease generally and suggestive of mesothelioma in particular (7).

Computed tomography has also been shown to differentiate between mesothelioma and other malignant pleural disease, although this task has generally been considered a more difficult radiologic challenge. In a series of 215 patients (99 with mesothelioma, 39 with metastatic pleural disease, and 77 with benign pleural disease), Metintas et al (8) used multivariate analysis to show that (1) the presence of a pleural rind, (2) mediastinal pleural involvement, and (3) pleural thickness greater than 1 cm were independent findings both for differentiating mesothelioma from metastatic pleural disease and for differentiating malignant pleural disease (i.e., mesothelioma and metastatic pleural disease) from benign pleural disease. The first two findings were also useful for the differentiation of mesothelioma from benign pleural disease. Nodular pleural thickening was common among the CT scans of mesothelioma patients, and although it was found to be an independent finding for the differentiation of mesothelioma or malignant pleural disease from benign pleural disease, nodular pleural thickening could not be used to differentiate mesothelioma from metastatic pleural disease (8).

Another important aspect of CT is its ability to depict ancillary findings in the lungs that typically accompany mesothelioma and are associated with prior asbestos exposure. These findings include ipsilateral atelectasis [observed in 74% of cases in the 70-patient series of Ng et al (13)], rounded atelectasis [observed in 9% of cases in this series (13)], and lung nodules [observed in 11% of cases (13)]. A CT finding of compressive atelectasis secondary to a large pleural effusion in a mesothelioma patient is shown in Figure 28.11.

Computed tomography has become a valuable tool for biopsy guidance. Closed pleural needle biopsy may be used in lieu of more invasive procedures (e.g., thoracoscopy or thoracotomy) to obtain pleural tissue or fluid samples for histopathologic diagnosis. In the absence of CT guidance, however, the sensitivity of closed pleural needle biopsy for the diagnosis of mesothelioma has been limited due to a typically small sample size and an inability to visualize the source of the acquired sample within the patient (15,16). The addition of CT guidance to the biopsy procedure greatly reduces these limitations. In a series of 30 patients, Metintas et al (15) correctly diagnosed mesothelioma in 83% of cases by use of CT-guided closed pleural needle biopsy, a figure that represents a substantial improvement in efficiency relative to the same biopsy procedure performed without CT.

Magnetic Resonance Imaging

Magnetic resonance imaging adds substantial information to the clinical evaluation of mesothelioma patients, particularly with regard to resectability (due to its ability to depict local tumor extent), diagnosis, staging, surgical planning, and follow-up. Most cases of mesothelioma

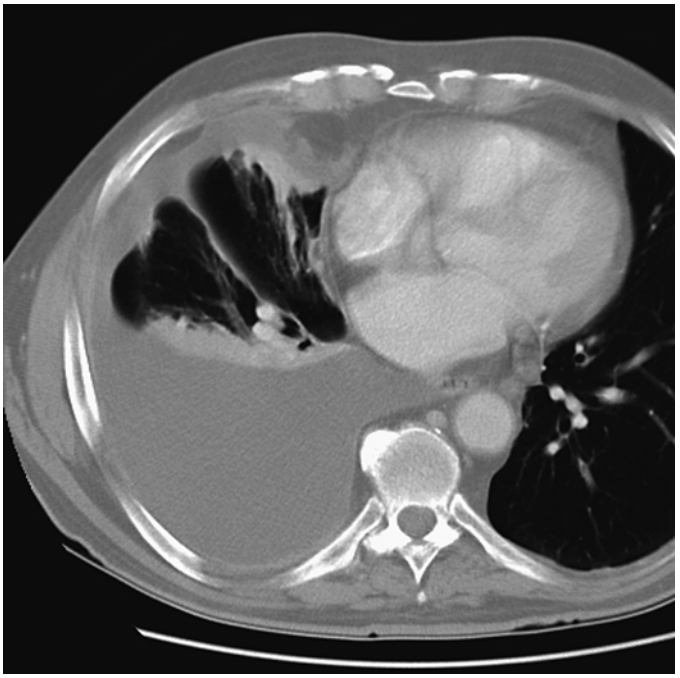


Figure 28.11. Enhanced CT in an 83-year-old man with malignant mesothelioma shows a large right-sided pleural effusion with underlying compressive atelectasis.

abut the ribs and chest wall, and a substantial percent also abut the pericardium and diaphragm. T1-weighted MRI may be used to identify edema in the ribs, a finding consistent with tumor invasion. Magnetic resonance imaging has an advantage over CT in its ability to image tissue planes; indeed, a clear fat plane between the inferior diaphragmatic surface and the adjacent abdominal organs, plus a smooth inferior diaphragmatic surface on MRI, is one of the most reliable indicators of resectability. Likewise, lack of tumor invasion into the mediastinal fat is another measure of resectability better demonstrated on MRI.

One generally recognized advantage of MRI over CT has been the multiplanar capabilities inherent in the MRI acquisition process. Although the spatial resolution of CT in the imaging plane exceeds that of MRI (pixel dimensions on the order of 0.7 mm versus 1.0 mm), CT image acquisition is constrained to the axial plane; postprocessing of the axially acquired data is possible to reformat sagittal and coronal image planes, but the anisotropy of traditional CT voxels renders such reformatted images with suboptimal quality compared with the axially reconstructed images. Magnetic resonance imaging, however, allows for the acquisition of images in arbitrary planes, a powerful capability for the evaluation of mesothelioma with its platelike growth pattern and propensity for chest wall invasion, diaphragmatic involvement, and extension into the interlobar fissures. The multiplanar aspects of MRI do not suffer from the partial volume effect that is characteristic

of axial CT images near curved structures such as the lung apices or the dome of a hemidiaphragm (17).

The multiplanar advantage of MRI, however, is waning in the face of newer multidetector row CT scanners. With 16 or more rows of detectors, rapid high-resolution acquisition has become possible with isotropic voxels so that no preferred plane exists for image reconstruction (18). In effect, all planes have equal resolution, and the radiologist or clinician may decide, after image acquisition, which visualization plane best meets the needs of the particular study. The diagnostic evaluation of mesothelioma is expected to benefit tremendously from this improvement in CT technology.

Magnetic resonance imaging has a further advantage over CT with regard to the information captured. Computed tomography predominantly records information about one physical characteristic of patient anatomy and pathology: attenuation coefficients. An x-ray beam generated by a CT scanner traverses the patient and is attenuated to a greater or lesser extent depending on the attenuation coefficients of the tissues encountered on its way to the detector; the chemical composition and physical density of the material, along with the energy spectrum of the x-ray beam, determine the fundamental appearance of the acquired image. The myriad pulse sequences available on MRI scanners, however, are designed to capture information about different physiologic and molecular processes within the patient. These processes include exchange of water on and off of macromolecules and membranes, water diffusion, and blood flow. The MRI pulse sequences exploit the characteristic differences between these processes in different tissues to provide the required image contrast necessary for tissue differentiation, which may be further enhanced through administration of contrast agents [such as gadolinium–diethylenetriamine pentaacetic acid (Gd-DTPA)] that advantageously alter relaxation and local magnetic susceptibility. In this context, prediction of mesothelioma response and degree of response to new antiangiogenic agents may be captured by MRI.

In a study of 26 paired MRI and CT scans of mesothelioma patients at various stages of disease, Knuuttila et al (17) directly compared the imaging findings of MRI and CT to identify the relative merits of each modality. They found that CT exceeded MRI in its ability to depict pleural calcifications and to detect enlarged lymph nodes with pathologic suspicion. Neither CT nor MRI, however, could be used to accurately assess lymph node staging due to low sensitivity and low specificity. The ability to depict invasion of the chest wall, mediastinum, and lung parenchyma was found to be equal for both modalities. Relative to CT, MRI more clearly indicated the spread of tumor into the interlobar fissures, the extension of tumor through the diaphragm (Fig. 28.12), and tumor invasion of ribs or vertebral bodies. MRI demonstrated an important ability to differentiate mesothelioma from the pleural fluid that usually accompanies it and often confounds the assessment of tumor burden (Fig. 28.13). The authors concluded that MRI was "better for evaluating the growth pattern and extent of

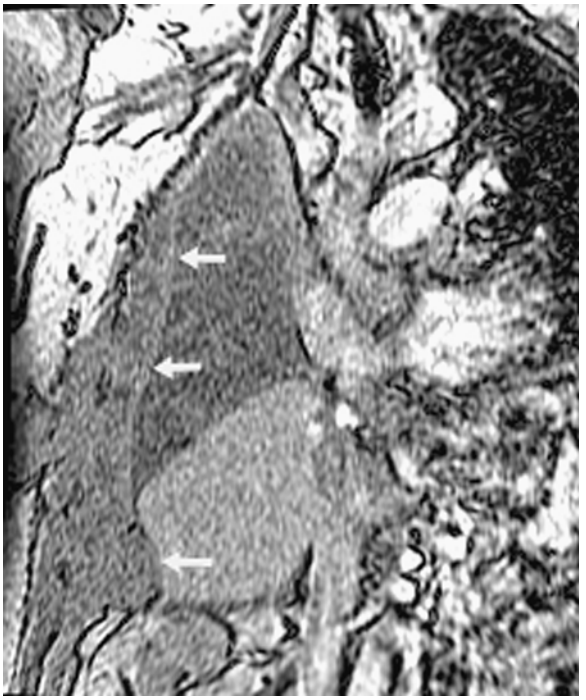


Figure 28.12. T2-weighted coronal MR section in a 70-year-old man with right-sided mesothelioma demonstrates transdiaphragmatic extension of tumor.

[mesothelioma] and should be more widely used, especially when evaluating tumor resectability and in research protocols when an accurate evaluation of disease extent is essential" (17).

Other authors have noted the increased signal strength of mesothelioma relative to the chest wall on T2-weighted MRI (5,19,20). Moreover, MRI may be used to exclude tumor invasion of the spinal canal (1). With regard to diaphragmatic effects of mesothelioma, neither chest radiography nor thoracic CT is capable of distinguishing between elevation and inversion of a hemidiaphragm to the extent possible with MRI, which, on coronal images, depicts the diaphragm as a distinct linear structure separating intrathoracic and intraabdominal structures (21).

A study by Knuuttila et al (22) compared the relative abilities of contrast-enhanced CT and MRI to differentiate mesothelioma from other pleural malignancies or benign pleural disease, although the imaging findings of the study were not verified surgically. In a study with 34 sets of paired CT and MRI scans, the findings of pleural fluid, pleural enhancement, focal pleural thickening, and enhancement of focal pleural thickening were observed statistically significantly more frequently in mesothelioma patients than in patients with other pleural malignancies or benign pleural disease. Focal thickening and enhancement of interlobar fissures occurred significantly more frequently in malignant pleural disease (mesothelioma or other malignancy) than in

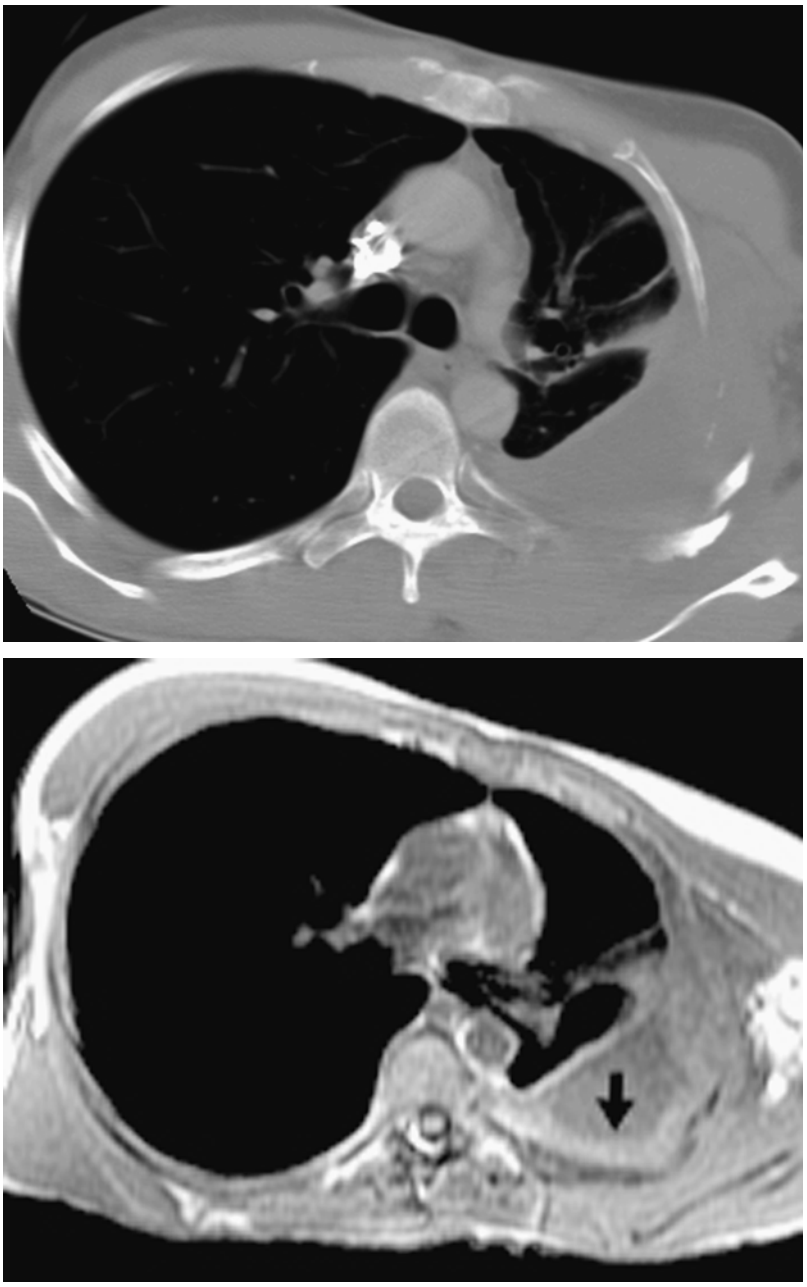


Figure 28.13. A: Enhanced CT in a 43-year-old man with mesothelioma reveals left-sided disease with invasion of the lateral chest wall. On CT it is difficult to ascertain whether the intrathoracic disease consists of only tumor, or if a pleural effusion is also present. B: Corresponding axial T1-weighted MRI section of the same patient acquired one day later depicts the ability of MRI to delineate pleural effusion from tumor; the left-sided disease clearly consists of both tumor and effusion.

benign pleural disease. Magnetic resonance imaging was able to depict abnormal enhancement of interlobar fissures better than CT, but CT better depicted pleural calcifications, although calcifications were not specific to mesothelioma, other pleural malignancy, or benign pleural disease. Compared with CT, MRI better depicted invasive tumor growth into the diaphragm, mediastinum, and chest wall, findings that were observed significantly more frequently in mesothelioma patients than in patients with other pleural malignancies, and MRI better depicted invasion of bony structures, a finding that was observed significantly more frequently in patients with other pleural malignancies than in mesothelioma patients. Neither modality was able to differentiate pathologic mediastinal lymph nodes (22).

The role of imaging in the staging of mesothelioma has gained interest in recent years. In the context of the International Mesothelioma Interest Group (IMIG) Staging System (23), Heelan et al (24) compared MRI and CT findings with surgical and pathologic staging for 65 patients who underwent one of the following procedures: extrapleural pneumonectomy, thoracotomy with partial pleural pleurectomy, thoracotomy with biopsy, laparoscopy with biopsy, or supraclavicular lymph node biopsy. Of the anatomic sites evaluated, only two demonstrated significant differences between the diagnostic capabilities of CT and MRI, with MRI demonstrating superiority over CT: invasion of the diaphragm and invasion of the endothoracic fascia or a single chest wall focus of involvement. Other anatomic sites that were evaluated under this staging system included scattered foci of visceral pleural involvement, confluent visceral pleural tumor, invasion of lung parenchyma, mediastinal fat involvement, pericardial involvement, chest wall invasion, and ipsilateral hilar or mediastinal lymph node involvement. Overall, both imaging modalities demonstrated fairly low diagnostic accuracies. These investigators suggested that the complex growth pattern of mesothelioma along pleural and fissural surfaces combined with the anatomic contiguity of the pleural tumor and the structures it eventually invades hinders the ability of cross-sectional imaging to stage mesothelioma with greater accuracy (24).

Positron Emission Tomography

Positron emission tomography with the fluorine-18-labeled analog of 2-deoxyglucose (F-18 fluorodeoxyglucose or FDG) as a radiotracer provides uniquely different information from other imaging modalities. The resulting functional images of metabolic activity have been used in oncology to differentiate malignant from benign lesions, to stage malignant disease, and to assess tumor response to therapy. The benefits of PET imaging in recent years have gained recognition for the evaluation of mesothelioma. In particular, its role as an adjunct to CT and MRI for the diagnosis of mesothelioma and the identification of the extent of disease has been explored.

Positron emission tomography images may be analyzed either qualitatively (i.e., visually) or through semiquantitative metrics, such as the standardized uptake value (SUV), which measures the ratio of decay-

corrected radiotracer uptake in a region (i.e., a lesion) to the injected dose normalized for body weight. In a study based on the visual interpretation of PET images from 15 patients, the presence of mesothelioma was detected by PET in all 11 positive cases, and the absence of disease was confirmed in the four negative cases (25). Of the 34 lesions from these cases that were biopsied, 28 of the 29 actually positive lesions were identified on PET (the one false negative measured 0.5 mm in diameter) and four of the five actually negative lesions were confirmed on PET (the one false positive was inflammatory pleuritis). Three patterns of FDG uptake were noted (focal or linear, diffuse, and heterogeneous), which corresponded to the structural findings observed at MRI or CT. Whereas PET identified all three patients with chest wall involvement, CT only provided evidence of such involvement in one of these patients; moreover, PET identified bilateral disease in three patients, while CT demonstrated bilateral involvement in only one of these patients (25).

Carretta et al (26) obtained a PET-based sensitivity of 92% for the identification of mesothelioma based on visual interpretation augmented by SUV values. The one false negative represented mesothelioma of the epithelial subtype, which tends to have low metabolic uptake (27). Since it measures tissue metabolic activity of any nature, FDG is not a specific tumor marker (27); therefore, PET is unable to discriminate mesothelioma from other malignant pleural disease and should not replace histologic diagnosis based on biopsy or thoracoscopy (26), although the disease activity demonstrated by PET may be used to guide biopsy site selection (27).

Using semiquantitative SUV values alone, Bénard et al (27) reported a 91% sensitivity and a 100% specificity for the differentiation of malignant and benign pleural disease by PET. The potential staging of the extent of mesothelioma by PET was also observed (27), although others have concluded that PET does not depict local extent of mesothelioma but is valuable for the identification of extrathoracic metastases (28). Bénard et al (29) later showed statistically significantly shorter survival times among patients in a high SUV group, concluding that patients with highly active mesothelioma on PET (i.e., more metabolically active disease and hence, a greater uptake of FDG) have a poorer prognosis. The extent to which this increased FDG uptake indicated inherent biologic characteristics of mesothelioma in these patients or simply reflected differences in tumor size remained an unanswered question (29).

Tumor Measurement

The notion of tumor response is fundamental in oncology. Assessment of disease progression or response to therapy is necessary for the clinical management of the oncology patient and critical for the evaluation of drug efficacy during clinical trials. Accordingly, the *diagnostic* role of imaging is replaced by a *surveillance* role once the presence of mesothelioma in a patient is confirmed. The importance of this surveillance role

must not be underestimated: the radiologic assessment of patients enrolled in clinical trials for the evaluation of novel therapeutic regimens has gained acceptance as a surrogate for patient survival outcomes during the regulatory approval process (30). Clinical trials thus may be conducted with smaller subject populations, a benefit that reduces both time and expense. This radiologic assessment, however, necessitates quantitative tumor measurements and the standardization of tumor response criteria based on such measurements.

The issue of standardization has evolved over the years. In 1981, the World Health Organization (WHO) recommended the radiologic quantification of solid tumors through bidimensional measurements on imaging studies (31). These measurements represent the product of (1) the length of the longest in-plane diameter of the lesion (as represented on the section that demonstrates the greatest extent of the lesion for CT or MRI scans) and (2) the length of the longest diameter that may be constructed perpendicular to the longest in-plane diameter. Tumor response then is determined from a comparison of lesion bidimensional measurements across temporally sequential imaging studies (31).

Nearly two decades later, the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines advocated the replacement of bidimensional tumor measurements with unidimensional measurements, specifically on CT or MRI scans: the length of the longest axial diameter of the lesion (on the CT section that demonstrates the greatest extent of the lesion) (32,33). Under these guidelines, a tumor is classified as demonstrating (1) partial response, when the sum of the unidimensional measurements of all lesions in a follow-up CT scan represents a decrease of more than 30% from the baseline scan sum; (2) progressive disease, when the unidimensional measurement sum in the follow-up CT scan represents an increase of more than 20% from the baseline scan sum (or if new lesions develop); (3) stable disease, when the extent of measurement reduction is not great enough to qualify as partial response or the extent of measurement increase is not great enough to qualify as progressive disease; or (4) complete response, when the follow-up scan demonstrates resolution of all lesions (33). These tumor response criteria were found to be in concordance with the WHO criteria of 50% reduction for partial response and 25% increase for progressive disease (32).

The measurement guidelines offered by WHO or RECIST, which were designed for compact tumors, are generally not as appropriate for mesothelioma with its circumferential growth pattern and often scalloped morphology (34,35). Accordingly, alternative CT measurement protocols, adapted from RECIST, have been proposed specifically for mesothelioma. For one such protocol that is gaining recognition, between one and three unidimensional measurements of pleural thickness are obtained on each of three CT sections (36,37). The sum of these unidimensional measurements is used to represent tumor burden. The RECIST guidelines for tumor response classification then are applied to the summed measurements obtained from temporally sequential CT scans.

The actual manner in which tumor measurement protocols are implemented raises issues of consistency and reproducibility. In studies

unrelated to mesothelioma, inter- and intraobserver variability in the selection and measurement of lesions in CT scans have been reported (38–40); the circumferential morphology and axial extent of mesothelioma, however, further complicate the measurement of this specific tumor. Such difficulties may impair accurate evaluation of patient prognosis and hinder an accurate evaluation of clinical trials. In a recent study, Armato et al (41) articulated a three-step process for the manual measurement of mesothelioma that involves (1) selection of a limited number of CT sections in which the disease is most prominent, (2) identification of specific locations within the selected sections that demonstrate the greatest extent of pleural thickening, and (3) the actual measurement of tumor thickness at those locations. With the first two of these steps held fixed, 95% limits of agreement for relative interobserver difference of mesothelioma tumor thickness measurements were found to span a range of 30% for a database of 22 CT scans. The investigators noted the expectation of increased variability had observers been allowed to implement all three steps of the measurement process and had temporally sequential scans of the patients been evaluated as they are in actual clinical practice (41). Such variability may lead to discordant tumor response classification, which may adversely affect the conduct of clinical trials.

Computed tomography provides an opportunity for computerized image analysis methods to facilitate implementation of tumor measurement protocols. Much progress has been made in the use of computers to analyze medical images, and the potential of semiautomated techniques for the measurement of tumor masses in CT has been shown (42). Armato et al (41) developed a computer interface and computerized techniques for the semiautomated generation of mesothelioma tumor thickness measurements. User-identified points along the chest wall or mediastinal boundary are automatically connected to the lung boundary to provide pleural thickness measurements. In a study of 22 CT scans from mesothelioma patients, the mesothelioma measurements generated by the semiautomated algorithms closely approximated the average measurements of five human observers. Of all semiautomated tumor thickness measurements, 83% were within 15% of the corresponding average manual measurements (41). Such computer-assisted approaches are expected to greatly enhance the utility of CT scans in the management of mesothelioma patients, to reduce data acquisition time during clinical trials, and to make the radiologic assessment of mesothelioma more efficient and consistent.

Despite the volumetric capabilities of CT, tumor volume is not considered in the present clinical evaluation of mesothelioma. Some investigators have begun to explore tumor volume. Pass et al (43), for example, showed a correlation between mesothelioma tumor volume and median survival in a series of 48 patients. Furthermore, Prasad et al (44) demonstrated that measurements of metastatic tumors based on volume yield tumor response classifications that differ from those obtained based on the RECIST guidelines, so that, in general, linear measurements may not be accurate surrogates for tumor volume. The fact that volume is *not* considered clinically, however, is out of neces-

sity, not out of need. Volume measurements are needed, but such measurements are exceedingly cumbersome and quite impractical to obtain through manual approaches, especially for mesothelioma; clinicians, therefore, have submitted to the more practical acquisition of a limited number of unidimensional measurements. The extent to which unidimensional measurements sufficiently capture the often asymmetric and nonuniform three-dimensional growth of a morphologically complex tumor such as mesothelioma is questionable, but volume measurements will certainly require some degree of automation. To this end, the power of the computer will be more fully realized by automated and semiautomated methods that evaluate the two- and three-dimensional characteristics of tumor area and volume.

References

1. Eibel R, Tuengerthal S, Schoenberg SO. The role of new imaging techniques in diagnosis and staging of malignant pleural mesothelioma. Curr Opin Oncol 2003;15:131–138.
2. Aberle DR, Balmes JR. Computed tomography of asbestos-related pulmonary parenchymal and pleural diseases. Clin Chest Med 1991;12:115–131.
3. Gefter WB, Epstein DM, Miller WT. Radiographic evaluation of asbestos-related chest disorders. Crit Rev Diagn Imaging 1984;21:133–181.
4. Wechsler RJ, Rao VM, Steiner RM. The radiology of thoracic malignant mesothelioma. Crit Rev Diagn Imaging 1984;20:283–310.
5. Marom EM, Erasmus JJ, Pass HI, Patz EF Jr. The role of imaging in malignant pleural mesothelioma. Semin Oncol 2002;29:26–35.
6. Wanebo HJ, Martini N, Melamed MR, Hilaris B, Beattie EJ Jr. Pleural mesothelioma. Cancer 1976;38:2481–2488.
7. Leung AN, Müller NL, Miller RR. CT in differential diagnosis of diffuse pleural disease. AJR 1990;154:487–492.
8. Metintas M, Ucgun I, Elbek O, et al. Computed tomography features in malignant pleural mesothelioma and other commonly seen pleural diseases. Eur J Radiol 2002;41:1–9.
9. Müller NL. Imaging of the pleura. Radiology 1993;186:298–309.
10. Yilmaz UM, Utkaner G, Yalniz E, Kumcuoglu Z. Computed tomographic findings of environmental asbestos-related malignant pleural mesothelioma. Respirology 1998;3:33–38.
11. Müller KM, Fischer M. Malignant pleural mesotheliomas: an environmental health risk in southeast Turkey. Respiration 2000;67:608–609.
12. Rabinowitz JG, Efremidis SC, Cohen B, et al. A comparative study of mesothelioma and asbestos using computed tomography and conventional chest radiography. Radiology 1982;144:453–460.
13. Ng CS, Munden RF, Libshitz HI. Malignant pleural mesothelioma: the spectrum of manifestations on CT in 70 cases. Clin Radiol 1999;54:415–421.
14. Kawashima A, Libshitz HI. Malignant pleural mesothelioma: CT manifestations in 50 cases. AJR 1990;155:965–969.
15. Metintas M, Özdemir N, Isiksoy S, et al. CT-guided pleural needle biopsy in the diagnosis of malignant mesothelioma. J Comput Assist Tomogr 1995; 19:370–374.
16. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. J Clin Oncol 1989;7:1157–1168.

17. Knuuttila A, Halme M, Kivisaari L, Kivisaari A, Salo J, Mattson K. The clinical importance of magnetic resonance imaging versus computed tomography in malignant pleural mesothelioma. Lung Cancer 1998;22:215–225.
18. Mezrich R. Sixteen-section multi-detector row CT scanners: this changes everything. Acad Radiol 2003;10:351–352.
19. Bonomo L, Feragalli B, Sacco R, Merlino B, Storto ML. Malignant pleural disease. Eur J Radiol 2000;34:98–118.
20. Lorigan JG, Libshitz HI. MR imaging of malignant pleural mesothelioma. J Comput Assist Tomogr 1989;13:617–620.
21. Kinoshita T, Ishii K, Miyasato S. Localized pleural mesothelioma: CT and MR findings. Magn Reson Imaging 1997;15:377–379.
22. Knuuttila A, Kivisaari L, Kivisaari A, Palomäki M, Tervahartiala P, Mattson K. Evaluation of pleural disease using MR and CT. Acta Radiol 2001;42: 502–507.
23. Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995;108:1122–1128.
24. Heelan RT, Rusch VW, Begg CB, Panicek DM, Caravelli JF, Eisen C. Staging of malignant pleural mesothelioma: comparison of CT and MR imaging. AJR 1999;172:1039–1047.
25. Gerbaudo VH, Sugarbaker DJ, Britz-Cunningham S, Di Carli MF, Mauceri C, Treves ST. Assessment of malignant pleural mesothelioma with ^{18}F-FDG dual-head gamma-camera coincidence imaging: comparison with histopathology. J Nucl Med 2002;43:1144–1149.
26. Carretta A, Landoni C, Melloni G, et al. 18-FDG positron emission tomography in the evaluation of malignant pleural diseases—a pilot study. Eur J Cardiothorac Surg 2002;17:377–383.
27. Bénard F, Sterman D, Smith RJ, Kaiser LR, Albelda SM, Alavi A. Metabolic imaging of malignant pleural mesothelioma with fluorodeoxyglucose positron emission tomography. Chest 1998;114:713–722.
28. Flores RM, Akhurst T, Gonen M, Larson SM, Rusch VW. Positron emission tomography defines metastatic disease but not locoregional disease in patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2003;126:11–16.
29. Bénard F, Sterman D, Smith RJ, Kaiser LR, Albelda SM, Alavi A. Prognostic value of FDG PET imaging in malignant pleural mesothelioma. J Nucl Med 1999;40:1241–1245.
30. Saini S. Radiologic measurement of tumor size in clinical trials: past, present, and future. AJR 2001;176:333–334.
31. Miller AB, Hogestraeten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207–214.
32. James K, Eisenhauer E, Christian M, et al. Measuring response in solid tumors: unidimensional versus bidimensional measurement. J Nat Cancer Inst 1999;91:523–528.
33. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Nat Cancer Inst 2000;92:205–216.
34. Monetti F, Casanova S, Grasso A, Cafferata MA, Ardizzoni A, Neumaier CE. Inadequacy of the new Response Evaluation Criteria in Solid Tumors (RECIST) in patients with malignant pleural mesothelioma: report of four cases. Lung Cancer 2004;43:71–74.
35. van Klaveren RJ, Aerts JGJV, de Bruin H, Giaccone G, Manegold C, van Meerbeeck JP. Inadequacy of the RECIST criteria for response evaluation in patients with malignant pleural mesothelioma. Lung Cancer 2004;43: 63–69.

36. Vogelzang NJ, Rusthoven J, Symanowski J, et al. A phase III study of pemetrexed in combination with cisplatin vs. cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–2644.
37. Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Ann Oncol 2004;15:257–260.
38. Hopper KD, Kasales CJ, van Slyke MA, Schwartz TA, TenHave TR, Jozefiak JA. Analysis of interobserver and intraobserver variability in CT tumor measurements. AJR 1996;167:851–854.
39. Thiesse P, Ollivier L, Di Stefano-Louineau D, et al. Response rate accuracy in oncology trials: reasons for interobserver variability. J Clin Oncol 1997; 15:3507–3514.
40. Erasmus JJ, Gladish GW, Broemeling L, et al. Interobserver and intraobserver variability in measurement of non-small-cell carcinoma lung lesions: implications for assessment of tumor response. J Clin Oncol 2003;21:2574–2582.
41. Armato SG III, Oxnard GR, MacMahon H, et al. Measurement of mesothelioma on thoracic CT scans: a comparison of manual and computer-assisted techniques. Med Physics 2004;31:1105–1115.
42. Schwartz LH, Ginsberg MS, DeCorato D, et al. Evaluation of tumor measurements in oncology: use of film-based and electronic techniques. J Clin Oncol 2000;18:2179–2184.
43. Pass HI, Temeck BK, Kranda K, Steinberg SM, Feuerstein IR. Preoperative tumor volume is associated with outcome in malignant pleural mesothelioma. J Thorac Cardiovasc Surg 1998;115:310–317.
44. Prasad SR, Jhaveri KS, Saini S, Hahn PF, Halpern EF, Sumner JE. CT tumor measurement for therapeutic response assessment: comparison of unidimensional, bidimensional, and volumetric techniques—initial observations. Radiology 2002;225:416–419.

29

Endoscopic Imaging

Gian Franco Tassi and Gian Pietro Marchetti

The origin of mesothelioma from the serous membranes and its typical local growth, with subsequent invasion of adjacent tissues, gives the exploration of the pleural cavity (thoracoscopy) and of the peritoneal cavity (laparoscopy) a crucial role in diagnosis.

Direct exploration of the serous membranes often facilitates the identification of lesions with a neoplastic aspect that can be sampled. It is also possible to perform multiple biopsies essential for correct diagnosis using different techniques: histochemical, immunohistochemical, and ultrastructural. Endoscopic examination is also important to establish the endocavitary extent of the tumor, giving data that complete those from radiologic imaging—computed tomography (CT) in particular, but also magnetic resonance imaging (MRI) and positron emission tomography (PET) in certain situations.

Given the more frequent occurrence of pleural mesothelioma, thoracoscopy is discussed first, followed by laparoscopy and its application in the less frequent occurrence of peritoneal mesothelioma.

Thoracoscopy

Although the history of malignant pleural mesothelioma as a distinct nosologic entity is relatively recent (c. 1920), the role of thoracoscopy for diagnosis is even more recent. The first publications appeared in the 1960s (1), and the use of the method on a large scale occurred in the late 1980s (2,3). Thoracoscopy is now the standard examination to obtain the histologic diagnosis of mesothelioma in vivo (4) because endoscopic biopsies under direct vision yield a diagnosis in most cases (5), thereby equaling the accuracy previously produced only by thoracotomy.

The procedure not only allows adequate and abundant tissue sampling for immunohistochemical staining, which is necessary for differential diagnosis from adenocarcinoma, but also allows staging of the disease which is a useful prognostic factor and an important element in deciding therapy. In the initial stages, where neoplastic presence is

restricted to the parietal and diaphragmatic pleura, local treatment with immunomodulators can be effective, while in more advanced cases it is possible to perform a palliative talc pleurodesis (6).

The exploration of the pleural cavity can now be considered all but obligatory when mesothelioma is suspected, not only for its singular diagnostic importance but also for its prognostic and therapeutic value.

Technique

Medical thoracoscopy and surgical thoracoscopy are quite different (7), both in anesthesia and instrumentation, and in the way they are performed. Medical thoracoscopy is carried out in an endoscopy room under local anesthesia or neuroleptoanalgesia, with one or two points of entry and 7-mm instruments. Surgical thoracoscopy is carried out in an operating room under general anesthesia, often with double lumen intubation, two to three points of entry, and 10-mm instruments. On the basis of our direct experience (250 cases examined between 1983 and 2002), we consider medical thoracoscopy to be the best way for the diagnosis of mesothelioma, given its simple execution, lower financial cost, and good tolerance by patients.

The normal instrumentation includes a rigid endoscope with a video camera, connected to a cold-light source using a 7-mm-diameter direct (0 degree) and lateral (50 degree) optic, and optical forceps for the biopsy of the parietal and visceral pleura.

The exploration of the pleural cavity is possible only when a pneumothorax has been created, which in medical thoracoscopy in cases of effusion, occurs on the entry of ambient air after the aspiration of the liquid; in the absence of effusion, it is induced artificially before the exam (8). A trocar is then inserted, generally in the fifth or sixth intercostal space in the midaxillary line, through which the thoracoscope is passed. The pleural cavity is explored first with the direct optic and then with the lateral optic, which is essential for the proper study of the thoracic angles. If necessary, a second trocar can be positioned to carry out lung biopsies using coagulating forceps.

In the case of mesothelioma, to prevent tumor seeding after the procedure, it is necessary to carry out local radiotherapy (9), which has also proved an effective treatment in our experience.

Samples of the parietal and diaphragmatic pleura are taken with double-spoon forceps, which remove the tissue without tearing it. Samples from the lungs are taken through the second point of entry using coagulating forceps to ensure hemostasis of the lung and the closure of the pleural breach. Multiple biopsy samples—at least 10 and of a reasonable size (minimum 4 mm)—should be taken (4), both of suspect neoplastic lesions such as nodules and masses, and in different pleural areas.

To improve the already good tolerance by patients to this method, we have recently developed a mini-invasive technique that we have called minithoracoscopy (10), which is particularly well tolerated because of the small caliber of the instruments. This technique can be carried out without problems under local anesthesia and is suitable in

particular for patients with either a small thorax or restricted intercostal spaces, and in treating minor, especially loculated, effusions. Two points of entry are used (one for the endoscope and one for the bioptic forceps) positioned about 4 cm from each other, achieving a good exploration of the thoracic cavity and enabling bioptic samples of 5×10 mm.

Diagnostic Value

If we compare data regarding the sensitivity of the methods used prior to thoracoscopy, the clear advantage of the latter is quite evident. Our experience, similar to the results of larger case studies (6), demonstrates that mesothelioma can be diagnosed by thoracoscopy in over 90% of cases, much higher than the diagnostic yield of about 40% achieved using pleural needle biopsy and fluid cytology (6). The small percentage of false negatives present in all the studies is generally due to the incomplete exploration of the pleural cavity caused by adhesions that cannot be divided endoscopically. It should also be emphasized that this is usually the case when an endoscopic exam is delayed too long and is performed several months after the appearance of an effusion. It therefore can be supposed that prompt use of the method in the investigation of pleurisies that have not been diagnosed using other means can further increase the sensitivity of the technique.

Thoracoscopic Findings

To achieve a correct interpretation of thoracoscopic findings, it is first necessary to establish if there are adhesions between the lungs and the thoracic walls, and whether these adhesions can be resected. If they cannot be resected, they can render the exploration of the pleural cavity incomplete and therefore inadequate. However, even an incomplete exploration can be sufficient if it shows the presence of macroscopically neoplastic pleura and significant biopsies are obtained.

Good knowledge of the endoscopic appearance of normal and abnormal pleura is always necessary to identify aspects that are clearly tumoral or generically inflammatory. Tumoral lesions are nodules, masses, pleural thickenings, and pachypleuritis with a neoplastic aspect. *Nodules* (Fig. 29.1) are small solid lumps between 1 and 10 mm in diameter and can be either isolated and infrequent, or, more often, numerous and widespread. *Masses* are larger pathologic formations (>10 mm) and often confluent. *Pleural thickenings* with a neoplastic aspect are areas with a thickness of several millimeters with whitish, poorly vascularized tissue and an irregular surface with ill-defined limits and an infiltrative aspect. *Malignant pachypleuritis* (Fig. 29.2) presents a diffuse, usually whitish, thickening with an irregular surface.

Lesions that can present in isolation are nodules, masses, and limited thickenings, even though in many cases they are present as multiple lesions. Simple inflammation, in which the presence of mesothelioma is a histologic "surprise," is less frequent [6.5% in Boutin and Rey's (2) case studies and 1.2% in ours (Table 29.1)]. It is important to be aware of and recognize such occurrences in order to avoid the risk of under-

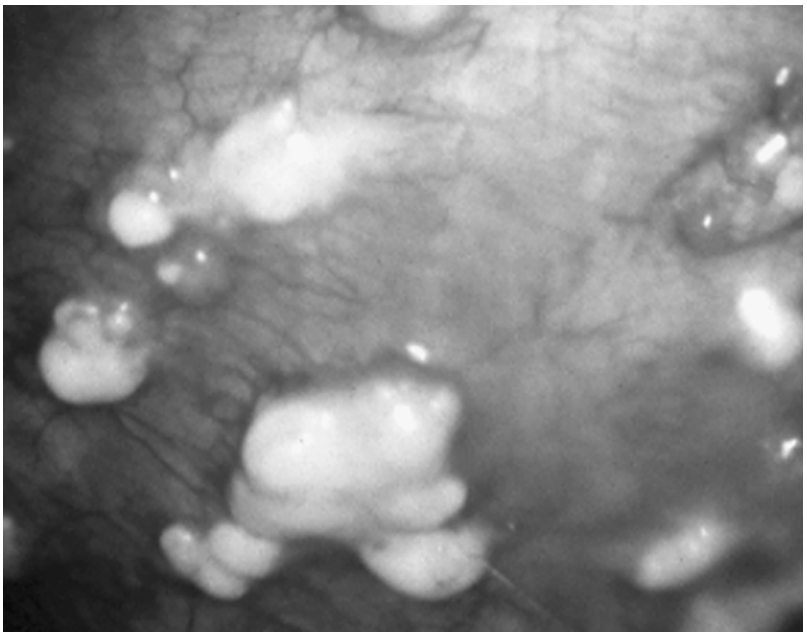


Figure 29.1. Neoplastic parietal nodules in T1a epithelial mesothelioma.

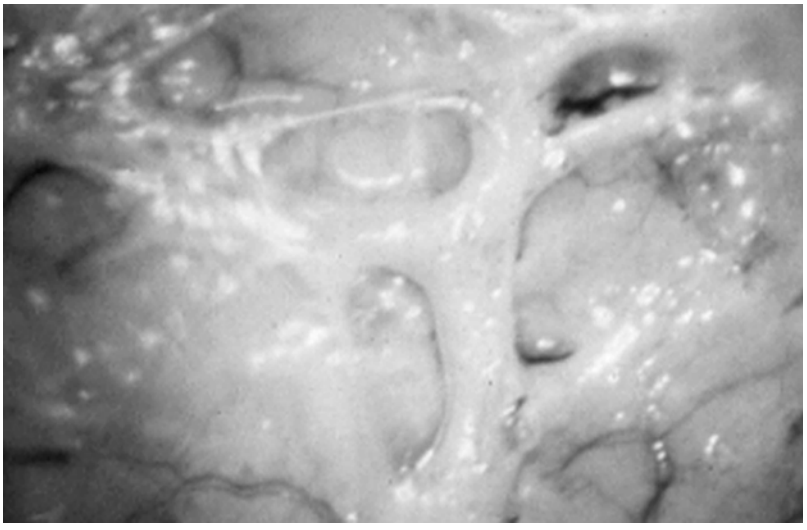


Figure 29.2. Pachypleuritic pattern in sarcomatous mesothelioma.

Table 29.1. Thoracoscopic findings in 250 cases of mesothelioma

Finding	Number	Percent
Nodules	99	39.6
Masses	14	5.6
Pleural thickenings	28	11.2
Pachypleuritis	26	10.4
Inflammation	3	1.2
Multiple lesions	80	32

estimating their importance, justifying the need for multiple biopsies in all cases of indeterminate effusion in subjects with possible exposure to asbestos.

The endoscopic findings described can be divided into three broad categories correlating to clinical and radiological data: pachypleuritic, multinodular, and "aspecific" patterns. *Pachypleuritis* can be predicted in the presence of a retraction of the hemithorax and with linear thickenings, visible on the circumference with CT, and often with the absence of pleural effusion. Endoscopically the costal pleura is rigid with decreased respiratory movements, and the intercostal spaces, when visible, are considerably reduced. Hard whitish tissue, poorly vascularized, covers the surface of all the pleural cavity, in particular, the parietal and diaphragmatic, but often also the visceral pleura, in particular, in the area of the fissures. It is simple to take a bioptic sample and it is often possible to decorticate extensive areas of the tissue. After the biopsy of the pleural thickening, it is always advisable to repeat the sampling in the decorticated areas to verify the extent of neoplastic infiltration in the subpleural tissues.

The *multinodular* pattern is normally identifiable with CT because the nodular structures and masses absorb the contrast medium that highlights them against the liquid of the effusion, which is almost always present. A reddish color is predominant in endoscopy because the nodules and masses, usually multiple, have increased vascularity, and while affecting various surfaces, tend to predominate in the lower middle regions of the costal pleura. Often a dense and viscous liquid flows from them after biopsy.

The "*aspecific*" pattern is characterized by findings of relative normality by CT, which shows only the pleural effusion. In an endoscopic examination, simple inflammation can be seen together with spots of thickening in the lower parietal regions and small areas with a granular surface, indicating lymphangitis (6).

In the presence of neoplastic lesions, it is not possible to differentiate between mesothelioma and pleural metastases of other tumors because the modifications are often similar. However, in our experience some endoscopic patterns are more frequent with mesothelioma (Table 29.2).

An important aspect for diagnosis is represented by the presence of fibrohyaline plaques (irregular surface with nodules with the appear-

Table 29.2. Differential endoscopic diagnosis between mesothelioma and neoplasic metastases

Finding	Mesothelioma	Metastases
Asbestotic fibrohyaline plaques	**Yes**	**No**
Emidiaphragm involved	**Always**	**Occasional**
Costal sectors involved	**Middle/lower**	**All**
Involvement limited to fissures	**Yes**	**No**
Rigid emidiaphragm	**Yes**	**No**
Multiple lesions	**Frequent**	**Rare**
"Hard" parietal pleura	**Yes**	**No**
"Grape-like" aspect	**Frequent**	**Rare**

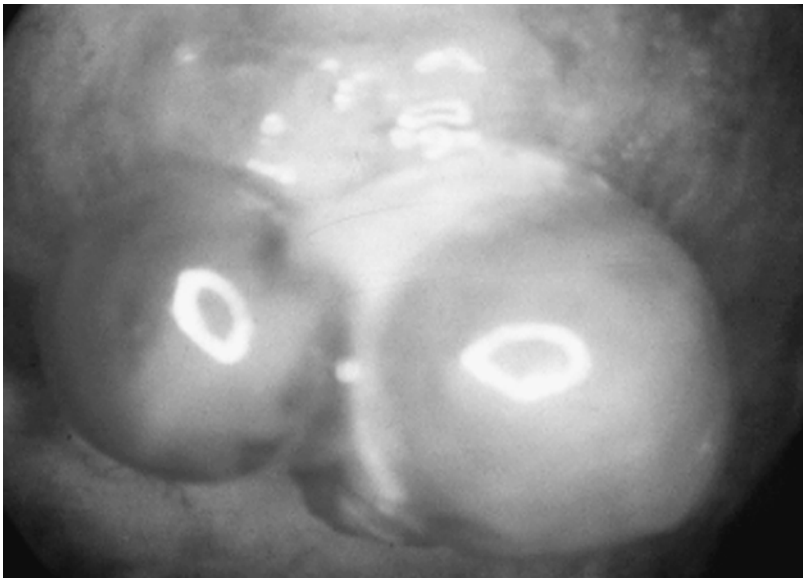


Figure 29.3. Grape-like translucent confluent nodules in biphasic pleural mesothelioma.

ance of "candle-wax drops"), which we observed in 36 (14.4%) of our 250 patients. These plaques, which indicate previous exposure to asbestos (11), together with obvious neoplastic lesions (nodules, masses, thickenings), clearly point to a diagnosis of mesothelioma. In our experience of 1000 thoracoscopies in neoplastic pleurisy, we have never found these plaques associated with other tumors. Another distinctive aspect is the invasion of the diaphragm, which is frequent with mesothelioma, but almost always absent with metastases, the latter also being characterized by a widespread invasion of the costal pleura. Neoplastic visceral invasions limited to the fissures can be observed in mesothelioma, while with metastases there is a larger visceral invasion. The primitive neoplasia more often provides a "mixed" endoscopic picture with more elementary lesions and "hard" parietal pleura on bioptic sampling, while metastatic tumors are more often monomorphous. The respiratory movements of the lung and diaphragm are also different, being reduced in mesothelioma and normal in metastases.

Some authors describe as a characteristic endoscopic sign, even if not exclusive to mesothelioma, confluent nodulations that are white or yellowish, translucent, with a "grape-like" appearance (Fig. 29.3), from which a dense and viscous liquid flows after biopsy (2).

Endoscopic evaluation, besides examining the macroscopic aspects of the pleura, should define neoplastic or inflammatory character, and in cases of tumorous pleura, identify the extent of neoplastic invasion and the type and dimensions of the lesions.

The extent of the neoplasia should be identified in the different parts of the parietal pleura (costal, diaphragmatic, mediastinal), the visceral

pleura, and the pericardium, to map out the pleural cavity in order to identify the points where biopsies are performed and also for its predictive value. There are reports that cases with involvement of less than a third of the pleural cavity have a better prognosis (3). The identification of visceral pleura involvement is crucial to establish the stage of development of the disease (see Endoscopic Staging, below), and in this case, it is often necessary to perform pulmonary biopsies. A simple and effective technique is to use electrically isolated coagulating forceps, which are introduced through the point of entry for the thoracoscope or through a second trocar (7). This technique has the advantage of sealing the surface of the lung and thus avoiding an air leak. The exploration of the visceral pleura should be carried out in minute detail, with particular attention to the fissures where neoplastic nodules can be found, which are not immediately evident. A pulmonary biopsy, however, should be avoided in the area of the fissures because of the risk of vascular injury. The type and dimensions of the lesions should also be accurately recorded, since inflammatory and nodular patterns appear to have a better prognosis (3).

Benign Asbestos-Induced Pleural Lesions

In endoscopic evaluation of mesothelioma, the important benign lesions, when considering a possible correlation with previous exposure to asbestos, are pleural hyaline plaques and parietal "black spots." Both are alterations caused by the migration of asbestos fiber in the pleural space (translocation) and from their accumulation in the areas where lymphatic tissues are more numerous (11).

Pleural plaques are now considered highly specific in determining exposure to asbestos, and their extent appears directly correlated to the intensity of exposure (11,12). Endoscopically they present as whitish elevations with a smooth surface, sometimes with pearl-like nodulations and characteristic hard consistency (Fig. 29.4). In particular, their fibrous consistency and well-defined borders, and their clearly visibility and elevation from the surrounding pleura, means that they can be distinguished from neoplastic plaques whose borders infiltrate the pleural tissue. They are more often located in the middle posterior area of the costal pleura and in the tendinous portion of the diaphragm. They are covered with normal mesothelium and originate from submesothelial tissues (13). Their presence together with neoplastic lesions strongly suggests mesothelioma. However, it has not been demonstrated that malignant mesothelioma develops from these plaques (14). Parietal "black spots" are circular or irregular, less often linear, spots that can be 3 to 10 mm in diameter, rarely larger than 50 mm, clustered in groups of three to five or scattered as single areas. They are found predominantly in the lower areas of the costal, paravertebral, and axillary pleura and on the diaphragm. These are deposits of carbon particles and other types of dust and correspond to the anatomic distribution of structures involved in pleral cavity clearance, like subpleural lymphatic lacunae (13).

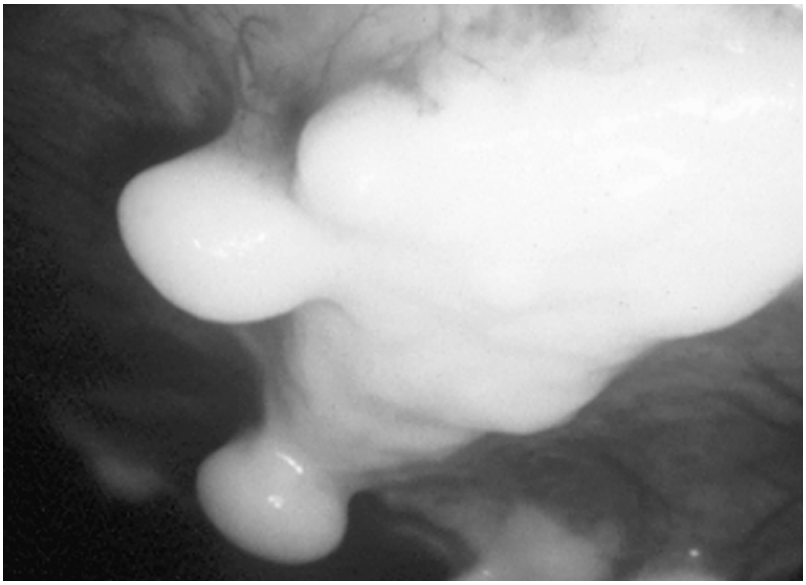


Figure 29.4. Pearly white asbestotic pleural plaque over the parietal pleura.

The interest in mesothelioma in these black spots comes from both the experimental and clinical demonstration of the presence in these anatomic structures of asbestos fibers (15) and of their coexistence with hyaline plaques, even though their spatial and topographic distribution are inversely correlated. It therefore has been proposed that these black spots, developing independently from hyaline plaques, can initiate asbestos-related inflammatory and neoplastic modifications of the pleura (15). They should be examined and sampled during thoracoscopy as they can undergo mineralogic analysis.

Endoscopic Staging

The principal contribution of pleural endoscopy to staging of mesothelioma is the identification of involvement of the visceral pleura, which can strongly influence the prognosis. It has been demonstrated that cases evolve differently (3) depending on whether the involvement is restricted exclusively to the parietal and diaphragmatic pleura, as opposed to the visceral pleura, and in the same way, on whether the visceral involvement is limited or extensive. The proposal to subdivide stage I (tumor limited to the ipsilateral pleura) into Ia (parietal and diaphragmatic pleura) and Ib (visceral pleura focally involved) (3) was accepted in the tumor, node, metastasis (TNM) classification drawn up by the International Mesothelioma Interest Group (IMIG) (16). The IMIG defines T1a as "a tumor limited to the ipsilateral parietal including mediastinal and diaphragmatic pleura" and T1b as a "tumor involving parietal, mediastinal, and diaphragmatic pleura with scattered foci of tumor also involving the visceral pleura" (16).

In practice, thoracoscopy is the only method that allows the identification of visceral pleura small nodules (1–3 mm), which are missed by diagnosis using CT. It is also possible with thoracoscopy to perform biopsies of the visceral pleura in suspect areas such as limited thickenings to define the true extent of the disease.

Conclusion

In recent years, thoracoscopy has become notably widespread thanks to its simplicity, safety, and diagnostic effectiveness, which makes its use indispensable in all pleural disease that cannot otherwise be diagnosed. This is in particular the case of mesothelioma in which there are frequently difficulties and delays in achieving a definitive diagnosis. The endoscopic exam has completely changed the approach to this dreadful disease. It has not only allowed diagnosis in almost all cases, but has also identified the initial phases of the disease that can be treated effectively.

Laparoscopy

There is a substantial difference between pleural and peritoneal mesothelioma, indicated by the fact of the greater rarity of the latter (5,17), a rarity that has prevented the creation of sufficient case studies to provide significant data on the endoscopic patterns of the disease. However, in the presence of ascites with the clinical suspicion of mesothelioma, laparoscopy represents the quickest and least invasive means to obtain a diagnosis.

Technique

Diagnostic laparoscopy can be performed under either local or general anesthesia. The first approach is preferred by gastroenterologists, and in general, for laparoscopy in internal medicine, in which the exam is performed with the patient under conscious sedation with cardiopulmonary monitoring (18). Surgical laparoscopy, performed under general anesthesia with tracheal intubation, is undoubtedly more invasive, but necessary for an exploration extended to the omental bursa and the perivascular regions, as in staging laparoscopy of abdominal or pelvic tumors (18).

An endoscopy table is essential so that the patient can be put in the Trendelenburg and reverse Trendelenburg positions during the exam in order to move the ascitic fluid and the bowel (19). The instrument normally used is a rigid endoscope with a video camera, connected to a cold-light source, using 7- or 10-mm optics with a visual angle of 0 and 30 degrees.

To explore the peritoneal cavity, it is necessary to create an adequate pneumoperitoneum, which is induced by insufflating CO_2 or N_2O through a Veress needle inserted near the umbilicus. At the same point a trocar is then introduced and the laparoscope is passed through it to inspect the peritoneal cavity. A second trocar can be positioned under

visual control for the bioptic forceps and accessory instruments. It is important to place the trocars along the midline of the abdominal wall (linea alba), which is less vascularized than other areas of the abdominal wall, to reduce the spread of neoplasia in the points of entry and to allow their excision in the event of proceeding with cytoreductive surgery.

Biopsies are performed, using optical forceps or with forceps introduced through a second trocar, on suspect neoplastic lesions, such as nodules or masses, and should be multiple in order to provide sufficient tissue for histopathologic examination.

For some years a technique known as *minilaparoscopy* has been used with small-caliber instruments (20), which is minimally invasive and facilitates good-quality sampling. It is ideal for carrying out the exam under local anesthesia, does not leave scars, and is relatively painless (21).

Diagnostic Value

A recent clinical classification of peritoneal mesothelioma established a methodologic basis for the use of laparoscopy, helping to identify patients for whom this exam is appropriate (17). Two clinical types of the disease were identified: the "wet" type, which presents with symptoms of neoplastic ascites, and the "dry-painful" type, characterized by painful mass lesions.

Laparoscopy is used for patients with ascites, who represent from 65% (17) to 90% (22) of cases. Cytologic examination of the ascitic liquid is rarely diagnostic (5,17). Laparoscopy facilitates both a visual examination of the neoplastic lesions and multiple sampling, which is essential for correct pathologic diagnosis. This diagnosis is difficult, since many abdominal and pelvic tumors show very similar peritoneal seeding. Therefore, it is first necessary to distinguish mesothelioma from carcinoma, and then, if dealing with mesothelioma, it is necessary to distinguish the usual more frequent subtypes—epithelial, biphasic, and sarcomatous—from well-differentiated papillary and cystic mesotheliomas, which seem to represent a separate disease with a better prognosis (5).

The endoscopic appearance of mesothelioma—nodules (Fig. 29.5), plaques, or reddish masses that cover the parietal and visceral peritoneum—is indistinguishable from that of metastatic tumors. The only distinctive characteristic appears to be the absence of hepatic parenchymal metastases (23). Laparoscopy cannot provide a proper evaluation of the true extent of the disease because the neoplastic growth provokes an encasement of the viscera and extensive adhesions. However, there is not yet an established staging system for peritoneal mesothelioma.

Laparoscopy is not advisable for the "dry-painful" type of the disease, which presents in the form of masses without ascites. Only laparotomy can provide an accurate diagnosis, because the observable lesions can be thought to represent adenocarcinoma or intraabdominal abscesses (17); needle aspiration, besides exposing the patient to the risk of neoplastic seeding, is not able to provide a sufficient quantity of tissue.

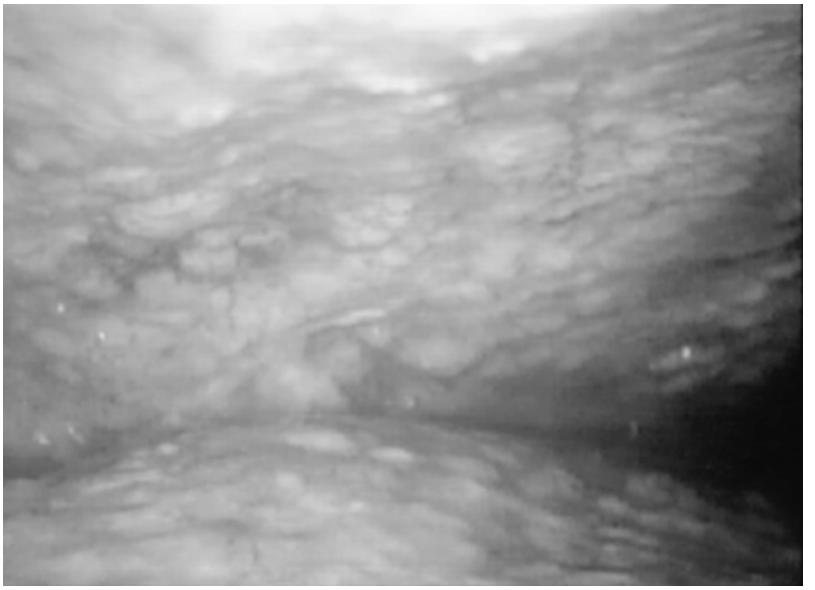


Figure 29.5. Diffuse parietal (top) and visceral (bottom) nodules in peritoneal epithelial mesothelioma.

It should be noted that laparoscopy can be used for preoperative evaluation of pleural mesothelioma when CT has been unable to exclude diaphragmatic invasion, the presence of which would hinder surgical treatment (24). The use of MRI, and more recently, multisliced CT with reconstruction of coronal images, has made this application of laparoscopy an exception.

Conclusion

Laparoscopy has a diagnostic role exclusively in peritoneal mesothelioma with ascites. While considering some encouraging therapeutic results that have been obtained with a combination of intraperitoneal chemotherapy and cytoreductive surgery, laparoscopy should always be performed with particular attention to the risk of neoplastic invasion of the parietal scars to avoid patients becoming untreatable by local-regional therapeutic strategies.

References

1. Sattler A. Zur Problematik des Pleuramesothelioms. Wien Klin Wochenschr 1965;77:668–670.
2. Boutin C, Rey F. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: diagnosis. Cancer 1993; 72:389–393.
3. Boutin C, Rey F, Gouvernet J, et al. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 2: prognosis and staging. Cancer 1993;72:394–404.

4. Ruffié P, Lehmann M, Galateau-Salle F, et al. Malignant mesothelioma. Br J Cancer 2001;84(suppl 2):49–50.
5. British Thoracic Society Standards of Care Committee. Statement on malignant mesothelioma in the United Kingdom. Thorax 2001;56:250–265.
6. Boutin C, Schlesser M, Frenay C, et al. Malignant pleural mesothelioma. Eur Respir J 1998;12:972–981.
7. Mathur PN, Astoul P, Boutin C. Medical thoracoscopy. Technical details. Clin Chest Med 1995;16:479–486.
8. Loddenkemper R. Thoracoscopy-state of the art. Eur Respir J 1998;11:213–221.
9. Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. Chest 1995;108:754–758.
10. Tassi GF, Marchetti GP. Minithoracoscopy: a less invasive approach to thoracoscopy. Chest, in press.
11. Gevenois PA, de Maertelaer V, Madani A, et al. Asbestosis, pleural plaques and diffuse pleural thickening: three distinct benign responses to asbestos exposure. Eur Respir J 1998;11:1021–1027.
12. Bianchi C, Brollo A, Ramani L, et al. Pleural plaques as risk indicators for malignant pleural mesothelioma: a necropsy-based study. Am J Ind Med 1997;32:445–449.
13. Mitchev K, Dumortier P, De Vuyst P. "Black spots" and hyaline pleural plaques on the parietal pleura of 150 urban necropsy cases. Am J Surg Pathol 2002;26:1198–1206.
14. Greenberg SD. Benign asbestos-related pleural disease. In: Roggli VL, Greenberg SD, Pratt PC, eds. Pathology of Asbestos-Associated Diseases. Boston: Little, Brown, 1992:165–187.
15. Boutin C, Dumortier P, Rey F, et al. Black spots concentrate oncogenic asbestos fibers in the parietal pleura. Thoracoscopic and mineralogic study. Am J Respir Crit Care Med 1996;153:444–449.
16. Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995;108:1122–1128.
17. Sugarbaker PH, Acherman YIZ, Gonzalez-Moreno S, et al. Diagnosis and treatment of peritoneal mesothelioma: The Washington Cancer Institute experience. Semin Oncol 2002;29:51–61.
18. Schneider ARJ, Eickhoff A, Arnold JC, et al. Diagnostic laparoscopy. Endoscopy 2001;33:55–59.
19. Greene FL. Laparoscopic maneuvers in the presence of ascites. J Surg Oncol 1997;65:141–142.
20. Helmreich-Becker I, Meyer zum Buschenfelde KH, Lohse AW. Safety and feasibility of a new minimally invasive diagnostic laparoscopy technique. Endoscopy 1998;30:756–762.
21. Kovacs GT, Baker G, Dillon M, et al. The microlaparoscope should be used routinely for diagnostic laparoscopy. Fertil Steril 1998;70:698–701.
22. Borow M, Conston A, Livornese L, et al. Mesothelioma following exposure to asbestos: a review of 72 cases. Chest 1973;64:641–646.
23. Piccigallo E, Jeffers LJ, Reddy KR, et al. Malignant peritoneal mesothelioma. A clinical and laparoscopic study of ten cases. Dig Dis Sci 1988;33: 633–639.
24. Conlon KC, Rusch VW, Gillern S. Laparoscopy: an important tool in the staging of malignant pleural mesothelioma. Ann Surg Oncol 1996;3:489–494.

30

Benign Mesotheliomas, Mesothelial Proliferations, and Their Possible Association with Asbestos Exposure

Giovan Giacomo Giordano and Oscar Nappi

Over the past several years the spectrum of mesothelial pathology has greatly increased (1). Nevertheless, benign mesotheliomas and mesothelial proliferations represent a rather broad category, encompassing clearly defined lesions, aspecific reactive patterns, and proliferating lesions that cannot yet be specifically defined. As any other human tissue, mesothelial epithelium and submesothelial mesenchymal tissue react to injuries with reproducible patterns (2–4). In particular, benign epithelial lesions can express one or more of several growth patterns, which can be divided into papillary, adenomatoid, micro- or macrocystic, and solid or nodular (Table 30.1); malignant epithelial mesotheliomas also exhibit a similar microarchitecture. The range of benign submesothelial (mesenchymal) proliferations is much more restricted and basically includes reactive fibrous and fibrosclerosing changes and the solitary fibrous tumor (formerly called benign fibrous mesothelioma).

Several contributions concerning differential diagnostic criteria between mesothelial reactive changes and malignant mesotheliomas on small specimens (5,6) and cytologic material (7) have been published; immunohistochemically, a strong linear membrane reactivity for EMA and a nuclear reactivity for p53 are considered suspicious for malignancy, but to a lesser extent both can be found also in reactive proliferations (8). The evaluation of anamnestic data and clinical presentation always have to be considered.

Malignant Mesothelioma In Situ

For mesothelial proliferations showing frankly atypical cytologic features without stromal invasion, the term *malignant mesothelioma in situ* has been introduced; these changes are often found in proximity of invasive mesothelioma or, rarely, before its development, and have been considered morphologic precursors (9). Considering the poor effectiveness of any treatment of invasive mesotheliomas, the recognition of

Table 30.1. Epithelial growth patterns of mesothelium as expressed in benign proliferations

Epithelial growth pattern	Benign mesothelial proliferations
Papillary	R A, WDPM
Adenomatoid	R A, AT
Solid-nodular	R A, NHMH
(Micro-macro) cystic	R A, AT, PMM

RA, reactive aspecific; WDPM, well-differentiated papillary mesothelioma; AT, adenomatoid tumor; NHMH, nodular histiocytic/mesothelial hyperplasia; PMM, peritoneal multicystic mesothelioma.

a stage 0 mesothelioma, on the basis of validated and universally accepted criteria, should represent a critical step in the management of this tumor. Unfortunately, in our experience, so far, reliable criteria for this diagnosis have not been codified. Therefore, since mesothelial reactive proliferations often show several degrees of cellular atypia, a diagnosis of malignant mesothelioma in situ should be made with extreme caution considering the radical surgical therapy following this diagnosis. At the present time we think that only if there is a history of asbestos exposure without evidence of recognized recent inflammatory pathology and a multifocal or rather extensive mesothelial surface cell proliferations with consistent nuclear atypias, a diagnosis of malignant mesothelioma in situ should be suggested; otherwise, the term *atypical mesothelial hyperplasia* should be used for these lesions (6) and a follow-up with periodic observations could be preferable. Nevertheless, it is noteworthy that cases of superficial atypical mesothelial changes associated with infiltrating mesothelioma have been reported having an inmmunoreactivity for EMA and p53 similar to that of invasive mesothelioma (8); these features could represent an additional support for a diagnosis of malignant mesothelioma in situ.

Epithelial Benign Mesotheliomas and Mesothelial Proliferation

Papillary Pattern

A papillary pattern is not uncommon in epithelial benign and malignant mesothelial proliferations. Papillary-like cell aggregates are found also in cytologic samples from serosal effusions secondary to malignant as well as benign mesothelial pathology.

Well-differentiated papillary mesothelioma is an entity characterized by a papillary growth pattern.

Well-Differentiated Papillary Mesothelioma

Well-differentiated papillary mesothelioma (WDPM) is a rare and intriguing tumor (10–12). It is currently considered a low-grade malignant tumor, more so than a fully benign tumor, in light of the wide spectrum of lesions that are morphologically similar but biologically different, including benign proliferations and aggressive lesions that merge with true malignant mesothelioma.

The large majority of the cases arise in the peritoneum of women 30 to 40 years of age. However, sporadic cases also have been described in the peritoneum of male patients, as well as in the pericardium, pleura, and tunica vaginalis (13).

Clinically, the usual presentation is pain and serous effusion. Macroscopically, the lesion generally exhibits a superficial, sometimes multifocal vegetative proliferation. Microscopically, a papillary proliferation characterized by papillae with a fibrovascular stalk lined by bland, single mesothelial cells, is present (Fig. 30.1); no mitosis is usually detected; in some case, psammomas bodies have been described. Papillary proliferation is superficial but occasionally adenomatoid-like microtubules in underlined stroma have been observed.

Immunohistochemically, proliferating cells are reactive for cytokeratin (CK) and mesothelial markers (calretinin, HMBE-1). Carcinoembryonic antigen (CEA) is always negative. Especially on small biopsies, WDPMs have to be differentiated from aspecific reactive mesothelial hyperplasia, epithelial malignant mesothelioma with a prominent papillary pattern, and serous papillary carcinoma of the ovary and peritoneum. Clinical presentation is important in differentiating WDPM from reactive mesothelial hyperplasia that usually is not mass forming and from malignant mesothelioma in which the cytomorphologic features are also significant. Immunoreactivity for B72.3, Ber-Ep4, CA19.9, and Leu-M1 and negativity for calretinin and HMBE-1 are useful markers in differentiating WDPM, as well as malignant

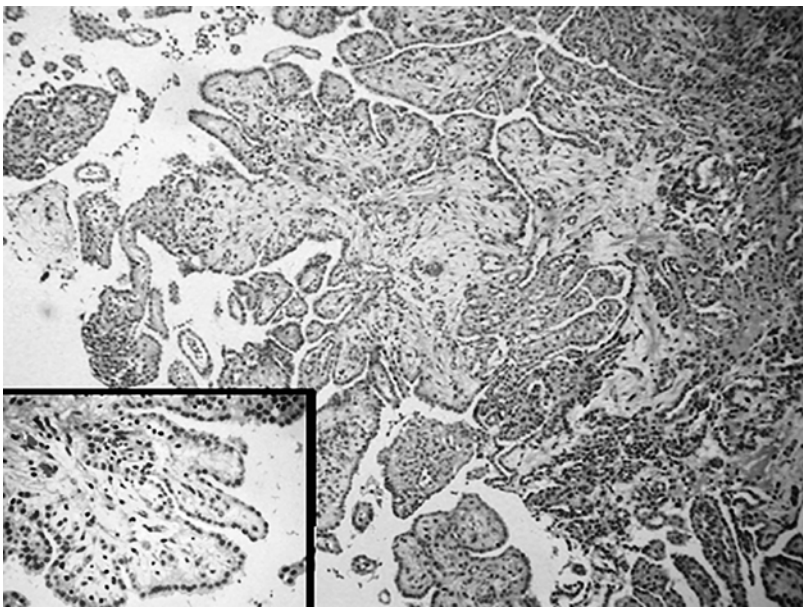


Figure 30.1. Well-differentiated papillary mesothelioma of peritoneum. This field shows several papillary projections with a fibrovascular stalk lined by bland, flat, single mesothelial cells. In the underneath stroma, a proliferation of duct-like structures is seen. Inset: a papillary structure with a broad stalk seen at high power.

mesotheliomas, from papillary serous carcinoma of the peritoneum or of the ovary; CEA immunoreactivity is also an excellent negative marker for mesothelial neoplasias but is not always detected in papillary carcinoma (14,15).

Adenomatoid (Pseudoglandular, Tubular) Pattern

The adenomatoid pattern is also frequently expressed by proliferating epithelial mesothelium as well as epithelial mesothelial tumors. Sometimes associated with a microcystic pattern, this pattern is typically represented by adenomatoid tumors. The so-called mesothelioma of the atrioventricular node also shows a similar pattern but it does not have a mesothelial origin.

Adenomatoid Tumor

Adenomatoid tumor (AT) is a benign mesothelial tumor with a dominant tubular pattern. This tumor usually arises from the mesothelium of the paratesticular region (16), from the serosal surface of the uterus wall (17) and, much less frequently, from that of the ovary, salpynx, and broad ligament. Exceptionally, it can arise also from the pleura (18) and pericardium (19). Macroscopically, AT is usually a small nodule, often found incidentally if it is less than 1 cm. Microscopically, its growth pattern is characterized by bland, flat, epithelioid cells arranged in tubules, gland-like structures, microcysts, or cords (Fig. 30.2). Not infrequently, a cytoplasmatic vacuolization is present with a signet ring–like feature. A mesothelial origin of the AT has been definitively confirmed by ultrastructural and immunohistochemical studies (20,21); this tumor always is immunoreactive for CK/cocktail, EMA, and calretinin.

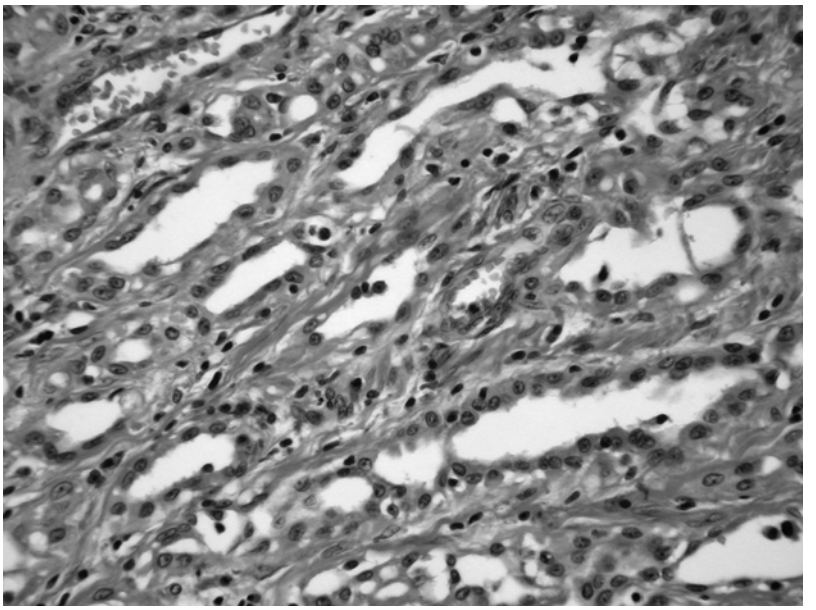


Figure 30.2. Adenomatoid tumor of rete testis. A proliferation of duct-like and glandular-like structures in a fibrous stroma is shown.

Sometimes AT has to be histologically differentiated from adenocarcinomas, vascular tumors, and on rare occasions, from malignant epithelial mesotheliomas with a dominant tubular/glandular pattern. Appropriate immunohistochemical reactions, such as CEA and other possible markers for specific adenocarcinomas as well as endothelial markers, usually help to clarify the diagnosis in selected cases.

Rarely, cases of AT having an infiltrating local pattern have been reported, but the behavior of AT is usually indolent and benign.

Mesothelioma of the Atrioventricular Node

The so-called mesothelioma of the atrioventricular node is not a true mesothelioma. This definition is a misnomer based on historical observations regarding the similarity of the proliferative cells with mesothelial cells and the lesion's pattern with that of an adenomatoid tumor. Today, it has been accepted that it arises from a congenital heterotopia of endodermal tissue (22–24). The large majority of these tumors has been detected during autopsy (some sporadic cases have been reported in transplanted hearts) and most of them, although inconspicuous, range in size from a few millimeters to 1 to 2 cm and have been considered the cause of death in cardiac arrest or ventricular fibrillation (23).

Macroscopically, this tumor often exhibits micropolycystic features in the area of the atrioventricular node. Microscopically, microcystic spaces are lined by bland, flat, mesothelioid cells that are immunoreactive for CK; positivity for CEA also has been reported.

Cystic/Microcystic Pattern

Although cases of AT with a dominant microcystic pattern have been reported, the best example of a lesion characterized by this pattern is the peritoneal multicystic mesothelioma.

Peritoneal Multicystic Mesothelioma

Peritoneal multicystic mesothelioma (PMM) arises almost exclusively from the peritoneum (2,25); exceptional cases have been described in the testis (26) and pleura (27). Like adenomatoid tumor, the histogenesis of PMM has also been controversial, the true mesothelial origin having been confirmed only recently by ultrastructural and immunohistochemical studies. Cystic mesotheliomas, arising from serosal peritoneal membranes, can apparently involve the parenchyma of single peritoneal and pelvic organs. The common clinical setting is the pelvic peritoneum of young female patients; on the basis of the size of the proliferation, it can be accidentally detected, present vague symptoms, or show a palpable abdominal mass and pain; ascitis is rarely present. It can be also multifocal with synchronous or metachronous proliferating lesions in several parts of the abdomen and pelvis. Macroscopically, one cyst (in this case, terms such as *cystic mesothelioma* and *mesothelial cyst* seem more appropriate) or several cysts with thin walls and variable size are present; cysts usually have a fluid content (2,25).

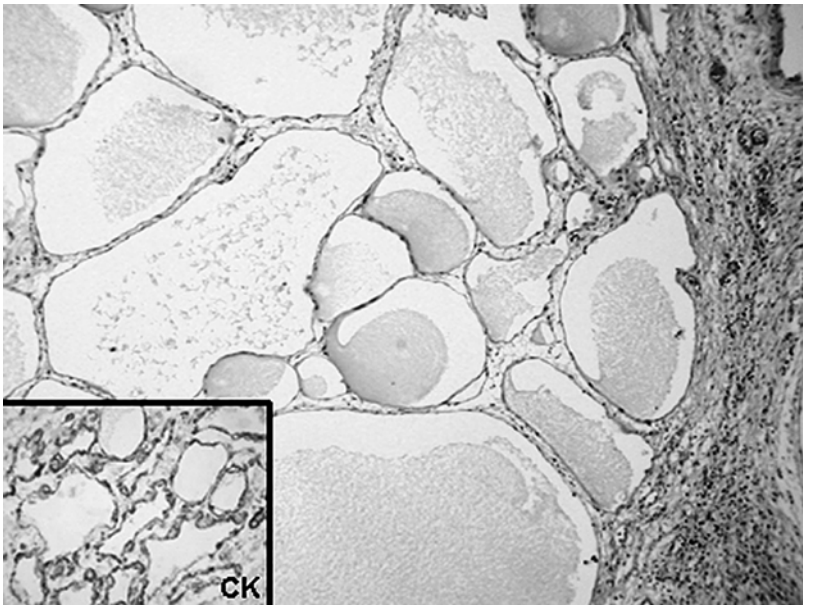


Figure 30.3. Peritoneal multicystic mesothelioma. Several cystic structures internally lined by flat mesothelioid cells are shown. Inset: mesothelioid cells stained with cytokeratin (CK).

Microscopically, the internal cystic surface is lined by bland, flat, endothelioid cells (Fig. 30.3); immunoreactivity for cytokeratin/cocktail, calretinin, or HMBE-1 is diagnostically present. A common, sometimes difficult, differential diagnosis is with (multi)cystic lymphangiomas; nevertheless, immunoreactivity for endothelial markers and immunonegativity for cytokeratin usually permit a correct diagnosis.

Basically, PMM is a benign tumor, but radical surgery is mandatory because of the possibility of recurrences; follow-up is needed also because of multifocality. Cases of malignant cystic mesothelioma have been reported, but in the majority of cases cytologic and clinical features generally clarify the diagnosis. It is important to remember that in the spectrum of mesothelial proliferations, cystic is not always synonymous with benign (2,25).

Solid/Nodular Pattern

Within reactive hyperplastic changes of the mesothelium, a solid pattern can be focally or extensively present. Moreover, there are some peculiar clinical settings in which this pattern is characteristically observed:

- Inguinal or umbilical hernia sacs, following chronic injury or incarceration of mesothelium (Fig. 30.4): This feature, not infrequently found in hernia sacs of children but also adults, has been defined as nodular mesothelial hyperplasia and has sometimes led to a misdiagnosis of malignancy (28).

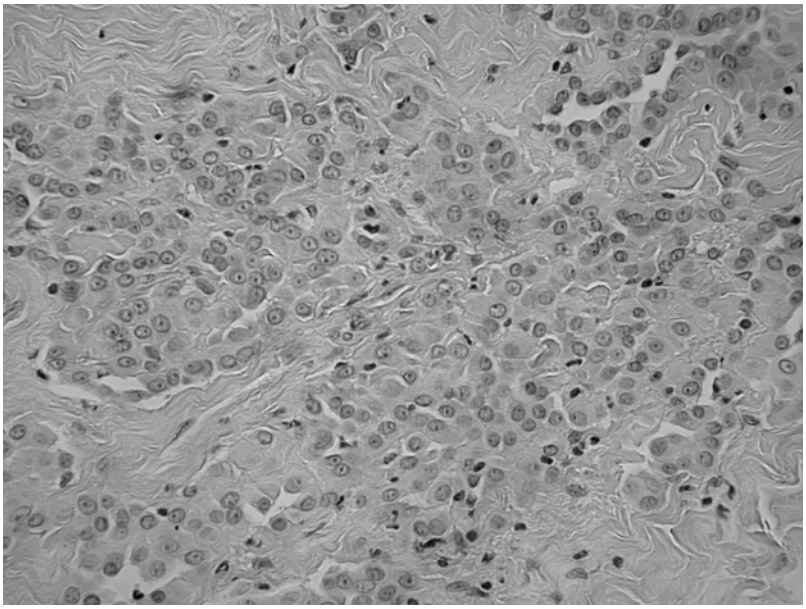


Figure 30.4. Nodular mesothelial hyperplasia in the inguinal hernia sac. A nodular aggregation of mesothelioid cells in the stroma is seen.

- Cardiac excrescences variously interpreted and called histiocytoid hemangioma-like lesions (HHLL) (29) or mesothelial/monocytic incidental cardiac excrescence (MICE) (30): These are characterized by nests of round mesothelioid cells usually detected during cardiac surgery; some authors have considered these lesions to be artifactually determined by aspirated mesothelial cells during cardiotomy suction (31).
- Nodular aggregates found in transbronchial biopsies: These can represent a potential source of misdiagnosis, especially versus neuroendocrine tumors (32).

The immunohistochemical evidence that many, if not most, of the round mesothelioid cells present in these lesions are immunoreactive for CD 68, a hystiocytic marker, and not for cytokeratin, which is positive only in other cells, has suggested that in most cases of the abovementioned pathologic findings, a mixed proliferation of histiocytes and mesothelial cells is present; alternatively, such evidence suggests that the mesothelial cells are entrapped and not proliferating. Consequently, a diagnosis of nodular histiocytic/mesothelial hyperplasia has been suggested for all of them (33).

Mesenchymal Benign Mesothelial Tumors and Mesothelial Proliferations

Benign mesothelial lesions characterized by proliferations of mesenchymal tissue are basically represented by reactive submesothelial fibrosclerotic proliferations and the localized fibrous tumor. Submesothelial

fibrosclerotic proliferations are relatively common reactions of serosal membranes to chronic injuries. Pleural plaques (PPs) and chronic fibrous pleurisy, with the so-called fibrothorax at the extremity of the spectrum, are the best clinically defined of them. Pleural plaques are firm, sometimes calcified lesions, present in the parietal pleura, microscopically characterized by hypocellular collagen-rich mesenchymal proliferation with a distinctive basket-weave pattern. They can present as single or multiple lesions ranging from a few millimeters to several centimeters. A relation with asbestos exposure is widely accepted and sufficiently documented; in selected cases, association with malignant mesothelioma has been described as well. Other pneumoconioses have been reported to be associated with PP; in our experience, in spite of different reported considerations, clinical presentation, number of lesions, and microscopic morphology of these cases substantially overlap those that are secondary to asbestos exposure.

Chronic Fibrous Pleurisy

Chronic fibrous pleurisy (CFP), especially in case of small biopsies, presents serious problems of differential diagnosis with sarcomatous mesotheliomas if a consistent spindle cell proliferation is present, or with desmoplastic malignant mesotheliomas (DMMs) if, as occurs more frequently, the lesion is sclerotic and paucicellular (Fig. 30.5).

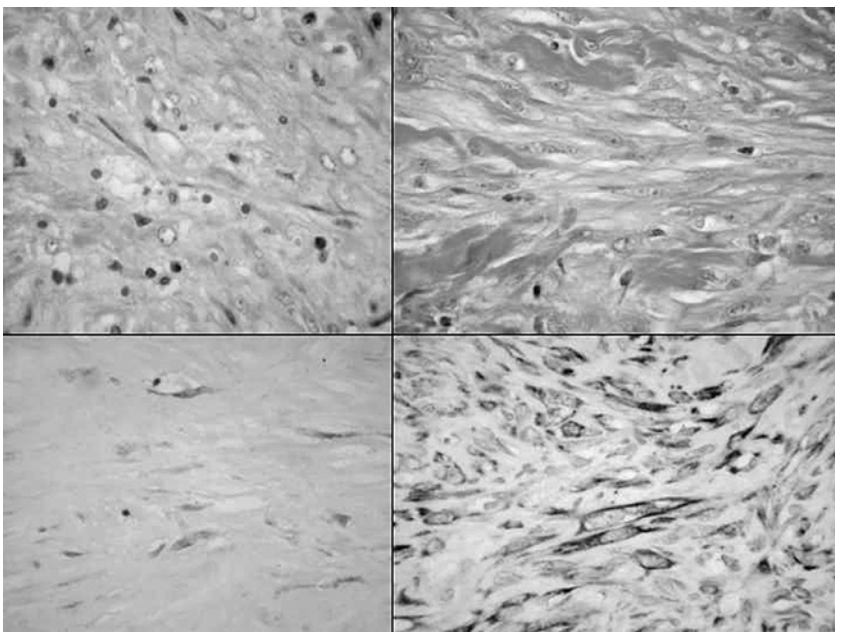


Figure 30.5. Fibrosing pleurisy (FP) (left) and desmoplastic malignant mesothelioma (DMM) (right) are shown side by side. The two lesions are impressively similar; DMM (upper right) shows some degree of nuclear atypia. Cytokeratin is strongly expressed in DMM (lower right) but a focal and weak positivity is also present in FP (lower left).

Reliable criteria of malignancy, in absence of frank sarcomatous overgrowths, are currently being considered (6): absence of a zonal effect (consisting of a superficial high cellularity and deep paucicellularity, usually present in chronic fibrotic reactions); invasion of surrounding tissues (adipose tissue, skeletal muscle, lung parenchyma); the so-called bland necrosis, typical of DMM and consisting of circumscribed areas in which necrosis is demonstrated by a poorly stained eosin; and absence of an elongated capillary vessel perpendicular to the serosal surface as an expression of the reactive granulation tissue usually present in CFP.

Immunohistochemistry is usually of little help; it is remarkable that, after an injury causing denudation of mesothelial layers, submesothelial fibroblasts that normally expressed vimentin only acquire immunoreactivity for low molecular weight cytokeratin. For this reason, in the presence of mesenchymal mesothelial proliferations, the positivity for cytokeratin should not be considered as diagnostic of desmoplastic mesothelioma (2,6). Nevertheless, a clear immunonegativity, or a weakly focal positivity for cytokeratin, favors the diagnosis of fibrosing pleurisy, and the immunopositivity of fibrosclerosing proliferation infiltrating lung parenchyma or striated muscle favors that of DMM.

Localized Fibrous Tumor of the Pleura

Localized fibrous tumor (LFT) of the pleura, although variously named, has been thought for many decades to arise from surface mesothelial cells and, therefore, to be a benign mesothelioma. Today, it is considered a pleural localization of a potentially ubiquitous lesion of mesenchymal origin (34). It can arise in the pleura of patients of both sexes and of any age. In about 50% of cases, the tumor is asymptomatic and incidentally found; otherwise, cough, pain, and dyspnea are common symptoms. Typically, LFT is separated from the (generally visceral) pleura by a peduncle, resulting in a polypoid mass, which can also reach great size (up to 40 cm) and consistent weight. The cut surface is firmly fibrous.

Microscopically, several features have been described: sclerosing, myxoid, and hemangiopericytomatous. Typically LFT is immunoreactive for CD 34 (35); the positivity for this marker is needed to confirm the diagnosis (Fig. 30.6). Bcl-2 (36) and CD 99 (37), both positive in the majority of cases, are considered useful to distinguish LFT from sarcomatoid malignant mesothelioma, which is only sporadically immunoreactive for them (37,38).

Some cases of LFT have a malignant behavior; histological criteria for selecting them are similar to those of other mesenchymal neoplasias: an increase in cellularity, nuclear atypias, an infiltrative pattern, and a greater mitotic index (more than 4× high-power fields). Solitary fibrous tumors have been described everywhere, including the pericardium (39), vaginalis testis (16) and the peritoneum (40).

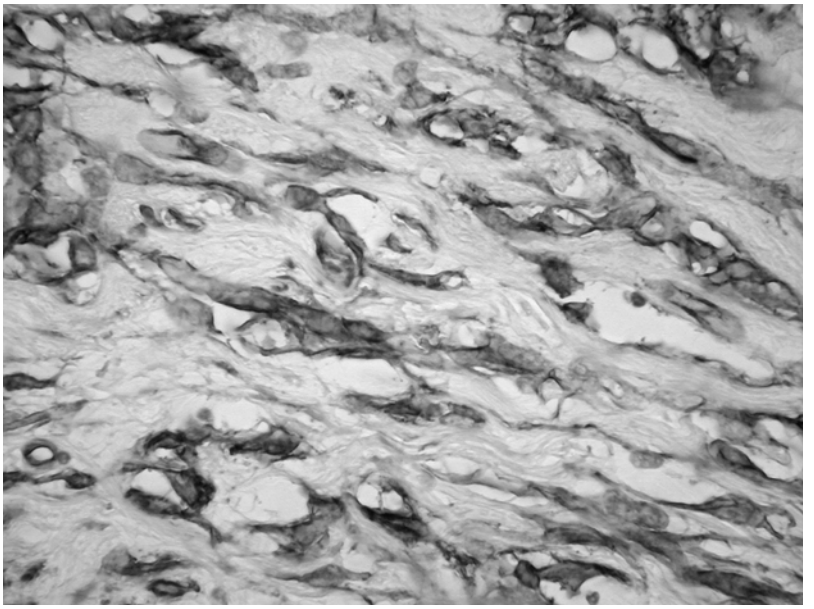


Figure 30.6. Solitary fibrous tumor of the pleura. The diagnostic positivity for CD 34 is shown.

Possible Relation of Benign Mesotheliomas and Mesothelial Proliferations to Asbestos Exposure

To the best of our knowledge, excluding some sporadic and questionable reported cases, in none of the benign mesotheliomas described, above does the asbestos exposure play a statistically significant role; the only mesothelial reactive change generally considered secondary to asbestos exposure is the fibrosis macroscopically expressed by pleural plaques (2).

References

1. Wick MR, Mill SE. Mesothelial proliferations: an increasing morphologic spectrum. Am J Clin Pathol 2000;113:619–622.
2. Battifora H, McCaughey WTE. Tumors of the Serosal Membranes. Atlas of Tumors Pathology, 3rd series, fascicle 15. Washington, DC: Armed Forces Institute of Pathology, 1995.
3. Bolen JW, Hammar SP, McNutt MA. Reactive and neoplastic serosal tissue. A light microscopic, ultrastructural and immunohistochemical study. Am J Surg Pathol 1986;10:34–37.
4. Bolen JW, Hammar SP, McNutt MA. Serosal tissue: reactive tissue as a model for understanding mesotheliomas. Ultrastruct Pathol 1987;11:251–262.
5. Henderson DW, Shilkin KB, Whitaker D. Reactive mesothelial hyperplasia vs mesothelioma, including mesothelioma in situ. Am J Clin Pathol 1998; 110:397–4004.

6. US-Canadian Mesothelioma Reference Panel. The separation of benign and malignant mesothelial proliferations. Am J Surg Pathol 2000;24:1183–1200.
7. Koss LG. Cytology and Its Histopathologic Basis, 4th ed. Philadelphia: JB Lippincott, 1992.
8. Cury PM, Butcher DN, Corrin B, Nicholson AG. The use of histological and immunohistochemical markers to distinguish pleural malignant mesothelioma and in situ mesothelioma from reactive mesothelial hyperplasia and reactive pleural fibrosis. J Pathol 1999;189:251–257.
9. Whitaker D, Henderson DW, Shikin KB. The concept of mesothelioma in situ: implications for diagnosis and histogenesis. Semin Diagn Pathol 1992; 9:151–161.
10. Addis BJ, Fox H. Papillary mesothelioma of ovary. Histopathology 1983; 7:287–298.
11. Butnor KJ, Sporn TA, Hammar SP, Roggli VL. Well differentiated papillary mesothelioma. Am J Surg Pathol 2001;25:1304–1309.
12. Daya D, McCaughey WTE. Well differentiated papillary mesothelioma of the peritoneum: a clinicopathologic study of 22 cases. Cancer 1990;65:292–296.
13. Xiao S-Y, Rizzo P, Carbone M. Benign papillary mesothelioma of the tunica vaginalis testis. Arch Pathol Lab Med 2000;144:143–147.
14. Attanoos RL, Webb R, Dojcinov SD, Gibbs AR. Value of mesothelial and epithelial antibodies in distinguishing diffuse peritoneal mesothelioma in females from serous papillary carcinoma of the ovary and peritoneum Histopathology 2002;40:237–244.
15. Ordonez NG. Role of immunohistochemistry in distinguishing epithelial peritoneal mesotheliomas from peritoneal and ovarian serous carcinoma. Am J Surg Pathol 1998;22:1203–1214.
16. Perez-Ordonez B, Srigley JR. Mesothelial lesions of the paratesticular region. Semin Diagn Pathol 2000;17:294–306.
17. Nogales FF, Isaac MA, Hardisson D, et al. Adenomatoid tumors of the uterus: an analysis of 60 cases. Int J Gynecol Pathol 2002;21:344.
18. Handra-Luca A, Couvelard A, Abd Alsamad I, et al. Adenomatoid tumor of the pleura. Case report. Ann Pathol 2000;20:369–372.
19. Natarajan S, Luthringer DJ, Fishbein MC. Adenomatoid tumor of the heart: report of a case. Am J Surg Pathol 1997;21:1378–1380.
20. Lehto VP, Miettinen M, Virtanen I. Adenomatoid tumor: immunohistological features suggesting a mesothelial origin. Virchows Arch B Cell Pathol Mol Pathol 1983;42:153–159.
21. Mackay B, Bennington JL, Skoglund RW. The adenomatoid tumor: fine structural evidence for a mesothelial origin. Cancer 1971;27:109–115.
22. Duray PH, Mark EJ, Barwick KW, Madri JA, Strom RL. Congenital polycystic tumor of the atrioventricular node. Autopsy study with immunohistochemical findings suggesting endodermal derivation. Arch Pathol Lab Med 1985;109:30–34.
23. McAllister HA Jr, Fenoglio JJ Jr. Tumors of the Cardiovascular System. Atlas of Tumor Pathology, 2nd series, fascicle 15. Washington, DC: AFIP 1978.
24. Monma N, Satodate R, Tashiro A, Segawa I. Origin of so-called mesothelioma of the atrioventricular node. An immunohistochemical study. Arch Pathol Lab Med 1991;115:1026–1029.
25. Weiss SW, Tavassoli FA. Multicystic mesothelioma: an analysis of pathologic findings and biologic behaviour in 37 cases. Am J Surg Patholo 1983; 12:737–746.
26. Lane TM, Wilde M, Schofield J, et al. Case report: benign cystic mesothelioma of the tunica vaginalis. Br J Urol Int 1999;84:533–534.

27. Ball NJ, Urbanski SJ, Green FH, et al. Pleural multicystic mesothelial proliferation: the so called multicystic mesothelioma. Am J Surg Pathol 1990; 14:375–378.
28. Rosai J, Dehner LP. Nodular mesothelial hyperplasia in hernia sac: a benign reactive condition simulating a neoplastic process. Cancer 1975;35:165–175.
29. Luthringer DJ, Virmani R, Weiss SW, Rosai J. A distinctive cardiovascular lesion resembling histiocytoid (epithelioid) hemangioma: evidence suggesting mesothelial participation. Am J Surg Pathol 1996;14:993–1000.
30. Veinot JP, Tazelaar HD, Edwards WD, Colby TV. Mesothelial/monocytic incidental cardiac excrescences (cardiac MICE). Mod Pathol 1995;7:9 –16.
31. Courtice RW, Stinson WA, Walley VM. Tissue fragments recovered at cardiac surgery masquerading as tumoral proliferations. Evidence suggesting iatrogenic or artefactual origin and common occurrence. Am J Surg Pathol 1994;18:167–174.
32. Chan JKC, Loo KT, Yau BKC, Lam SY. Nodular histocytic/mesothelial hyperplasia: a lesion potentially mistaken for a neoplasm in transbronchial biopsy. Am J Surg Pathol 1997;21:658–663.
33. Ordonez NG, Ro JY, Ayala AG. Lesion described as nodular mesothelial hyperplasia are primarily composed of histiocytes. Am J Surg Pathol 1998; 22:285–292.
34. Ordonez NG. Localized (solitary) fibrous tumor of the pleura. Adv Anat Pathol 2000;6:327–340.
35. van de Rijn M, Lombard CM, Rouse RV. Expression of CD 34 by solitary fibrous tumors of the pleura, mediastinum and lung. Am J Surg Pathol 1994;18:814–820.
36. Chilosi M, Facchetti F, Dei Tos AP, et al. Bcl-2 expression in pleural and extrapleural solitary fibrous tumors. J Pathol 1997;181:362–367.
37. Renshaw AA. 013 (CD 99) in spindle cell tumors: reactivity with hemangiopericytoma, solitary fibrous tumor, synovial sarcoma and meningioma but rarely with sarcomatoid mesothelioma. Appl Immunohistochem 1995; 3:250–256.
38. Seyers K, Ramael M, Singh SK, et al. Immunoreactive for bcl-2 protein in malignant mesothelioma and non-neoplastic mesothelium. Virchows Arch 1994;424:631–634.
39. Altavilla G, Blandamura S, Gardiman M, et al. Solitary fibrous tumor of the pericardium. Pathologica 1995;87:82–86.
40. Young RH, Clement PB, McCaughey WTE. Solitary fibrous tumors ("fibrous mesotheliomas") of the peritoneum. Arch Pathol Lab Med 1990;114:493–495.

31

Cytology of Malignant Mesothelioma

Richard M. DeMay

Because patients with mesotheliomas frequently present with effusions, cytologic examination of the effusion fluid may be the first diagnostic study. The fluid is often yellowish, but many are bloody (1–3). It is characteristically thick and mucoid owing to hyaluronic acid content. The cell count is high, and typically remains high after repeated taps, which is unusual in benign effusions.

In theory, the diagnosis of mesothelioma is easy, based on malignant cells that look like mesothelial cells (Figs. 31.1 and 31.2). There is no "foreign," alien, extra, or discrete population of tumor cells, in contrast with metastatic malignancy (4–7). Instead, there is a morphologic "kinship" of the malignant cells with native mesothelial cells, forming a continuous spectrum from apparently benign to atypical to malignant-appearing mesothelial cells (4–13).

In practice, the diagnosis of mesothelioma can be difficult. For example, the "kinship" that is so characteristic of mesothelioma is a two-edged sword (4). This same morphologic feature that makes the diagnosis possible can also make the diagnosis impossible in some cases. Some mesotheliomas show only subtle cytologic abnormalities (5,9,14). The malignant cells can be indistinguishable from benign, reactive mesothelial cells or even macrophages (12,15,16). At the other end of the spectrum, in poorly differentiated tumors, the diagnosis of malignancy may be obvious, but the mesothelial origin may not be (4).

A key observation in the cytologic diagnosis of mesothelioma—one that can be made at low microscopic power—is the presence of "more and bigger cells, in more and bigger clusters" (4). Extreme mesothelial cellularity suggests the diagnosis of mesothelioma (9–11,17,18), although abundant mesothelial cells can also occur in some benign conditions and not all mesotheliomas yield highly cellular specimens (4). Furthermore, too many large clusters of cells suggest a diagnosis of malignancy, particularly in pleural effusions, although again, not every case has this feature (19).

Cell groups can range from small to large; large groups are sometimes composed of hundreds of cells (12,13,20). Single cells are usually present in mesothelioma, and predominate in some cases (6,8,9,11,12,14,21).

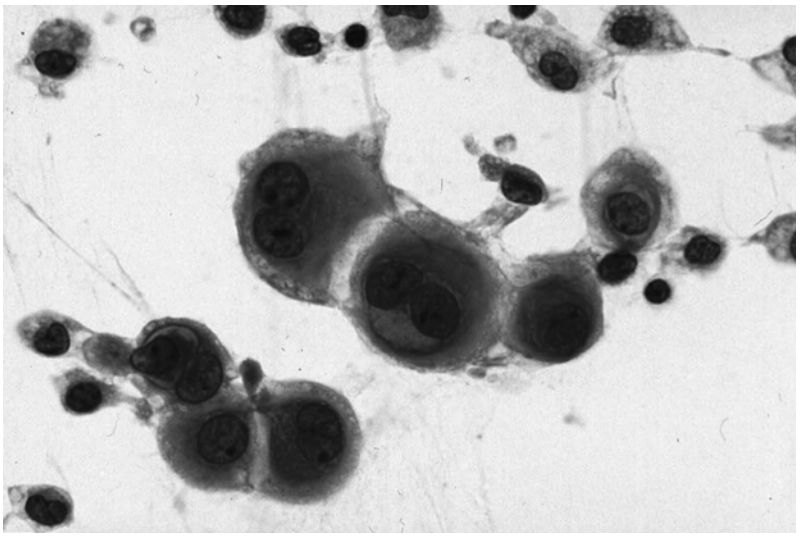


Figure 31.1. Malignant mesothelioma: malignant mesothelial–like cells. Pap stain (400×).

When single cells predominate, the diagnosis can be more difficult; a possible clue may be the sheer number of mesothelial cells (14).

Cell aggregates in mesothelioma characteristically are large, and have knobby, flower-like, berry-like, or highly complex contours (Fig. 31.3) (4,11,17). In contrast, cell aggregates in benign effusions are fewer, smaller, and less complex (13,18). Cell aggregates in adenocarcinoma can be large, but tend to have smooth, community borders (5). The clus-

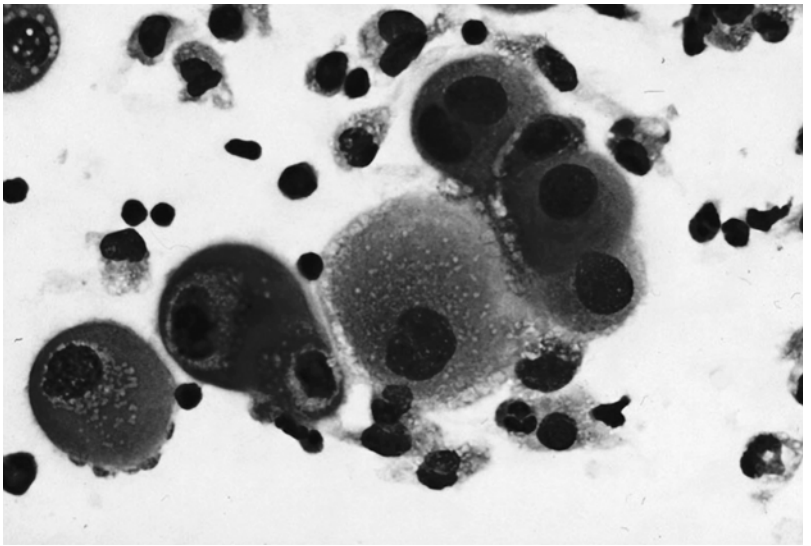


Figure 31.2. Malignant mesothelioma: malignant mesothelial–like cells. Romanovsky stain (400×).

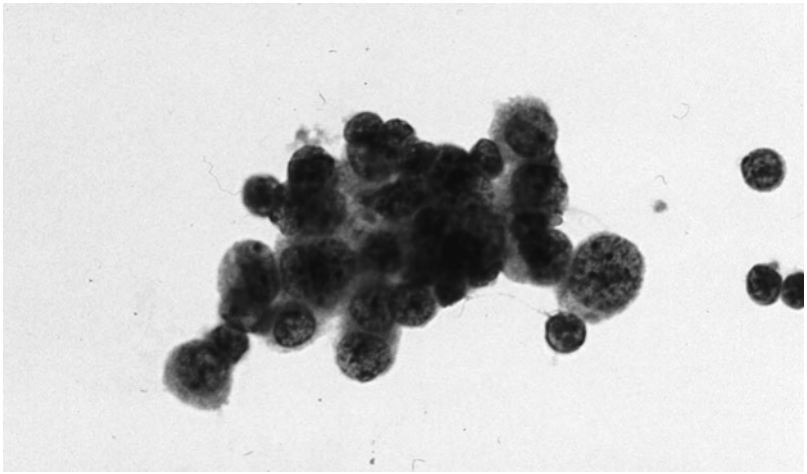


Figure 31.3. Malignant mesothelioma: complex aggregate of malignant mesothelial cells. Pap stain (200×).

ters are usually solid in mesothelioma, but can be hollow, mimicking glandular acini (5,11,18). True acinar formation is characteristic of adenocarcinoma. Cell aggregates with central cores of collagen usually indicate mesothelial cells (benign or malignant) (22). Collagen cores are rare in adenocarcinoma. The collagenous material is homogeneous and translucent. It stains cyanophilic in the Papanicolaou (Pap) stain and is slightly metachromatic in Romanovsky stains (Fig. 31.4). Papillary aggregates, though nonspecific, are more common in mesothelioma than in either adenocarcinoma or benign effusions (16,21,23).

"Cell-in-cell" patterns (also known as "cell-embracing," "pincer-like grip," "cell engulfment," and "cannibalism") (Fig. 31.5) are more

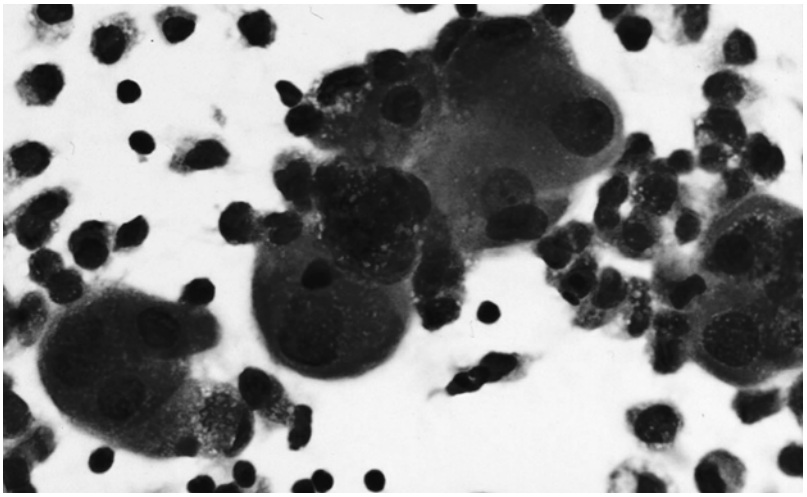


Figure 31.4. Malignant mesothelioma: metachromatic collagen core. Romanovsky stain (400×).

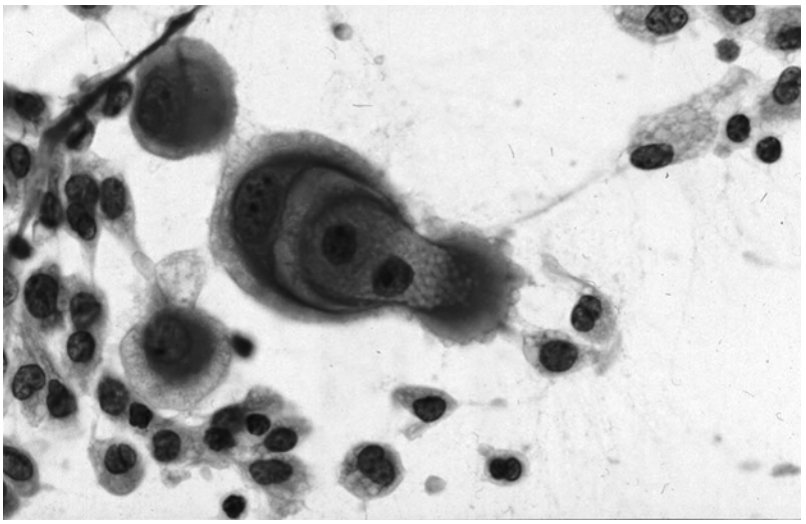


Figure 31.5. Malignant mesothelioma: cell-in-cell pattern, "pincer-like grip," characteristic of mesothelial cells. Pap stain (400×).

common and more complex in mesothelioma than benign effusions (4,5,7,14,17,23). "Windows" (openings between adjacent cells) are a characteristic feature of (benign or malignant) mesothelial cells, and are more common in mesothelioma than in adenocarcinoma (24) (Fig. 31.1). Unfortunately, there are exceptions to these general rules presented above, such as community borders in mesothelioma and knobby contours in adenocarcinoma (5).

Malignant mesothelial cells are typically more variable in size than benign mesothelial cells and frequently larger and sometimes even giant (Fig. 31.6) (5,25). However, frankly bizarre-appearing cells favor

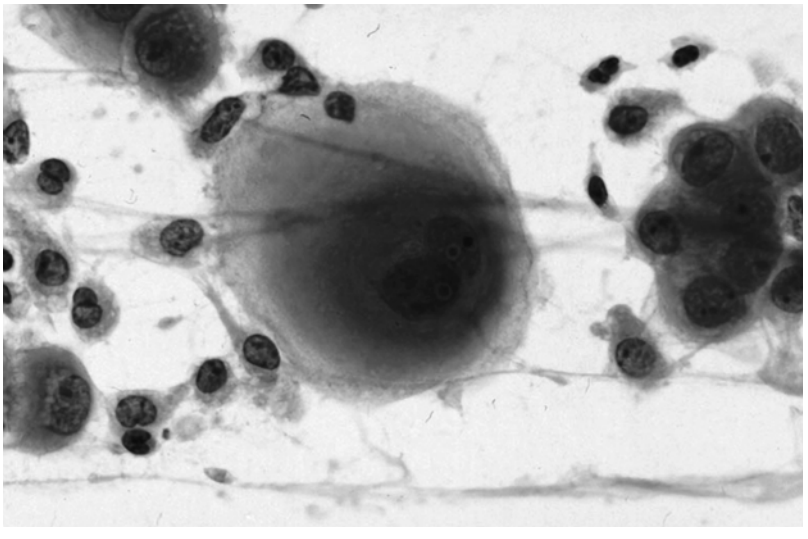


Figure 31.6. Malignant mesothelioma: giant mesothelial cell. Pap stain (400×).

a diagnosis of carcinoma. Classically, the nucleus (N) and cytoplasm (C) tend to change in size proportionately, so that the N/C ratio remains relatively constant. This imparts a certain degree of uniformity to mesothelioma, not usually seen in adenocarcinoma (4,26).

The cytoplasm of mesothelial cells is characteristically dense in the center (endoplasm) and delicate toward the edges (ectoplasm) (Figs. 31.1 and 31.2) (9). A characteristic "two-tone" staining pattern is sometimes seen, in which the endoplasm stains pink to orange and the ectoplasm blue to green (4,9,19). Sometimes, dense, coagulated mummified cells (similar to Councilman bodies in the liver) are seen in mesothelioma. These cells look a bit like dyskeratocytes, with pink-to-orange cytoplasm and dark, pyknotic nuclei (Fig. 31.7) (4). Though rare, they are a marker for mesothelioma (exclude squamous cell carcinoma) (14,19).

Microvilli can sometimes be appreciated in the Pap stain as fine cytoplasmic projections that are metachromatic (pink) in Romanovsky stains (4). Microvilli are rarely seen on adenocarcinoma cells in effusions, and even when they are, the microvilli are stubby and only present on the "luminal" surface (in cell blocks). Similarly, cytoplasmic blebs (prominent cytoplasmic outpouchings, probably degenerated microvilli that coalesce) are often accentuated in malignant mesothelial cells, compared with benign ones, and are uncommonly seen in adenocarcinomas (23).

Several kinds of vacuoles can be seen in mesothelioma, including tiny lipid vacuoles, larger glycogen vacuoles, hard-edged hyaluronic acid vacuoles, and clear, degenerative vacuoles. Both the number of vacuoles and the number of vacuolated cells are highly variable. Adenocarcinoma can also have lipid, glycogen, mucin, or degenerative vacuoles. Cytoplasmic vacuoles are often best appreciated with

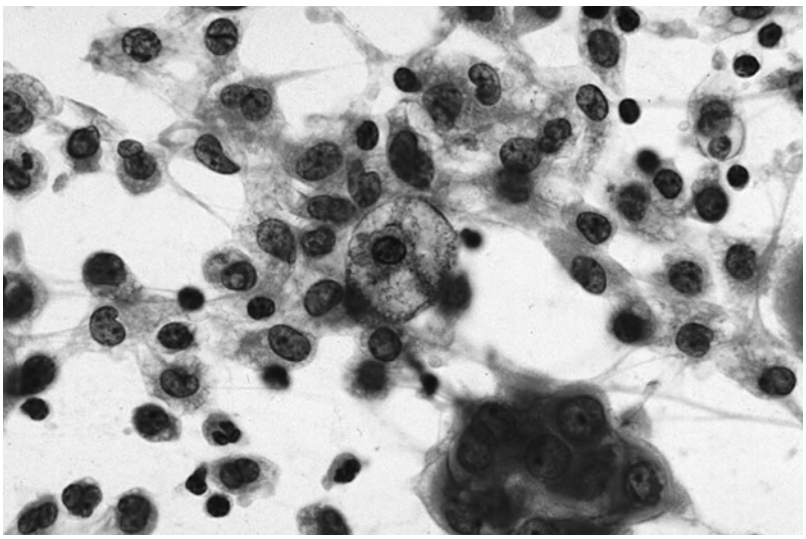


Figure 31.7. Malignant mesothelioma: "mummified" mesothelial cell. Pap stain (400×).

Romanovsky stains (4,12,23,27). Special stains can be helpful in differential diagnosis of vacuoles.

Mesothelial nuclei are usually located near the center of the cell, while eccentrically located nuclei are more characteristic of adenocarcinoma (4,8,12). Binucleation and trinucleation are fairly common, and multinucleation occurs, although these are nonspecific findings. Although the usual nuclear criteria of malignancy (pleomorphism, enlargement, abnormal chromatin, nucleoli, etc.) apply in the diagnosis of mesothelioma (19), nuclear atypia can be subtle in some cases (6,18). Conversely, marked nuclear atypia can be seen in benign conditions such as hepatitis, uremia, pancreatitis, and postradiation, as well as adenocarcinoma. Degeneration can cause changes that mimic malignancy.

Marked nuclear membrane irregularity is associated with malignancy, but may not be a prominent feature. Irregular nuclear membranes can also be seen in benign mesothelial cells, particularly in washing specimens ("daisy cells"). Intranuclear cytoplasmic invaginations, rare in benign mesothelial cells, can be seen in either mesothelioma or adenocarcinoma (4,12,28,29). Chromatin abnormalities range from subtle to obvious. However, marked hyperchromasia is usually absent, unless the cells are degenerated (which usually gives the chromatin a smudgy, homogeneous appearance) (14). Nucleoli are usually seen in mesothelioma and may be enlarged, multiple, or irregular in outline (30). Macronucleoli, if present, suggest malignancy (19). Mitoses are uncommon, and not helpful in diagnosis unless they are clearly abnormal (9,12,14).

The hyaluronic acid that is characteristic of mesothelioma can sometimes be seen in cytologic specimens as a flocculent material in the background of the slide (4,9). In Romanovsky stains it resembles synovial fluid: coarsely granular, pink (metachromatic) precipitate (16). In the Pap stain, it ranges from granular to fluffy to bubbly in appearance (Fig. 31.8) (31).

Psammoma bodies or marked lymphocytic infiltration can occur in mesothelioma (12,32); both are nonspecific (23). Necrosis and debris are not common in mesothelioma, but favor malignancy when seen (with exceptions, particularly infections) (12,33).

False-negative diagnoses are well known in mesothelioma. Most false negatives are due to inadequate sampling (i.e., unsatisfactory specimens with few or no mesothelial cells) (19,34). Sarcomatous mesothelioma is rarely diagnosed in exfoliative cytology, since few or no diagnostic cells are exfoliated (35,36). Benign mesothelial proliferations with reactive ("atypical") mesothelial cells can be difficult to distinguish from mesotheliomas (see below) (30). Conversely, cytologically bland mesotheliomas, composed of cells resembling benign mesothelial cells or histiocytes, are difficult to recognize as malignant (15,16,37). At the other extreme, sometimes the malignant cells are highly abnormal appearing, with clearly malignant features. In such cases, the diagnosis of malignancy may be obvious, but the cell of origin may not be evident (13,38).

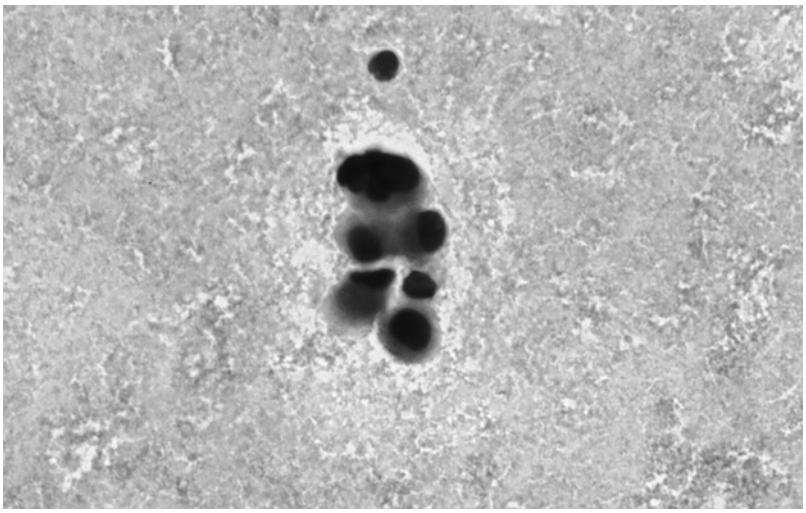


Figure 31.8. Malignant mesothelioma: flocculent background, hyaluronic acid. Pap stain (400×).

False-positive diagnoses of mesothelioma are rare, but can occur (14,19). Although large clusters of benign mesothelial cells can occasionally be seen in benign effusions, particularly in pericardial effusions or ascites, they are unusual (19,39–42). Diagnosis of mesothelioma based on complex aggregates of atypical mesothelial cells is reliable, but diagnosis based on single atypical mesothelial cells is more difficult (19).

References

1. Antman KH. Clinical presentation and natural history of benign and malignant mesothelioma. Semin Oncol 1981;8:313–319.
2. Pisani RJ, Colby TV, Williams DE. Malignant mesothelioma of the pleura. Mayo Clin Proc 1988;63:1234–1244.
3. Ribak J, Lilis R, Suzuki Y, et al. Malignant mesothelioma in a cohort of asbestos insulation workers: clinical presentation, diagnosis, and causes of death. Br J Ind Med 1988;45:182–187.
4. DeMay RM. The Art and Science of Cytopathology. Vol I: Exfoliative Cytology, Vol II: Aspiration Cytology. Chicago: American Society of Clinical Pathologists, 1966.
5. Ehya H. The cytologic diagnosis of mesothelioma. Semin Diagn Pathol 1986;3:196–203.
6. Triol JH, Conston AS, Chandler SV. Malignant mesothelioma: cytopathology of 75 cases seen in a New Jersey community hospital. Acta Cytol 1984; 28:37–45.
7. Whitaker D, Shilkin KB. The cytology of malignant mesothelioma in Western Australia. Acta Cytol 1978;22: 67–70.
8. Klempman S. The exfoliative cytology of diffuse pleural mesothelioma. Cancer 1962;15:691–704.

9. Naylor B. The exfoliative cytology of diffuse malignant mesothelioma. J Pathol Bact 1963;86:293–298.
10. Nguyen G-K. Cytopathology of pleural mesotheliomas. Am J Clin Pathol 2000;114(suppl 1, Pathol Patterns Rev):S68–S81.
11. Roberts GH, Campbell GM. Exfoliative cytology of diffuse mesothelioma. J Clin Pathol 1972;25:577–582.
12. Sherman ME, Mark EJ. Effusion cytology in the diagnosis of malignant epithelioid and biphasic pleural mesothelioma. Arch Pathol Lab Med 1990; 114:845–851.
13. Whitaker D, Shilkin KB. Diagnosis of pleural malignant mesothelioma in life—a practical approach. J Pathol 1984;143:147–175.
14. Whitaker D. The cytology of malignant mesothelioma. Cytopathology 2000;11:139–151.
15. Guffanti MC, Faleri ML. Benign-appearing mesothelioma cells in a serous effusion. Acta Cytol 1985;29:90–92.
16. Spriggs AI, Grunze H. An unusual cytologic presentation of mesothelioma in serous effusions. Acta Cytol 1983;27:288–292.
17. DiBonito L, Falconieri G, Colautti I, et al. Cytopathology of malignant mesothelioma: a study of its patterns and histological bases. Diagn Cytopathol 1993;9:25–31.
18. Leong AS-Y, Stevens MW, Mukherjee TM. Malignant mesothelioma: cytologic diagnosis with histologic, immunohistochemical, and ultrastructural correlation. Semin Diagn Pathol 1992;9:141–150.
19. Whitaker D, Shilkin KB, Sterrett GF. Cytological appearances of malignant mesothelioma. In: Henderson DW, Shilkin KB, Langlois SLP, et al, eds. Malignant Mesothelioma. New York: Hemisphere, 1991:167–182.
20. Berge T, Gröntoft O. Cytologic diagnosis of malignant pleural mesothelioma. Acta Cytol 1965;9:207–212.
21. Kho-Duffin J, Tao L-C, Cramer H, et al. Cytologic diagnosis of malignant mesothelioma, with particular emphasis on the epithelial noncohesive cell type. Diagn Cytopathol 1999;20:57–62.
22. Takagi F. Studies on tumor cells in serous effusion. Am J Clin Pathol 1954; 24:663–675.
23. Stevens MW, Leong AS-Y, Fazzalari NL, et al. Cytopathology of malignant mesothelioma: a stepwise logistic regression analysis. Diagn Cytopathol 1992;8:333–341.
24. Yu GH, Baloch ZW, Gupta PK. Cytomorphology of metastatic mesothelioma in fine-needle aspiration specimens. Diagn Cytopathol 1999;20:328–332.
25. Kwee W-S, Veldhuizen RW, Alons CA, et al. Quantitative and qualitative differences between benign and malignant mesothelial cells in pleural fluid. Acta Cytol 1982;26:401–406.
26. Adams VI, Unni KK. Diffuse malignant mesothelioma of pleura: diagnostic criteria based on an autopsy study. Am J Clin Pathol 1984;82:15–23.
27. Boon ME, Veldhuizen RW, Ruinaard C, et al. Qualitative distinctive differences between the vacuoles of mesothelioma cells and of cells from metastatic carcinoma exfoliated in pleural fluid. Acta Cytol 1984;28:443–449.
28. Baddoura FK, Varma VA. Cytologic findings in multicystic peritoneal mesothelioma. Acta Cytol 1990;34:524–528.
29. Herrera GA, Wilkerson JA. Ultrastructural studies of malignant cells in fluids. Diagn Cytopathol 1985;1:272–275.
30. Gupta PK, Frost JK. Cytologic changes associated with asbestos exposure. Semin Oncol 1981;8:283–289.

31. Whitaker D. Hyaluronic acid in serous effusions smears. Acta Cytol 1986; 30:90–91.
32. Japko L, Horta AA, Schreiber K, et al. Malignant mesothelioma of the tunica vaginalis testis: report of first case with preoperative diagnosis. Cancer 1982;49:119–127.
33. U.S.–Canadian Mesothelioma Reference Panel. The separation of benign and malignant mesothelial proliferations. Am J Surg Pathol 2000;24:1183–1200.
34. Renshaw AA, Dean BR, Antman KH, et al. The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. Chest 1997;111: 106–109.
35. Kobayashi TK, Teraoka S, Tsujioka T, et al. Ciliated ovarian adenocarcinoma cells in ascitic fluid cytology: report of a case with immunocytochemical features. Diagn Cytopathol 1988;4:234–238.
36. Tao L-C. The cytopathology of mesothelioma. Acta Cytol 1979;23:209–213.
37. Boon ME, Posthuma HS, Ruiter DJ, et al. Secreting peritoneal mesothelioma: report of a case with cytological, ultrastructural, morphometric and histologic studies. Virchows Arch Pathol Anat 1981;392:33–44.
38. Whitaker D. The validity of a cytological diagnosis of mesothelioma. Aust NZ J Med 1987;17(S2):519 (abstract).
39. Becker SN, Pepin DW, Rosenthal DL. Mesothelial papilloma: a case of mistaken identity in a pericardial effusion. Acta Cytol 1976;20:266–268.
40. Rosai J, Dehner LP. Nodular mesothelial hyperplasia in hernia sacs: a benign reactive condition simulating a neoplastic process. Cancer 1975;35: 165–175.
41. Selvaggi SM, Migdal S. Cytologic features of atypical mesothelial cells in peritoneal dialysis fluid. Diagn Cytopathol 1900;6:22–26.
42. Spriggs AI, Jerrome DW. Benign mesothelial proliferation with collagen formation in pericardial fluid. Acta Cytol 1979;23:428–430.

32

Immunohistochemistry

Betta Pier-Giacomo

The variegated histologic pattern peculiar to malignant mesothelioma (MM) makes a morphology-based distinction from either pleural metastases (notably carcinomas) or benign reactive mesothelial proliferations difficult (1,2), especially when only pleural effusion fluid or small tissue samples obtained through percutaneous needle biopsy or medical thoracoscopy are submitted for pathologic assessment. For this reason, diagnostic techniques ancillary to conventional light microscopy, such as histochemistry, electron microscopy, and immunohistochemistry (IHC), have been recommended. The aim is to provide the pathologist with a more objective and possibly conclusive support in order to identify (1) the mesothelial character and (2) the malignant biology of the pleural lesion in question, these being the prerequisites for the pathologic diagnosis of MM, which, if definite, leads to a discouraging prognosis and may raise legal issues.

Over the past 20 years, IHC has certainly been the most extensively investigated technique in the search for a reliable ancillary diagnostic tool in the field of tumors of the serosal membranes, considering that a recent Medline search under "mesothelioma and IHC" yielded 573 items for the period 1979 to 2002, that is, since Singh et al (3) first reported the usefulness of an anti–mesothelial cell serum in confirming MM diagnosis. In spite of this intensive research effort, no single immunostain that is entirely conclusive for either MM or serosal metastatic tumor currently exists, and for most antibodies recorded in the literature and made commercially available both the diagnostic value of each one and their various combinations in immunohistochemical panels are still under debate (4–6).

There are several causes for these conflicting results; they partly originate from the lack of uniformity of immunostaining procedures (i.e., fixation, processing and antigen retrieval), partly from the varying sensitivity and specificity of the primary antibodies used, partly from the heterogeneous forms of samples submitted to immunostaining (effusion fluids, small biopsies, surgical resections, autopsy material), and partly from the interpretation of immunostaining (cut-off positivity value, reactivity patterns). It is worth adding to this list of drawbacks

the prevailingly low methodologic quality in study case recruitment, since the preliminary verification of the diagnostic accuracy of MM in agreement with the traditional reference standard (electron microscopy or postmortem histology) is infrequent; on the other hand, the inclusion of only morphologically paradigmatic and unequivocal cases implies considerable uncertainty as to the real proportion of tumors positive for a given antibody in the population. Finally, as far as statistical analysis is concerned, most of these immunohistochemical studies are based on a rather small number of cases. It follows that the 95% confidence intervals (although these are not usually reported by the authors) are wide and hinder the conclusion that the authors tend toward—the superiority of one antibody over another. In fact, these conclusions could be supported only by much wider studies or through a meta-analysis of several studies.

However, I would like to emphasize the real, inherent difficulties in defining a unique, firmly reproducible immunoprofile for a tumor, such as MM, whose hallmark is phenotypic versatility (1,2), even within a single case, as shown not only at the level of the three-tiered histologic grade of the classic epithelial and sarcomatoid patterns, but also by the additional components (myoblastic, chondroblastic, osteoblastic, angioblastic) peculiar to embryonic mesoderm differentiation (7). Hence, a variegated pattern of antigen expression may result, as demonstrated by the differing rates of positive reactivity for the antimesothelial antibodies calretinin, thrombomodulin, cytokeratin 5/6, and CD44H, with reference to the different epithelial MM histologic patterns, i.e., tubulopapillary, adenomatoid, solid, small cell, and pleomorphic (8). In addition, when assessing the specificity and sensitivity of antimesothelial antibodies, it must be borne in mind that pooling pleural metastases from different primary sites in the non-MM group, compared with MM, brings with it a variability in immunostaining results related to the types of neoplasms included in the study [e.g., the varying reactivity for cytokeratin 5/6 (9), usually absent in pulmonary adenocarcinoma and frequently observed in nonpulmonary adenocarcinoma]. The pathologist should be aware that the choice of the proper antibody or antibodies should be suggested not only by the diagnostic dilemma to be resolved, but also by knowledge of tissue reactivity with respect to the specific morphologic phenotype of the lesion under examination.

Therefore any new data pertaining to the IHC of MM requires prudence and rigorous evaluation when it is considered for possible diagnostic application in daily practice. However, many pathologists are unfamiliar with the histology of MM, due to the low overall incidence of this tumor, at least until recently, and this has led to an overestimation of the adjunct role of IHC to the point that a diagnosis of MM has become almost entirely an immunodiagnosis instead of being based mainly on the microscopic picture highlighted by a conventional good-quality hematoxylin and eosin (H&E) stain.

It was not until the mid-1990s that the reproducibility of the immunohistologic diagnosis of MM was evaluated by a panel of five Italian pathologists expert in asbestos-related diseases, which included

the author (10). A battery of commercial negative mesothelial markers was used in the study. In addition, no attempt was made to standardize a priori the criteria to be used in the interpretation of the immunostained slides, and each pathologist was left free to perform as if he were in his own daily diagnostic setting. It was found that "the information additionally contributed by IHC did not seem to change the pathologists' diagnoses very much in comparison with those made by routine H&E stain" (10). At that time the authors also remarked that "until highly specific and sensitive probes for the positive identification of MM become available, a careful scrutiny of routinely stained preparations still remains the most rewarding component of the diagnostic pathway" (10). The primacy of morphology in the diagnostic approach is still valid, since limitations of the specificity or sensitivity also affect the recently marketed positive mesothelial markers, more prominent for some (thrombomodulin and CD44H) and less marked for others (calretinin, cytokeratin 5/6 and Wilms' tumor gene product 1) (4–6).

The above general remarks concerning IHC and MM, as well as the following immunostaining data, result from my professional experience as a pathologist working for most of my career in the province of Alessandria, northwest Italy, where the 1988 to 1997 mortality rate from malignant pleural neoplasms, standardized on the Italian population in 1991, is the highest in the country (number of cases: 400; standardized rate × 100,000: 6.59) and mostly related to occupational or environmental exposure to asbestos mainly due to the asbestos-cement industry in Casale Monferrato. Because of this local MM "epidemic," 306 cases of MM (>80% from thoracoscopy or percutaneous needle biopsy) were personally diagnosed for the first time or reviewed for a second opinion during the period of 1990 to 2002. All these cases with their relevant histologic and immunohistochemical details have been entered into a database, which has been used both to outline a short history of the developments of the IHC of MM in the past decade and to assess in brief the diagnostic value of at least some of the several different antibodies purported to contribute to an accurate identification of MM.

As earlier stated, there are three main problem areas in the pathologic assessment of any effusion fluid or tissue specimen submitted with a clinical suspicion of MM, and each area is characterized by a specific microscopic pattern that requires the distinction between

epithelial MM and metastatic carcinoma,
sarcomatoid MM and sarcoma (primary or metastatic) or sarcomatoid carcinoma, and
malignant and benign mesothelium.

As for the capacity of IHC to provide possible clues to a more accurate diagnosis of MM, first it is necessary to underline that the contribution of IHC cannot be generalized, but differs in relation to each diagnostic setting, each with its own peculiar set of morphologic and immunophenotypic variables.

Immunohistochemistry and Epithelial Malignant Mesothelioma vs. Metastatic Carcinoma

Negative Markers

The main study interest has always focused on the role of IHC in the distinction between epithelial MM and adenocarcinoma, this being the most frequent and impelling diagnostic dilemma since the epithelial type, either alone or in a composite epitheliomesenchymal pattern, accounts for 75% to 90% of MMs. Until recently, immunohistologic diagnosis was mainly a process of exclusion, since immunostaining for confirmation of MM involved the use of antibodies to epithelial glycoproteins, which, when absent, allowed the pathologist to rule out pleural metastatic carcinoma and, to a lesser extent, confirm epithelial MM. Carcinoma markers of greatest value on both cytologic (usually cell-block) and histologic specimens included carcinoembryonic antigen (CEA) (Fig. 32.1), BerEP4 (Fig. 32.2), Leu M1 (Fig. 32.3), and B72.3, and the use of these markers combined in a panel was recommended to enhance diagnostic accuracy in the lack of absolute specificity and sensitivity for each one, and the frequent absence of overlapping expression of these glycoproteins.

Carcinoembryonic antigen has always been recognized as an irreplaceable marker in any standard panel for MM because of its very high specificity and sensitivity, again confirmed by three studies (11–13) published in 2002, which reported a reactivity for MM ranging

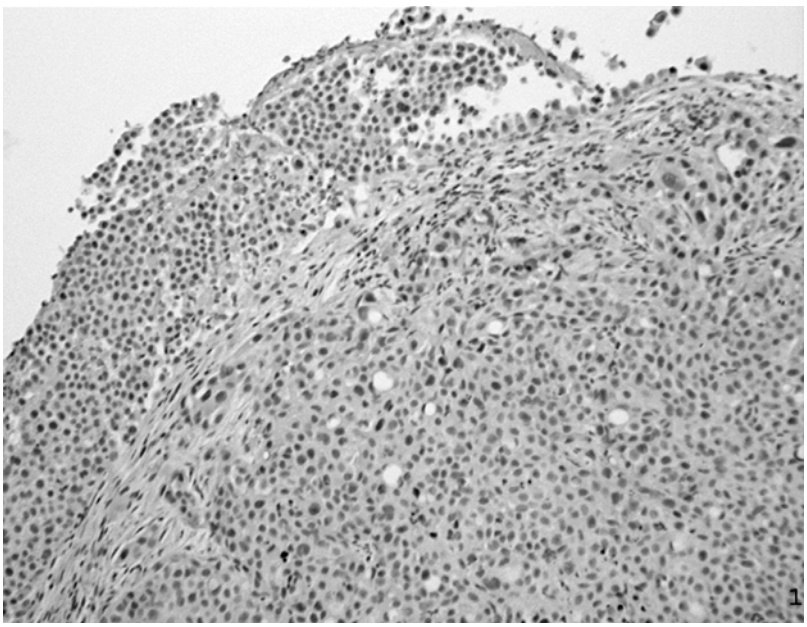


Figure 32.1. Complete negative immunostaining for carcinoembryonic antigen (CEA) monoclonal antibody in an epithelial malignant mesothelioma (MM) (solid type). ABC technique (100×).

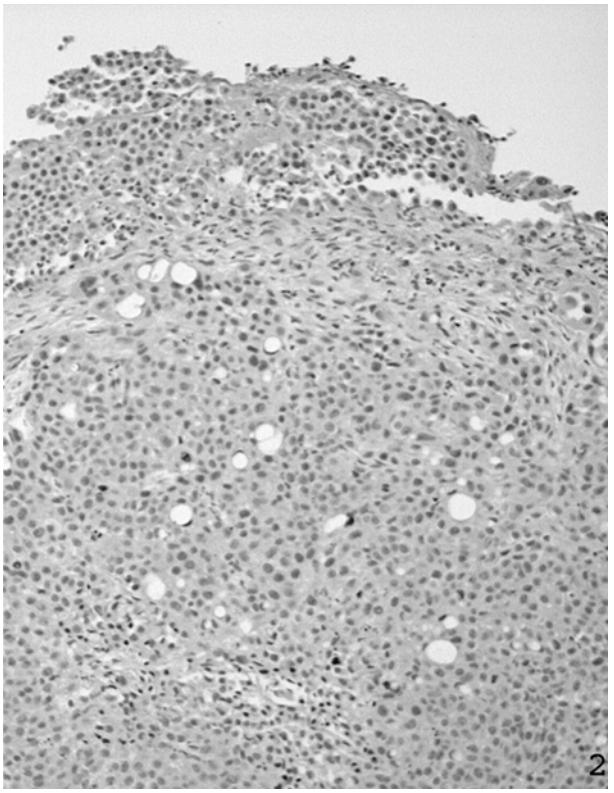


Figure 32.2. No immunoreactivity for BerEP4 in the same case as in Figure 32.1. ABC technique (100×).

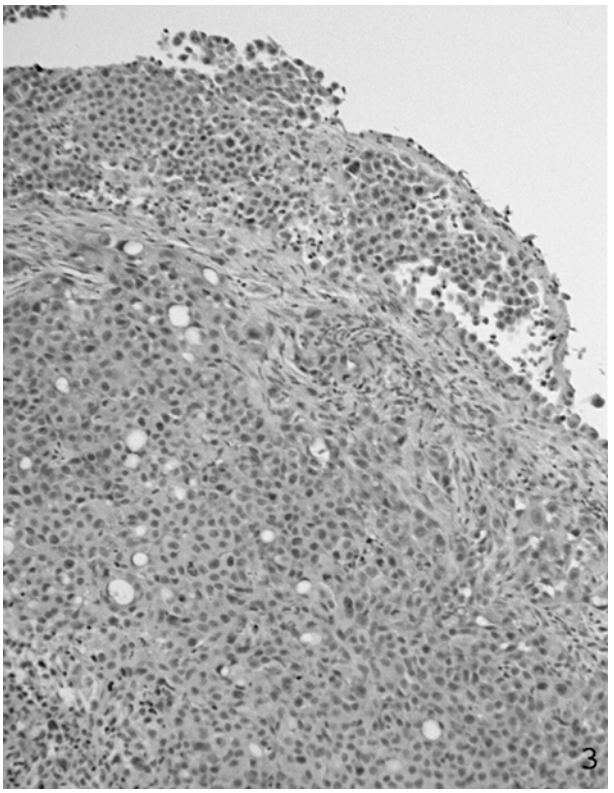


Figure 32.3. Absence of labeling for CD15 (Leu M1) in the same case as in Figure 32.1. ABC technique (100×).

from 0% (11) to 2% (12) to 5.4% (13), although a staining of only weak intensity was responsible for the last slightly worse result. In my practice, 274 (89.5%) of 306 MMs were immunostained for CEA. When only the epithelial and biphasic types of MM ($n = 248$) were considered, i.e., the histologic categories for which the use of CEA is specifically intended, the rate of CEA-positive ($n = 1$) and equivocal ($n = 2$) cases was 1.2%. The three cases consisted of small tissue specimens and two of them occurred when the polyclonal CEA with its inherent drawbacks of cross-reactivity was still being used. Both the equivocal cases featuring weak and focal cytoplasmic staining were of the epithelial type with a mesothelial character on morphology, and the additional adenocarcinomatous markers, namely BerEP4, Leu M1, and B72.3, were all absent in the former, whereas in the latter, BerEP4 was clearly positive. The CEA-positive epithelial MM case consisted of a tiny needle specimen with crush artifacts that was negative for all epithelial markers and positive for the mesothelial marker calretinin (see below). Twenty sequential "definitely-not-MM" cases (i.e., pleural metastatic carcinomas) were retrieved from my consultation file for comparison; a homogeneous, diffuse, cytoplasmic reaction was found in 14 (87.5%) out of 16 cases immunostained for CEA. From this review of personal findings, CEA appears to be a highly reliable negative marker for MM (overall negativity rate: 98.9%), and probably the best; in the very few CEA-positive or equivocal cases the other exclusionary markers concurrently assessed provided the necessary confidence for a diagnosis of MM qualified as certain or highly probable.

The additional negative markers—BerEP4, Leu M1, and B72.3—have shown a level of diagnostic accuracy lower than CEA, but still acceptable, in my experience, in agreement with many other published series [see Ordóñez (6) for a review], and by universal consent have always been considered as useful reagents. The standard panel in my laboratory included BerEP4, Leu M1, and B72.3, together with CEA from 1992 to 1996; then B72.3 was replaced with calretinin, a second-generation positive marker (see below).

Focal positive (i.e., membrane staining in >5% of tumor cells, not necessarily restricted to the lateral surfaces of the cells) and equivocal (i.e., membrane staining in ≤5% of tumor cells) BerEP4 reactivity was observed in 29 (14.1%) of 206 epithelial and biphasic MMs and was always weak or moderate in intensity. Conversely, 12 (92.3%) of 13 pleural metastatic adenocarcinomas from the consultation file were BerEP4 positive, usually with a diffuse and intense reactivity. These percentages are in agreement with those reported by some of the latest papers (11,14).

Leu M1 (CD15) monoclonal antibody was used in 184 (60.1%) of 306 MMs. Positive (i.e., focal, intense, and predominantly cytoplasmic in >5% of tumor cells) labeling was shown by seven and equivocal (i.e., focal and prevailingly weak in ≤1% of tumor cells) by 12 of 171 epithelial and biphasic MMs for an overall 11.1% rate. On the other hand, 14 (87.5%) of 16 pleural metastatic carcinomas were Leu M1 positive. These results show the percentage values of Leu M1 reactivity for both MMs and adenocarcinomas to be a little higher than those most

recently published, even if recorded from smaller case series [positivity in MM: 0% (0/41) according to Abutaily et al (12) and 6.3% (7/112) according to Roberts et al (15)]. No MM in my series stained positively with both BerEP4 and Leu M1 except for one epithelial "probable" MM, which, however, was both CEA- and B72.3-negative and positive for AMAD-2 (see below).

B72.3 monoclonal antibody, during the period in which I used it, proved a valuable negative marker since only one epithelial MM was positive and six MM (five epithelial and one biphasic) exhibited an equivocal reactivity, the remaining 80 cases (92%) being definitely negative. The use of this antibody was discontinued simply for cost/benefit reasons when the inclusion of at least one new positive mesothelial marker in the diagnostic panel required a reduction in the number of negative markers applied up to that time.

Newer antibodies proposed for the exclusion of MM include E-cadherin, MOC-31, and thyroid transcription factor (TTF)-1. E-cadherin is still under evaluation in my laboratory, where, however, its diagnostic effectiveness seems to be less promising with respect to the initial claims (16) (see below). Following an early positive test on cytologic preparations in the late 1980s, MOC-31 was rediscovered in the mid-1990s and has lately received favorable assessments on the part of several groups (13,14,17), according to whom it outperforms BerEP4 and Leu M1 on histologic material to the point that it is now recommended among the first-line exclusionary markers (6). TTF-1 has been demonstrated to be selectively expressed by pulmonary carcinomas, irrespective of their histologic subtypes, whereas MM and nonpulmonary carcinomas are consistently negative (12,18). Therefore, TTF-1 could be reserved to the specific setting of the distinction between epithelial MM and lung adenocarcinoma, but this possible role requires further investigation before including the antibody in a standard panel.

Positive Markers

The search for positive markers for MM has been the focus of an intense and long-lasting research activity. It has always been recognized that it would be better if the diagnosis of MM were made on positive identification of this tumor by means of consistently specific antimesothelial antibodies rather than by exclusion after the accumulation of negative immunostaining results, as was done until the mid-1990s.

Even if a certain number of both polyclonal and monoclonal antibodies have been generated using normal or neoplastic mesothelial cells or mesothelial cytoplasmic proteins as immunogens [see Donna et al (19) for a review], only two antibodies have been marketed in recent years, namely, AMAD-2 and HBME-1. On the other hand, several novel antibodies exhibiting only partial specificity for MM, as not raised specifically against mesothelial or MM cells, have been marketed in the past decade. These antibodies react with calretinin, cytokeratin (CK) 5/6, Wilms' tumor gene (*WT1*), thrombomodulin, N-cadherin, and the receptor for hyaluronic acid (CD44S), and have in

common the capacity to label MM cells on formalin-fixed and paraffin-embedded material. All these antibodies can be regarded as belonging to the group of the second-generation or positive markers for MM.

I contributed personally to the development of AMAD-2 polyclonal antibody (19), which was raised against a recombinant protein specific to the cytoplasm of human MM cells and provided with growth-factor–like activity within the frame of an autocrine loop controlling mesothelial cell proliferation (20). AMAD-2 was able to label all MM histologic types and demonstrated a 100% specificity and 92% sensitivity. AMAD-2 was marketed for a short time but did not become popular in surgical pathology practice because of a severe lack of inter-laboratory reproducibility due to technical difficulties. In fact, its effectiveness on routinely processed material required the use of paraffin wax with a melting point of ≤52°C, in order to preserve the structural integrity of the specific antigen to be detected for diagnostic purposes. However, most laboratories make use of paraffin wax with a higher melting point, which damages the antigen, thus interfering with its specific binding to AMAD-2 and causing high background staining and subsequent false-positive results.

HBME-1 is a monoclonal antibody generated against a suspension of cells from a well-differentiated epithelial MM. Seventy-one (95.9%) of 74 epithelial and biphasic MMs and six (14.3%) of 42 pleural carcinomatous metastases reacted with the antibody in a personal comparative study with AMAD-2 (19). A lower specificity was reported by Miettinen and Kovatich (21), who, however, emphasized the thick membrane staining pattern in MM (Fig. 32.4) as a possible clue in its

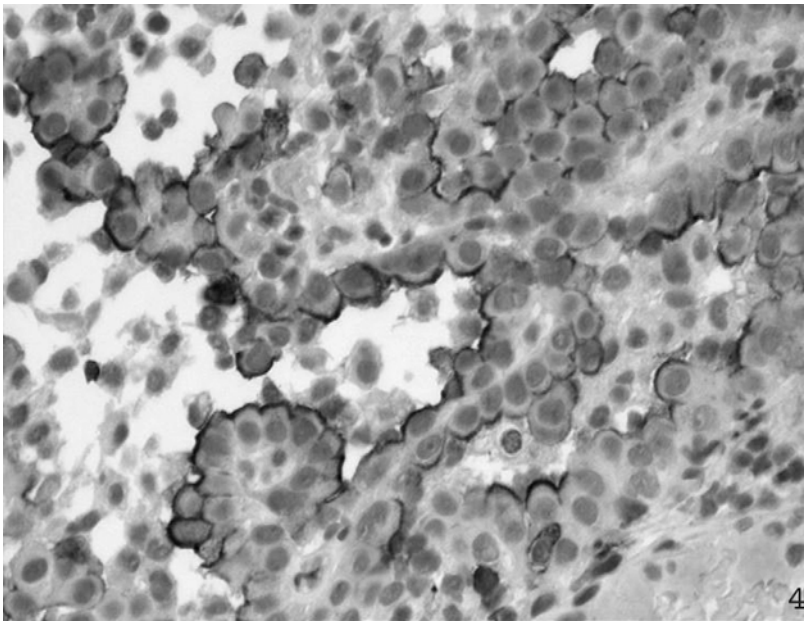


Figure 32.4. Positive membrane staining for HBME in the same case as in Figure 32.1 at higher magnification. ABC technique (400×).

distinction from adenocarcinoma, in which, conversely, a prevailingly cytoplasmic reactivity with a possible concurrent thin surface staining is observed. In my experience, the use of HBME-1 has often given rise to equivocal results related to these overlapping reactivity patterns. The low specificity reported by other investigators in the past has been confirmed with rates ranging from 27% (17) to 56% (15), even though these findings seem to be disproved by the 91.3% specificity for MM reported by Gonzales-Lois et al (14).

I have acquired quite extensive experience with calretinin, having used it as a positive mesothelial marker in the standard diagnostic panel for several years, and the results gathered up to now confirm reports from other investigators that the antibody against recombinant human calretinin manufactured by Zymed Laboratories is highly reliable [see Ordóñez (6) for a review]. Lower sensitivity rates have been obtained by laboratories where the anticalretinin antibody manufactured by Chemicon was employed. In my practice, 177 sequential MMs were immunostained for calretinin; 117 were epithelial, 49 biphasic, and 11 sarcomatoid. The staining was usually diffuse and strong and the pattern was nuclear (Figs. 32.5 and 32.6) and, to a lesser extent, cyto-

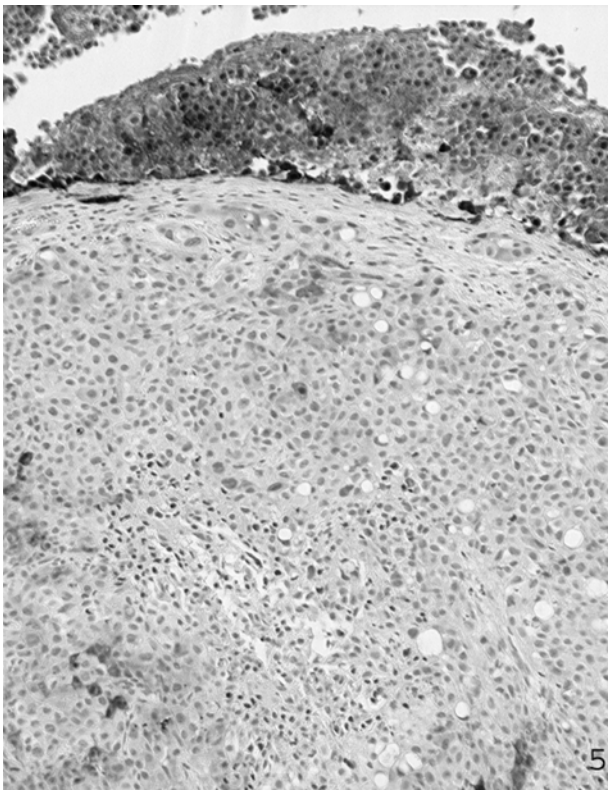


Figure 32.5. Calretinin expression is more intense and diffuse at the surface than in the deep portion of the same MM as in Figure 32.1. ABC technique (100×).

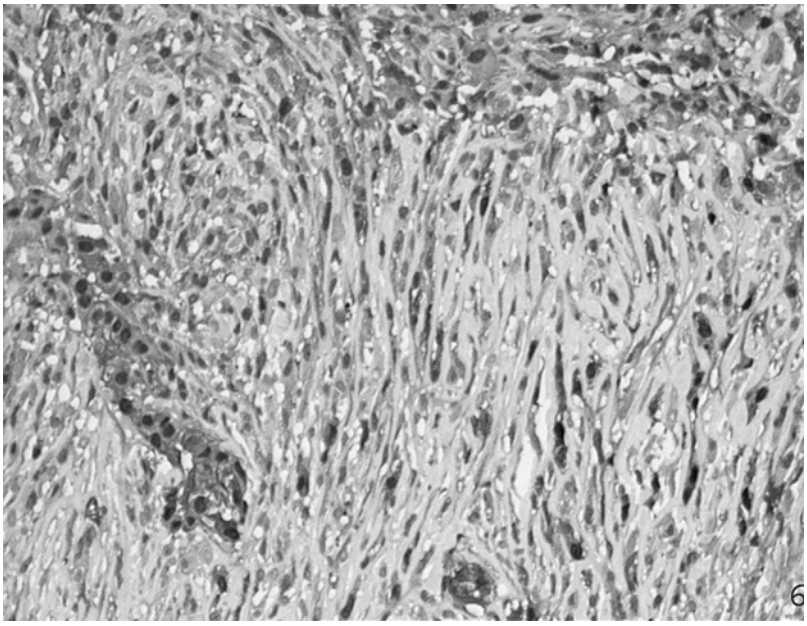


Figure 32.6. Calretinin immunopositivity is seen in the nuclei of MM cell of the same case as in Figure 32.1. ABC technique (200×).

plasmic; however, only the nuclear reactivity was recorded for diagnostic purposes. The overall rate of calretinin-positive cases was 87% (154/177), which rose to 90.4% (150/166) for the epithelial plus biphasic types and decreased to 36.4% (4/11) for the sarcomatoid type, in which, additionally, the number of labeled neoplastic cells per case was always <30%. Only eight MMs (three epithelial, one biphasic, and four sarcomatoid) were negative, whereas 15 cases showed equivocal staining (i.e., weak, focal, or prevailing cytoplasmic). The concurrent high specificity of the antibody was supported by the analysis of 16 pleural metastatic carcinoma, in which calretinin was negative in all cases. These findings are in agreement with those recently published [sensitivity for MM: 80% (12) to 97% (11); specificity for MM: 90% (11) to 94% (12)], and all of them confirm the early results from Doglioni et al (22) (sensitivity: 100%; specificity: 90%). The only noticeable limit to the diagnostic value of calretinin is during differential diagnosis between MM and pleural synovial sarcoma (23) (primary or metastatic), and between MM and the rare primary thymic epithelial tumors of the pleura (24), since the potentially shared calretinin patterns in these three entities require the use of other diagnostic markers. Finally, when calretinin was applied in the setting of the diagnostic cytopathology of serous effusion, estimates of diagnostic accuracy were still positive, with the only disappointing report on effectiveness of the antibody being hampered by the small number of investigated cases ($n = 7$) (25).

Cytokeratin 5/6 and *WT1* are two positive markers of mesothelial differentiation, which warrant a brief comment, considering the

promising results they have shown in the studies published so far [see Ordóñez (6) for a review]. These results cannot be evaluated in the light of my own experience, which is still not sufficiently extensive to be used as a reference.

Cytokeratin 5 is expressed by normal and neoplastic mesothelial cells and its possible role as a useful positive mesothelial marker was already highlighted in the late 1980s, when a monoclonal antibody, AE14, selectively specific for CK 5 and effective on frozen material, was developed (26). The subsequent availability of antibodies working on routine material allowed several laboratories to evaluate the diagnostic accuracy of this marker on larger series. The latest results confirm CK 5/6 as a useful aid to distinguish epithelial and biphasic MM from pulmonary adenocarcinoma, but warn the pathologist not to trust this probe for the differential diagnosis between MM and either serosal metastatic carcinomas of nonpulmonary origin (9) or the rare primary thymic epithelial tumors of the pleura (24), both of which may express CK 5.

WT1, originally identified as a tumor suppressor gene, is overexpressed in a variety of malignancies, including MM. Nuclear *WT1* expression has been assessed as a diagnostic tool on both histologic and cytologic material, and the results from the latest studies concur about its diagnostic usefulness in the appropriate clinical setting. However, *WT1* seems less sensitive for MM (70–75%) with respect to calretinin (23,27,28), since reactivity occurs also in serous ovarian carcinomas (29), and its use is not recommended on autopsy material (17).

Thrombomodulin is a transmembrane glycoprotein involved in the regulation of anticoagulant activity. It has been reported to be neither as sensitive nor as specific as the three above-mentioned positive mesothelial markers [see Ordóñez (6) for a review]. It has attained a 83% sensitivity only in the small cell variant of MM (8). In addition, its expression is focal in distribution and occurs also in blood and lymphatic vessels, thus causing difficulties in interpreting immunostaining results.

CD44H has generated conflicting results in terms of diagnostic accuracy and it is now recognized as having no practical value in the diagnosis of MM [see Ordóñez (6) for a review].

Cadherins

Cadherins are transmembrane glycoproteins that mediate extracellular calcium-dependent cell–cell adhesion that is important in embryonic development and maintenance of normal tissue differentiation. The cadherin family consists of more than 30 members widespread in normal tissues, although the individual classic members, such as E (epithelial), N (neural), and P (placental) cadherins, display remarkable tissue expression selectivity, which can allow the differentiation of morphologically similar but histogenetically distinct tumors. In particular N-cadherin is expressed by the pleural mesothelial cells and E-cadherin by the epithelial cells of the lung. Diagnostic immunohistochemistry has relied on this finding reminiscent of the specific N-cadherin expres-

sion in the embryonic mesoderm and consistent with the evidence from embryology that the mesothelium is made of a coelomic epithelium developed from the mesoderm (7). However, the use of antibodies to N- and E-cadherins for the histopathologic diagnosis of MM has provided conflicting results up to now [see Ordóñez (6) for a review]. The potential diagnostic relevance of the cadherin immunoprofile in MM has been extensively investigated in my laboratory, where immunostaining and biochemical techniques, both at the cell line and tissue levels in a series of pleural MMs and newborn hernial sacs, were done. N-cadherin was found in eight of nine MM cell lines (Figs. 32.7D and 32.8) as well as in five normal mesothelial cell lines (Figs. 32.7B and 32.8). E-cadherin was detected in six (66.7%) of nine MM cell lines (Figs. 32.7C and 32.8), whereas none of the five normal mesothelial cell lines stained for E-cadherin (Figs. 32.7A and 32.8). Expression of E- and N-cadherin was also investigated in the corresponding MM or mesothelial tissue specimens in order to prove that expression of E-cadherin was not induced by the in vitro culture environment. The pattern and the intensity of expression of E- and N-cadherins in the tissue specimens closely mirrored those observed in MM cell lines. This study appears to confirm that the expression of cadherins in MM is more heterogeneous and less mutually exclusive with respect to the normal or reactive mesothelium. These results are at variance with those recently reported by Müller et al (30) and Abutaily et al (12). In fact, the former authors observed that nearly all adenocarcinomas (17/18) stained positively for E-cadherin, while there was only a weak staining reaction in six (13.6%) of 44 MMs; the latter authors reported a 22% sensitivity of E-cadherin for epithelial MM that, combined with a 100% sensitivity for adenocarcinomas, led them to consider this antibody as a first-line reagent with TTF-1 in the immunohistochemical approach to pleural biopsy. In my opinion, the diagnostic value of E-cadherin is questionable in distinguishing between epithelial MM and adenocarcinoma, whereas it is worth investigating the possible role of E-cadherin expression in distinguishing malignant from benign mesothelium.

Immunohistochemistry and Sarcomatoid Mesothelioma vs. Spindle Cell Malignancies

The main neoplastic entities in the setting of the differential diagnosis of sarcomatoid MM include sarcoma (primary or secondary) and metastatic spindle cell (sarcomatoid) carcinoma (1). It is said that conventional sarcomatous MMs are not a diagnostic problem, provided the densely packed spindle cells are mesothelial (2). The overall contribution of IHC to this discrimination process is again valuable, but to a lesser extent, and it relies predominantly on the use of antikeratin antibodies, as spindle-type mesothelial cells are keratin-positive. The sarcomatoid type reached a total of 34 cases (11.1%) in my series of MMs, 18 qualified as definite and 16 highly probable, respectively. Immunohistology data showed positivity for either cytokeratins 8 and 18 (Cam 5.2) or broad-spectrum cytokeratins (AE1/AE3) in 29 of 30 cases

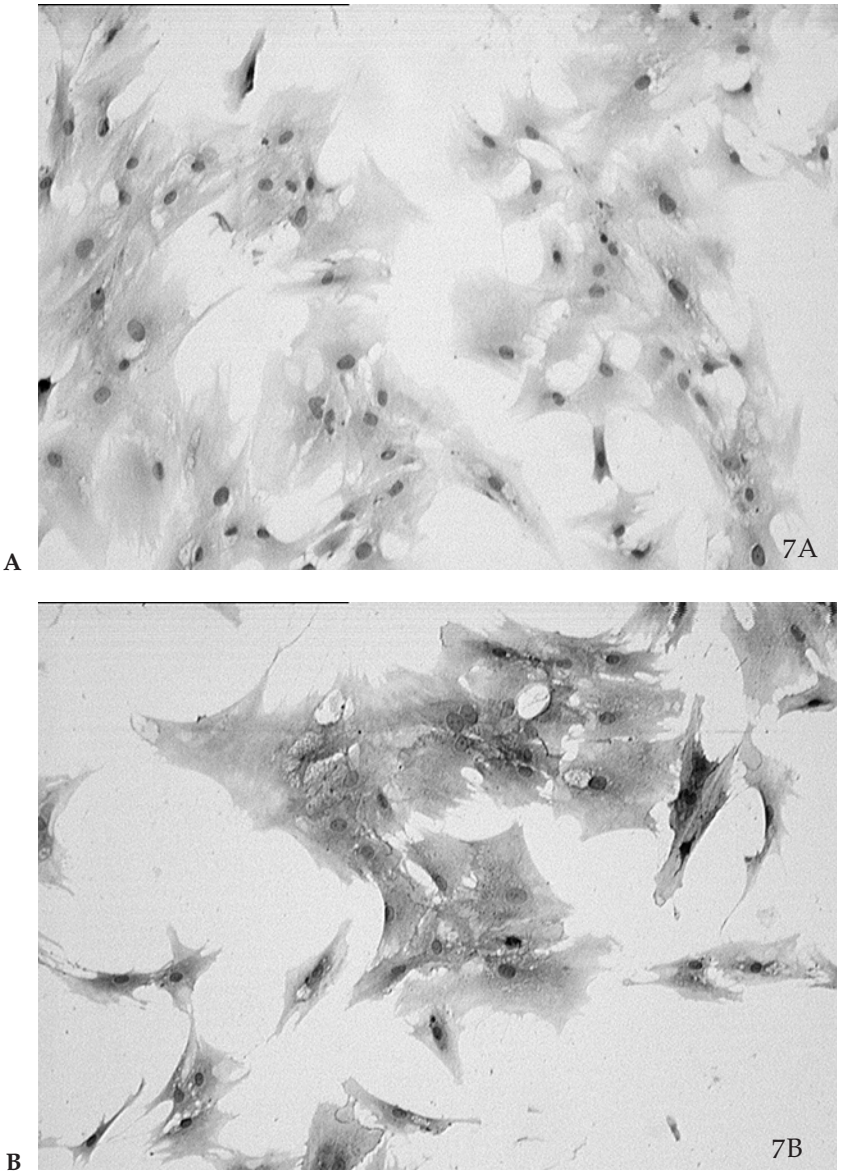
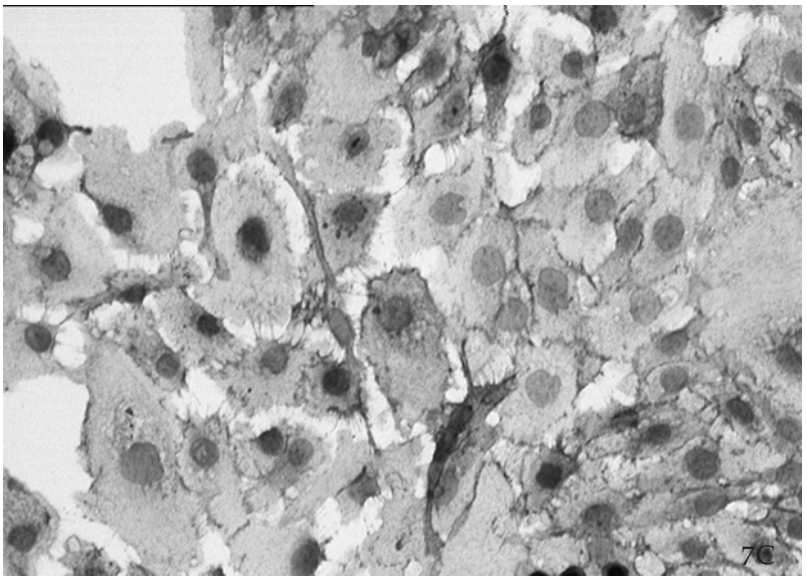


Figure 32.7. In vitro cultured human mesothelial cells appear negative for E-cadherin (A) but are reacting with anti–N-cadherin antibody (B) in a prevailingly cytoplasmic pattern. An MM cell line shows membrane reactivity for both E-cadherin (C) and N-cadherin (D). ABC technique (480×).

(96.7%), and calretinin-positivity in four of 11 cases (36.4%), including all the cytokeratin-positive ones. These findings are in agreement with all previous reports, some already published in the late 1980s, according to which the consistent cytokeratin immunostaining appears to be a sensitive and useful method to discriminate sarcomatoid MM from both sarcomas of different histogenetic types and solitary fibrous tumors of the pleura, the latter being characterized by positive stain-

C



D

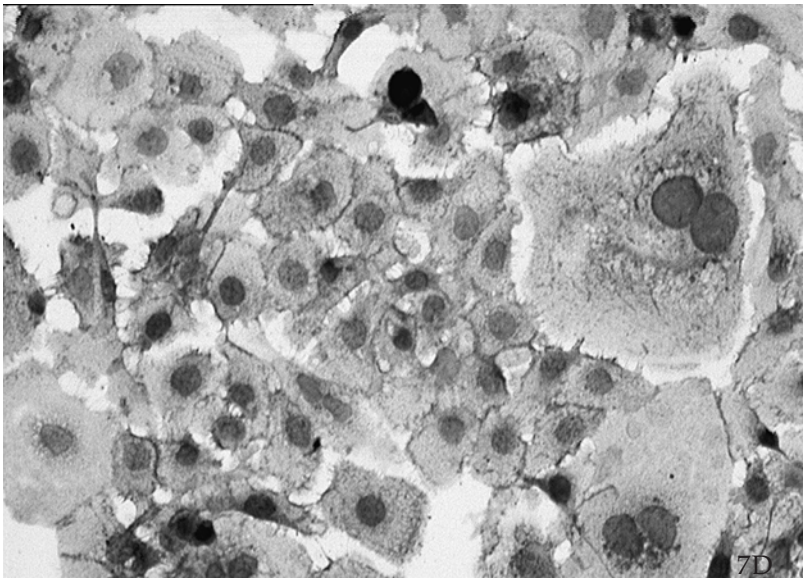


Figure 32.7. *Continued*

ing for CD-34, an hematopoietic progenitor cell antigen, and bcl-2. The occasional expression of muscle markers (31,32) (desmin, smooth muscle actin, and muscle-specific actin) or neural markers (33) (S-100 protein and neuron-specific enolase), in addition to cytokeratins, may occur in sarcomatoid MM—a not surprising finding in light of the above-mentioned differentiative capacity of MM, which is in keeping with the mesodermal nature of the mesothelium (7). Therefore, only the nonexpression of cytokeratin associated with the presence of mesenchymal tissue markers should lead the pathologist not to favor the

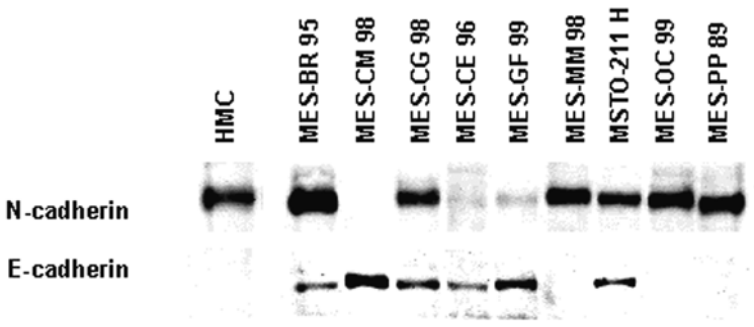


Figure 32.8. Western blot analysis of N- and E-cadherin expression in nine MM cell lines compared with a human mesothelial cell line (HMC).

diagnosis of sarcomatoid MM when dealing with a pleura-based spindle-cell sarcoma. However, it is worth reminding the reader that both cytokeratin-positive sarcomas and cytokeratin-negative sarcomatoid MMs, especially the desmoplastic variant, are not infrequent, and in the latter instance, I make the diagnosis of probable MM, provided that the full set of universally recognized criteria (2) is fulfilled.

Obviously positive results for cytokeratins do not help to distinguish sarcomatoid MM from metastatic spindle-cell carcinoma. In this setting of differential diagnosis, the possible contribution of novel antimesothelial markers (calretinin, CK 5/6, and thrombomodulin) has been exploited by Attanoos et al (34), and in spite of the low sensitivity of each of them for sarcomatoid MM, when they were concurrently used in a panel, 63% of the sarcomatoid MMs were stained by at least one antibody, and conversely no spindle-cell carcinoma was labeled. From these results, the use of this panel in addition to broad-spectrum keratins seems to be worthy of investigation in the problem of spindle-cell neoplasms affecting the serosal membranes.

Immunohistochemistry and Benign vs. Malignant Mesothelium

Finally, IHC is currently of no value in distinguishing between malignant and reactive mesothelium, since both epithelial and spindle mesothelial cells are keratin- and calretinin-positive, irrespective of their biologic nature. No antigen exclusively expressed by either benign or malignant mesothelium has been validated for possible diagnostic use so far. A recent preliminary study appears to indicate a possible role for telomerase reverse transcriptase (TERT), which has been detected immunohistochemically in all but one of 68 MMs, but only in one of 19 benign mesothelial lesions using routinely processed histologic material (35); however, larger studies are needed. Immunostaining for epithelial membrane antigen (EMA) and p53 protein were proposed in the past as a possible adjunct in this setting of differential diagnosis, but in my own experience, these two markers were never helpful and were abandoned following their careful evaluation on both

cytologic and histologic material. This differential diagnosis is typically an H&E diagnosis to which keratin immunostaining can contribute only in order to assess the possible invasion of submesothelial and adjacent tissues by the tumor mesothelial cells, an important diagnostic criterion (2) especially when MM is paucicellular and provided with bland cytologic features.

References

1. Battifora H, McCaughey WTE. Tumours of the Serosal Membranes. Atlas of Tumour Pathology, Third Series, Fascicle 15. Washington, DC: Armed Forces Institute of Pathology, 1994.
2. Cagle P, Corson J, Gibbs AR, et al. The separation of benign and malignant mesothelial proliferations. Am J Surg Pathol 2000;24:1183–1200.
3. Singh G, Whiteside TL, Dekker A. Immunodiagnosis of mesothelioma: use of anti-mesothelial cell serum in an indirect immunofluorescence assay. Cancer 1979;43:2288–2296.
4. Moran CA, Wick MR, Suster S. The role of immunohistochemistry in the diagnosis of malignant mesothelioma. Semin Diagn Pathol 2000;17:178–183.
5. King JE, Hasleton PS. Immunohistochemistry and the diagnosis of malignant mesothelioma. Histopathology 2001;38:471–476.
6. Ordóñez NG. Immunohistochemical diagnosis of epithelioid mesotheliomas: a critical review of old markers, new markers. Hum Pathol 2002;33: 953–967.
7. Donna A, Betta PG. Mesodermomas: a new embryological approach to primary tumours of coelomic surfaces. Histopathology 1981;5:31–44.
8. Attanoos RL, Webb R, Dojcinov SD, Gibbs AR. Malignant epithelioid mesothelioma: anti-mesothelial marker expression correlates with histological pattern. Histopathology 2001;39:584–588.
9. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology 2002;40:403–439.
10. Betta PG, Andrion A, Donna A, et al. Malignant mesothelioma of the pleura. The reproducibility of the immunohistological diagnosis. Pathol Res Pract 1997;193:759–765.
11. Osborn M, Pelling N, Walker MM, et al. The value of "mesothelium-associated" antibodies in distinguishing between metastatic renal cell carcinomas and mesotheliomas. Histopathology 2002;41:301–307.
12. Abutaily AS, Addis BJ, Roche WR. Immunohistochemistry in the distinction between malignant mesothelioma and pulmonary adenocarcinoma: a critical evaluation of new antibodies. J Clin Pathol 2002;55(9):662–668.
13. Gumurdulu D, Zeren EH, Cagle PT, et al. Specificity of MOC-31 and HBME-1 immunohistochemistry in the differential diagnosis of adenocarcinoma and malignant mesothelioma: a study on environmental malignant mesothelioma cases from Turkish villages. Pathol Oncol Res 2002;8:188–193.
14. Gonzalez-Lois C, Ballestin C, Sotelo MT, et al. Combined use of novel epithelial (MOC-31) and mesothelial (HBME-1) immunohistochemical markers for optimal first line diagnostic distinction between mesothelioma and metastatic carcinoma in pleura. Histopathology 2001;38:528–534.
15. Roberts F, Harper CM, Downie I, et al. Immunohistochemical analysis still has a limited role in the diagnosis of malignant mesothelioma. A study of thirteen antibodies. Am J Clin Pathol 2001;116:253–262.

16. Han AC, Peralta-Soler A, Knudsen KA, et al. Differential expression of N-cadherin in pleural mesotheliomas and E-cadherin in lung adenocarcinomas in formalin-fixed, paraffin-embedded tissues. Hum Pathol 1997;28: 641–645.
17. Oates J, Edwards C. HBME-1, MOC-31, WT1 and calretinin: an assessment of recently described markers for mesothelioma and adenocarcinoma. Histopathology 2000;36:341–347.
18. Lau SK, Luthringer DJ, Eisen RN. Thyroid transcription factor-1: a review. Appl Immunohistochem Mol Morphol 2002;10:97–102.
19. Donna A, Betta PG, Chiodera P, et al. Newly marketed tissue markers for malignant mesothelioma: immunoreactivity of rabbit AMAD-2 antiserum compared with monoclonal antibody HBME-1 and a review of the literature on so-called antimesothelioma antibodies. Hum Pathol 1997;28: 929–937.
20. Donna A, Betta PG, Ribotta M, et al. Mitogenic effects of a mesothelial cell growth factor: evidence for a potential autocrine regulation of normal and malignant mesothelial cell proliferation. Int J Exp Pathol 1992;73:193–202.
21. Miettinen M, Kovatich AJ. HBME-1. A monoclonal antibody useful in the differential diagnosis of mesothelioma, adenocarcinoma, and soft-tissue and bone tumours. Appl Immunohistochem 1995;3:115–122.
22. Doglioni C, Tos AP, Laurino L, et al. Calretinin: a novel immunocytochemical marker for mesothelioma. Am J Surg Pathol 1996;20:1037–1046.
23. Miettinen M, Limon J, Niezabitowski A, et al. Calretinin and other mesothelioma markers in synovial sarcoma: analysis of antigenic similarities and differences with malignant mesothelioma. Am J Surg Pathol 2001; 25:610–617.
24. Attanoos RL, Galateau-Salle F, Gibbs AR, et al. Primary thymic epithelial tumours of the pleura mimicking malignant mesothelioma. Histopathology 2002;41:42–49.
25. Yu GH, Soma L, Hahn S, et al. Changing clinical course of patients with malignant mesothelioma: implications for FNA cytology and utility of immunocytochemical staining. Diagn Cytopathol 2001;24:322–327.
26. Moll R, Dhouailly D, Sun TT. Expression of keratin 5 as a distinctive feature of epithelial and biphasic mesotheliomas. An immunohistochemical study using monoclonal antibody AE14. Virchows Arch B Cell Pathol Incl Mol Pathol 1989;58:129–145.
27. Ordonez NG. Value of thyroid transcription factor-1, E-cadherin, BG8, WT1, and CD44S immunostaining in distinguishing epithelial pleural mesothelioma from pulmonary and non-pulmonary adenocarcinoma. Am J Surg Pathol 2000;24:598–606.
28. Foster MR, Johnson JE, Olson SJ, et al. Immunohistochemical analysis of nuclear versus cytoplasmic staining of WT1 in malignant mesotheliomas and primary pulmonary adenocarcinomas. Arch Pathol Lab Med 2001;125: 1316–1320.
29. Hecht JL, Lee BH, Pinkus JL, et al. The value of Wilms tumour susceptibility gene 1 in cytologic preparations as a marker for malignant mesothelioma. Cancer 2002;96:105–109.
30. Muller AM, Weichert A, Muller KM. E-cadherin, E-selectin and vascular cell adhesion molecule: immunohistochemical markers for differentiation between mesothelioma and metastatic pulmonary adenocarcinoma? Virchows Arch 2002;441:41–46.
31. Mayall FG, Goddard H, Gibbs AR. Intermediate filaments expression in mesotheliomas: leiomyoid mesotheliomas are not uncommon. Histopathology 1992;21:453–457.

32. Kung IT, Thallas V, Spencer EJ, et al. Expression of muscle actins in diffuse mesotheliomas. Hum Pathol 1995;26:567–570.
33. Hurlimann J. Desmin and neural marker expression in mesothelial cells and mesotheliomas. Hum Pathol 1994;25:753–757.
34. Attanoos RL, Dojcinov SD, Webb R, et al. Anti-mesothelial markers in sarcomatoid mesothelioma and other spindle-cell neoplasms. Histopathology 2000;37:224–231.
35. Kumaki F, Kawai T, Churg A, et al. Expression of telomerase reverse transcriptase (TERT) in malignant mesotheliomas. Am J Surg Pathol 2002;26: 365–370.

33

Malignant Mesothelioma Electron Microscopy

Raoul Fresco

In spite of recent advances in immunocytochemistry, electron microscopy continues to be the "gold standard" for the differential diagnosis of mesothelioma from other tumors affecting serosal surfaces (1). Three major morphologic subtypes of mesothelioma are well established: epithelial, sarcomatoid, and mixed or biphasic. Of these three types, electron microscopy has been the most contributory in the diagnosis of the epithelial variant and is less helpful in the sarcomatoid mesotheliomas (2,3).

Epithelial Mesothelioma

This subtype, which is the most common, is also the one most difficult to differentiate at the light microscopic level from peripheral lung or metastatic adenocarcinoma. At the ultrastructural level, the most characteristic feature of the neoplastic epithelial cells in the epithelial and mixed types of mesotheliomas is the distinctive morphology of its microvilli (4,5).

Microvilli

Unlike adenocarcinomas (6,7), which usually have relatively scarce short blunt microvilli (Fig. 33.1), mesotheliomas are characterized by the presence of abundant long, slender, undulating, and often bifurcating microvilli. These lack filamentous cores, core rootlets, and surface glycocalyx (fuzz); they measure approximately 0.1 μm in diameter and up to 3 μm in length (Fig. 33.2). Some investigators (8–10) combined the quantitative and qualitative features of tumor cell microvilli and developed a length to diameter ratio (MLDR) as a useful measurement to help distinguish mesotheliomas from adenocarcinoma. Thus, MLDR values over 15 are highly suggestive of mesothelioma, while most adenocarcinomas have MLDR values of under 10. However, some overlapping exists, and other ultrastructural criteria are used to confirm the diagnosis. Profuse microvilli frequently cover the entire tumor surface, best seen in cells aggregated in a papillary

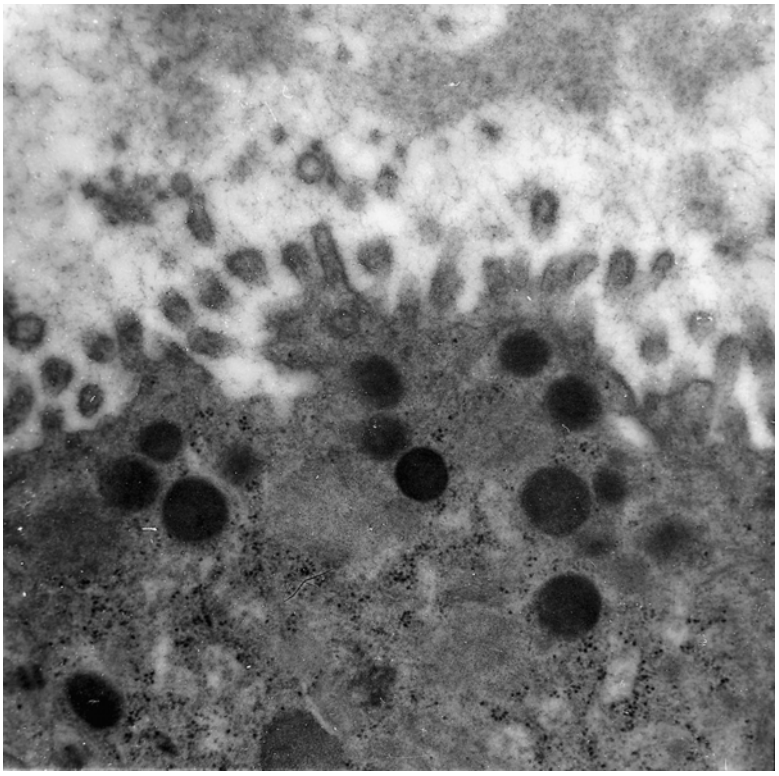


Figure 33.1. Lung adenocarcinoma. Relatively scarce, short, blunt microvilli covered by fuzzy glycocalyx. Zymogen-like electron dense secretory granules are present, but no intermediate filaments are seen.

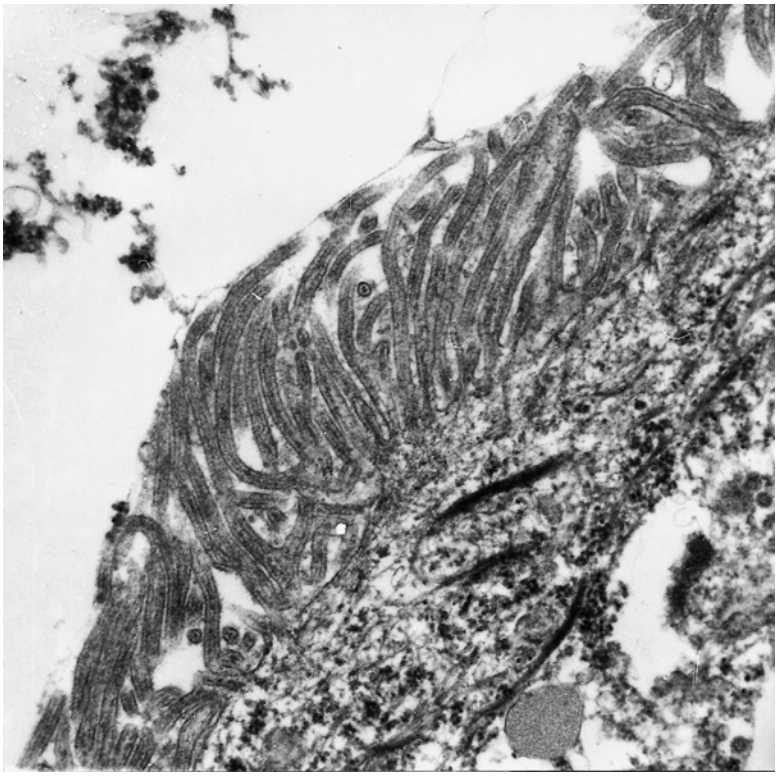


Figure 33.2. Epithelial mesothelioma. Abundant, long, slender at times, bifurcating microvilli. Cytoplasm shows bundles of intermediate filaments.

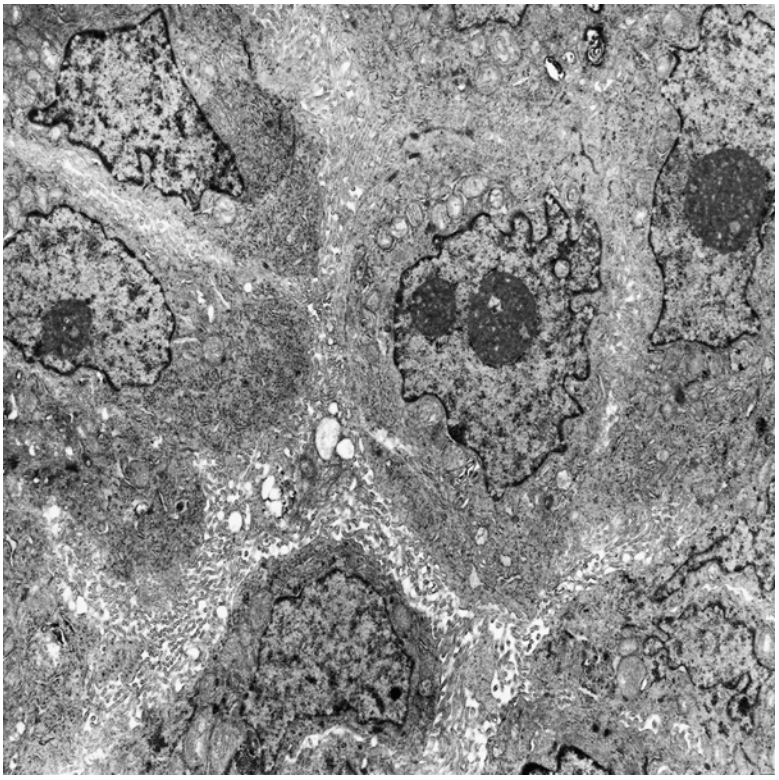


Figure 33.3. Epithelial mesothelioma, papillary pattern. Profuse microvilli covering most of the cellular surfaces. One or more prominent nucleoli are present in the nuclei.

pattern (Fig. 33.3). When the tumor cells form a tubular pattern, the microvilli line the luminal surfaces (Fig. 33.4). At times, these microvilli are seen to contact collagen fibrils without the interposition of basement membranes, a phenomenon first described by Dewar et al (7) and termed microvillus-collagen association by Ghadially et al (11,12) (Figs. 33.5 and 33.6). This interaction was once believed to be a diagnostic feature of mesotheliomas, and though it is most often seen in these tumors, it has also been reported to occur in adenocarcinomas (11).

Intermediate Filaments

Tonofilaments are present in the cytoplasm of a majority of mesotheliomas (Fig. 33.2). These are often aggregated in fibrillary bundles arising in relation to desmosomes or in the perinuclear zone, whereas they are rarely seen in adenocarcinomas of the lung (9,10,13).

Cellular Junctions

While desmosomes occur in both mesotheliomas and adenocarcinomas, long (more than 1 μm), so-called giant desmosomes, when present, favor a diagnosis of mesothelioma (Fig. 33.7) (14,15).

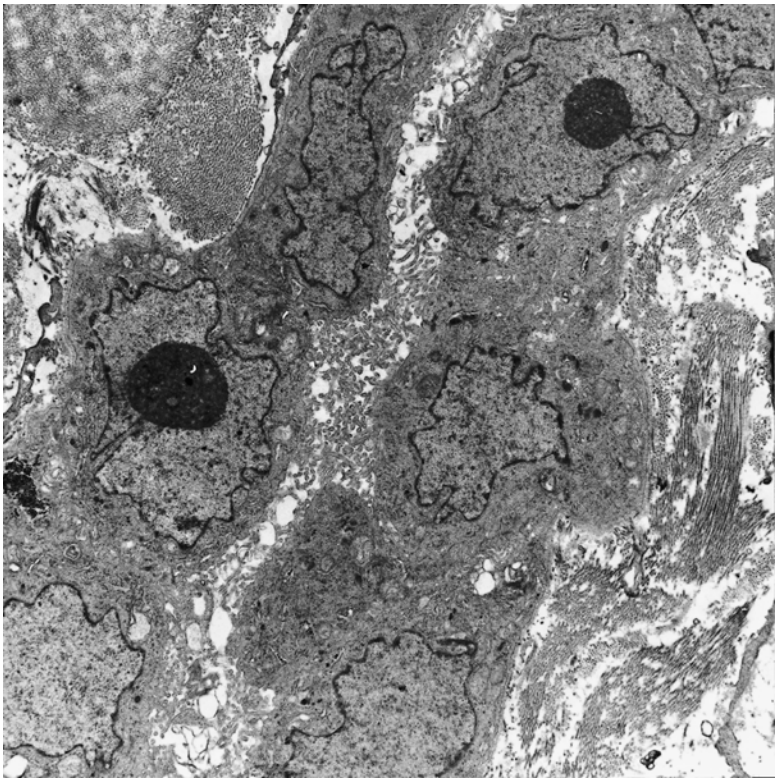


Figure 33.4. Epithelial mesothelioma, tubular pattern. Numerous microvilli line the luminal surfaces. The abluminal surfaces show a basal lamina, beyond which are bundles of collagen fibers.

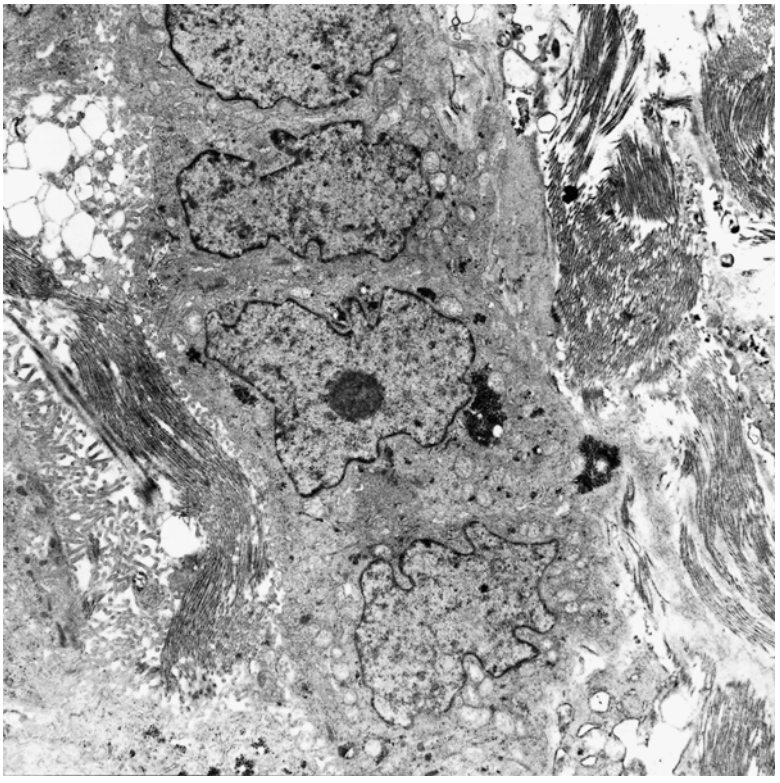


Figure 33.5. Epithelial mesothelioma, tubular pattern. Collagen fibers are seen in direct contact with the microvilli lining the lumen.

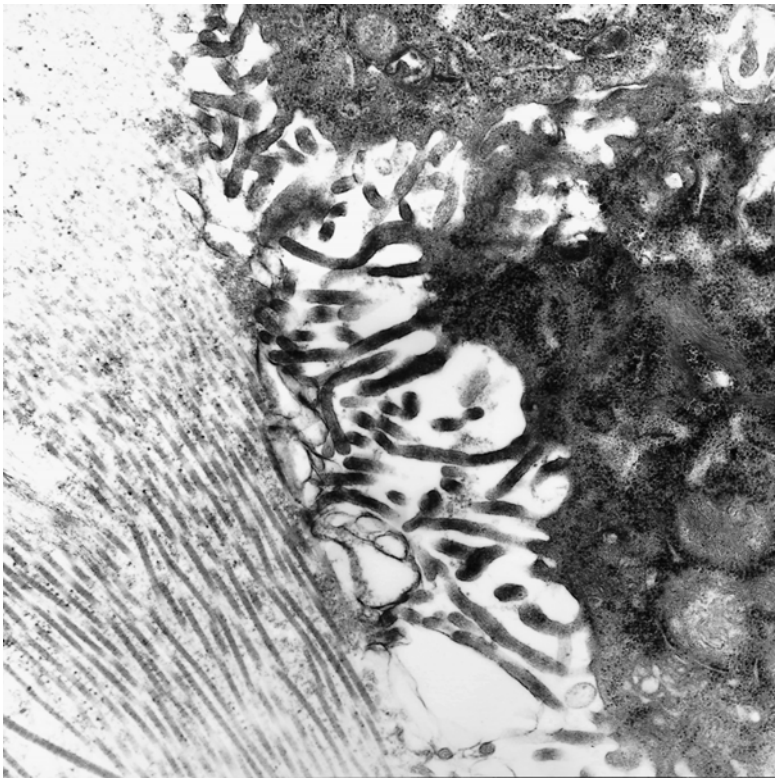


Figure 33.6. Epithelial mesothelioma. Microvillus-collagen association.

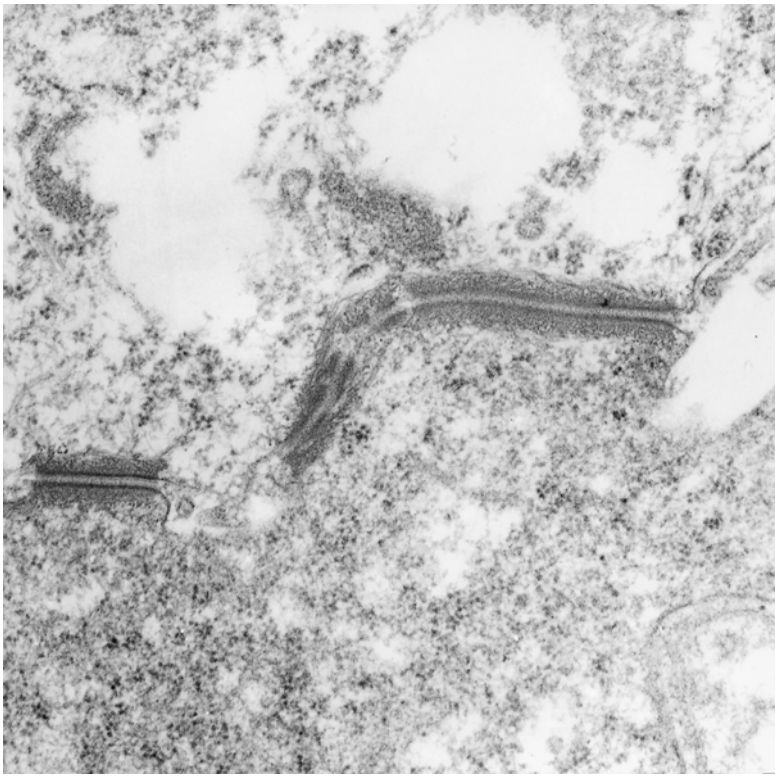


Figure 33.7. Epithelial mesothelioma. Giant desmosome.

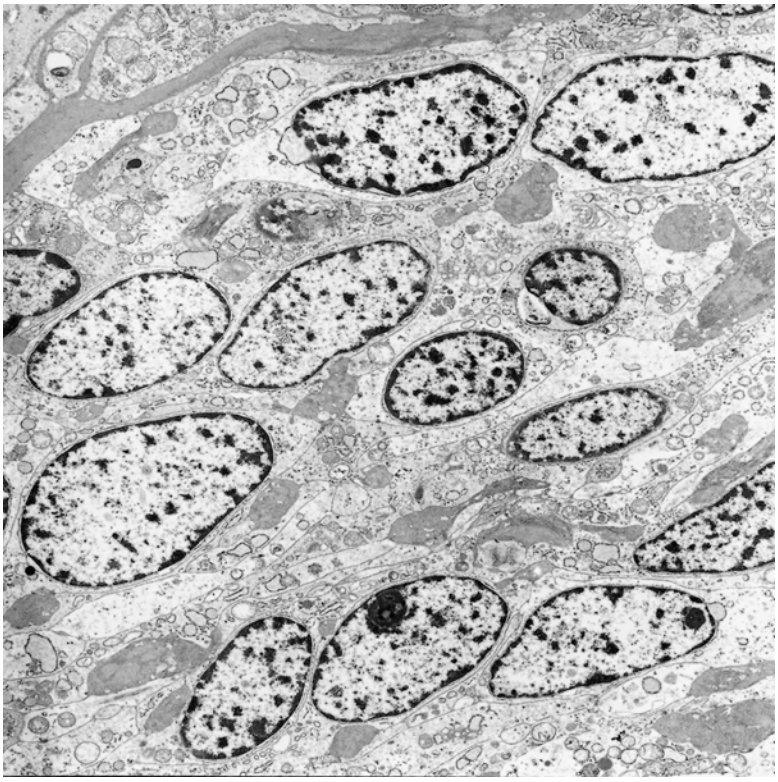


Figure 33.8. Sarcomatoid mesothelioma. Spindle-shaped tumor cells with elongated nuclei and abundant cisternae of rough surfaced endoplasmic reticulum.

Sarcomatoid Mesothelioma

While the epithelial type of mesothelioma can be confused with adenocarcinoma when examined with the light microscope, the sarcomatoid variant is likely to be confused with fibrosarcoma, even at the ultrastructural level. The mesenchymal-looking cells forming these tumors resemble fibroblasts; they have elongated nuclei with clumped chromatin and their cytoplasm contains numerous cisternae of rough endoplasmic reticulum (Fig. 33.8). No basal laminae, desmosomes, or microvilli can be recognized, but rudimentary intercellular junctions are often seen (16). Some of the cells may show myofibroblastic differentiation, with peripheral bundles of filaments bearing focal densities (17).

Mixed (Biphasic-Transitional) Mesothelioma

As their names imply, these tumors have features that are found in both epithelial and sarcomatoid subtypes. The two components may be closely intermixed or may occur in different parts of the same tumor,

features that may present diagnostic difficulties, even at the ultrastructural level, in view of the small size of samples usually studied by electron microscopy. This morphologic diversity demonstrates the multidirectional differentiation capability of mesothelial cells (18,19).

Technical Considerations

In the previous paragraph we alluded to what is a well-known problem confronting diagnostic ultrastructural pathologists, namely the inherent sampling error due to the small area available for examination under the electron beam. Often, the need for electron microscopy is realized only after all the biopsy material has been embedded in paraffin for light microscopy with no portion kept for ultrastructural studies. In such a case, a selected area of the paraffin block can be scooped out, deparaffinized in xylene, and transferred to absolute and then descending grades of alcohol and finally water, at which point it can be reprocessed for electron microscopy (20). The resulting ultrastructural preservation is in great part dependent on the quality of the primary formalin fixation. Our use of this methodology over

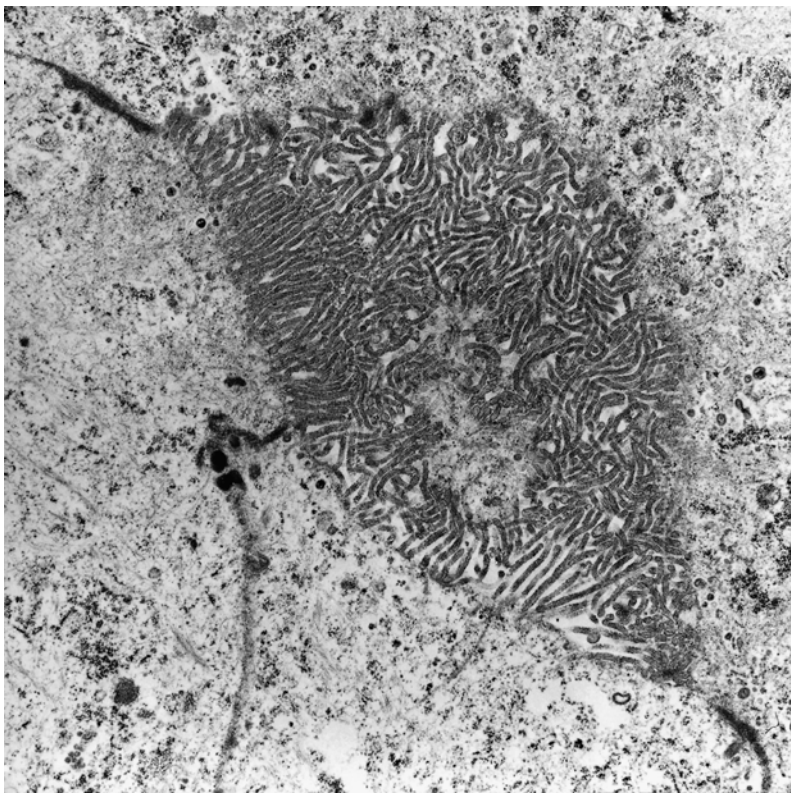


Figure 33.9. Mesothelioma. Formalin fixed, deparaffinized biopsy. Microvillus morphology and prominent desmosomes show excellent preservation, allowing correct diagnosis.

the past few years has exceeded our expectations, and while we do not recommend it routinely as a substitute for conventional electron microscopic techniques, it certainly should be considered when optimally processed tissue is not available and diagnostic problems arise at the light microscopic level (Fig. 33.9).

Viral Pathogenesis

In view of the increasing evidence that at least some cases of mesotheliomas are associated with simian virus 40 (SV40) infection (21), it is interesting to note that some authors have detected tubuloreticular structures in two cases of pleural mesotheliomas (22). These intracytoplasmic structures, first believed to be viral in nature (23), can be traced to be continuous with the endoplasmic reticulum, suggesting a host–cell response (24). They have been shown to be induced by α-interferon, and besides autoimmune diseases, where they were first reported, they are present in many viral infections (25,26).

References

1. Hammar SP, Bockus DE, Remington FL, Rohrbach KA. Mucin-positive epithelial mesotheliomas: a histochemical, immunohistochemical, and ultrastructural comparison with mucin-producing pulmonary adenocarcinomas. Ultrastruct Pathol 1996;20:293–325.
2. Battifora H. The pleura. In: Sternberg SS, ed. Diagnostic Surgical Pathology. New York: Raven Press, 1989:829–855.
3. Battifora H, McCaughey WTE. Tumors of the Serosal Membranes: Atlas of Tumor Pathology, 3rd series, fascicle 15. Washington, DC: Armed Forces Institute of Pathology, 1995.
4. Ghadially FN. Ultrastructural Pathology of the Cell and Matrix. London: Butterworths, 1982:1240–1251.
5. Henderson DW. Transmission electron microscopy of malignant mesothelioma. MSA Bull 1993;23:288–297.
6. Comin CE, de Klerk NH, Henderson DW. Malignant mesothelioma: current conundrums over risk estimates and whither electron microscopy for diagnosis? Ultrastruct Pathol 1997;21:315–320.
7. Dewar A, Valente M, Ring NP, et al. Pleural mesothelioma of epithelial type and pulmonary carcinoma: an ultrastructural and cytochemical comparison. J Pathol 1987;152:309–316.
8. Burns TR, Greenberg SD, Mace ML, et al. Ultrastructural diagnosis of epithelial malignant mesothelioma. Cancer 1985;56:2036–2040.
9. Warhol MJ, Corson JM. An ultrastructural comparison of mesotheliomas with adenocarcinomas of the lung and breast. Hum Pathol 1985;16:50–55.
10. Warhol MJ, Hickey WF, Corson JM. Malignant mesothelioma: ultrastructural distinction from adenocarcinoma. Am J Surg Pathol 1982;6:307–314.
11. Ghadially FN, McCaughey WT, Perkins DG, Rippstein P. Diagnostic value of microvillus-matrix associations in tumors. J Submicrosc Cytol Pathol 1992;24:103–108.
12. Ghadially FN, McCaughey WT, Perkins DG, et al. Morphogenesis and frequency of microvillus-matrix associations in mesotheliomas and adenocarcinomas. MSA Bull 1993;23:281–287.

13. Warhol MJ, Corson JM. An ultrastructural comparison of mesotheliomas with adenocarcinomas of the lung and breast. Hum Pathol 1985;16:50–55.
14. Burns T, Johnson E, Cartwright J Jr, et al. Desmosomes of epithelial malignant mesothelioma. Ultrastruct Pathol 1988;12:385–388.
15. Ghadially FN, Rippstein PU, Cavell S, Venance SL. Giant desmosomes in tumors. Ultrastruct Pathol 1995;19:469–474.
16. Klima M, Bossart MI. Sarcomatous type of malignant mesothelioma. Pathology 1983;4:349–358.
17. D'Andiran G, Gabbiani G. A metastasizing sarcoma of the pleura composed of myofibroblasts. Prog Surg Pathol 1980;2:31–40.
18. Ghadially FN. Diagnostic Electron Microscopy of Tumours, 2nd ed. London: Butterworths, 1985:96–105.
19. Oury TD, Hammar SP, Roggli VL. Ultrastructural features of diffuse malignant mesotheliomas. Human Pathol 1998;29:1382–1392.
20. Widehn S, Kindblom LG. A rapid and simple method for electron microscopy of paraffin-embedded tissue. Ultrastruct Pathol 1988;12:131–136.
21. Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 1996;9:1781–1790.
22. Bernaudin JF, Soler P, Basset F. Intracytoplasmic tubuloreticular structures in 2 cases of sarcoidosis and 2 cases of malignant pleural mesothelioma. Pathol Eur 1973;8:277–285.
23. Grausz H, Earley LE, Stephens BG, Lee JC, Hopper J Jr. Diagnostic import of virus-like particles in the glomerular endothelium of patients with systemic lupus erythematosus. N Engl J Med 1970;283:506–511.
24. Uzman BG, Saito H, Kasac M. Tubular arrays in the endoplasmic reticulum in human tumor cells. Lab Invest 1971;24:492–498.
25. Mills AE, Emms M. Frequent occurrence of microtubuloreticular complexes encountered during routine ultrastructural examination at a children's hospital. Ultrastruct Pathol 1988;12:599–604.
26. Sidhu GS. The ultrastructural markers of AIDS. MSA Bull 1993;23:276–280.

34

Rare Variants of Mesothelioma

Markku Miettinen

This chapter describes the essential clinicopathologic features of rare variants of mesothelioma. These variants include benign or potentially malignant multicystic peritoneal and well-differentiated papillary mesothelioma, and fully malignant, deciduoid, desmoplastic, lymphohistiocytoid, and small cell variants of diffuse malignant mesothelioma. The former term *fibrous mesothelioma* refers to a mesenchymal, nonmesothelial neoplasm now designated as solitary fibrous tumor or localized fibrous tumor; it is beyond the scope of this chapter.

Multicystic (Benign) Peritoneal Mesothelioma (Benign Cystic Mesothelioma, Multilocular Peritoneal Inclusion Cyst)

This clinicopathologically distinctive, rare, mesothelial proliferation was named multicystic peritoneal mesothelioma by Mennenmeyer and Smith (1). Fewer than 100 cases have been reported in the English-language literature, including four series (2–5). Although usually benign, occasional reports on malignant or progressive behavior may warrant exclusion of the word *benign* from the designation of this tumor. Reported examples of this entity may represent a mixture of benign, possibly nonneoplastic mesothelial proliferations and true neoplasms, although a relatively common tendency to recur may suggest neoplastic nature. The above synonyms reflect the differences in understanding of this process.

Clinical Features

Multicystic peritoneal mesothelioma typically occurs in young and middle-aged women in the peritoneal cavity, most often in the pelvis. Less than 20% of the cases have occurred in men, in whom the tumors have often occurred around the right hemicolon. The median age for the largest series was 38 years for women and 47 years for men, and a small number of these tumors occurred in children (5).

Clinically, the patients with peritoneal lesions present with long-standing abdominal pain, increase of abdominal girth, and, rarely, with an acute abdomen. Some examples have been diagnosed incidentally. Occurrence together with endometriosis has been reported (6), and one tumor was associated with both endometriosis and disseminated peritoneal leiomyomatosis (7). One tumor was diagnosed in a patient with ruptured appendiceal mucinous cystadenoma and pseudomyxoma peritonei (8).

The prognosis is generally good, but local recurrences occur commonly, in some cases repeatedly. Some cases have been managed by repeated aspirations instead of surgery (9,10). The two fatalities reported in the largest series in 1988 were one infant whose tumor also contained areas of malignant epithelial mesothelioma, and a man whose tumor was left unoperated (5).

Similar lesions in the pleural cavity were reported in a 37-year-old woman with a history of smoking and early asbestosis exposure. The four apparently separate lesions involved both parietal and visceral pleura, and a brief follow-up of 8 months was uneventful (11).

Pathology

Grossly, the lesions form multiple, confluent cysts or a solitary multicystic mass (Fig. 34.1). The lesion usually involves the pelvis, where it can form a dominant mass or multiple separate cystic lesions on the external surface or pelvic organs. The omentum can also be involved. The cysts vary from a few millimeters to several centimeters in diameter and typically contain clear fluid.

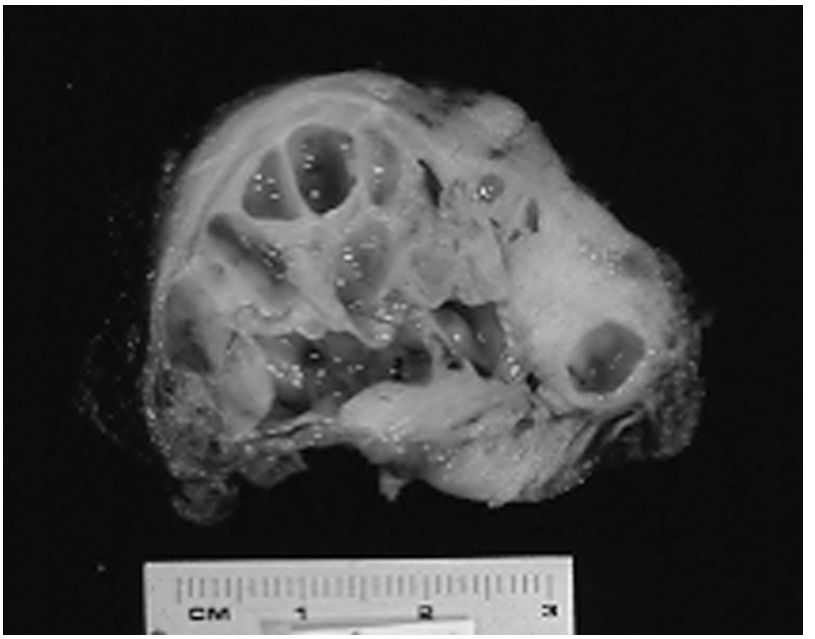


Figure 34.1. Grossly, this example of multicystic peritoneal mesothelioma forms a mass composed of multiple cystic spaces.

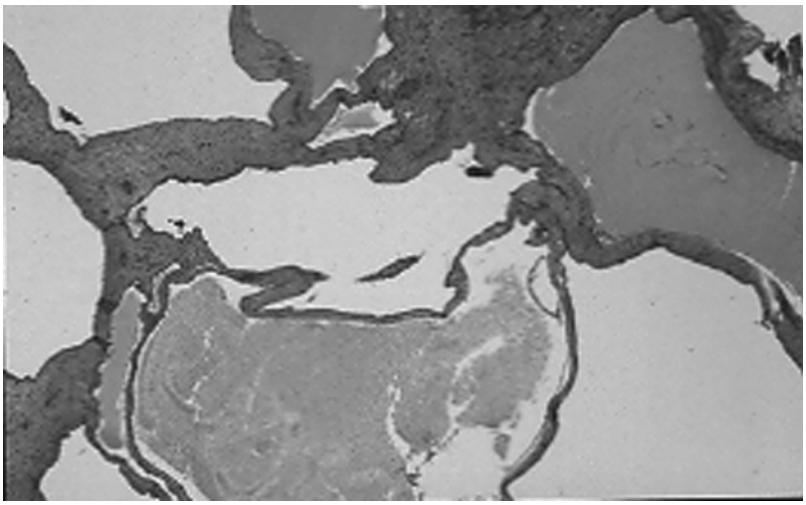


Figure 34.2. Histologically, multicystic peritoneal mesothelioma contains thin- and thick-walled cysts containing proteinaceous fluid.

Histologically, the cysts are lined by cuboidal mesothelial cells and contain proteinaceous fluid (Fig. 34.2). The intervening stroma contains reactive myofibroblasts and may contain focal chronic inflammatory infiltration. The mesothelial cyst lining may have proliferative features with slight papillary or microcystic (Fig. 34.3) or sometimes adenomatoid tumor-like pattern. Immunohistochemically the cyst-lining epithelial components are positive for keratins and calretinin.

Multicystic mesothelioma has to be distinguished from cystic lymphangioma, which can also for a solid multicystic peritoneal mass. However, in the lymphangioma the lining cells are attenuated endothe-

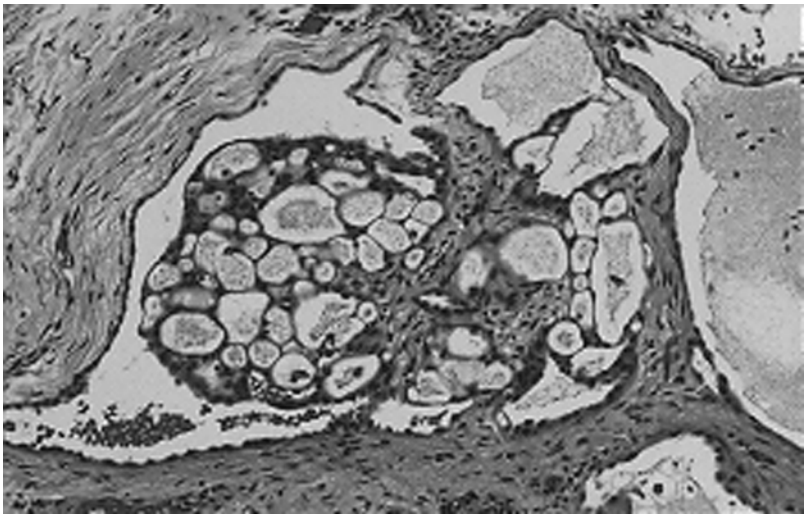


Figure 34.3. The cyst lining in multicystic peritoneal mesothelioma is mainly cuboidal mesothelial cells, but proliferative foci can be present.

lial cells positive for endothelial markers such as CD31 and CD34 and negative for mesothelial markers. However, in my experience lymphangioma lining cells can be positive for keratins 7 and 18.

Well-Differentiated Papillary Mesothelioma of Peritoneum

This rare tumor typically forms multiple papillary lesions on peritoneal surfaces covered with minimally atypical mesothelial cells.

Clinical Features

Two series have reported these tumors, one as a "benign papillary mesothelioma" (12), and another as a "well-differentiated papillary mesothelioma" (13). These tumors usually occur in young and middle-aged women as clusters of multiple peritoneal papillary projections or rarely as a single lesion. Only rarely has there been a history of asbestosis exposure. Most lesions are small (<2 cm), and have been incidental findings during surgery of other conditions or rarely manifested by a symptomatic tumor torsion and occasionally with ascites. Although the prognosis is generally good, disease progression to fully malignant mesothelioma has been observed in some cases (14). Therefore, there may be a continuum between this tumor and a well-differentiated papillary diffuse malignant mesothelioma.

Pathology

Grossly, the lesions appear as peritoneal nodules that often show papillary projections and may contain calcifications. Rarely, there is a diffuse, mat-like pattern.

Histologically, typical cases show well-differentiated papillary projections lined by a single layer of cuboidal mesothelial cells with limited atypia and a pauciellular, often edematous fibrous core (Fig. 34.4). Lesions with more complex patterns may approach the features of well-differentiated malignant mesothelioma, and in some cases this distinction can be arbitrary. Large lesion size and the presence of solid areas with increased atypia and mitotic activity should lead to the diagnosis of malignant mesothelioma.

Deciduoid Mesothelioma

Deciduoid mesothelioma is a designation for a rare variant of malignant epithelial mesothelioma with deciduas-like epithelial cells with abundant cytoplasm. It seems to account for 2% to 5% of malignant mesotheliomas.

Clinical Features

This mesothelioma variant was originally reported by Talerman et al (15) in the peritoneum of a 13-year-old and named deciduoid mesothe-

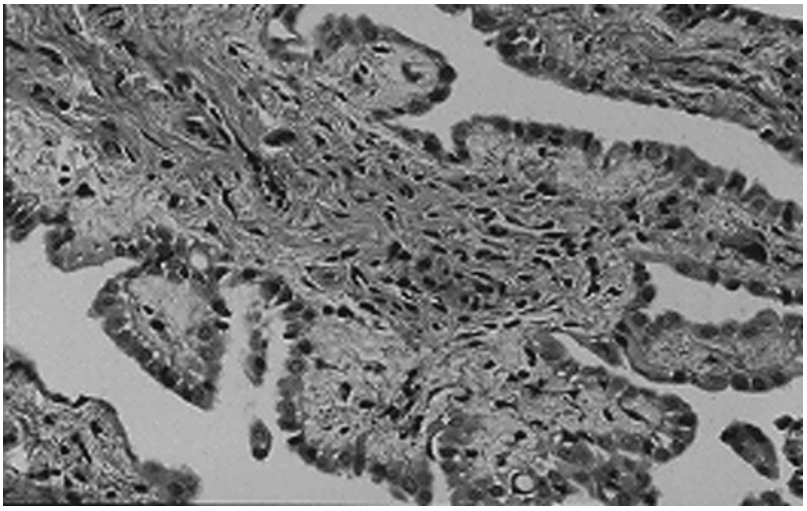


Figure 34.4. Well-differentiated papillary mesothelioma is composed of multiple papillary projections lined by a simple layer of mildly proliferative mesothelia.

lioma. Nascimento et al (16) reported it in two young females without an asbestosis history. Orosz et al (17) reported another case in a peritoneal cavity of a young woman. Subsequent reports have shown a wider clinicopathologic spectrum including occurrence in older subjects (median age 53 years), in the pleura, and in connection with asbestosis (18–21). However, based on these reports, deciduoid mesothelioma has a predilection for females and for the abdominal cavity (>50% of cases). The age range is wide and includes children and young adults more often than the series of conventional mesotheliomas. Prognosis does not significantly differ from conventional mesotheliomas, and most patients with follow-up data have died of disease within 3 years, with the median survival being 7 months (21).

Pathology

Histologically, the deciduoid mesothelioma is composed of diffuse sheets of large, epithelioid cells with abundant pale to brightly eosinophilic cytoplasm, giving the tumor cells a decidua-like appearance (Fig. 34.5). Ultrastructural features are similar to conventional diffuse malignant mesothelioma, with slender and tall cell surface microvilli. Immunohistochemically the tumor cells in this variant are positive for the simple epithelial keratins 7, 8, 18, and 19, and for keratin 5, HBME-1 and calretinin.

Desmoplastic Malignant Mesothelioma

Desmoplastic mesothelioma is defined as diffuse malignant mesothelioma having an acellular/paucicellular connective tissue component comprising more than 50% of the tumor volume and containing malig-

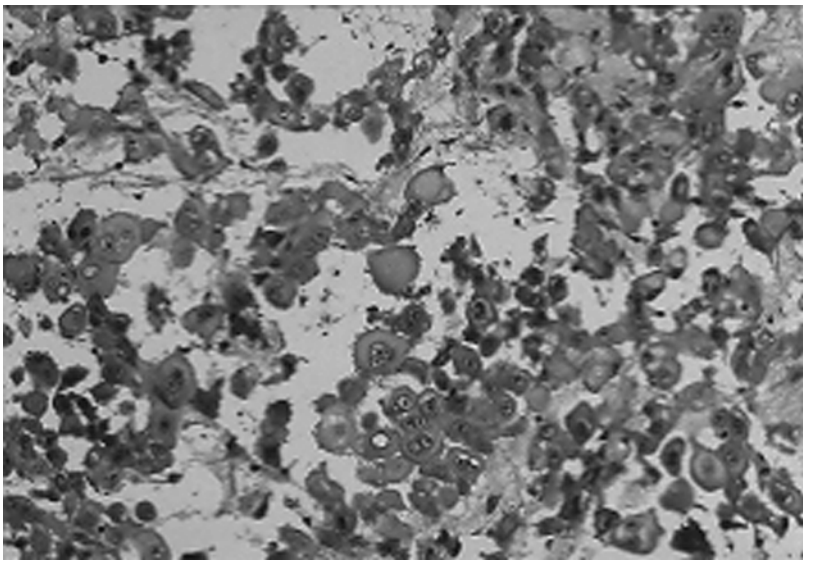


Figure 34.5. Deciduoid mesothelioma is composed of large cells with variably eosinophilic cytoplasm; the cellular cohesion varies.

nant sarcomatous or epithelial components or both. Therefore, it may also be considered a variant of sarcomatous, epithelial, and biphasic mesothelioma.

Clinical Features

This relatively rare variant of diffuse malignant mesothelioma typically occurs in pleura, almost always in patients with significant asbestosis exposure. It comprises 5% to 10% of all pleural mesotheliomas and principally occurs in older adults with a marked male predominance. This tumor is practically uniformly fatal and the mean survival is only 6 to 12 months. Distant metastases in liver and kidneys are more common in this tumor than in conventional malignant epithelial mesothelioma (22,23).

Pathology

Grossly, desmoplastic mesothelioma typically forms a fibrous encasing around the lung with the principally involved side, and it can also involve pericardium and be bilateral in a minority of cases.

Histologically, this mesothelioma is characterized by a dominance of fibrous stroma, and areas of the tumor may resemble a benign fibrosing inflammatory process or pleural plaque when densely fibrous and nearly acellular. However, other areas contain atypical spindled cells with a sarcomatoid pattern, often arranged in a slightly storiform manner (Fig. 34.6) with the high magnification demonstrating clear atypia (Fig. 34.7). Malignant epithelial components are present in a minority of cases.

The differential diagnosis from a benign, fibrosing process requires demonstration of stromal invasion, an overt sarcomatous component,

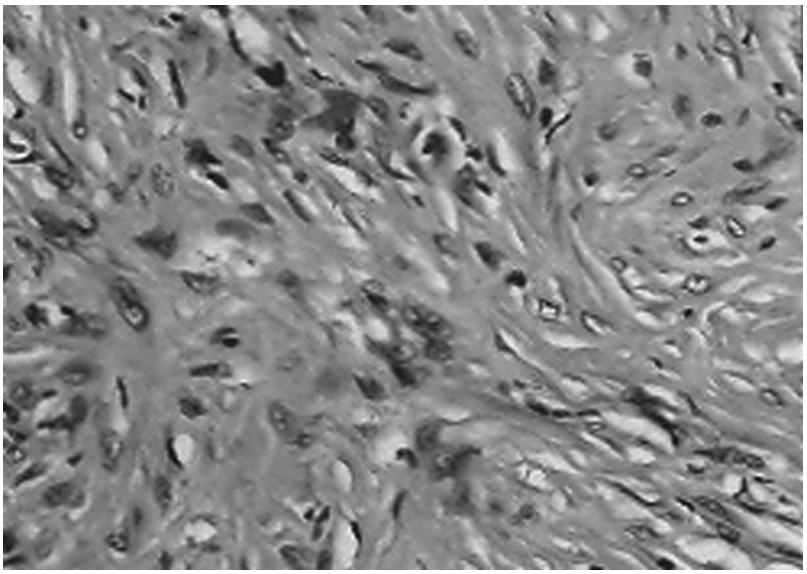


Figure 34.6. Desmoplastic mesothelioma has a vague storiform, spindle cell pattern.

an atypical component, or clinically invasive or metastatic behavior. The presence of tumor cell necrosis or collagen necrosis is supportive of malignancy, although it is not specific (24,25). Obviously, the diagnostic difficulties are magnified when only a small biopsy is available, and such material can be nondiagnostic in this entity.

Immunohistochemical demonstration of strong keratin expression (including keratins 7, 8, 18, and 19) is diagnostically helpful (Fig. 34.8), whereas mesothelioma markers, such as calretinin, HBME-1, and *WT1*, are inconsistently expressed in the sarcomatous areas of these tumors

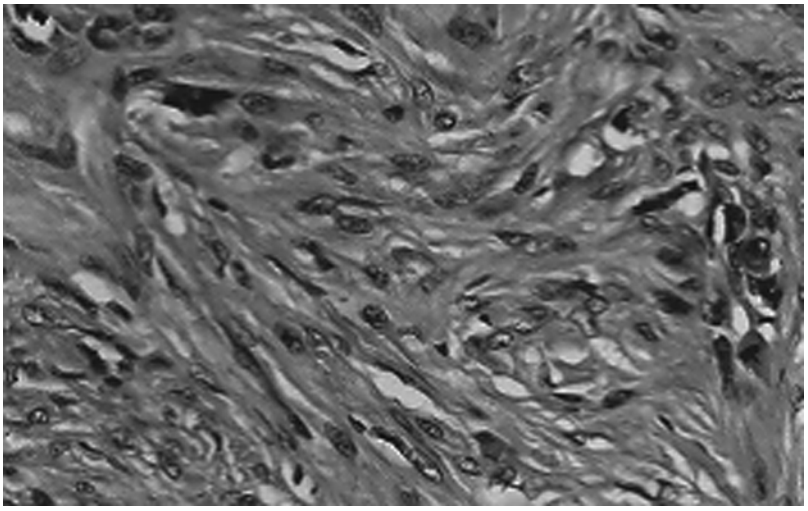


Figure 34.7. More atypical tumor cells are at least focally seen in desmoplastic mesothelioma.

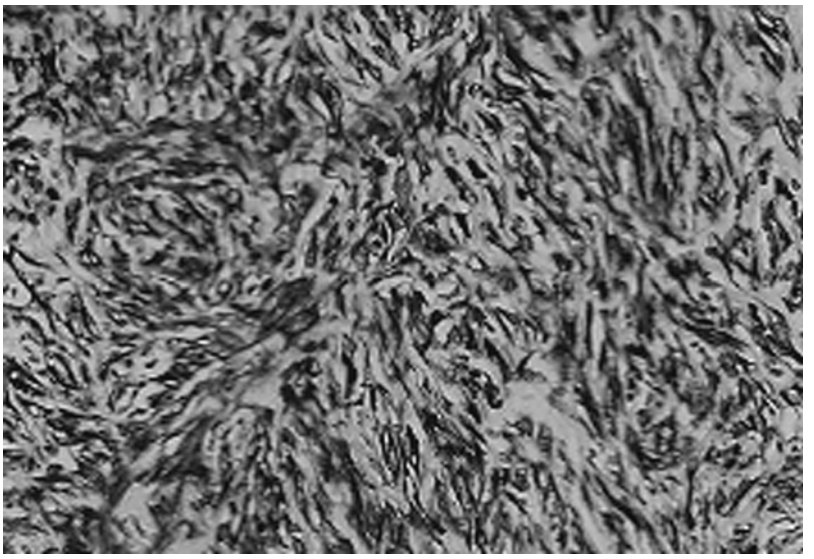


Figure 34.8. All spindle cells in desmoplastic mesothelioma are strongly positive for keratins.

in our experience. It should be noted that the presence of keratin-positive cells is not sufficient for this diagnosis, because pleural plaques typically also contain keratin-positive spindle cells. Nuclear immunostaining for cell cycle marker TP53 (p53) has not been conclusive or reproducible in the diagnosis according to one study (25).

Lymphohistiocytoid Mesothelioma

This designation refers to a rare variant of diffuse sarcomatoid mesothelioma that can histologically resemble a lymphoma or a true histiocytic sarcoma. Three such pleural tumors were originally identified among 394 cases entered as mesotheliomas in the Australian Mesothelioma Surveillance Program (<1% of all mesotheliomas). All patients had a history of asbestosis and died in 4 to 8 months (26).

Histologically distinctive are significant lymphohistiocytoid infiltration and lymhoplasma-cytic and histicytic infiltration; the latter may lead to the false assumption that all neoplastic cells are lymphoid or histiocytic. Also, the scattered large neoplastic cells among mixed lymphocytic and histiocytic population may lead to the misdiagnosis of Hodgkin's disease. Immunohistochemically the lesional cells are positive for keratins and usually for calretinin (27). In contrast, only the background lymphoid and histiocytic populations are positive for lymphoid and histiocytic markers.

Small Cell Mesothelioma

This description pertains to a very rare variant of diffuse malignant mesothelioma with a predominantly small cell histologic pattern. With proper sampling, areas of conventional diffuse mesothelioma are

almost always identified in these tumors. A study by Mayall and Gibbs (28) identified 10 such cases among 160 mesotheliomas in autopsy material (6%). The question can be raised whether the possibility has been ruled out that at least in some cases, this pattern could represent postmortem artifact (cell shrinkage). Immunohistochemical studies on this variant are scant, but it was reported to consist of keratin-positive and chromogranin A negative cells; this reports predated the availability of calretinin (28).

Other small cell neoplasms may have been historically confused with small cell mesothelioma. It is likely that older reports of this entity may have referred to desmoplastic small round cell tumor, a primitive epithelial, polyphenotypic neoplasm. This tumor typically occurs in the abdominal cavity of 5- to 30-year-old males. A minority of cases have occurred in women, and well-documented examples have been reported in the pleura. Irregularly shaped islands of small round tumor cells in desmoplastic stroma, immunohistochemical coexpression of keratins and desmin, and the presence of *WT1-EWS* gene fusion with the t(11;22) translocation are the key diagnostic features of this tumor (29).

References

1. Mennenmeyer R, Smith M. Multicystic peritoneal mesothelioma: a report with electron microscopy of a case mimicking intra-abdominal cystic hygroma (lymphangioma). Cancer 1979;44:692–698.
2. Katsube Y, Mukai K, Silverberg SG. Cystic mesothelioma of the peritoneum: a report of five cases and review of literature. Cancer 1982;50: 1615–1622.
3. Carpenter HA, Lancaster JR, Lee RA. Multilocular cysts of the peritoneum. Mayo Clin Proc 1982;57:634–638.
4. Schneider V, Partridge JR, Gutierrez F, Hurt WG, Maizels MS, Demay RM. Benign cystic mesothelioma involving the female genital tract: report of four cases. Am J Obstet Gynecol 1983;145:355–359.
5. Weiss SW, Tavassoli FA. Multicystic mesothelioma. An analysis of pathological findings and biologic behavior in 37 cases. Am J Surg Pathol 1988; 12:737–746.
6. Groisman GM, Kerner H. Multicystic mesothelioma with endometriosis. Acta Obstet Gynecol Scand 1992;71:642–644.
7. Zotalis G, Nayar R. Hicks DG. Leiomyomatosis peritonealis disseminata, endometriosis, and multicystic mesothelioma: an unusual association. Int J Gynecol Pathol 1998;17:178–182.
8. Kusuyama T, Fujita M. Appendiceal mucinous cystadenoma associated with pseudomyxoma peritonei and multicystic peritoneal mesothelioma: report of a case. Surg Today 1995;25:745–749.
9. van der Klooster JM, Lambers MD, van Bommel EF, Scholten PC. Successful catheter drainage of recurrent benign multicystic mesothelioma of the peritoneum. Neth J Med 1997;50:246–249.
10. Inman DS, Lambert AW, Wilkins DC. Multicystic peritoneal inclusion cysts: the use of CT guided drainage for symptom control. Ann R Coll Surg Engl 2000;82:196–197.
11. Ball NJ, Urbanski SJ, Green FH, Kieser T. Pleural multicystic mesothelial proliferation. The so-called multicystic mesothelioma. Am J Surg Pathol 1990;14:375–378.

12. Goepel JR. Benign papillary mesothelioma of the peritoneum: a histological, histochemical and ultrastructural study of six cases. Histopathology 1981;5:21–30.
13. Daya D, McCaughey WTE. Well-differentiated papillary mesothelioma of the peritoneum. A clinicopathologic study of 22 cases. Cancer 1990;65:292–296.
14. Battifora H, McCaughey WTE. Tumors of the Serous Membranes. Washington, DC: Armed Forcers Institute of Pathology, 1995.
15. Talerman A, Montero JR, Chilcote RR, Okagaki T. Diffuse malignant peritoneal mesothelioma in a 13-year-old girl: report of a case and review of the literature. Am J Surg Pathol 1985;9:73–80.
16. Nascimento AG, Keeney GL, Fletcher CDM. Deciduoid peritoneal mesothelioma: an unusual phenotype affecting young females. Am J Surg Pathol 1994;18:439–445.
17. Orosz Z, Nagy P, Szentirmay Z, Zalatnai A, Hauser P. Epithelial mesothelioma with deciduoid features. Virchows Arch 1999;434:263–266.
18. Shanks JH, Harris M, Banerjee SS, et al. Mesotheliomas with deciduoid morphology. A morphologic spectrum and a variant not confined to young females. Am J Surg Pathol 2000;24:285–294.
19. Ordonez NG. Epithelial mesothelioma with deciduoid features. A report of four cases. Am J Surg Pathol 2000;24:816–823.
20. Serio G, Scattone A, Pennella A, et al. Malignant deciduoid mesothelioma of the pleura: report of two cases with long survival. Histopathology 2002; 40:348–352.
21. Shia JA. Deciduoid mesothelioma: a report of five cases and literature review. Ultrastruct Pathol 2002;26:355–363.
22. Cantin R, Al-Jabi M, McCaughey WTE. Desmoplastic diffuse mesothelioma. Am J Surg Pathol 1982;6:215–222.
23. Wilson GE, Hasleton PS, Chatterjee AK. Desmoplastic malignant mesothelioma: a review of 17 cases. J Clin Pathol 1992;45:295–298.
24. Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferations. Am J Surg Pathol 2000;24:1183–1200.
25. Mangano WE, Cagle PT, Churg A, Vollmer RT, Roggli VL. The diagnosis of desmoplastic malignant mesothelioma and its distinction from fibrous pleurisy: a histologic and immunohistochemical analysis of 31 cases including p53 immunostaining. Am J Clin Pathol 1998;110:191–199.
26. Henderson DW, Attwood HD, Constance TJ, Shilkin KB, Steele RH. Lymphohistiocytoid mesothelioma: a rare lymphomatoid variant of predominantly sarcomatoid mesothelioma. Ultrastruct Pthol 1988;12:367–384.
27. Khalidi HS, Medeiros LJ, Battifora H. Lymphohistiocytoid mesothelioma. An often misdiagnosed variant of sarcomatoid mesothelioma. Am J Clin Pathol 2000;113:649–654.
28. Mayall FG, Gibbs AR. The histology and immunohistochemistry of small cell mesothelioma. Histopathology 1992;20:47–51.
29. Gerald WL, Ladanyi M, de Alava E, et al. Clinical, pathologic and molecular spectrum of tumors associated with t(11;22)(p13;q12): desmoplastic small round-cell tumor and its variants. J Clin Oncol 1998;16:3026–3036.

35

Differentiating Sarcomas from Mesotheliomas

Oliver Kim and Thomas Krausz

Despite the abundant literature data on the topic, the diagnosis of malignant mesothelioma remains challenging. Its frequent phenotypic heterogeneity and diverse architectural patterns underline the capacity of mesothelioma to mimic other neoplasms, notably adenocarcinoma and sarcomas. Immunohistochemical markers facilitate solving differential diagnostic problems; however, in some cases, a definitive diagnosis of malignant mesothelioma is still a challenge. The pleura or other serosal surfaces can be involved by a number of neoplastic conditions, ranging from benign to malignant. Furthermore, metastatic neoplasms commonly involve these sites. Although malignant mesothelioma is a relatively uncommon tumor, it is the most common primary malignancy of the pleura and can develop at other sites, including the peritoneum, pericardium, and tunica vaginalis (1). Histologically, the major subtypes of malignant mesothelioma are epithelioid, sarcomatoid, and mixed. Therefore, the differential diagnosis varies according to histologic type. Correlation between clinical, radiographic, and pathologic findings is critical to make a correct diagnosis.

Although distinguishing mesothelioma from an adenocarcinoma is the more common problem, differentiating either a metastatic or primary sarcoma from a malignant mesothelioma can have important therapeutic consequences. Primary mesenchymal tumors, primarily solitary fibrous tumors and sarcomas, of the pleura and other serosal membranes are rare (1). Most sarcomatous tumors of the pleura are manifestations of either a metastatic sarcoma or a sarcomatoid mesothelioma. In addition, sarcomas arising from the chest wall or lung can also involve the pleura. Both primary and metastatic sarcomas can mimic the characteristic clinical, radiologic, and pathologic findings of a malignant mesothelioma. Distinguishing sarcomatoid mesothelioma from morphologically similar sarcomas is a diagnostic challenge. This chapter outlines a practical approach in distinguishing mesothelioma from sarcomas.

Sarcoma Versus Malignant Mesothelioma: General Differential Diagnostic Considerations

The pathologic features of sarcomatoid mesothelioma are not entirely specific and often overlap with other primary and secondary serosal-based sarcomas and spindle cell carcinomas. Furthermore, depending on the degree of collagen deposition (desmoplastic mesothelioma), a more frequent problem is distinguishing sarcomatoid mesothelioma from benign fibrous pleurisy. The growth pattern of sarcomatoid mesothelioma is diverse. It may be storiform with similarity to the so-called malignant fibrous histiocytoma. The tumor cells may display a fibrosarcoma-like appearance with long fascicles exhibiting a herring-bone pattern. Leiomyoid differentiation has been described in which the cells have oval, elongated nuclei, eosinophilic cytoplasm, and coexpress actin and desmin (2,3). In addition, heterologous elements, including osteoid (4), chondroid (4), and rhabdomyoblastic differentiation (5), rarely can be identified. Depending on the histologic features present, diagnostic considerations can include fibrosarcoma, so-called malignant fibrous histiocytoma, malignant peripheral nerve sheath tumor (6), rhabdomyosarcoma, leiomyosarcoma (7), synovial sarcoma (8), angiosarcoma (9–11), liposarcoma (12), malignant solitary fibrous tumor (13–15), and chondrosarcoma (16), all of which have been reported to arise primarily in the pleura. Metastatic neoplasms, including sarcomatoid carcinoma, malignant melanoma, and thymoma, have all been documented and should be diagnostic considerations (17). Gastrointestinal stromal tumor (GIST), either as a metastasis or an extraintestinal primary, can also histologically resemble a sarcomatoid mesothelioma. C-KIT immunoreactivity defines this tumor, and is negative in mesotheliomas.

Cytokeratin expression is most useful in distinguishing most sarcomas from sarcomatoid mesothelioma. Characteristically, nearly all mesotheliomas of epithelioid, sarcomatoid, or mixed type exhibit strong cytokeratin expression (18,19). Both low and high molecular weight cytokeratins are detectable in most mesotheliomas, especially low molecular weight cytokeratins.

Although cytokeratin can be utilized to distinguish sarcomatoid mesothelioma from most sarcomas, there are a few caveats. First, investigators have reported variable immunoreactivity with cytokeratins. Although all epithelioid mesotheliomas express cytokeratin, the percentage of sarcomatoid mesotheliomas reported in the literature to express cytokeratin is variable. Some investigators have detected cytokeratins in 100% of their sarcomatoid mesothelioma cases examined (18,19). In contrast, others failed to detect cytokeratins in up to 40% of sarcomatoid mesotheliomas (4,5,13,20–24). In our experience, all cases of sarcomatoid mesothelioma exhibit immunoreactivity with cytokeratin antibody CAM 5.2. Thus, these results stress the importance of utilizing, in addition to cytokeratins, a panel of other immunomarkers.

Second, cytokeratin expression can also be occasionally seen in sarcomas (25,26), including monophasic synovial sarcoma, angiosarcoma, malignant peripheral nerve sheath tumor, and leiomyosarcoma. But cytokeratin immunoreactivity seen in these sarcomas is usually focal. Monophasic synovial sarcomas tend to express either or both cytokeratins 7 and 19, whereas malignant peripheral nerve sheath tumors do not (27). Malignant melanoma can metastasize to the pleura and simulate a mesothelioma. Rare melanomas are keratin-positive (28,29) but typically express S-100 and HMB-45 antigen.

Third, cytokeratin does not discriminate sarcomatoid mesothelioma from metastatic sarcomatoid carcinoma, primary pleural thymoma, pseudomesotheliomatous carcinoma of lung, or even metastatic epithelioid sarcoma. Cytokeratin immunoreactivity is typically strong in all of these tumors. In these cases, positivity with any of the mesothelial-specific markers or absence of carcinoma markers would favor sarcomatoid mesothelioma. However, many of the allegedly specific mesothelial markers have cross-reactivity with other tumors, especially metastatic carcinoma. Hence, the importance of utilizing a broad panel of carcinoma and mesothelial markers is emphasized, in addition to obtaining clinical history. Of important note, thymomas can rarely occur in the pleura without evidence of an associated mediastinal tumor (30). They may occur as a localized tumor or, more rarely, as a diffuse pleural thickening mimicking a mesothelioma (30–32). Histologically, they may be confused with a sarcomatoid mesothelioma with a lymphocytic infiltrate or a lymphohistiocytoid mesothelioma. The neoplastic cells exhibit strong cytokeratin immunoreactivity. As opposed to mesotheliomas, which can have a mixed population of plasma cells and T and B lymphocytes, thymomas contain a population of precursor T lymphocytes, commonly coexpressing CD1, CD2, CD3, CD99 (MIC-2) (33), bcl-2 (34), and terminal deoxynucleotidyl transferase (35) antigens. Epithelioid sarcoma, both the conventional and proximal variant, can metastasize to the lung and pleura and be difficult to differentiate from the epithelioid mesothelioma. They typically exhibit strong immunoreactivity to both low and high molecular weight keratins. Although the conventional or distal variant of epithelioid sarcoma tends to occur in young adults, the proximal variant occurs in the older age group. Thus, epithelioid sarcoma should be considered in the differential diagnosis. The differentiating factor would be the strong positivity for CD34 in a high percentage of epithelioid sarcomas, for which mesotheliomas are negative.

Ultrastructural features of sarcomatoid mesothelioma are nonspecific and overlap with those of fibroblasts (5,36). The tumor cells contain variable amounts of rough endoplasmic reticulum, Golgi apparatus, intermediate filaments, and extracellular collagen. Occasionally, the tumor cells exhibit actin filaments in the cell periphery, resembling myofibroblasts. Epithelial differentiation can also be identified on occasion, which includes the presence of intercellular junctions, rare surface microvilli, aggregates of tonofilaments, and incomplete formation of basal lamina. Differentiation of sarcomatoid mesotheliomas from other

sarcomatoid neoplasms can be made by the presence of specific ultrastructural components in other tumors that are typically lacking in mesotheliomas. Thus, the diagnosis continues to rely on a multimodal approach incorporating clinical history, gross and microscopic features, immunohistochemistry, and electron microscopy to arrive at a definitive diagnosis.

Synovial Sarcoma Versus Malignant Mesothelioma

Synovial sarcoma is a rare tumor most commonly found in the soft tissues of the extremities but other sites, including the head and neck (37–39), mediastinum (40–42), lung (43–48), heart (49), esophagus (50), and vulva (51), have also been reported. There have been reports of synovial sarcoma arising in the pleural cavity (8,52,53). The rarity of this tumor at this site makes it a potentially overlooked diagnosis, especially since the histologic characteristics of synovial sarcoma can closely resemble malignant mesothelioma. Both tumors can present with either purely sarcomatoid or mixed sarcomatoid/epithelioid components. Thus, monophasic synovial sarcoma must be distinguished from sarcomatoid malignant mesothelioma, and biphasic synovial sarcoma from mixed malignant mesothelioma. However, there are both clinical and histologic features that aid in making a diagnosis of synovial sarcoma arising within the pleural cavity.

In contrast to malignant mesothelioma, the majority of pleural synovial sarcomas tend to occur in children, adolescents, and young adults. Although presenting with effusions is common, as in mesothelioma cases, synovial sarcomas tend to be localized and relatively circumscribed mass lesions with rapid growth, rather than a diffusely infiltrating mass, as is characteristic for mesothelioma. Pleural synovial sarcomas exhibit similar histomorphologic, immunohistologic, and ultrastructural features as synovial sarcomas occurring in other sites. Reported cases of primary pleural synovial sarcomas correspond to both the biphasic (52,53) and monophasic (44,54,55) variants of synovial sarcoma. Thus, synovial sarcoma can be confused with both the biphasic and sarcomatoid malignant mesothelioma (Figs. 35.1 and 35.2). In synovial sarcoma, the neoplastic epithelial cells tend to express "carcinoma" markers, including keratins (Fig. 35.1), epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and BerEP4. The cytokeratin staining pattern of the glandular cells of synovial sarcoma is diffuse as opposed to the perinuclear keratin-staining pattern seen in mesothelioma. In addition, the secretions from the epithelial component are typically positive for neutral mucins, which are periodic acid-Schiff (PAS) positive and diastase-resistant, and Alcian blue positive and hyaluronidase resistant. This histochemical and immunophenotypic reaction strongly argues against a malignant mesothelioma. Monophasic synovial sarcoma and the sarcomatoid component of biphasic synovial sarcoma characteristically express cytokeratins, focally differentiating it from the typically strong and diffuse cytokeratin positivity seen in sarcomatoid mesothelioma (Fig. 35.2). Epithelial

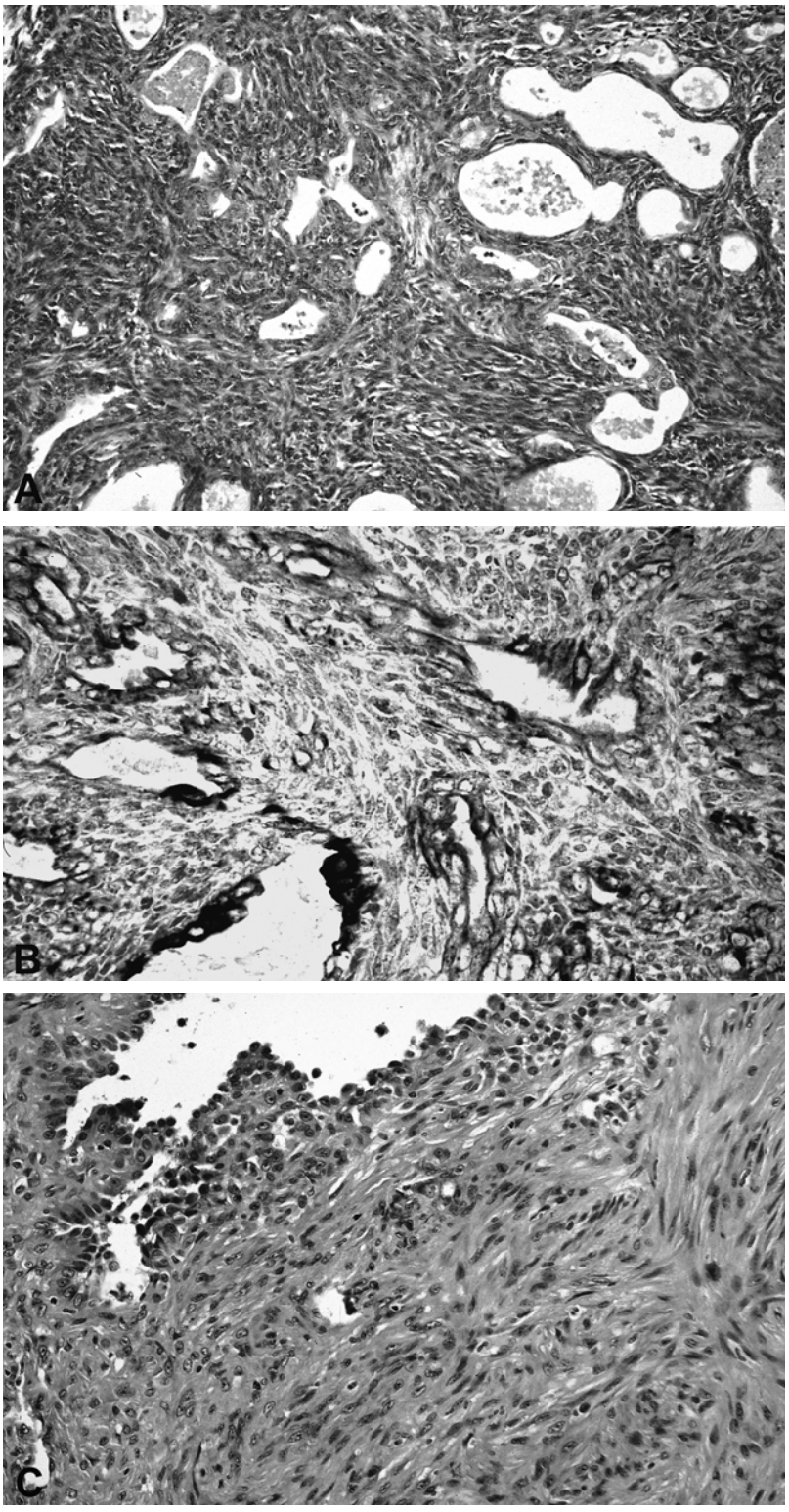


Figure 35.1. Biphasic synovial sarcoma [hematoxylin and eosin (H&E)] (A) exhibiting strong keratin immunoreactivity (B) of the epithelioid (glandular) component. Biphasic malignant mesothelioma (H&E) (C) showing both epithelioid and sarcomatoid components.

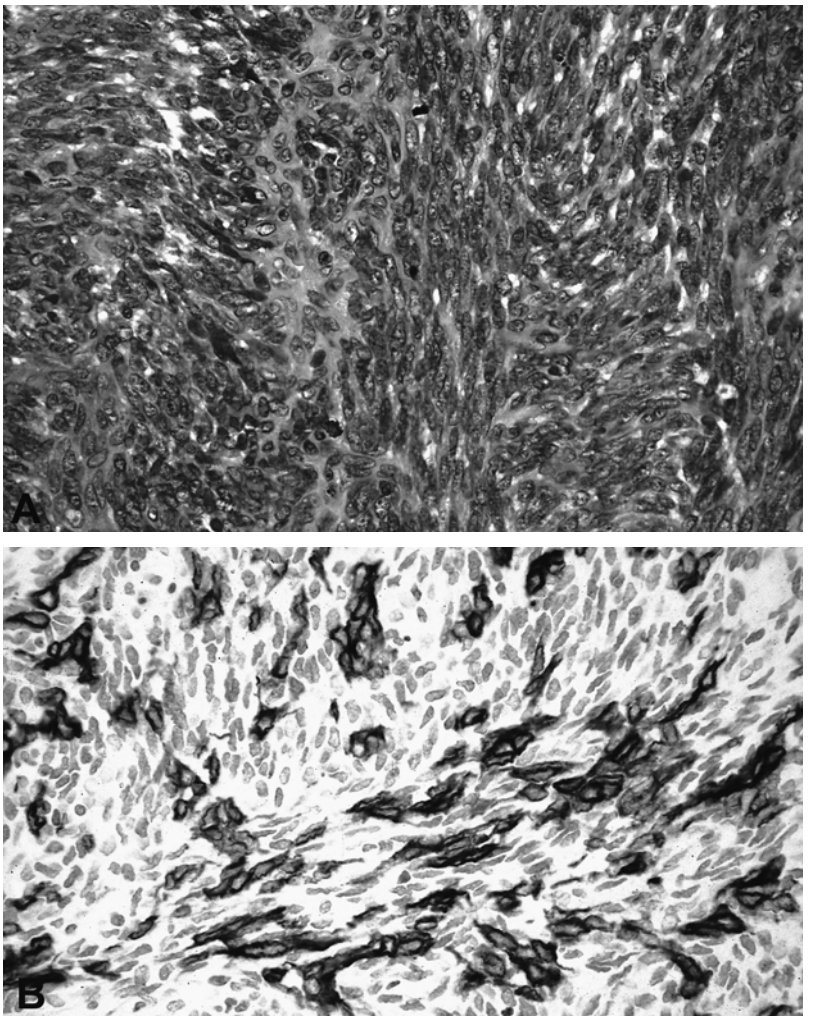


Figure 35.2. Monophasic synovial sarcoma (H&E) (A) expressing focal keratin immunoreactivity (B) in the spindle cells. In comparison, a sarcomatoid malignant mesothelioma (H&E) (C) exhibiting strong and diffuse keratin immunoreactivity (D).

membrane antigen also reacts in a similar pattern as cytokeratins in synovial sarcomas except it is expressed in a more prominent, often linear fashion. MIC-2 (CD99) and bcl-2 have been shown to be useful markers in the diagnosis of synovial sarcoma, exhibiting immunoreactivity in the majority of cases (24,56,57). However, a few reports have shown variable positivity for both markers in cases of mesothelioma (58,59). It is important to keep in mind when the differential diagnoses include malignant peripheral nerve sheath tumor and spindle cell melanoma that S-100 protein may be detected in approximately 30% of synovial sarcomas. Rare cells in malignant mesothelioma can also express S-100 antigen. Ultrastructurally, the epithelial component exhibits features similar to adenocarcinoma (60), including terminal bar and

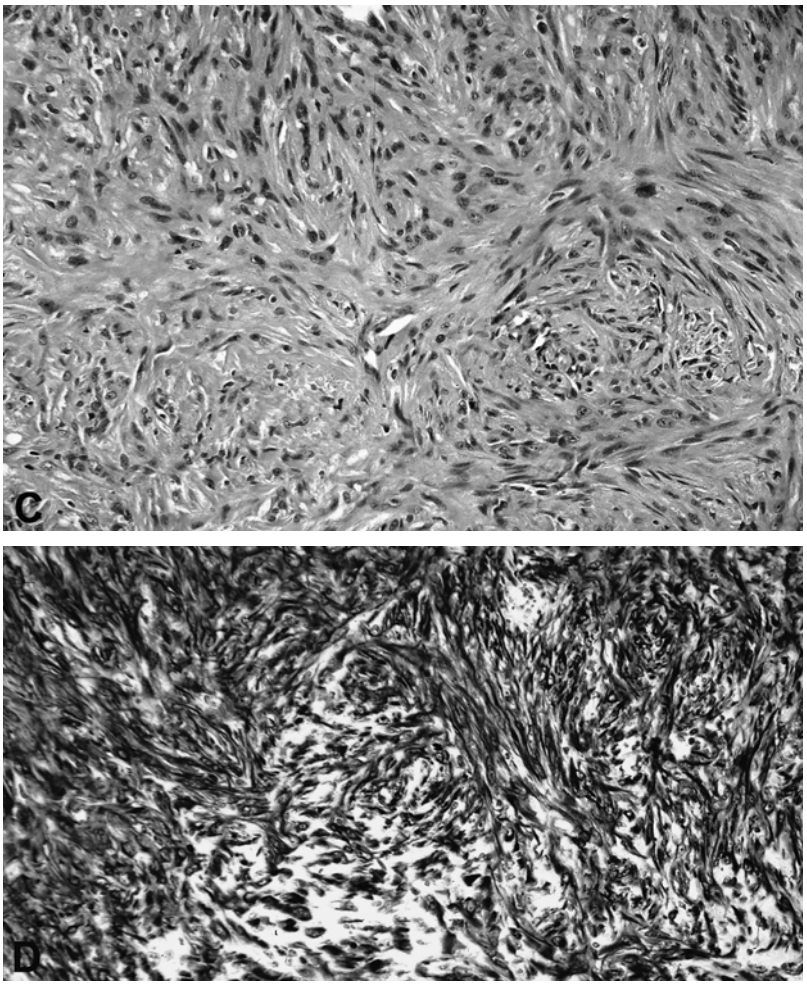


Figure 35.2. *Continued*

desmosomal junctional complexes, external lamina, and surface microvilli protruding into the glandular lumen. In the monophasic synovial sarcoma, the ultrastructural features may be nonspecific, but occasional abortive lumina with projecting microvilli may be seen with thorough examination. Of course, t(X;18)(p11;q11) is the cytogenetic hallmark of synovial sarcoma, being present in nearly all cases (61,62).

Epithelioid Hemangioendothelioma/Angiosarcoma Versus Malignant Mesothelioma

Although primary malignant vascular tumors of the serous membranes are rare, both epithelioid hemangioendothelioma and angiosarcoma of the pleura have been described (10,11,63,64). Endothelial tumors arising near serous membranes can mimic an epithelioid malignant mesothelioma histologically and clinically (Fig. 35.3). Although these

are regarded as morphologically and biologically distinct entities, representing the low- and high-grade forms of malignant vascular tumors, it is increasingly recognized that it is difficult to distinguish between an epithelioid angiosarcoma and high-grade epithelioid hemangioendothelioma. In addition, both tumors, when arising within the pleura, have been associated with high morbidity and mortality (10). However, the diagnostic distinction may still be important as further studies determine which histologic features of epithelioid hemangioendothe-

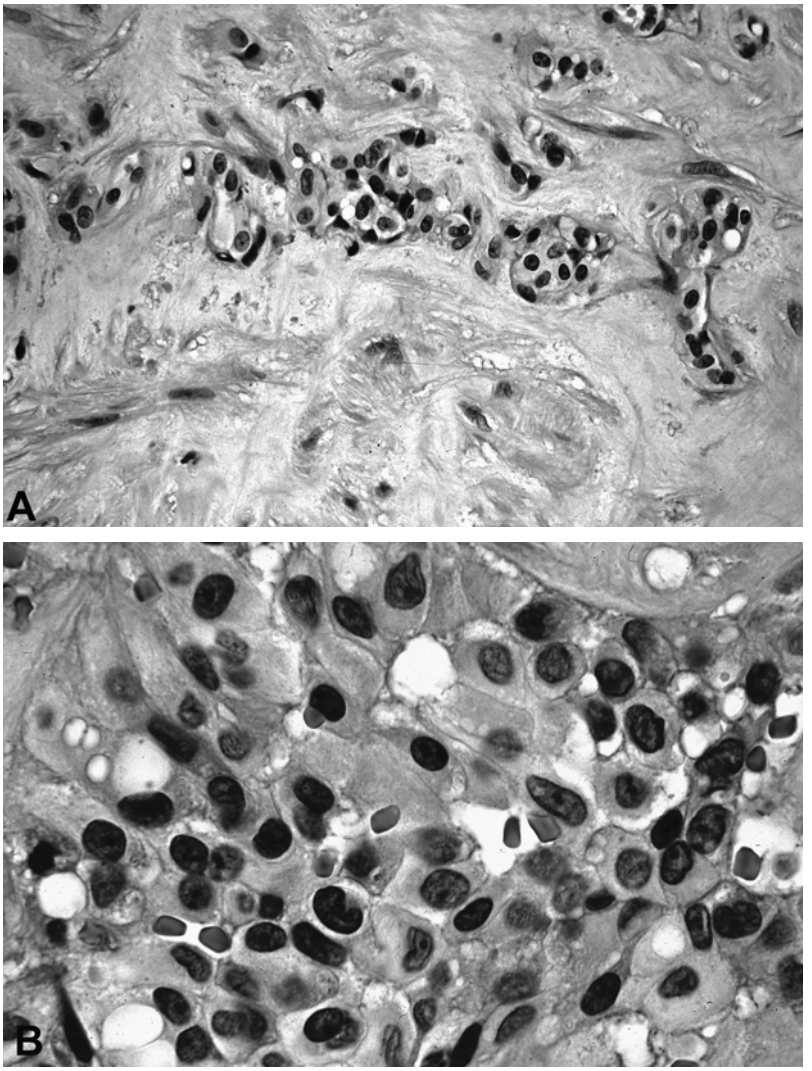


Figure 35.3. Epithelioid hemangioendothelioma showing the typical myxohyaline matrix (H&E) (A) with aggregates of epithelioid tumor cells exhibiting intracytoplasmic lumina with entrapped red blood cells (H&E) (B). Strong CD31 immunoreactivity (C) reflects the endothelial nature of the epithelioid tumor cells. Note the cytohistologic similarities to an epithelioid malignant mesothelioma (H&E) (D).

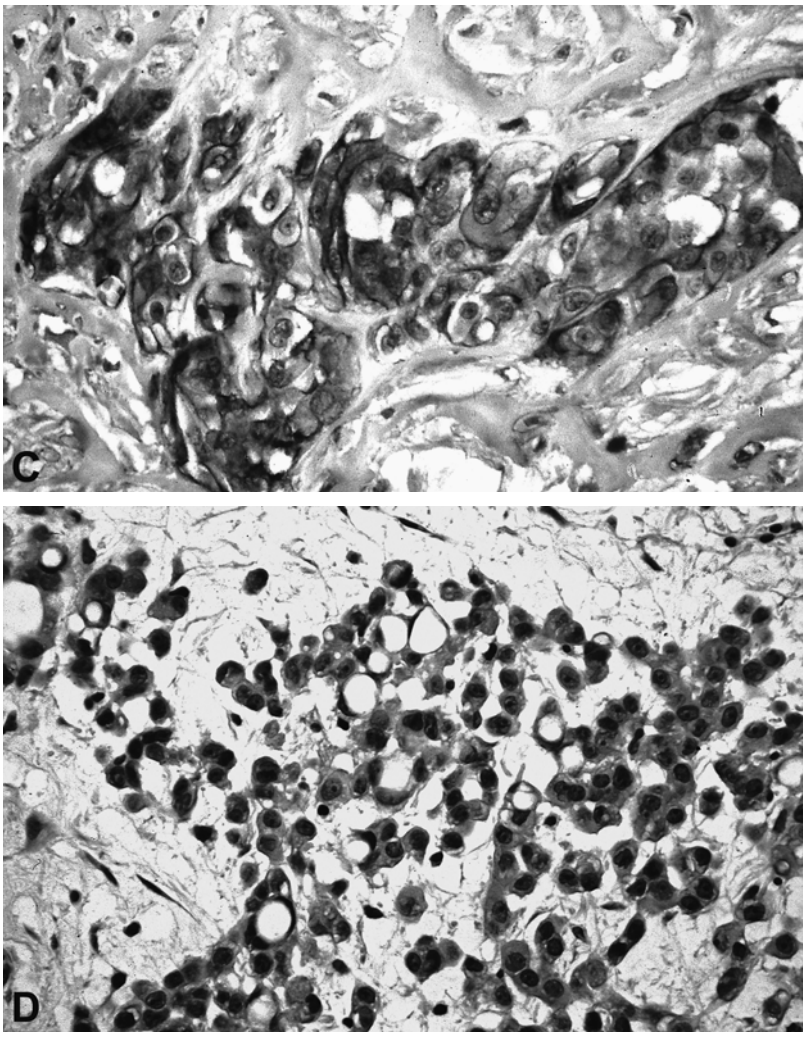


Figure 35.3. *Continued*

lioma have an impact on prognosis. We have seen a case of epithelioid hemangioendothelioma where the patient survived 8 years after pleurectomy. Epithelioid tumor cells with prominent nucleoli and cytoplasmic vacuoles characterize both tumors. The vacuoles do not contain hyaluronic acid, glycogen, lipid, or mucin, but may contain an entrapped red blood cell, reflecting primitive lumen formation (Fig. 35.3). Epithelioid angiosarcoma is histologically a more aggressive-looking tumor with large, vesicular nuclei and frequent mitotic figures. The myxohyaline matrix is typical for epithelioid hemangioendothelioma and not seen in epithelioid angiosarcoma. Both tumors may exhibit a tubulopapillary growth pattern with diffuse extension over the pleural surfaces similar to that seen in an epithelioid mesothelioma. Hence, the term *pseudomesotheliomatous angiosarcoma* or *hemangioendothelioma* was coined. As opposed to epithelioid mesotheliomas,

epithelioid angiosarcoma or hemangioendothelioma shows only focal and inconsistent immunoreactivity to cytokeratin antibodies (63,65). The tumor cells also coexpress endothelial markers, including CD31 (Fig. 35.3), CD34, and von Willebrand factor. Ultrastructurally, the tumor cells demonstrate abundant intermediate filaments, micropinocytotic vesicles, abortive lumena, interrupted basal lamina, and Weibel-Palade bodies.

Solitary Fibrous Tumor Versus Malignant Mesothelioma

Solitary fibrous tumor is the most common benign mesenchymal pleural neoplasm and must be distinguished from a sarcomatoid malignant mesothelioma. Although first described by Klemperer and Rabin (66) as a localized fibrous mesothelioma, subsequent studies demonstrated that the tumor cells did not exhibit features of mesothelial cells but showed fibroblastic differentiation (56,67,68). The tumor most often occurs in adults between the fifth and sixth decade of life. Extrapleural solitary fibrous tumors are also well recognized. Most patients are asymptomatic but may experience hypoglycemia, which is characteristically associated with some cases of solitary fibrous tumor (69). As opposed to mesotheliomas, solitary fibrous tumors are typically well circumscribed and often partially encapsulated, or may be attached to the pleura by a short pedicle. Histologically, solitary fibrous tumors exhibit a so-called patternless architecture characterized by a combination of alternating hypocellular, collagenous, and hypercellular spindle cell areas with hemangiopericytoma-like vasculature (Fig. 35.4).

The tumor is composed of bland spindle cells with scant cytoplasm and vesicular nuclei. The spindle cell component can adopt a variety of growth patterns that may be mistaken for other tumors, including a storiform pattern reminiscent of fibrohistiocytic tumors; hemangiopericytic, angiofibromatous, herringbone, neural-type, and fascicular patterns similar to fibrosarcoma; and malignant peripheral nerve sheath tumor or monophasic synovial sarcoma. The hypocellular areas can exhibit prominent myxoid change, and, rarely, metaplastic bone formation, mature adipocytes, and multinucleated stromal giant cells. Mitotic activity is generally low. Malignant solitary fibrous tumors are usually hypercellular with cytologic atypia, necrosis, infiltrative margins, and increased mitotic activity (more than four mitoses per 10 high-power fields), although the cut-off point between a benign and malignant solitary fibrous tumor is not established. On a small biopsy, it can be extremely difficult to differentiate a solitary fibrous tumor from a mesothelioma or other mesenchymal tumors. Immunohistochemistry has an important diagnostic role in this differentiation. Tumor cells in solitary fibrous tumors are characteristically immunoreactive with CD34 (70–73) (Fig. 35.4). In addition, MIC-2 (CD99) is present in a high percentage of cases (74). Variable positivity is seen with bcl-2 (56,75) and smooth muscle actin. Focal and limited reactivity is occasionally seen for S-100, and desmin. Mesothelioma is

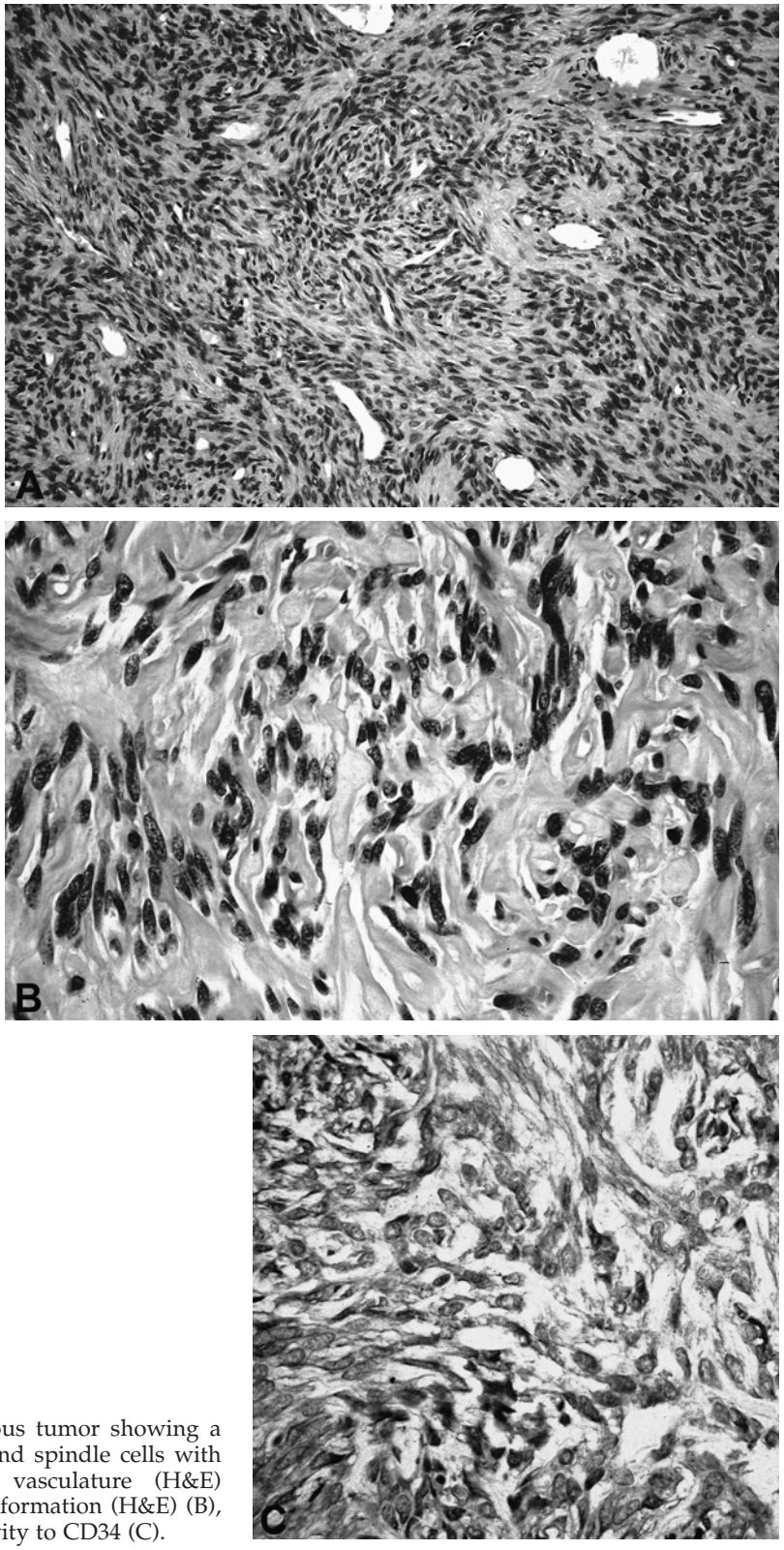


Figure 35.4. Solitary fibrous tumor showing a hypercellular focus of bland spindle cells with hemangiopericytoma-like vasculature (H&E) (A), intercellular collagen formation (H&E) (B), and strong immunoreactivity to CD34 (C).

typically CD34-negative and strongly positive for cytokeratin. A diagnostic problem may arise when entrapped mesothelial cells in a solitary fibrous tumor immunoreact strongly with a cytokeratin immunostain. Ultrastructurally, solitary fibrous tumors are nonspecific and often demonstrate features of fibroblasts, myofibroblasts, or, arguably, pericytes (71). Electron microscopy is of limited use in distinguishing solitary fibrous tumor from sarcomatoid mesothelioma.

References

1. Battifora H, McCaughey WTE. Tumor and pseudotumors of the serosal membrane. In: Rosai J, Sobin L, eds. Atlas of Tumor Pathology, 3rd ed. Washington DC: Armed Forces Institute of Pathology, 1995:37–47.
2. Hurlimann J. Desmin and neural marker expression in mesothelioma cells and mesothelioma. Hum Pathol 1994;25:753–757.
3. Mayall FG, Goddard H, Gibbs AR. Intermediate filament expression in mesotheliomas: leiomyoid mesotheliomas are not uncommon. Histopathology 1992;21:453–457.
4. Yousem SA, Hochholzer L. Malignant mesotheliomas with osseous and cartilaginous differentiation. Arch Pathol Lab Med 1987;111:62–66.
5. Roggli JL, Colbeck J, Sanfilipo F, et al. Pathology of human mesothelioma. Aetiologic and diagnostic consideration. Pathol Ann 1987;22:91–131.
6. Woodruff JM, Christiansen WN. Glandular peripheral nerve sheath tumors. Cancer 1993;72:3618–3628.
7. Moran CA, Suster S, Koss MN. Smooth muscle tumours presenting as pleural neoplasms. Histopathology 1995;27:227–234.
8. Nicholson AG, Goldstraw P, Fisher C. Synovial sarcoma of the pleura and its differentiation from other primary pleural tumours: a clinicopathological and immunohistochemical review of three cases. Histopathology 1998; 33:508–513.
9. Attanoos RL, Dallimore NS, Gibbs AR. Primary epithelioid haemangioendothelioma of the peritoneum: an unusual mimic of mesothelioma. Histopathology 1997;30:375–377.
10. Lin B, Colby T, Gown AM, et al. Malignant vascular tumors of the serous membranes mimicking mesothelioma. Am J Surg Pathol 1996;20:1431–1439.
11. McCaughey WTE, Dardick I, Barr JR. Angiosarcoma of serous membranes. Arch Pathol Lab Med 1983;107:304–307.
12. Wong WW, Pluth JR, Grado GL, Schild SE, Sanderson DR. Liposarcoma of the pleura. Mayo Clin Proc 1994;69:882–885.
13. Carter D, Otis CN. Three types of spindle cell tumors of the pleura. Fibroma, sarcoma, and sarcomatoid mesothelioma. Am J Surg Pathol 1988; 12:747–753.
14. England DM, Hochholzer L, McCarthy MJ. Localized benign and malignant fibrous tumors of the pleura. Am J Surg Pathol 1989;13:640–658.
15. Moran CA, Suster S, Koss MN. The spectrum of histologic growth patterns in benign and malignant fibrous tumors of the pleura. Semin Diagn Pathol 1992;9:169–180.
16. Luppi G, Cesinaro AM, Zoboli A, Morandi U, Piccinini L. Mesenchymal chondrosarcoma of the pleura. Eur Respir J 1996;9:840–843.
17. Chernow B, Sahn SA. Carcinomatous involvement of the pleura. An analysis of 96 patients. Am J Med 1977;63:695–702.

18. Battifora H. The pleura. In: Sternberg SS, ed. Diagnostic Surgical Pathology, vol 1. New York: Raven Press, 1989:829–855.
19. Montag AG, Pinkus GS, Corson JM. Keratin protein immunoreactivity of sarcomatoid and mixed types of diffuse malignant mesotheliomas: an immunoperoxidase study of 30 cases. Hum Pathol 1988;19:336–342.
20. Al-Izzi M, Thurlow NP, Corrin B. Pleural mesothelioma of connective tissue type, localized fibrous tumour of the pleura, and reactive submesothelial hyperplasia: an immunohistochemical comparison. J Pathol 1989; 157:41–44.
21. Azumi N, Battifora H, Carlson G, et al. Sarcomatous mesothelioma of pleura: immunohistochemical study. Lab Invest 1989;60:4.
22. Blobel GA, Moll R, Franke WW, et al. The intermediate filament cytoskeleton of malignant mesotheliomas and its diagnostic significance. Am J Pathol 1985;121:235–247.
23. Mayall FG, Goddard H, Gibbs AR. The diagnostic implications of variable cytokeratin expression in mesotheliomas. J Pathol 1993;170:165–168.
24. Wirth PR, Legler J, Wright GL. Immunohistochemical evaluation of seven monoclonal antibodies for differentiation of pleural mesothelioma from lung adenocarcinoma. Cancer 1991;67:655–662.
25. Brown DC, Theaker JM, Banks PM, et al. Cytokeratin expression in smooth muscle and smooth muscle tumours. Histopathology 1987;11:477–486.
26. Miettinen M, Lehto VP, Virtanen I. Keratin in the epithelial-like cells of classic biphasic synovial sarcoma. Virchow Arch 1982;40:157–161.
27. Smith TA, Machen SK, Fisher C, Goldblum JR. Usefulness of cytokeratin subsets for distinguishing monophasic synovial sarcoma from malignant peripheral nerve sheath tumor. Am J Clin Pathol 1999;112:641–648.
28. Bishop PW, Menasce LP, Yates AJ, et al. An immunophenotypic survey of malignant melanomas. Histopathology 1993;23:159–166.
29. Zarbo RJ, Gown AM, Nagle RB, et al. Anomalous cytokeratin expression in malignant melanoma: one- and two-dimensional Western blot analysis and immunohistochemical survey of 100 melanomas. Mod Pathol 1990;3: 494–501.
30. Moran CA, Travis WD, Rosado-de-Christenson ML, Koss ML, Rosai J. Thymomas presenting as pleural tumors: report of eight cases. Am J Surg Pathol 1992;16:138–144.
31. Payne CB, Morningstar WA, Chester EH. Thymoma of the pleura masquerading as diffuse mesothelioma. Am Rev Respir Dis 1966;94:441–446.
32. Shih D, Wang J, Tseng H, et al. Primary pleural thymoma. Arch Pathol Lab Med 1997;121:79–82.
33. Chan JK, Tsang WY, Seneviratne S, Pau MY. The MIC2 antibody 013. Practical application for the study of thymic epithelial tumors. Am J Surg Pathol 1995;10:1115–1123.
34. Brocheriou I, Carnot F, Briere J. Immunohistochemical detection of bcl-2 protein in thymoma. Histopathology 1995;27:251–255.
35. Robertson PB, Neiman RS, Worapongpaiboon S, John K, Orazi A. 013 (CD99) positivity in hematologic proliferations correlates with TdT positivity. Mod Pathol 1997;10:277–282.
36. Oury TD, Hammar SP, Roggli VL. Ultrastructual features of diffuse malignant mesotheliomas. Hum Pathol 1998;29:1382–1392.
37. Amble FR, Olsen KD, Nascimento AG, Foote RL. Head and neck synovial sarcoma. Otolaryngol Head Neck Surg 1992;107:631–637.
38. Grayson W, Nayler SJ, Jena GP. Synovial sarcoma of the parotid gland. A case report with clinicopathologic analysis and review of the literature. A Afr J Surg 1998;36:32–34.

39. Shmookler BM, Enzinger FM, Brannon RB. Orofacial synovial sarcoma. A clinicopathologic study of 11 new cases and review of the literature. Cancer 1982;50:267–276.
40. Cataldi A, Orlassino R, Novero D. Synovial sarcoma of the mediastinum. Report of a case. Radiol Med 1994;87:719–721.
41. Peoch M, LeMarchardour F, Bost F, et al. Primary pulmonary sarcoma of the mediastinum. A case report with immunohistochemistry, ultrastructural, and cytogenetic study. Ann Pathol 1995;15:203–206.
42. Witkin GB, Miettinen M, Rosai J. A biphasic tumor of the mediastinum with feature of synovial sarcoma. A report of four cases. Am J Surg Pathol 1989; 13:490–499.
43. Hisaoka M, Hashimoro H, Iwamasa T, Ishikawa K, Aoki T. Primary synovial sarcoma of the lung: report of two cases confirmed by molecular detection of SYT-SSX fusion gene transcripts. Histopathology 1999;34:205–210.
44. Kaplan MA, Goodsman MD, Satish J, Bhagavan BS, Travis WD. Primary pulmonary sarcoma with morphologic features of monophasic synovial sarcoma and chromosome translocation t(X;18). Am J Clin Pathol 1996; 105:195–199.
45. Keel SB, Bacha E, Mark EJ, Nielsen SP, Rosenberg AE. Primary pulmonary sarcoma: a clinicopathologic study of 26 cases. Mod Pathol 1999;12:1124–1131.
46. Koss M, Travis W, Moran C. Pulmonary sarcomas, blastoma, carcinosarcomas, and teratomas. In: Hasleton PS, ed. Spencer's Pathology of the Lung, 5th ed. New York: McGraw Hill, 1996:1065–1109.
47. Roberts CA, Seemayer TA, Neff JR, Alonso A, Nelson M, Bridge JA. Translocation (X;18) in primary synovial sarcoma of the lung. Cancer Genet Cytogenet 1996;88:49–52.
48. Yoon GS, Park SY, Kang GH, Kim OJ. Primary pulmonary sarcoma with morphologic features of biphasic synovial sarcoma: a case report. J Korean Med Sci 1998;13:71–76.
49. Nicholson AG, Rigby M, Lincoln C, Meller S, Fisher C. Synovial sarcoma of the heart. Histopathology 1997;30:349–352.
50. Billings SD, Meisner LF, Cummings DW, Tejada E. Synovial sarcoma of the upper digestive tract: a report of two cases with demonstration of the (X;18) translocation by fluorescence in situ hybridization. Mod Pathol 2000;13: 68–76.
51. Nielson GP, Shaw PA, Rosenberg AE, Dickersin GR, Young RH, Scully RE. Synovial sarcoma of the vulva: a report of two cases. Mod Pathol 1996;9: 970–974.
52. Gaertner E, Zeren H, Colby TV, et al. Biphasic synovial sarcomas arising in the pleural cavity: a clinicopathologic study of five cases. Am J Surg Pathol 1996;20:36–45.
53. Jawahar DA, Vuletin JC, Gorecki P, et al. Primary biphasic synovial sarcoma of the pleura. Respir Med 1997;91:568–570.
54. Essary LR, Vargas SO, Fletcher CDM. Primary pleuropulmonary synovial sarcoma: reappraisal of a recently described anatomic subset. Cancer 2002;94:459–469.
55. Zeren H, Moran CA, Suster S, Fishback NF, Koss MN. Primary pulmonary sarcomas with features of monophasic synovial sarcomas: a clinicopathological, immunohistochemical, and ultrastructural study of 25 cases. Hum Pathol 1995;26:474–480.
56. Chilosi M, Facchetti F, Tos APD, et al. bcl-2 expression in pleural and extrapleural solitary fibrous tumours. J Pathol 1997;181:362–367.

57. Dei Tos AP, Wadden C, Calonje E, et al. Immunohistochemical demonstration of glycoprotein p30/32^{MIC2} (CD99) in synovial sarcoma. A potential cause of diagnostic confusion. Appl Immunohistochem 1995;3:168–173.
58. Segers K, Ramael M, Singh SK, et al. Immunoreactivity for bcl-2 protein in malignant mesothelioma and non-neoplastic mesothelium. Virchows Arch 1994;424:631–634.
59. Stevenson AJ, Chatten J, Bertoni F, Miettinen M. (p30/32^{MIC2}) neuroectodermal/Ewing's sarcoma antigen as an immunohistochemical marker: review of more than 600 tumors and the literature experience. Appl Immunohistochem 1994;2:231–240.
60. Fisher C. Synovial sarcoma: ultrastructural and immunohistochemical features of epithelial differentiation in monophasic and biphasic tumors. Hum Pathol 1986;17:996–1008.
61. Limon J, Mrozek K, Mandahl N, et al. Cytogenetics of synovial sarcoma. Presentation of 10 new cases and review of the literature. Genes Chromosomes Cancer 1991;3:338–345.
62. Smith S, Reeves BR, Wong L, Fisher C. A consistent chromosome translocation in synovial sarcoma. Cancer Genet Cytogenet 1987;26:179–180.
63. Falconieri G, Bussani R, Mirra M, Zanella M. Pseudomesotheliomatous angiosarcoma: a pleuropulmonary lesion simulating malignant pleural mesothelioma. Histopathology 1997;30:419–424.
64. Zhang PJ, Livolsi VA, Brooks JJ. Malignant epithelioid vascular tumors of the pleura: a report of a series and literature review. Hum Pathol 2000;31:29–34.
65. Gray MH, Rosenberg AE, Dickersin GR, et al. Cytokeratin expression in epithelioid vascular neoplasms. Hum Pathol 1990;21:212–217.
66. Klemperer P, Rabin CB. Primary neoplasms of the pleura. Report of five cases. Arch Pathol 1937;11:385–412.
67. Burrig KF, Kastendieck H. Ultrastructural observations on the histogenesis of localized fibrous tumors of the pleura (benign mesothelioma). Virchows Arch 1984;403:413–424.
68. Scharifker D, Kaneko M. Localized fibrous "mesothelioma" of pleura (submesothelial fibroma): a clinicopathologic study of 18 cases. Cancer 1979;43:627–635.
69. Dotan ZA, Mor Y, Olchovsky D, et al. Solitary fibrous tumor presenting as perirenal mass associated with hypoglycemia. J Urol 1999;162:2087–2088.
70. Brunnemann RB, Ro JY, Ordonez NG, Mooney J, El Naggar AK, Ayala AG. Extrapleural solitary fibrous tumor: a clinicopathologic study of 24 cases. Mod Pathol 1999;12:1034–1042.
71. Mentzel T, Bainbridge TC, Katenkamp D. Solitary fibrous tumor: clinicopathological, immunohistochemical, and ultrastructural analysis of 12 cases arising in soft tissues, nasal cavity and nasopharynx, urinary bladder and prostate. Virchows Arch 1997;430:445–453.
72. Nielson GP, O'Connell JX, Rosenberg AE. Solitary fibrous tumor of soft tissue: a report of 15 cases, including 5 malignant examples with light microscopic, immunohistochemical, and ultrastructural data. Mod Pathol 1997;10:1028–1037.
73. Suster S, Nascimento AG, Mietinnen M, Sickel JZ, Moran CA. Solitary fibrous tumors of soft tissue. A clinicopathologic and immunohistochemical study of 12 cases. Am J Surg Pathol 1995;19:1257–1266.
74. Renshaw AA. 013 (CD99) in spindle cell tumors. Reactivity with hemangiopericytoma, solitary fibrous tumor, synovial sarcoma, and meningioma

but rarely with sarcomatoid mesothelioma. Appl Immunohistochem 1995; 3:250–256.

75. Suster S, Fisher C, Moran CA. Expression of bcl2 oncoprotein in benign and malignant spindle cell tumors of soft tissue, skin, serosal surfaces, and gastrointestinal tract. Am J Surg Pathol 1998;22:863–872.

36

Diagnosis of Synovial Sarcoma of the Pleura and Differentiation from Malignant Mesothelioma

Amy Powers and Michele Carbone

Synovial sarcomas (SSs) are soft tissue tumors that occur primarily in adolescents and young adults between the ages of 15 and 40 (1). The tumors comprise 5% to 10% of all soft tissue sarcomas, and most commonly arise in extremities in the vicinity of large joints. Rare cases have also been reported in virtually every anatomic site, including the head and neck, lung, heart, mediastinum, abdominal wall, central nervous system (CNS), prostate, and pleura. Synovial sarcomas do not arise from synovium, as the name implies. Instead, they are thought to arise from primitive mesenchymal cells, which explains their development in locations devoid of synovium (1).

Synovial sarcomas of the pleura usually represent metastatic disease, but more than 20 primary SSs of the pleura have been reported in the English-language literature, making these tumors a rare but important diagnostic consideration (Table 36.1) (2–10). The origin of pleural synovial sarcomas may be undifferentiated submesothelial mesenchyme, which could undergo differentiation toward epithelial or spindle cells. The rarity of primary synovial sarcoma of the pleura and its morphologic similarity to malignant mesothelioma (MM), the most common primary malignant pleural lesion, make it a difficult and easily overlooked diagnosis. This chapter discusses the differences between the two entities.

Clinical History

Clinical features can be useful in distinguishing between MM and SS of the pleura. However, because there is considerable overlap between these two entities, clinical findings alone are not always reliable in making a diagnosis of SS versus MM.

Synovial sarcomas have no significant gender predilection, while mesotheliomas are more common in males (1). Synovial sarcomas also tend to occur in younger patients. Of 23 primary SSs of the pleura reported in the literature, the average age was 37 (range 9–77). Mesothe-

Table 36.1. Primary pleural synovial sarcomas: clinical and pathological features

Author (reference)	Patient age	Sex	Histopathology	Molecular studies
Jawahar et al (3)	18	F	Biphasic	t(X;18)
Gaertner et al (4)	17	F	Biphasic	NP
	17	F	Biphasic	NP
	50	M	Biphasic	NP
	9	M	Biphasic	NP
	32	F	Biphasic	NP
Aubry et al (2)	33	M	Monophasic	t(X;18)
	41	M	Monophasic	t(X;18)
	41	M	Monophasic	t(X;18)
	49	F	Monophasic	t(X;18)
	69	M	Monophasic	t(X;18)
Nicholson et al (5)	42	M	Biphasic	NP
	28	M	Monophasic	NP
	42	M	Monophasic	NP
Carbone et al (10)	74	M	Biphasic	t(X;18)
Colwell et al (6)	39	M	Monophasic	Negative
	23	F	Monophasic	t(X;18)
	33	M	Biphasic	Indeterminant
Essary et al (7)	30	M	Monophasic	NP
	32	F	Monophasic	NP
Hirano et al (9)	46	F	Biphasic	NP
Ng et al (11)	15	M	Monophasic	t(X;18)
Chan et al (8)	77	F	Not Stated	NP

NP, not performed.

liomas, in contrast, typically develop in patients 50 to 70 years of age and are rarely seen in adolescents and young adults. Occasional primary pleural SSs have been reported in older adults, however. Aubry et al (2) reported a monophasic SS in a 69-year-old man, and Carbone et al (10) detailed the development of a biphasic SS in a 74-year-old man. A primary pleural SS in an old individual was also reported by Chan et al (8), but the histologic subtype was not specified. Thus, a diagnosis of SS should not be ruled out based on old age alone. Chan et al suggested that SSs in older individuals (>60 years of age) are more likely to have unusual histologic patterns and poorly differentiated morphology, which can make diagnosis more challenging. In addition, these higher grade lesions are typically associated with aggressive behavior and frequent metastasis.

Synovial sarcomas typically grow at a faster rate and present radiologically as a discrete, localized mass with or without associated pleural thickening. A pseudocapsule is occasionally present (4). In contrast, MMs grow slowly, more commonly present as diffuse pleural thickening or multiple pleural nodules, and do not have capsules or pseudocapsules. Localized MMs are extremely rare, and the presence of a localized pleural-based mass should instead raise suspicion of a soft tissue tumor. Pleural effusions, although more common in MM, have been reported in both malignancies and do not reliably rule out SS (4).

A clinical history of asbestos exposure in an individual with a pleural-based tumor should raise the suspicion of an MM. However, pleural SSs have also been reported to occur in asbestos-exposed individuals, and one should be cautious not to jump to a diagnosis of MM based on history alone (10).

Gross Pathology

A thorough macroscopic examination must be performed when differentiating between these malignancies, as they tend to have distinct gross morphologies. Gross examination of 22 reported cases of pleural synovial sarcoma demonstrated both solid and cystic masses ranging in size from 4.5 to 25 cm (2–7,9–11). Occasionally, pleural SSs have been reported to encase a lobe (2) or even an entire hemithorax (10), but they typically form localized, pleural-based masses with or without pedicles. In rare cases of SS, multifocal patterns have also been reported (6). Synovial sarcomas tend to be gray-white and fleshy, and frequently have associated hemorrhage, necrosis, and calcification. Pseudocapsulation was reported in several tumors. These tumors may have associated pleural thickening, and one tumor was associated with pleural plaques (10).

Malignant mesotheliomas, in contrast, typically present as multiple nodules covering the pleura or as a diffuse sheet-like pleural thickening that can encase and compress the lungs (1). The tumor may extend superficially into the lungs, or along needle biopsy tracts. Localized, solitary discrete masses, in contrast to SS, are extremely rare. The appearance of MM is also typically gray-white, and can vary from firm and rubbery to soft and gelatinous. There may be foci of hemorrhage and necrosis, and this tumor is typically associated with pleural plaques due to its strong association with asbestos exposure (1).

Histopathology

Malignant mesotheliomas of the pleura typically have an epithelioid, biphasic, or sarcomatoid pattern. Synovial sarcomas, like MMs, also exhibit biphasic or sarcomatoid morphology. Theoretically, a monophasic epithelial SS should exist, but this variant could be reliably diagnosed only by using cytogenetic data (1). Since no monophasic epithelial SS of the pleura have been reported, only the biphasic and sarcomatoid variants of these tumors are considered in this discussion.

Histologically, biphasic SS and biphasic MM exhibit subtle differences (4,12). Biphasic SS (Fig. 36.1) have a long interweaving spindled component that is compact and cellular, with little stromal collagen. Foci of hemiangiopericytomatous architecture (Figs. 36.1A and 36.2) and of microcalcification (Fig. 36.1D) are characteristic, and hyaline fibrosis can be present. Mast cells are often prominent, but glycogen is sparse. In contrast, the spindled component of biphasic MM consists of shorter, looser fascicles of blunt spindle cells with more stromal collagen. Hyaline fibrosis and hemangiopericytomatous architecture are

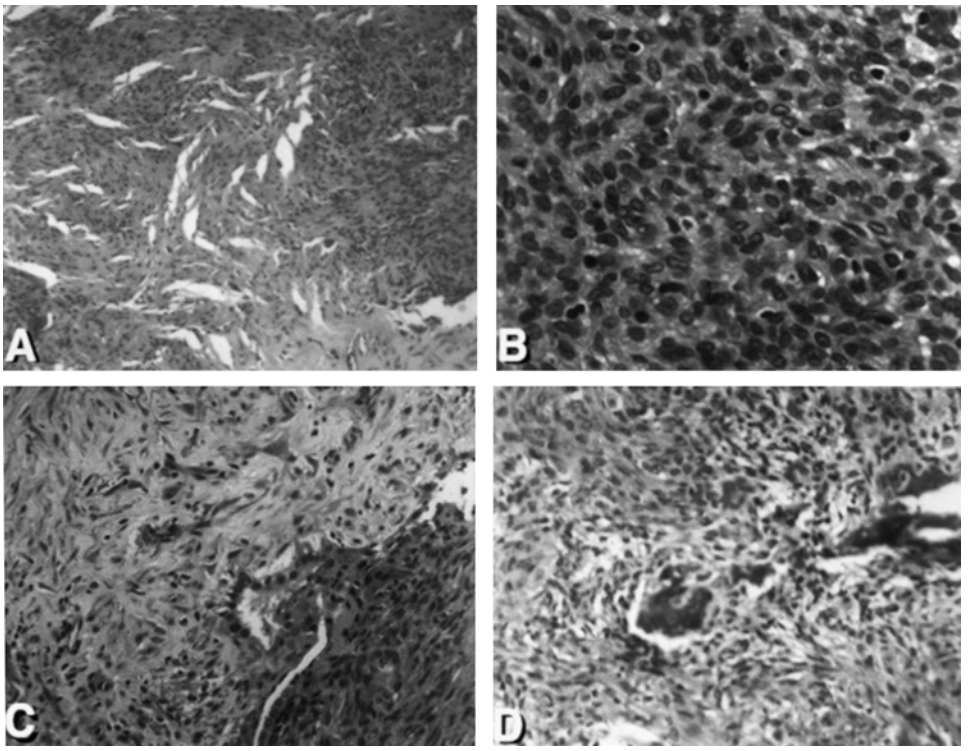


Figure 36.1. Histology of synovial sarcoma (SS). A: Hemgiopericytomatous appearance (100×). B: Sarcomatoid SS. C: Biphasic SS, focus of epithelioid differentiation. D: Sarcomatoid SS, focus of microcalcification.

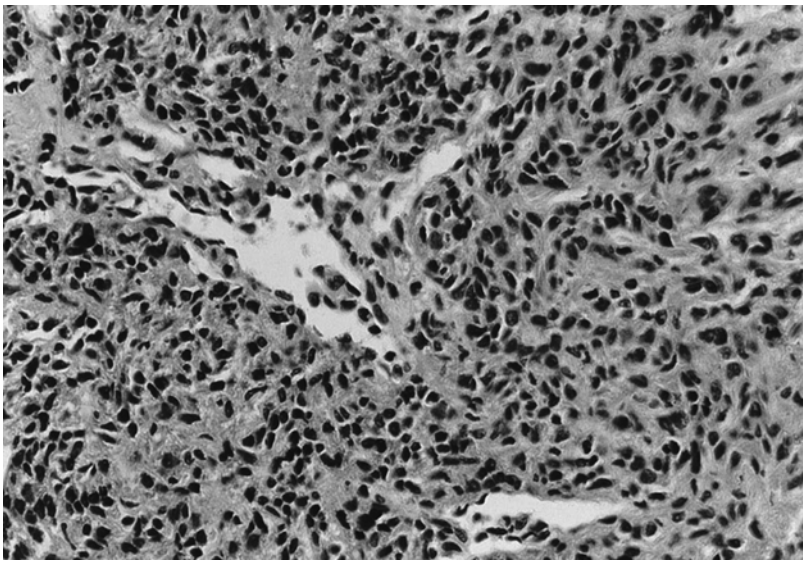


Figure 36.2. Histology of SS. Hemangiopericytomatous appearance at high magnification (400×).

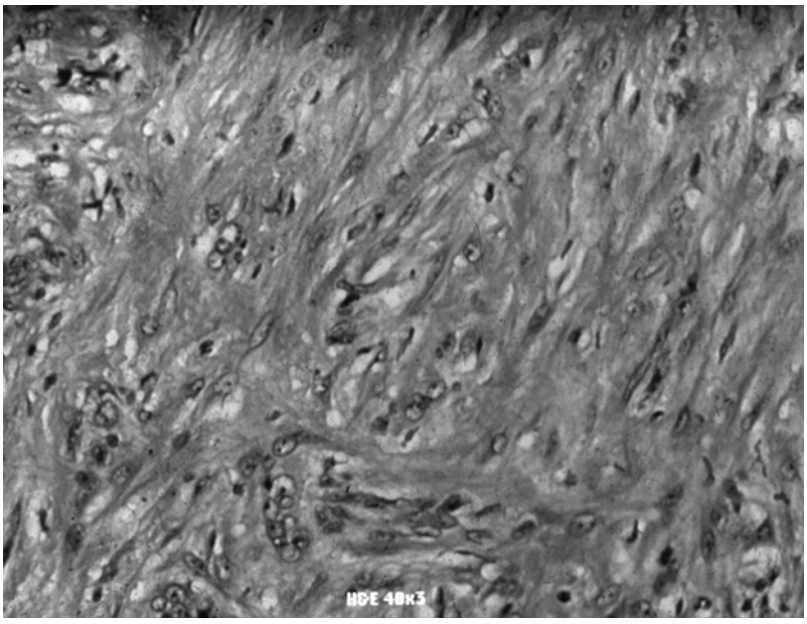


Figure 36.3. Desmoplastic mesothelioma. Compare with histology of sarcomatoid SS shown in Figure 36.1.

rare. Mast cells are also fewer in number, but glycogen is abundant (4,12). The epithelial component of biphasic SS typically consists of epithelial cells forming cleft-like glandular spaces and tubulopapillary structures. The epithelial component of well-differentiated biphasic mesotheliomas can also be tubulopapillary, but there is typically a gradual transition between the sarcomatous and epithelial elements in these tumors, while there is a sharp abutment of these areas in SS (1).

Sarcomatoid MMs, similar to the spindle component in biphasic variants, usually consist of short blunt fascicles of pleomorphic tumor cells (Fig. 36.3). The fascicles may be poorly formed, and cells can have abundant eosinophilic cytoplasm. Sarcomatoid MM rarely displays a fibrosarcomatous or hemangipericytomatous pattern. This is distinct from monophasic SSs, which are composed of longer interweaving fascicles of densely packed, mildly pleomorphic, and overlapping spindle cells with a high mitotic rate. Moreover, abundant dense collagen deposition among sparse sarcomatoid and/or gland-like epithelioid structures, characteristic of desmoplastic mesothelioma (Fig. 36.3), is not seen in SS. Monophasic SSs may exhibit a fibrosarcomatous or hemangiopericytomatous pattern, and the presence of either of these two patterns in a pleural-based lesion should immediately raise suspicion for SS (1,12).

Mucin

Mucin staining is typically not performed in the differentiation between pleural SS and MM, but some authors have observed useful differences in staining. In contrast to MM, pleural SSs contain secretions

that are mucicarmine positive and hyaluronidase resistant, and periodic acid-Schiff (PAS) positive and diastase resistant. Rare MM may exhibit mucicarmine or PAS staining, but it is eliminated with hyaluronidase or diastase digestion (4,12).

Immunohistochemistry

Immunohistochemistry (IHC) plays a limited role in the distinction of SS from MM, since there are presently no immune markers that are unique for either entity. We recommend that a panel of markers be used to support a diagnosis of either of these tumors, which should include cytokeratins, calretinin, WT-1, Bcl-2, CD56, and CD99.

Both SS and MM display immunoreactivity for vimentin and pancytokeratin. The former, although nonspecific is useful to verify the immunoreactivity of the tissue (i.e., almost everything stains for vimentin, Fig. 36.4A). It is our experience and that of others that nearly 100% of MMs are diffusely positive for cytokeratin (10,13); >90% of SSs (Fig. 36.4B) also display focal reactivity, which is most pronounced in the epithelioid component (1). While poorly differentiated SSs are less likely to exhibit cytokeratin positivity, as many as 50% have been

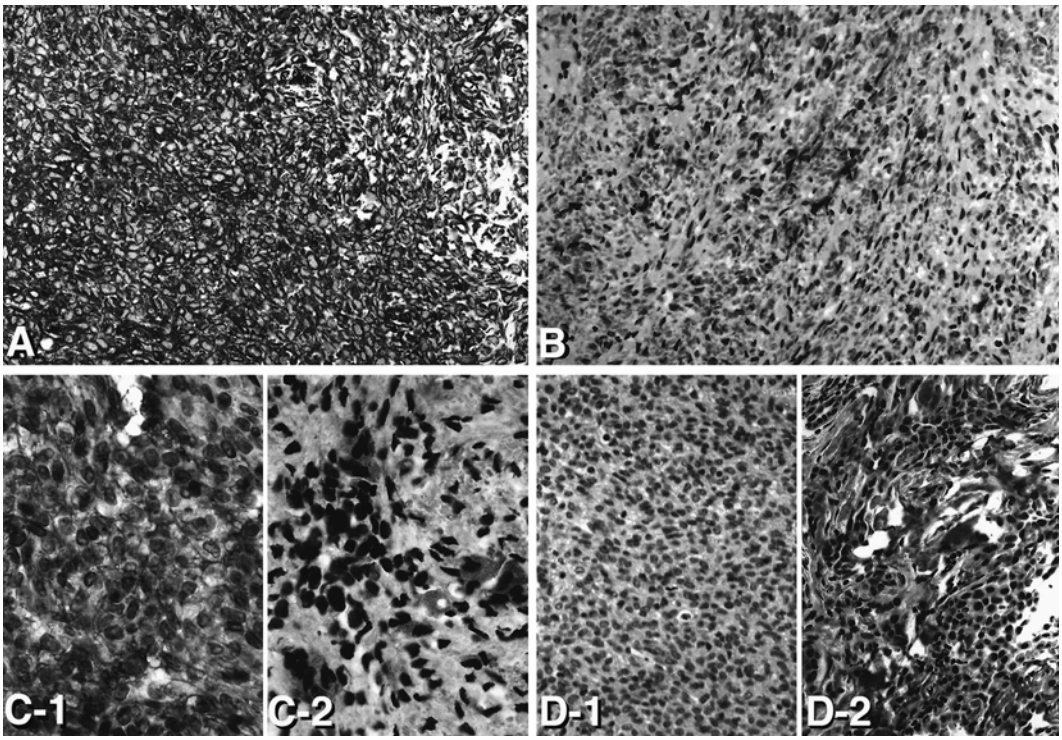


Figure 36.4. Immunoreactivity of SS. A: Vimentin, positive (200×). B: Cytokeratin 5/6. Note positivity of superficial and entrapped reactive mesothelial cells. Tumor cells are mostly negative (100×). C1: CD99, positive (400×). C2: Bcl-2, focally positive on spindle tumor cells (200×). D1: WT-1, negative (200×). D2: WT-1, fibrous MM positive control (200×).

shown to express focal keratin positivity (14). Thus, it appears that while the value of cytokeratin alone is limited in differentiating between these two tumors, focal positivity, rather than diffuse staining, is suggestive of SS.

Like the cytokeratins, the use of calretinin to differentiate between these two tumors is limited. It is well established that both epithelioid and sarcomatoid MM express calretinin. Aubry et al (2) observed calretinin reactivity in 44 of 44 mesotheliomas (36 epithelial, five biphasic, and four sarcomatoid). In biphasic mesotheliomas, staining was seen in both the epithelial (3–4+) and spindle cells (2+). Like MM, SS can also express this marker. Miettinen et al (15) demonstrated calretinin positivity in 71% of biphasic SS, 52% of monophasic SS, and 56% of poorly differentiated SS.

While both SS and MM express calretinin, it has been suggested that this marker may be of some value in differentiating between biphasic variants of these tumors. Cappello and Barnos (12) observed calretinin reactivity (2–3+) in the epithelial component of four of four biphasic MMs. The spindled component was negative in four of four MMs. In contrast, they observed staining (1–2+) in the spindled component in four of four biphasic SSs, while the epithelial component was weakly positive (1+) in only one of four. Thus, they concluded that strong diffuse calretinin staining in the epithelial component of a biphasic tumor with or without staining of the spindle cells is more indicative of an MM than an SS.

In contrast to cytokeratin and calretinin, WT-1 appears to be a more useful marker in differentiating between SS and MM (Fig. 36.4D1,2). Miettinen et al (15) found that none of 18 biphasic SSs, none of 31 monophasic SSs, and none of 11 poorly differentiated SSs expressed WT-1. In contrast, 12 of 17 epithelioid MMs expressed WT-1. Similarly, Amin et al (16) found that 95% of MMs expressed WT-1, including sarcomatous variants. Thus, WT-1 reactivity supports a diagnosis of MM rather than SS.

Like WT-1, Bcl-2, a protein involved in apoptosis, appears useful in discriminating between MM and SS of the pleura. Bcl-2 staining was found in the spindle component of 79% to 100% of SSs (Fig. 36.4C2) but only in 0 to 10% of MMs (12). In a direct comparision, Cappello and Barnes (12) observed Bcl-2 reactivity (3+) in the spindle component of four of four biphasic SSs, but only weak positivity (1–2+) in four of four biphasic MMs. The epithelial component of two of four SSs and four of four MMs was also positive (1–2+). Cappello and Barnes suggested that strong spindle cell Bcl-2 staining is more indicative of SS.

The use of Ber-Ep4 is controversial. Reports of Ber-Ep4 staining in MM are variable. Gaffey et al (17) studied 49 MMs, including epithelioid and biphasic variants, and found that 10 (20%) exhibited focal (<25%) epithelioid staining. In contrast, Sheibani et al (18) found that of 115 MMs (including epithelioid, biphasic, and sarcomatoid variants), only one (0.9%) biphasic tumor stained with Ber-Ep4. Cappello and Barnes (12) found that four of four biphasic MMs were negative for Ber-Ep4, while two of four biphasic SSs displayed focal positivity in the epithelioid component. In the study by Gaetner et al (4), five of five

pleural-based biphasic SSs exhibited epithelioid staining with Ber-Ep4, while four of five showed staining in the spindle cell component.

HBME-1 is of limited value in the distinction between SS and MM, as HBME-1 positivity is seen in epithelioid components of MM as well as biphasic and monophasic SS (15). Staining for epithelial membrane antigen (EMA) may also be seen in both, and occasional S-100 positivity is also seen in SS and MM (4). CD99 (Fig. 36.4C1), the product of the *MIC2* gene, has been observed at similar frequencies in both SS and MM (5). CD56 staining is frequent in SS (1). Both SS and MM typically demonstrate no reactivity with B72.3, LeuM1, or CD34.

Overall, the use of immunohistochemistry in the distinction between MM and SS of the pleura is challenging and limited at best. Panels of markers are recommended since no single marker is diagnostic of either MM or SS. However, coexpression of Bcl-2, CD56, and CD99 with negative staining for calretinin, WT-1, and focal cytokeratin positivity strongly suggests the diagnosis of SS rather than MM.

Electron Microscopy

Ultrastructurally, MMs are characterized by several unique features that can be useful when trying to differentiate these tumors from SSs. Classically, biphasic and epithelioid MMs are characterized by long, slender, tortuous branching microvilli (Fig. 36.5A), but this finding may be diminished or lost in poorly differentiated neoplasms. Abundant intracytoplasmic glycogen is also seen. SSs, in contrast, have shorter blunt microvilli (Fig. 36.5B), and glycogen is sparse to absent (1,9,10).

Molecular Studies

Overall, while clinical history, gross and microscopic examination, and IHC may suggest a diagnosis of pleural SS, molecular diagnostic studies are considered the only definite way to differentiate SS from MM of the pleura. Regardless of histologic subtype, the chromosomal translocation t(X;18)(p11.2;q11.2) is characteristic of synovial sarcomas (10). A variety of techniques have been used to detect this translocation including conventional and molecular cytogenetics and reverse-transcription polymerase chain reaction (RT-PCR). While this translocation has been occasionally reported in other tumor types, particularly fibrosarcomas and malignant fibrous histiocytomas, these cases more likely represent misdiagnosed SS (1).

The characteristic translocation results in fusion of the *SYT* gene on chromosome 18 to the *SSX* gene on chromosome X (Fig. 36.6). This translocation has been convincingly demonstrated in primary pleural SSs. Aubry et al (2), using RT-PCR, confirmed the presence of this translocation in five of five sarcomatoid primary SSs of the pleura. Carbone et al (10) and Ng et al (11) also confirmed the presence of this translocation in biphasic and monophasic pleural SSs, respectively. Overall, of 11 cases of primary pleural SS, nine (82%) contained the

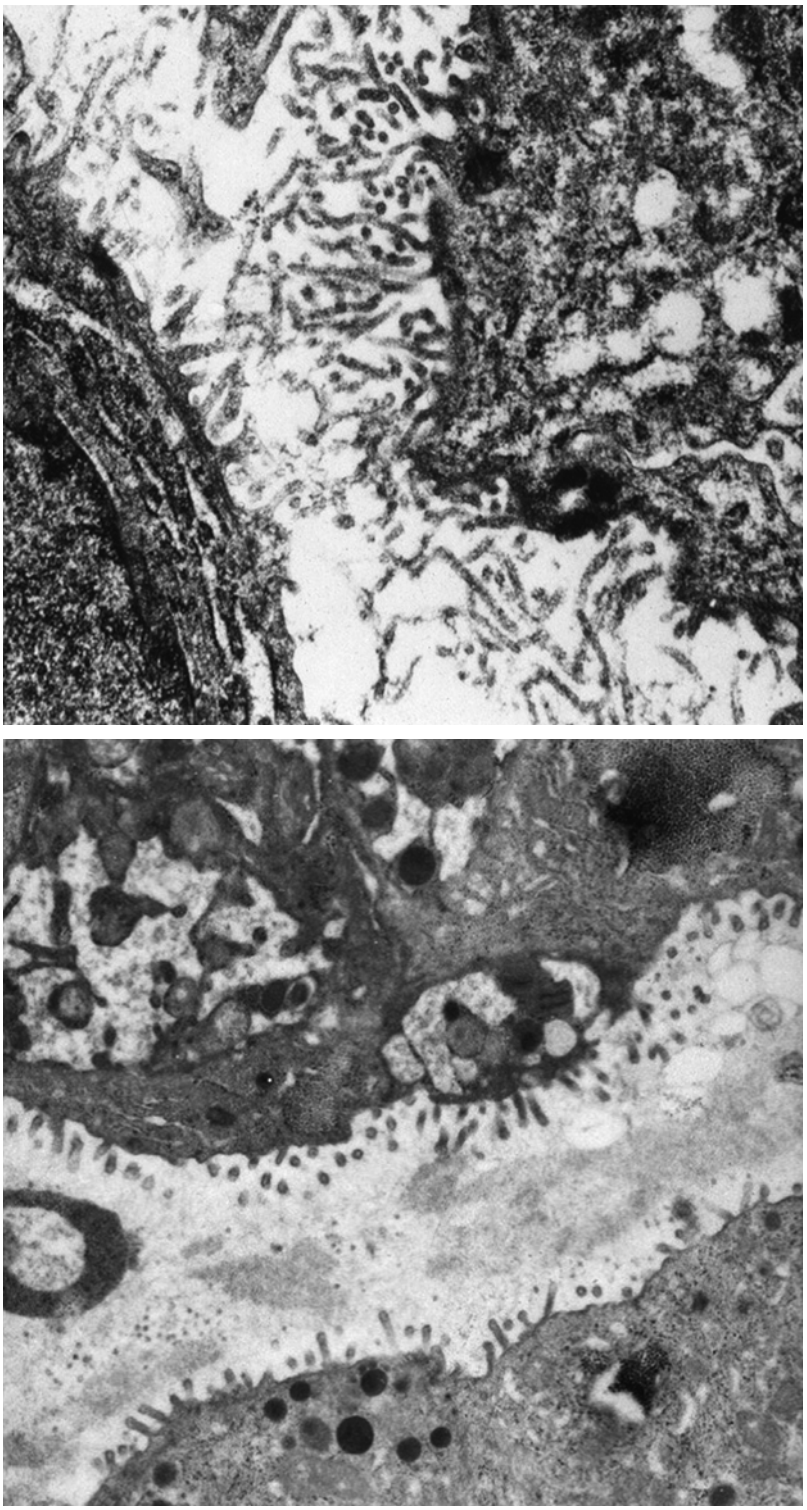


Figure 36.5. Electron microscopy characteristics of mesothelioma (A) characterized by long branching microvilli (12,000×) and of SS (B) showing short blunt microvilli (8,000×).

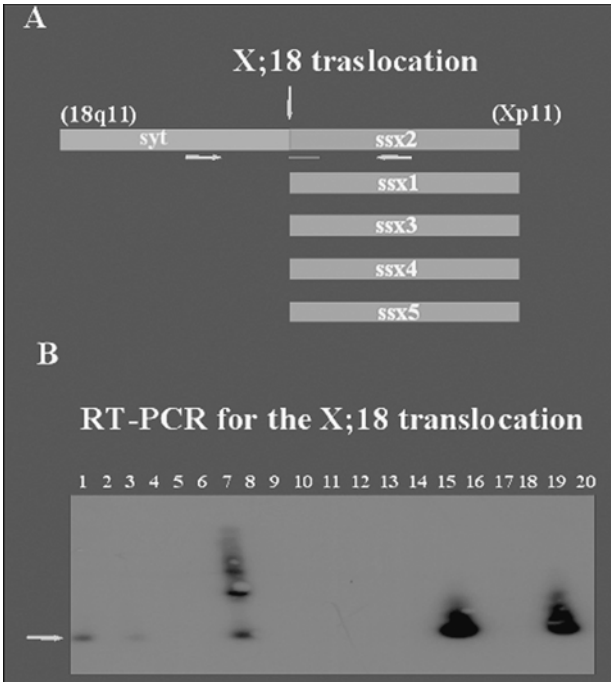


Figure 36.6. X;18 translocation. A: The different possible molecular rearrangements described in this type of translocation. The translocation detected in this particular tumor involves the *SYT* gene on chromosome 18, and the *SSX*2 gene on chromosome X. B: Southern blot hybridization showing the X:18 translocation detected in this tumor. Lane 1: Patient RNA extraction purified on Qiagen column. Lane 3: RNA extraction not purified; (10 µl). Lane 5: RNA extraction not purified (2 µl). Lane 7: SS-positive control (courtesy of Dr. Lasota). Lanes 9, 11, 13: Negative controls. Lane 14: Molecular weight. Lanes 15 and 19: SS-positive controls from our collection of SS. Lane 17: MM negative control. Lanes 2, 4, 6, 8, 10, 12, 16, and 18 are empty. RT-PCR.

translocation, one (9%) did not, and one (9%)was indeterminant. While MM can harbor multiple cytogenetic abnormalities, including partial loss of chromosome 1 (1p11–p22), chromosome 3 (3p14–p25), and chromosome 9 (9p), there have been no reports of the X;18 translocation in MM (1,10). Thus, molecular diagnostics is currently an extremely valuable tool in the differential diagnosis of a pleural-based mass when there is suspicion of SS.

The type of *SYT-SSX* fusion gene detected in SS appears to correlate with both tumor morphology and prognosis. Kawai et al (19) observed that biphasic morphology correlated with the *SYT-SSX2* fusion transcript, and monophasic morphology correlated with the *SYT-SSX1* fusion transcript. Furthermore, those patients with biphasic tumors expressing the *SYT-SSX2* fusion transcript had a better survival rate than those with *SYT-SSX1* monophasic SS. Nilsson et al (20) also found that SS containing the *SYT-SSX1* fusion transcripts had poorer outcomes.

Conclusion

The true incidence of primary SS of the pleura is unknown. Although it is a rare tumor, it is likely underdiagnosed and frequently mistaken for MM, the most common malignant pleural lesion. Diagnosis of pleural SS can be extremely challenging. The apparent rarity of the tumor in this location makes it an easily overlooked diagnosis. In addition, this tumor can be reliably distinguished from a MM only by using cytogenetics, since these entities have overlapping clinical, gross, histologic, and immunohistochemical features. This diagnostic problem has been compounded by the fact that the molecular tools to diagnose the unique X;18 translocation have only recently become available. Furthermore, few laboratories have the resources in place to identify this translocation (4,10,12,15).

While challenging, the distinction between MM and SS of the pleura is essential, since these entities have distinct treatments and prognosis. Synovial sarcomas can be responsive to chemotherapy, particularly to ifosfamide-based regimens, while sarcomatoid mesotheliomas are chemoresistant (1,21). As a result, synovial sarcomas are treated aggressively, while patients with sarcomatoid mesotheliomas are often given supportive therapy only. Furthermore, patients with MM have an average survival of less than 12 months, while patients with SS can have longer survival rates. In a series of primary biphasic SS of the pleura (4), patients survived an average of 35 months, with a range of 12 to more than 96 months. With chemotherapy, some authors have reported 5-year survivals as high as 57% in patients with SS (6). Finally, a diagnosis of MM often has important legal consequences, due to its strong association with asbestos exposure; SS has not been associated with asbestos, and when mistaken for a MM, it can result in unnecessary legal fees and settlements.

References

1. Weiss SW, Goldblum JR, eds. Soft Tissue Tumors, 4th ed. St. Louis: Mosby, 2001.
2. Aubry MC, Bridge JA, Wickert R, Tazelaar HD. Primary monophasic synovial sarcoma of the pleura. Am J Surg Pathol 2001;25:776–781.
3. Jawahar DA, Vuletin JC, Gorecki P, Persechino F, Macera M, Magazeh P. Primary biphasic synovial sarcoma of the pleura. Respir Med 1997;91: 568–570.
4. Gaertner E, Zeren H, Fleming M, Colby T, Travis W. Biphasic synovial sarcomas arising in the pleural cavity. A clinicopathologic study of five cases. Am J Surg Pathol 1996;20:36–45.
5. Nicholson AG, Goldstraw P, Fisher C. Synovial sarcoma of the pleura and its differentiation from other primary pleural tumors: a clinicopathological and immunohistochemical review of three cases. Histopathology 1998;33: 508–513.
6. Colwell AS, D Cunta J, Vargas S, et al. Synovial sarcoma of the pleura: a clinical and pathologic study of three cases. J Thorac Cardiovasc Surg 2002; 124:828–832.

7. Essary LR, Vargas SO, Fletcher CD. Primary pleuropulmonary synovial sarcoma: reappraisal of a recently described anatomic subset. Cancer 2002;94:459–469.
8. Chan JA, McMenamin ME, Fletcher CDM. Synovial sarcoma in older patients: clinicopathological analysis of 32 cases with emphasis on unusual histological features. Histopathology 2003;43:72–83.
9. Hirano H, Kizaki T, Sashikata T, et al. Synovial sarcoma arising from the pleura: a case report with ultrastructural and immunohistochemical studies. Med Electron Microsc 2002;35:102–108.
10. Carbone M, Rizzo P, Powers A, et al. Molecular analyses, morphology and immunohistochemistry together differentiate pleural synovial sarcomas from mesotheliomas: clinical implications. Anticancer Res 2002;22:3443–3448.
11. Ng SB, Ahmed Q, Tien SL, et al. Primary pleural synovial sarcoma. A case report and review of the literature. Arch Pathol Lab Med 2003;127:85–90.
12. Cappello F, Barnes L. Synovial sarcoma and malignant mesothelioma of the pleura: review, differential diagnosis and possible role of apoptosis. Pathology 2001;33:142–148.
13. Battifora H, McCaughey WTE. Atlas of Tumor Pathology. Tumors of the Serous Membrane. Washington, DC: Armed Forces Institute of Pathology, 1995:17–89.
14. Folpe AL, Schmidt RA, Chapman D, Gown AM. Poorly differentiated synovial sarcoma: immunohistochemical distinction from primitive neuroectodermal tumors and high grade malignant peripheral nerve sheath tumors. Am J Surg Pathol 1998;22:673–682.
15. Miettinen M, Limon J, Niezabitowski A, Lasota J. Calretinin and other mesothelioma markers in synovial sarcoma. Am J Surg Pathol 2001;25:610–617.
16. Amin KM, Litzky LA, Smythe WR, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. Am J Pathol 1995;146:344–356.
17. Gaffey MJ, Milles SE, Swanson PE, et al. Immunoreactivity for Ber-Ep4 in adenocarcinomas, adenomatoid tumors, and malignant mesotheliomas. Am J Surg Pathol 1992;18:593–599.
18. Sheibani K, Shin SS, Kezirian J, et al. Ber-Ep4 antibody as a discriminant in the differential diagnosis of malignant mesothelioma versus adenocarcinoma. Am J Surg Pathol 1991;15:779–784.
19. Kawai A, Woodruff J, Healey JH, et al. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. N Engl J Med 1998;228:153–160.
20. Nilsson G, Skytting B, Xie Y, et al. The SYT-SSX1 variant of synovial sarcoma is associated with a high rate of tumor cell proliferation and poor clinical outcome. Cancer Res 1999;59:3180–3184.
21. Pass HI, Robinson BW, Testa JR, Carbone M. Emerging translational therapies for mesothelioma. Chest 1999;116:455S–460S.

37

Pitfalls in the Diagnosis of Malignant Mesothelioma

Donald G. Guinee, Jr. and William D. Travis

The pathologic assessment of pleural lesions encompasses a variety of neoplastic and reactive conditions that may be difficult to distinguish (Table 37.1). The most common diagnostic problems involve the distinction of an epithelial malignant mesothelioma from adenocarcinoma, and the distinction of reactive epithelial or fibrous proliferations from epithelial or sarcomatoid mesothelioma, respectively. Pleural involvement by benign or malignant processes may sometimes simulate mesothelioma. Likewise, some types of mesotheliomas may simulate either benign processes or other types of malignancies. Integration of histologic, clinical, and radiographic data is important in arriving at an accurate diagnosis.

Epithelial Mesothelioma Versus Mesothelial Hyperplasia

In some cases, the differential diagnosis between mesothelioma and an organizing pleural effusion with reactive mesothelial hyperplasia may be exceedingly difficult (1–3). The strongest criterion of malignancy is the presence or absence of stromal invasion. Benign mesothelial proliferations associated with organizing pleuritis lack invasion. Glands or cells may become incorporated into the thickened pleura, but these tend to be oriented parallel to the surface. There is often a gradation of cellularity from higher (toward the pleural cavity or subpleural) to lower (or sclerotic) toward the chest wall or lung (Fig. 37.1). Mesothelioma, on the other hand, may either show glands and cells with no particular orientation to the surface (Fig. 37.2A,B) and no particular gradient of cellularity or with increased cellularity toward the chest wall or lung. The presence of mesothelial cells within fat or muscle of the chest wall or within the lung parenchyma is consistent with invasion and strongly supports the interpretation of malignant mesothelioma. True papillary formations deep within tissue are also usually a sign of invasion and consistent with malignancy. Immunohistochemical staining for cytokeratin may be helpful in confirming the presence

Table 37.1. Problems in the differential diagnosis of malignant mesothelioma

Epithelial mesothelioma vs. mesothelial hyperplasia
Organizing pleuritis vs. sarcomatoid or desmoplastic mesothelioma
Benign processes or neoplasms mimicking mesothelioma
Fibrous pleurisy (chronic fibrous pleuritis)
Reactive eosinophilic pleuritis
Nodular mesothelial hyperplasia
Xanthomatous pleuritis
Adenomatoid tumor
Thymoma
Metastatic or primary malignancies of the pleura mimicking malignant mesothelioma
Carcinoma simulating mesothelioma ("pseudomesotheliomatous carcinoma")
Vascular tumors simulating mesothelioma
Epithelioid hemangioendothelioma
Angiosarcoma
Primary pleural synovial sarcoma
Metastatic melanoma simulating mesothelioma
Primary effusion lymphoma and pyothorax associated lymphoma
Mesothelioma simulating other malignancies
Localized mesothelioma
Desmoplastic mesothelioma simulating sclerosing mediastinitis
Mucin-positive mesothelioma
Lymphohistiocytoid mesothelioma
Deciduoid mesothelioma
Mesothelioma of low malignant potential
Well-differentiated papillary mesothelioma
Diagnostic pitfalls in metastatic mesothelioma
Mesothelial cells in mediastinal lymph nodes

of invasion into chest wall or in illustrating the pattern of glands within a thickened pleura (Fig. 37.2C). Pathologists should be cautious in the interpretation of invasion as mesothelial cells within fibrinous exudates or tangential sections of pleura with reactive mesothelial cells may simulate invasion.

Besides invasion, other features suggesting malignancy include marked cytologic atypia, lack of inflammation, atypical mitoses, and tumor necrosis (Tables 37.2 and 37.3) (2,3). Cytologic atypia should be assesed with caution as reactive mesothelial cells are often atypical in an inflammatory background. Tumor necrosis must also be distinguished from necrotic inflammatory exudates, which may complicate pleural diseases. Nonetheless, a pathologist can render a diagnosis of malignant mesothelioma in the absence of stromal invasion if biopsies from a solid tumor mass show conclusive cytologic features of malignancy. These features are summarized in Tables 37.2 and 37.3.

In addition to histologic features, some authors have suggested other ancillary techniques to aid in the distinction of benign from malignant mesothelial proliferations. These efforts have included counting the silver nucleolar organizer regions, and staining for p-glycoprotein, p53, and telomerase reverse transcriptase (3–7). However, the International Mesothelioma Panel has not recommended these techniques for

diagnosis, and additional studies are needed to further establish their utility.

When unable to make a definitive diagnosis, the biopsy should be considered an "atypical mesothelial proliferation" to indicate the uncertainty. Definitive diagnosis may be more difficult in small biopsies.

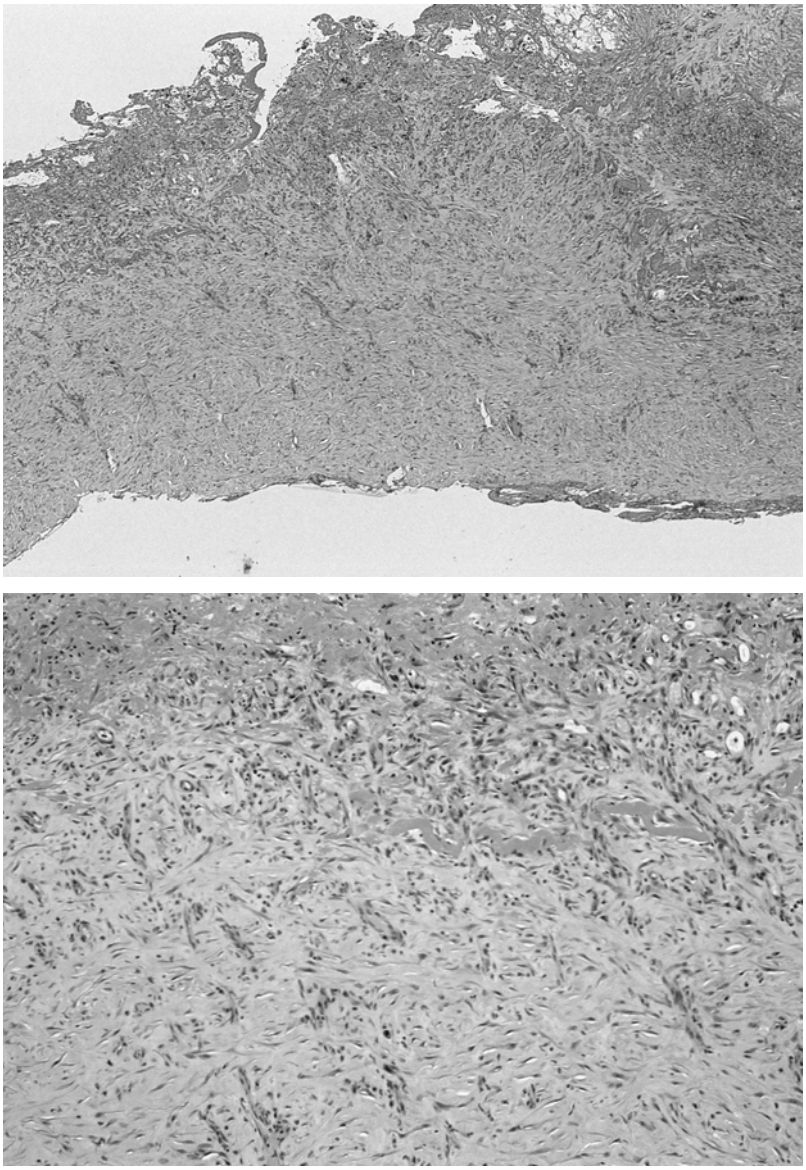


Figure 37.1. A,B: Organizing pleural effusion. There is a gradation of cellularity from higher (underneath pleural surface) to lower (or sclerotic) toward the chest wall. Mesothelial cells, when entrapped, tend to be arranged parallel to the pleural surface.

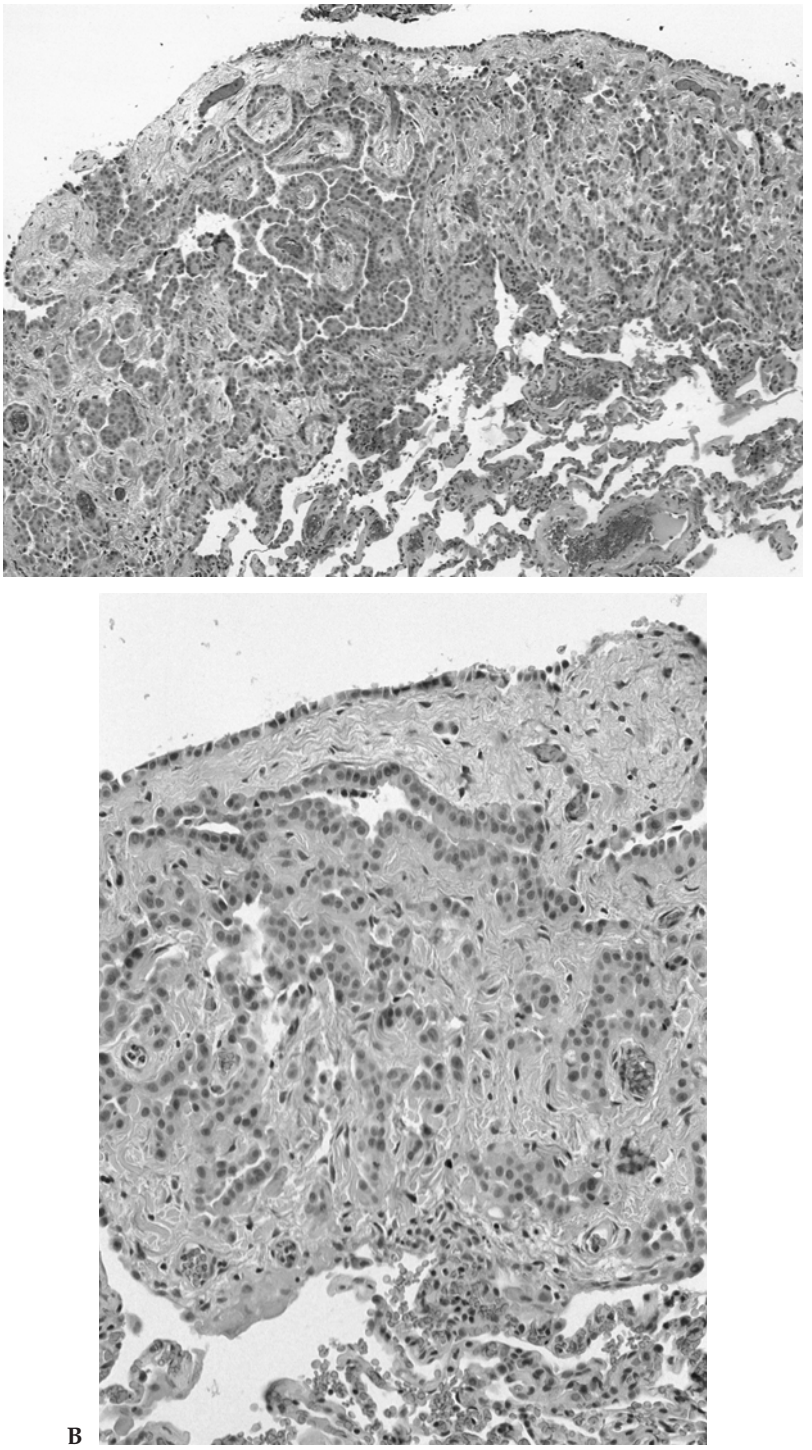
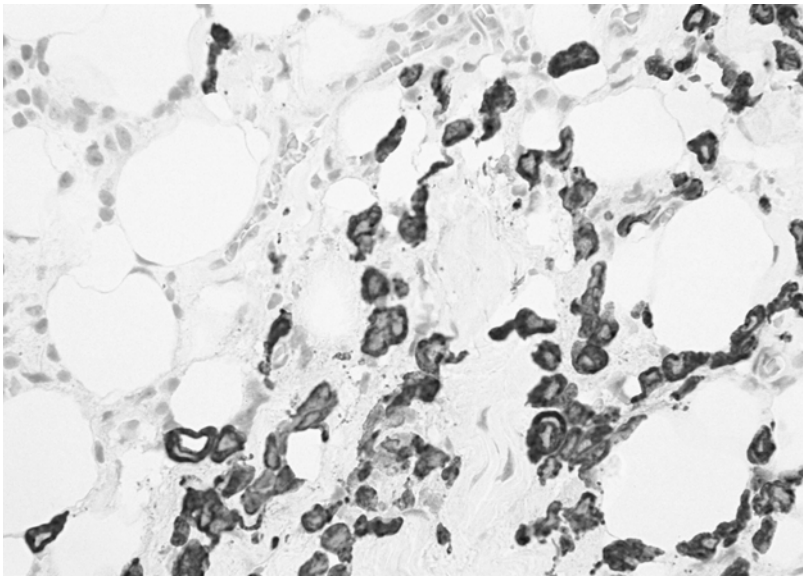


Figure 37.2. Malignant mesothelioma, epithelial type. A,B: A thickened pleura is infiltrated by a haphazard proliferation of irregular glands and cells. C: The presence of mesothelial cells within fat of the chest wall is highlighted by immunohistochemical staining for cytokeratin, consistent with invasion.



C

Figure 37.2. *Continued*

Table 37.2. Features favoring malignancy in epithelial mesothelial proliferations

True stromal invasion
Infiltration of (1) chest wall fat or skeletal muscle or (2) visceral pleural connective tissue or interlobular septa
True papillary formations within tissue
Irregular orientation of nests of mesothelial cells with respect to pleural surface
Marked cytologic atypia
Diffusely high cellularity without gradation
Atypical mitoses
Tumor necrosis

Table 37.3. Features favoring reactive mesothelial proliferation associated with an organizing pleural effusion

No stromal invasion
No infiltration of fat or skeletal muscle of chest wall
No true papillary formations within tissue
Parallel orientation of nests of mesothelial cells with respect to pleural surface (presumably representing entrapped cells within an organizing pleural effusion)
Only mild to moderate nuclear atypia
Gradation of cellularity from higher (toward the pleural cavity or subpleural) to lower or sclerotic (toward chest wall or lung)
Mitoses confined to cells outside of tissue; no atypical mitoses
Absence of tumor necrosis

Organizing Fibrous Pleuritis Versus Sarcomatoid or Desmoplastic Mesothelioma

As in the distinction of reactive epithelial mesothelial proliferations from epithelial mesothelioma, the distinction of reactive fibrous pleural proliferations from sarcomatoid mesotheliomas may be difficult. Mesotheliomas in which there is a prominent collagenous stroma, termed desmoplastic mesotheliomas, may be especially problematic as they may contain only scattered cytologically atypical cells within a prominent sclerotic background (8,9). As with epithelial mesothelial proliferations, invasion of the chest wall or visceral pleura strongly supports malignancy (Fig. 37.3). Immunohistochemical staining for cytokeratin is helpful in highlighting infiltration of chest wall fat or skeletal muscle by atypical spindled mesothelial cells. Keratin expression is also useful in highlighting whether the pattern of a spindled cell proliferation is orderly or disorderly (2,9–11). Caution should be used, however, in more superficial areas of the biopsy as subserosal fibroblasts may show keratin expression in reactive conditions (12).

Besides invasion, frankly sarcomatous foci, necrosis, pattern of cellularity, and the relative absence of associated inflammation are also helpful in distinguishing desmoplastic mesothelioma from reactive processes. Frankly sarcomatous areas are consistent with malignancy, whereas organizing fibrous pleuritis tends to show a gradient of cellularity ("zonation") from higher toward the pleural cavity (subpleural), and lower toward the chest wall or the lung. Foci of bland necrosis in a spindled cell proliferation or the presence of distant metastases are also consistent with malignancy. Desmoplastic mesotheliomas typi-

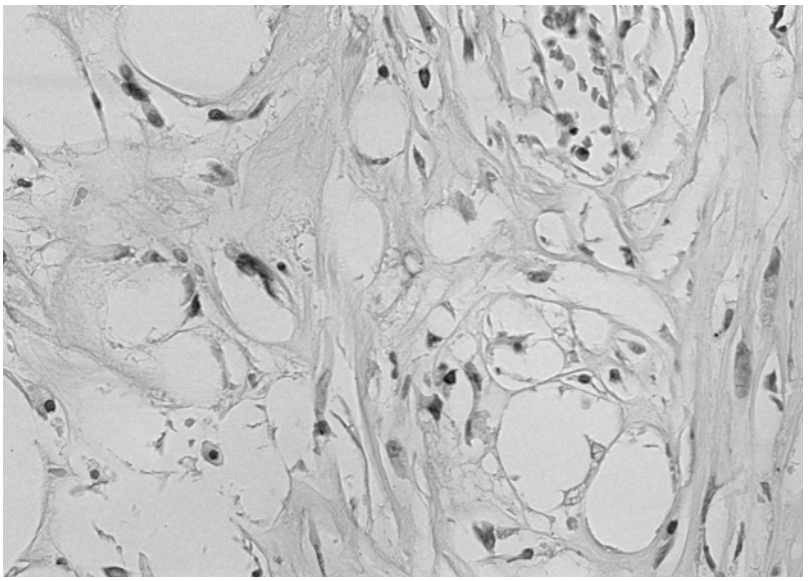


Figure 37.3. Malignant mesothelioma, desmoplastic type. Invasion of fat of chest wall by scattered atypical cells of desmoplastic mesothelioma.

Table 37.4. Features favoring malignancy in spindle cell proliferations involving the pleura

Invasion of the chest wall
Atypical cytokeratin positive cells within fat or skeletal muscle
Frankly sarcomatous areas
Areas of bland necrosis
Distant metastases
Storiform pattern

cally show a "storiform" pattern of spindled cells that sometimes form nodules with increased cellularity. In contrast, reactive pleural fibrosis or pleural plaques show a different pattern. Reactive pleural fibrosis often demonstrates a parallel arrangement of blood vessels oriented perpendicular to the pleural surface (2,3,9–11). Pleural plaques show a basket-weave pattern of dense fibrous tissue with slit-like spaces unlike the haphazard or storiform arrangement in desmoplastic mesothelioma. These histologic features are summarized in Tables 37.4 and 37.5.

Benign Processes Mimicking Mesothelioma

Some types of benign processes, both inflammatory and neoplastic, can mimic mesothelioma histologically. Inflammatory processes that may mimic or enter into the differential diagnosis of mesothelioma include chronic fibrous pleuritis, reactive eosinophilic pleuritis, so-called nodular mesothelial hyperplasia, and pleural inflammation, which contains numerous foamy macrophages ("xanthomatous pleuritis"). Benign neoplasms or neoplasms of indeterminate malignancy that may mimic malignant mesothelioma include adenomatoid tumors and sometimes thymomas.

Chronic pleuritis, when severe, may cause marked diffuse pleural fibrosis. This entity, termed fibrous pleurisy or chronic fibrous pleuritis, can mimic mesothelioma radiographically. Histologically, biopsies may show a haphazard growth pattern and focal cytologic atypia of mesothelial cells, especially in the presence of inflammation. Although tongues of fibrous tissue may extend into parietal pleural fat, this extension does not constitute invasion and keratin stains do not demonstrate invasive growth of mesothelial cells (2).

Table 37.5. Features favoring reactive organizing pleuritis in spindle cell proliferations involving the pleura

No chest wall invasion
Gradation of cellularity from high (toward pleural cavity or subpleural) to low (toward chest wall or lung)
No areas of necrosis within spindle cell proliferation
No distant metastases
Absence of a storiform pattern
Parallel orientation of blood vessels oriented perpendicular to the pleural surface

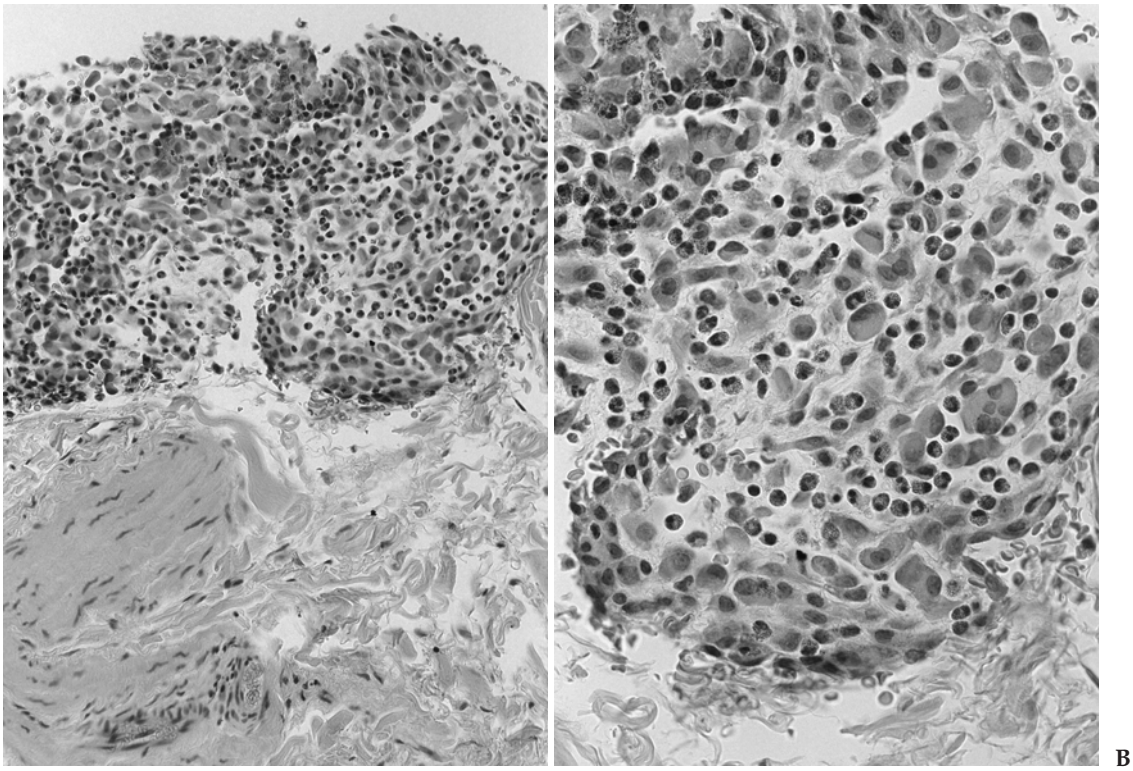


Figure 37.4. A,B: Reactive eosinophilic pleuritis. An exuberant inflammatory infiltrate is present along the pleural surface consisting of numerous eosinophils admixed with histiocytes, lymphocytes, and occasional multinucleated giant cells.

Reactive eosinophilic pleuritis (Fig. 37.4) is a condition originally described by Askin et al (13), in which there is a pleural inflammatory infiltrate consisting of sheets or nodules containing numerous eosinophils, reactive mesothelial cells, histiocytes, lymphocytes, and occasional giant cells (13,14). This reaction is usually an incidental histologic finding that often follows pneumothorax, and when exuberant, may enter into the histologic differential diagnosis of malignant mesothelioma. Radiographic findings are often of pneumothorax, but diffuse pleural thickening or multiple pleural nodules are not present. Tissue is received in pathology typically after resection of pulmonary blebs or bullae. Histologically, there is no evidence of invasion. If uncertain, immunohistochemical stains for histiocytic markers (CD45 and CD68) will highlight the histiocytic cells and confirm the diagnosis.

Nodular mesothelial hyperplasia is a condition first described by Rosai and Dehner (15) in 1975 as a distinct nodular lesion occurring in hernia sacs. The authors attributed the lesions to nodular collections of reactive mesothelial cells and noted their benign nature despite their sometimes worrisome histologic appearance. Chan et al (16) subsequently reported the observation of similar lesions consisting of cellular nodules in two patients in transbronchial biopsies. These lesions occurred in proximity to strips of mesothelium presumably from bits

of visceral pleura sampled by the biopsy. Ordonez et al (17) also reported a similar finding in the pleural biopsies from two patients. Grossly, these are focal nodular lesions usually lacking the diffuse pleural thickening typical of mesothelioma. Histologically, pleural nodular mesothelial hyperplasia is characterized by nodules of cohesive polygonal cells with nuclear grooves. The mononuclear cells may sometimes contain large intracytoplasmic vacuoles (Fig. 37.5). Although initially thought to represent collections of mesothelial cells, positive staining for CD68 in most cells supports a histiocytic reaction (16,17) and can be used to support the diagnosis.

Occasional pleural biopsies may show sheets of foamy macrophages that may mimic mesothelioma, metastatic melanoma, or metastatic carcinoma. These findings, which we term "xanthomatous pleuritis," typically occur in a background of pleural thickening and fibrosis with variable numbers of admixed lymphocytes, plasma cells, neutrophils, and eosinophils (Fig. 37.6). Clinically, patients may present with pleural effusion(s) or sometimes empyema. Positive immunohistochemical staining for histiocytic markers (CD68) and negative staining for cytokeratin confirms the diagnosis in uncertain cases.

Adenomatoid tumors are small tumors often found incidentally during pelvic surgery in the male or female genital tracts (18,19). They are considered a form of benign mesothelioma. Four cases have been

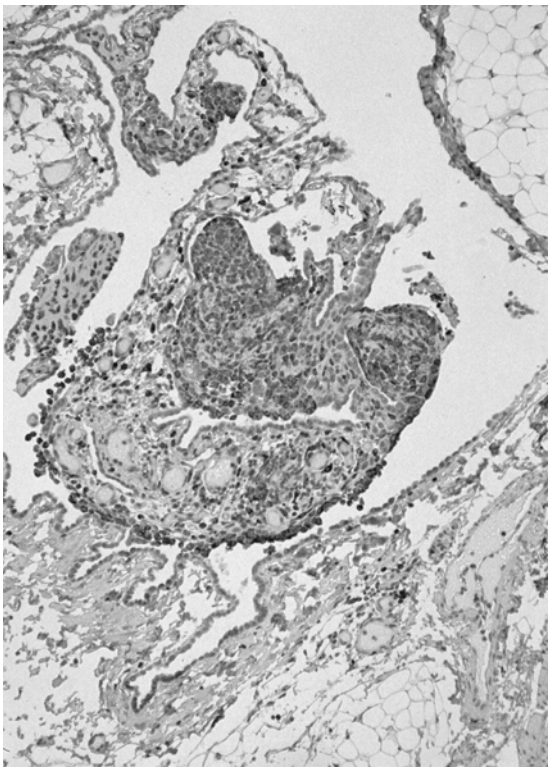


Figure 37.5. Nodule of histiocytes along pleural surface. Immunohistochemical staining for CD68 highlights a nodular aggregate of histiocytes along the surface of the pleura. (Courtesy of Dr. David Dail.)

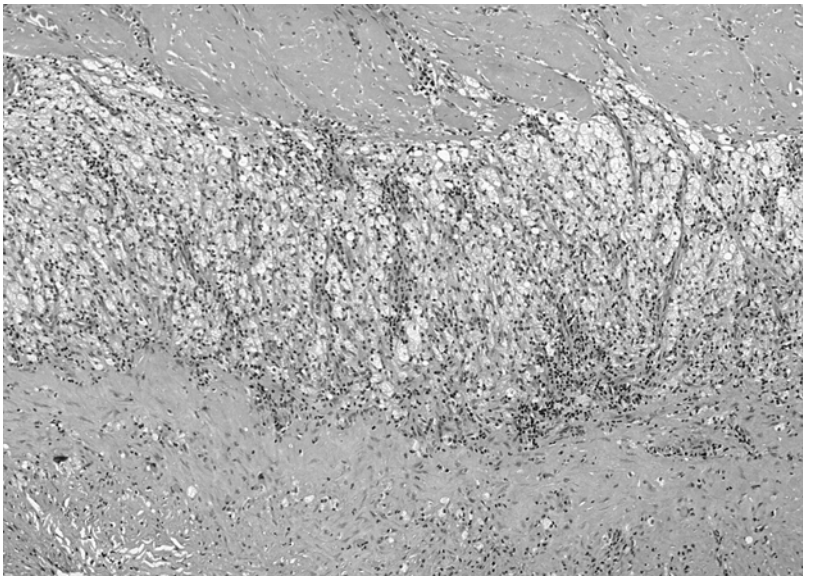


Figure 37.6. Xanthomatous pleuritis. Sheets of foamy macrophages in a background of chronic pleuritis may mimic metastatic carcinoma, metastatic melanoma, or clear cell mesothelioma.

reported in the pleura consisting of small nodules ranging from 0.5 to 3.0 cm found incidentally during surgery for lung masses (20–22). Histologically, the tumors consisted of irregularly arranged tubules and glands lined by epithelioid cells. Individual cells were also present, sometimes containing cytoplasmic vacuoles (Fig. 37.7). The tumor cells stain similarly to mesothelial cells. They stain positively for cytokeratin, calretinin, and HBME-1, and negatively for carcinoembryonic

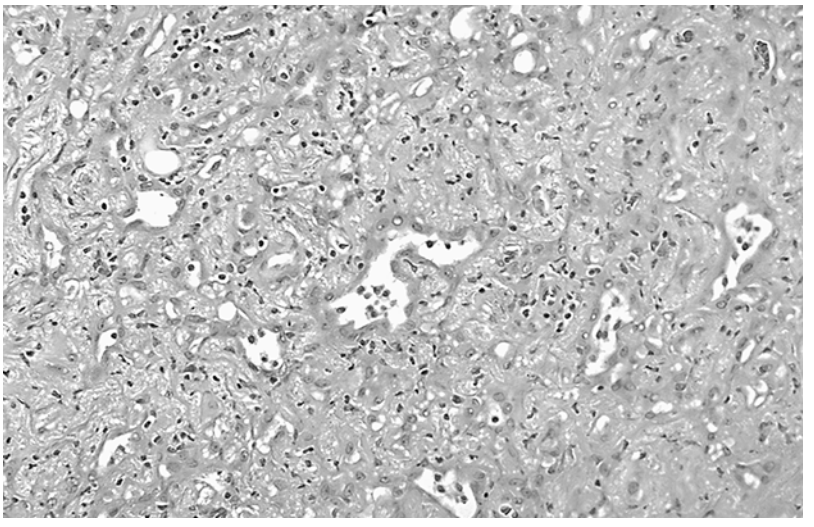


Figure 37.7. Adenomatoid tumor. A nodular aggregate of irregularly arranged tubules and glands lined by epithelioid cells.

antigen (CEA), BER-EP4, B72.3, and CD15 by immunohistochemistry (19,22). Ultrastructural examination of one case showed slender microvilli typical of mesothelial cells (22). Although their histologic features overlap somewhat with mesothelioma, the presence of abundant fibrous stroma and a bland appearance of the cells favor adenomatoid tumor. In uncertain cases, adenomatoid tumors are usually readily distinguished from mesotheliomas by their clinical characteristics. Adenomatoid tumors present as a small incidental solitary nodule usually found during another surgery. Malignant mesothelioma, on the other hand, presents with diffuse pleural thickening or multifocal pleural nodules.

Thymoma may occasionally present as a pleural tumor (23,24) either primary or as secondary involvement from a mediastinal origin. The diagnosis of primary pleural thymoma requires exclusion of a mediastinal mass. Clinically, these patients may be asymptomatic or present with respiratory difficulty, weight loss, or fever. Chest radiograph may show a localized mass or diffuse pleural thickening with encasement of the lung. Nodular thickening with extension along fissures was noted on one case. While thymomas may resemble mesothelioma clinically, they can usually be distinguished on histologic grounds. Histologically, these lesions are identical to thymomas presenting in the anterior mediastinum and consist of a variable mixture of lymphocytes and epithelial cells, subdivided into lobules by broad fibrous bands (Fig. 37.8). Mesotheliomas, on the other hand, often have an absent or inconspicuous lymphoid component and lack the lobulations characteristic of thymomas. Tubules or papillary formations are not usually found in thymoma, but they are typical of mesothelioma. Immunohistochemical staining for cytokeratin and lymphoid markers (especially CD45 and CD3) highlights the admixed epithelial and lymphoid cells. Available follow-up suggests a variable course with prolonged survival in some patients (24).

Metastatic or Primary Malignancies of the Pleura Mimicking Malignant Mesothelioma

Metastatic or primary malignant processes may mimic mesothelioma clinically, pathologically, or both. Peripheral lung carcinoma ("pseudomesotheliomatous carcinomas"), indeterminate and malignant vascular neoplasms, primary sarcomas, metastatic melanoma, and primary pleural lymphomas may be confused with mesothelioma. As in the preceding discussion, these entities are distinguished by integration and consideration of clinical, radiographic, and pathologic features.

Peripheral lung carcinomas may sometimes diffusely involve the pleural surface in a manner similar to that of mesothelioma (25–27). These patients are typically older men who present with nonspecific respiratory complaints including dyspnea, cough, and chest pain. A minority of patients have previous exposure to asbestos. A unilateral pleural effusion with or without pleural masses is the most common

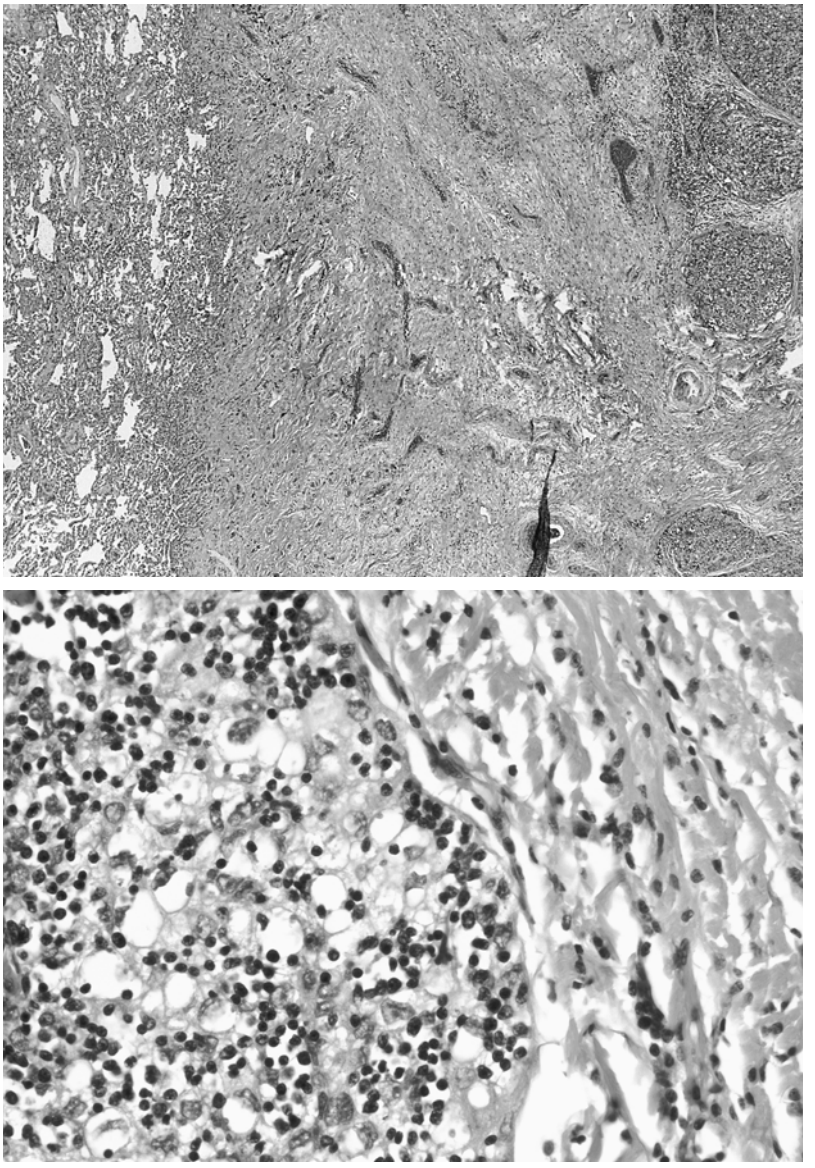


Figure 37.8. A,B: Thymoma. Lobular aggregates of lymphocytes and epithelial cells similar to mediastinal thymomas.

abnormality on chest radiograph. Unlike typical forms of lung cancer, hemoptysis is an uncommon presenting symptom. At thoracotomy, the pleura is often diffusely thickened or has multiple nodules. Histologically, these tumors consist of a mixture of glands, nests, papillary structures, or sheets of malignant cells (Fig. 37.9). Some tumors may have areas of spindled cells. If a portion of lung is included, foci of subpleural adenocarcinoma may be present (25,26).

Special stains and immunohistochemistry are helpful in the distinction of pseudomesotheliomatous carcinomas from mesotheliomas.

Unlike mesothelioma, periodic acid-Schiff (PAS)-positive, diastase-resistant mucin is present in most pseudomesotheliomatous carcinomas. This mucin resists hyaluronidase pretreatment. Although traditionally recognized as "adenocarcinomas," the 1999 World Health Organization (WHO) definition of poorly differentiated adenocarcinoma requiring the presence of at least five mucin-positive cells in two high-power fields would lead to the reclassification of many of these tumors as large-cell carcinomas. Positive immunohistochemical staining for two or more markers recognizing carcinoma such as CEA, CD15, MOC31, BER-EP4, and B72.3 support the diagnosis (25,26,28). Markers of mesotheliomas such as calretinin and cytokeratin (CK) 5/6 are typically negative. Expression of thyroid transcription factor-1 (TTF-1) in pseudomesotheliomatous carcinomas may also be helpful in confirming a pulmonary origin (28–30). The International Mesothelioma Panel and 2003 WHO classification recommend the following workup as a minimum in the distinction of pseudomesotheliomatous carcinoma from mesothelioma: two markers of carcinoma (e.g., CEA and B72.3), two markers of mesothelioma (e.g., calretinin and CK 5/6), a pancytokeratin, TTF-1, and a mucin stain.

The prognosis of pseudomesotheliomatous carcinoma is as dismal as that of mesothelioma. Median survival was reported at 8 months. Attempts at radiotherapy and chemotherapy have been largely unsuccessful (26).

Vascular neoplasms on occasion may mimic mesothelioma clinically and pathologically (31). Epithelioid hemangioendothelioma or epithelioid angiosarcoma may diffusely involve the pleural, peritoneal, or

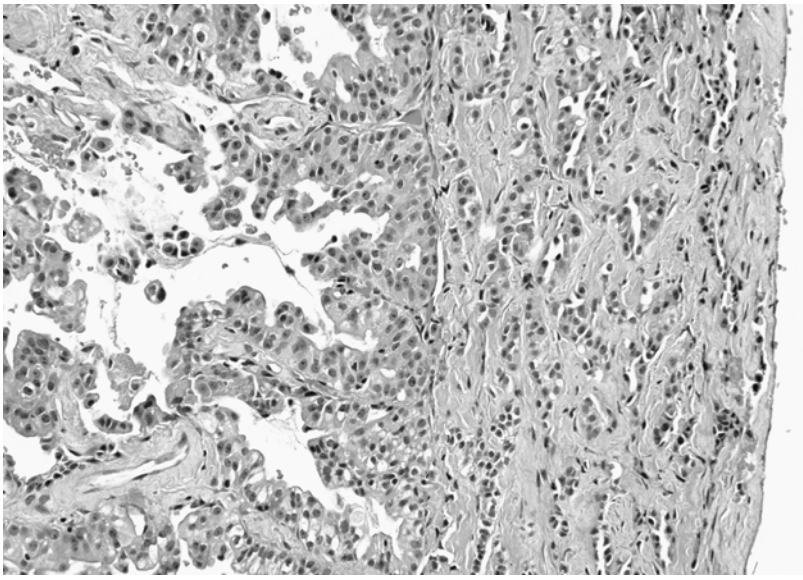


Figure 37.9. Pseudomesotheliomatous adenocarcinoma. Peripheral lung adenocarcinoma diffusely involving the pleura in a manner similar to mesothelioma.

pericardial cavities either primarily or secondarily and present with a unilateral pleural effusion or diffuse pleural or peritoneal thickening. Histologically, these tumors are often biphasic consisting of nests of epithelioid cells with varying amounts of a spindle cell stroma. Other features of vascular differentiation are present such as intracytoplasmic vascular lumina (sometimes containing entrapped erythrocytes), and microcystic or vascular spaces lined by tumor cells. A tubulopapillary pattern reminiscent of mesothelioma may be present (Fig. 37.10). Greater nuclear atypia should prompt consideration of epithelioid angiosarcoma (Fig. 37.11). Immunohistochemical stains may help in the distinction of these tumors from malignant mesothelioma and in the confirmation of their vascular origin. In Lin et al's (31) series of 14 patients, immunohistochemical staining for cytokeratin was variable ranging from weak to moderate. Mesotheliomas, on the other hand, are typically strongly positive for cytokeratin. Immunohistochemical staining for factor VIII, CD34, or CD31 help to confirm the interpretation. The prognosis of these tumors is dismal. Most patients die of their disease.

Synovial sarcomas are rare tumors that may occasionally present in the pleura and cause confusion with malignant mesothelioma (32–34). Distinction of synovial sarcoma from malignant mesothelioma rests on consideration of clinical, radiographic, and histologic features. Clinically, synovial sarcoma typically occurs in younger patients (average age 25), grows at a faster rate than mesotheliomas, and appears radiographically as a pleural-based mass that is usually localized and only rarely is associated with diffuse pleural thickening. Mesothelioma, on the other hand, typically presents in older patients, grows slowly over years, and presents radiographically as diffuse pleural thickening or

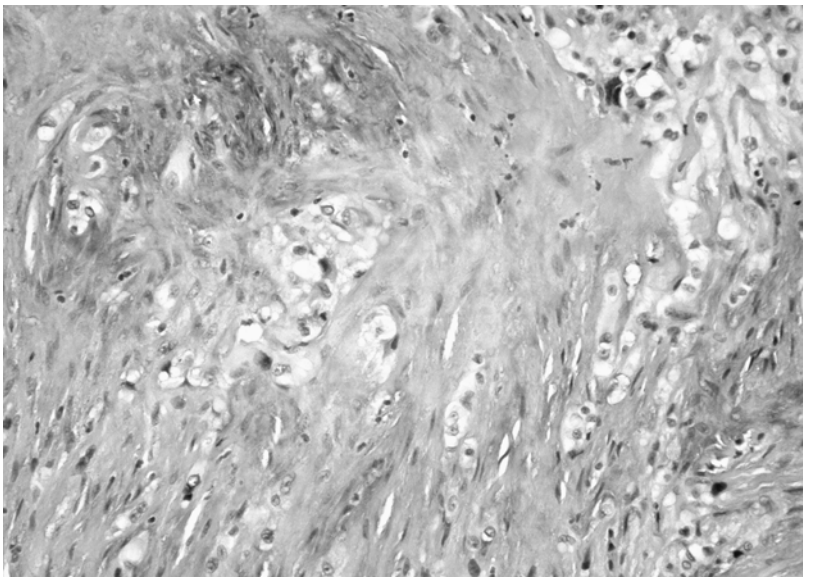


Figure 37.10. Epithelioid hemangioendothelioma. Nests of epithelioid cells with occasional cytoplasmic vacuoles. (Courtesy of Dr. David Dail.)

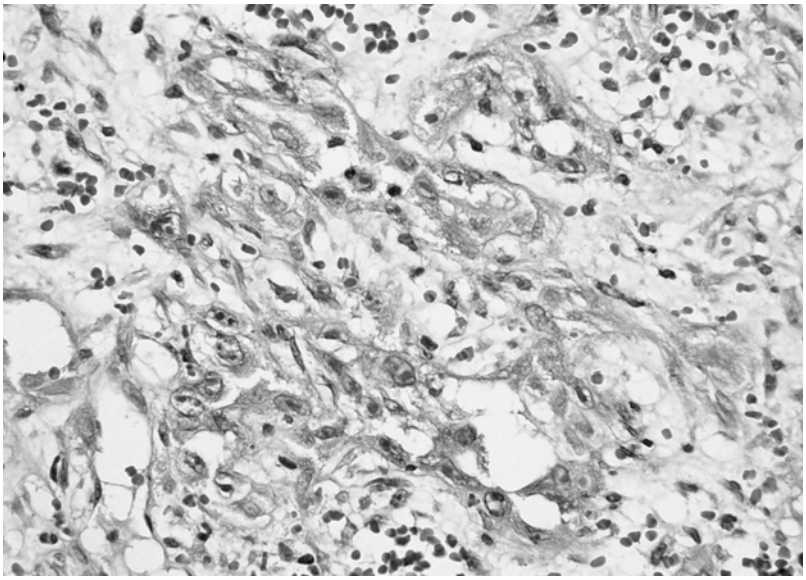


Figure 37.11. Angiosarcoma. Irregular anastomosing cords of malignant cells and associated hemorrhage present within a thickened pleura. (Courtesy of Dr. David Dail.)

multiple pleural nodules. Histologically, synovial sarcomas are similar to their counterparts in soft tissue consisting of a biphasic proliferation of epithelioid and spindled areas or as a monophasic proliferation of spindle cells (Fig. 37.12). In contrast to mesothelioma, gland-like spaces often stain positively for mucin by PAS with diastase or mucicarmine. Synovial sarcomas are usually weakly positive for pancytokeratin, CK 7, and epithelial membrane antigen (EMA). In contrast to mesotheliomas, synovial sarcomas may show staining of the glandular component for BER-EP4, but usually lack WT-1 by immunohistochemistry (35). Calretinin expression should be interpreted with caution as most biphasic synovial sarcomas have shown variable expression (35). Electron microscopy also may be useful in difficult cases as features of mesothelioma such as long, thin, slender microvilli are absent in synovial sarcoma (32,33). Finally, fluorescent in situ hybridization may identify the characteristic chromosomal translocation t(X;18)(p11.2;q11.2) and help to confirm the diagnosis (34). Since synovial sarcoma commonly metastasizes to the lung, a thorough search should be performed for an extrathoracic primary before accepting the pleura as the site of origin.

We have also seen rare cases of malignant melanoma metastatic to the pleura mistaken for malignant mesothelioma. While there may be overlap in the histologic features of mesothelioma, melanomas are more likely to show cellular pleomorphism, high mitotic rate, and nuclear cytoplasmic inclusions. Cytoplasmic melanin pigment can be highlighted on a Fontana-Masson stain. Negative immunohistochemical staining for cytokeratin and positive staining for melanoma markers

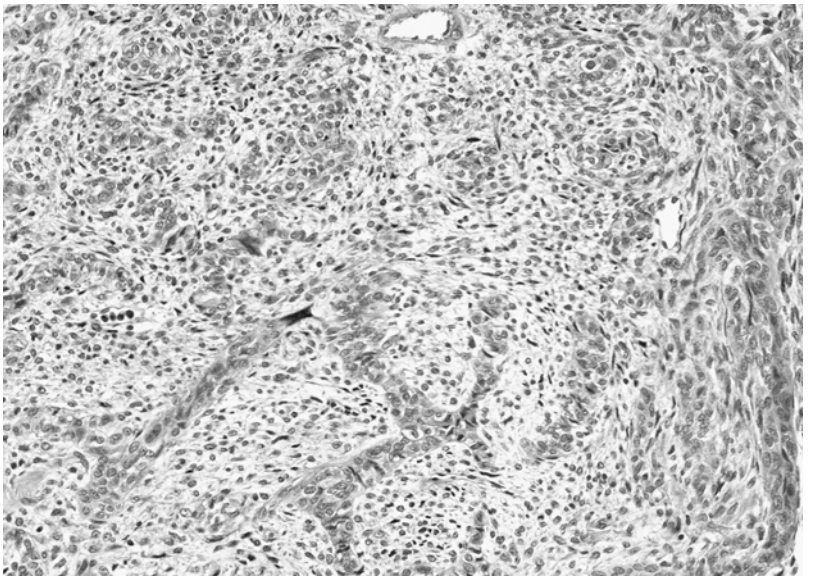


Figure 37.12. Synovial sarcoma. Biphasic proliferation of malignant epithelioid and spindled cells.

such as HMB-45, S-100, and tyrosinase are helpful in confirming the diagnosis.

Pleural effusion lymphomas and pyothorax-associated lymphomas are types of lymphomas that present in the pleura and enter into the differential diagnosis of malignant mesothelioma. Pleural effusion lymphoma is a recently described entity that occurs as a lymphomatous effusion involving the pleural, pericardial, or peritoneal space in patients with advanced AIDS. They usually do not have a clinically apparent mass throughout their course (36–39). Smears from the pleural fluid show round to ovoid malignant lymphoid cells with large round nuclei. Occasional cells are pleomorphic with multiple nuclei (Fig. 37.13). Mitotic figures are numerous. The neoplastic lymphoid cells lack expression of T- or B-cell antigens on flow cytometry, but do express leukocyte common antigen (CD45) and activation markers [CD30, CD38, human leukocyte antigen (HLA)-DR, and CD71]. The malignant cells consistently show immunoglobulin heavy chain gene rearrangements on Southern blot hybridization. Human herpes virus 8 (HHV8) may be identified in all cases by polymerase chain reaction (PCR) or Southern blot analysis (36,38). In most cases, there is co-infection with Epstein-Barr virus (EBV). Response to chemotherapy in AIDS patients with pleural effusion lymphomas has been poor. Most patients survive only several months.

Pleural effusion lymphomas are distinct from pyothorax-associated lymphomas that arise in the setting of long-standing pleural inflammation in mine workers and after artificial pneumothorax or tuberculous pleuritis (40–43). In contrast to pleural effusion lymphomas, pyothorax-associated lymphomas usually have an associated pleural mass, and are associated only with EBV but not HHV8 (44). These are

large B-cell lymphomas, sometimes with a prominent background of T cells.

Both pleural effusion lymphomas and pyothorax-associated lymphomas are easily distinguished from mesothelioma by immunohistochemical stains. In contrast to mesotheliomas, both of these tumors lack staining for cytokeratin but express leukocyte common antigen (CD45), confirming their lymphoid origin.

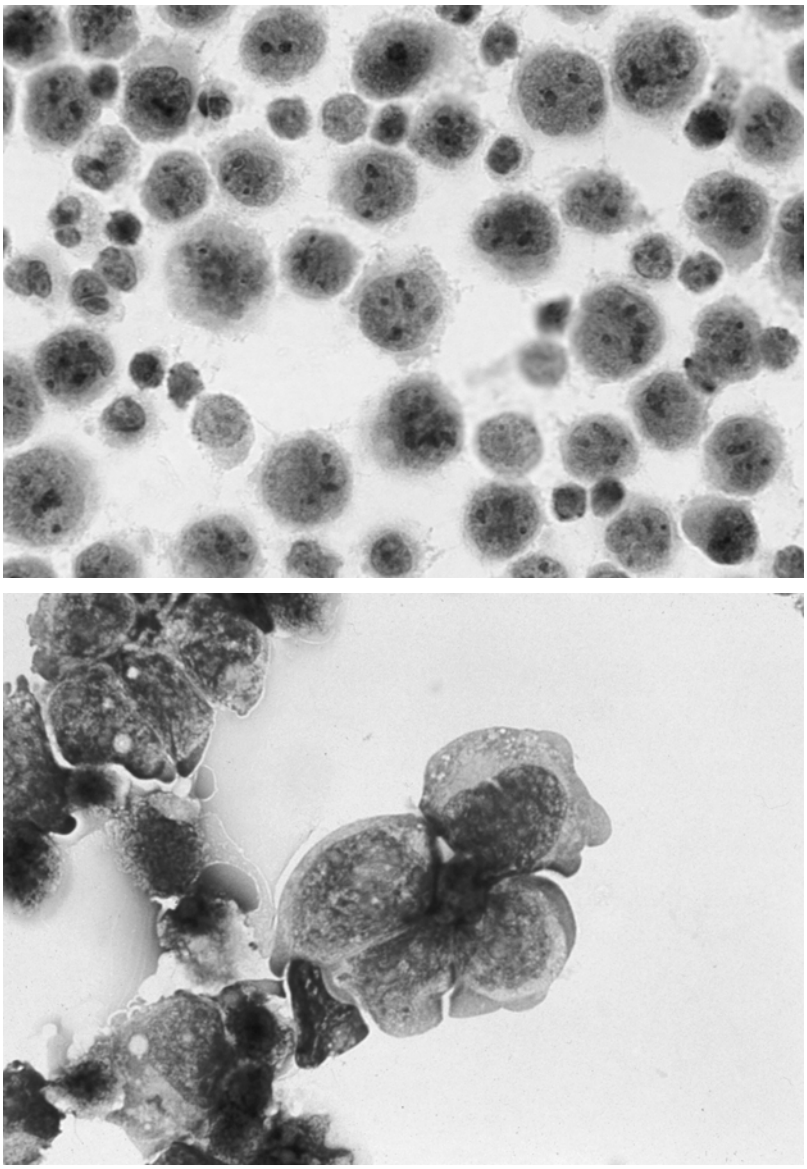


Figure 37.13. Pleural effusion lymphoma. Papanicolaou stained (A) and Wright Giemsa air dried (B) smears of pleural fluid with large malignant lymphoid cells, some containing multiple nuclei.

Mesotheliomas as Mimickers of Other Diseases

Mesotheliomas may simulate either inflammatory conditions or other neoplasms clinically, histologically, or both. Examples of such diagnostic dilemmas include "localized mesothelioma" mimicking adenocarcinoma, desmoplastic mesothelioma simulating sclerosing mediastinitis, mucin-positive mesothelioma, the distinction of lymphohistiocytoid mesotheliomas from lymphomas, and the distinction of deciduoid mesothelioma from exuberant decidual reactions. As in prior discussions, accurate diagnosis relies on integration of the clinical, radiographic, and histologic findings.

Rarely, mesotheliomas may present as a solitary localized pleural mass. This type of presentation may cause confusion as it is much more typical of adenocarcinomas. Patients with these tumors are often asymptomatic when a solitary extrapulmonary mass is discovered on routine chest x-ray. Computed tomography (CT) scan confirms the pleural location and solitary nature of these masses. Grossly, these tumors are sessile or pedunculated ranging up to 10 cm in maximum dimension. Histologically, they are similar to more typical mesotheliomas with epithelial or spindle cell components. Histochemical, immunohistochemical, and ultrastructural findings are also consistent with a mesothelial origin. Unlike the typical presentation of mesothelioma, complete surgical resection may be curative in some cases. Five of six patients in Crotty et al's (45) series had long-term tumor-free survival after surgical excision alone (46).

Crotty et al (47) reported a case of desmoplastic mesothelioma that simulated sclerosing mediastinitis. Sclerosing mediastinitis is an exuberant fibroinflammatory reaction within the mediastinum that typically occurs as a late sequela of *Histoplasma capsulatum* infection. The diagnostic difficulty in this case stems from the unusual presentation of mesothelioma as a localized mass in the mediastinum rather than as diffuse pleural thickening or nodularity. Overlap of histologic findings may also cause confusion as areas of fibrosis are a prominent feature of both desmoplastic mesothelioma and sclerosing mediastinitis. The findings of focal frankly sarcomatous foci allowed the final diagnosis in this case (47). Cytokeratin expression in atypical spindle cells also supported the interpretation of desmoplastic mesothelioma. Although reactive subserosal fibroblasts may express cytokeratin (12), the dense fibrous tissue proliferation deep to the pleural surface in sclerosing mediastinitis should be keratin negative unless entrapped epithelial structures such as thymic epithelium are present.

The demonstration of neutral mucin (e.g., by mucicarmine or PAS with diastase) within a malignant epithelial pleural neoplasm has been regarded as proof of adenocarcinoma. However, rare cases of mucin-positive mesothelioma have been reported (48,49). These cases show mucicarmine and PAS-positive, diastase-resistant staining within cytoplasmic vacuoles of malignant cells. This staining is unaffected by pretreatment with hyaluronidase. The cases reported were similar in all other respects pathologically to more typical mesotheliomas. Thus, although rare, neutral mucin may occur in mesotheliomas (48,49).

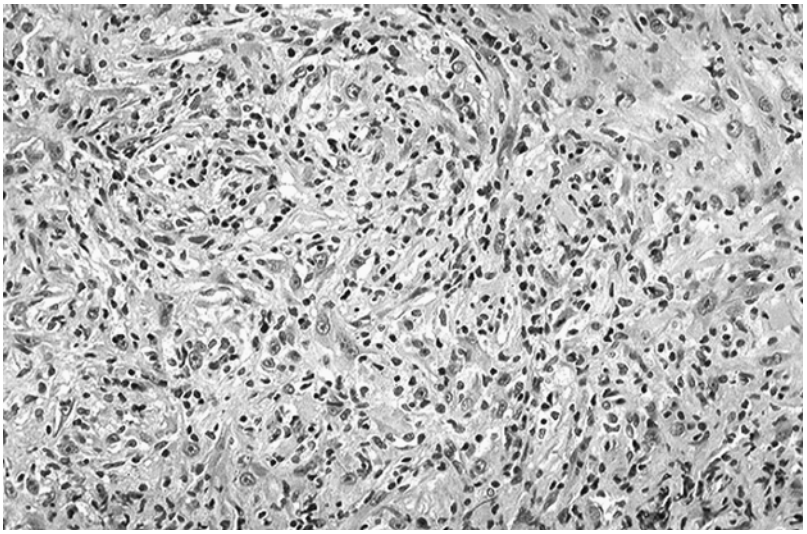


Figure 37.14. Lymphohistiocytoid mesothelioma. "Histiocytoid cells" with a prominent chronic inflammatory infiltrate superficially resembling lymphoma. (Courtesy of Dr. Elisabeth Brambilla.)

However, in such cases the diagnosis of mesothelioma should be made only if all other morphologic, immunohistochemical, and ultrastructural features are consistent with this interpretation. Electron microscopy may be particularly helpful in supporting the interpretation of mesothelioma in these cases.

"Lymphohistiocytoid mesothelioma" is a rare variant of sarcomatoid mesothelioma that can sometimes be confused with lymphoma. Clinically, patients with these neoplasms present with pleural thickening, effusion, or nodularity similar to other types of mesotheliomas. Histologically they are characterized by variable numbers of ovoid to spindle-shaped mesothelial cells that somewhat resemble histiocytes with a prominent admixed chronic inflammatory infiltrate consisting of lymphocytes and plasma cells (Fig. 37.14). Although superficially resembling lymphoma, they are readily distinguished by positive immunohistochemical staining of the histiocytoid mesothelial cells for cytokeratin. While background lymphocytes consist predominantly of mature T cells ($CD45^+$, $CD3^+$), the mesothelial cells lack staining with lymphoid markers. The mesothelial cells stain positively for cytokeratin and calretinin and stain negatively for other markers typically found in adenocarcinoma such as CEA, BER-EP4, and B72.3 (50,51).

"Deciduoid mesothelioma" is another rare variant of mesothelioma that may be mistaken for florid decidual reactions. Although initially reported in the peritoneum of young women (52), similar tumors have more recently been reported in the pleura in both adult men and women (53,54). Grossly, these tumors present similar to other mesotheliomas as multiple pleural nodules or as a diffuse rind encasing the underlying lung parenchyma. Histologically, they consist of sheets or trabeculae of large polygonal or ovoid cells with large vesicular nuclei

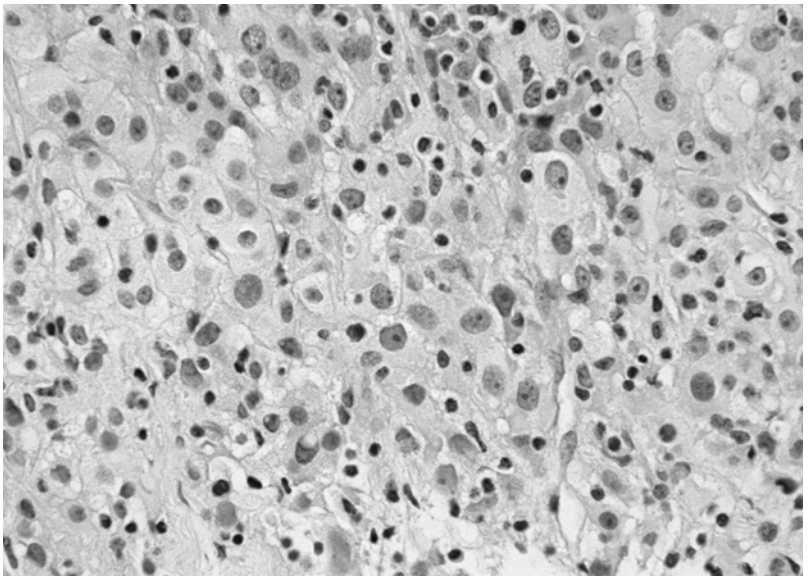


Figure 37.15. Deciduoid mesothelioma. Large polygonal cells with abundant cytoplasm, large vesicular nuclei and prominent nucleoli. (Courtesy of Dr. David Dail.)

and prominent nucleoli resembling decidua (Fig. 37.15). Deciduoid mesotheliomas may be differentiated from deciduoid reactions by their greater cytologic atypia, greater mitotic activity, and ultrastructural features. While staining for cytokeratin in decidual reactions has been variably reported as absent or focally positive, deciduoid mesotheliomas are consistently diffusely strongly positive for cytokeratin 5/6. These tumors also stain positively for markers of mesothelial differentiation (HBME-1 and calretinin) and negatively for markers expressed by adenocarcinoma (CEA, BER-EP4, and Leu M1) (52–54).

Rare Variants of Mesothelioma with Indeterminate Behavior

Well-differentiated papillary mesothelioma is a rare variant of mesothelioma originally thought to be restricted to the peritoneum, but more recently reported in the pleura (55,56). This variant is important to distinguish from the more typical diffuse malignant mesothelioma because of its generally better prognosis. Patients often present with dyspnea or recurrent pleural effusions. The effusions may be accompanied by nodular pleural thickening or a solitary mass radiographically. Histologically, the tumors consist of thin fibrovascular papillary cores lined by a single layer of bland cuboidal to flattened mesothelial cells. Mitoses are generally absent and invasion into the underlying pleura is uncommon or focal. The lining cells stain similarly to other mesothelial cells with positive staining for cytokeratin, HBME-1, and calretinin and negative staining for markers of adenocarcinoma (CEA,

B72.3, and Leu M1). Ultrastructural features are similar to those of other mesotheliomas with long slender microvilli. Well-differentiated papillary mesotheliomas may be distinguished from the more typical diffuse malignant mesotheliomas by the diffusely infiltrative nature of the latter. While initial reports of well-differentiated papillary mesothelioma suggested an entirely benign prognosis, more recent studies have suggested a variable course, with resolution in some patients and progression in others. Progression in some patients may be due to the presence of an unsampled or unrecognized diffusely infiltrative component (56).

Diagnostic Pitfalls in the Diagnosis of Metastatic Mesothelioma

Hyperplastic mesothelial cells may occasionally involve the sinuses of mediastinal or pelvic lymph nodes and cause confusion with metastatic mesothelioma or carcinoma (57–61). This finding usually occurs in the setting of a patient with pleural or pericardial inflammation or effusions. Some affected patients have had constrictive pericarditis or coronary artery disease. Histologically, there are small clusters or single cells within the lymph node sinus. Occasional cases with numerous cells can occur. Rare cases with cells in extranodal sinuses have also been reported (57). Individual cells are bland with eosinophilic cytoplasm and a central nucleus. While these cases can often be distinguished from metastatic carcinoma by immunohistochemical stains, distinction from metastatic mesothelioma may be more difficult and often requires careful clinicopathologic correlation (57–61).

References

1. Churg A. Diseases of the pleura. In: Thurlbeck WM, Churg AM, eds. Pathology of the Lung, 2nd ed. New York: Thieme, 1995:1067–1109.
2. Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferations. Am J Surg Pathol 2000;24:1183–1200.
3. Henderson DW, Shilkin KB, Whitaker D. Reactive mesothelial hyperplasia vs mesothelioma, including mesothelioma in situ: a brief review. Am J Clin Pathol 1998;110:397–404.
4. Mayall FG, Goddard H, Gibbs AR. P53 immunostaining in the distinction between benign and malignant mesothelial proliferations using formalin-fixed paraffin sections. J Pathol 1992;168:377–381.
5. Ayres JG, Crocker JG, Skilbeck NQ. Differentiation of malignant from normal and reactive mesothelioma cells by the argyrophil technique for nucleolar organizer region associated proteins. Thorax 1988;43:366–370.
6. Ramael M, van den Bossche J, Buysse C, et al. Immunoreactivity for P-170 glycoprotein in malignant mesothelioma and in non-neoplastic mesothelium of the pleura using the murine monoclonal antibody JSB-1. J Pathol 1992;167:5–8.
7. Kumaki F, Kawai T, Churg A, et al. Expression of telomerase reverse transcriptase (TERT) in malignant mesotheliomas. Am J Surg Pathol 2002;26: 365–370.

8. Kannerstein M, Churg J. Desmoplastic diffuse malignant mesothelioma. In: Fenoglio CM, Wolff M, eds. Progress in Surgical Pathology, vol 2, 1st ed. New York: Masson, 1980:19–29.
9. Wilson GE, Hasleton PS, Chatterjee AK. Desmoplastic malignant mesothelioma: a review of 17 cases. J Clin Pathol 1992;45:295–298.
10. Mangano WE, Cagle PT, Churg A, Vollmer RT, Roggli VL. The diagnosis of desmoplastic malignant mesothelioma and its distinction from fibrous pleurisy: a histologic and immunohistochemical analysis of 31 cases including p53 immunostaining. Am J Clin Pathol 1998;110:191–199.
11. McCaughey WTE, Al-Jabi M. Differentiation of serosal hyperplasia and neoplasia in biopsies. Pathol Ann 1986;21:271–293.
12. Bolen JW, Hammar SP, McNutt MA. Reactive and neoplastic serosal tissue. A light-microscopic, ultrastructural, and immunocytochemical study. Am J Surg Pathol 1986;10:34–47.
13. Askin FB, McCann BG, Kuhn C. Reactive eosinophilic pleuritis. A lesion to be distinguished from pulmonary eosinophilic granuloma. Arch Pathol Lab Med 1977;101:187–192.
14. McDonnell TJ, Crouch EC, Gonzalez JG. Reactive eosinophilic pleuritis. A sequela of pneumothorax in pulmonary eosinophilic granuloma. Am J Clin Pathol 1989;91:107–111.
15. Rosai J, Dehner LP. Nodular mesothelial hyperplasia in hernia sacs: a benign reactive condition simulating a neoplastic process. Cancer 1975;35: 165–175.
16. Chan JK, Loo KT, Yau BK, Lam SY. Nodular histiocytic/mesothelial hyperplasia: a lesion potentially mistaken for a neoplasm in transbronchial biopsy. Am J Surg Pathol 1997;21:658–663.
17. Ordonez NG, Ro JY, Ayala AG. Lesions described as nodular mesothelial hyperplasia are primarily composed of histiocytes. Am J Surg Pathol 1998; 22:285–292.
18. Golden A, Ash JE. Adenomatoid tumors of the genital tract. Am J Pathol 1945;21:63–79.
19. Nogales FF, Isaac MA, Hardisson D, et al. Adenomatoid tumors of the uterus: an analysis of 60 cases. Int J Gynecol Pathol 2002;21:34–40.
20. Handra-Luca A, Couvelard A, Abd AI, et al. Adenomatoid tumor of the pleura. Case report. Ann Pathol 2000;20:369–372.
21. Ikuta N, Tano M, Iwata M, et al. A case of adenomatoid mesothelioma of the pleura. Nihon Kyobu Shikkan Gakkai Zasshi. Jpn J Thorac Dis 1989; 27:1540–1544.
22. Kaplan MA, Tazelaar HD, Hayashi T, Schroer KR, Travis WD. Adenomatoid tumors of the pleura. Am J Surg Pathol 1996;20:1219–1223.
23. Fukayama M, Maeda Y, Funata N, et al. Pulmonary and pleural thymoma. Diagnostic application of lymphocyte markers to the thymoma of unusual site. Am J Clin Pathol 1988;89:617–621.
24. Moran CA, Travis WD, Rosado-de-Christenson M, Koss MN, Rosai J. Thymomas presenting as pleural tumors. Am J Surg Pathol 1992;16:138–144.
25. Koss M, Travis W, Moran C, Hochholzer L. Pseudomesotheliomatous adenocarcinoma: a reappraisal. Semin Diagn Pathol 1992;9:117–123.
26. Koss MN, Fleming M, Przygodzki RM, Sherrod A, Travis W, Hochholzer L. Adenocarcinoma simulating mesothelioma: a clinicopathologic and immunohistochemical study of 29 cases. Ann Diagn Pathol 1998;2:93–102.
27. Harwood TR, Gracey DR, Yokoo H. Pseudomesotheliomatous carcinoma of the lung. A variant of peripheral lung cancer. Am J Clin Pathol 1976; 65:159–167.

28. Ordonez NG. The immunohistochemical diagnosis of epithelial mesothelioma. Hum Pathol 1999;30:313–323.
29. Di Loreto C, Puglisi F, Di Lauro V, Damante G, Beltrami CA. TTF-1 protein expression in pleural malignant mesotheliomas and adenocarcinomas of the lung. Cancer Lett 1998;124:73–78.
30. Abutaily AS, Addis BJ, Roche WR. Immunohistochemistry in the distinction between malignant mesothelioma and pulmonary adenocarcinoma: a critical evaluation of new antibodies. J Clin Pathol 2002;55:662–668.
31. Lin BTY, Colby T, Gown AM, et al. Malignant vascular tumors of the serous membranes mimicking mesothelioma. A report of 14 cases. Am J Surg Pathol 1996;20:1431–1439.
32. Gaertner E, Zeren EH, Fleming MV, Colby TV, Travis WD. Biphasic synovial sarcomas arising in the pleural cavity. A clinicopathologic study of five cases. Am J Surg Pathol 1996;20:36–45.
33. Cappello F, Barnes L. Synovial sarcoma and malignant mesothelioma of the pleura: review, differential diagnosis and possible role of apoptosis. Pathology 2001;33:142–148.
34. Colwell AS, D'Cunha J, Vargas SO, Parker B, Cin PD, Maddaus MA. Synovial sarcoma of the pleura: a clinical and pathologic study of three cases. J Thorac Cardiovasc Surg 2002;124:828–832.
35. Miettinen M, Limon J, Niezabitowski A, Lasota J. Calretinin and other mesothelioma markers in synovial sarcoma: analysis of antigenic similarities and differences with malignant mesothelioma. Am J Surg Pathol 2001; 25:610–617.
36. Ansari MQ, Dawson DB, Nador R, et al. Primary body cavity-based AIDS-related lymphomas. Am J Clin Pathol 1996;105:221–229.
37. Jaffe ES. Primary body cavity-based AIDS-related lymphomas. Am J Clin Pathol 1996;105:141–142.
38. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 1995;332:1186–1191.
39. McNiff JM, Cooper D, Howe G, et al. Lymphomatoid granulomatosis of the skin and lung. An angiocentric T-cell-rich B-cell lymphoproliferative disorder. Arch Dermatol 1996;132:1464–1470.
40. Martin A, Capron F, Liguory-Brunaud MD, De Frejacques C, Pluot M, Diebold J. Epstein-Barr virus-associated primary malignant lymphomas of the pleural cavity occurring in longstanding pleural chronic inflammation. Hum Pathol 1994;25:1314–1318.
41. Ibuka T, Fukayama M, Hayashi Y, et al. Pyothorax-associated pleural lymphoma. A case evolving from T-cell-rich lymphoid infiltration to overt B-cell lymphoma in association with Epstein-Barr virus. Cancer 1994;73: 738–744.
42. Molinie V, Pouchot J, Navratil E, Aubert F, Vinceneux P, Barge J. Primary Epstein-Barr virus-related non-Hodgkin's lymphoma of the pleural cavity following long-standing tuberculous empyema. Arch Pathol Lab Med 1996; 120:288–291.
43. Nakatsuka S, Yao M, Hoshida Y, Yamamoto S, Iuchi K, Aozasa K. Pyothorax-associated lymphoma: a review of 106 cases. J Clin Oncol 2002; 20:4255–4260.
44. Said JW, Tasaka T, Takeuchi S, et al. Primary effusion lymphoma in women: report of two cases of Kaposi's sarcoma herpes virus-associated effusion-based lymphoma in human immunodeficiency virus-negative women. Blood 1996;88:3124–3128.

45. Crotty TB, Myers JL, Katzenstein ALA, Tazelaar HD, Swensen SJ, Churg AJ. Localized malignant mesothelioma. Am J Surg Pathol 1994;18:357–363.
46. Ojeda HF, Mech KJ, Hicken WJ. Localized malignant mesothelioma: a case report. Am Surg 1998;64:881–885.
47. Crotty TB, Colby TV, Gay PC, Pisani RJ. Desmoplastic malignant mesothelioma masquerading as sclerosing mediastinitis: a diagnostic dilemma. Hum Pathol 1992;23:79–82.
48. MacDougall DB, Wang SE, Zidar BL. Mucin-positive epithelial mesothelioma. Arch Pathol Lab Med 1992;116:874–880.
49. Hammar SP, Bockus DE, Remington FL, Rohrbach KA. Mucin-positive epithelial mesotheliomas: a histochemical, immunohistochemical, and ultrastructural comparison with mucin-producing pulmonary adenocarcinomas. Ultrastruct Pathol 1996;20:293–325.
50. Khalidi HS, Medeiros LJ, Battifora H. Lymphohistiocytoid mesothelioma. An often misdiagnosed variant of sarcomatoid malignant mesothelioma. Am J Clin Pathol 2000;113:649–654.
51. Henderson DW, Attwood HD, Constance TJ, Shilkin KB, Steele RH. Lymphohistiocytoid mesothelioma: a rare lymphomatoid variant of predominantly sarcomatoid mesothelioma. Ultrastruct Pathol 1988;12:367–384.
52. Nascimento AG, Keeney GL, Fletcher CD. Deciduoid peritoneal mesothelioma. An unusual phenotype affecting young females. Am J Surg Pathol 1994;18:439–445.
53. Ordonez NG. Epithelial mesothelioma with deciduoid features: report of four cases. Am J Surg Pathol 2000;24:816–823.
54. Shanks JH, Harris M, Banerjee SS, et al. Mesotheliomas with deciduoid morphology: a morphologic spectrum and a variant not confined to young females. Am J Surg Pathol 2000;24:285–294.
55. Daya D, McCaughey WT. Well-differentiated papillary mesothelioma of the peritoneum. A clinicopathologic study of 22 cases. Cancer 1990;65: 292–296.
56. Butnor KJ, Sporn TA, Hammar SP, Roggli VL. Well-differentiated papillary mesothelioma. Am J Surg Pathol 2001;25:1304–1309.
57. Isotalo PA, Veinot JP, Jabi M. Hyperplastic mesothelial cells in mediastinal lymph node sinuses with extranodal lymphatic involvement. Arch Pathol Lab Med 2000;124:609–613.
58. Parkash V, Vidwans M, Carter D. Benign mesothelial cells in mediastinal lymph nodes. Am J Surg Pathol 1999;23:1264–1269.
59. Clement PB, Young RH, Oliva E, Sumner HW, Scully RE. Hyperplastic mesothelial cells within abdominal lymph nodes: mimic of metastatic ovarian carcinoma and serous borderline tumor—a report of two cases associated with ovarian neoplasms. Mod Pathol 1996;9:879–886.
60. Brooks JS, LiVolsi VA, Pietra GG. Mesothelial cell inclusions in mediastinal lymph nodes mimicking metastatic carcinoma. Am J Clin Pathol 1990;93: 741–748.
61. Rutty GN, Lauder I. Mesothelial cell inclusions within mediastinal lymph nodes. Histopathology 1994;25:483–487.

38

Management of Benign Variants of Mesothelioma

Raja M. Flores

Benign pleural plaques, solitary fibrous tumors of the pleura, and malignant pleural mesothelioma are discussed together in this chapter. Although solitary fibrous tumors are not considered to be of mesothelial origin, the clinical and radiologic presentation of these tumors and a spectrum of other pleural-based processes may be quite similar. Distinguishing these conditions from diffuse malignant pleural mesothelioma is critical to ensure proper management (1). It is imperative to obtain the correct histologic diagnosis by surgical (thoracoscopic) biopsy—utilizing immunohistochemical staining and electron microscopy when necessary—because treatment options range from observation only for pleural plaques, to limited resection for solitary fibrous lesions, to a major operation such as extrapleural pneumonectomy for diffuse malignant pleural mesothelioma.

Non-neoplastic Lesions of the Pleura

Pleural Plaques

Pleural plaques, which usually result from asbestos exposure, can present as diffuse thickening of the visceral and parietal pleural layers. These lesions can vary in appearance from scattered nodular lesions on the pleural surface to lesions as wide as 6 cm. The coalescence of pleural surfaces and the propensity for the lower hemithorax can cause these lesions to be mistaken for diffuse malignant pleural mesothelioma. Plaques are thought to be formed by lymphatic transport of asbestos fibers from the visceral to the parietal pleura, with the fibers undergoing phagocytosis by macrophages that secrete substances stimulating submesothelial fibroblasts (2). It is important to remember that both mesothelioma and pleural plaques may be present simultaneously.

The distinction between benign pleural plaques and mesothelioma generally can be made by computed tomography (CT) scan. Pleural calcifications are often present in patients with a history of asbestos exposure, and extensive calcification usually indicates benign pleural

pathology (3). Pleural plaques also have a relatively smooth contour with a plateau-like appearance on the pleural surface. By contrast, malignant mesotheliomas appear more irregular and nodular on CT scan. In certain cases, positron emission tomography (PET) scan may distinguish benign from malignant pleural pathology (4). However, when the diagnosis is uncertain, surgical biopsy should be performed, preferably by videothoracoscopy (VATS).

Pleural Fibrosis

Pleural fibrosis may result from a variety of diseases, including rheumatoid arthritis and other connective tissue disorders, silicosis, pneumoconiosis, hypersensitivity pneumonitis, or endometriosis. However, the most frequent cause of pleural fibrosis is bacterial pneumonia, especially empyema. It is very difficult to distinguish clinically between exclusive benign pleural fibrosis and desmoplastic mesothelioma. The pathologic distinction between these two entities cannot be made by frozen section and requires immunohistochemical staining or even electron microscopy. Severe reactions to foreign bodies such as talc, mineral oil, or starch can also give a similar clinical appearance to mesothelioma (1).

Although pleural fluid cytology or percutaneous pleural biopsy may demonstrate cells suspicious for mesothelioma, they often fail to establish a definitive tissue diagnosis. Formal surgical resection via extrapleural pneumonectomy or pleurectomy/decortication should not be undertaken until a definitive tissue diagnosis is obtained by VATS or open pleural biopsy. Only then can appropriate decisions be made about treatment.

Treatment

Pleural plaques are benign lesions and do not necessarily portend the development of malignant pleural mesothelioma. However, because of the association between pleural plaques and prior asbestos exposure, it is reasonable to keep patients who have plaques under CT scan surveillance. Although there is no standard recommendation for follow-up, our policy at Memorial Sloan-Kettering Cancer Center is to obtain CT scans annually. Progressive pleural fibrosis may infrequently require decortication to improve lung function.

Solitary Fibrous Tumor of the Pleura

These tumors were previously called localized or solitary mesothelioma, pleural fibroma, localized fibrous tumor, submesothelial fibroma, or localized fibrous mesothelioma. They differ significantly from diffuse malignant mesothelioma in origin and behavior. Whereas

diffuse malignant mesothelioma is thought to develop from mesothelial cells, the localized fibrous tumor arises from the submesothelial connective tissue (5). In contrast to the histologic features of diffuse malignant pleural mesothelioma, fibrous tumors of the pleura do not have epithelial features, do not stain for cytokeratins on immunohistochemical analysis, and do not have multiple long branching microvilli on electron microscopy (6).

The etiology of solitary fibrous tumors is unknown and they are not associated with prior asbestos exposure. They are rare, with approximately 800 reported cases in the literature. They may occur at any age (although predominantly during the sixth and seventh decades), are seen with equal frequency in men and women, and may develop anywhere in the chest with no particular affinity for any hemithorax or lobe.

Approximately 50% of benign fibrous tumors of the pleura are asymptomatic and are identified incidentally on routine chest x-ray. They generally appear as a well-circumscribed, homogeneous lesion located at the periphery or between fissures on CT scan (Fig. 38.1). Occasionally, pedunculated lesions may be demonstrated radiographically and can change position in association with body position and respiration (Fig. 38.2). Symptomatic patients may present with chest pain, cough, dyspnea, and fever. Pierre-Marie-Bamberg syndrome (pulmonary osteoarthropathy and clubbing) has been described in approximately 15% of cases and is related to the production of hyaluronic acid by the tumor (7). Doege-Potter syndrome (refractory hypoglycemia) has been described in approximately 5% of cases and is caused by tumor secretions of an insulin-like substance (8). Resolution of symptoms occurs after tumor resection, and recurrence of symptoms usually signals tumor recurrence.

Solitary fibrous tumors of the pleura may be either benign or malignant (Table 38.1). Benign fibrous tumors are usually pedunculated, measure less than 10 cm, arise from the visceral pleura, are relatively acellular, and have few mitotic figures. The average tumor is about 6 cm in size, but may range from subcentimeter to 40 cm, and some have weighed as much as 3 kg. In contrast, malignant fibrous tumors are larger on average and are frequently nonpedunculated tumors that arise from the parietal, mediastinal, or diaphragmatic pleura. They display increased cellularity, pleomorphism, and frequent mitotic figures (Table 38.2).

The distinction between benign and malignant solitary fibrous tumors is occasionally difficult. The histologic features and the number of mitotic figures do not necessarily reflect the tumor's propensity to recur because malignant degeneration of benign solitary fibrous tumors has been reported (9). In fact, benign tumors may recur as histologically more malignant tumors years after the initial resection (10). However, the clinical presentation and benign versus malignant histology generally appear to be the best predictors of outcome. A comprehensive summary of published data on outcome was recently reported by de Perrot et al (10) (Table 38.3).

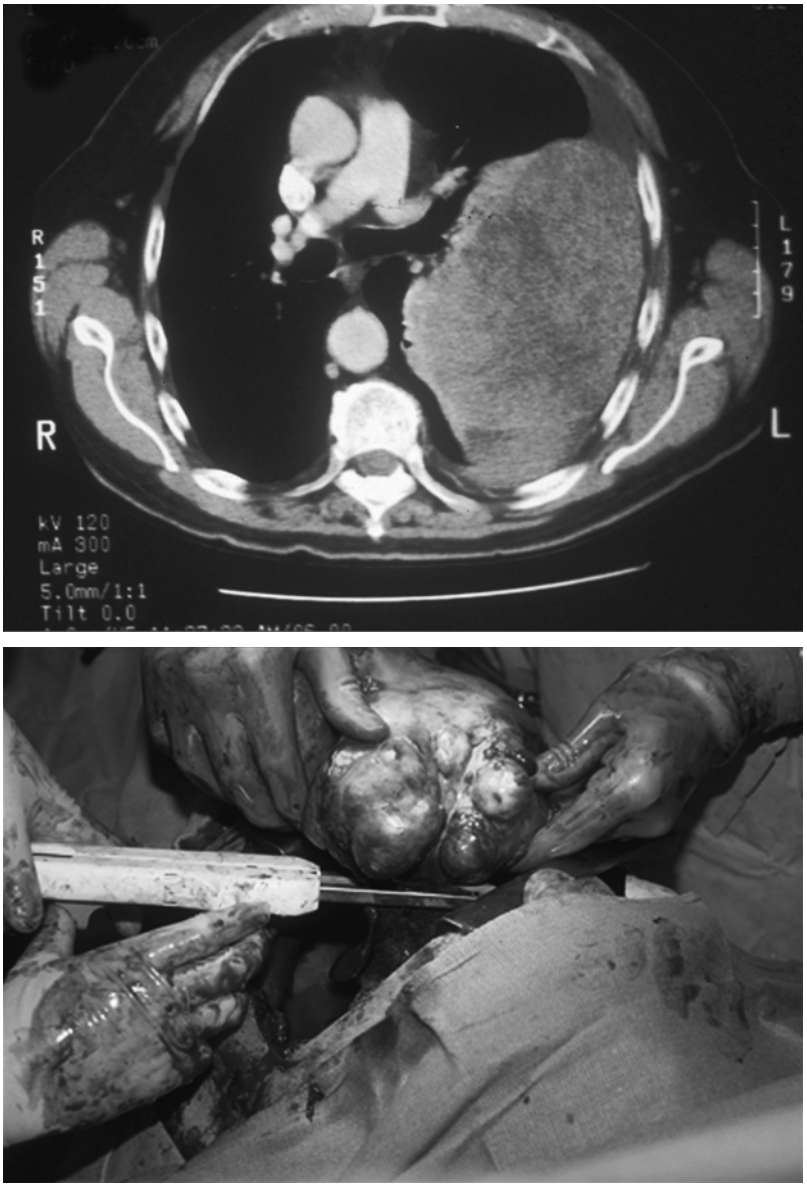
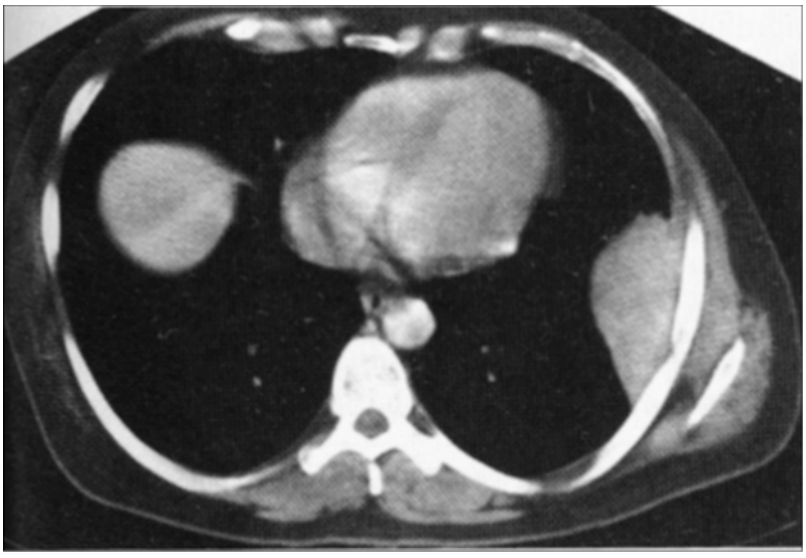


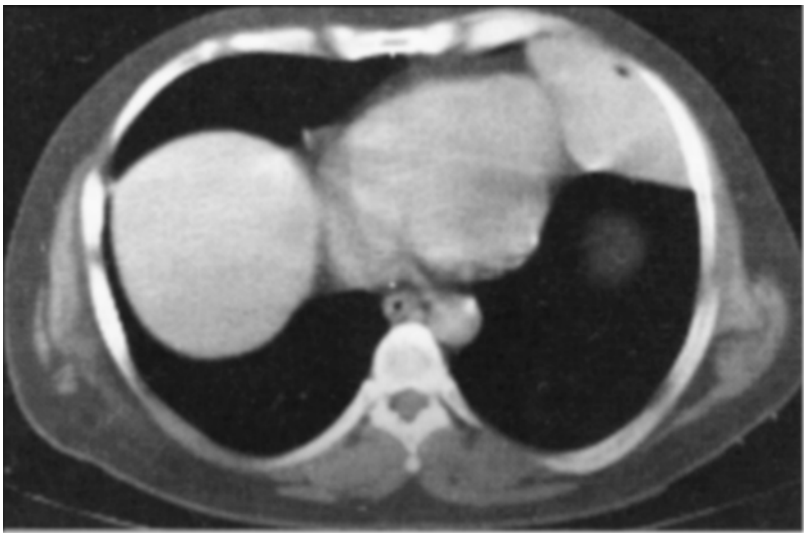
Figure 38.1. A: Computed tomography (CT) scan of large solitary fibrous tumor of the pleura. B: Thoracotomy view of solitary fibrous tumor.

Treatment

The mainstay of treatment for either benign or malignant solitary fibrous tumors is complete surgical resection. Given the relatively benign nature of the majority of solitary fibrous tumors, it is important to recognize them preoperatively in order to plan the operative approach. Since the average diameter is approximately 6 cm, VATS resection of these lesions is usually possible. Most benign solitary



A



B

Figure 38.2. A: CT of benign pedunculated solitary fibrous tumor. B: Change in tumor position with movement and respiration.

Table 38.1. Pathologic features that distinguish benign from malignant localized fibrous tumors of pleura

	Benign (n = 141)		Malignant (n = 82)	
Feature	**No.**	**(%)**	**No.**	**(%)**
Gross				
Pedunculated	**73**	**(52)**	**21**	**(26)**
Atypical location	**67**	**(48)**	**55**	**(67)**
Size (>10 cm)	**34**	**(24)**	**45**	**(55)**
Necrosis and hemorrhage	**21**	**(15)**	**53**	**(65)**
Microscopic				
Increased cellularity	**18**	**(13)**	**62**	**(76)**
Pleomorphism	**14**	**(10)**	**69**	**(84)**
Mitosis (>4 mf/10 hpf)	**2**	**(1)**	**63**	**(77)**

Source: Data from Englund et al (16).

Table 38.2. Summary of reported features in 360 cases of solitary fibrous tumors of the pleura

Years	No. of patients	Age range (y)	sex M/F	Symptomatic patients (% of total)	Prevalence of Various Symptoms (%)						Pleural laterality (right/ left)	Clinical origin (visceral/ parietal)	Behavior: (benign/ lethal)
					Cough	Chest pain	Dyspnea	PO	Fever	Other			
1942–1972	190	12–82	84/106 (mean 50)	72	39	40	26	47	24	37	61/60	51/28	147/20
1973–1980	170	5–87	81/89 (mean 53)	54	54	51	49	22	25	25	78/68	90/23	142/18
Total	360	5–87	165/195 (mean 51)	64	46	44	37	35	24	32	139/128	141/51	289/38

PO, Pulmonary osteoarthropathy.
Source: Data from Briselli et al (5).

Table 38.3. Summary of recent publications on solitary fibrous tumors of pleura (SFTP)*

	Malignant sessile	Malignant pedunculated	Benign sessile	Benign pedunculated
Total number of patients	**43**	**15**	**62**	**65**
Number of patients alive without recurrence	**16 (37%)**	**13 (87%)**	**57 (92%)**	**64 (98%)**
Number of patients alive with at least one recurrence	**14 (33%)**	**1 (7%)**	**4 (6%)**	**—**
Number of deaths related to the tumor	**13 (30%)**	**1 (7%)**	**1 (2%)**	**1 (2%)**
<24 months	**10**	**1**	**—**	**—**
>24 months	**3**	**—**	**1**	**1**

* Includes all series reporting adequate follow-up for patients with a diagnosis of SFTP proven by histology and immunohistochemistry.
Source: Data from dePerrot et al (14), with permission.

fibrous tumors of the pleura arise as pedunculated masses from the visceral pleura and can be removed by simple wedge resection of the underlying pulmonary parenchyma (Figs. 38.3 and 38.4). It is important to resect the tumor completely, including the stalk, to prevent local recurrence. If there is any doubt about residual tumor or of an inadequate resection by VATS, then a standard thoracotomy should be performed to complete the resection.

In addition, a thorough exploration of the entire pleural cavity, including the pleural, pericardial, and diaphragmatic surfaces, is essential because contact metastasis to adjacent structures has been described (11). Based on a review of the literature, de Perrot et al (10) constructed an algorithm of recurrence risk based on clinical presentation and benign versus malignant histology (Fig. 38.5). The recurrence rate is highest after resection of malignant sessile solitary fibrous tumors. Although most recurrences occur locally within the hemithorax, extrathoracic metastasis has also been described (9,12).

An extensive surgical resection should be performed when treating primary tumors that are adherent to surrounding structures and local recurrences as well. En bloc chest wall, pericardial, or diaphragmatic resection is warranted when lesions are adherent to these areas. Lobectomy, pneumonectomy, or even extrapleural pneumonectomy may be required in some cases, especially in cases of recurrent malignant fibrous tumor of the pleura (Fig. 38.4).

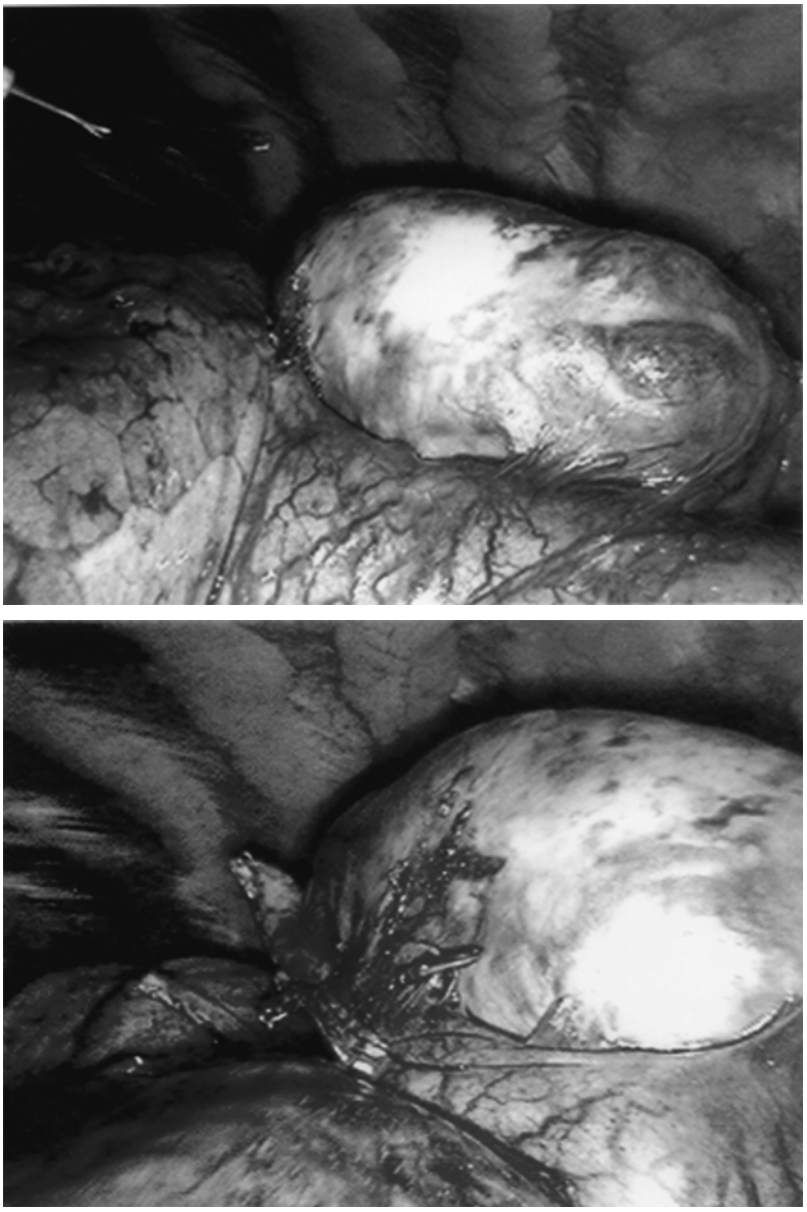
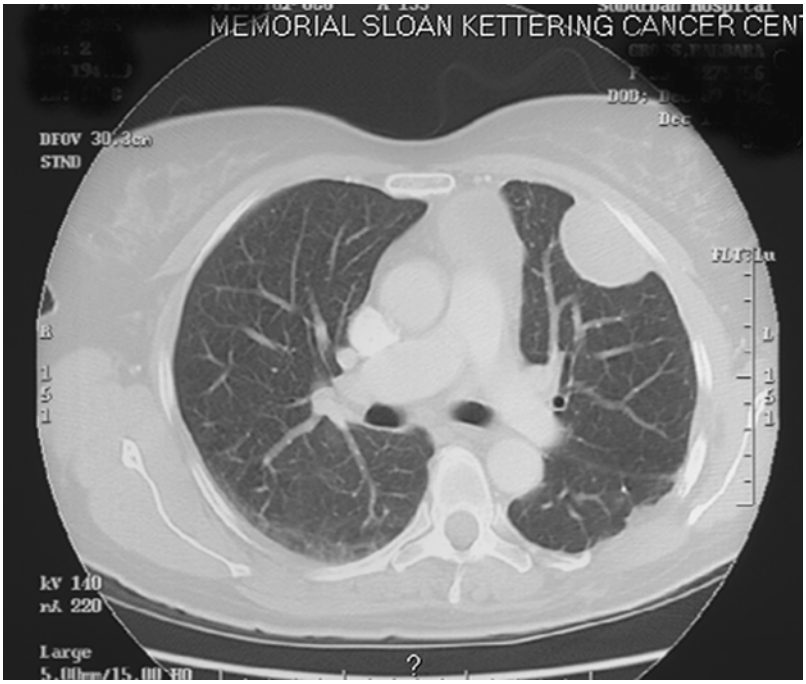
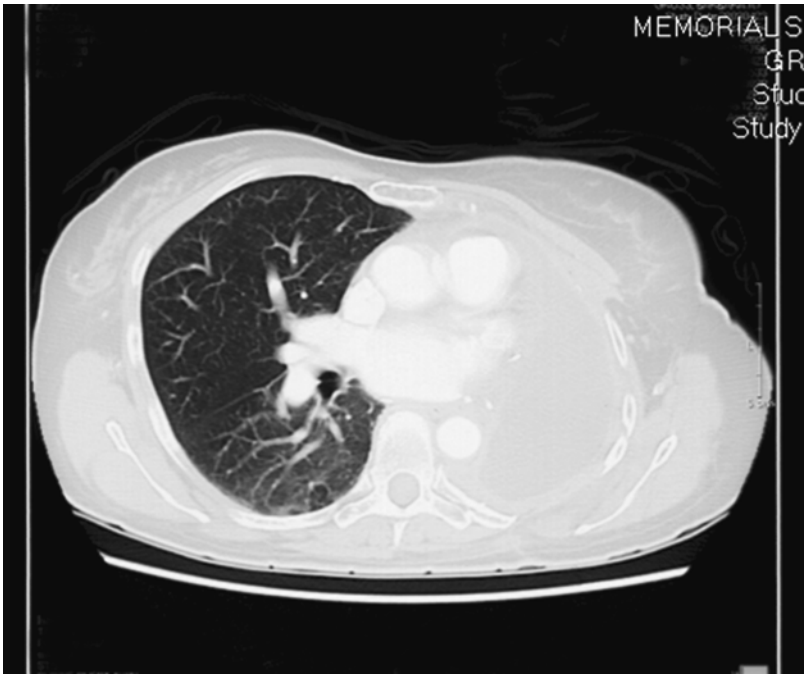


Figure 38.3. A: Thoracoscopic view of malignant fibrous tumor. B: Thoracoscopic resection of malignant fibrous tumor, note stalk.

The role of adjuvant therapy is unclear. There have been anecdotal reports of resection followed by radiotherapy (7,12) and chemotherapy (13). We have successfully used postoperative high-dose (54 Gy) hemithoracic radiation after pneumonectomy or extrapleural pneumonectomy for recurrent malignant fibrous tumors to decrease the risk of local recurrence.



A



B

Figure 38.4. A: Malignant fibrous tumor recurrent after resection of benign fibrous tumor at 2-year follow-up. B: Extrapleural pneumonectomy with adjuvant high-dose hemithoracic radiation for recurrent malignant fibrous tumor.

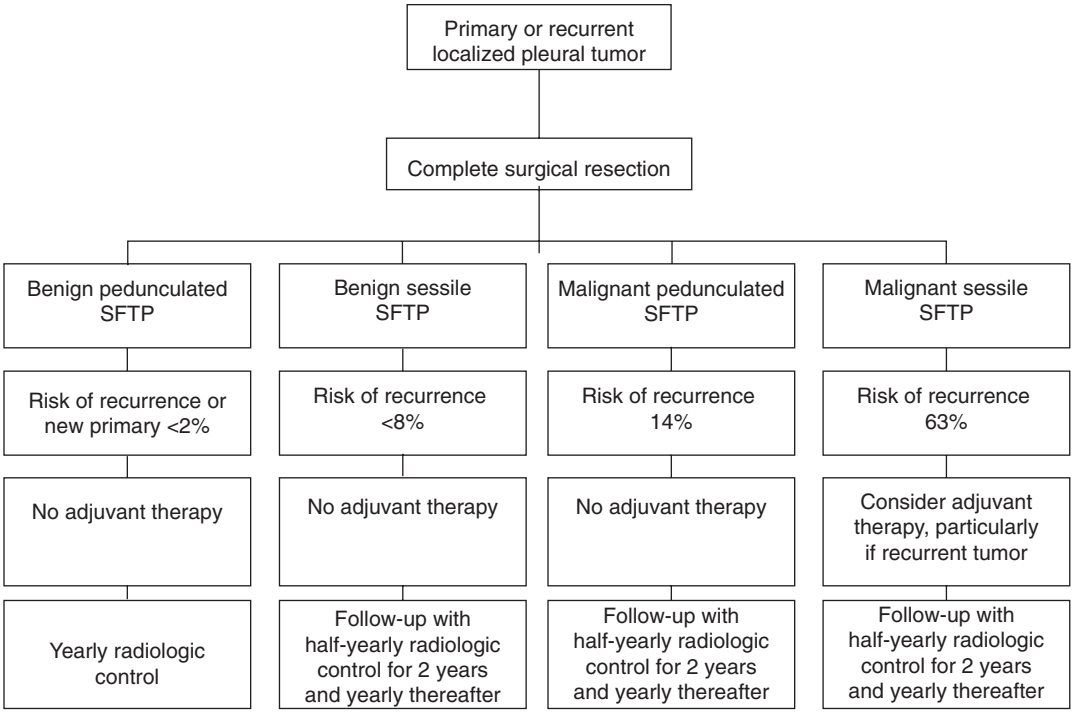


Figure 38.5. Treatment plan and follow-up according to the type of solitary fibrous tumor of the pleura (SFTP). *Source*: Data from dePerrot et al (14), with permission.

Other Less Common Tumors of the Pleura

Other primary tumors of the pleura are exceedingly rare and include lipomas, endotheliomas, angiomas, angiosarcomas, and cysts. Neurogenic sarcoma, synovial sarcoma, fibrosarcoma, and malignant fibrous histiocytoma may be mistakenly diagnosed as solitary fibrous tumors because of the dense spindle cell proliferation (14). The majority of these are thought to arise from the subpleural tissues rather than from the pleura itself. Lipomas are the most common among these rare tumors, and a review of 7751 CT examinations of the chest found pleural lipomas to be present incidentally in 0.14% of cases (15). Lipomas have a characteristic appearance on chest radiograph and usually present as a smooth, well-defined mass flattened against the chest wall. Pleural cysts usually arise at the pleuropericardial angle and are seen on x-ray as discrete unilocular masses. In the past, these uncommon tumors have been surgically excised for diagnosis rather than symptoms. However, if a definitive diagnosis can be made based on CT scan characteristics, such lesions may be observed rather than resected.

Summary

The distinction between benign pleural plaques and malignancy can usually be made by simple radiographic findings. When there is uncertainty, surgical biopsy, preferably by a VATS approach, should be performed. Biopsy should be performed separately from the definitive resection because immunohistochemistry and electron microscopy are usually required to distinguish benign pleural lesions from diffuse malignant pleural mesothelioma.

Benign or malignant solitary fibrous tumors, when pedunculated and free of adjacent structures, may be easily treated by VATS surgical excision. Careful evaluation of adjacent structures and the entire pleural cavity is essential to minimize the risk of recurrence. All solitary fibrous tumors, regardless of a benign or malignant histology, must be approached with caution because of the risk of local recurrence. The initial treatment of choice for solitary fibrous tumors or pleural tumors mimicking this lesion is en bloc surgical resection. Patients should be followed closely postoperatively and recurrences managed by extensive surgical resection with or without adjuvant radiation.

References

1. Battifora H, McCaughey W. Tumors of the serosal membranes. In: Battifora H, McCaughey W, eds. Atlas of Tumor Pathology, vol 15. Washington, DC: Armed Forces Institute of Pathology, 1995.
2. Hillerdal G. The pathogenesis of pleural plaques and pulmonary asbestosis: possibilities and impossibilities. Eur J Respir Dis 1980;61:129–138.
3. Titola M, Kivisaari L, Zitting A, et al. Computed tomography of asbestos-related pleural abnormalities. Int Arch Occup Environ Health 2002;75:224–228.
4. Benard F, Sterman D, Smith RJ, et al. Metabolic imaging of malignant pleural mesothelioma with fluorodeoxyglucose positron emission tomography. Chest 1998;114:713–722.
5. Briselli M, Mark E, Dickersin R. Solitary fibrous tumors of the pleura: eight new cases and review of 360 cases in the literature. Cancer 1981; 47:2678.
6. Said J, Nash G, Banks-Schlegel S, et al. Localized fibrous mesothelioma: an immunohistochemical and electron microscopic study. Hum Pathol 1984; 15:440.
7. Cardillo G, Facciolo F, Cavazzana A, et al. Localized (solitary) fibrous tumors of the pleura: an analysis of 55 patients. Ann Thorac Surg 2000;70: 1808–1812.
8. Chamberlain M, Taggart D. Solitary fibrous tumor associated with hypoglycemia: an example of the Doege-Potter syndrome. J Thorac Cardiovasc Surg 2000;119:185–187.
9. Yokoi T, Tsuzuki T, Yatabe Y, et al. Solitary fibrous tumor: significance of p53 and CD34 immunoreactivity in its malignant transformation. Histopathology 1998;32:423–432.

10. dePerrot M, Kurt A, Robert J, et al. Clinical behavior of solitary fibrous tumors of the pleura. Ann Thorac Surg 1999;67:1456–1459.
11. Nomori H, Horio H, Fuyuno G, et al. Contacting metastasis of a fibrous tumor of the pleura. Eur J Cardiothorac Surg 1997;12:928–930.
12. Suter M, Gebhard S, Boumghar M, et al. Localized fibrous tumors of the pleura: 15 new cases and review of the literature. Eur J Cardio Thorac Surg 1998;14:453–459.
13. Veronesi G, Spaggiari L, Mazzarol G, et al. Huge malignant localized fibrous tumor of the pleura. J Cardiovasc Surg 2000;41:781–784.
14. dePerrot M, Fischer S, Brundler M, et al. Solitary fibrous tumor of the pleura. Ann Thorac Surg 2002;74:285–293.
15. Christmas T, Manning L, Davis M, et al. HLA antigen expression and malignant mesothelioma. Am J Respir Cell Mol Biol 1991;5:213.
16. England D, Hochholzer L, McCarthy M. Localized benign and malignant fibrous tumors of the pleura: a clinicopathologic review of 223 cases. Am J Surg Pathol 1989;13:640.

39

First-Line Chemotherapy for Malignant Pleural Mesothelioma

Pasi A. Jänne

The Impact of Systemic Chemotherapy

The true impact of systemic chemotherapy in mesothelioma has been difficult to evaluate because of the rarity of the disease, the paucity of randomized studies, the lack of uniform staging, heterogeneity within the pathologic subclasses of mesothelioma, the imbalance of prognostic factors, and the difficulties in assessing response to therapy using computed tomography (CT) and other radiographic imaging modalities. It presently is not clear whether chemotherapy prolongs survival in patients with mesothelioma compared to supportive care alone.

The natural history of mesothelioma can be variable and thus benefits seen in clinical trials may be biased by patient selection. Some attempts to define the natural history of mesothelioma have been made. Merritt and colleagues examined 101 patients who were not candidates for aggressive surgical therapy between 1987 and 1999. Seventy of these patients received a pleurodesis, 30 had palliative radiation for chest pain, and 9 received chemotherapy as a radiation sensitizer. The median survival in this group of patients was 7 months [213 days; 95% confidence interval (CI), 137–289 days]. Unfortunately, staging information is not available in these patients. A retrospective review of 332 patients with mesothelioma from Canada from 1965 to 1984, identified 176 patients who were treated with palliative care alone without any surgery, chemotherapy, or radiation therapy (1). In this group of patients the media survival was 6.8 months. Although neither of these studies was randomized, they do provide some data on the natural history of the disease in the absence of aggressive surgery or systemic chemotherapy. In contrast, the median survival of 337 patients in 11 mesothelioma clinical trials conducted by the Cancer and Leukemia Group B (CALGB) between 1984 and 1994 was also only 7 months (2).

To date no randomized studies have been performed using supportive care alone as a control arm. The British Thoracic Society and the British Medical Research Council are conducting the first such randomized study (Fig. 39.1) (3). In this study, patients with malignant

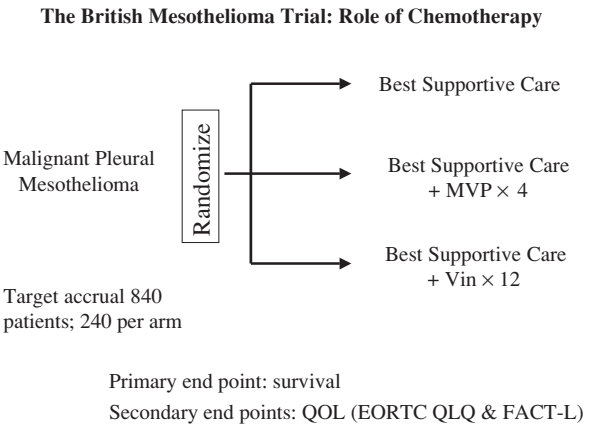


Figure 39.1. Phase III trial evaluating the role of chemotherapy in the treatment of mesothelioma. MVP, mitomycin, vinblastine, cisplatin; Vin, vinorelbine; QOL, quality of life.

mesothelioma will be randomized to receive best supportive care (BSC) alone, BSC and four cycles of mitomycin, vinblastine, and cisplatin (MVP), or BSC and 12 cycles of vinorelbine. The choice of the chemotherapy arms is based on prior phase II studies that demonstrated an improvement in quality of life in patients receiving these chemotherapy regimens (4,5). This randomized study will assess the impact of chemotherapy not only on overall survival but also on patient's quality of life, which will be assessed by either the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ) C30 and LC13 or the Functional Assessment of Cancer Therapy–Lung (FACT-L) questionnaire (6). The sample size for this study is 840 patients (280 per arm) with a 90% power at a significance level of .05 to detect a median survival difference of 3 months (9 vs. 12 months) in patients receiving chemotherapy (3). The estimated accrual time is 4 years and preliminary data suggest that this design is feasible (7). This is a very important study, and it should provide definitive data on the benefits of chemotherapy and on survival and quality of life for patients with malignant mesothelioma. Unfortunately, the trial is experiencing accrual difficulties.

Evaluating the Impact of Chemotherapy

The evaluation of the impact of therapy is also challenging in mesothelioma. In addition to survival analyses, the main methods of evaluating the benefits of chemotherapy include radiographic tumor assessment, and evaluations of symptom control and improvements in the quality of life. Radiographic assessment, even by CT, of the pleural rind is sometimes difficult, especially when the tumor rind is accompanied by a pleural effusion or when the rind thickness is less than 1 cm.

Patients with pleural mesothelioma are also often symptomatic. They present with chest pain, shortness of breath, dyspnea, weight loss, and night sweats, among other symptoms. The impact of chemotherapy on the symptoms of mesothelioma is poorly characterized. Very few studies exist in which quality of life has been evaluated as a result of chemotherapy. Steele and colleagues (4) performed a phase II study using vinorelbine in patients with chemotherapy-naive malignant mesothelioma. This study also used the Rotterdam Symptom Checklist to assess quality of life. The partial radiographic response rate was 24%, but 48% of patients reported improvements in respiratory symptoms and 76% in psychosocial functioning. Importantly, quality of life improvements were not limited to patients achieving radiographic benefits but were also observed in patients without radiographic tumor regression.

Nowak and colleagues (8) performed a phase II multicenter study of cisplatin/gemcitabine in chemotherapy-naive patients with mesothelioma. The partial response rate to therapy was 17% (Table 39.1). Quality of life was assessed by the EORTC QLQ-C30 questionnaire and pulmonary function was assessed by measuring forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) (6). In patients who responded to chemotherapy there was a significant change in FVC over baseline ($p = .002$). There was no change in nonresponding patients. Patients who had stable disease as their best response to therapy were not examined as a separate group. Similarly, quality of life improved significantly in the responding compared to nonresponding (partial response and stable disease) patients ($p = .006$). Quality of life was also assessed in the phase III trial of cisplatin/pemetrexed versus cisplatin using a modified Lung Cancer Symptom Scale (LCSS) for patients with mesothelioma (9,10). Significant improvements in quality of life were seen as early as week 12 in cough, dyspnea, and pain, favoring those who received cisplatin/pemetrexed (10). By week 18 all measures of quality of life were statistically significant in favor of patients who received cisplatin/pemetrexed. This study also examined changes in vital capacity during chemotherapy treatment (11). There was a statistically significant improvement in vital capacity ($p = .034$ at cycle 4 and $p = .002$ at cycle 6) favoring patients receiving cisplatin/pemetrexed (12). Changes in pulmonary function tests (PFTs), slow vital capacity (SVC), FVC, and FEV_1 were also examined in relationship to radiographic response to chemotherapy (13). Patients who achieved a radiographic response to chemotherapy had a significant improvement in PFTs when compared to those with disease progression. However, patients with stable disease as their best radiographic response also had a statistically significant improvement in SVC, FVC, and FEV_1 compared to patients with disease progression (13). These studies underscore the importance of including the assessments of quality of life and pulmonary function, in addition to survival end points, in future chemotherapy studies of mesothelioma. Additional studies should also help confirm whether quality of life benefits are limited to just those with radiographic regressions or are also observed in patients with stable disease as a result of treatment.

Table 39.1. Combination chemotherapy studies in mesothelioma

Agent(s)	No. of patients	Response rate (95% CI)	Median survival (95% CI)	Reference
Anthracycline based				
Doxorubicin/Ifosfamide	24	32% (13–51%)	7 (NA)	57.
Doxorubicin/Ifosfamide	17	13% (2–38%)	7.8 (NA)	58.
Doxorubicin/cisplatin/cylophosphamide	23	30% (19–36%)	13.8 (NA)	59.
Doxorubicin/5azacytidine	36	22% (NA)	13 (NA)	60.
Doxorubicin/cisplatin/mitomycin c	24	21% (7–42%)	NA	61.
Doxorubicin/cyclophosphamide	36	11% (6–21%)	6.7 (NA)	62.
Doxorubicin/cylophosphamide/DTIC	40	13% (6–21%)	5.5 (NA)	62.
Doxorubicin/IFN α	25	16% (8–30%)	11 (3–19)	63.
Platinum based				
Cisplatin/DHAC	36	14% (5–36%)	6.4 (4.3–10.1)	64.
Cisplatin/irinotecan	15	27% (8–55%)	6.5 (NA)	65.
Cisplatin/vinblastine	20	25% (NA)	NA	66.
Cisplatin/doxorubicin	35	14% (5–30%)	8.8 (NA)	67.
Cisplatin/doxorubicin	26	25% (10–47%)	10 (NA)	68.
Cisplatin/doxorubicin/IFN α	37	29% (15–47%)	9.3 (NA)	69.
Cisplatin/mitomycin c	35	26% (12–43%)	7.7 (NA)	67.
Cisplatin/etoposide	27	12% (NA)	NA	70.
Cisplatin/mitomycin c/IFN α	20	10% (NA)	15 (NA)	71.
Cisplatin/mitomycin c/IFN α	43	23% (11–36%)	11.5 (10.2–12.8)	72.
Cisplatin/IFN α	26	36% (20–60%)	12 (NA)	73.
Cisplatin/IFN α	30	27% (NA)	NA	74.
Cisplatin/mitomycin c/etoposide/5FU	45	38% (24–53%)	16 (9.8–21.5)	75.
Cisplatin/mitomycin c/vinblastine	39	20% (NA)	6 (NA)	55.
Cisplatin/gemcitabine	21	48% (26–69%)	9.5 (NA)	29.
Cisplatin/gemcitabine	53	33% (20–46%)	11.2 (NA)	88.
Cisplatin/gemcitabine	32	16% (1–31%)	9.6 (8–12)	30.
Carboplatin/gemcitabine	50	26% (15–40%)	15.2 (NA)	31.
Cisplatin/paclitaxel	18	6% (0–24%)	12 (NA)	76.
Carboplatin/IFNa	15	7% (0–20%)	5.8 (NA)	77.
Carboplatin/pemetrexed	27	32% (NA)	14.8 (NA)	78.
Oxaliplatin/raltitrexed	55	20% (11–31%)	7.2 (5.3–9.2)	79.
Oxaliplatin/vinorelbine	17	12% (NA)	NA	80.
Oxaliplatin/gemcitabine	25	40% (21–61%)	13 (NA)	81.

CI, confidence interval; NA, not available; DTIC, dimethyltriazeno imidazole carboxamide; 5-FU, 5-fluorouracil; IFN, interferon; DHAC, dihydro-azacytidine.

Single-Agent Chemotherapy

Virtually every chemotherapy agent has been evaluated in patients with mesothelioma. The response rates to single-agent therapy have been variable, and only a few agents have demonstrated consistent response rates of 10% to 20%. The most efficacious single chemotherapy agents for mesothelioma are the anthracyclines, the antimetabolites, and the platinum analogues (Table 39.2). Agents with little to no antitumor activity include the taxanes, topoisomerase inhibitors, and vinca alkaloids apart from vinorelbine (14–18).

Of the anthracyclines, doxorubicin was one of the first agents to be tested in mesothelioma. The Eastern Cooperative Oncology Group (ECOG) performed a retrospective review of its experience with doxorubicin and chemotherapy regimens containing doxorubicin. The

response rate was 14%, including two complete responses, with a median survival of 7.5 months (19). However, subsequent studies failed to demonstrate similar antitumor activity of doxorubicin (20). Analogues of doxorubicin and liposomal formulations have also been examined and to date, appear to offer no additional benefit (Table 39.2). The response rates and median survivals have been quite variable, ranging from 0 to 43% and 4.4 to 17 months, respectively (Table 39.2). The largest single study of doxorubicin to date was a phase III trial comparing doxorubicin to ranpirnase (Onconase; Alfacell Corp., Bloomfield, NJ), an antitumor ribonuclease, in patients with mesothelioma (Table 39.3). Preliminary findings from the study have been presented; in the intention-to-treat population, the median survival for patients who received doxorubicin was 8.2 months (21). Single-agent cisplatin and carboplatin have also been investigated in at least four studies. The response rates are low (but consistent at 7% to 16%) and the median survivals range from 5 to 8 months. Cisplatin was the reference arm of the phase III trial comparing cisplatin to cisplatin/pemetrexed (Table 39.3) (12). In that study the median survival of patients treated on the cisplatin arm was 9.3 months (12). Thus for single-agent doxorubicin and cisplatin, these two phase III studies establish a benchmark with a median survival range of 8 to 9 months. A meta-analysis of 83 clinical studies of chemotherapy in the treatment of mesothelioma, conducted between 1983 and 2001, has also recently been performed (22). Cisplatin was identified as the most active single-agent treatment with response rate of 23.2% (95% CI, 19.7–26.8%) for cisplatin-containing regimens compared with 11.6 % (95% CI, 10–13.3%; $p < .001$) for non-cisplatin-containing regimens. Cisplatin is also the reference arm of an ongoing phase III trial comparing cisplatin to the combination of cisplatin and raltitrexed.

The other class of agents with consistent antitumor activity are the antimetabolites including the antifolates and nucleoside analogues (Table 39.2). The response rates range from 5% to 37% (apart from one study of gemcitabine with a 0% response rate) and the median survivals also range from 4.7 to 11 months (Table 39.2). The antifolates appear to be one of the most active group of agents in the treatment of mesothelioma (Table 39.2). The mechanism(s) behind these observations have not been completely elucidated. Antifolates can be transported into tumor cells through the alpha folate receptor, the reduced folate carrier, and via a newly identified pemetrexed transporter (23–25). This latter transporter, yet to be identified, is a high-affinity and specific pemetrexed transport mechanism found on mesothelioma cell lines. Future studies need to be performed to further characterize this mechanism, but these observations may in part explain the sensitivity of mesotheliomas to pemetrexed. An alternative, although not mutually exclusive, hypothesis is the presence of a common genetic deletion in mesotheliomas. A homozygous deletion of 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAP) located on chromosome 9p21 has been observed in approximately 70% of mesotheliomas.[26] Tumor cell lines that are MTAP deficient are more dependent on *de novo* purine biosynthesis and *in vitro* more sensitive to the effects of antifolates compared to those containing a wild type MTAP gene.[27,28] Thus

Table 39.2. Selected single agent chemotherapy studies in mesothelioma

Agent	No. of patients	Response rate (95% CI)	Median survival (95% CI)	Reference
Anthracyclines				
Doxorubicin	15	0%	NA	20.
Mitoxantrone	46	2.5% (0–13%)	4.4 (NA)	32.
Mitoxantrone	30	7% (NA)	NA	33.
Epirubicin	63	15% (6.1–27.8%)	9.2 (NA)	34.
Epirubicin	23	5% (NA)	7.5 (NA)	35.
Pirarubicin	35	8.6% (NA)	10 (NA)	36.
Detorubicin	35	43% (NA)	17 (NA)	37.
Menogaril	22	5% (0–23%)	NA	38.
Liposomal doxorubicin	32	6% (0–20%)	13 (NA)	39.
Liposomal doxorubicin	24	0%	8.5 (NA)	40.
Liposomal daunorubicin	11	0%	6.1 (NA)	41.
Platinums				
Cisplatin	25	13% (4–31%)	5 (NA)	42.
Cisplatin	35	14% (NA)	7.5 (NA)	43.
Carboplatin	31	16% (5.4–34%)	8 (NA)	44.
Carboplatin	41	7% (2–21%)	7.1 (NA)	45.
Antimetabolites-Antifolates				
Methotrexate	63	37% (NA)	11 (NA)	46.
Trimetrexate	17	12% (2–33%)	5.0 (1.9–9.6)	47.
Trimetrexate	34	12% (7–29%)	8.9 (6.5–13.8)	47.
Edatrexate	20	25% (9–49%)	9.6 (NA)	48.
Edatrexate + leucovorin	40	16% (6–31%)	6.6 (NA)	48.
Di-Deazafolic acid	18	5% (0.1–27.3%)	NA	49.
5-Fluorouracil	20	5% (NA)	5 (NA)	50.
5-dihydro azacytadine	41	17% (7–32%)	6.7 (5.0–9.6)	51.
Raltitrexed	24	21% (7–42%)	7 (5.5–18.7)	52.
Pemetrexed	64	14% (7–25%)	10.7 (7.7–14.5)	53.
Antimetabolites-Others				
Gemcitabine	17	0%	4.7 (3.1–12.9)	54.
Gemcitabine	27	7% (1–24%)	8 (5–12)	55.
Gemcitabine	16	31% (NA)	NA	56.
Others				
Vinorelbine	29	24% (10–44%)	10.6 (NA)	44.

Table 39.3. Phase III studies in malignant mesothelioma

Agent(s)	No. of patients	Response rate	Median survival	Reference
Ranpirnase	84	NA	7.7 (NA)	21
Doxorubicin	70	NA	8.2 (NA)	
Cisplatin	228	17% (NA)	9.3 (NA)	12
Cisplatin/pemetrexed	228	41% (NA)	12.1 (NA)*	

* $p < .05$.

mesotheliomas containing homozygous MTAP deletions may be more sensitive to antifolates. This hypothesis needs to be validated in clinical studies of antifolates.

Combination Chemotherapy

Many combination chemotherapy studies have been performed, but only a few have evaluated the benefits of combination chemotherapy compared to single-agent chemotherapy. The two most common combinations include either doxorubicin or platinum (cisplatin, carboplatin, and oxaliplatin) (Table 39.1). In general, the response rates are higher for both doxorubicin- (11–32% for combination vs. 0–14% for single-agent) and platinum- (6–48% for combination vs. 7–16% for single-agents) containing combinations than those observed with single-agent therapy (Tables 39.1 and 39.2). However, the median survivals are not clearly superior for the combination studies. A meta-analysis evaluating chemotherapy for malignant mesothelioma demonstrated that combinations containing both cisplatin and doxorubicin were associated with the highest response rate (28.5%; 95% CI, 21.3–35.7%) (22). In this same analysis combination studies were associated with a significantly better response rate compared to single-agent studies (22.6% vs. 11.6%; $p < .001$) (22).

The combination of cisplatin or carboplatin and gemcitabine is commonly used in the treatment of patients with malignant mesothelioma. Three phase II studies of cisplatin/gemcitabine have been published to date (Table 39.1). Two of the studies examined giving cisplatin 100 mg/m^2 on day 1 with gemcitabine at 1000 mg/m^2 on days 1, 8, and 15 of a 28-day cycle (8,26). With this schedule, the day 15 gemcitabine dose was reduced or omitted in 58% (126/219) of patients with a mean relative dose intensity of 75% (8). The response rates and median survivals in these studies were 48% and 9.5 months and 33% and 11.2 months, respectively (Table 39.1) (8,26). The study by van Haarst and colleagues (27) used a 21-day schedule with cisplatin 80 mg/m^2 on day 1 and gemcitabine 1250 mg/m^2 on days 1 and 8. Most patients received their planned chemotherapy and mean relative dose intensity of gemcitabine was 94%. The response rate was slightly lower with this schedule (16%), but the median survival was comparable (9.6 months; Table 39.1). A single study of carboplatin/gemcitabine has also been performed (28). In this study, 50 patients were treated with carboplatin [area under the curve (AUC) = 5] on day 1 and gemcitabine 1000 mg/m^2 on days 1, 8, and 15. Similar to the 28-day cisplatin/gemcitabine combination schedules, the day 15 gemcitabine was reduced or omitted in 44% of patients. The response rate and median survival in this study were 26% (95% CI, 15–40%) and 15.2 months, respectively. These studies demonstrate consistent antitumor activity of the cisplatin/carboplatin and gemcitabine combinations and hence, represent a combination chemotherapy option for patients with mesothelioma. Other platinum-based phase II studies, including oxaliplatin-based combination studies, have also been performed (Table 39.1). Non–

platinum-based combination chemotherapy regimens, including gemcitabine and pemetrexed, are presently being evaluated.

The largest combination chemotherapy study to date in mesothelioma is the phase III study comparing single-agent cisplatin to the combination of cisplatin and pemetrexed (Table 39.3) (11). This study, representing the first large randomized study comparing combination chemotherapy to single-agent therapy, randomized 456 patients, 228 to receive either cisplatin or cisplatin/pemetrexed, with survival as the primary end point. Patients receiving the combination of cisplatin/pemetrexed had both a better response rate (41% vs. 17%) and median survival [12.1 vs. 9.3; $p < .020$; log rank (Fig. 39.2)] (11). The combination was also associated with better lung function and subjective quality of life. This study establishes the superiority of the benefits of combination cisplatin-based chemotherapy compared to cisplatin alone. A similar phase III study comparing cisplatin to cisplatin and raltitrexed is ongoing.

There are many active combination chemotherapy regimens. It is not clear at this time whether one is superior to the other, and definitive proof would require additional phase III studies. However, given the limited numbers of patients with mesothelioma and the potential small incremental benefits in survival such results might yield (compare, for example, the many phase III studies of doublet chemotherapies in non–small-cell lung cancer), such studies should be avoided. The choice of chemotherapy regimens for the treating oncologist and the patient with mesothelioma will ultimately depend on the availability of the drugs, their side-effect profiles, cost, and convenience of administration.

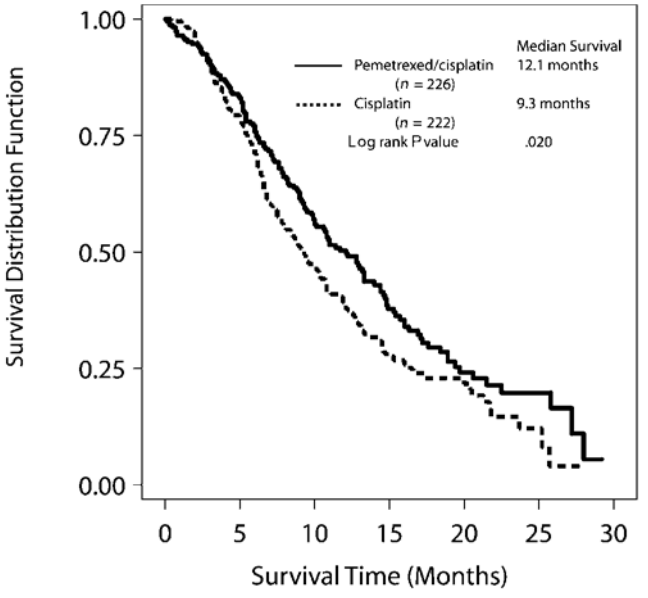


Figure 39.2. Kaplan-Meier estimates of overall survival for all patients treated with cisplatin/pemetrexed vs. cisplatin alone.

Conclusions and Future Directions

Progress has been made in the treatment of mesothelioma with chemotherapy and mesothelioma is no longer a chemotherapy-resistant disease. Anthracyclines, platinum agents, and antimetabolites have demonstrated consistent, albeit low level, single-agent antitumor activity. Combination platinum-based chemotherapies are associated with a higher response rate and are commonly used in clinical practice. However, questions remain unanswered, including the survival benefits of chemotherapy. The rarity of the disease has limited the size of many of the clinical trials, and only one phase III clinical trial has been completed. The combination of cisplatin and pemetrexed is likely to set a new standard for chemotherapy in mesothelioma. Efforts should now focus on combination studies (such as with a molecular agent) with cisplatin/pemetrexed, integration of active chemotherapy combinations into multimodality approaches, use of novel therapeutic agents such as receptor tyrosine kinase inhibitors, and second-line studies.

Summary

Most patients with malignant mesothelioma are candidates for systemic chemotherapy during the course of their disease, but no standard regimen has been established. Several phase II single-agent and combination chemotherapy studies have been performed over the past two decades. Although the true impact of chemotherapy in mesothelioma remains to be determined, agents with consistent antitumor activity include the platinum agents and the antifolates. Phase II studies of combination chemotherapy are associated with higher response rates but not necessarily longer median survivals. Recent data from a large randomized phase III clinical trial established the superiority of cisplatin/pemetrexed compared to cisplatin alone. Data from this and other ongoing studies will help establish standard chemotherapy regimens for mesothelioma and provide a basis for combination studies with molecular agents and incorporation of these regimens into multimodality treatment approaches.

References

1. Ruffie P, Feld R, Minkin S, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. J Clin Oncol 1989;7:1157–1168.
2. Herndon JE, Green MR, Chahinian AP, et al. Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the Cancer and Leukemia Group B. Chest 1998;113:723–731.
3. Girling DJ, Muers MF, Qian W, et al. Multicenter randomized controlled trial of the management of unresectable malignant mesothelioma proposed by the British Thoracic Society and the British Medical Research Council. Semin Oncol 2002;29:97–101.

4. Steele JP, Shamash J, Evans MT, et al. Phase II study of vinorelbine in patients with malignant pleural mesothelioma. J Clin Oncol 2000;18:3912–3917.
5. Middleton GW, Smith IE, O'Brien ME, et al. Good symptom relief with palliative MVP (mitomycin-C, vinblastine and cisplatin) chemotherapy in malignant mesothelioma. Ann Oncol 1998;9:269-273.
6. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 1993;85:365–376
7. Muers MF, Rudd R, Hodson A, et al. A feasibility study of active symptom control (ASC) with or without chemotherapy in malignant pleural mesothelioma. In: Hansen HH, ed. 10th World Conference on Lung Cancer. Vancouver, Canada: International Association for the Study of Lung Cancer, 2003:S11.
8. Nowak AK, Byrne MJ, Williamson R, et al. A multicentre phase II study of cisplatin and gemcitabine for malignant mesothelioma. Br J Cancer 2002;87: 491–496.
9. Hollen PJ, Gralla RJ, Liepa AM, et al. Validation of a quality of life instrument in patients with pleural mesothelioma: Lung Cancer Symptom Scale (LCSS). In: Grunberg SM, ed. American Society of Clinical Oncology. San Francisco: American Society of Clinical Oncology, 2001.
10. Gralla RJ, Hollen PJ, Liepa AM, et al. Improving quality of life in patients with malignant pleural mesothelioma: results of the randomized pemetrexed + cisplatin vs. cisplatin trial using LCSS-meso instrument. In: Grunberg SM, ed. American Society of Clinical Oncology. Chicago: American Society of Clinical Oncology, 2003.
11. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–2644.
12. Vogelzang NJ, Rusthoven J, Paoletti P, et al. Phase III single-blinded study of pemetrexed + cisplatin vs. cisplatin alone in chemonaive patients with malignant pleural mesothelioma. Proc Am Soc Clin Oncol 2002;21:abstract 5.
13. Paoletti P, Pistolesi M, Rusthoven JJ, et al. Correlation of pulmonary function tests with best tumor response status: results from the phase III study of pemetrexed + cisplatin vs. cisplatin in malignant pleural mesothelioma. In: Grunberg SM, ed. American Society of Clinical Oncology. Chicago: American Society of Clinical Oncology, 2003.
14. Maksymiuk AW, Marschke RF Jr, Tazelaar HD, et al. Phase II trial of topotecan for the treatment of mesothelioma. Am J Clin Oncol 1998;21:610–613.
15. Kindler HL, Herndon JE, Vogelzang NJ, et al. CPT-11 in malignant mesothelioma: a phase II trial by the Cancer and Leukemia Group B (CALGB 9733). In: Perry MC, ed. American Society of Clinical Oncology. New Orleans: American Society of Clinical Oncology, 2000.
16. Martensson G, Sorenson S. A phase II study of vincristine in malignant mesothelioma—a negative report. Cancer Chemother Pharmacol 1989;24: 133–134.
17. Cowan JD, Green S, Lucas J, et al. Phase II trial of five day intravenous infusion vinblastine sulfate in patients with diffuse malignant mesothelioma: a Southwest Oncology Group study. Invest New Drugs 1998;6:247–248.
18. van Meerbeeck J, Debruyne C, van Zandwijk N, et al. Paclitaxel for malignant pleural mesothelioma: a phase II study of the EORTC Lung Cancer Cooperative Group. Br J Cancer 1996;74:961–963.

19. Lerner HJ, Schoenfeld DA, Martin A, et al. Malignant mesothelioma. The Eastern Cooperative Oncology Group (ECOG) experience. Cancer 1983;52: 1981–1985.
20. Sorensen PG, Bach F, Bork E, et al. Randomized trial of doxorubicin versus cyclophosphamide in diffuse malignant pleural mesothelioma. Cancer Treat Rep 1985;69:1431–1432.
21. Vogelzang N, Taub R, Shin D, et al. Phase III randomized trial of onconase (onc) vs. doxorubicin (dox) in patients (pts) with unresectable malignant mesothelioma (umm): analysis of survival. In: Perry MC, ed. American Society of Clinical Oncology. New Orleans: American Society of Clinical Oncology, 2000:577a.
22. Berghmans T, Paesmans M, Lalami Y, et al. Activity of chemotherapy and immunotherapy on malignant mesothelioma: a systematic review of the literature with meta-analysis. Lung Cancer 2002;38:111–121.
23. Bueno R, Appasani K, Mercer H, et al. The alpha folate receptor is highly activated in malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;121:225–233.
24. Khokhar NZ, Lam AF, Rusch VW, et al. Despite some expression of folate receptor alpha in human mesothelioma cells, internalization of methotrexate is predominantly carrier mediated. J Thorac Cardiovasc Surg 2002;123: 862–868.
25. Wang Y, Zhao R, Chattopadhyay S, et al. A novel folate transport activity in human mesothelioma cell lines with high affinity and specificity for the new-generation antifolate, pemetrexed. Cancer Res 2002;62:6434–6437.
26. Illei PB, Rusch VW, Zakowski MF, et al. Homozygous deletion of CDKN2A and codeletion of the methylthioadenosine phosphorylase gene in the majority of pleural mesotheliomas. Clin Cancer Res 2003;9:2108–2013.
27. Chen ZH, Olopade OI, Savarese TM. Expression of methylthioadenosine phosphorylase cDNA in p16-, MTAP-malignant cells: restoration of methylthioadenosine phosphorylase-dependent salvage pathways and alterations of sensitivity to inhibitors of purine de novo synthesis. Mol Pharmacol 1997;52:903–911.
28. Hori H, Tran P, Carrera CJ, et al. Methylthioadenosine phosphorylase cDNA transfection alters sensitivity to depletion of purine and methionine in A549 lung cancer cells. Cancer Res 1996;56:5653–5658.
29. Byrne MJ, Davidson JA, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant mesothelioma: a phase II study. J Clin Oncol 1999;17:25–30.
30. van Haarst JM, Baas P, Manegold C, et al. Multicentre phase II study of gemcitabine and cisplatin in malignant pleural mesothelioma. Br J Cancer 2002;86:342–345.
31. Favaretto AG, Aversa SM, Paccagnella A, et al. Gemcitabine combined with carboplatin in patients with malignant pleural mesothelioma: a multicentric phase II study. Cancer 2003;97:2791–2797.
32. van Breukelen FJ, Mattson K, Giaccone G, et al. Mitoxantrone in malignant pleural mesothelioma: a study by the EORTC Lung Cancer Cooperative Group. Eur J Cancer 1991;27:1627–1629.
33. Eisenhauer EA, Evans WK, Raghavan D, et al. Phase II study of mitoxantrone in patients with mesothelioma: a National Cancer Institute of Canada Clinical Trials Group Study. Cancer Treat Rep 1986;70:1029–1030.
34. Mattson K, Giaccone G, Kirkpatrick A, et al. Epirubicin in malignant mesothelioma: a phase II study of the European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group. J Clin Oncol 1992;10:824–828.
35. Magri MD, Veronesi A, Foladore S, et al. Epirubicin in the treatment of malignant mesothelioma: a phase II cooperative study. The North-Eastern

Italian Oncology Group (GOCCNE)—Mesothelioma Committee. Tumori 1991;77:49–51.
36. Kaukel E, Koschel G, Gatzemeyer U, et al. A phase II study of pirarubicin in malignant pleural mesothelioma. Cancer 1990;66:651–654.
37. Colbert N, Vannetzel JM, Izrael V, et al. A prospective study of detorubicin in malignant mesothelioma. Cancer 1985;56:2170–2174.
38. Hudis CA, Kelsen DP. Menogaril in the treatment of malignant mesothelioma: a phase II study. Invest New Drugs 1992;10:103–106.
39. Baas P, van Meerbeeck J, Groen H, et al. Caelyx in malignant mesothelioma: a phase II EORTC study. Ann Oncol 2000;11:697–700.
40. Oh Y, Perez-Soler R, Fossella FV, et al. Phase II study of intravenous Doxil in malignant pleural mesothelioma. Invest New Drugs 2000;18:243–245.
41. Steele JP, O'Doherty CA, Shamash J, et al. Phase II trial of liposomal daunorubicin in malignant pleural mesothelioma. Ann Oncol 2001;12: 497–499.
42. Mintzer DM, Kelsen D, Frimmer D, et al. Phase II trial of high-dose cisplatin in patients with malignant mesothelioma. Cancer Treat Rep 1985;69: 711–712.
43. Zidar BL, Green S, Pierce HI, et al. A phase II evaluation of cisplatin in unresectable diffuse malignant mesothelioma: a Southwest Oncology Group Study. Invest New Drugs 1988;6:223–226.
44. Raghavan D, Gianoutsos P, Bishop J, et al. Phase II trial of carboplatin in the management of malignant mesothelioma. J Clin Oncol 1990;8:151–154.
45. Vogelzang NJ, Goutsou M, Corson JM, et al. Carboplatin in malignant mesothelioma: a phase II study of the Cancer and Leukemia Group B. Cancer Chemother Pharmacol 1990;27:239–242.
46. Solheim OP, Saeter G, Finnanger AM, et al. High-dose methotrexate in the treatment of malignant mesothelioma of the pleura. A phase II study. Br J Cancer 1992;65:956-960.
47. Vogelzang NJ, Weissman LB, Herndon JE II, et al. Trimetrexate in malignant mesothelioma: a Cancer and Leukemia Group B Phase II study. J Clin Oncol 1994;12:1436–1442.
48. Kindler HL, Belani CP, Herndon JE II, et al. Edatrexate (10-ethyldeaza-aminopterin) (NSC #626715) with or without leucovorin rescue for malignant mesothelioma. Sequential phase II trials by the cancer and leukemia group B. Cancer 1999;86:1985–1991.
49. Cantwell BM, Earnshaw M, Harris AL. Phase II study of a novel antifolate, N10-propargyl-5,8 dideazafolic acid (CB3717), in malignant mesothelioma. Cancer Treat Rep 1986;70:1335–1336.
50. Harvey VJ, Slevin ML, Ponder BA, et al. Chemotherapy of diffuse malignant mesothelioma. Phase II trials of single-agent 5-fluorouracil and adriamycin. Cancer 1984;54:961–964.
51. Vogelzang NJ, Herndon JE II, Cirrincione C, et al. Dihydro-5-azacytidine in malignant mesothelioma. A phase II trial demonstrating activity accompanied by cardiac toxicity. Cancer and Leukemia Group B. Cancer 1997;79: 2237–2242.
52. Baas P, Ardizzoni A, Grossi F, et al. The activity of raltitrexed (Tomudex) in malignant pleural mesothelioma: an EORTC phase II study (08992). Eur J Cancer 2003;39:353–357.
53. Scagliotti GV, Shin DM, Kindler HL, et al. Phase II Study of Pemetrexed With and Without Folic Acid and Vitamin B12 as Front-line Therapy in Malignant Pleural Mesothelioma. J Clin Oncol 2003;21:1556–1561.
54. Kindler HL, Millard F, Herndon JE II, et al. Gemcitabine for malignant mesothelioma: a phase II trial by the Cancer and Leukemia Group B. Lung Cancer 2001;31:311–317.

55. van Meerbeeck JP, Baas P, Debruyne C, et al. A Phase II study of gemcitabine in patients with malignant pleural mesothelioma. European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group. Cancer 1999;85:2577–2582.
56. Bischoff HG, Manegold C, Knopp M, et al. Gemcitabine (Gemzar) may reduce tumor load and tumor associated symptoms in malignant pleural mesothelioma. In: Perry MC, ed. American Society of Clinical Oncology. Los Angeles, CA, American Society of Clinical Oncology, 1998.
57. Dirix LY, van Meerbeeck J, Schrijvers D, et al. A phase II trial of dose-escalated doxorubicin and ifosfamide/mesna in patients with malignant mesothelioma. Ann Oncol 1994;5:653–655.
58. Carmichael J, Cantwell BM, Harris AL. A phase II trial of ifosfamide/mesna with doxorubicin for malignant mesothelioma. Eur J Cancer Clin Oncol 1989;25:911–912.
58. Shin DM, Fossella FV, Umsawasdi T, et al. Prospective study of combination chemotherapy with cyclophosphamide, doxorubicin, and cisplatin for unresectable or metastatic malignant pleural mesothelioma. Cancer 1995;76:2230–2236.
60. Chahinian AP, Pajak TF, Holland JF, et al. Diffuse malignant mesothelioma. Prospective evaluation of 69 patients. Ann Intern Med 1982;96:746–755.
61. Pennucci MC, Ardizzoni A, Pronzato P, et al. Combined cisplatin, doxorubicin, and mitomycin for the treatment of advanced pleural mesothelioma: a phase II FONICAP trial. Italian Lung Cancer Task Force. Cancer 1997;79: 1897–1902.
62. Samson MK, Wasser LP, Borden EC, et al. Randomized comparison of cyclophosphamide, imidazole carboxamide, and adriamycin versus cyclophosphamide and adriamycin in patients with advanced stage malignant mesothelioma: a Sarcoma Intergroup Study. J Clin Oncol 1987;5:86–91.
63. Upham JW, Musk AW, van Hazel G, et al. Interferon alpha and doxorubicin in malignant mesothelioma: a phase II study. Aust N Z J Med 1993;23: 683–687.
64. Samuels BL, Herndon JE 2nd, Harmon DC, et al. Dihydro-5-azacytidine and cisplatin in the treatment of malignant mesothelioma: a phase II study by the Cancer and Leukemia Group B. Cancer 1998;82:1578–1584.
65. Nakano T, Chahinian AP, Shinjo M, et al. Cisplatin in combination with irinotecan in the treatment of patients with malignant pleural mesothelioma: a pilot phase II clinical trial and pharmacokinetic profile. Cancer 1999;85:2375–2384.
66. Tsavaris N, Mylonakis N, Karvounis N, et al. Combination chemotherapy with cisplatin-vinblastine in malignant mesothelioma. Lung Cancer 1994; 11:299–303.
67. Chahinian AP, Antman K, Goutsou M, et al. Randomized phase II trial of cisplatin with mitomycin or doxorubicin for malignant mesothelioma by the Cancer and Leukemia Group B. J Clin Oncol 1993;11:1559–1565.
68. Ardizzoni A, Rosso R, Salvati F, et al. Activity of doxorubicin and cisplatin combination chemotherapy in patients with diffuse malignant pleural mesothelioma. An Italian Lung Cancer Task Force (FONICAP) Phase II study. Cancer 1991;67:2984–2987.
69. Parra HS, Tixi L, Latteri F, et al. Combined regimen of cisplatin, doxorubicin, and alpha-2b interferon in the treatment of advanced malignant pleural mesothelioma: a Phase II multicenter trial of the Italian Group on Rare Tumors (GITR) and the Italian Lung Cancer Task Force (FONICAP). Cancer 2001;92:650–656.

70. Eisenhauer EA, Evans WK, Murray N, et al. A phase II study of VP-16 and cisplatin in patients with unresectable malignant mesothelioma, An NCI Canada Clinical Trials Group Study. Invest New Drugs 1988;6:327–329.
71. Tansan S, Emri S, Selcuk T, et al. Treatment of malignant pleural mesothelioma with cisplatin, mitomycin C and alpha interferon. Oncology 1994;51: 348–351.
72. Metintas M, Ozdemir N, Ucgun I, et al. Cisplatin, mitomycin, and interferon-alpha2a combination chemoimmunotherapy in the treatment of diffuse malignant pleural mesothelioma. Chest 1999;116:391–398.
73. Soulie P, Ruffie P, Trandafir L, et al. Combined systemic chemoimmunotherapy in advanced diffuse malignant mesothelioma. Report of a phase I-II study of weekly cisplatin/interferon alfa-2a. J Clin Oncol 1996;14: 878–885.
74. Trandafir L, Ruffie P, Borel C, et al. Higher doses of alpha-interferon do not increase the activity of the weekly cisplatin-interferon combination in advanced malignant mesothelioma. Eur J Cancer 1997;33:1900–1902.
75. Kasseyet S, Astoul P, Boutin C. Results of a phase II trial of combined chemotherapy for patients with diffuse malignant mesothelioma of the pleura. Cancer 1999;85:1740–1749.
76. Fizazi K, Caliandro R, Soulie P, et al. Combination raltitrexed (Tomudex(R))-oxaliplatin: a step forward in the struggle against mesothelioma? The Institute Gustave Roussy experience with chemotherapy and chemoimmunotherapy in mesothelioma. Eur J Cancer 2000;36:1514–1521.
77. O'Reilly EM, Ilson DH, Saltz LB, et al. A phase II trial of interferon alpha-2a and carboplatin in patients with advanced malignant mesothelioma. Cancer Invest 1999;17:195–200.
78. Hughes A, Calvert P, Azzabi A, et al. Phase I clinical and pharmacokinetic study of pemetrexed and carboplatin in patients with malignant pleural mesothelioma. J Clin Oncol 2002;20:3533–3544.
79. Fizazi K, Doubre H, Le Chevalier T, et al. Combination of raltitrexed and oxaliplatin is an active regimen in malignant mesothelioma: results of a phase II study. J Clin Oncol 2003;21:349–354.
80. Steele JP, Shamash J, Evans MT, et al. Phase II trial of vinorelbine and oxaliplatin ("VO") in malignant pleural mesothelioma (MPM). In: Grunberg SM, ed. American Society of Clinical Oncology. San Francisco, California, American Society of Clinical Oncology, 2001:335a.
81. Schuette W, Blankenburg T, Lauerwald K, et al. A multicenter phase II study of gemcitabine and oxaliplatin for malignant pleural mesothelioma. Clinical Lung Cancer 2003;4:294–297.

40

Second-Line Chemotherapy

Nick Pavlakis and Nicholas J. Vogelzang

Chemotherapy trials for malignant pleural mesothelioma have almost exclusively focused on chemotherapy-naive patients. Until 2000, the literature generally consisted of a plethora of phase II studies (1,2) in which interpretation of efficacy has been hampered by small sample size and heterogeneity in reporting of response outcomes. A recent systematic review of this literature indicated the most active single agent to be cisplatin (3).

In 2002 the report of the pivotal randomized phase III trial (pemetrexed and cisplatin compared with cisplatin alone), the largest clinical study in mesothelioma to date ($n = 456$), confirmed an improvement in response rate, survival, quality of life, and lung function for this combination in the first-line setting (4). After setting a new standard of care for first-line therapy and confirming the value of palliative chemotherapy in malignant pleural mesothelioma, clinical research questions can now focus on second-line chemotherapy, previously not worthy of investigation. Indeed clinical trials in this patient population are rare. Recently, however, a dedicated second-line phase II study has been published, confirming the feasibility of conducting clinical trials in this setting (5). Furthermore, the literature, which contains anecdotal reports and a number of phase II studies that specifically allowed entry of patients with prior chemotherapy, suggests the potential efficacy of second-line chemotherapy (6,7). Last, Manegold et al (8) recently reported that post-study (second-line) chemotherapy in the abovementioned phase III trial, usually with gemcitabine or doxorubicin, was identified as a significant predictor of extended survival ($p < .01$). This finding could not be explained by a retrospective analysis of patient risk factors by Cox Multiple Regression analysis (8). This chapter reviews the use of second-line chemotherapy for malignant pleural mesothelioma and suggests future directions for clinical care and research activity in this area.

Review of the Literature

There have been at least 16 studies or reports published that describe the inclusion of patients with malignant mesothelioma treated in the second-line setting (Table 40.1). Only one of these, Giaccone et al (5), is a dedicated phase II study focusing solely on a second-line population. In most series, about 30% of patients were pretreated. While the inclusion of pretreated patients in clinical studies commenced as early as 1983, the efficacy reports of second-line treatment can only be considered as anecdotal given the small number of patients included. Nonetheless, one can see that responses were consistently observed (Table 40.2), the most notable being of responses with carboplatin in the 1980s (9,10). Subsequently, with the emergence of new active agents

Table 40.1. Studies or reports of second-line chemotherapy in pretreated patients with malignant mesothelioma

Drug(s)	Study design	*n*	No. of second-line patients	Percent receiving second-line therapy	Reference
Gemcitabine, vinorelbine, doxorubicin, and others	Report of outcome of post-study chemotherapy in phase III study of Pemetrexed (Pem) and cisplatin (cis) v cis	456	82 in pemetrexed and cisplatin arm; 104 in cisplatin arm	38–48	8
Raltitrexed and oxaliplatin	Phase II, two centers	70	15	21	7
ZD0473	Phase II, multicenter	47	47	100	5
Ranpirnase	Phase II, multicenter	105	39	37	16
Doxil	Phase II, single center	15	5	33	19
Raltitrexed and oxaliplatin	Phase I, with expanded mesothelioma cohort	17	10	59	8
Doxil	Phase II	24	12	50	20
Cisplatin and gemcitabine	Letter	NA	3	100	6
Cisplatin, 5-FU, leucovorin, mitomycin-C, etoposide	Phase II	45	10	22	21
Ifosfamide	Phase II, multicenter	30	8	27	22
Carboplatin	Phase II, two centers	31	5	16	10
Diaziquone	Phase II	20	4	20	23
Carboplatin	Phase II	17	3	18	24
Carboplatin	Phase II	16	6	37	9
Cisplatin	Phase II	25	7	28	25
Vindesine	Phase II	20	6	30	26

5-FU, 5-fluorouracil; NA, not available.

Table 40.2. Summary of reported clinical/efficacy outcomes in second-line treated patients (the outcomes reported are from the studies listed in Table 40.1)

Drug(s)	No. treated	Type of prior chemotherapy	Response	Median TTP	Median survival	Reference
Gemcitabine, vinorelbine, doxorubicin, others	186	Pem/Cis or Cis (186)	ns	ns	Post-study chemotherapy prolonged survival	8
Raltitrexed and Oxaliplatin	15	Cisplatin (15)	3/15	27 weeks (95% CI 13–31) (18 weeks)	44 weeks (95% CI 24–40) (31 weeks)	7
ZD0473	47	Cisplatin (39)	5/43*	77 days (95% CI 44–105)	203 days (95% CI 165–277)	5
Ranpirnase	39	Platinum (24) Doxorubicin (16)	2**	102 days overall (95% CI 64–161)	61% overall 1-year survival	16
Doxil	5	Doxorubicin (2)	1/5	ns	ns	19
Raltitrexed and Oxaliplatin	10	ns	4/6 (all platinum refractory)	ns	ns	13
Doxil	12	Dox/Cis (9)	0/24	ns	ns	20
Cisplatin and gemcitabine	3	Doxorubicin (3)	1/3	ns	ns	6
Cisplatin, 5-FU, leucovorin, mitomycin-C, etoposide	10	5-FU (3) Immunotherapy (7)	ns	ns	ns	21
Ifosfamide	8	ns	1/8	ns	ns	22
Carboplatin	5	Anthracyclines	1/5	ns	ns	10
Diaziquone	4	ns	0/4	ns	ns	23
Carboplatin	3	ns	0/3	ns	ns	24
Carboplatin	6	ns	1/6	ns	ns	9
Cisplatin	7	ns	0/7	ns	ns	25
Vindesine	6	ns	ns	ns	ns	26

* All minor responses in 43 evaluable patients.

** One partial, one minor response.

TTP, time to progression; ns, not specified.

and drug combinations, the total number of patients reported as demonstrating drug activity in the second-line setting has increased.

The gemcitabine/cisplatin regimen is one such combination that has emerged as an active first-line therapy with reported response rates of 31% to 48%, with associated improvement in lung function and global quality of life, and a surprisingly low (<10%) progressive disease rate during the study treatment (11,12). The first report of potential second-line activity of the new cisplatin/gemcitabine regimen was in three patients previously treated within a randomized trial comparing the amphibian ribonuclease Onconase (Ranpirnase), to doxorubicin (6). All three patients had failed prior doxorubicin and were treated with cisplatin and gemcitabine. One patient demonstrated "radiological regression" of tumor and symptomatic improvement, while another obtained pain relief after commencement of chemotherapy. No subsequent trials of the gemcitabine/cisplatin as second-line therapy have been reported.

The next combination in which activity was seen in chemotherapy-pretreated patients was with the thymidilate synthase inhibitor raltitrexed (Tomudex) combined with oxaliplatin (7,13) (Tables 40.1 and 40.2). Oxaliplatin is a new platinum analogue demonstrating only partial cross-resistance with cisplatin or carboplatin, first demonstrated in platinum-resistant leukemia models (14). In a phase I study evaluating this new combination, impressive results were seen in an initial cluster of patients with mesothelioma (13). Therefore, the investigative team decided to recruit further mesothelioma patients into the phase I study in order to determine potential activity in this subset. Overall, six of the 17 patients [35%] with mesothelioma demonstrated an objective partial response in that phase I trial. An even more interesting finding was that four of these six responding patients were considered "platinum refractory," i.e., having disease progression during or within 6 months of cisplatin-based therapy. These responses were confirmed by independent radiologic review. Ten of the 17 patients had received prior chemotherapy; four were responders, three had stable disease, and only three patients had disease progression at the first assessment. These findings suggested activity with this combination, which was then explored in a phase II study specifically focused on mesothelioma (7) (Tables 40.1 and 40.2).

The phase II study evaluated the efficacy of raltitrexed and oxaliplatin in 70 patients with diffuse malignant pleural mesothelioma, 15 of whom had received prior chemotherapy (7). Patients received raltitrexed 3 mg/m^2 and oxaliplatin 130 mg/m^2 i.v. q21 days. All 15 previously pretreated patients had received cisplatin. The overall response rate was 20%. The same response rate was observed in the pretreated patients. In pretreated patients, the median time to progression was 27 weeks [95% confidence interval (CI), 13–31 weeks] compared with 17 weeks in the chemotherapy-naive population (95% CI, 11–21 weeks). Median survival from the start of treatment in pretreated patients was 44 weeks (95% CI, 24–40 weeks) and 226 weeks (95% CI, 63–292 weeks) from the diagnosis of mesothelioma. This compares with a median survival from the start of treatment in chemotherapy-naive patients of 31 weeks (95% CI, 23–40 weeks) and 49 weeks (95% CI, 40–52 weeks) from the diagnosis of mesothelioma. One-year survival was 22% in chemotherapy-naive patients (95% CI, 10.9–33.2%) compared with 40% in the pretreated patients (95% CI, 15.2–64.8%). Toxicity was manageable, with the most common adverse events being asthenia, nausea/vomiting, and paresthesia. No toxic deaths occurred. Improvement in one or more symptom dimensions occurred in 13% to 40% of the 15 pretreated patients compared with 18% to 34% in the chemotherapy-naive patients.

In this post hoc subset analysis, the prolonged survival seen in the cisplatin-pretreated patients most likely represents a selection bias in favor of better prognosis in this group (i.e., those with more slowly growing tumors survive long enough to receive second-line therapy). An alternative hypothesis is that there are mesotheliomas that are inherently chemotherapy sensitive. Similar to patients with high-grade lymphomas and germ cell tumors, such patients owe their prolonged

survival to effective second- and third-line drug therapy, not to a slow growth rate of the tumor. Although detailed prognostic classification comparing pretreated and chemotherapy-naive patients was not presented, overall, 66% of patients had epithelial histology and 81.4% had a World Health Organization (WHO) performance status of 0 or 1 (7). Still, it is remarkable to see tumor responses with a platinum analogue in cisplatin-pretreated patients. These data suggest the non–cross-resistance (or lack of resistance) to the raltitrexed and oxaliplatin combination in mesothelioma. Non–cross-resistance or inherent drug sensitivity, apart from patient-based favorable prognostic characteristics, would appear to be a key factor in predicting second-line activity of chemotherapy combinations in many diseases where second-line chemotherapy is effective (non–small-cell lung cancer, lymphomas, ovarian and breast cancers, germ-cell tumors, etc.).

Another novel agent showing promise in mesothelioma is the antitumor ribonuclease Onconase (Bloomfield, NJ) (ranpirnase). It acts by binding to the cell surface and then penetrating through to the cytosol where it degrades transfer RNA (tRNA), which may result in cell death either through an apoptotic switch or by interruption of cell growth and proliferation through protein synthesis inhibition (15). Results from a multicenter phase II study, which included pretreated patients, have been published (16) (Tables 40.1 and 40.2). A randomized trial comparing first-line doxorubicin with Onconase has also been completed (17). The phase II study enrolled 105 patients, 39 (37%) of whom had prior chemotherapy (16) (Table 40.2). In 11 of the 39 patients, a cisplatin-containing regimen was used, in three, a doxorubicin-containing regimen was used, and in 13, a doxorubicin/cisplatin combination was used. The remaining 12 patients were treated with a variety of other agents. All patients received Onconase 480 $\mu g/m^2$ i.v. weekly. Survival was the primary end point, and outcomes were assessed according to their Cancer and Leukemia Group B (CALGB) prognostic group classification. Of the 81 patients evaluable for a response, four partial responses and two minor responses were seen, and 35 stabilizations of previously progressive disease were noted. In the 39 pretreated patients, one partial response, one minor response, and 11 stabilizations of previously progressive disease were seen. Overall median survival was 6 months (95% CI, 4.7–10 months), with 1- and 2-year survival of 34.3% and 21.6%, respectively. The breakdown of survival between previously treated and first-line–treated patients was not reported, so the true survival impact of Onconase in the second-line setting is not known. Survival was prolonged in patients with either tumor response or stable disease, even when analyzed by CALGB prognostic group. Furthermore, as 37% of patients were previously treated, it is likely that chemotherapy-pretreated patients demonstrating nonprogressive disease had a survival gain, particularly given the 95% confidence limits around the median survival estimate.

The only dedicated second-line study of chemotherapy in malignant mesothelioma investigated the activity and tolerability of a novel platinum analogue ZD0473 (5). This was an open-label, multicenter phase II study that recruited 47 patients from six different countries

with a median age of 59 (range 37–75). All patients had received prior chemotherapy, and 39 of 47 (83%) had received a prior platinum-based protocol. The WHO performance status was 0, 1 in 36 patients, and 2 in 11 patients; 80% of patients had advanced disease (International Mesothelioma Interest Group stages II to IV). All patients had relapsed or progressive disease at study enrollment. The mesothelioma histologic classification was not reported.

ZD0473 was administered at a starting dose of 120 mg/m^2 in 14 patients, six of whom tolerated subsequent escalation to 150 mg/m^2. The remaining 33 patients received a starting dose of 150 mg/m^2. Forty-three patients were evaluable for response using the revised WHO-RECIST criteria. No complete or partial responses were seen, but there were five minor responses (≥10% lesion shrinkage), and 19 patients had stable disease. Median time to progression was 77 days (95% CI, 44–105 days) and median survival was 203 days (95% CI, 165–277 days). The median number of cycles received overall was 3.0 (range 1–6) and six patients received more than six cycles. All patients eventually withdrew from the study, five due to adverse events and 29 due to progressive disease. The main toxicity was hematologic (thrombocytopenia, 36% grade III) with no grade IV hematologic or nonhematologic toxicity observed. Quality of life was assessed using the Functional Assessment of Cancer Therapy–Lung (FACT-L) questionnaire. Little change was reported in patient overall quality of life throughout the trial. Although no major responses were seen with ZD0473, this study was important in confirming the feasibility of clinical trials of second-line chemotherapy.

With over 40% of patients responding to pemetrexed/cisplatin as first-line treatment for malignant mesothelioma, patients and their treating physicians now have higher expectations for chemotherapy in this disease. These expectations have fueled a need for clinical trials in the second-line setting. A recent updated report on the pemetrexed/cisplatin phase III trial may shine some light and give impetus to answer the question of survival with second-line chemotherapy (8). In this report, the impact of second-line post-study chemotherapy on the survival of patients within the pemetrexed/cisplatin phase III trial was explored. The analysis of post-study chemotherapy was not a part of study design, occurred in selected patients, and should be considered a hypothesis-generating exercise. The percentage of patients receiving post-study chemotherapy was 48% in the cisplatin treatment arm compared with 38% in the pemetrexed/cisplatin arm. The majority of patients received gemcitabine, navelbine, or doxorubicin monotherapy. Multiple regression analysis indicated that post-study chemotherapy had a significant correlation with prolonged survival ($p < 0.01$). One of the key findings of this report was the survival advantage observed in the pemetrexed/cisplatin treatment in spite of the imbalance in post-study chemotherapy favoring the cisplatin arm. This strengthens the argument further for the efficacy of first-line pemetrexed/cisplatin but also indicates the potential for improved survival with second-line treatment.

Future Directions

The value of second-line chemotherapy on patient survival and quality of life should be tested in a randomized controlled trial, ideally against a best supportive care control arm. Eli Lilly Inc. has embarked upon such a study comparing single-agent pemetrexed to best supportive care in the second-line setting. The trial is designed to accrue 240 patients but is accruing slowly and predominantly in the United Kingdom, Europe, and South Africa (Paolo Paoletti, M.D., personal communication, May 2003). Whether it will complete accrual is uncertain.

The question as to which agent or drug combination is best tested as second-line treatment depends to a great extent on which agents are used as first-line treatment. Gemcitabine alone was the most commonly used post-study chemotherapy agent in the context of prior cisplatin with or without pemetrexed, as reported in the Manegold et al (8) study (19.5% vs. 29.8%, pemetrexed/cisplatin vs. cisplatin arms, respectively). Gemcitabine in combination was the second most commonly used regimen (15.9% vs. 14.4%), followed by single-agent vinorelbine (9.8% vs. 4.8%) and single doxorubicin (8.5% vs. 10.6%). The variable single agent activity of gemcitabine as a single agent in the first-line setting (0–31% response rate across three phase II studies) and its greater efficacy in combination with cisplatin would argue for a gemcitabine combination rather than a single-agent study (18). The potential efficacy of oxaliplatin in patients previously treated with cisplatin suggests it may be a suitable agent to explore in combination with gemcitabine after failure of pemetrexed/cisplatin. Furthermore, given the benefit of doublet chemotherapy shown with pemetrexed/cisplatin, other doublets would be easily studied. The gemcitabine/doxorubicin doublet has shown good efficacy in metastatic urothelial malignancy and would be expected to be relatively non–cross-resistant with the pemetrexed/cisplatin regimen. Other potential doublets include vinorelbine and a platinating agent. The oxaliplatin/raltitrexed combination is yet another that has demonstrated some second-line activity in a small cohort of patients. Most important, formal phase II studies of such second-line chemotherapy regimens are required before a phase III trial could be considered. For example, CALGB has undertaken a phase II study of the novel antiangiogenesis/tyrosine kinase receptor antagonist BAY43–9006 as second-line therapy (H. Kindler, personal communication, May 2003). If the study accrues well and shows activity, a phase III study could be planned using that agent alone compared to best supportive care.

This population of pretreated high-performance status mesothelioma patients is an ideal population in which to study new drugs. At the University of Chicago, for example, open phase II trials available in this population include imatinib (Gleevec) (27) and SDX-101 (l-alanosine). Planned trials include an m-TOR (mammalian target of rapamycin) inhibitor and an inhibitor of multiple tyrosine kinases.

Conclusion

The role of chemotherapy in malignant mesothelioma has changed since the late 1990s with the emergence of new active regimens, improved image reporting, and high-quality data from large multicenter randomized studies. A review of the literature indicates increasing reports of efficacy of second-line chemotherapy in selected fit patients. The activity of pemetrexed and cisplatin in the first-line setting and the apparent value of post-study chemotherapy within the context of that phase III study have confirmed the sensitivity of some mesotheliomas to chemotherapy. Those data, in turn, have opened the door for exploration of chemotherapy or other novel therapies in the second-line setting for malignant mesothelioma. Last, novel phase II studies are required, indeed vital, to determine active agents and combinations in this setting. Careful analysis of the results of such studies by risk/prognostic group assignment and prior response duration will need to be done. Ultimately the most promising agent(s) will need to be evaluated in a randomized comparison against the current standard of best supportive care.

References

1. Baas P. Chemotherapy for malignant mesothelioma: from doxorubicin to vinorelbine. Semin Oncol 2002;29(1):62–69.
2. Tomek S, Emri S, Krejcy K, Manegold C. Chemotherapy for malignant pleural mesothelioma: past results and recent developments. Br J Cancer 2003;88:167–174.
3. Berghman T, Paesmans M, Lalami Y, et al. Activity of chemotherapy and immunotherapy on malignant mesothelioma: a systematic review of the literature with meta-analysis. Lung Cancer 2002;38:111–121.
4. Vogelzang NJ, Rusthoven J, Paoletti P, et al. Phase III single-blinded study of pemetrexed + cisplatin vs. cisplatin alone in chemonaive patients with malignant pleural mesothelioma. J Clin Oncol 2003; 21(14):2636–2644.
5. Giaccone G, O'Brien MER, Byrne MJ, Bard M, Kaukel E, Smit B. Phase II trial of ZD0473 as second-line therapy in mesothelioma. Eur J Cancer 2002; 38(suppl 8):S19–24.
6. Vogelzang NJ. Gemcitabine and cisplatin: second-line chemotherapy for malignant mesothelioma? J Clin Oncol 1999;17(8):2626–2627.
7. Fizazi K, Doubre H, Le Chevalier T, et al. Combination of raltitrexed and oxaliplatin is an active regimen in malignant mesothelioma: results of a Phase II study. J Clin Oncol 2003;21(2):349–354.
8. Manegold C, Symanowski J, Gatzemeier U, et al. Secondary (post-study) chemotherapy in the phase III study of pemetrexed + cisplatin vs. cisplatin in malignant pleural mesothelioma (MPM) is associated with longer survival. Proc ASCO 2003;2684a.
9. Cantwell BMJ, Franks CR, Harris AL. A phase II study of the platinum analogues JM8 and JM9 in malignant pleural mesothelioma. Cancer Chemother Pharmacol 1986;18:286–288.
10. Raghavan D, Gianoutsos P, Bishop J, et al. Phase II trial of carboplatin in the management of malignant mesothelioma. J Clin Oncol 1990;8(1):151–154.

11. Byrne M, Davidson A, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant pleural mesothelioma: a phase II study. J Clin Oncol 1999;17:25–30.
12. Nowak AK, Byrne MJ, Williamson R, et al. A multicentre phase II study of cisplatin and gemcitabine for malignant mesothelioma. Br J Cancer 2002;87(5):491–496.
13. Fizazi K, Ducreux M, Ruffie P, et al. Phase I, dose-finding, and pharmacokinetic study of raltitrexed combined with oxaliplatin in patients with advanced cancer. J Clin Oncol 2000;18(11):2293–2300.
14. Connors TA, Jones M, Ross WC, et al. New platinum complexes with antitumor activity. Chem Biol Interact 1972;5:415–424.
15. Iordanov MS, Ryabinina OP, Wong J, et al. Molecular determinants of apoptosis induced by the cytotoxic ribonuclease Onconase: evidence for cytotoxic mechanisms different from inhibition of protein synthesis. Cancer Res 2000;60:1983–1994.
16. Mikulski SM, Costanzi JJ, Vogelzang NJ, et al. Phase II trial of a single weekly intravenous dose of ranpirnase in patients with unresectable malignant mesothelioma. J Clin Oncol 2002;20(1):274–281.
17. Vogelzang N, Taub R, Shin D, et al. Phase III randomized trial of Onconase (ONC) vs. doxorubicin (DOX) in patients (Pts) with unresectable malignant mesothelioma (UMM): analysis of survival. Proc ASCO 2000;2274a.
18. Kindler HD, van Meerbeeck JP. The role of gemcitabine in the treatment of malignant mesothelioma. Semin Oncol 2002;29(1):70–76.
19. Skubitz KM. Phase II trial of pegylated-liposomal doxorubicin (Doxil) in mesothelioma. Cancer Invest 2002;20(5&6):693–699.
20. Oh Y, Perez-Soler R, Fossella FV, et al. Phase II study of intravenous Doxil in malignant pleural mesothelioma. Invest New Drugs 2000;18:243–245.
21. Kasseyet S, Astoul P, Boutin C. Results of a phase II trial of combined chemotherapy for patients with diffuse malignant mesothelioma of the pleura. Cancer 1999;85(8):1740–1749.
22. Zidar BL, Metch B, Balcerzak SP, et al. A phase II evaluation of ifosfamide and mesna in unresectable diffuse malignant mesothelioma. Cancer 1992; 70(10):2547–2551.
23. Eagen RT, Frytak S, Richardson RL, Creagan ET, Nichols WC. Phase II trial of diaziquone in malignant mesothelioma. Cancer Treat Rep 1986;70(3):429.
24. Mbidde EK, Harland SJ, Calvert AH, Smith IE. Phase II trial of carboplatin (JM8) in treatment of patients with malignant mesothelioma. Cancer Chemother Pharmacol 1986;18:284–285.
25. Mintzer DM, Kelsen D, Frimmer D, Heelan R, Gralla R. Phase II trial of high-dose cisplatin in patients with malignant mesothelioma. Cancer Treat Rep 1985;69(6):711–712.
26. Kelsen D, Gralla R, Cheng E, Martini N. Vindesine in the treatment of malignant mesothelioma: a phase II study. Cancer Treat Rep 1983;67(9): 821–822.
27. Villano JL, Husain AN, Stadler WM, Hanson LL, Vogelzang NJ, Kindler HL. A Phase II trial of imatinib mesylate in patients (pts) with malignant mesothelioma (MM). Proc Amer Soc Clin Oncol 2004;7200:663.

41

Treatment of Mesothelioma with Radiotherapy

Ryan P. Smith and Stephen M. Hahn

General Principles of Radiation Therapy

Radiation therapy is a therapeutic modality that uses ionizing radiation to treat cancers and some nonmalignant conditions. Ionizing radiation kills cells by damaging DNA (1). Approximately two thirds of the DNA damage caused by ionizing radiation is from indirect action, that is, damage caused by free radicals generated mostly from the ionization of water (1). The remainder of the damage caused by radiation is through direct ionization of DNA. Radiation damage is also highly dependent on the presence of oxygen. The cytotoxicity of radiation is approximately three times greater in the presence of oxygen than that which occurs in an anoxic environment (1). Hypoxia in human tumors has been extensively investigated and may be a physiologic cause for radiation resistance (2–4).

The absorbed dose of radiation therapy is prescribed in units called gray (Gy). The clinical use of radiation therapy in the United States is usually fractionated, that is, delivered in small doses daily. In general, for definitive or curative radiation therapy courses, a daily dose of 180 or 200 cGy per day is used. A total dose of radiation therapy for a curative course is usually between 5000 and 7000 cGy. For palliative courses of radiation therapy, daily doses between 250 and 400 cGy are used for a total dose of between 2000 to 3500 cGy. The biologic basis for fractionation is that greater tumor cell kill can be achieved with fractionation relative to normal tissue effects by exploiting the 4 Rs: cellular *repair*, *reassortment* of tumor cells into sensitive phases of the cell cycle, *reoxygenation* of tumor cells, and *repopulation* of normal tissues. The total dose of radiation therapy used clinically is, in general, dependent on the radiation doses that the normal tissues within the radiation field can tolerate (so-called tolerance doses). There are some tumors that are quite radioresponsive, and for these tumors, doses below normal tissue tolerances can be used. However, for most solid tumors, including mesothelioma, the dose of radiation therapy that is prescribed is based on the tolerance of the normal tissues. In the case of mesothelioma, normal tissues that are sensitive to the effects of radiotherapy are

within the radiation field. These normal tissues limit the doses that can be administered and include the lungs, heart, esophagus, spinal cord, liver, and stomach. In general, the acute side toxicities of radiation during radiotherapy for mesothelioma include skin redness, esophagitis, fatigue, and nausea. The potential long-term toxicities of radiotherapy for mesothelioma include radiation pneumonitis, cardiac damage, radiation myelitis, and radiation damage to the liver.

Specialized radiation treatment planning methods can be used to shape the radiation field with the goal of increasing the dose to the tumor and reducing the dose to normal tissues. The radiation treatment planning process involves the identification of a tumor or region that requires treatment followed by an evaluation of the radiation dose distributions provided by different field arrangements. Advances in computer hardware and software have enhanced the efficiency of this process. It is considered standard in many clinical situations to use three-dimensional radiation treatment planning for the delivery of three-dimensional conformal radiation therapy (3DCRT), which allows the physician to conform the radiation dose to a three-dimensional target volume (the tumor plus any additional tissues that require treatment) while also minimizing the dose to the surrounding normal tissues. In the typical situation, 3DCRT is delivered using a number of fixed radiation beams that are shaped using blocks or a multileaf collimator. The intensity of the radiation beam across the radiation field is mostly uniform (there are exceptions to this).

A new version of the treatment planning process called intensity modulated radiation therapy (IMRT) is being used by some centers to treat complex regions and tumors (5). It involves a treatment planning process and delivery that uses nonuniform radiation beam intensities across the radiation field. Computer optimization techniques are used by the physician, radiation physicists, and radiation dosimetrists to determine the appropriate radiation dose distribution.

The potential advantage of IMRT over conventional 3DCRT is the ability to deliver higher doses of radiation to the tumor while further minimizing the doses to adjacent normal tissues compared to conventional 3DCRT. Early reports of IMRT for prostate cancer, head and neck cancer, and brain tumors suggest that tumor radiation dose escalation may be feasible without increasing acute or late normal tissue side effects (6,7). However, there are several issues that have not been completely resolved with IMRT, which may be significant drawbacks to this technique (8). First, IMRT involves significantly more time and effort from physicians, physicists, dosimetrists, and radiation therapists. Second, there may be an increased risk of error because of the complexity of the treatment planning and delivery process; this requires a substantial program of quality assurance and verification of the radiation treatment fields. Third, the treatment times for patients are longer than for conventional radiation therapy. Typical treatment times for conventional 3DCRT are 3 to 5 minutes, while IMRT treatment times may range from 15 to 30 minutes. Fourth, although the immediately adjacent normal tissues may receive a lower dose of radiation compared to the tumor, a significantly greater normal tissue volume

receives a low to moderate radiation dose compared to standard approaches. This higher whole-body dose of low-dose radiation may increase the risk of second cancers. There are no long-term data currently available to address this issue.

Radiation therapy can be delivered externally as photons or electrons usually from a linear accelerator. Photons are a more penetrating type of radiation and can be used to deliver radiation to deep-seated tumors. Electrons are a superficial type of radiation through which the dose can be deposited to superficial structures such as the skin. Intraoperative radiation involves the use of external beam radiation (usually electrons) during the surgical procedure when exposure of a tumor bed is at its maximum. Radiation therapy can also be delivered with radioactive isotopes, which is called brachytherapy. This includes radiation seeds or implants, which are placed directly into the site of the tumor. One of the advantages of brachytherapy is that high doses of radiation are delivered to a localized area with relative sparing of the surrounding normal tissues. One form of brachytherapy that has been used for mesothelioma is the instillation of a radioactive ^{32}P or ^{198}Au solution within the pleura in order to expose the entire pleural surface to radiation (9,10).

Curative Radiation Therapy as a Single Modality

Treatment of malignant pleural mesothelioma, with definitive radiotherapy as a single modality is not a curative treatment strategy. The main limitation of radiotherapy in this setting is the inability to treat a large volume of disease in the chest with a curative radiation dose (>60 Gy) because of the risks of severe normal tissue toxicity. Several groups have reported their results with definitive radiotherapy. Law et al (11) administered radiation using a rotational technique to deliver 5000 to 5500 cGy to the pleural space. Survival in this group of patients ranged from 3 to 10 months, with the exception of one patient who was alive and well 4 years after the completion of treatment. The authors concluded that radiotherapy had a palliative benefit in a small number of patients but that it was of no value in other patients. Ball and Cruickshank, (12) from the MacCallum Cancer Institute in Australia, reported the results of radical radiotherapy in 12 patients with malignant pleural mesothelioma. These patients were treated with 5000 cGy to the entire hemithorax. Median survival of these patients was 17 months compared to 7 months for those offered palliative treatment only. This difference is likely the result of a selection bias, with those fit enough to undergo a full course of radiation likely to have a greater survival regardless of treatment given. In addition, in these 12 patients, two had toxicity that led to their deaths, one with radiation hepatitis and one with radiation myelopathy. The authors concluded that radiotherapy did not appear to be effective in prolonging survival in patients with mesothelioma.

A larger study of patients with malignant pleural mesothelioma, which included patients treated with definitive radiotherapy, was

reported by Ruffie et al (13). Radiation was given to a total of 49 patients. In those patients where the dose exceeded 4500 cGy, the course of radiation was defined as radical. The median survival was 9.8 months in patients treated with radical radiotherapy, which was no different from those treated with palliative radiation. Alberts et al (14) used a split course of radiotherapy in patients with pleural mesothelioma. In this study, 13 patients were treated with definitive radiation alone. Patients received variable schedules of radiation. Some received 1000 cGy in 1 week (five 200-cGy fractions) every 6 weeks up to a maximum of four courses. Others received 150-cGy fractions for 10 days, followed by a rest period of 2 weeks. This was followed by an additional 3000 cGy in 2 weeks, using 300-cGy fractions. Hence, they received a total of 4500 cGy throughout the entire course. One patient had a complete response to radiation, one patient had a partial response (≥50% reduction in the size of disease), and three patients had stability of their disease. The median duration of response was 133 days. Though direct comparisons were difficult to make between the groups of patients in this nonrandomized study, the duration of response to radiation was much shorter than with other treatment modalities. Holsti et al (15) have also reported their results with radical hemithoracic radiotherapy in patients with pleural mesothelioma. Fifty-seven patients were treated with a variety of fractionation schedules. The 2-year survival rate of the group overall was 21% and the 5-year survival rate was 9%. Two patients were reported to be long-term survivors. This group has reported that the toxicity to the intact lung is severe, with total loss of function on the irradiated side (16).

Definitive radiotherapy alone is not curative therapy in malignant pleural mesothelioma and is associated with substantial toxicities. The basic problem is that it is not possible to deliver curative doses to the hemithorax given the geometric limitations of the thoracic cavity and the sensitivity of the surrounding normal tissues.

Combined Chemotherapy and Definitive Radiotherapy

The poor results reported for definitive radiotherapy (RT) alone have led to studies evaluating the combination of chemotherapy and radiation. Alberts et al (14) treated patients with a variety of chemotherapy regimens, all concurrent with RT. These included RT plus doxorubicin, RT plus cyclophosphamide, RT plus procarbazine, and RT plus cyclophosphamide, vincristine, and actinomycin D. Radiation was given in a variety of schedules as well. Some received 1000 cGy in a week (in five 200-cGy fractions) every 6 weeks up to a maximum of four courses. Others received 150-cGy fractions for 10 days, followed by a rest period of 2 weeks. This was followed by an additional 3000 cGy in 2 weeks, using 300-cGy fractions. Hence, they received a total of 4500 cGy throughout the entire course. Though the response rates and response durations were increased with the addition of chemotherapy compared to radiation alone, the median survival was not significantly increased over those patients who received radiation alone. Ruffie et al

(13) treated mesothelioma patients with chemotherapy consisting of doxorubicin-based regimens and other combination chemotherapy regimens. There was a significant increase in survival in those patients receiving chemotherapy (median survival time of 12.3 months vs. 7.3 months) compared to those who did not receive chemotherapy. Although the chemoradiation group only had nine patients, their median survival was 14.2 months, among the highest of any group in the study. Median survivals were higher in doxorubicin groups (14.7 months) compared to those in groups using the chemotherapy regimens (11.9 months). Linden et al (17) treated patients with hemithoracic radiotherapy (40 Gy in 20 daily fractions) followed by chemotherapy in good performance status patients. The chemotherapy consisted of doxorubicin and cyclophosphamide. In this nonrandomized trial, the response rates were no different in patients treated with radiotherapy alone, chemotherapy alone, or combined treatment. The median survival was highest (13 months) in patients treated with combined modality therapy. The differences in survival among the different treatment groups are likely the result of a selection bias in favor of the combined modality group.

Some investigators have evaluated the addition of radiation sensitizers with definitive radiation therapy (18,19). Herscher et al, (18) from the U.S. National Cancer Institute, studied the use of a 5-day continuous infusion of paclitaxel with radical radiotherapy in patients with mesothelioma and non–small-cell lung cancer. In mesothelioma patients, hemithoracic radiation was delivered initially. This was followed by a boost of radiotherapy to the gross tumor volume for a total dose of 5760 to 6300 cGy. The maximally tolerated dose of paclitaxel in combination with radiation was 105 mg/m^2 as a 120-hour continuous infusion. The toxicities were neutropenia, nausea and vomiting, grade 2 lung injury, and persistent cough. The authors concluded that this treatment was well tolerated. Chen et al (19) evaluated pulsed paclitaxel delivered during radiotherapy in a phase I trial. A 12% complete response rate and an 88% partial response rate were reported for disease within the radiotherapy field. The authors reported that the treatment was well tolerated. Although these approaches are interesting, it is not likely that the addition of radiation sensitizers to radical radiotherapy will be a curative. Definitive chemoradiotherapy should be considered unproven for patients with mesothelioma.

Combined Surgical Resection and Definitive Radiotherapy

Surgical resection, when feasible, is the desired treatment for patients with malignant pleural mesothelioma. Surgery alone, however, is unlikely to sterilize the hemithorax. Adjuvant, postoperative external beam radiotherapy is one approach that has been used to eradicate residual microscopic disease after surgical resection (20). The rationale behind this approach is that debulking the tumor mass maximizes the effectiveness of radiation (21). After an extrapleural pneumonectomy,

radical radiotherapy can be administered without concern for damage to the underlying ipsilateral lung since it has been removed surgically. However, radical radiotherapy after a pleurectomy continues to place the ipsilateral lung at risk for substantial loss of function.

Law et al (11) reported the results of decortication followed by radiation therapy to a dose of 5000 to 5500 cGy in eight patients with pleural mesothelioma. The median survival for these patients was 18 months, which was not increased from patients treated with decortication alone (20 months) or those patients who did not receive treatment (18 months). Toxicities from this regimen were minimal, with nausea and malaise in six patients, transient radiation hepatitis in one patient, and mild esophagitis in one patient. Ruffie et al (13) reported the treatment of 12 patients with surgery followed by radiation. The median survival was 11.7 months, which was no different from the survival reported for those treated with radiation alone, with chemotherapy alone, with surgery and chemotherapy, or with trimodality therapy.

Some investigators have used brachytherapy or intraoperative external beam radiation in combination with surgery. Hilaris et al treated 41 patients with pleural mesothelioma after a parietal pleurectomy. Using this surgical procedure, however, resulted in residual disease being left behind in the majority of patients. Either brachytherapy or radioisotopes were used to eradicate gross residual disease. Permanent ^{125}I brachytherapy implants were used in patients who had measurable gross residual disease. If the residual disease was too diffuse, temporary ^{192}Ir implants were placed 3 to 5 days after the pleurectomy. If gross disease was noted on the surface of the lung, a solution of ^{32}P was instilled into the pleural cavity 5 to 7 days after thoracotomy. External beam radiation to a dose of 4500 cGy was delivered to the pleural surface 4 to 6 weeks after surgery via a combination of photons and electron. There was no mortality and minimal toxicity from this treatment strategy. Six patients (15%) developed complications from treatment. Two patients developed subcutaneous emphysema, one patient developed pneumonitis, one developed pulmonary fibrosis, one developed pericardial effusion, and one developed esophagitis. The median survival was 21 months, with 1-year and 2-year survivals of 65% and 40%, respectively. Only 17% of the patients failed locally, which may be a reflection of the aggressive local therapy. The authors' conclusions were that, while aggressive surgical resection is an essential portion of treatment, it is often very difficult to remove all sites of disease. By the results reported in this study, intraoperative brachytherapy followed by external beam radiation therapy was an effective method of controlling local recurrence. It is not clear if an increased local control rate will translate into a survival advantage.

Rusch and colleagues (22) at the Memorial Sloan-Kettering Cancer Center completed a phase II trial of surgery followed by postoperative radiation in patients with pleural mesothelioma. Eighty-eight patients with biopsy-confirmed mesothelioma were treated. Twenty-one patients were unresectable and taken off study. The majority of patients (n = 62) underwent an extrapleural pneumonectomy (EPP), followed by 54 Gy delivered through anterior and posterior fields in 30 fractions

of 1.8 Gy. Five patients were treated with a pleurectomy, which was followed by intraoperative radiation therapy to a dose of 15 Gy, using a high-dose iridium applicator. This was followed by 54 Gy to the hemithorax via anterior and posterior fields, in the same fractionation schedule as those who underwent EPP. There were seven postoperative deaths, all primarily related to pulmonary complications in patients who had undergone an EPP. A total of 33 patients had some complications, with the most common being atrial arrythmias ($n = 17$), respiratory failure (six), pneumonia (five), and empyema (five). In general, radiation was well tolerated, with grade 3 toxicities mainly related to fatigue, nausea, and esophagitis. There were five grade 4 toxicities, the most serious being an esophagopleural fistula. Only the patients who underwent EPP were considered for survival analysis. The median survival was 17 months, with an overall survival of 27% at 3 years. Only 13% had locoregional recurrence, with the majority of patients failing with distant metastases. The authors concluded that their approach of aggressive surgery with EPP, followed by high-dose radiation to the entire hemithorax, provided a favorable outcome for those patients who were able to complete the therapy compared to historical data. It should be noted that almost one quarter of the patients in this study were unresectable and were not included in the survival analysis, which introduces a bias in the reported results. An additional number of patients were unable to complete radiation. Therefore, although this treatment regimen appears to be associated with excellent clinical outcome, it is difficult to evaluate the relative impact of the treatment regimen from the patient selection factors.

Lee et al (23) recently retrospectively reviewed the efficacy and toxicity of surgery with intraoperative radiotherapy followed by chemotherapy. Twenty-six patients with malignant pleural mesothelioma were included in the analysis. Twenty-four patients were treated with surgery consisting of a pleurectomy/decortication followed by intraoperative radiotherapy, consisting of 4 to 9 MeV electrons to median dose of 15 Gy (range, 5–15 Gy). External beam radiation was delivered by 3DCRT in 14 patients and IMRT in 10 patients. The goal of the external beam radiation therapy was to treat the pleural surface of the lung and all surgical scars while sparing the underlying lung parenchyma. The median dose of radiation delivered was 41.4 Gy (range, 30.1–48.8 Gy). Chemotherapy consisting of cisplatin, doxorubicin, and cyclophosphamide was administered to selected patients beginning 1 to 2 months after radiation was completed. There were no deaths caused by the therapy and few postoperative complications (three cases of atrial fibrillation and one patient with a persistent air leak). Radiation was also well tolerated, with symptoms of pneumonitis noted in only four patients and pericarditis in one patient. In all cases, these symptoms resolved with conservative management. The median overall survival was 18.1 months and the median progression-free interval was 12.2 months. Locoregional relapse was the most common site of failure. The authors concluded that this approach was a potential treatment option for adjuvant radiotherapy in patients who were unable to tolerate an EPP.

Intensity modulated radiation therapy offers the potential for administering higher doses of radiotherapy to the hemithorax while minimizing normal tissue toxicities (5). Stevens et al, at the M.D. Anderson Cancer Study, have treated 28 patients with IMRT after EPP (24,25). The hemithorax was treated with doses of 4500 to 5000 cGy. Some regions of the hemithorax were boosted to a total dose of 6000 cGy. Radiation dose homogeneity to the entire hemithorax was excellent. Side effects included nausea, vomiting, dyspnea, and esophagitis. The median follow-up is 9 months and the local control rate is 100%. One year survival is 65%. These early results are encouraging and are worthy of additional study.

Prevention of Scar Recurrences

Malignant seeding along thoracentesis tracts, biopsy tracts, chest tube sites, and surgical incisions is a common complication of procedures in patients with malignant mesothelioma (26). The frequency of malignant seeding has been reported to occur in approximately 20% to 50% of mesothelioma patients who undergo these procedures (13,27–29). Cutaneous recurrences usually present as painful subcutaneous nodules and may be unresponsive to conventional therapies. Boutin et al (26) have investigated the use of radiation to prevent malignant seeding after invasive diagnostic procedures. Forty patients were randomized, after an invasive diagnostic procedure, to either radiotherapy or no treatment. The radiotherapy regimen consisted of 21 Gy in 3 days delivered with electrons to healing biopsy tracts and thoracoscopic sites and was delivered 10 to 15 days after the procedure. No patient in the radiation treatment group developed subcutaneous nodules. Alternatively, eight of 20 patients in the untreated group developed metastases. Tolerance to this local radiotherapy was excellent. This study supports the use of radiotherapy to the chest wall after diagnostic procedures to prevent cutaneous tumor recurrences.

Palliation

Palliation of symptoms takes on increasing importance in tumors such as mesothelioma in which curative treatment options do not exist for the majority of patients. Palliation of patients with mesothelioma commonly involves the management of dyspnea and chest pain. Dyspnea most often results from intractable pleural effusions, which can trap the lung. Uncontrolled local tumor can also cause encasement of the lung with tumor growing into the lung parenchyma. Chest pain is often the result of tumor invasion of chest wall structures including ribs, muscles, and intercostal nerves (30). Radiotherapy is most commonly used to palliate pain in patients with advanced mesothelioma (31). Investigators from the Netherlands have reported using palliative radiotherapy to treat painful chest wall metastases in patients with

mesothelioma (31). A greater degree of palliation was reported in patients who were treated with fractions of 400 cGy compared with patients who were treated with 300 cGy. Unfortunately, pain recurrence within the treated field remained a significant problem. The authors reported that a total dose of 36 Gy in 400-cGy fractions provided local palliation in at least 50% of patients. Ball and Cruickshank (12) reported a 72% rate of symptom improvement using palliative courses of radiation therapy. These investigators reported that short courses of radiation (20 Gy in five fractions) were as efficacious for symptom relief as more protracted courses of radiation (30–40 Gy in 10–15 fractions). Ruffie et al (13) reported the results of palliative radiation therapy in 85 patients with mesothelioma. Palliation was often not achieved with radiation because adequate doses of radiation were not delivered to some patients. When doses greater than 4500 cGy were used, pain relief was attained in over 50% of the cases. An additional study by Gordon et al (32) reported results that supported the dose response relationship suggested by Ruffie et al. These authors found that radiotherapy provided a 38% palliation rate overall, and suggested that higher doses of 40 to 50 Gy were needed to obtain pain relief. One of the largest studies of palliative radiotherapy in mesothelioma was reported by Davis et al (33). Of 111 patients who were followed, 71 were treated with radiation for symptoms. Although pain was the most common symptom requiring palliation, other symptoms related to superior vena caval obstruction, mass effect, and effusion were also treated. The authors found that greater than 60% of patients had some symptomatic benefit from radiation therapy. Unlike previous studies, the authors reported that the palliative response did not vary with dose. Therefore, the authors' standard approach is to offer patients short courses of treatment (20 Gy in five fractions) rather than longer courses of radiotherapy. These studies support the use of radiation as a useful tool in the palliation of mesothelioma.

Peritoneal Mesothelioma

Peritoneal mesothelioma is an uncommon presentation of mesothelioma. Radiation therapy is not commonly used because of the large volume of tissue that would require treatment when the peritoneal cavity is involved with disease. The toxicities to the small bowel, liver, kidneys, and other abdominal organs would preclude the delivery of curative doses of radiation in the majority of cases. The largest series of radiation therapy in peritoneal mesothelioma was reported by investigators at the Memorial Sloan-Kettering Cancer Center (34). Twenty-five patients with peritoneal mesothelioma in this series underwent surgical debulking. Seven patients received external beam radiotherapy and six patients received external beam radiation combined with the intracavitary instillation of a ^{32}P solution. The median survival was 12 months from the time of diagnosis. Only four patients survived more than 5 years, and all four of these patients were treated with intracavitary ^{32}P and external beam radiation therapy.

Conclusion

The role of radiation therapy in the treatment of mesothelioma has yet to be fully defined. Definitive radiotherapy as a single modality therapy is not curative and is not of clear benefit to patients. Additional study is needed to evaluate definitive radiotherapy in combination with novel radiation sensitizers and chemotherapy in patients with unresectable disease. Postoperative external radiation therapy may have a role as adjuvant treatment after extrapleural pneumonectomy in selected patients. Every effort should be made to deliver radiation doses of 45 to 60 Gy in standard fractions of 180 to 200 cGy. The complex geometry of the thoracic cavity after surgical resection complicates the delivery of radiotherapy and underscores the need to treat these patients with modern treatment planning techniques. Three-dimensional conformal radiation therapy planning is needed to plan radiation therapy in this patient population. The early results with intensity-modulated radiation therapy appear encouraging and should be investigated further. Postoperative radiotherapy in patients who have undergone a pleurectomy/decortication is more complicated because of the underlying lung. The preliminary results of using intraoperative external beam radiation or brachytherapy warrant further evaluation. The major use of radiation therapy in the treatment of mesothelioma currently continues to be in palliation and in the reduction of scar recurrences.

References

1. Hall E. Radiobiology for the Radiologist, 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2000.
2. Evans S, et al. Detection of hypoxia in human squamous cell carcinoma by EF5 binding. Cancer Res 2000;60:2018–2024.
3. Evans SM, et al. Hypoxic heterogeneity in human tumors: EF5 binding, vasculature, necrosis, and proliferation. Am J Clin Oncol 2001;24(5):467–472.
4. Brizel DM, et al. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. Radiother Oncol 1999; 53(2):113–117.
5. Intensity-modulated radiotherapy: current status and issues of interest. Int J Radiat Oncol Biol Phys 2001;51(4):880–914.
6. Zelefsky MJ, et al. High dose radiation delivered by intensity modulated conformal radiotherapy improves the outcome of localized prostate cancer. J Urol 2001;166(3):876–881.
7. Eisbruch A, et al. Intensity-modulated radiation therapy for head and neck cancer: emphasis on the selection and delineation of the targets. Semin Radiat Oncol 2002;12(3):238–249.
8. Glatstein E. Intensity-modulated radiation therapy: the inverse, the converse, and the perverse. Semin Radiat Oncol 2002;12(3):272–281.
9. Brady LW. Mesothelioma—the role for radiation therapy. Semin Oncol 1981;8(3):329–334.
10. Richart R, Sherman CD. Prolonged survival in diffuse pleural mesothelioma treated with Au198. Cancer 1959;12:799.

11. Law MR, et al. Malignant mesothelioma of the pleura: a study of 52 treated and 64 untreated patients. Thorax 1984;39(4):255–259.
12. Ball DL, Cruickshank DG. The treatment of malignant mesothelioma of the pleura: review of a 5-year experience, with special reference to radiotherapy. Am J Clin Oncol 1990;13(1):4–9.
13. Ruffie P, et al. Diffuse malignant mesothelioma of the pleura in Ontario and Quebec: a retrospective study of 332 patients. J Clin Oncol 1989;7(8):1157–1168.
14. Alberts AS, et al. Malignant pleural mesothelioma: a disease unaffected by current therapeutic maneuvers. J Clin Oncol 1988;6(3):527–535.
15. Holsti LR, et al. Altered fractionation of hemithorax irradiation for pleural mesothelioma and failure patterns after treatment. Acta Oncol 1997;36(4): 397–405.
16. Mattson K, et al. Multimodality treatment programs for malignant pleural mesothelioma using high-dose hemithorax irradiation. Int J Radiat Oncol Biol Phys 1992;24(4):643–650.
17. Linden CJ, et al. Effect of hemithorax irradiation alone or combined with doxorubicin and cyclophosphamide in 47 pleural mesotheliomas: a nonrandomized phase II study. Eur Respir J 1996;9(12):2565–2572.
18. Herscher LL, et al. Phase I study of paclitaxel as a radiation sensitizer in the treatment of mesothelioma and non-small-cell lung cancer. J Clin Oncol 1998;16(2):635–641.
19. Chen Y, et al. Schedule-dependent pulsed paclitaxel radiosensitization for thoracic malignancy. Am J Clin Oncol 2001;24(5):432–437.
20. Kindler H, Vogelzang N. Mesothelioma. In: Vokes E, Golomb H, eds. Oncologic Therapies. Berlin: Springer-Verlag, 1999:635–651.
21. Sugarbaker DJ, et al. Extrapleural pneumonectomy in the multimodality therapy of malignant pleural mesothelioma. Results in 120 consecutive patients. Ann Surg 1996;224(3):288–294; discussion 294–296.
22. Rusch VW, et al. A phase II trial of surgical resection and adjuvant high-dose hemithoracic radiation for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;122(4):788–795.
23. Lee TT, et al. Radical pleurectomy/decortication and intraoperative radiotherapy followed by conformal radiation with or without chemotherapy for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2002;124(6): 1183–1189.
24. Ahamad A, et al. 100% local control of malignant pleural mesothelioma after extrapleural pneumonectomy followed by intensity modulated radiotherapy (IMRT) to the chest. Int J Radiat Oncol Biol Phys 2003;in press.
25. Forster KM, et al. Intensity-modulated radiation therapy following extrapleural pneumonectomy for the treatment of malignant mesothelioma: clinical implementation. Int J Radiat Oncol Biol Phys 2003;in press.
26. Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. Chest 1995;108(3):754–758.
27. Law MR, Hodson ME, Turner-Warwick M. Malignant mesothelioma of the pleura: clinical aspects and symptomatic treatment. Eur J Respir Dis 1984; 65(3):162–168.
28. Brenner J, et al. Malignant mesothelioma of the pleura: review of 123 patients. Cancer 1982;49(11):2431–2435.
29. Adams VI, Unni KK. Diffuse malignant mesothelioma of pleura: diagnostic criteria based on an autopsy study. Am J Clin Pathol 1984;82(1):15–23.
30. Sterman DH, Kaiser LR, Albelda SM. Advances in the treatment of malignant pleural mesothelioma. Chest 1999;116(2):504–520.

31. de Graaf-Strukowska L, et al. Factors influencing the outcome of radiotherapy in malignant mesothelioma of the pleura—a single-institution experience with 189 patients. Int J Radiat Oncol Biol Phys 1999;43(3): 511–516.
32. Gordon W Jr, et al. Radiation therapy in the management of patients with mesothelioma. Int J Radiat Oncol Biol Phys 1982;8(1):19–25.
33. Davis SR, Tan L, Ball DL. Radiotherapy in the treatment of malignant mesothelioma of the pleura, with special reference to its use in palliation. Australas Radiol 1994;38(3):212–214.
34. Brenner J, et al. Malignant peritoneal mesothelioma: review of 25 patients. Am J Gastroenterol 1981;75(4):311–313.

42

Intrapleural Chemotherapy with and Without Surgery in Malignant Pleural Mesothelioma (MPM)

Bilal Piperdi, Dong M. Shin, and Roman Perez-Soler

Although malignant pleural mesothelioma (MPM) is a rare disease, its annual incidence is believed to be increasing. Approximately 2000 to 3000 new cases of MPM are diagnosed annually in the United States (1). It is a very aggressive disease with median survival of approximately 6 to 8 months. The majority of the patients at the time of diagnosis are surgically unresectable due to extensive local disease. Even in patients who undergo aggressive surgical debulking, the majority of them, in some series up to 80%, have local relapse despite aggressive adjuvant therapy (2). Systemic chemotherapy until recently has not been shown to improve survival in patients with MPM.

Interapleural therapy can be an attractive option for MPM, particularly in early, nonbulky disease, which is easily accessible from the pleural cavity. Inrapleural drug delivery can achieve increased and prolonged local drug concentration with less systemic toxicity. Several agents, both chemotherapy and immunotherapy, have been evaluated as intrapleural therapy. Intrapleural immunotherapy is reviewed in Chapter 43. This chapter reviews the experience with intrapleural chemotherapy alone or as adjuvant to debulking surgery and discusses our encouraging results with liposome-entrapped platinum derivative, cis-Bis-neodecanoato-trans-R, R-1, 2-diaminocyclohexane platinum (II) (L-NDDP), which is structurally similar to oxalipatin, as intrapleural therapy in patients with MPM.

Intrapleural Chemotherapy

Delivering chemotherapy directly to tumor-involved body cavities has always been an attractive option. Theoretically, it has the advantage of delivering the drug directly to the tumor with fewer systemic side effects. Dedrick et al (3) in 1978 proposed a mathematical model of the pharmacokinetics of intraperitoneal chemotherapy. The patient was divided into two compartments: the peritoneal cavity and the remainder of the body. The concentration of a drug in the peritoneal cavity is

inversely proportional to its rate of clearance from both compartments. In general, the clearance rates are determined by (1) the characteristics of the cavity, (2) the characteristics of the drug itself, (3) the concentration of the drug delivered, (4) the route of entry into the plasma, (5) the site of metabolism, and (6) the mode of excretion. Depending on all of these factors, the intracavitary levels of the drug can be manyfold higher than systemic levels. Indeed, intraperitoneal levels of 21-fold higher than serum levels have been reported after intraperitoneal administration of cisplatin (4). Intraperitoneal cisplatin has been proven superior to intravenous cisplatin in a large randomized study of ovarian cancer (5).

The ideal antitumor drug designed for intrapleural therapy should have two properties: prolonged residence time in the cavity, and the ability to penetrate easily into the tumor tissue from its surface. Prolonged residence of the medication in the pleural cavity and, therefore, delayed absorption into the systemic circulation result in peak systemic levels of the drug well below that of the pleural levels. This ensures a subtherapeutic and subtoxic range of cytotoxic medication systemically and hence, an increased therapeutic index. As in any form of topical therapy, the lipid solubility of the drug and its penetration into the tumor is a critical factor in determining the efficacy of the drug administered intrapleurally. Unfortunately, most classic chemotherapeutic agents tested as intrapleural chemotherapy in the clinical trials are hydrophilic and lack these properties.

The initial studies with intrapleural therapy were done in patients with malignant pleural effusion. Rusch et al (6) reported intrapleural cisplatin and cytarabine in patients with malignant pleural effusion. Only one of 37 patients developed grade 4 renal toxicity and three of 27 patients had grade 3 hematologic toxicity. The overall response rate at 3 weeks was 49%. The median duration of response for six patients with a CR was 9 months but the median overall survival for the whole group was, disappointingly, 5.7 months. The majority of the patients died of progressive systemic disease.

Malignant pleural mesothelioma is an ideal candidate for intrapleural therapy because the disease tends to remain confined to the pleural cavity. It is usually resistant to radiotherapy, and systemic chemotherapy provides only a modest increase in survival. Death in patients with MPM is usually due to aggressive local growth and invasion. Intrapleural cisplatin has been evaluated in conjunction with debulking surgery in several trials as reviewed below. We have conducted a phase II study with single-agent intrapleural liposome-entrapped platinum derivative with encouraging results.

Intrapleural Chemotherapy with Surgical Debulking

The three surgical procedures that have been used for the treatment or palliation of MPM are pleurodesis, pleurectomy/decortication (P/D), and extrapleural pneumenectomy (EPP). None of these procedures as a single modality has resulted in significant prolongation of median

Table 42.1. Phase II trials of intrapleural chemotherapy following surgical debulking in patients with malignant pleural mesothelioma

Author	Number of patients	Type of surgery	Intrapleural chemotherapy	Systemic chemotherapy	Median survival	2-year survival
Rusch et al (8)	36	P/D	Cisplatin and mitomycin C	Cisplatin and mitomycin C	17 months	40%
Rice et al (9)	19	9 P/D 10 EPP	Cisplatin and mitomycin C	Cisplatin and mitomycin C	13 months	22%
Sauter et al (10)	13	P/D	Cisplatin and cytarabine	Cisplatin and mitomycin C	9 months	15%
Juturi et al (11)	22	9 P/D 12 EPP	Paclitaxel	Paclitaxel and radiation therapy	9 months	NA

EPP, extrapleural pneumonectomy; NA, not available; P/D, pleurectomy/decortication.

survival, although EPP can "cure" 10% to 20% of patients (see Chapters 46 and 47). Intrapleural therapy has been evaluated as a part of multimodality treatment in patients who can undergo debulking surgery (7).

Rusch et al (8) reported a phase II trial of P/D followed by immediate intrapleural cisplatin and mitomycin C and systemic therapy with the same agents 3 to 5 weeks postoperatively (Table 42.1). Thirty-six patients were enrolled, and 28 patients underwent P/D and intrapleural chemotherapy. There was one perioperative death (3.6%) and two episodes of grade 4 renal failures. Twenty-three of the 27 surviving patients received systemic chemotherapy. The 1- and 2-year survival rates were 68% and 40%, respectively, and the median survival was 17 months. Despite intrapleural therapy, locoregional recurrence was seen in 80% of the patients.

Rice et al (9), from Cleveland Clinic, used a similar regimen in 19 patients with stage I disease. Surgical debulking was one with P/D in nine patients and EPP in 10 patients. The P/D patients received immediate intrapleural cisplatin and mitomycin C postprocedure, while the intrapleural therapy was done 1 to 2 weeks postprocedure for patients undergoing EPP. There was one perioperative death (5%), and 15 patients received cisplatin-based systemic chemotherapy. The median survival was 13 months and the median disease-free survival was 11 months.

Sauter et al (10), from Fox Chase Cancer Center, reported their experience of multimodality treatment including intrapleural therapy in 20 patients with MPM. All patients had subtotal pleurectomy and 13 patients with preoperative diagnosis of MPM were enrolled in a phase II study with intrapleural cisplatin and cytarabine after subtotal pleurectomy followed by intravenous cisplatin and mitomycin C. There was one treatment-related death due to cisplatin-induced nephrotoxicity. The median survival and time to progression were 9 and 6 months, respectively, in patients who received intrapleural therapy, and was significantly lower than in seven other patients who did not receive intrapleural therapy.

Juturi et al (11) reported their experience with intracavitary paclitaxel as a part of multimodality treatment in 22 patients with MPM. Nine

patients underwent P/D and 12 patients underwent EPP. This was followed by intrapleural paclitaxel 125 mg/m^2 on D1 postoperatively for P/D patients or on D7 for EPP patients. Concurrent chemoradiation with intravenous paclitaxel was begun 4 to 8 weeks after the surgery. The toxicity of the regimen was considerable, including empyema, wound dehiscence, mucositis, neutropenia, neutropenic sepsis, and one toxic death. The median survival was, disappointingly, only 9 months. Despite the aggressive multimodality treatment, locoregional relapse was seen in 64% of the patients.

Hyperthermic Intrapleural Chemotherapy

One of the major problems with intrapleural therapy has been the quick reabsorption of chemotherapy from the pleural surface into the systemic circulation. Pharmacokinetic studies have shown that intrapleural and intraperitoneal cisplatin is quickly reabsorbed with peak plasma level at 15 to 20 minutes, similar to those achieved with intravenous cisplatin. This accounts for the increased toxicity and limits the maximum dose that can be delivered, and hence, decreases the efficacy. Several methods have been explored to overcome this limitation.

One way to increase the local tumor cell kill is to deliver hyperthermic chemotherapy. Ratto et al (12) studied hyperthermic intrapleural perfusion in MPM. Three patients underwent P/D and normothermic cisplatin, three patients underwent P/D and hyperthermic cisplatin, and four patients underwent EPP and hyperthermic perfusion. The cisplatin dose was 100 mg/m^2. There was higher systemic absorption of the cisplatin with P/D than with EPP. Higher local tissue concentrations were achieved with hyperthermic infusion than with normothermic infusion.

Sugarbaker et al (13) reported the phase I data of intraoperative intracavitary hyperthermic chemotherapy with EPP in 50 patients with MPM. After EPP, the exposed hemithorax and lower abdomen were lavaged for 1 hour with cisplatin at 44°C. Thiosulfate was infused intravenously for 6 hours to bind systemically absorbed cisplatin. In their dose-escalating study, the maximum tolerated dose (MTD) for hyperthermic cisplatin delivered was 250 mg/m^2. Operative mortality was 2% (1/50). Constrictive pericarditis was seen in 10% (5/50) with 8% (4/50) requiring reoperation, all in patients with left EPP. Empyema developed in 8% (4/50) of the patients. Grade II renal insufficiency developed in 6% (3/50) and grade II hematologic toxicity in 33% (16/48) of the patients. Substantial gradient of cisplatin was noted between systemic and local levels. Efficacy was not reported (13, 14).

Liposome-Entrapped Platinum Derivative

Another way to enhance the local effect with fewer systemic complications is to use a favorable delivery vehicle. We have studied the intrapleural therapy with L-NDDP, which is a lipophilic and non–cross-

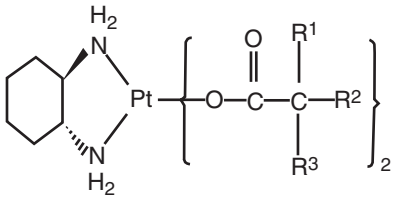


Figure 42.1. Structure of cis-Bis-neodecanoato-trans-R,R-1,2-diaminocyclohexane platinum (L-NDDP).

resistant platinum compound that is formulated in large multilamellar liposomes (1–3 μm) (Fig. 42.1). Because of its lipophilicity, its transmembrane transport has been shown to be much higher than that of cisplatin, and hence its tissue penetration is markedly increased. Because of the large liposomes used to deliver L-NDDP, its intracavitary residence is much more prolonged than that of cisplatin (15).

In our phase I study in patients with malignant pleural effusion, the MTD of intrapleural L-NDDP was 450 mg/m^2. The dose-limiting toxicity was chemical pleuritis with rapid pleural fluid reaccumulation. The only toxicity observed at MTD was grade 1 to 2 nausea and vomiting without premedication. Neither myelosuppression nor nephrotoxicity was observed. In one patient with MPM, the pleural effusion disappeared without evidence of recurrence for 19+ months. Two other patients with MPM had >50% reduction in pleural fluid size (16).

These encouraging results prompted us to perform a phase II study of intrapleural L-NDDP in patients with MPM (Table 42.2). The L-NDDP dose was 450 mg/m^2 and the treatment was given every 3 to 4 weeks. To date 34 patients have been enrolled. In the initial eight patients, the first course of L-NDDP was given at the time of thoracoscopy. There were two treatment-related deaths, most probably due to the method of drug infusion. One patient died of pneumonia with peritonitis and the other due to sepsis secondary to chest wall cellulitis at the thoracoscopy site. In subsequent patients, the initial course of L-NDDP was delivered via a Tenkoff or Denver catheter, 1 week after the placement of the catheter and repeated every 3 to 4 weeks. No significant side effects were observed in subsequent patients except mild to moderate pleuritic chest pain, transient fever, mild nausea and vomiting, fatigue, and allergic reaction (grade 1–3). No significant renal

Table 42.2. Phase II trial of intrapleural L-NDDP in patients with MPM

Number of patients enrolled	**34**
Evaluable patients	**23**
Number of patients not treated	**4**
Patients with no follow-up biopsies	**7**
Complete pathologic response	**13/23 (57%)**
Complete cytologic response	**15/18 (83%)**

toxicity was observed. There were two grade 3 thrombocytopenias and two grade 3/4 neutropenias and one grade 3 anemia.

Thoracoscopy-guided biopsies and cytologic examination of the pleural fluid were performed at baseline and after one or two courses of therapy to assess the pathologic response. Among 23 evaluable patients who had pre- and posttreatment biopsies, *13 (57%) had* a complete pathologic response after treatment. Among 18 evaluable patients with positive cytology, 15 (83%) showed complete cytologic response. These results are very promising, especially when taking into account the modest systemic and local toxicity seen with the treatment (17).

Conclusion and Future Directions

Intrapleural delivery is an effective way of delivering chemotherapy in patients with MPM. Intrapleural chemotherapy can be particularly effective early, when the disease is limited to the pleural surface. However, the current data with the adjuvant intrapleural chemotherapy is inconclusive and should not be routinely used except in the setting of clinical trials. Our results with intrapleural L-NDDP are very encouraging and warrant further studies either as a neoadjuvant or an adjuvant to debulking surgery or in combination with intravenous chemotherapy.

References

1. Connelly RR, Spirtas R, Myers MH, et al. Demographic patterns for mesothelioma in the United States. J Natl Cancer Inst 1987;78:1053–1060.
2. Baldini EH, Recht A, Strauss GM, et al. Patterns of failure after trimodality therapy for malignant pleural mesothelioma. Ann Thorac Surg 1997; 63:334–338.
3. Dedrick RL, Myers CE, Bungay PM, et al. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 1978;62:1–10.
4. Howell SB, Pfeifle CL, Wung WE, et al. Intraperitoneal cis-diaminedichloroplatinum with systemic thiosulfate protection. Cancer Res 1983;43: 1426–1431.
5. Albert DS, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 1996;335:1950–1955.
6. Rusch V, Figlin R, Godwin D, Piantadosi S. Intrapleural cisplatin and cytarabine in the management of malignant pleural effusion: a Lung Cancer Study Group Trial. J Clin Oncol 1991;9:313–319.
7. Zellos L, Sugarbaker D. Multimodality treatment of diffuse malignant pleural mesothelioma. Semin Oncol 2002;29:41–50.
8. Rusch V, Saltz L, Venkatraman E, et al. A phase II trial of pleurectomy/decortication followed by intrapleural and systemic chemotherapy for malignant mesothelioma. J Clin Oncol 1994;12:1156–1163.
9. Rice TW, Adelstein DJ, Kirby TJ, et al. Aggressive multimodality therapy for malignant pleural mesothelioma. Ann Thorac Surg 1994;58:24–29.

10. Sauter ER, Langer C, Coia LR, et al. Optimal management of malignant mesothelioma after subtotal pleurectomy: revisiting the role of intrapleural chemotherapy and postoperative radiation. J Surg Oncol 1995;60:100–105.
11. Juturi JV, Adelstein DJ, Rice TW, et al. Intracavitary paclitaxel in the multimodality management of malignant pleural mesothelioma. Proc Am Soc Clin Oncol 2001; 20:366a (abstr 1460).
12. Ratto GB, Civalleri D, Esposito M, et al. Pleural space perfusion with cisplatin in the multimodality treatment of malignant mesothelioma: a feasibility and pharmacokinetic study. J Thorac Cardiovasc Surg 1999;117: 759–765.
13. Sugarbaker D, Zellos L, Capalbo L, et al. Clinical results of phase I trial of intraoperative intracavitary hyperthermic chemotherapy with EPP for MPM. Proc Am Soc Clin Oncol 2001;20:370a (abstr 2097).
14. Wright J, Tretyakov O, Frei E, et al. Pharmacological phase I study of heated, intraoperative, intrapleural and intraperitoneal cisplatin during EPP for MPM. Proc Am Soc Clin Oncol 2001;20:370a (abstr 2094).
15. Perez-Soler R, Francis K, Al-Baker S, et al. Preparation and characterization of a liposomal preparation containing a lipophilic cisplatin derivative for clinical use. J Microencapsulation 1994;11:41–54.
16. Perez-Soler R, Shin DM, Siddik ZH, et al. Phase I clinical and pharmacological study of liposome-entrapped NDDP administered intrapleurally in patients with malignant pleural effusion. Clin Cancer Res 1997;3:373–379.
17. Perez-Soler R, Walsh GL, Swisher SG, et al. Phase II study of a liposome-entrapped cisplatin analog (L-NDDP) administered intrapleurally in patients with MPM. Proc Am Soc Clin Oncol 1999;18 (abstr).
18. Shin DM, et al. Pathologic response with liposomal-entrapped cisplatin analog (L-NDDP) administered in patients with MPM: phase II clinical study. Proc Int Lung Cancer Cong 2000 (abstr 3690).

43

Management of Pleural Effusions in Mesothelioma

Shahriyour Andaz and Robert B. Cameron

Although malignant pleural mesothelioma (MPM) is nearly always incurable, palliative therapy is often necessary. Pleural effusions are probably the most common cause of symptoms requiring palliative treatment in patients with MPM. Treatment of pleural effusions in patients with MPM frequently is difficult, and one must keep the goals of therapy (i.e., control of dypsnea and patient comfort), the wishes of the patient, and the nature of the underlying process in perspective. Since MPM-related pleural effusions can severely impair patients' quality of life and can quickly relapse if treated inadequately, prompt and effective measures should be taken to control this morbid problem. The principles guiding therapy of MPM effusions also can be applied in the management of other malignant pleural effusions.

Indications for Palliation

Palliation of MPM pleural effusions is indicated in any patient with symptoms of dyspnea and significant pleural fluid. Furthermore, palliation may be indicated in patients without current symptoms or minimal pleural fluid in anticipation of increasing pleural fluid collections. Most frequently, this opportunity occurs at the time of diagnosis, particularly when thoracoscopy is used. Palliative measures can and should be used even if surgical resection is being contemplated, since palliative measures do not interfere with the surgeon's ability to resect all gross tumor with either an extrapleural pneumonectomy or pleurectomy with decortication. Most often, palliative therapy for pleural effusions is employed in patients with medical contraindications for major surgical resection, contralateral recurrence following initial surgery, advanced disease (stage III/IV), or sarcomatous pathology, or in patients who refuse surgical intervention.

Methods of Palliation

Palliative measures for pleural effusions in MPM include pleurodesis, drains, shunts, as well as other palliative therapies. By far the most common technique for palliation is pleurodesis, but in selected patients the use of external drains, internal shunts, and other palliative methods all play a key role in accomplishing the goal of maximal palliation of patient symptoms.

Pleurodesis

Pleurodesis is the mainstay of palliative treatment options for patients with MPM and pleural effusions. Pleurodesis requires the apposition of the visceral and parietal pleurae. Loculated pleural fluid and tumor may prevent this critical condition and, therefore, render attempts at pleurodesis ineffective. Prior to any attempt at pleurodesis, every effort should be made to maximize the expansion of the involved lung for greatest benefit. Adjuvant surgical procedures may be required, such as thoracoscopy, to drain loculated fluid collections and to debride fibrinous exudates on the lung. Patients with chronic effusions (i.e., those present for greater than 4 to 6 weeks), however, may have developed a layer of tumor covering the visceral pleura that prevents full reexpansion. This situation, termed "trapped lung" (Fig. 43.1), can prevent apposition of a large enough portion of pleura that pleurodesis alone will not prevent reaccumulation of pleural effusions. In these cases, additional procedures, such as external drainage, pleuroperitoneal shunts, and, in selected patients, palliative decortication, must be considered. In cases of minimal trapped lung, pleurodesis still may be performed, anticipating that only a small asymptomatic loculated area of fluid will accumulate in the area of pleural nonapposition. The possible development of trapped lung should be kept in mind when deciding on treatment strategies for pleural effusions associated with MPM. Prompt treatment, including drainage and pleurodesis, produces results far superior to an approach of observation or even intermittent thoracocentesis.

Chemical Pleurodesis

The most common technique used to control pleural effusion associated with MPM is chemical pleurodesis. Like other forms of pleural symphysis, chemical pleurodesis is worthwhile only if the underlying lung expands and pleural apposition is achieved. As soon as the effusion is drained and the underlying lung expands fully, pleurodesis may be performed. Currently, the most widely used compound is sterile, asbestos-free talc, administered either as a powder or slurry, but other "chemicals" also are used, including doxycycline (tetracycline is no longer available in a sterile parenteral form), bleomycin, and nitrogen mustard.

Talc Pleurodesis: Talc as a sclerosing agent was first used by Bethune (1) in 1935 prior to surgery for tuberculosis to avoid pulmonary collapse. Talc is a pulverized, natural, foliated, hydrated magnesium silicate that is passed through a mesh filter to eliminate large particles. Talc

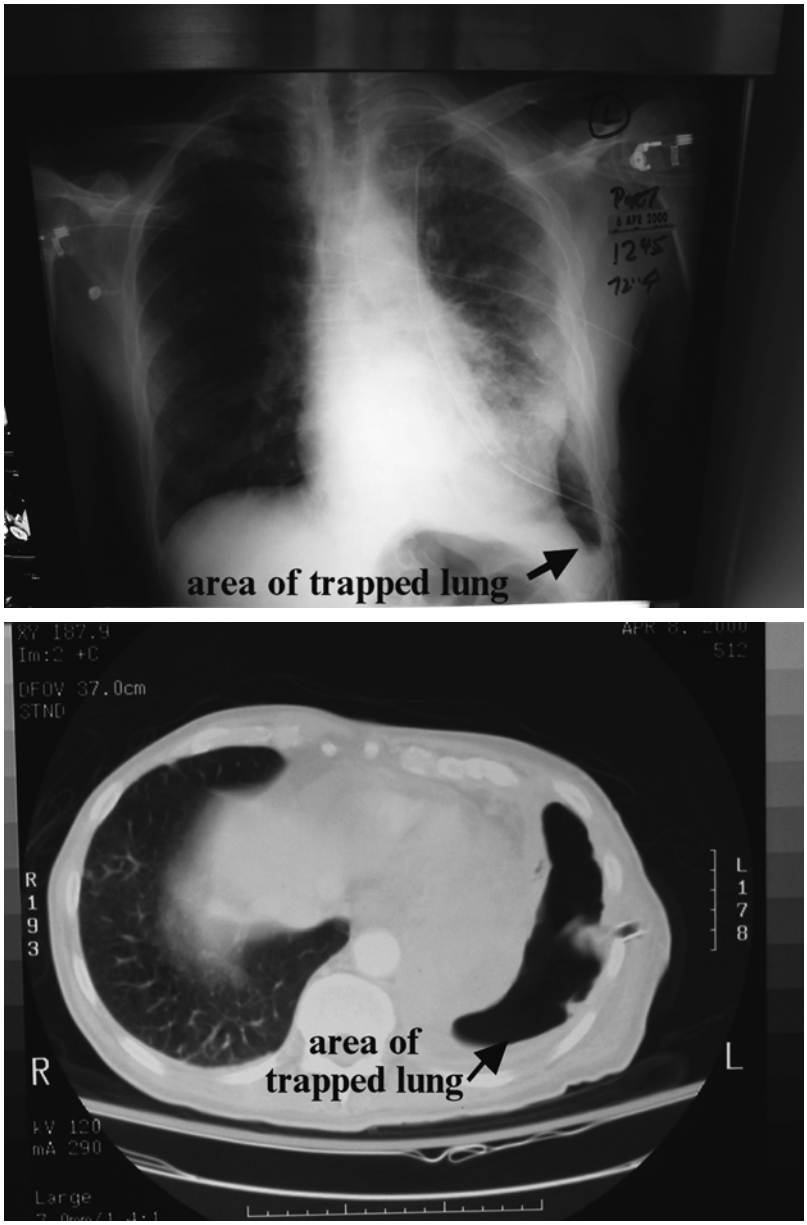


Figure 43.1. Posteroanterior (PA) chest x-ray (A) and computer tomography (CT) scan (B) showing intrapleural space caused by trapped lung.

preparations vary in calcium, aluminum, and iron composition, depending on the origin of the talc deposit, but have the approximate chemical formula of $Mg_3(Si_2O_5)_2(OH)_2$.

Mechanism. Upon contact with the pleural surface, talc induces an interleukin-8 (IL-8)–mediated neutrophil influx into the pleural space. Subsequently, macrophages accumulate (2), and release IL-8 as well as macrophage chemoattractant protein 1.

Increased expression of adhesion molecules on mesothelial cells may amplify the inflammatory response (3). Furthermore, talc instillation is associated with a rapid and marked rise in mesothelial cell–derived basic fibroblast growth factor (bFGF) in pleural fluid (4). Finally, with successful talc pleurodesis, pleural fibrinolytic activity declines, suggesting an important role of the coagulation cascade. When extensive tumor covers both pleural surfaces, pleurodesis is less effective, further supporting the key role of the mesothelial cell in pleural fibrosis.

Cost. Talc is the least expensive of all agents available for pleurodesis. The cost for 5 g of nonsterile talc is approximately $0.30, compared with $86.00 for 500 mg of doxycycline and $1,140.00 for 70 units of bleomycin (5). Talc sterilization can be problematic, with anaerobic *Bacillus* species cultured from talc received from six manufacturers in the United States (6). Fortunately, however, sterilization generally can be accomplished using several techniques, including dry heat, ethylene oxide gas, and λ-irradiation. All sterilization methods are economical, with dry heat being the least expensive ($4.74 for 5 g). Once sterilized, talc remains bacterium free for at least 1 year.

Technique. Talc pleurodesis may be accomplished either by insufflating dry talc powder (poudrage) directly into the chest during surgery (most commonly during thoracoscopy) or by injecting a talc "slurry" through an existing chest tube. Intraoperative talc poudrage is relatively straightforward and is facilitated by using aerosolized talc preparations. In the absence of other indications for surgery (i.e., loculated effusion, need for pathology confirmation, incomplete expansion, etc.), however, talc pleurodesis can and should be performed using a talc slurry at the bedside, since this can be done without general anesthesia and with a very high (>90%) success rate (see Efficacy, below). Pleurodesis using talc slurry generally requires that a chest tube at least 20 French in size is used. Once complete drainage of the pleural fluid and apposition of the pleural surfaces is accomplished, 5 to 6 g of sterile talc slurry in 120 to 180 mL of normal saline containing 200 mg of lidocaine (20 mL of 1.0% lidocaine solution) is instilled into the chest tube. Usually the patient is medicated with a narcotic and often a benzodiazepine to maximize comfort during the procedure. The chest tube is then clamped, and the patient is rotated into six different positions: right and left lateral decubitus, supine, prone, sitting, and head down (Trendelenburg) position every 15 to 20 minutes (1½ to 2 hours total time) to achieve uniform distribution of the talc slurry. The chest tube is unclamped and placed to continuous suction. This process may be repeated in 2 to 3 days if significant chest tube drainage continues, although this is usually not necessary. When the drainage is less than 100 to 150 cc/24 hours, the chest tube may be removed. Patients usually can be discharged from the hospital within 72 hours if the procedure is performed expeditiously. In the past, it was customary to wait until the daily chest drainage was low before performing any pleurodesis; however, this delays treatment often for days, is associated with the development of adhesions, which may interfere with maximal pleurodesis, and is completely unnecessary. Talc pleurodesis can be

successfully accomplished with instillation of talc immediately after radiographic complete lung expansion even if the initial drainage volume exceeds 150 mL/24 hours. Talc pleurodesis has been performed on occasion on an outpatient basis.

Morbidity. Chest pain and fever are the most common adverse effects of talc as well as all pleurodesing agents. The intensity of chest pain with talc ranges from minimal to severe discomfort. Although initially patients always describe a "tight" or "burning" sensation, generally medication prior to the pleurodesis with a narcotic and a benzodiazepine minimizes any discomfort. Because of the intense inflammatory response, talc frequently causes fever (usually >38°C) occurring characteristically 4 to 12 hours after instillation and lasting for no longer than 72 hours. These initial "inflammatory" fevers generally are not signs of infection and are treated simply with Tylenol. True infectious complications (empyemas) have been reported in 0 to 11% of talc slurry procedures (7), but should be uncommon if sterile techniques are employed. Empyemas may occur if there is a preexisting pleural infection or if a chronic indwelling chest tube is used for pleurodesis. If there is any suspicion that a pleural infection exists, another agent should be used for the pleurodesis. Finally, local chest tube site infections are rare (8). Cardiovascular complications, such as arrhythmias (9), cardiac arrest (10), chest pain (11), myocardial infarction (12), and hypotension (13) rarely have been reported. In many instances, it has been difficult to distinguish complications arising from talc administration from those occurring as a result of simultaneous surgical procedures. Most patients also have associated comorbid conditions that increase the risk of developing complications.

Respiratory Insufficiency. Respiratory failure presenting as adult respiratory distress syndrome (ARDS) has been reported with both talc poudrage and talc slurry. Rinaldo and colleagues (14) reported the development of ARDS in three patients who received 10 g of talc as a slurry via a chest tube (two of the three patients recovered). Todd et al (12) reported respiratory failure or pneumonia in seven patients. The true incidence and pathophysiology of this catastrophic problem are unknown. Contributing factors include the intense local and systemic inflammatory responses to talc administration. A systemic as well as a local inflammatory response has been shown to develop shortly after pleurodesis with upregulation of systemic matrix metalloproteinases-2 and -9 in a dose-dependent manner (15). Another contributing factor is inadequate surfactant production. Rapid reexpansion of a collapsed lung may exceed its ability to produce enough surfactant to cover the much larger surface area of the expanded lung's alveoli, thereby resulting in acute (noncardiogenic) pulmonary edema. Gradual reexpansion of a lung, chronically compressed by a significant pleural effusion, over at least 4 to 6 hours allows adequate time for surfactant production.

The talc preparation may be a contributing factor in the development of talc-associated ARDS. The mean particle diameter in talc preparations varies from 10.8 to 33.6 μm (16), while lymphatic channels of pleural membranes in sheep have been shown to be 8 to 10 μm in diam-

eter (17). Talc, like most chemicals instilled into the pleural space, has been documented in bronchoalveolar lavage fluid of patients (18) and may enter the circulation through lymphatic channels in an inflamed pleural membrane or directly through the lung alveoli. Preparations using particles of smaller diameter may predispose to the development of ARDS. It is interesting that the United States, which has the highest reported incidence of ARDS following talc administration, also has the smallest mean particle diameter in talc preparations (17). Talc dose also may be a factor contributing to the development of ARDS. A rabbit model comparing two doses of talc, 200 and 50 mg/kg, found an increased incidence of systemic talc deposition, fibrotic visceral pleural thickening, and foreign-body granulomas with the higher dose (19). Finally, other contributing factors could include excessive sedation, chronic obstructive airway disease, and restrictive lung disease due to infiltration of the lung parenchyma by the malignant process or pulmonary fibrosis. In summary, although the pathophysiology of talc-associated ARDS remains unclear, it is doubtful that the method of administration plays a role. Talc dose and particulate size, however, may be important factors, and for this reason, it would be prudent to avoid talc preparations with small particle size and limit the total dose to 5 to 6 g. Due to the dosage concerns, bilateral, simultaneous talc pleurodesis procedures are not recommended.

Efficacy. There is a dearth of specific information regarding the efficacy of talc pleurodesis in patients with MPM; however, there are many studies utilizing talc for malignant pleural effusions of all types. In prospective randomized trials, talc controlled malignant effusion in over 90% of cases and was superior when compared to doxycycline in a series of 33 patients both in the short ($p = 0.009$) and long term ($p = 0.00003$) (20). A review of the English-language literature from 1966 to 1994 identified 1168 patients treated with different chemical agents for malignant pleural effusions. Talc was noted to be the most effective overall, with a success rate of 93%, compared with *Corynebacterium parvum* (CP) (76%), tetracycline (67%), doxycycline (72%), and bleomycin (54%) (21). In another review of 32 case series of predominantly malignant effusions, talc was effective either completely or partially in 659 (91%) of 723 cases (22). Cardillo et al (23) reported 93% control of recurrent mesothelioma-related effusions with thoracoscopic insufflation of 5 g of sterile purified talc. Operative mortality rate was less than 1%, and there were no episodes of ARDS. Similar results were reported by Schulze et al (24) in 105 patients with malignant pleural effusion in whom video-assisted thoracic surgery (VATS) with talc insufflation resulted in relief of dyspnea in 92% of cases. Effective palliation of MPM-associated pleural effusions can be associated with survival of up to 7 months, particularly with epithelioid tumors (25).

Other Chemical Agents: Chemical agents (other than talc) used for pleurodesis include bleomycin, doxycycline, oxytetracycline, silver nitrate, and nitrogen mustard. "Chemical" pleurodesis also can be accomplished with live agents, such as CP. Senyigit et al (26) compared the efficacy of oxytetracycline (OT), CP, and nitrogen mustard (NM) in 117

patients who had at least stage II malignant mesothelioma according to the Butchart staging system. At 1- and 3-month follow-up, OT (81%, 90 days) and CP (86%, 79.3%) led to a significantly higher rate of successful pleurodesis as compared to NM (48.2%, 41%). Patients receiving NM had a higher incidence of nausea-vomiting and hypotension (47%) compared to OT (4%) and CP(5%). These results suggest that OT and CP may be used as effective sclerosing agents for pleurodesis in the control of pleural effusions in patients who otherwise are not candidates for the use of talc. Other inexpensive alternatives, like silver nitrate, also have been tried with few side effects, but the success rates have been inferior to that of talc (27).

Since "chemical" pleurodesis is mediated through cytokine production, direct intrapleural injection of cytokine(s) might avoid the need to use chemicals to induce pleural inflammation. Lee et al (28,29) demonstrated that transforming growth factor-β (TGF-β) could cause pleural adhesions without a severe inflammatory response in an animal model. Despite a dose-related increase in the intrapleural fluid occurring in the first 72 hours, the amount of pleural fluid decreased by day 5. In addition, the white blood cell content of the pleural fluid was significantly less than with talc or doxycycline administration, indicating a less severe inflammatory response. However, TGF-β is too expensive to use routinely.

Mechanical Pleurodesis

Some patients undergoing surgery, i.e., thoracoscopic biopsy or drainage, may not be candidates for talc pleurodesis (due to active pleural infection, etc.). These patients may be treated best with mechanical methods of inducing pleural symphysis.

Mechanical Abrasion: Mechanical methods of pleurodesis require surgical intervention. Pleurodesis at the time of thoracoscopy may be carried out by mechanical abrasion of the pleura, inciting an inflammatory process similar to that of chemically induced reactions described above and facilitating pleural symphysis (30). Mechanical pleurodesis for pneumothorax has been well described (31). The parietal pleura can be mechanically abraded with gauze, the rough surface of a cautery scratch pad, or a mechanical rotary brush (32). In selected cases of malignant pleural effusions requiring pleurodesis when simultaneous pleural infections are suspected, loculated pleural fluid can be drained, lung can be partially decorticated and expanded, and parietal pleura can be mechanically abraded as part of a single procedure.

Limited Pleurectomy: In a small group of highly selected patients, inadequate lung expansion leaves a residual pleural space that leads to failure of all attempts at pleurodesis due to visceral pleural encasement and lack of pleural apposition. Symptoms may persist and may not be palliated well by other means of treatment (i.e., large-bore drainage, etc.). In these patients, a limited palliative decortication may be considered if the expected patient survival is significant. Martin-Ucar et al (33) found that decortication was required in 66% of a series of 51 consecutive patients with unresectable mesothelioma. Significant improve-

ment in dyspnea was obtained up to 3 months following surgery. The procedure, however, was associated with a 30-day mortality rate of 7.8% and significant morbidity. One-year survival was only 31% but was significantly higher in those with epithelial tumors and without preoperative weight loss. Thoracoscopic pleurectomy may offer significant advantages in terms of surgical morbidity and mortality. A combination of thoracoscopy cytoreductive pleurectomy and lung mobilization was found to effectively obliterate the pleural space in nearly 75% of cases (34). Cardillo et al (23) recently reported the use of thoracoscopy in 29 patients to remove visceral pleural tumor that limited lung expansion after drainage of pleural effusions. Significantly better effusion control was reported than with talc poudrage alone (11).

Drainage Procedures

Following drainage of MPM-related pleural effusions, there may be significant limitation of lung expansion by encasement of the visceral pleural with tumor. This finding is termed "trapped lung" (Fig. 43.1). The resulting pleural space that is not filled by lung parenchyma, if significant, leads to failure of attempts at pleurodesis, reaccumulation of large amounts of pleural fluid, and often recurrent or persistent symptoms of dyspnea. In many of these patients, drainage of the space, despite persistence of the trapped lung, alleviates or markedly improves symptoms. Such palliation can be accomplished through a number of different drainage approaches.

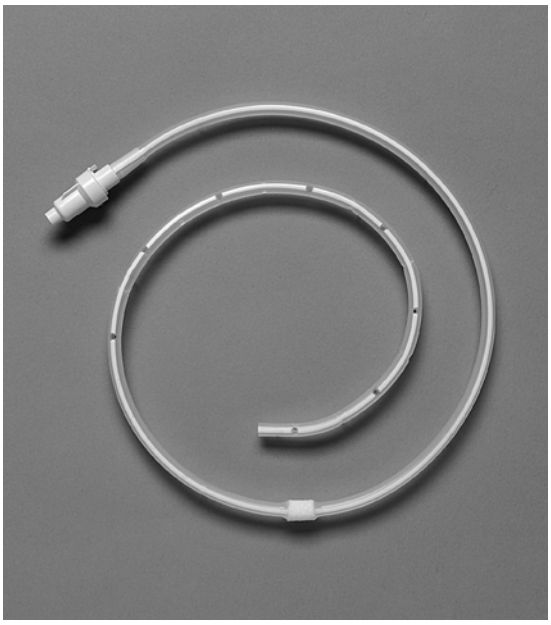
Tube Thoracostomy

Except for short-term drainage, the use of standard chest tubes to drain persistent pleural spaces associated with trapped lung is mentioned only to be condemned. These types of chest tubes are uncomfortable, inconvenient, and a source of infection (empyema). Their use does not provide good palliation in these terminal patients.

Pleurx Catheter Drainage

Patients determined to have significant trapped lung following drainage of pleural effusions can be treated with a Pleurx pleural catheter drainage system (Denver Biomedical, Golden, CO; Fig. 43.2). The Pleurx catheter is a soft Silastic catheter with multiple fenestrations. The catheter is placed into the pleural space with the Seldinger technique under local anesthesia utilizing a dilator and peel-away sheath. The catheter itself has a Teflon cuff that provides a barrier to ascending infections and a one-way valve that is accessed with a sterile plastic catheter for drainage. With a catheter in place, pleural effusions can be drained intermittently at home by trained family members or caregivers. Pleural catheters are a treatment option that allows terminal patients to be treated on an outpatient basis (35). The advantages of pleural drainage catheters (PDCs) are outlined in Table 43.1.

The use of pleural catheters has been described by Ohm et al (36) for trapped lung following drainage of malignant pleural effusions. They compared the outcomes, safety, and efficacy with that of thoracoscopy



A

B

Figure 43.2. A: Pleurx catheter. B: Drainage bulb. (Photographs reprinted with permission of Denver Biomedical, Golden, CO.)

Table 43.1. Pleurx benefits (compared to standard chest tube drainage and pleurodesis)

Allows outpatient/ambulatory management of malignant pleural effusions
Palliates symptoms of dyspnea even in patients with trapped lung
Decreases or eliminates hospitalization for effusion-related problems
Decreases or eliminates the need for repeated thoracenteses for recurrent effusions

and talc pleurodesis (TP). Forty-one consecutive patients with symptomatic effusions were enrolled and those without full lung expansion had pleural catheter placement. Thoracoscopy with TP was performed in seven patients, and 34 patients had PDC placement. In 19 (56%) PDC patients the length of stay was less than 2 days. Although a significant number (68%) of patients died during the follow-up period, the PDC system still appeared to be efficacious, with a shorter length of stay (LOS) and increased quality of life. Pien (37) describes inserting soft pleural catheter for outpatient drainage in 11 patients with pleural effusions, with the catheters remaining in place until death, from 15 to 234 days (mean 115 days). One patient required revision after catheter occlusion, and other complications included catheter infection, localized skin breakdown, and cellulitis. Ten of 11 patients reported symptomatic benefit with the pleural catheters. Putnam et al (38) conducted a prospective randomized trial comparing the effectiveness and safety of the Pleurx catheter with chest tube and doxycycline pleurodesis. There was no treatment-related mortality or major morbidity. Pleurx catheter–treated patients had initial hospital treatment success of 92% with spontaneous pleurodesis occurring in 70%. The Pleurx catheter was found to be safe and as efficacious as chest tube and sclerosis with considerably shorter LOS (1 day versus 6.5 days).

The use of pleural catheter systems also has been shown to be cost-effective (39). Outpatients treated with pleural catheters (none required admission) had mean charges of $3400 as compared to inpatients, whose mean charges ranged from $7000 to $11,000. Outpatient pleural catheter drainage was confirmed to be safe, cost-efficient, and successful without significant morbidity.

Pleuroperitoneal Shunt

Pleuroperitoneal shunts have been implanted in symptomatic patients with trapped lung for chronic drainage of effusions (40). Use of these shunts is limited to patients who can manually compress the pump (internal or external; Fig. 43.3) up to 100 times a day. Tumor implantation into the peritoneal cavity is an anticipated consequence and can adversely affect patients' course. Baeyens and Berrisford (41) reported extensive subcutaneous tunnel infiltration within 9 weeks of placement of Denver shunts (Denver Biomaterials, Surgimed Inc., Golden, CO; Fig. 43.3). Advanced cases with extremely poor overall survival, however, are unlikely to be affected by tumor seeding. Schulze et al (24) reported a small series of cases with thoracoscopic placement of Denver shunts between pleural and peritoneal cavities. Despite placement under direct vision, surgical reintervention was required in two of 14 patients (14%) for shunt dysfunction. A similar rate was reported in a larger series from the Brompton Hospital (42). Other complications included shunt occlusion, requiring revision or replacement; skin erosion; infection; and malignant seeding along the chest wall at the site of shunt insertion. Peritoneal seeding was not detected as a clinical problem. In this series, patients survived somewhat longer, with a median survival of 10.1 months, and effective palliation was achieved in 95%.

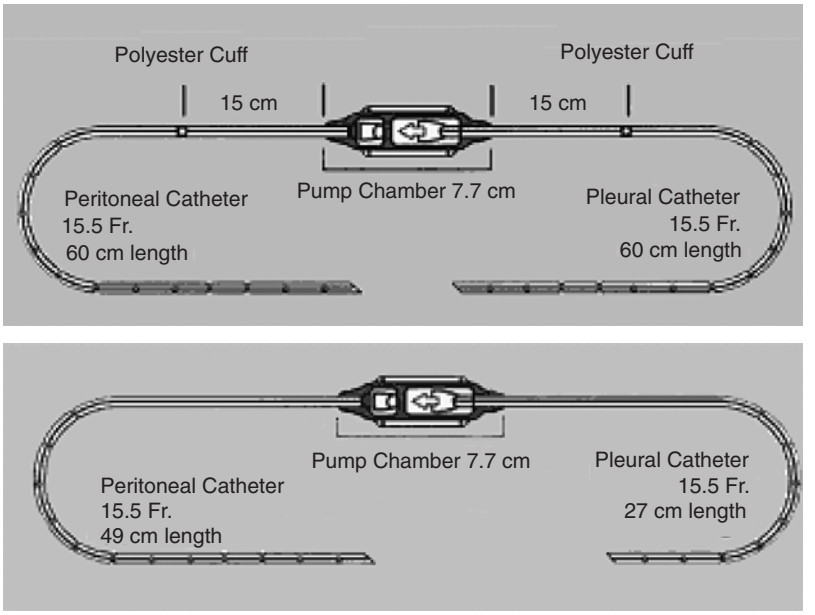


Figure 43.3. A: Denver shunt with internal pump. B: Denver shunt with external pump.

Other Therapies

Intracavitary Chemotherapy and Hyperthermia

For patients with malignant pleural effusions whose disease is not readily responsive to systemic chemotherapy, local measures may be necessary. Intrapleural chemotherapy has the potential advantage of treating the underlying malignancy in addition to providing local control. Concentration is related to the clearance rates, which is determined by the characteristics of the cavity and the drug (molecular weight, lipid-water coefficient, association constant, concentration of the drug, sites of metabolism and mode of excretion). The intracavitary levels of a chemotherapeutic agent administered into the pleural space can be manyfold higher than the systemic levels. Studies have found that the concentration of the intrapleural agents was threefold to fivefold greater than those given systemically (43).

One of the earliest papers addressing this treatment in mesothelioma patients described the use of weekly doxorubicin for 4 weeks followed by monthly administration (44). Mean survival was 21 months. Kermani et al (45) treated 17 patients with intrapleural cisplatin with only two responders.

In 1994 forty-six patients with cytologically proven symptomatic and previously untreated malignant pleural effusions were entered by the Lung Cancer Study Group (LCSG) in a study incorporating intrapleural cisplatin and cytarabine (46). Cisplatin as a single dose and cytarabine were instilled into the pleural space via a chest tube that was then immediately removed. The overall response rate, complete plus

partial, at 3 weeks was 49%. Toxic complications included reversible grade 3 renal toxic reactions in one patient, grade 3 hematologic toxic reactions in three patients, and grade 3 cardiopulmonary toxic reactions in five patients. Median length of response was 9 months for a complete remission and 5.1 months for a partial remission.

Although chemotherapy has the potential advantage of treating the underlying malignancy in addition to controlling the malignant effusion, intracavitary cisplatin and cytarabine therapy as administered, appears inferior to existing sclerosing agents for the control of malignant pleural effusions. Although administration is safe, it cannot be recommended for the standard control of malignant pleural effusions, but it may have a role incorporated into combination modality therapies for diseases such as malignant pleural mesothelioma.

Intracavitary chemotherapy is more effective in treating peritoneal mesothelioma (23% response rate) than lung mesothelioma (12% response rate). Intracavitary cisplatin therapy requires intravenous sodium thiosulfate to prevent cisplatin-induced nephrotoxicity. Mitomycin C, doxorubicin, and epirubicin have also been effective intraperitoneally.

A median survival period of 403 days was recently reported in a study in Japan examining the effectiveness of repeated intrapleural chemotherapy using an implantable access system (INFUSE-A-PORT; Horizon Medical Products; Manchester, GA) (47). 5-fluorouracil (5-FU; 250 mg per body) and cisplatin (10 mg per body) were administered through the implantable access biweekly at the outpatient clinic. Excessive fluid was drained when symptomatic. The treatment was well tolerated with no treatment-related mortality, renal dysfunction, bone marrow suppression, or infection. One patient experienced a hemothorax after eight intrapleural administrations. The port and the catheter were involved with the tumor in one patient.

The addition of hyperthermia to chemotherapy in the treatment of pleural mesothelioma was done in the hope of enhancing results (48). Rusch et al (49) reported that 16 of 20 patients experienced a locoregional relapse in a series of 28 patients who were treated with intrapleural cisplatin and mitomycin following pleural decortication. Although the overall survival in this series of primarily early-stage patients was 17 months, there were serious toxicities. Theoretically, hyperthermia improves the efficacy of chemotherapy by increasing drug absorption, by its effects on DNA synthesis, and by cell membrane permeability (50).

Carry et al (51) reported a series of five patients using hyperthermic chemotherapy. Three patients with mesothelioma were treated with heated mitomycin C, and two received cisplatin following pleurectomy. Two of the three mesothelioma patients were dead at 4 and 11 months, and both of those who were resected developed early ipsilateral pleural recurrence.

The role of intracavitary chemotherapy needs to be further evaluated using newer chemotherapeutic agents with fewer systemic side effects. The role of intracavitary chemotherapy needs to be further defined.

Radiotherapy

Most patients presenting with MPM are suitable only for palliative treatment. Radiotherapy has not been shown to improve survival in patients with this disease, but it is of use in the palliation of symptoms. In a retrospective review of 111 patients with MPM referred to the Peter MacCallum Cancer Institute, the palliative effect of radiotherapy was analyzed. More than half of the patients whose response could be assessed had some symptomatic relief from the radiotherapy treatment. No dose-response relationship could be found (52).

Radiotherapy has also been used to prevent recurrence after a percutaneous procedure on mesothelioma patients. A randomized prospective study assessed the efficacy of local radiotherapy in preventing malignant seeding along with invasive diagnostic procedures (cytology, needle biopsy, thoracoscopy, or chest tube placement). Forty consecutive patients with histologically proven malignant mesothelioma were enrolled. Twenty patients received three daily sessions of radiotherapy at a dosage of 7 Gy 10 to 15 days after thoracoscopy. The other 20 patients did not receive radiotherapy. None of the 20 patients treated developed entry-tract metastasis. In contrast, eight of the 20 (40%) patients who were not treated developed metastases. These findings confirm the efficacy and safety of early local radiotherapy in preventing malignant seeding after invasive diagnostic procedures in patients with MPM (53).

Chest pain is a frequent symptom, and radiotherapy can provide palliation in 50% to 68% of these patients (54). Although there was early symptomatic relief after 30 Gy delivered in 10 fractions, symptoms recurred in nearly all patients within 5 months after radiotherapy (55). Radiation-induced damage to the mediastinum and spine prohibits the use of large doses of radiation, suggesting the use of high fraction size and small field. Pain was better controlled in patients receiving a 4-Gy/fraction scheme versus those receiving fractions of less than 4 Gy (50% vs. 39%) (56). Due to limited palliation provided by radiotherapy alone, the possibility of a combination of radiotherapy with cytotoxic agents or cytokines needs to be evaluated in future clinical trials.

Immunotherapy

Studies have shown that asbestos fibers (amosite, crocidolite, and particularly, chrysotile) suppress natural killer (NK) activity of nylon wool, nonadherent, human blood lymphocytes in a dose-dependent fashion (57,58). This suppression of NK activity can be fully restored by exposure to recombinant IL-2 (59,60). Other work suggests that MPM inhibits the function of tumor infiltrating lymphocytes as measured by decreased interferon-γ (IFN-γ) production through the release of TGF-β (60). Transfection of the IFN-γ gene into established mesothelioma tumors resulted in tumor infiltration with $CD4^+$ and $CD8^+$ T lymphocytes and significant regression in a murine model (61). The use of IFN-γ itself has been shown to directly inhibit MPM cell growth in 16 of 32 mesothelioma cell lines (62). Interferon-α (IFN-α) similarly inhibits growth of mesothelioma cell lines (63) and has been shown to reverse the depression of lymphocyte activities, enhance the number

of tumor-infiltrating lymphocytes and macrophages, and attenuate both IL-6 messenger RNA (mRNA) expression and serum IL-6 levels in mice with MPM (64). These data suggest that modulation of the immune milieu of the pleural space may lead to improved host responses to MPM and possibly to improved control of pleural effusions. This concept has been explored primarily with two types of cytokines, interferons and interleukins.

Interleukins: Although there are a number of reports using IL-2 to treat MPM (65,66), data on the use of IL-2 specifically to palliate pleural effusions associated with MPM is limited. A phase I trial of intrapleural recombinant IL-2 as passive immunotherapy for malignant pleural effusions included 15 patients with MPM (67). A maximum tolerated dose (MTD) was determined to be 24×10^6 IU/m^2/day for the 5-day continuous infusion. There was one complete response and six partial responses for an overall response rate of 46.67% (7/15). Side effects occurred in 50% of the patients treated at the MTD and consisted mostly of fluid retention. A phase II trial was reported involving 31 patients given intrapleural IL-2 (9×10^6 IU twice weekly for 4 weeks) followed by subcutaneous IL-2 (3×10^6 IU) for up to 6 months in responding patients (68). The median number of injections was seven (range 2–8), and side effects included neuropathy, weight gain, fever, heart failure, and gastrointestinal symptoms. Using the Paladine criteria, there was no recurrence of effusion within 1 month in 39% (12/31) and minimal asymptomatic fluid in another 52% (16/31). There was an accompanying response in solid tumor disease in 22% (one complete and six partial responses) and stabilization in another 32% (10/31).

Interferons: Although interferons (-α, -γ, and -β) have been used as potential treatments for MPM, very little data exist expressly related to the palliation and control of MPM-related effusions. Monnet et al (69) conducted a multicentric pilot phase II study to evaluate the tolerance and the activity of intrapleural interferon-γ and autologous human activated macrophages (AMϕs) in patients with stage IA, IB, and IIA malignant pleural mesothelioma; AMϕs were injected intrapleurally and followed 3 days later with intrapleural infusion of interferon-γ (9×10^6 IU). This was repeated once a week for 8 weeks. Fever was the most frequent toxicity, mainly after IFN-γ injection. During follow-up, if there was progression of disease, patients received additional chemotherapy. The overall tumor response rate was 14%, but there was "pleural symphysis" in 50% (7/14) of patients who completed the therapy but only in 36.8% (7/19) of all patients entered into the study. A prospective multiinstitutional study evaluated the efficacy of intrapleural treatment with INF-γ in 89 patients with Butchart stages I and II (70). The overall tumor response rate was 29% with eight complete responses confirmed by histology and nine partial responses. Interferon-α is perhaps the most widely studied cytokine in the treatment of mesothelioma. Information regarding the control of MPM effusions, however, is still scarce. Recombinant interferon-α_{2b} has been used in the management of malignant pleural effusions with success rates

of up to 100%, but these studies did not include patients with mesothelioma (71,72). One study comparing IFN-α (20×10^6 IU) with IFN-β (6×10^6 IU) and with IL-2 (6×10^6 IU) given weekly for 2 to 3 weeks found that 56% (39/70) of pleural effusions were controlled (73). There was no difference in efficacy between IFN-α and IFN-β; however, both were inferior to IL-2 therapy.

Special Considerations

At times an empyema can mimic a malignant mesothelioma (74) and the surgeon is confronted with a mesothelioma. A limited palliative pleurectomy may be carried out (75) at the time of the initial exploration; however, in the bigger picture, this is detrimental and should be strongly discouraged, as it significantly interferes with subsequent more definitive treatment and procedures.

Summary

The mainstay of unresectable malignant mesothelioma remains the control of patients' symptoms. Most patients survive only a few months. Talc is the single most commonly used agent for pleurodesis either as slurry or by poudrage. The use of talc is not without complications. Cytokines hold promise, but additional work and reduced costs are necessary. The search for an ideal agent for pleurodesis continues.

References

1. Bethune N. Pleural poudrage: new technique for deliberate production of pleural adhesions as preliminary to lobectomy. J Thorac Surg 1935;4:251.
2. Van den Heuvel MM, Smith HJM, Barbierato SB, Havenith CEG, Beelan RHJ, Postmus PE. Talc-induced inflammation in the pleural cavity. Eur Respir J 1998;12:1419–1423.
3. Nasreen N, Hartman DL, Mohammed KA, Antony VB. Talc-induced expression of C-C and C-X-C chemokines and intercellular adhesion molecule-1 in mesothelial cells. Am J Respir Crit Care Med 1998;158:971–978.
4. Antony VB, Kamal MA, Godbey S, Loddenkemper RW. Talc induced pleurodesis: role of basic fibroblast growth factor (bFGF). Eur Respir J 1997; 10:403S.
5. Walker-Renard PB, Vaughan LM, Sahn SA. Chemical pleurodesis for malignant pleural effusions. Ann Intern Med 1994;120:56–64.
6. Kennedy L, Vaughn LM, Steed LL, Sahn SA. Sterilization of talc for pleurodesis. Available techniques, efficacy and cost analysis. Chest 1995;107: 1032–1034.
7. Sorensen PG, Svendsen TL. Treatment of malignant pleural effusion with drainage, with and without instillation of talc. Eur J Respir Dis 1984;65: 131–135.
8. Prorok J, Nealon TF. Pleural symphysis by talc poudrage in the treatment of malignant pleural effusion. Bull Soc Int Chir 1968;6:630–635.
9. Ohri SK, Oswal SK, Townsend ER, Fountain SW. Early and late outcome after diagnostic thoracoscopy and talc pleurodesis. Ann Thorac Surg 1992; 53:1038–1041.

10. Fentiman IS, Rubens RD, Hayward JL. A comparison of intracavitary talc and tetracycline for the control of pleural effusions secondary to breast cancer. Eur J Cancer Clin Oncol 1986;22:1079–1081.
11. Hartman DL, Gaither JM, Kesler KA, Mylet DMBJ, Mathur PN. Comparison of insufflated talc under thoracoscopic guidance with standard tetracycline and bleomycin pleurodesis for the control of malignant pleural effusions. J Thorac Cardiovasc Surg 1993;105:743–748.
12. Todd TRJ, Delarue NC, Ilves R, Pearson FG, Cooper JD. Talc poudrage for malignant pleural effusion. Chest 1980;78:542–543(abstr).
13. Adler RH, Sayek I. Treatment of malignant pleural effusion: a method using tube thoracostomy and talc. Ann Thorac Surg 1976;22:8–15.
14. Rinaldo JE, Owens GR, Rogers RM. Adult respiratory distress syndrome following intrapleural instillation of talc. J Thorac Cardiovasc Surg 1983;85: 523–526.
15. Montes JF, Ferrer J, Villarino MA, Baeza B, Crespo M, Garcia-Valero J. Influence of talc dose on extrapleural talc dissemination after talc pleurodesis. Am J Respir Crit Care Med 2003;168(3):348–355.
16. Ferrer J, Villarino MA, Tura JM, et al. Talc preparations used for pleurodesis vary markedly from one preparation to another. Chest 2001;119:1901–1905.
17. Broaddus VC, Light RW. Disorders of the pleura; general principles and diagnostic approach. In: Murray JF, Nadel JA, eds. Textbook of Respiratory Medicine, 3rd ed. Philadelphia: WB Saunders, 2000:995–2012.
18. Milanez JRC, Werebe EC, Vargas FS, et al. Respiratory failure due to insufflated talc. Lancet 1997;349:251–252.
19. Montes JF, Ferrer J, Villarino MA, Baeza B, Crespo M, Garcia-Valero J. Influence of talc dose on extrapleural talc dissemination after talc pleurodesis. Am J Respir Crit Care Med 2003;168(3):348–355.
20. Kuzdzal J, Sladek K, Wasowski D, et al. Talc powder vs doxycycline in the control of malignant pleural effusion: a prospective, randomized trial. A Med Sci Monit 2003;9(6):PI54–59.
21. Walker-Renard PB, Vaughan LM, Sahn SA. Chemical pleurodesis for malignant pleural effusions. Ann Intern Med 1994;120:56–64.
22. Kennedy L, Sahn SA. Talc pleurodesis for the treatment of pneumothorax and pleural effusion. Chest 1994;106:1215–1222.
23. Cardillo G, Carbone FL, Corzani RF, et al. Long-term follow-up of video-assisted talc pleurodesis in malignant recurrent pleural effusions. Eur J Cardiothorac Surg 2002;21:302–306.
24. Schulze M, Boehle AS, Kurdow R, et al. Effective treatment of malignant pleural effusion by minimal invasive thoracic surgery: thoracoscopic talc pleurodesis and pleuroperitoneal shunts in 101 patients. Ann Thorac Surg 2001;71:1809–1182.
25. Merritt N, Blewett CJ, Miller JD, et al. Survival after conservative (palliative) management of pleural malignant mesothelioma. J Surg Oncol 2001; 78:171–174.
26. Senyigit A, Bayram H, Babayigit C, Topcu F, Balci AE, Satici O. Comparison of the effectiveness of some pleural sclerosing agents used for control of effusions in malignant pleural mesothelioma: a review of 117 cases. Respiration 2000;67(6):623–629.
27. Vargas FS, Teixeira LR, Antonangelo L, et al. Experimental pleurodesis in rabbits induced by silver nitrate or talc: 1-year follow-up. Chest 2001;119: 1516–1520.
28. Lee YC, Lane KB, Parker RB, et al. Transforming growth factor-b_2 (TGF-b_2) produces effective pleurodesis in sheep with no systemic complications. Thorax 2000;55:1058–1062.

29. Lee YCG, Teixeira LR, Devin CJ, et al. Transforming growth factor-beta(2) induces pleurodesis significantly faster than talc. Am J Respir Crit Care Med 2001;163:640–644.
30. LoCicero J. Thoracoscopic management of malignant pleural effusion. Ann Thorac Surg 1993;56(3):641–643.
31. Casadio C, Rena O, Giobbe R, Rigoni R, Maggi G, Oliaro A. Stapler blebectomy and pleural abrasion by video-assisted thoracoscopy for spontaneous pneumothorax. J Cardiovasc Surg (Torino) 2002;43(2):259–262.
32. Maier A, Anegg U, Renner H, et al. Four-year experience with pleural abrasion using a rotating brush during video-assisted thoracoscopy. Surg Endosc 2000;14(1):75–78.
33. Martin-Ucar AE, Edwards JG, Rengajaran A, et al. Palliative surgical debulking in malignant mesothelioma. Predictors of survival and symptom control. Eur J Cardiothorac Surg 2001;20:1117–1121.
34. Grossebner MW, Arifi AA, Goddard M, et al. Mesothelioma—VATS biopsy and lung mobilization improves diagnosis and palliation. Eur J Cardiothorac Surg 1999;16:619–623.
35. Brubacher S, Gobel BH. Use of the Pleurx pleural catheter for the management of malignant pleural effusions. Clin J Oncol Nurs 2003;7(1):35–38.
36. Ohm C, Park D, Vogen M, et al. Use of an indwelling pleural catheter compared with thorascopic talc pleurodesis in the management of malignant pleural effusions. Am Surg 2003;69(3):198–202; discussion 202.
37. Pien GW. Use of an implantable pleural catheter for trapped lung syndrome in patients with malignant pleural effusion. Chest 2001;119(6):1641–1646.
38. Putnam JB Jr, Light RW, Rodriguez RM, et al. A randomized comparison of indwelling pleural catheter and doxycycline pleurodesis in the management of malignant pleural effusions. Cancer 1999;86:1992–1999.
39. Putnam JB Jr, Walsh GL, Swisher SG, et al. Outpatient management of malignant pleural effusion by a chronic indwelling pleural catheter. Ann Thorac Surg 2000;69:369–375.
40. Weese JL, Schouteh JT. Pleural peritoneal shunts for the treatment of malignant pleural effusions. J Thorac Cardiovasc Surg 1992;103:881.
41. Baeyens I, Berrisford RG. Pleuroperitoneal shunts and tumor seeding. J Thorac Cardiovasc Surg 2001;121(4):813.
42. Genc O, Petrou M, Ladas G, et al. The long-term morbidity of pleuroperitoneal shunts in the management of recurrent malignant effusions. Eur J Cardiothorac Surg 2000;18:143–146.
43. Taub RN, Antman KH. Chemotherapy for malignant mesothelioma. Semin Thorac Cardiovasc Surg 1997;9(4):361–366.
44. Markman M, Cleary S, Pfeifle C. Cisplatin administered by intracavitary route as the treatment for malignant mesothelioma. Cancer 1986;58:18–21.
45. Kirmani S, Clearly SM, Mowry J. Intracavitary cisplatin for malignant mesothelioma: an update. Proc Am Soc Clin Oncol 1998;5:273.
46. Figlin R, Mendoza E, Piantadosi S, Rusch V. Intrapleural chemotherapy without pleurodesis for malignant pleural effusions: LCSG Trial 861. The Lung Cancer Study Group: Final Analysis. Chest 1994;106(6):363S.
47. Shoji T, Tanaka F, Yanagihara K, Inui K, Wada H. Phase II study of repeated intrapleural chemotherapy using implantable access system for management of malignant pleural effusion. Chest 2002;121(3):821–824.
48. van Ruth S, Baas P, Haas RLM, Rutgers EJTh, Verwaal VJ, Zoetmulder FAN. Cytoreductive surgery combined with intraoperative hyperthermic intrathoracic chemotherapy for stage I malignant pleural mesothelioma. Ann Surg Oncol 2003;10:176–182.

49. Rusch VW, Niedzwiecki D, Tao Y, et al. Intrapleural cisplatin and mitomycin for malignant mesothelioma following pleurectomy: pharmacokinetic studies. J Clin Oncol 1992;10:1001–1006.
50. Christophi C, Winkworth A, Muralihdaran V, Evans P. The treatment of malignancy by hyperthermia. Surg Oncol 1998;7:83–90.
51. Carry PY, Brachet A, Gilly FN, et al. A new device for the treatment of pleural malignancies: intrapleural chemohyperthermia preliminary report. Oncology 1993;50:348–352.
52. Davis SR, Tan L, Ball DLAF. Radiotherapy in the treatment of malignant mesothelioma of the pleura, with special reference to its use in palliation. Australas Radiol 1994;38(3):212–214.
53. Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. Chest 1995;108(3):754–758.
54. Davis SR, Tan L, Ball DL. Radiotherapy in the treatment of malignant mesothelioma of the pleura, with special reference to its use in palliation. Australas Radiol 1994;38:212–214.
55. Bisset D, Macbeth FR, Cram I. The role of palliative radiotherapy in malignant mesothelioma. Clin Oncol 1991;3:315–317.
56. de Graaf-Strukowska L, van der Zee J, van Putten W, et al. Factors influencing the outcome of radiotherapy in malignant mesothelioma of the pleura—a single institution experience with 189 patients. Int J Radiat Oncol Biol Phys 1999;43:511–516.
57. Robinson BWS. Asbestos and cancer: human natural killer cell activity is suppressed by asbestos fibers but can be restored by recombinant interleukin 2. Am Rev Respir Dis 1989;139:897–902.
58. Al Jarad N, Macey M, Uthayakumar S, et al. Lymphocyte subsets in subjects exposed to asbestos: changes in circulating natural killer cells. Br J Ind Med 1992;49:811–814.
59. Farace F, Angevin E, Dietrich PY, et al. Low-dose IL-2 treatment: activation of discrete T- and NK-cell subpopulations in vivo. Int J Cancer 1995;62(5): 523–528.
60. Jarnicki AG, Fitzpatrick DR, Robinson BW, Bielefeldt-Ohmann H, Altered CD3 chain and cytokine gene expression in tumor infiltrating T lymphocytes during the development of mesothelioma. Cancer Lett 1996;103(1):1–9.
61. Cordier Kellerman L, Valeyrie L, Fernandez N, et al. Regression of AK7 malignant mesothelioma established in immunocompetent mice following intratumoral gene transfer of interferon gamma. Cancer Gene Ther 2003; 10(6):481–490.
62. Zeng L, Buard A, Monnet I, et al. *In vitro* effect of recombinant human IFN γ on human MPM cell lines. Int J Cancer 1993;55:515–520.
63. Ohnuma T, Szrajer L, Holland JF, et al. Effects of natural interferon alpha, natural tumor necrosis factor alpha and their combination on human mesothelioma xenografts in nude mice. Cancer Immunol Immunother 1993;36(1):31–36.
64. Bielefeldt-Ohmann H, Marzo AL, Himbeck RP, et al. Interleukin-6 involvement in mesothelioma pathobiology: inhibition by interferon alpha immunotherapy. Cancer Immunol Immunother 1995;40(4):241–250.
65. Boutin C, Schlesser M, Frenay C, et al. Malignant pleural mesothelioma. Eur Respir J 1998;12:972–981.
66. Astoul P, Picat-Joossen D, Viallat JR, et al. Intrapleural administration of interleukin-2 for the treatment of patients with malignant pleural mesothelioma: a phase II study. Cancer 1998;83:2099–2104.

67. Astoul P, Viallat JR, Laurent JC, et al. Intrapleural recombinant IL-2 in passive immunotherapy for malignant pleural effusion. Chest 1993;103(1): 209–213.
68. Castagneto B, Zai S, Mutti L, et al. Palliative and therapeutic activity of IL-2 immunotherapy in unresectable malignant pleural mesothelioma with pleural effusion: results of a phase II study on 31 consecutive patients. Lung Cancer 2001;31:303–310.
69. Monnet I, Breau JL, Moro D, et al. Intrapleural infusion of activated macrophages and γ-interferon in malignant pleural mesothelioma. A phase II study. Chest 2002;121(6):1921–1927.
70. Boutin C, Nussbaum E, Monnet I, et al. Intrapleural treatment with recombinant gamma-interferon in early stage malignant pleural mesothelioma. Cancer 1994;74:2460–2467.
71. Wilkins HE, Connolly MM, Grays P, et al. Recombinant interferon alpha-2b in the management of malignant pleural effusions. Chest 1997;111:1597–1599.
72. Goldman CA, Skinnider LF, Maksymiuk AW. Interferon instillation for malignant pleural effusions. Ann Oncol 1993;4(2):141–145.
73. Lissoni P, Barni S, Tancini G, et al. Intracavitary therapy of neoplastic effusions with cytokines: comparison among interferon alpha, beta and interleukin-2. Support Care Cancer 1995;3(1):78–80.
74. Smith R, Nguyen GK. Pleural mesothelioma presenting initially as empyema. Diagn Cytopathol 2003;29(2):119–121.
75. Martin-Ucar AE, Edwards JG, Rengajaran A, Muller S, Waller DA. Palliative surgical debulking in malignant mesothelioma. Predictors of survival and symptom control. Eur J Cardiothorac Surg 2001;20(6):1117–1121.

44

Preoperative Chemotherapy and Surgery

Eric Vallières

Combined modality therapy has been shown to be of some therapeutic value in the management of selected patients with early-stage pleural mesothelioma (1–5). This approach to mesothelioma has largely been pioneered by the Brigham and Women's Hospital group in Boston, which reported combining upfront extrapleural pneumonectomy with sequential adjuvant chemotherapy and radiotherapy (2,4,6). Encouraged by these results, we initiated in 1997, a treatment program of early-stage mesotheliomas at the University of Washington Medical Center, combining the same three modalities but in a different sequence: induction chemotherapy, followed by extrapleural pneumonectomy, and then adjuvant hemichest radiation therapy (7).

In the postoperative setting, it was our impression that chemotherapy often had to be delayed, the dose reduced or terminated prematurely, and that it was a very difficult undertaking in most patients recovering from extrapleural pneumonectomy. Similarly, adjuvant radiotherapy was rarely delivered as planned when sequenced after postoperative chemotherapy in these patients.

As well, our and others' experiences with preoperative chemotherapy in the treatment of lung and esophageal cancers seemed encouraging at the time. In theory, the advantages of induction chemotherapy may be (1) improved tolerance of chemotherapy in the preoperative setting, (2) a better and easier delivery of chemotherapy when compared to postoperative therapy, (3) early treatment of micrometastases, and (4) cytoreduction with potentially improved resectability. In addition, giving radiotherapy alone in the postoperative setting may allow a better, earlier, and more consistent delivery.

The University of Washington Experience

Over the past 6 years, we have adhered to this sequence of chemotherapy first and then surgery and radiation. However, we have allowed modifications to the chemotherapy regimen used (8,9) and to the radiation therapy as well (10).

Initially in 1997, we favored the induction use of the cisplatin/methotrexate/vinblastine (PMV) triplet based on our own experience with this combination in patients with advanced nonsurgical disease (11). Since then, other combinations have been allowed: cisplatin/gemcitabine, carboplatin/gemcitabine, or, more recently, cisplatin/pemetrexed (Lilly Oncology–Compassionate Drug Use program) (8,9).

Early on, we favored the use of adjuvant fast neutron radiotherapy in these patients, based on our own experience with neutron radiation in sarcomas and other malignancies that shared many similarities with mesotheliomas. However, a large proportion of our patients came to Seattle from out of town, and many preferred to be treated in radiation therapy facilities located closer to home where neutrons were not available. Following the report by Rusch et al (10) in 2001, describing superb locoregional control rates with adjuvant external-beam high-dose hemithoracic photon radiation after extrapleural pneumonectomies, we have treated everyone as per the Memorial Sloan-Kettering Cancer Center protocol.

To date, we have screened over 150 patients with mesothelioma and have offered this trimodality treatment to approximately 40, 20% of whom preferred to be treated elsewhere or not to be treated at all. The majority of patients that were not offered this approach presented with nonsurgical disease or were considered too frail to withstand the combined treatments, the surgery, or both. As of September 2003, 33 patients had completed the initial two phases of their treatment. The great majority of patients had epithelial histology, with only three patients presenting with sarcomatous or mixed histology. Utilizing the Brigham's staging system, initial clinical stages at presentation were distributed as follows: stage I (n = 13), stage II (n = 15), and stage III (n = 5).

Preoperative Chemotherapy

By design, the goal was to give two cycles on induction chemotherapy and then reimage. If there was radiologic evidence of a response or stable disease and the agents were well tolerated by the patients, two more cycles of the same chemotherapy were administered. Of the first 19 patients who received preoperative PMV therapy, 15 received all four cycles as planned (79%). In later years, as other combinations became available, four other patients were initiated on PMV but were switched to different chemotherapy after one or two cycles because of cisplatin toxicity (two) or disease progression (two). Seven patients initiated cisplatin-gemcitabine chemotherapy, three completing all four cycles, one only three, and the other three patients switched to something different because of cisplatin toxicity (two) or progression (one). Our last three patients received the combination of cisplatin- pemetrexed preoperatively for four cycles in two and six cycles in one. Overall, of the 33 patients treated, 29 received three or more cycles of chemotherapy preoperatively (88%).

Clinical Response

Two patients were asymptomatic at the initiation of their treatment. Twenty-one patients (63.6%) showed an improvement of their disease symptoms on chemotherapy, while nine (27.3%) were unchanged.

Radiologic Response

Two patients had normal computed tomography (CT) of the chest at initiation and at completion of chemotherapy; hence, their radiologic response could not be assessed. Thirteen patients (39.4%) had an imaging response to the chemotherapy (less effusion, decreased tumor mass, and/or less nodal involvement) (Figs. 44.1 and 44.2), 12 patients' tumors remained stable (36.4%), and six showed progression of the disease (18.2%).

Morbidity of Induction Chemotherapy

Grade 3 or higher toxicity from the chemotherapy was seen in eight patients (24.2%), mainly hematologic; none were fatal. Grade 1 or 2 complications occurred in 27 patients (81.8%).

Surgery

Four patients, unfortunately, were found to be unresectable at exploration. One had clinical stage III disease at presentation, showed no response to chemotherapy, and was found at thoracotomy to have extensive involvement of multiple ribs. A 78-year-old man initially with clinical stage I disease had questionable resectability along his descending aorta, and the surgery was aborted when dissection in the area resulted in a multilevel partial-thickness injury to his aortic wall. Two other patients had clinical stage I disease at presentation, failed to respond to chemotherapy, and were found to have extensive chest wall involvement at exploration associated with internal mammary nodal disease in one case and extrapleural paravertebral nodal disease in the other. The 29 other patients successfully completed the planned extrapleural pneumonectomy, 19 being right sided. Surgery times range from 235 to 470 minutes, with a mean of 318 minutes. Blood losses during surgery ranged from 250 to 1700 mL, with a mean of 750 mL. Length of stay in the hospital after extrapleural pneumonectomy varied from 5 to 19 days a mean of 8.9 days.

Thirty-Day Mortality of Extrapleural Pneumonectomy After Induction Chemotherapy

Only one death occurred during initial hospitalization or within 30 days of surgery (3% of all patients explored, 3.5% of patients undergoing extrapleural pneumonectomy). The one death was a 60-year-old man who had sustained atrial fibrillation after surgery and had a fatal stroke on the day of his planned discharge, 12 days after surgery.

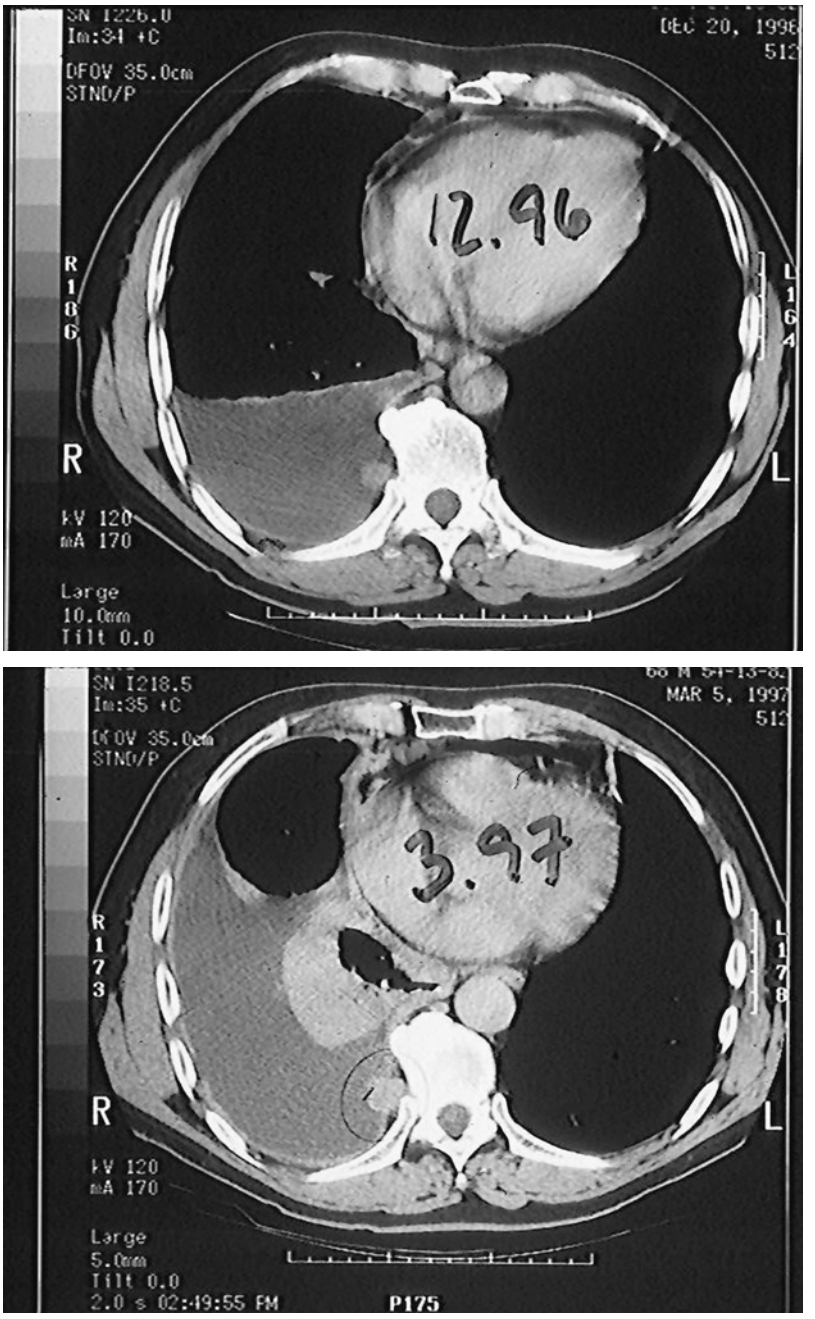
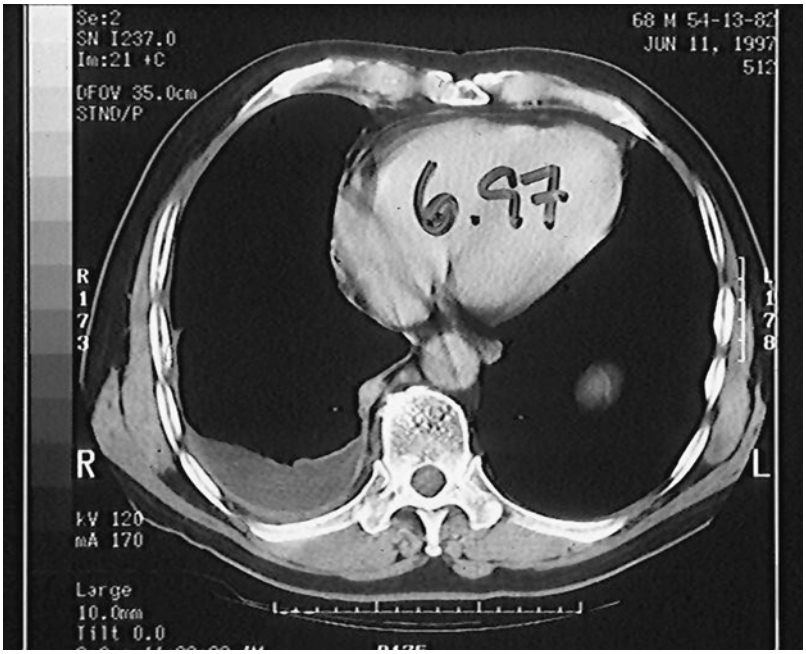
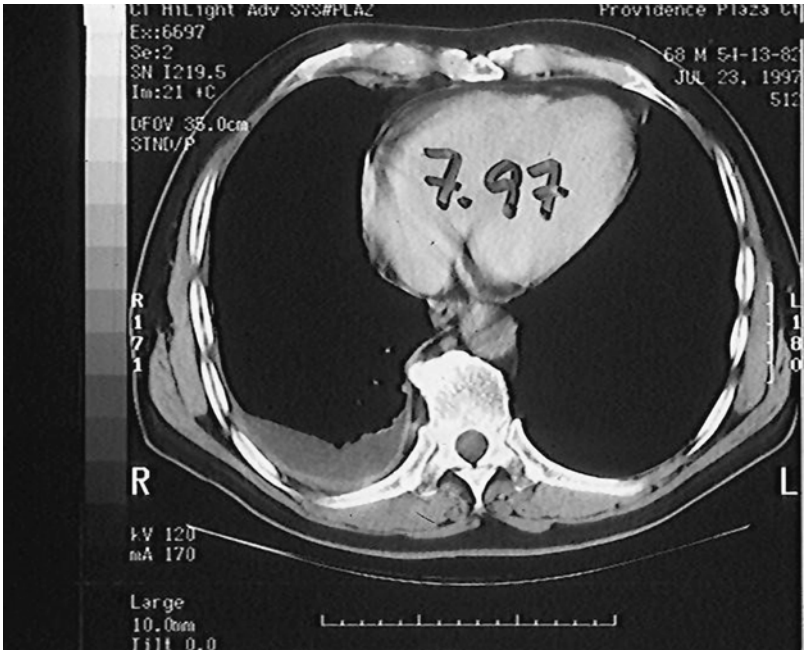


Figure 44.1. A 68-year-old man with early-stage epithelial right-sided mesothelioma, diagnosed in December 1996 (A) that was left untreated until March 1997, when he started cisplatin/methotrexate/vinblastine (PMV) induction chemotherapy (B). Chest imaging after three cycles of chemotherapy (C) and preoperatively after four cycles (D) demonstrates a decrease in the size of his predominant pleural nodule and lessening of his pleural effusion. (No thoracocentesis or pleurodesis performed while on chemotherapy.)



C



D

Figure 44.1. *Continued*

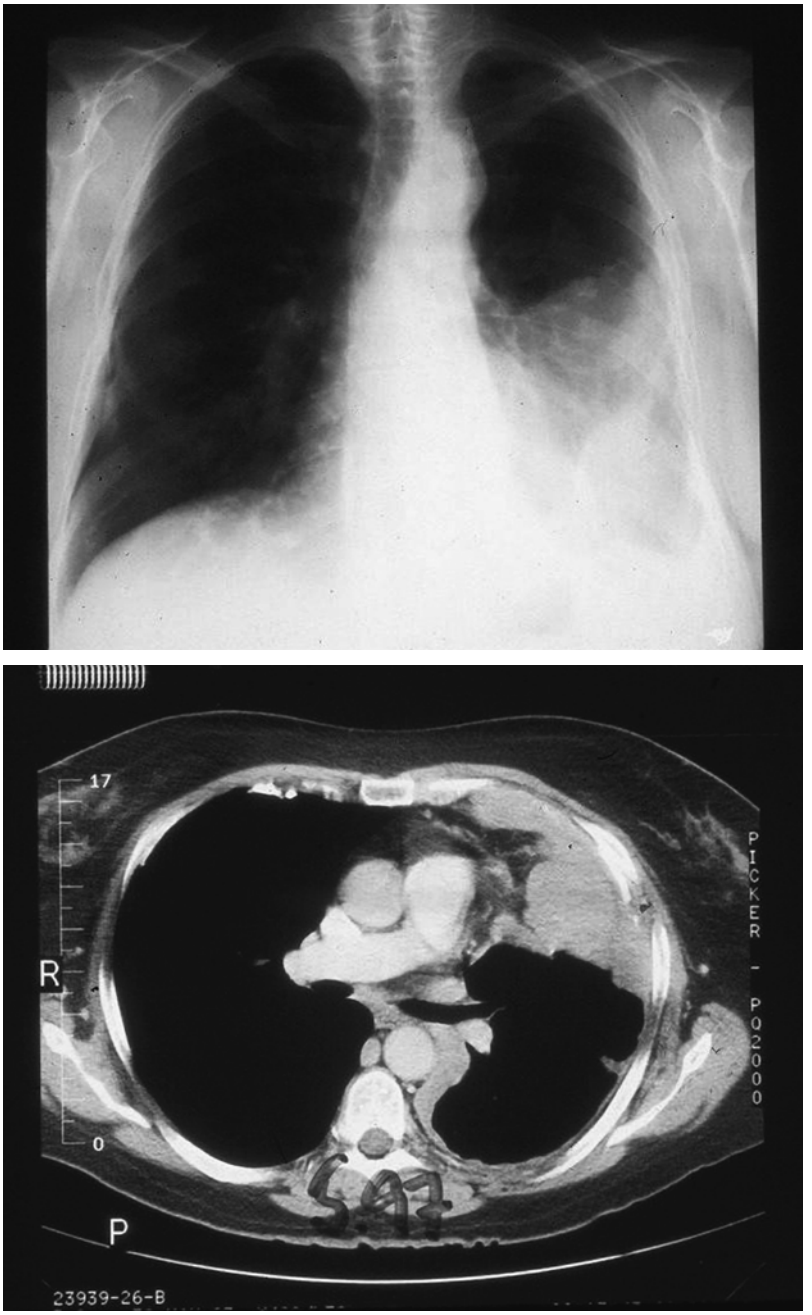
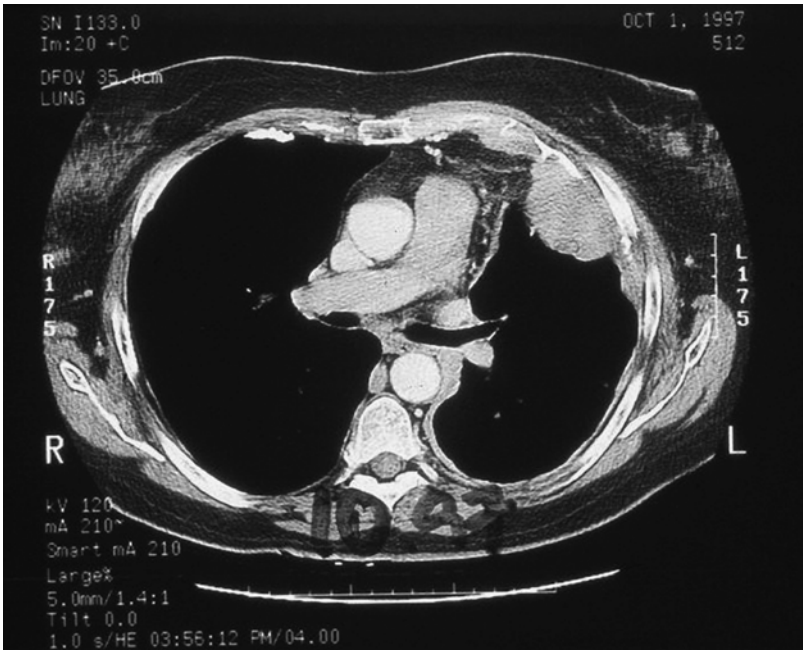
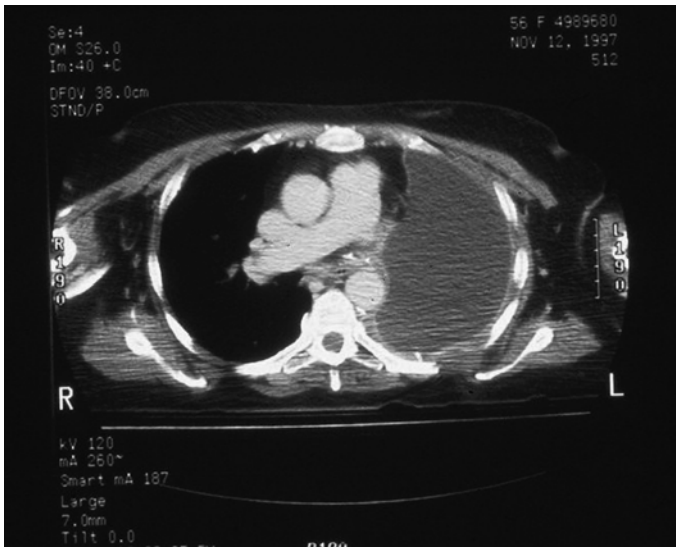


Figure 44.2. A 56-year-old woman with a clinical stage II left-sided epithelial pleural mesothelioma at presentation. A,B: Preoperative CT shows significant decrease in the size of her predominant mass after three cycles of PMV chemotherapy. C: Imaging of her chest 6 months after left extrapleural chemotherapy and adjuvant radiotherapy. Following surgery, her mesothelioma was staged T3N0M0R1. She lived for 48 months from the time of her diagnosis.



C



D

Figure 44.2. *Continued*

Ninety-Day Mortality of Extrapleural Pneumonectomy After Induction Chemotherapy

Two other deaths occurred from 30 to 90 days after surgery (3/33, 9%). Both patients had initially been discharged from the hospital in the first week after uncomplicated surgery. A 61-year-old man died on day 49 of complications of a pneumonia he sustained 3 weeks after surgery, having been previously discharged 6 days after an uneventful resection. A 58-year-old man died of presumed pulmonary emboli and

respiratory failure at home, 4 weeks after surgery. He had had an uncomplicated hospital course, having been discharged on day 7.

Early Complications of Extrapleural Pneumonectomy After Induction Chemotherapy

Following surgery, 10 patients sustained major early complications (34%) including one reexploration for bleeding and two for diaphragmatic dehiscence, one where the omentum had been carried through the right diaphragmatic reconstruction to cover the bronchial stump. Including these three patients, six patients required reintubation in their early postoperative management. Minor complications were seen in 80% of the patients, the most common being atrial fibrillation in 11 of the 29 patients (38%).

Delayed Complications of Extrapleural Pneumonectomy After Induction Chemotherapy

A significant number of patients needed to be readmitted following initial hospital discharge for a variety of reasons including poor pain control, pneumonia, or postpneumonectomy empyemas. Of the first 16 patients, six sustained postpneumonectomy empyemas, two with bronchopleural fistulae. We have since routinely been using mupirocin nasal ointment prophylaxis starting 3 to 5 days before resection in all patients and have seen only one postpneumonectomy empyema in the next 17 patients. This occurred 10 months after initial surgery and was not associated with a bronchial fistula.

Delivery of Radiotherapy

Five patients did not receive adjuvant radiotherapy because of early death (three), early development of brain metastases within 6 weeks of resection (one), or the presence of an empyema with bronchopleural fistula (one). The other 28 patients were able to start their planned adjuvant radiation therapy within 4 to 11 weeks after extrapleural pneumonectomy (median 6 weeks) (28/33, 85%).

Survival

To date, of the 29 patients who underwent resection, 12 patients are still alive 6 to 65 months from the date of their diagnosis, three having had their surgery in the past 6 months (mean survival 21 months, median 26 months). For the 33 patients, our mean survival to date is 22 months from the time of diagnosis.

Other Experiences Published

In 2002 we reported on the feasibility of this induction chemotherapy approach to mesothelioma, combining our series with the Mayo Clinic's in Rochester. At the time, 31 patients were reported (12). In this two-institution series, nearly 90% of the patients completed all three phases of therapy and there was only one early postoperative death.

The same year at ASCO, Kestenholz and colleagues of (13) the SAKK reported on 16 patients with mesothelioma treated in the same trimodality sequence. Induction chemotherapy consisted of three cycles of cisplatin and gemcitabine and was successfully delivered in 15 of the 16 patients (94%). Ten patients (62.5%) were reported as having had a response to chemotherapy or stable disease, and six progressed. Thirteen of the patients underwent extrapleural pneumonectomy (81%). There was no operative death and minimal operative morbidity. Ten patients received postoperative radiotherapy. At the time of this report, three patients were alive without evidence of disease at 18, 19, and 24 months. The 2-year survival was 30% with a median of 21 months (13). In 2003, at ASCO, Cacciari et al (14) reported on 10 patients with stages I and II epithelial mesotheliomas who received three cycles of induction cisplatin and gemcitabine chemotherapy followed by extrapleural pneumonectomy and then adjuvant mitoxantrone/methotrexate/mitomycin chemotherapy. Patients with nodal involvement or positive resection margins were also given adjuvant hemithorax radiotherapy. There was one perioperative death (10%). When reported, four patients were alive 15, 23, 28, and 31 months after the start of their treatment. These two European experiences are very similar to that of the Mayo Clinic and ours.

Conclusion

The sequence of induction chemotherapy, extrapleural pneumonectomy, and adjuvant radiotherapy is certainly feasible in selected patients with mesothelioma. This sequence can be completed as planned in a high proportion of patients. In this limited experience, induction chemotherapy does not appear to be increasing the perioperative mortality and complication risks of extrapleural pneumonectomy. Whether this multimodality sequence is superior to the strategy of surgery first followed by chemotherapy and radiotherapy will require further study and the experience of other centers.

References

1. Calavrezos A, Koschel G, Husselman H, et al. Malignant mesothelioma of the pleura: a prospective therapeutic study of 132 patients from 1981–1985. Klin Wochenschr 1988;66:607–613.
2. Sugarbaker DJ, Heher EC, Lee TH, et al. Extrapleural pneumonectomy, chemotherapy and radiotherapy in the treatment of diffuse malignant mesothelioma. J Thorac Cardiovasc Surg 1991;102:10–15.
3. Rice TW, Adelstein DJ, Kirby TJ, et al. Aggressive multimodality therapy for malignant pleural mesothelioma. Ann Thorac Surg 1994;58:24–29.
4. Sugarbaker DJ, Garcia JP, Richards WG, et al. Extrapleural pneumonectomy in the multimodality therapy of malignant pleural mesothelioma: results in 120 consecutive patients. Ann Surg 1996;224:288–294.
5. Maggi G, Casadio C, Cianci R, et al. Trimodality management of malignant pleural mesothelioma. Eur J Cardiothorac Surg 2001;19(3):346–350.

6. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117:54–65.
7. Vallières E, Hunt K, Stelzer K. Induction chemotherapy, extrapleural pneumonectomy and adjuvant fast neutron radiation therapy for pleural mesothelioma. Proc ASCO 2000;19:578a(abstr 2279).
8. Byrne MJ, Davidson JA, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant mesothelioma: a phase II study. J Clin Oncol 1999;17: 25–30.
9. Vogelzang NJ, Rusthoven J, Paoletti P, et al. Phase III single-blinded study of pemetrexed + cisplatin vs. cisplatin alone in chemo naive patients with malignant pleural mesothelioma. Proc ASCO 2002;21:2a(abstr 5).
10. Rusch VW, Rosenweiz K, Venkatraman E, et al. A phase II trial of surgical resection and adjuvant high-dose hemithoracic radiation for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;122:788–795.
11. Hunt KJ, Longton G, Williams MA, Treatment of malignant mesothelioma with methotrexate and vinblastine, with or without platinum chemotherapy. Chest 1996;109:1239–1242.
12. Miller DL, Vallières E, West HL, Okuno SH, Marks RS. Operative morbidity and mortality after induction therapy and extrapleural pneumonectomy for malignant pleural mesothelioma. European Association for Cardio-Thoracic Surgery, Monaco, September 24, 2002, abstract 149.
13. Kestenholz PB, Taverna C, Schneiter D, et al. Neoadjuvant chemotherapy followed by extrapleural pneumonectomy and adjuvant radiotherapy for malignant mesothelioma. Proc ASCO 2002;21:335a(abstr 1338).
14. Cacciari N, Pinto C, Sacco R, et al. Multimodality treatment of potentially resectable malignant pleural mesothelioma (mpm): a multicentric Italian study (SITMP1). Proc ASCO 2003;22:690(abstr 2773).

45

Photodynamic Therapy for Pleural Mesothelioma

Evelio Rodriguez, Paul Baas, and Joseph Friedberg

Malignant pleural mesothelioma (MPM) is still regarded as a terminal cancer with no treatment modality accepted as the standard of care. The disease commonly presents as a pleural effusion, which defies diagnosis. Although there are now batteries of immunohistochemical stains that are most consistent with the diagnosis of mesothelioma, it can still be difficult, even with pleural biopsies, to establish the diagnosis. The tumor tends to spread diffusely, generally within one hemithorax, eventually invading all local structures. Even though autopsy studies of patients with MPM revealed that more than 50% of patients had metastasis (1), patients usually succumb to the disease before the effect of the metastases become clinically evident. Surgery alone has little impact on the vast majority of mesothelioma cases and is associated with high local recurrence rates and low survival rates (2). As a result, combinations of surgery with adjuvant treatment aimed at residual microscopic disease remain the most aggressive and, arguably, the most effective treatment strategy. A number of innovative adjuvant treatments have been employed, including photodynamic therapy (PDT), a light-based cancer treatment. This chapter focuses on the role of PDT in the treatment of mesothelioma.

Photodynamic Therapy for Mesothelioma

Surgical Debulking

The concept behind the combination of surgery and PDT for mesothelioma is that the surgery is used for debulking of all gross disease and the PDT is used to treat the residual microscopic disease. It is naive to assume that there will not be at least microscopic disease remaining after even the most radical resection for a pleural mesothelioma that has presented in the usual diffuse manner. As a result, one can only achieve macroscopic debulking of the disease. This can be accomplished by a lung-sparing pleurectomy or an extrapleural pneumonectomy (EPP). Although it may be a challenging operation, EPP is the easiest way to achieve a complete debulking; however, it is likely to

have significant detrimental impact on the patient's hemodynamic and pulmonary reserve. Thus, our preference is to perform lung-sparing procedures whenever possible, the technique of which is described below.

Overview

Photodynamic therapy is a technique for killing tumors that utilizes a photosensitizer that is activated by visible light. Photosensitizers may be preferentially taken up by, or retained in, tumor cells (3,4). Once inside the cells, the photosensitizer is activated with a laser light of a wavelength specific to the sensitizer's absorption spectrum. Activation of the photosensitizer in the presence of molecular oxygen results in the production of excited species of oxygen capable of inducing cell death. Cell death occurs by apoptosis or from direct destruction of certain cellular elements (5,6). In addition, PDT may result in neovascular damage that may compromise the tumor's blood supply (7). Finally, PDT of tumors enhances the host antitumor immune system's response (8,9).

Photosensitizers

So far, only two commercially available photosensitizers have been used in patients with mesothelioma. Photofrin (di-hematoporphyrin derivate) was the first commercially available photosensitizer and has its major excitation wavelengths in the ultraviolet (UV) range (200–450 nm), and in the green range (510 nm), and there is a small absorption peak in the red range (630 nm). For clinical use, the peak at 630 nm is considered the most important, since the depth of penetration of visible light through tissue is proportional to the wavelength. At this wavelength, which results in a tissue penetration of about three fourths of a centimeter, the drug has a low singlet oxygen yield that may require illumination times up to several hours to achieve significant effect. Several groups have used this drug (10,11).

Meta-tetrahydroxyphenylchloride (Foscan) is the second drug that has been used for treating mesothelioma. It has major absorption peaks in the UV range (200–450 nm), and the green range (520 nm), and the highest peak is in the red range at 652 nm (3). The penetration depth of light at this wavelength is strongly dependent on tissue properties but can reach depths of 1 to 1.5 cm. The singlet oxygen yield is approximately 30 times higher in the red light band than with Photofrin. It is assumed that Foscan is a pure compound that is active in the administered form. The drug is minimally metabolized and excreted mainly by the bile.

Both drugs are administered intravenously but have different pharmacokinetic properties. Photofrin is usually given in a dose of 2.0 mg/kg 2 days before the illumination to achieve good tissue concentration. The drug concentrations generally remain high for several days (12). For Foscan the advised dose is 0.1 to 0.15 mg/kg with a drug–light interval of 2 to 4 days (13). Both drugs are thought to have some affinity for tumor cells but perhaps more importantly, they are

thought to be retained by the abnormal endothelial cells of the tumor's vessels. This effect of vascular closure by PDT is considered to have a significant secondary effect on tumor cell eradication.

The ubiquitous side effect of photosensitizer administration is cutaneous photosensitivity. For standard dosages of Foscan, this is generally less than 4 weeks, and for Photofrin® is at least 4 to 5 weeks. Other side effects are very uncommon (12,13).

Laser Equipment

For the treatment of large surface areas with PDT, high-power light sources are required. In general, it is necessary to use a laser to supply light at the appropriate wavelength and intensity. Tunable dye lasers, pumped by a larger, fixed wavelength, and green light lasers are commonly used to produce red light in the 7 W range. These lasers have the advantage that the dye modules can be interchanged to allow for a broad spectrum of wavelengths that can be produced. These lasers have the disadvantage of being relatively large and requiring high power supplies and water-cooling systems. Recently developed, are diode lasers that are more transportable and have power outputs of up to 6 W (in the red light wave band). They do not require the use of high power supplies or water-cooling systems, but have the disadvantage of producing only a single wavelength of light.

Dosimetry

Although there is an element of selectivity of photosensitizers for tumor tissue, it must be assumed that there has been partitioning of the sensitizers into all tissues. As a result, all structures that are illuminated can be injured. It is crucial, therefore, not to overdose normal tissues with light. Some investigators rely on "calculated" light doses (11,14). Based on experiments that have demonstrated that the measured and calculated light doses may vary widely in an anatomic environment (15), we believe that light dosing must be empiric and must rely on measured dosimetry. Light sensors are placed at strategic positions within the hemithorax and fed into a real-time dosimetry system that has a separate channel for each sensor. During PDT, the light source is moved around the chest cavity until each sensor has measured the desired dose of light.

There are currently two types of sensors in use: flat and isotropic. The flat sensors, originally used by Pass et al (10), clearly underestimate the total fluence delivered to tissue surfaces when compared to spherical isotropic detectors (16). Again, the safe dose of light must be determined empirically and any exchange of sensors requires a predetermined conversion factor.

Some investigators fill the hemithorax with diffuse intralipid solution to help scatter light as a light source is moved around the chest cavity (2,10). This is our preferred method for light delivery, regardless of the debulking technique, as it assures that there is no shielding of tissue by pooled blood and also allows direct manipulation of the costophrenic recesses, the most difficult areas to assure good illumina-

tion. Others have focused on integral illumination by using a bulb fiber, and they do not use a light diffusing medium (15). In this technique, a transparent sterile bag is placed in the chest cavity after EPP and filled with warm saline to facilitate flattening and expansion of the chest cavity structures. After partial closure of the surgical wound, a single spherical bulb fiber is placed in the center of the bag to allow an integral illumination of the entire cavity and enhance the reflection of light. This technique is not compatible with a lung-sparing procedure and may not be applicable if it does not appear that the bag will expand all crevices in the hemithorax.

Surgical Approach

Prior to undergoing surgical debulking and intraoperative PDT, it is our practice to perform an extensive metastatic workup, including brain magnetic resonance imaging (MRI), computed tomography (CT) of the chest/abdomen/pelvis, and a bone scan or positron emission tomography (PET) scan. Any suspicion of pericardial, cardiac, or great-vessel invasion is further evaluated with MRI studies or esophageal echocardiography. Invasive staging with a bronchoscopy, esophagoscopy, and laparoscopy with peritoneal washings and biopsies is also performed. We have used both bronchoscopy and laparoscopy to diagnose occult metastases that have evaded the radiographic workup. Mediastinoscopy can be used to stage the paratracheal lymph nodes and should be used if the results will affect enrollment in the protocol being employed. Every patient undergoes cardiac and pulmonary function evaluations prior to surgery. If no metastatic disease is detected, then the patient receives the photosensitizer at the appropriate time interval and is brought to the operating room for surgical debulking and photodynamic therapy. (The specific findings of our phase I trial are discussed below.)

Standard double-lumen endotracheal intubation is performed and the patient is placed in the lateral decubitus position. Pulse oximeters use a red light probe capable of activating photosensitizers, and consequently they can cause burns to the nail bed. To avoid this, the oximeter probes are rotated between the fingers every 15 to 30 minutes.

Prior to turning on the overhead operating lights and surgeons' headlights, a posterolateral thoracotomy incision is created using only the fluorescent room lights for illumination. The incision is dictated by the need to excise previous biopsy incisions and the desired entrance to the chest cavity. It is our routine to excise the seventh rib as it is still possible to perform the desired pulmonary procedure, and this lower incision provides better access to the costophrenic recesses, commonly the most difficult area of the hemithorax to access for debulking and light delivery. The skin is then shielded by sewing blue towels to the wound edges. Once all exposed skin has been covered with drapes and towels, the headlights and overhead lights may be turned on without fear of cutaneous burns. The overhead operating room lights and surgeon's headlamps are covered with yellow filter paper to reduce photosensitizer activation (Fig. 45.1).

Figure 45.1. Operating room light protected by yellow filter paper in order to reduce photosensitizer activation.

Although the PDT can be expected to affect tissue for a depth of at least several millimeters, it should be the goal of the surgeon to have no visible or palpable tumor remaining at the conclusion of the debulking. It is our bias to attempt at least partial-thickness preservation of both the pericardium and diaphragm to avoid tumor spillage into other body cavities, regardless of whether a pneumonectomy or decortication is performed. Details of this technique are described elsewhere (2). It is our belief that an advantage of PDT as an adjuvant treatment is that it offers the option of performing a pulmonary sparing procedure. This may be particularly advantageous for patients who are candidates for surgery, but are marginal candidates for pneumonectomy.

The technique of light delivery we have used is similar to the procedure described by Baas et al (17) and Pass et al (18). Briefly, the distribution and total dose of light delivered is monitored with isotropic light detectors (Rare Earth Medical, West Yarmouth, MA) with an accuracy of ±15%. Four probes are placed in the thoracic cavity at the following locations: apex, bronchial stump/esophagus, dorsal sinus of the diaphragm, and the pericardium. The light dosimetry system and light sensors are shown in Figure 45.2. This system is similar to that used by Baas et al (17). Light is delivered into the chest cavity via a flat cut fiber placed within a modified endotracheal tube sealed at both ends and filled with 10% intralipid solution. Laser light is generated using the KTP/532 Laser System pumping a model 630 XP Dye Module, (Laserscope, Inc., San Jose, CA). The retractors are removed from the chest cavity to avoid shielding and the hemithorax is filled with 0.1% intralipid solution. It is important for the chest cavity to be hemostatic as heme pigment absorbs light and causes a significant and noticeable decrease in the fluence. We aspirate and replace the warm intralipid

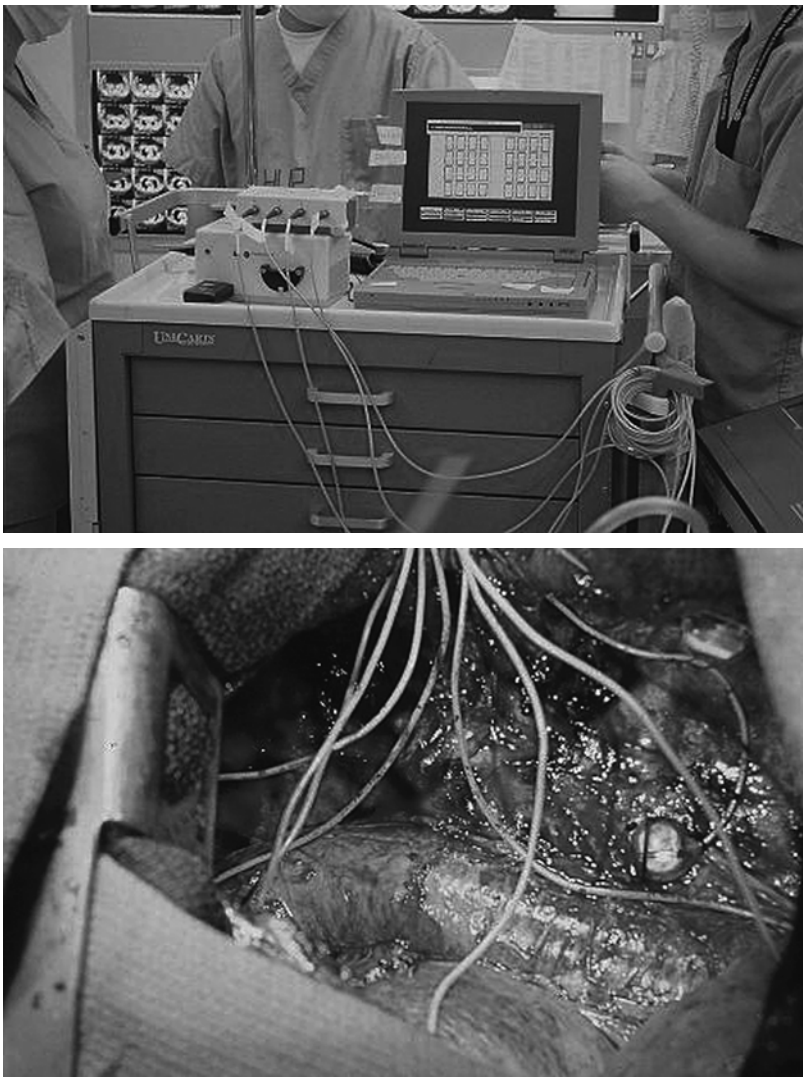


Figure 45.2. A: Light dosimetry system. B: Isotropic light sensors placed in left chest after pneumonectomy and pleurectomy. These sensors are connected to the dosimetry system. (Courtesy of Clinical Physics Department, Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.)

solution constantly throughout the procedure to facilitate light delivery (Fig. 45.3).

Clinical Studies

The first studies combining surgery and PDT were published in 1994 (10,11). Both investigators used Photofrin as the photosensitizer. The study by Takita et al (11) of the Roswell Park Cancer Institute, was later updated in 1998, and included a total of 40 patients with a 6-year follow-up (14). Patients were treated with pleurectomy alone (n = 28),

EPP ($n = 7$), or combined pleurectomy and lobectomy ($n = 5$) in order to achieve optimal cytoreduction. The morbidity and treatment-related mortality for this series were 45% and 7.5%, respectively. Complications included atrial fibrillation ($n = 15$), sepsis ($n = 11$), prolonged ventilation ($n = 10$), and bronchopleural fistulas ($n = 3$). In addition, five patients underwent reoperation for spontaneous splenic rupture, diaphragmatic dehiscence, esophageal perforation, empyema, and diaphragmatic hemorrhage. For the survival analysis, the three patients who died of treatment-related causes were excluded, leaving 37 patients. The median survival time and estimated 2-year survival percentage were 15 months and 23%, respectively. However, when patients were separated by their stage, the median survival time and 2-year survival percentage for stages I and II patients, were 36 months and 61% compared to 10 months and 0% for patients with stage III. The authors of this series concluded that surgical intervention and PDT offer good survival results in patients with stage I or II pleural mesothelioma.

Pass et al (10) of the National Cancer Institute, Bethesda, MD, performed a phase I study in 54 patients. A total of 12 patients could not be debulked to the prerequisite 5-mm residual tumor thickness and thus were excluded, leaving 42 patients for the study. The choice of surgical resection depended on the extent of the disease, but was kept as limited as possible: five lobectomy-pleurectomies, 19 modified pleuropneumonectomies, and 18 pleurectomies. The illumination procedure was performed with real-time dosimetry using seven flat photodiodes. The maximal tolerated dose (MTD) was declared as 30 J/cm^2 with a 24-hour dosing interval. Based on these results, Pass et al (18) conducted a phase III study comparing maximal debulking

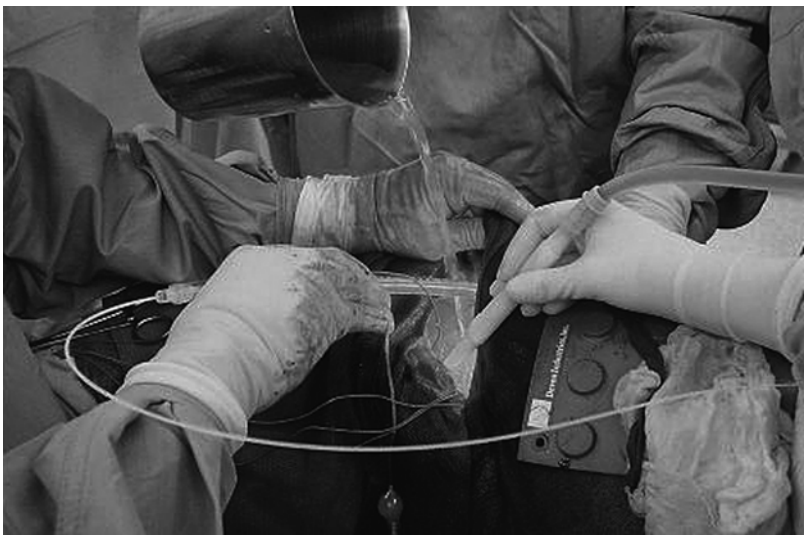


Figure 45.3. Light is delivered into the chest cavity while warm intralipid solution is aspirated and replaced constantly throughout the procedure.

surgery and postoperative cisplatin, interferon alpha-2a, and tamoxifen immunochemotherapy with or without intraoperative PDT. The type of resection for patients assigned to PDT ($n = 25$) included 11 pleurectomies and 14 pneumonectomies compared to the no-PDT group ($n = 23$), which underwent 12 pleurectomies and 11 pneumonectomies. There was one operative death secondary to hemorrhage, and each group had two bronchopleural fistulas. There were no differences in the median survival (PDT 14.4 months vs. no-PDT 14.1 months) or median progression-free time (PDT 8.5 months vs. no-PDT 7.7 months). The majority of the patients were stages III and IV ($n = 40$) and this may be the reason why a better survival was not observed in the PDT group, since PDT is mainly a treatment for local control.

Ris et al (19) of Switzerland, reported the first experience with the second-generation photosensitizer mTHPC in thoracic malignancies in 1991. In a later stage, they reported their experience in eight patients with thoracic malignancies in 1996 (20). The PDT was performed without real-time light dosimetry, so only an estimate of the delivered light doses could be made. Of the eight patients treated, three suffered severe postoperative complications: colonic perforation (one), bronchopleural fistula (one), and aspiration pneumonia (one). Several patients succumbed from distant manifestations of MPM. Whether the PDT resulted in local control was not reported.

A phase I/II study investigated optimal dose and toxicities of mTHPC for intraoperative PDT in resected MPM (21). In this study, doses of Foscan were escalated while the illumination times and surgical procedures were kept the same. Twenty-four patients had pleuropneumonectomies and the drug–light interval was 4 days for the majority of patients. The illumination was performed until a total fluence of $10\,J/cm^2$ was achieved at all sites. In this study, a total of 28 patients with performance scores of 0–1 [Eastern Cooperative Oncology Group (ECOG)] were entered. In two of these patients a pleuropneumonectomy could not be performed due to extrathoracic growth of the tumor. At the third dose level (0.15 mg/kg Foscan) dose-limiting toxicity was observed. Three patients died, one due to myocardial infarction, one due to bronchopleural fistula, and one due to incorrect placement of the isotropic detectors in the thoracic cavity. This resulted in an overdose of light at the mediastinal structures, leading to an esophagopleural fistula. The median survival for all 28 patients was 10 months. The conclusion of the authors was that Foscan-mediated PDT could not be recommended without further improvements in the PDT technique and better patient selection.

A more recent phase I study by Friedberg et al (2), investigated the toxicities and MTD of Foscan-mediated PDT and surgery in 36 patients with MPM. Four different PDT cohorts were studied in a total of 26 patients who completed treatment. Seven patients were debulked with an EPP and 19 were debulked with a lung-sparing pleurectomy-decortication. The reasons for the 10 patients not completing the study are shown in Table 45.1.

The most common grades III to V toxicities observed during this phase I trial included atrial dysrhythmia ($n = 13$), transient ventricular

Table 45.1. Exclusion criteria in Friedberg et al's phase I study of Foscan-mediated photodynamic therapy

Finding	Number of patients
Preoperative	
EKG changes	**1**
Subdiaphragmatic disease	**2**
Liver metastasis	**1**
Contralateral endobronchial mesothelioma	**1**
Perioperative	
Perioperative myocardia infarct	**1**
Unresectable, aortic involvement	**2**
Unresectable, subclavian artery involvement	**1**
Unresectable, stomach involvement (hiatal hernia)	**1**
Malignacy not confirmed; pt. ineligible	**1**

Source: From Friedberg et al (2).

dysrhythmia ($n = 2$), incisional third-degree burn ($n = 1$), esophageal perforation ($n = 1$), adult respiratory distress syndrome (ARDS) ($n = 1$), and pulmonary embolism ($n = 1$). Two patients' deaths occurred in the postoperative period among the 20 patients enrolled at the MTD. One patient had a pulmonary embolism and was appropriately anticoagulated and discharged. At home, the patient developed massive upper gastrointestinal bleeding and expired. The second patient died of complications after an iatrogenic esophageal perforation during endoscopy for upper gastrointestinal bleeding. The established MTD was 0.1 mg/kg of Foscan injected 6 days before surgery in combination with 10 J/cm^2 of 652-nm wavelength light. The dose-limiting toxicity was a systemic capillary leak syndrome that resulted in two PDT-related mortalities. Fourteen patients were treated at the MTD without significant complications. The median progression-free survival and overall survival was 12.4 months for all 20 patients enrolled at the MTD. Only three patients treated at the MTD developed isolated local recurrences. These results were especially encouraging, given that a significant proportion of patients had unfavorable histology and significant lymph node involvement, exclusion criteria for most surgical protocols. The authors concluded that a particular advantage of Foscan-mediated PDT was that it offered the option of performing a lung-sparing procedure as was performed in the last 17 of 19 patients enrolled. A phase II study was planned that would include chemotherapy to complement the excellent local control that was observed. This study has not been performed due to change in the management of the company producing Foscan.

Conclusion

The role, if any, for PDT in the treatment of mesothelioma has yet to be established. The number of centers exploring this technology is limited as it is very labor intensive and requires not only specialized equipment but also physicist support. The number of patients treated in the different trials is small and thus no definitive conclusions can be

drawn. Further complicating interpretation of published results are the number of variables (type of sensitizer, light dose, drug dose, drug–light interval, methods of light measurement, technique of light delivery, surgical debulking techniques) that differ between studies. In addition, the majority of reports are phase I and II studies. The final outcome of these studies, with respect to survival, is of limited value. The only phase III study, performed with an earlier generation photosensitizer, reported no advantage for the use of PDT in combination with surgery and immunochemotherapy. To date, the most that can be said is that intraoperative PDT can be performed safely in experienced centers and that there are some encouraging results, especially in patients with stage I and II MPM, particularly with the newer generation of photosensitizers.

Summary

The number of cases of malignant pleural mesothelioma diagnosed each year is increasing and it is expected to increase at least until the year 2010. The diagnosis of this disease is often difficult. There is no current standard of care for pleural mesothelioma and only a few trials using the combination of surgery and adjuvant therapies appear to have demonstrated any significant impact on the expected course of the disease. Newer diagnostic and treatment techniques are needed to improve survival in these patients with MPM. This chapter discussed the role of one of the adjuvant treatments, photodynamic therapy, in the management of mesothelioma.

References

1. van Ruth S, Baas P, Zoetmulder FA. Surgical treatment of malignant pleural mesothelioma. A review. Chest 2003;123:551–561.
2. Friedberg JS, Mick R, Stevenson J, et al. A phase I study of Foscan-mediated photodynamic therapy and surgery in patients with mesothelioma. Ann Thorac Surg 2003;75:952–959.
3. Berenbaum MC, Akande SL, Bonnett R, et al. Mesotetra(hydroxyphenyl) porphyrins, a new class of potent tumour photosensitizer with favourable selectivity. Br J Cancer 1986;54:717–725.
4. Young SW, Woodburn KW, Wright M, et al. Lutetium texaphyrin (PCI-0123): a near-infrared, water-soluble photosensitizer. Photochem Photobio 1996;63:892–897.
5. Godar DE. Light and death: photons and apoptosis. J Invest Dermatol Symp Proc 1999;4(1):17–23.
6. Oleinick NL, Evans HH. The photobiology of photodynamic therapy: cellular targets and mechanisms. Radiat Res 1998;150:146S–156S.
7. Fingar VH. Vascular effects of photodynamic therapy. J Clin Laser Med Surg 1996;14:323–328.
8. Korbelik M. Induction of tumor immunity by photodynamic therapy. J Clin Laser Med Surg 1996;14:329–334.
9. Gollnick SO, Vaughan L, Henderson BW. Generation of effective antitumor vaccines using photodynamic therapy. Cancer Res 2002;62(6):1604–1608.

10. Pass HI, Delaney T, Tochner Z, et al. Intrapleural photodynamic therapy: results of a phase I trial. Ann Surg Oncol 1994;1:28–37.
11. Takita H, Mang TS, Loewen GM, et al. Operation and intracavitary photodynamic therapy for malignant mesothelioma: a phase II study. Ann Thorac Surg 1994;58:995–998.
12. Axcan Scandipharm Inc. Prescribing information, 2003. http://www.photofrin.com/prescribinginfo.aspx?lang=en-us&n=4&m=110&id=photofrin.
13. Biolitec Pharma Ltd. Summary of products characteristics, 2002. http://www.biolitecpharma.com/public/smpc.asp?s=foscan.
14. Moskal TL, Dougherty TJ, Urschel JD, et al. Operation and photodynamic therapy for pleural mesothelioma: 6-year follow-up. Ann Thorac Surg 1998;66:1128–1133.
15. Murrer H, Mariginissen HP, Star WM. Ex vivo light dosimetry and Monte Carlo simulations for endobronchial photodynamic therapy. Phys Med Biol 1995;40(11):1807–1817.
16. Vulcan TG, Zhu TC, Rodriguez CE, et al. Comparison between isotropic and nonisotropic dosimetry systems during intraperitoneal photodynamic therapy. Lasers Surg Med 2000;26:292–301.
17. Baas P, Murrer H, Zoetmulder FA, et al. Photodynamic therapy as adjuvant in surgically treated pleural malignancies. Br J Cancer 1997;76:819–826.
18. Pass HI, Temecj BK, Kranda K, et al. Phase III randomized trial of surgery with or without intraoperative photodynamic therapy, and postoperative immunochemotherapy for malignant pleural mesothelioma. Ann Surg Oncol 1997;628–633.
19. Ris HB, Altermatt HJ, Inderbitzi, et al. Photodynamic therapy with chlorins for diffuse malignant mesothelioma: initial clinical results. Br J Cancer 1991;64:1116–1120.
20. Ris HB, Altermatt HJ, Nachbur B, et al. Intraoperative photodynamic therapy with mTHPC for chest malignancies. Lasers Surg Med 1996;18:39–45.
21. Schouwink H, Rutgers ET, van der Sijp J, et al. Intraoperative photodynamic therapy after pleuropneumonectomy in patients with malignant pleural mesothelioma. Dose finding and toxicity results. Chest 2001;120:1167–1174.

46

Surgery and Postoperative Radiotherapy

Raja M. Flores, Kenneth E. Rosenzweig, and Valerie W. Rusch

There is no universally accepted standard therapy for malignant mesothelioma of the pleura. Studies evaluating the efficacy of single-modality treatment with chemotherapy, radiotherapy, and surgery alone have not consistently demonstrated a significant prolongation of survival. Patients usually die from pulmonary complications because advanced local disease restricts diaphragmatic and intercostal muscle function, leading to pneumonia and subsequent sepsis. As a result, research efforts focus on local control.

Surgery is the single most effective manner of achieving immediate local control. Debate has existed over the role of extrapleural pneumonectomy versus pleurectomy/decortication. However, complete surgical resection of all gross tumor is possible only with extrapleural pneumonectomy for disease higher than stage I (stage II includes lung invasion). Since the majority of surgical candidates are in the stage II and III categories, extrapleural pneumonectomy is required in the majority of cases to achieve complete resection of all gross tumor. Surgery alone, however, does not yield satisfactory results. Therefore, combined modality therapy has included surgery and radiation in an effort to achieve optimal local control.

Palliative Radiotherapy

Chest pain is a frequent symptom in patients who are not surgical candidates because of advanced local disease. Radiotherapy can provide palliation in approximately half of these patients, but the effects are frequently short-lived. Bisset et al (1) performed a prospective study of wide-field radiotherapy in 22 patients with chest pain due to mesothelioma who received 30 Gy in 10 daily fractions to the involved hemithorax. Patients' symptoms were assessed before radiotherapy, 1 month after radiotherapy, and then every 2 months. Nineteen assessable patients were followed for at least 3 months after radiotherapy. Pain control improved in 13 of 19 patients at 1 month, but nine of 12 patients had worsening chest pain at 3 months, and at 5 months, pain control

had deteriorated in six of seven patients. Partial regression of chest wall masses was seen in five of nine patients, but radiotherapy did not appear to delay the progression of respiratory symptoms or radiologic changes. The median duration of survival after radiotherapy was 4 months.

De Graaf-Strukowska et al (2) performed a retrospective review from a single institution over a 20-year period. A total of 227 radiotherapy series were administered to 189 patients with mesothelioma. The median survival was 5 months from the start of radiotherapy, and only 17% of patients were alive at 1 year after treatment. Chest pain and painful chest wall metastases were the main indications for radiotherapy. A higher local response rate was seen for patients treated with a 4 Gy per fraction scheme, versus those receiving fractions of less than 4 Gy (50% vs. 39%). Pain recurrence occurred predominantly within the previous radiotherapy field, and pain recurred after a median of 69 days (range 32–363) in the group treated using 4-Gy fractions. Radiotherapy can relieve pain due to malignant pleural mesothelioma, but its effect at doses of 3 and 4 Gy is short-lived.

Prophylactic Radiotherapy

Because of the propensity for mesothelioma to spread along surgically manipulated areas, Boutin et al (3) performed a prospective randomized study to assess the efficacy of local radiotherapy in preventing malignant seeding along invasive diagnostic procedures, such as cytology, needle biopsy, thoracoscopy, or chest tube placement. Forty consecutive patients with histologically proven mesothelioma were enrolled. Twenty patients received three daily sessions of radiotherapy at a dosage of 7 Gy 10 to 15 days after thoracoscopy. The other 20 patients did not receive radiotherapy. None of the 20 radiotherapy-treated patients developed entry tract metastasis. In contrast, eight of the 20 (40%) patients who were not treated developed metastases. These findings indicate that local radiotherapy prevents malignant seeding after invasive diagnostic procedures in patients with malignant pleural mesothelioma.

Surgical Resection Plus Radiotherapy

Brachytherapy/Surgery/External Beam Radiation Therapy

The potential toxicity of external beam radiation therapy (EBRT) to surrounding thoracic structures prompted investigators at Memorial Sloan-Kettering Cancer Center (MSKCC) to use brachytherapy in combination with surgery to treat malignant pleural mesothelioma (MPM). From 1976 to 1982, 41 patients with MPM underwent surgical resection followed by implantation of ^{125}I permanent radioactive seeds to residual gross disease. Residual diffuse disease was implanted with either temporary ^{192}Ir or ^{32}P. Mixed-beam EBRT was delivered to the affected hemithorax at 45 Gy. Median survival was 21 months and

survival at year 1 and 2 was 65% and 40%, respectively. At that period in time, the survival was better than historical survival rates of 25% at 1 year, 7% at 2 years, and a median survival of 6 months (4).

An updated experience from MSKCC was again analyzed on a larger cohort of patients. From 1976 to 1988, 105 patients were treated by a similar approach. Fifty-four patients received implants, while 41 patients had minimal residual disease and therefore did not receive implants. Overall median survival was 12.6 months; 1-year and 2-year survival rates were 52% and 23%, respectively. Patients with implants at one site fared better than patients with implants at multiple sites. The impact of brachytherapy on the outcome independent of amount of residual disease could not be determined. The most significant impact on survival was the achievement of complete resection of all gross disease by the cytoreductive surgical procedure. However, local failure or disease progression occurred in 63% of patients (5).

Intraoperative Radiotherapy

Lee et al (6) performed a retrospective review of radical pleurectomy/decortication and intraoperative radiotherapy (IORT) followed by EBRT for diffuse MPM; IORT was performed on all areas difficult to encompass with conformal radiotherapy, including fissures, pericardium, and diaphragm. An average of 3.3 sites was treated in each patient, with a range of two to six sites. The median dose was 15 Gy (range, 5–15). A total of 32 patients with diffuse MPM were initially evaluated between January 1995 and September 2000. Three patients were excluded from analysis because of unresectable disease, two patients died postoperatively, and one patient had recurrent disease previously treated at an outside institution. Of the remaining 26 patients included in the analysis, 24 received IORT. External beam radiation therapy was generally started 1 to 2 months after resection and delivered by means of three-dimensional conformal radiation therapy or intensity-modulated radiation therapy. The median dose was 41.4 Gy. At the time of data analysis, five of 26 patients were alive. The median follow-up was 9.7 months (range, 2–67.6 months), and the median overall survival and progression-free interval from the time of the operation, were 18.1 and 12.2 months, respectively. The Kaplan-Meier estimates of overall survival and freedom from progression at 1 year were 64% and 50%, respectively. The site of failure was mostly locoregional. However, there were four abdominal failures and one contralateral lung failure. While demonstrating that debulking with IORT was feasible, there is no evidence of a significant survival advantage. Survival was inversely proportional to the number of IORT sites treated, which emphasizes the importance of residual gross disease as a significant predictor of poor survival.

Extrapleural Pnemonectomy/External Beam Radiation Therapy/Adjuvant Chemotherapy

At Brigham and Women's hospital from 1980 to 1997, 183 patients underwent extrapleural pneumonectomy followed by adjuvant chemo-

therapy and radiotherapy (7). The number of patients who completed adjuvant therapy and the resultant toxicities are unspecified. However, the median follow-up was 13 months. The perioperative mortality rate was 3.8% (seven deaths) and the morbidity was 50%. The seven perioperative deaths were excluded from the survival analysis, but survival in the 176 remaining patients was 38% at 2 years and 15% at 5 years (median 19 months). Univariate analysis identified three prognostic variables associated with improved survival: epithelial cell type (52% 2-year survival, 21% 5-year survival, 26-month median survival; $p = .0001$), negative resection margins (44% at 2 years, 25% at 5 years, median 23 months; $p = .02$), and extrapleural nodes without metastases (42% at 2 years, 17% at 5 years, median 21 months; $p = .004$). Using the Cox proportional hazards, the relative risk of death was calculated for nonepithelial cell type odds ratio (OR) 3.0, 95% confidence interval (CI) 2.0–4.5; $p < .0001$), positive resection margins (OR 1.7, 95% CI 1.2–2.6; $p = .0082$), and metastatic extrapleural nodes (OR 2.0, 95% CI 1.3–3.2; $p = .0026$). Thirty-one patients with three positive variables had the best survival (68% 2-year survival, 46% 5-year survival, median 51 months; $p = .013$). This study identifies a subgroup of patients who appear to benefit from a multimodality approach that includes extrapleural pneumonectomy. The influence of resection margins on survival also exemplifies the need for improvement in local cytoreduction.

Pattern of Recurrence

Baldini et al (8) evaluated 49 patients with MPM who underwent extrapleural pneumonectomy followed by chemotherapy and radiation. There were two perioperative deaths, and one patient died 5 weeks after extrapleural pneumonectomy. Thirty-five of the surviving patients received adjuvant chemotherapy (32/35 received cyclophosphamide, doxorubicin, and cisplatin) followed by a median dose of 30.6 Gy of hemithorax radiation therapy. Ten patients received chemotherapy but no radiation therapy, and one patient received no adjuvant therapy. Median follow-up time for the 23 living patients from the date of operation was 18 months. Of the 46 patients available for evaluation, 25 had recurrence (54%) with a median time to first failure of 19 months (range, 5 to 51 months). The sites of first recurrence were local in 35% of patients, abdominal in 26%, the contralateral thorax in 17%, and other distant sites in 8%. Conversely, some patients had recurrence in multiple sites simultaneously. The most common site of failure after trimodality therapy was the ipsilateral hemithorax. Isolated distant failures were uncommon. Again, this study emphasized the need to improve local tumor control.

Extrapleural Pneumonectomy/High-Dose External Beam Radiation Therapy

The use of radiation therapy is limited by the volume of the primary tumor, which involves the entire hemithorax, and by proximity of the tumor to many vital structures that are very radiosensitive. In general,

the amount of radiation to the affected hemithorax has been limited to 45 Gy or less to minimize toxicity to the heart, lung, esophagus, and spinal cord. Maasilta (9) has documented the severe pulmonary toxicity caused by higher dose hemithoracic radiation. However, with the lung removed by extrapleural pneumonectomy, the amount of radiation to the hemithoracic area can be administered in very large doses without its attendant pulmonary toxicity.

Rusch et al (10) performed a phase II trial of high-dose hemithoracic radiation after complete resection to determine feasibility and to estimate rates of local recurrence and survival. Patients were eligible if they had a resectable tumor, as determined by computed tomographic (CT) scanning, and adequate cardiopulmonary function for extrapleural pneumonectomy or pleurectomy/decortication. After complete resection, patients received hemithoracic radiation (54 Gy) and then were followed up with serial CT scanning. From 1995 to 1998, 88 patients (73 men and 15 women; median age, 62.5 years) were entered into the study. The operations performed included 62 extrapleural pneumonectomies (70%) and five pleurectomies/decortications; procedures for exploration-only were performed in 21 patients. Seven (8.0%) patients died postoperatively. Adjuvant radiation was administered to 57 patients (54 undergoing extrapleural pneumonectomy and three undergoing pleurectomy/decortication) at a median dose of 54 Gy and was well tolerated (grade 0–2 fatigue, esophagitis) except for one late fatal esophageal fistula. The median survival was 33.8 months for stage I and II tumors but only 10 months for stage III and IV tumors ($p = .04$) (Fig. 46.1). For the patients undergoing extrapleural pneumonectomy, the sites of recurrence were locoregional in two, locoregional and distant in five, and distant-only in 30. This study demonstrated that hemithoracic radiation after complete surgical resection at a high dose

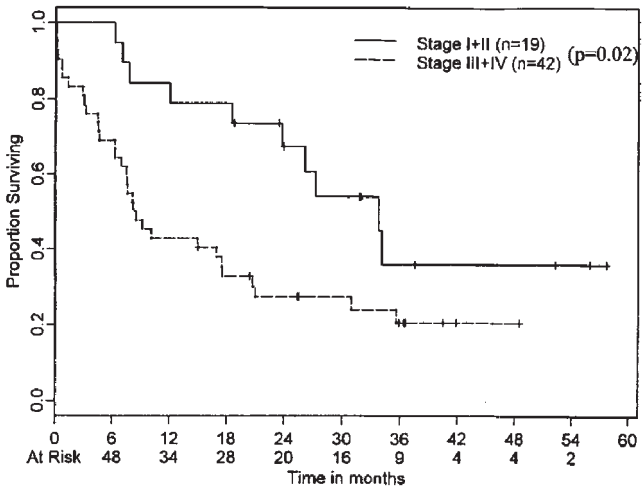


Figure 46.1. Kaplan-Meier survival analysis of extrapleural pneumonectomy (EPP) and high-dose radiotherapy by stage. [*Source*: Rusch et al (10), with permission.]

of 54 Gy was feasible. This approach dramatically reduced local recurrence and was associated with prolonged survival for early-stage tumors. But higher stage disease had a high risk of early distant relapse and is currently undergoing investigation in trials of systemic therapy added to this regimen of resection and radiation.

Intensity-Modulated Radiation Therapy

Poor local control after conventional radiotherapy may be due to the low dose of radiation that has been administered or to restriction of the target volume to avoid critical organs. Intensity-modulated radiation therapy (IMRT) has the potential to overcome these geometric/dosimetric constraints. Many patients are referred for radiation with intact lung following biopsy or pleurectomy. Delivery of efficacious doses of radiation to the pleural lining, while avoiding lung parenchyma toxicity, has been a difficult technical challenge. Using opposed photon fields produces doses in lungs that result in moderate-to-severe pulmonary toxicity in 100% of patients treated. Combined photon-electron beam treatment, at total doses of 4250 cGy to the pleural surface, results in two thirds of the lung volume receiving over 2100 cGy. A technique using intensity-modulated photon arc therapy significantly improves the dose distribution to the pleural surface with concomitant decrease in dose to lung parenchyma compared to traditional techniques. Intensity-modulated radiation therapy of the pleural lining consists of segments of photon arcs that can be intensity modulated with varying beam weights and multileaf positions to produce a more uniform distribution to the pleural surface, while at the same time reducing the overall dose to the lung itself. Computed tomography simulation is critical for precise identification of target volumes as well as critical normal structures (lung and heart). Rotational arc trajectories and individual leaf positions and weightings are then defined for each CT plane within the patient. Intensity-modulated radiation therapy may have improved potential for sparing dose effects to the critical structures of the lung, heart, and spinal cord (11). However, due to the oblique angle of the treatment portals, organs that are typically not in the radiation field may receive a significant dose of radiation. Specifically, special attention must be paid to the dose delivered to the contralateral lung and heart.

Intensity-Modulated Radiation Therapy/Extrapleural Pneumonectomy

Forster et al (12) evaluated the role of IMRT in combination with extrapleural pneumonectomy (EPP). Seven patients with MPM who had an EPP were treated with adjuvant IMRT. The clinical target volume (CTV) included the surgically violated area inside the chest wall with particular attention to the insertion of the diaphragm, pleural reflections, and the deep margin of the thoracotomy incision. Treatment was delivered by intensity-modulated 6-MV photon beams using dynamic multileaf collimation. The CTV ranged from 2667 to 7286 mL. The average CTV covered to 50 Gy was 94% (range, 92% to 98%). Res-

piratory motion was minimal. The average volume of the boost areas covered by 60Gy was 92% (range, 82% to 99%). Dose-volume constraints for normal tissue were met in almost all cases and acute toxicity was mild to moderate. The most severe side effects were anorexia, nausea or vomiting, and dyspnea. Esophagitis was absent or mild. After a minimum of 13 months of follow-up care, there were no cases of disease recurrence within the ipsilateral hemithorax. Input from the radiologist and from the surgeon in the planning process facilitates definition of the high-dose volumes (12,13).

Surgery

The limitations of chemotherapy and radiation alone have made surgery the mainstay of treatment for MPM. Operations for MPM are categorized as those performed for palliation and those performed with curative intent. The palliative procedures include talc pleurodesis via tube thoracostomy or video-assisted thoracic surgery (VATS). In some cases, thoracotomy and partial pleurectomy may be required to allow lung reexpansion. The operations performed with curative intent include EPP and pleurectomy/decortication. The intention of these procedures is to remove all gross tumor from the affected hemithorax.

The skepticism about performing EPP stems from its high mortality, initially reported as 31% by Butchart et al (14) in 1976. In addition, patients were inaccurately staged because CT was not yet used in clinical practice and there was no accepted staging system. However, over time with better patient selection and improvement in technique, this operation is being performed with outcomes similar to those of standard pneumonectomy (15). The largest series have demonstrated the mortalities of 4% to 5% (14,16,17) (Table 46.1).

Extrapleural Pneumonectomy

Extrapleural pneumonectomy usually entails an en bloc resection of the pleura, lung, pericardium, and hemidiaphragm. The theory is to maintain the integrity of the pleural envelope and thereby keep the malignant tumor within the confines of this envelope in an effort to remove the lesions without leaving gross residual tumor behind. It is performed via an extended S-shaped posterolateral thoracotomy incision and the best exposure is gained when the anterior portion of the incision is carried to the costal margin (Fig. 46.2). Excision of the sixth rib facilitates exposure to the extrapleural plane and both the apical and diaphragmatic surfaces of the hemithorax. The extrapleural plane lies

Table 46.1. Mortality rates of extrapleural pneumonectomy (7,14–16)

Study	Number of patients	Mortality
Butchart et al (1976)	29	31
Allen et al (1994)	40	8
Sugarbaker et al (1999)	183	4
Rusch (1999)	115	5

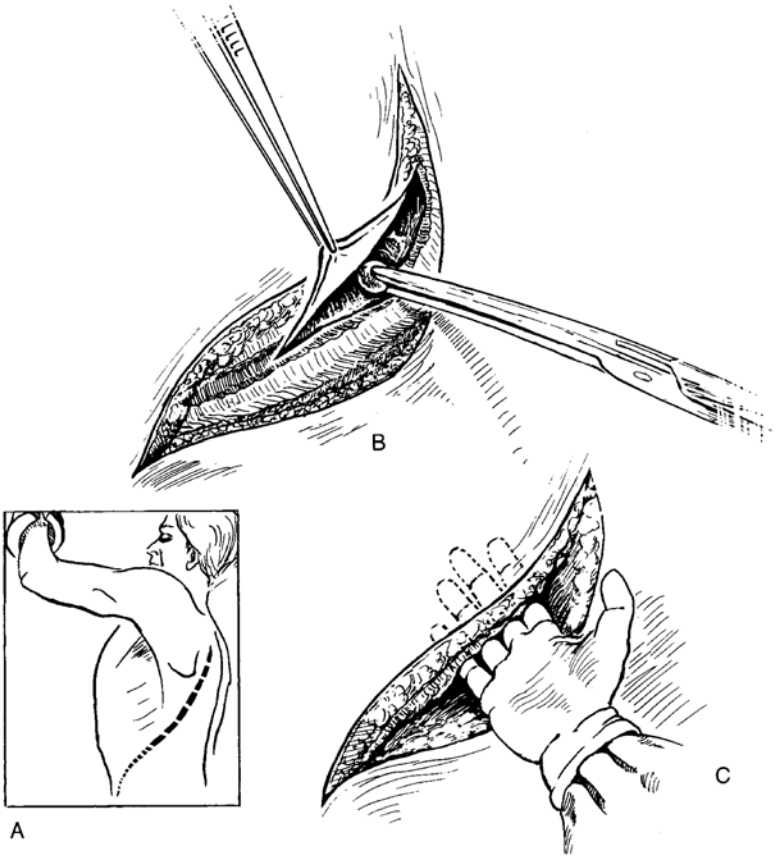


Figure 46.2. S-shaped incision for EPP. [*Source*: Rusch (21) with permission.]

between the endothoracic fascia and the outer surface of the parietal pleura. This is a very vascular plane, requiring continuous packing with lap pads to minimize blood loss, but it can be developed by a combination of both blunt and sharp dissection.

Once enough parietal pleura have been mobilized (usually three ribs superiorly and three ribs inferiorly), a chest retractor is inserted to help with exposure (Fig. 46.3). The dissection is carried superiorly to the subclavian vessels; care must be taken near the mammary vessels since these may be avulsed at their origin from the subclavian vessels. The pleura are mobilized away from the mediastinum anteriorly and posteriorly. On the left side, care must be taken not to injure the esophagus or the aorta, especially the branches of the intercostal vessels. On the right side, the superior vena cava may be adherent to the pleura. Once the superior dissection down to the bronchus has been completed, the posterior aspect of the hilum is dissected. The subcarinal lymph nodes are then dissected and sent to pathology as a separate specimen for accurate staging and better exposure of the main-stem bronchus. There may be a clean plane of dissection between the anterior portion of the hilum and the pericardium in some cases. In others,

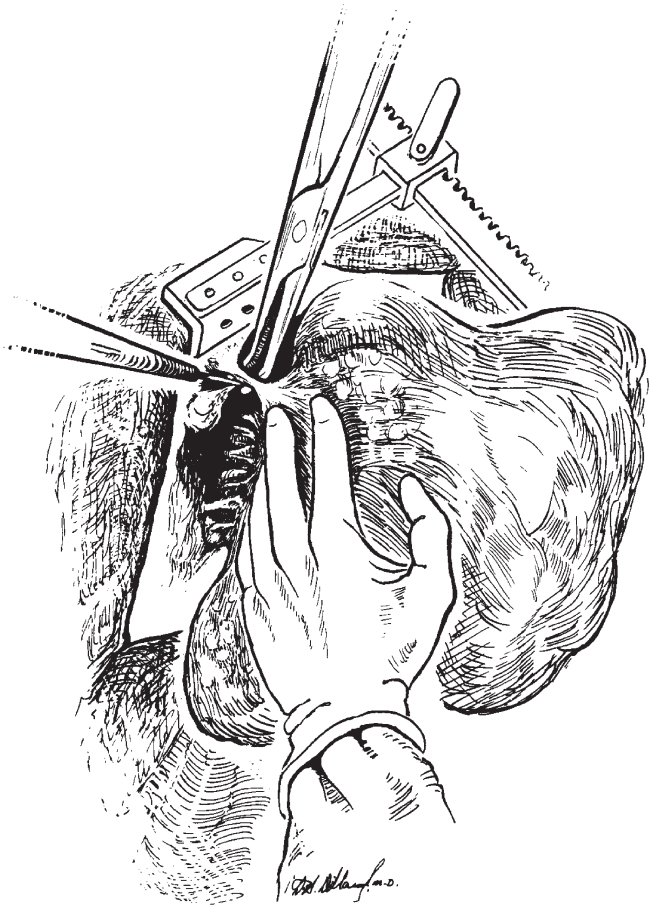


Figure 46.3. Extrapleural plane. [*Source*: Rusch (21) with permission.]

this plane is obliterated and an en bloc resection with the pericardium is required.

The lower hemithorax and diaphragm are then dissected. A palpable shelf can usually be palpated at the junction of the tumor and the diaphragmatic muscle. A hand may be placed beneath this shelf and the diaphragmatic muscle cauterized peripherally in a similar fashion to the Kocher maneuver of the duodenum. Once the diaphragm is mobilized from the posterior costophrenic angle, it may be rotated into the thoracotomy incision, thereby allowing further dissection posteriorly. If involvement of the diaphragm is extensive, it should be removed in its entirety. If involvement of the diaphragm is superficial, dissection may be carried through the diaphragmatic muscle using electrocautery (Figs. 46.4 and 46.5). The peritoneum should be peeled away from the back of the diaphragm by means of a sponge stick. It may be difficult to avoid entering the peritoneum at the level of the central tendon, but tiny defects should be repaired immediately to avoid potential seeding of the peritoneal cavity.

Once the entire extrapleural lung and diaphragm are completely mobilized, the pericardial interface is assessed. If resection of the peri-

cardium is required, traction sutures are useful to aid in visualization of the hilar structures and to minimize the hemodynamic instability and arrhythmias that may be precipitated by the changes in positioning of the heart (Fig. 46.6). On the right side, the hilar vessels are transected intrapericardially. On the left side, it is easiest to transect the pulmonary artery in its extrapericardial extrapleural position and transect the veins intrapericardially. The specimen consisting of pleura, lung, and diaphragm, with or without pericardium, is removed en bloc. Sampling of the paratracheal lymph nodes on the right and the aortopulmonary window on the left are submitted for accurate staging. Reconstruction of the diaphragm is then performed.

On the right side, Dexon mesh is preferred because the liver prevents herniation of intraabdominal contents. On the left side, 2-mm-thick Gore-Tex is used because it is more durable at resisting herniation. The prosthesis is secured by placing sutures around the ribs laterally

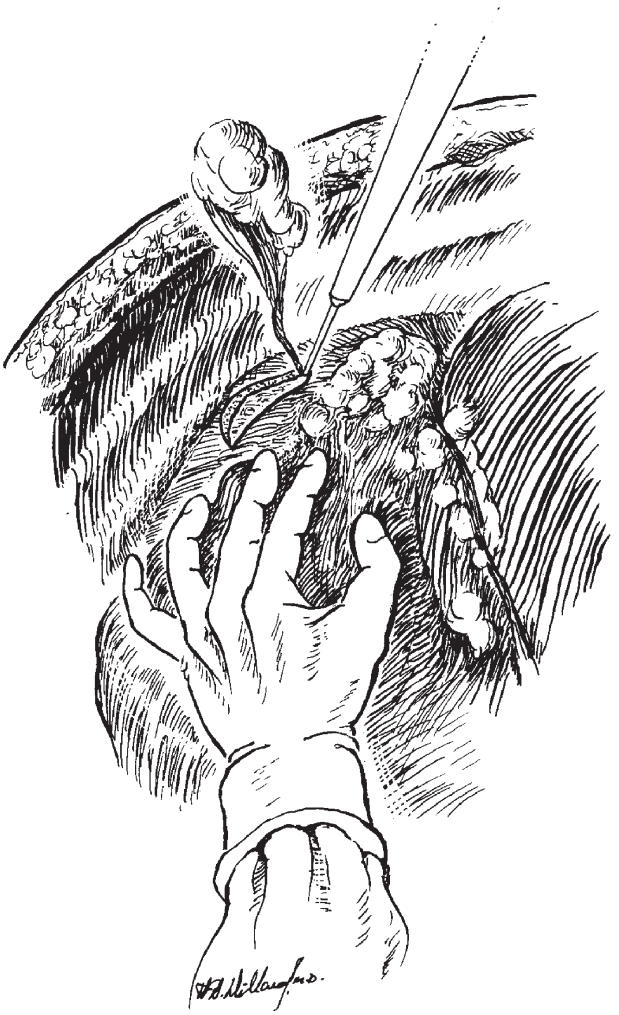


Figure 46.4. Diaphragmatic incision. [*Source*: Rusch (21) with permission.]

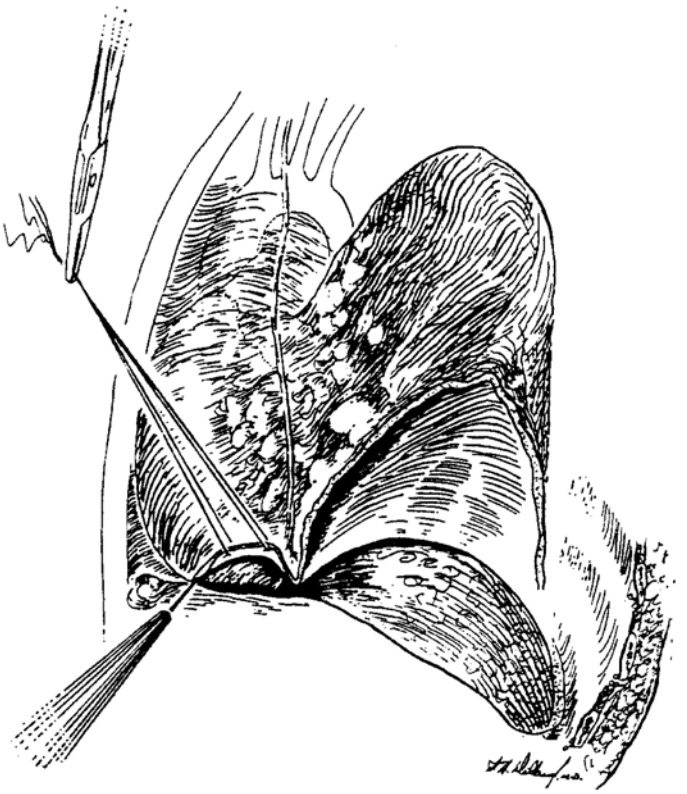


Figure 46.5. Placement of pericardial traction sutures. [*Source*: Rusch (21) with permission.]

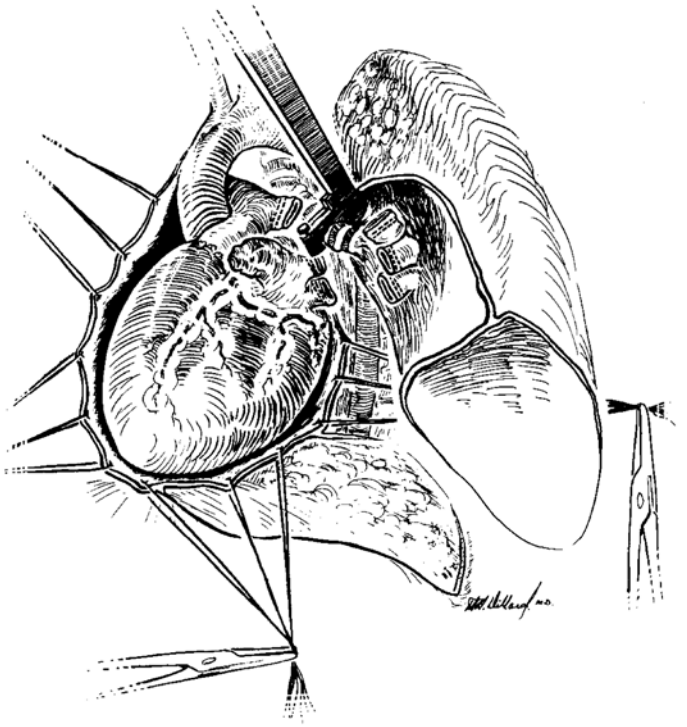


Figure 46.6. Transection of hilar structures. [*Source*: Rusch (21) with permission.]

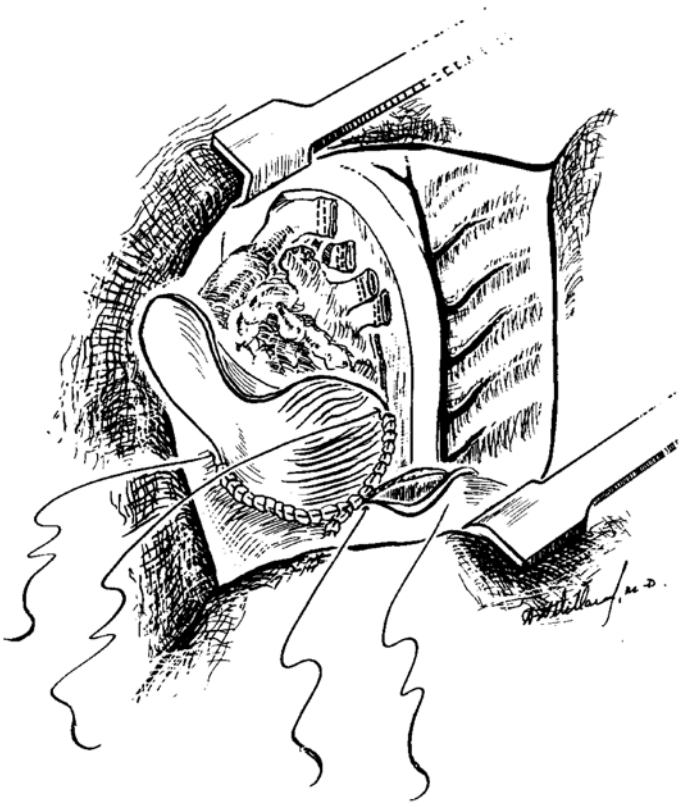


Figure 46.7. Pericardial reconstruction. [*Source*: Rusch (21) with permission.]

(Figs. 46.7 and 46.8). It is secured to the crus posteriorly and sewn to the edge of the pericardium medially. If the pericardium has been resected, the reconstruction is performed with Dexon mesh. This prevents cardiac herniation and maintains the heart in the central position to facilitate the administration of postoperative radiation. Hemostasis is aided by the use of the argon beam electrocoagulator. We prefer Dexon to Gore-Tex for the pericardial reconstruction because, in the event of an empyema, the Dexon does not require extirpation from the cardiac surface and potential injury. In addition, since Dexon is in mesh form, fenestration is unnecessary.

Pleurectomy/Decortication

This procedure should be reserved for patients with a limited amount of disease and a functional status that is prohibitive to tolerate an EPP. The procedure is performed in the same manner as an EPP, by mobilizing the entire lung. The extent of tumor is usually such that complete resection of the diaphragm or pericardium is not necessary but may be removed if required. Once the entire area has been mobilized, the tumor is then decorticated off the underlying lung (Fig. 46.9). Theoretically, pleurectomy decortication should remove all gross disease by surgical resection of the parietal pleura. To accomplish the goal of

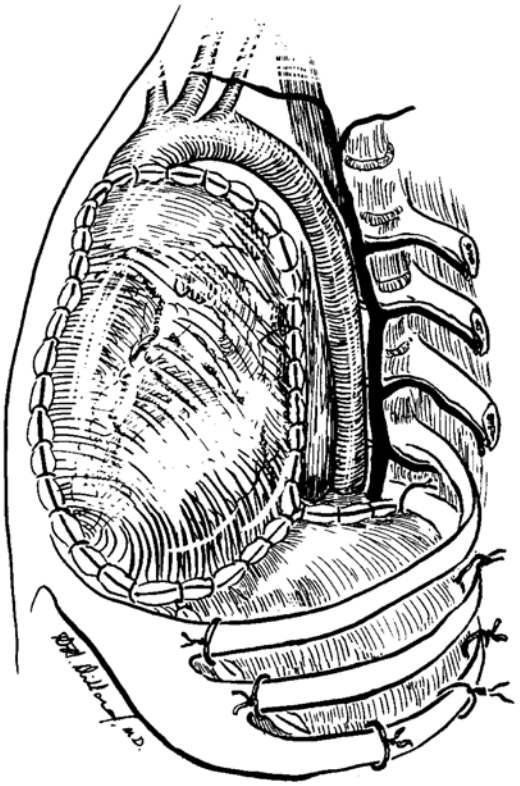


Figure 46.8. Pericardial and diaphragmatic reconstruction. [*Source*: Rusch (21) with permission.]

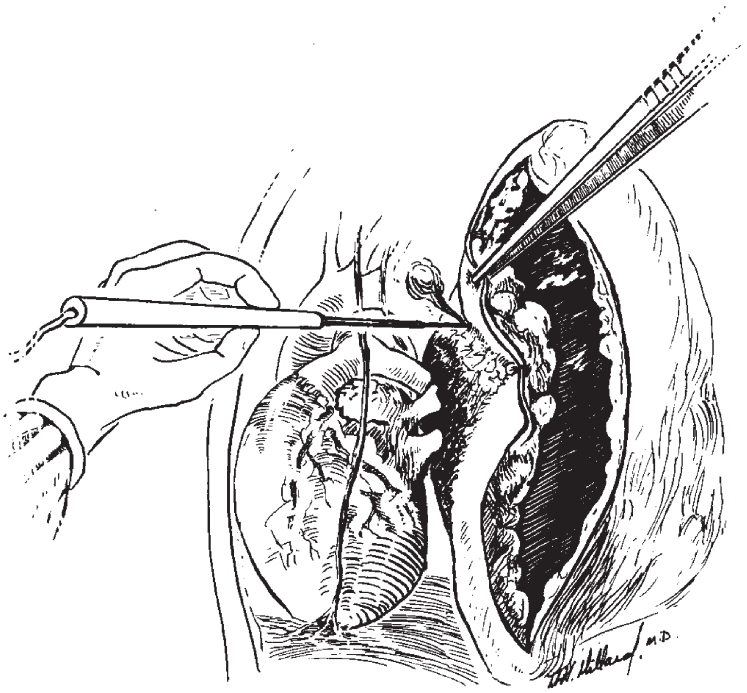


Figure 46.9. Pleurectomy decortication of tumor from underlying lung. [*Source*: Rusch (21) with permission.]

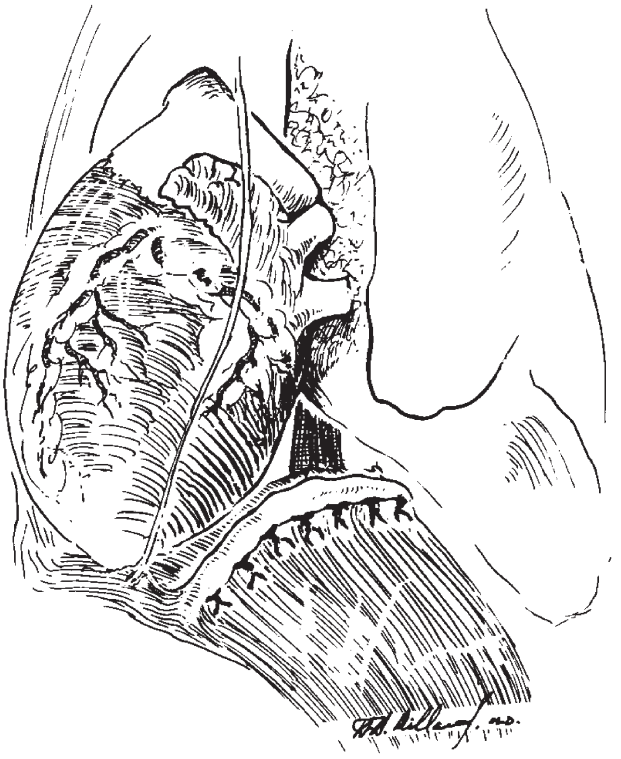


Figure 46.10. Diaphragmatic plication. [*Source*: Rusch (21) with permission.]

removal of all gross tumor, the patient must have stage 1a disease or a small amount of 1b disease (indicating visceral pleura involvement; the visceral pleura is a structure more difficult to delineate than the parietal pleura and is intimate with the underlying lung as opposed to the parietal pleura, which is intimate with the chest wall). Once there is invasion of lung parenchyma, the goal of complete removal of all gross tumor is impossible without removal of the underlying lung (i.e., EPP). If there is involvement of the pericardium or diaphragm, these structures may also be removed with a pleurectomy decortication.

An important maneuver that should be performed with pleurectomy/decortication when the majority of the diaphragm has been left in place is diaphragmatic plication. This is an important step even if the phrenic nerve is anatomically intact because the diaphragm has often been physiologically defunctionalized and will rise high into the hemithorax, causing lower lobe atelectasis postoperatively. In addition, diaphragmatic plication allows a greater field for radiation by maintaining the abdominal contents low (Fig. 46.10).

High-Dose Hemithoracic Radiation Treatment Plan

After all gross tumor has been removed by surgical resection, patients should then undergo postoperative radiation therapy. The rationale for this treatment strategy has been outlined above. For patients undergo-

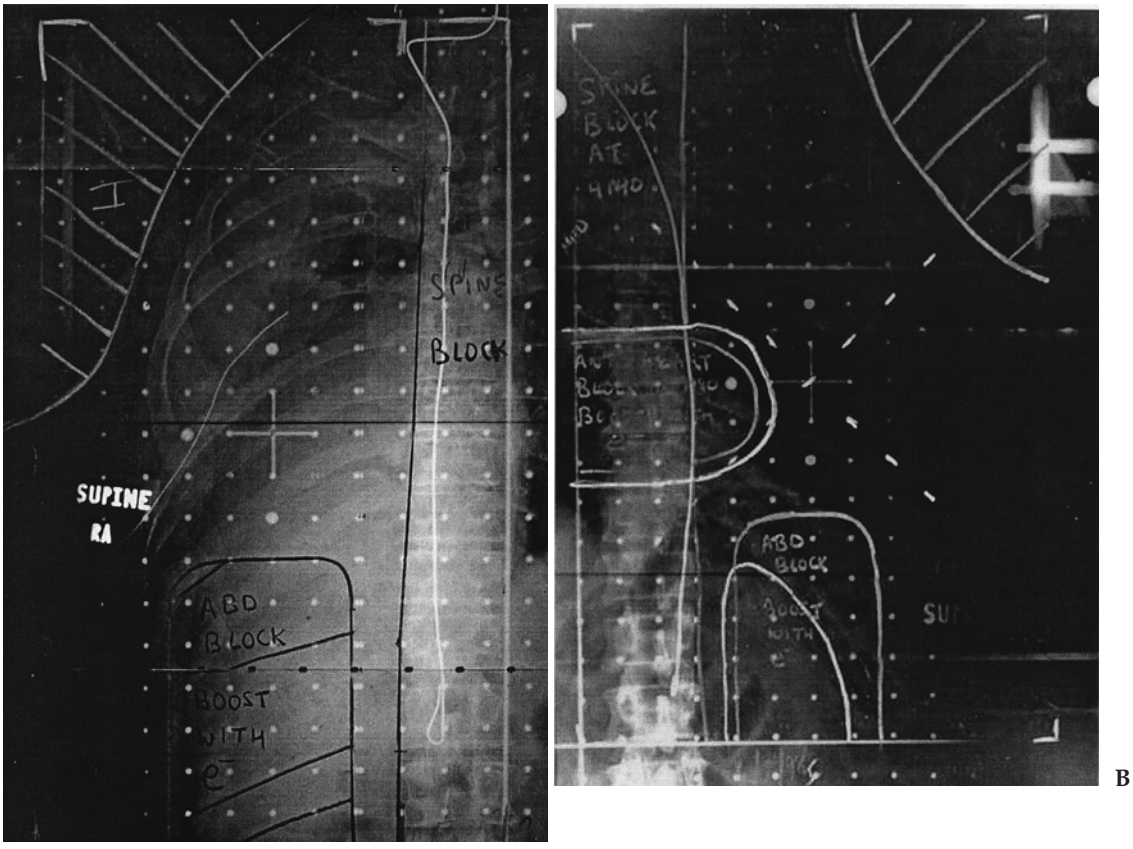


Figure 46.11. (A,B) Simulation films for high-dose external beam radiation therapy. [*Source*: Yajnik et al (22) with permission.]

ing EPP, adjuvant external beam radiation is started 3 to 6 weeks postoperatively. The target volume includes the entire hemithorax and the thoracotomy incision. A total of 54 Gy is delivered through anterior and posterior fields in 30 daily fractions of 1.8 Gy by using 6 MV or greater photons. The spinal cord is protected after 41.4 Gy. Cerrobend blocks are used to limit the dose to the liver, heart, and stomach when these organs are in the treatment field. Electrons are used in the blocked regions to prevent underdosing to the pleura and diaphragm.

Figure 46.11 demonstrates a simulation film of the right hemithorax after EPP. Field borders are T1 inferiorly and L2 superiorly. Laterally, the borders are the edge of the contralateral vertebral body and flash on skin. A block is placed over the abdomen to shield the liver and stomach from photon irradiation. A block covers the area where the diaphragm abuts the abdominal wall, facilitating treatment of this area with electrons. The daily dose of electron irradiation was 153 cGy, with the assumption that there is a 15% scatter under the blocks from the photon fields. Figure 46.11 demonstrates a simulation film of the left hemithorax after EPP. Field borders are T1, L2, 1.5 cm beyond the con-

tralateral edge of the vertebral body, and skin. The blocks covering the diaphragm and abdomen are similar to the right; however, a block is also placed anteriorly over the heart starting at 1980 cGy and the blocked area is treated with electron irradiation. The daily dose of electron irradiation is 153 cGy, again assuming that a 15% scatter will occur under the blocks from the photon field. The left kidney is contoured.

Patients undergoing pleurectomy/decortication in our study received intraoperative radiation with a previously described high-dose iridium applicator (17,18). A dose of 15 Gy was delivered to the mediastinum and diaphragm, reducing this to 10 Gy over the heart and esophagus. For these patients, EBRT was also started 3 to 5 weeks postoperatively. The dose administered was 45 to 54 Gy and the target volume included the perimeter of remaining lung tissue with a 0.5- to 1.0-cm margin, the chest wall with a 1.0-cm margin, the diaphragm, and the mediastinum. Currently, pleurectomy/decortication patients receive only EBRT, due to the high toxicity seen after IORT.

Summary

When evaluating a patient with MPM for surgical resection one must take into account the stage, functional status, and the surgical procedure. While pleurectomy/decortication may be the procedure of choice for patients with poor functional status and early-stage disease, the presence of lung parenchyma limits the amount of radiation that may be administered. In the majority of cases, an EPP is also required to obtain complete gross removal of tumor.

The study by Rusch et al (10) shows that high-dose hemithoracic radiation at a dose of 54 Gy can be administered to the entire hemithorax after EPP with an acceptable toxicity. This treatment regimen is associated with a very low risk of local recurrence. The few local recurrences in this study appear to have been failures at the margins of the radiation field, emphasizing the importance of including the diaphragm, costophrenic sulcus, and ipsilateral half of the mediastinum. It is notable that the radiation used at this dosage essentially eliminates the risk of tumor recurrence in the chest wall that is commonly seen in patients with MPM and previous thoracic incisions.

This study is the basis of our standard of care at Memorial Sloan-Kettering Cancer Center, and our results clearly indicate that now the greatest challenge is preventing the development of metastatic disease. With recent improvements in systemic therapy, it is now possible to add preoperative chemotherapy to the treatment regimen of patients (19,20). Both single-institution and multicenter trials are currently in progress for testing the efficacy of induction chemotherapy, followed by EPP and adjuvant hemithoracic radiation. It is hoped that active chemotherapy agents including cisplatin, gemcitabine, and pemetrexed will enhance the results of the excellent local treatment now available with EPP and adjuvant radiotherapy by decreasing the risk of distant relapse.

References

1. Bissett D, Macbeth FR, Cram I. The role of palliative radiotherapy in malignant mesothelioma. Clin Oncol 1991;3(6):315–317.
2. de Graaf-Strukowska L, van der Zee J, van Putten W, Senan S. Factors influencing the outcome of radiotherapy in malignant mesothelioma of the pleura—a single-institution experience with 189 patients. Int J Radiat Oncol Biol Phys 1999;43(3):511–516.
3. Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy. Chest 1995;108(3):754–758.
4. Hilaris BS, Nori D, Kwong E, Kutcher GJ, Martini N. Pleurectomy and intraoperative brachytherapy and postoperative radiation in the treatment of malignant pleural mesothelioma. Int J Radiat Oncol Biol Phys 1984;10(3): 325–331.
5. Mychalczak BR, et al. Results of treatment of malignant pleural mesothelioma with surgery, brachytherapy, and external beam irradiation. (abstract). Endocurie Hypertherm Oncol 1989;5:245.
6. Lee TT, Everett DL, Shu HK, et al. Radical pleurectomy/decortication and intraoperative radiotherapy followed by conformal radiation with or without chemotherapy for malignant pleural mesothelioma. J Thor Cardiovasc Surg 2002;124(6):1183–1189.
7. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117(1):54–63; discussion 63–65.
8. Baldini EH, Recht A, Strauss GM, et al. Patterns of failure after trimodality therapy for malignant pleural mesothelioma. Ann Thorac Surg 1997; 63(2):334–338.
9. Maasilta P. Deterioration in lung function following hemithorax irradiation for pleural mesothelioma. Int J Radiat Oncol Biol Phys 1991;20(3):433–438.
10. Rusch VW, Rosenzweig K, Venkatraman E, et al. A phase II trial of surgical resection and adjuvant high-dose hemithoracic radiation for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;122(4):788–795.
11. Tobler M, Watson G, Leavitt DD. Intensity-modulated photon arc therapy for treatment of pleural mesothelioma. Med Dosimetry 2002;27(4):255–259.
12. Forster KM, Smythe WR, Starkschall G, et al. Intensity-modulated radiotherapy following extrapleural pneumonectomy for the treatment of malignant mesothelioma: clinical implementation. Int J Radiat Oncol Biol Phys 2003;55(3):606–616.
13. Ahamad A, Stevens CW, Smythe WR, et al. Intensity-modulated radiation therapy: a novel approach to the management of malignant pleural mesothelioma. Int J Radiat Oncol Biol Phys 2003;55(3):768–775.
14. Butchart EG, Ashcroft T, Barnsley WC, Holden MP. Pleuropneumonectomy in the management of diffuse malignant mesothelioma of the pleura. Experience with 29 patients. Thorax 1976;31(1):15–24.
15. Rusch VW. Indications for pneumonectomy: extrapleural pneumonectomy. Chest Surg Clin North Am 1999;9(2):327–338.
16. Allen KB, Faber LP, Warren WH. Malignant pleural mesothelioma. Extrapleural pneumonectomy and pleurectomy. Chest Surg Clin North Am 1994; 4(1):113–126.
17. Harrison LB, Enker WE, Anderson LL. High-dose-rate intraoperative radiation therapy for colorectal cancer. Part I. Oncology 1995;9(7):679–683.

18. Harrison LB, Enker WE, Anderson LL. High-dose-rate intraoperative radiation therapy for colorectal cancer. Part II. Oncology 1995;9(7):737–748.
19. Byrne MJ, Davidson JA, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant mesothelioma: a phase II study. J Clin Oncol 1999; 17(1):25–30.
20. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21(14):2636–2644.
21. Rusch VW. Mesothelioma and less common pleural tumors. In: Pearson FG, Cooper JD, Deslauries J, et al, eds. Thoracic Surgery. Philadelphia: Churchill Livingstone, an imprint of Elsevier Science, 2002:1241–1263.
22. Yajnik S, Rosenzweig KE, Mychalczak B, et al. Hemithoracic radiation after extrapleural pneumonectomy for malignant pleural mesothelioma. Int J Radiat Oncol Biol Phys 2003;56(5):1319–1326.

47

The Development of the Brigham and Women's Multimodality Treatment Plan for Malignant Pleural Mesothelioma: A Model for Improving the Treatment of Rare Diseases

Michael T. Jaklitsch, Daniel Wiener, Raphael Bueno, and David J. Sugarbaker

Mesothelioma of the pleural space is an uncommon disease. The current incidence within the United States is 2000 to 3000 cases per year as compared with esophageal and lung cancer, which are at least four and 50 times more common, respectively (1). Few physicians treat more than a handful of cases of malignant pleural mesothelioma (MPM) over the course of their professional careers. Even fewer academic centers in North America and Europe have been able to acquire a collective experience large enough to develop new treatment protocols for this devastating disease.

The Brigham and Women's Hospital (BWH) and Dana-Farber Cancer Institute (DFCI) in Boston, Massachusetts, are a combined cancer center with a large experience treating MPM. The diagnosis and management of this disease has become a major interest of the academic thoracic surgeons, medical oncologists, radiation oncologists, pathologists, pulmonologists, respiratory therapists, nurses, and educational house staff of our hospital over the past 20 years. Current treatments are based on two decades of research and experience, and new treatments are being developed.

This chapter traces the historical development of the current treatment of MPM at BWH, explains the development of our working paradigm of this disease, and serves as a template for other surgical innovators to design unique treatment algorithms for similarly uncommon diseases.

Improved treatment of a rare disease depends on three critical elements coexisting at the same place and time: a relative high frequency

of the disease, the commitment of a multidisciplinary research-oriented team and institution, and the ensuing dynamic growth of professional expertise. The BWH experience with MPM includes all three of these elements.

Historical Context

The distinctiveness of the BWH/DFCI experience with mesothelioma can be best appreciated within a historical context of the disease. The recognition of mesothelioma as a cancer and the development of treatment options are recent developments in the context of medical history.

In 1960, Wagner et al (2) published the first mesothelioma case series, reporting on 33 patients from a South African asbestos mining town with known occupational and environmental crocidolite exposure. In the 1970s, a landmark study by Selikoff (3) established a firm link between asbestos exposure and mesothelioma. The author followed 17,800 asbestos insulation workers in the United States and Canada for a period of up to 50 years and found that the incidence of mesothelioma within this group increased rapidly starting 20 to 25 years after the first exposure. Peak incidence occurred at 40 to 45 years after exposure. Seven percent of all deaths in this group of asbestos workers were due to mesothelioma, a shockingly high incidence for a rare cancer.

The association between mesothelioma and asbestos is well established (4). The causative role of asbestos exposure has been investigated extensively and its pathophysiology has been described in detail (3,5). Persons at the highest risk include those who work directly with asbestos in mines, mills, or shipyards. This risk extends to people residing in areas surrounding these sites. Family members of asbestos workers also have a substantial increased risk, termed "bystander risk," thought to be secondary to exposure to hair and clothes brought into the home (6).

Early efforts at surgical and nonsurgical treatments were disappointing. Worn (7) published one of the first series of patients undergoing extrapleural pneumonectomy in 1974, reporting a 5-year survival rate of 10% and a median survival of 19 months. Butchart et al (8) published their initial experience with extrapleural pneumonectomy for maximal surgical debulking of pleural mesothelioma in 1976. Extrapleural pneumonectomy had previously been used for tuberculous empyema, but was an operative technique that had always been associated with a high perioperative mortality. In Butchart et al's series, extrapleural pneumonectomy for MPM had a perioperative mortality rate of 31%, a 5-year survival of 3.5%, and a median survival of 10 months.

Initial studies investigating adjuvant chemotherapy and radiation therapy repeatedly showed little to no activity against the disease. These poor results were partly due to the lack of an accurate way to measure response rate prior to the advent of computed tomography (CT) scan for the chest. Furthermore, early trials were poorly designed, with too few patients and without stratification by histologic subtype.

Median survival of patients enrolled in therapeutic trials varied from 3 to 17 months, with the majority falling in the 6- to 10-month range (9).

Early attempts at radiation therapy were very limited as there was no way to avoid injuring the underlying lung parenchyma and nearby vital structures. Several early studies failed to show an added benefit when radiation was used in combination with surgery or chemotherapy (10).

Considering these relatively ineffective treatments and the seemingly persistently dismal prognosis, it is not difficult to see why there has been a fair degree of skepticism among physicians treating patients with mesothelioma. Fortunately, a few clinicians were not deterred, including a distinct group of health care providers at BWH/DFCI. This chapter describes the evolution of our institutions' current understanding and approach to MPM.

Frequency of Disease in New England

New England has had a rich maritime military history. In August 1776, regiments from Marblehead and Salem, Massachusetts, rowed George Washington's army to safety across Long Island Sound after the defeat on Brooklyn Heights. Three of the first six frigates built by the fledgling United States were built in New England or New York. The large whaling and cod fishing fleets from New Bedford, Nantucket, and Gloucester have provided sailors to the United States Navy for over 200 years.

The pace of production of United States naval ships during World War II reached one ship per week in the large shipyards of New England and New York. Asbestos slurry was sprayed upon the bulkheads of the ships to insulate the compartments against the cold of the North Atlantic and against fire within individual sections of the ship. Although quickly and easily applied to the bulkheads, this asbestos slurry would flake, and particles of asbestos dust would be suspended in the air once it had dried. Unaware of the long-term complications of this exposure, the shipyard workers did not wear protective clothing or masks. Many mesothelioma patients who served on these ships describe a cloud of white dust below decks whenever the large guns of the warship were fired. Thus, a large proportion of the New England population came into contact with substantial quantities of asbestos by either working within the New England shipyards or serving in the navy. Asbestos was also commonly used to insulate heaters within the home, exposing an even larger New England population. The consequence of this exposure is reflected in current geographical trends in the prevalence of mesothelioma (Fig. 47.1).

The long latency period from exposure to development of the cancer has contributed to the high frequency of pleural mesothelioma in the greater Boston area during the past two decades. Prospective studies following people with known asbestos exposure have demonstrated a rapid rise in the incidence of malignant mesothelioma beginning at 20 years postexposure and a peak incidence of approximately 0.6% per

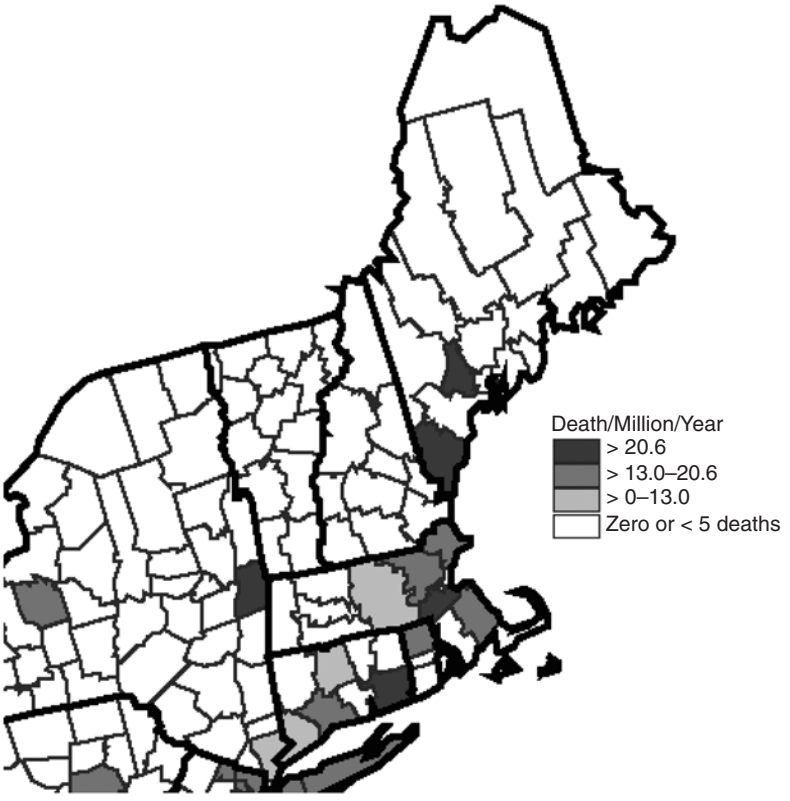


Figure 47.1. Malignant mesothelioma: age-adjusted mortality rates by county, United States residents age 15 and over, 1999. *Note*: Age-adjusted rates are not calculated for those counties with one to four deaths. (*Sources*: National Center for Health Statistics. Multiple causes of death data. Population estimates from U.S. Bureau of the Census. Work Related Lung Disease Surveillance Report 2002. Division of Respiratory Disease Studies National Institute for Occupational Safety and Health. U.S. Department of Health and Human Services. Center for Disease Control and Prevention. December 2002. http://www.cdc.gov/niosh/docs/2003-111/2003-111.html.)

year 40 to 45 years after exposure. As discussed above, asbestos mining and shipbuilding steadily increased to accommodate the war needs of the United States Navy during the late 1930s and 1940s. The Surveillance, Epidemiology, and End Results (SEER) Program data regarding the incidence of malignant mesothelioma between 1973 and 2000 (Fig. 47.2) depicts a trend that correlates with this exposure pattern and the known latency of disease.

Asbestos continued to be used in manufacturing for many years. In the United States, it wasn't until 1986 that the Toxic Substance Control Act addressed the health risks of asbestos, giving the Environmental Protection Agency (EPA) broad authority to regulate the manufacture, use, distribution in commerce, and disposal of the carcinogenic substance.

When one considers the timing of these federal regulations, the latency of the disease, the geographic distribution of asbestos exposure,

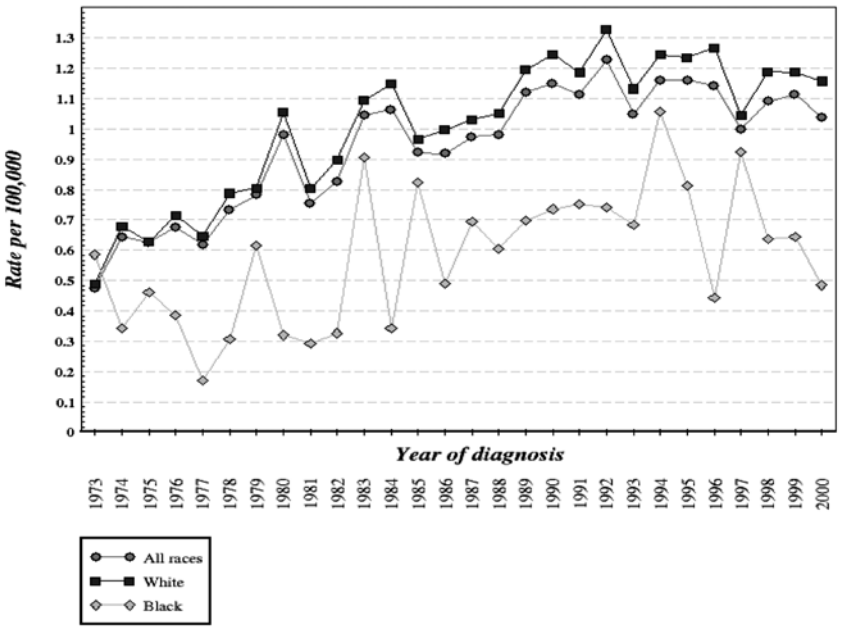


Figure 47.2. Surveillance, Epidemiology, and End Results (SEER) incidence age-adjusted rates for malignant mesothelioma, nine registries, 1973–2000. [*Source*: SEER Program (www.seer.cancer.gov). SEER Stat Database: Incidence—SEER Nine Registries Public Use, November 2002 submission (1973–2000). National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2003, based on the November 2002 submission.]

and the history of asbestos use, it is no coincidence that BWH has become an epicenter of treatment for MPM.

The 1980s: Diagnosis and Recognition of the Disease

The Sydney Farber Cancer Institute was founded in 1949, and originally treated only childhood cancers. In 1969 it expanded its mission to treat adult malignancies, and received federal designation as a regional comprehensive cancer center in 1973. It was renamed the Dana-Farber Cancer Institute in 1983. Located in Boston, Massachusetts, across the street from both the Peter Bent Brigham Hospital (which became the Brigham and Women's Hospital in 1980), and the Harvard Medical School, it soon became a major referral center for both aggressive and unusual malignancies within the New England area. Mesothelioma was among these cancers.

In March 1980, Karen Antman et al (11) published in the *American Journal of Medicine* the experience with the first 40 malignant mesothelioma patients treated at the Sydney Farber Cancer Institute. These patients had been treated between 1965 and 1978. Thirty-four of the patients had the pleural form of the disease and six patients had peritoneal mesothelioma. Sixty-three percent of these patients either

reported an asbestos exposure or were employed in New England shipyards, generally during World War II. In this series, Adriamycin (doxorubicin hydrochloride)-containing chemotherapy regimens induced a partial remission in 40% of the previously untreated patients. Yet, despite these remissions, the majority of patients (78%) ultimately died of local disease. Subtotal resection in this series and others (12) resulted in prolonged survival. Specifically, the 10 patients in Antman et al's review who underwent subtotal resections had a median survival of 15 months, compared to 8.5 months for the 20 patients who underwent only diagnostic operations and other treatments. Further analysis revealed that the median survival was a mere 4.2 months for patients who were diagnosed with limited disease but chose only supportive care.

Though Stout and Murray had distinguished mesothelioma from sarcoma in the 1940s, the treatment for the two diseases remained quite similar. Based on the findings published in their 1980 paper, however, Antman et al concluded that mesothelioma was sufficiently different from sarcomas to warrant treatment as a separate entity. Notwithstanding the biases inherent in this type of retrospective review, their evidence suggested an advantage to aggressive intervention. Therefore, the authors advocated a multimodality approach incorporating maximal surgical resection with adjuvant chemotherapy and radiation therapy.

In 1984, Antman organized a prospective multimodality protocol for malignant pleural mesothelioma at DFCI. This ambitious protocol started with an extrapleural pneumonectomy, as had been previously described by both Worn and Butchart. When possible, chemotherapy was started 4 to 6 weeks after surgery. Chemotherapy consisted of cyclophosphamide at a dose of 600 mg/m^2, combined with Adriamycin 60 mg/m^2, to a cumulative dose of 450 mg/m^2. After 1985, patients also received cisplatinum at 75 mg/m^2 (CAP chemotherapy). Radiation directed at previous sites of bulky disease was given to a dose of 5500 rad after the chemotherapy.

The accurate pathologic diagnosis of malignant mesothelioma also proved to be a barrier to treatment development. The distinction between lung adenocarcinoma and MPM is an important surgical issue, as surgical treatment of these two illnesses is radically different. Stimulated by the need to differentiate between these two histologically similar tumors, the pathology department of BWH drew on the large source of explanted tumors at our institution. In 1987, the department showed that staining for mucin and carcinoembryonic antigen and a predominantly peripheral pattern of staining for keratin proteins were highly characteristic of lung adenocarcinoma and allowed the distinction from malignant mesothelioma (13). In 1988, the department identified monoclonal antibodies to AE1/AE3 keratin proteins as being a sensitive method for the pathologic diagnosis of the sarcomatoid form of diffuse MPM (14). Further work in 1990 showed that the monoclonal antibody ME1 was reactive in frozen tissue sections with normal mesothelial cells and the epithelial type of malignant mesotheliomas (15). The ability to differentiate MPM from lung adenocarcinoma, sarcomas, and other pleural diseases made the BWH pathology depart-

ment a major referral center for tissue blocks from around the world. In turn, this process facilitated the additional accumulation of pleural mesothelioma cases from areas outside of New England.

The Early 1990s: Development of Multidisciplinary Expertise

Surgery Staff

The Brigham and Women's Hospital had a limited experience with extrapleural pneumonectomy for MPM from 1980 to 1987 (Fig. 47.3). Several cardiothoracic surgeons participated in these early efforts. Similar to other institutions, the initial experience with the operation was associated with high perioperative mortality and few long-term survivors. After the board of trustees created the Division of Thoracic Surgery at BWH in 1988, however, experience dramatically accelerated. This separate academic division was to be dedicated to the care of patients with noncardiac thoracic diseases. The work of this surgical division began with David Sugarbaker's return to Boston from his Toronto General Hospital thoracic surgical training. The Toronto program had become one of the most sought after thoracic surgical residencies in North America during the 1980s. A large and dynamic faculty, under the direction of Griffith Pearson, had developed a rich clinical practice, which included the first successful human lung trans-

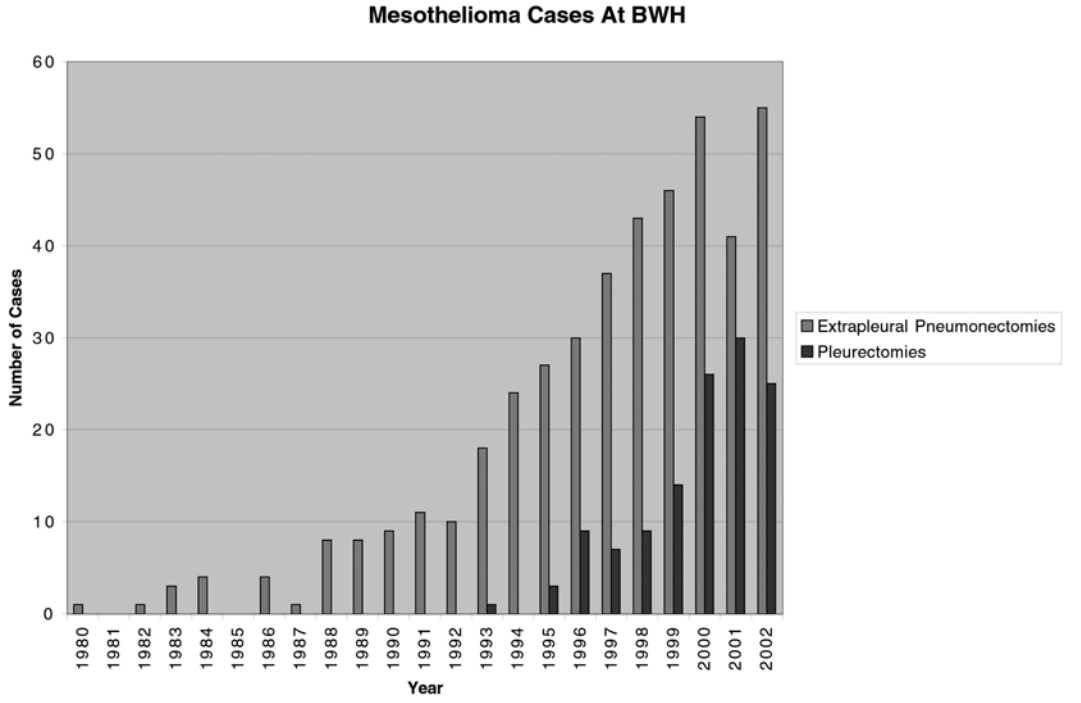


Figure 47.3. Mesothelioma cases at Brigham and Women's Hospital from 1980 to 2002 by type of resection.

plants, extensive esophageal surgery, lung cancer surgery, and the application of extrapleural pneumonectomy for MPM.

Steven Mentzer, a second alumnus from the Toronto program, joined Dr. Sugarbaker in 1990 and the two surgeons produced a dramatic increase in the volume of noncardiac thoracic operations performed at BWH, including extrapleural pneumonectomies. Over the next 12 years, they were joined by surgeons Malcolm DeCamp, Jr., David Harpole, Scott Swanson, Raphael Bueno, Jeanne Lukanich, Michael Jaklitsch, Yolonda Colson, Philip Linden, Lambros Zellos, and Michael Chang. Thus, only 12 surgeons have contributed to the BWH experience with extrapleural pneumonectomy, preserving the uniformity of the operation. At the same time, this group of surgeons congregating within a single institution sped the process of technical and clinical modifications, which reduced the expected operative mortality in a short period of time. Drs. Harpole, DeCamp, and Swanson currently lead thoracic surgical programs at other institutions, leaving nine full-time attending surgeons at BWH.

Intraoperative Expertise

Many contributions to the development of the modern extrapleural pneumonectomy as it is currently performed at BWH, were made through extensive discussions about surgical technique within the extended international thoracic surgical community. It has always been a goal of the members of this division to extensively describe potential pitfalls of the operation to all surgeons interested in learning this surgical technique. This information has been disseminated through written and oral presentations, with detailed illustrations. An early forthright discussion of our operative technique, as well as some of the technical difficulties with the operation, appeared in the 1992 publication by Sugarbaker et al (16).

As the operation begins, the patient, with a double-lumen endotracheal tube in place, is administered general anesthesia. The patient is placed in the lateral decubitus position. An extended posterolateral thoracotomy incision is made over the course of the sixth rib. A subperiosteal resection of the sixth rib is performed, and a plane is developed between the parietal pleura and the overlying rib cage.

The extrapleural dissection is begun superiorly toward the apex of the lung using both blunt and sharp techniques. Dissection is then begun in a similar fashion inferiorly and laterally to the sulcus between the pleura and the diaphragm (Fig. 47.4). The mediastinal pleura is then separated from the underlying structures down to the level of the azygos vein on the right and beneath the aortic arch on the left. Care is taken to prevent avulsion of the internal mammary vessels, the subclavian artery, and the azygos vein, as well as to keep the pleural envelope intact. During blunt dissection of the pleura in the left paravertebral sulcus, care must be taken to identify the correct plane. An incorrect retroaortic plane can produce bleeding from avulsing intercostal vessels.

The pericardium is opened and the serosal surface is inspected to ensure there is no direct invasion of tumor into the pericardial space.

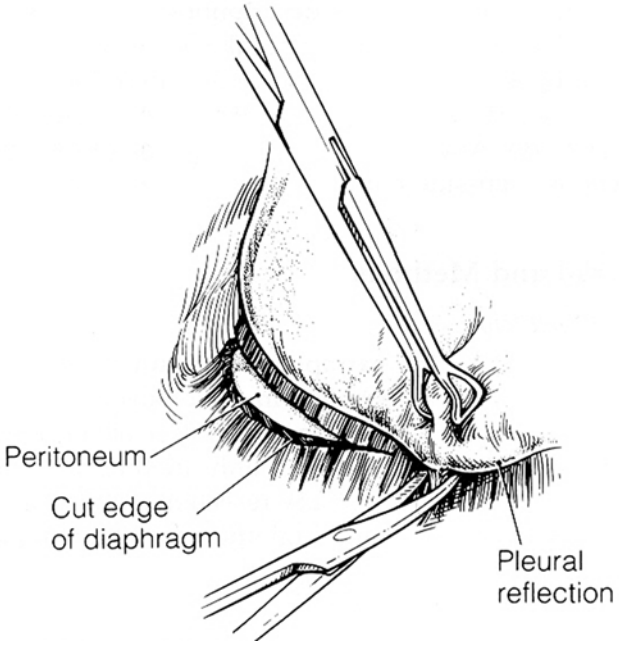


Figure 47.4. Extrapleural pneumonectomy: dissection inferiorly at the diaphragm (see text for details).

The diaphragm is divided in a circumferential fashion close to the chest wall. Care is taken when dividing the lateral bands of the diaphragm to preserve the underlying peritoneum. The diaphragm is separated from the peritoneum up to the lateral border of the pericardium. The crus of the diaphragm is divided in such a way as to prevent buttonholing of the inferior extent of the posterolateral pleura as it extends into the posterior diaphragmatic sulcus. The phrenic vessels are divided along the undersurface of the diaphragmatic crus.

The phrenic nerve is divided, and the pericardium is opened back to the level of the pulmonary vessels (Fig. 47.5). The pulmonary artery is dissected free from surrounding structures within the pericardium on the right, and just outside the pericardium on the left. Once the pulmonary artery has been divided, the pulmonary veins are likewise isolated from surrounding structures and divided. The posterior pericardium is mobilized to the level of the bronchus. The bronchus is divided with a heavy gauge bronchial stapler, and the specimen is passed off. A frozen section is obtained on the bronchial margin, and other areas of suspicion for margin involvement by tumor. A pericardial fat pad or pericardial flap is used to buttress the bronchial stump. The diaphragm and the pericardium are reconstructed with Gore-Tex patches.

Specific details learned through experience were highlighted in the 1992 description (16), including the importance of the careful dissection of the internal mammary vessels. These vessels, which are often tightly adherent to the pleura, can be inadvertently avulsed with posterior retraction of the tumor-filled parietal pleura. The placement of a

nasogastric tube to facilitate the identification of the esophagus was included. Specific dissection techniques to laterally divide the diaphragm while keeping the underlying peritoneum intact were described. The recommendation to divide the pulmonary artery trunk within the pericardium on the right and outside the pericardium on the left were included. Finally, specific details regarding the diaphragm and the structures piercing that muscle were given.

We have seen an evolution of the BWH techniques over the past decade. Early publications described the use of a running monofilament suture to anchor the prosthetic patches. This was abandoned when the beating action of a posteriorly displaced left atrium onto a Prolene knot produced an atrial laceration. The patches are now sewn into place with a soft braided permanent Ethibond suture leaving the knots outside the pericardium. Likewise, in our early experience, we would place a prosthetic pericardial patch only on the right side, since we believed that cardiac herniation was not possible on the left side. After a small number of cases of an entrapment syndrome of epicardial granulation tissue following heated chemotherapy, we changed our practice and now place pericardial patches for all patients (17). We observed a pattern of recurrence at previous chest tube sites, especially if the patient had been treated with talc poudrage in the past and the chest tube had been in place for greater than 2 days. In response, we added the prophylactic excision of all previous pleuroscopy and chest tube sites.

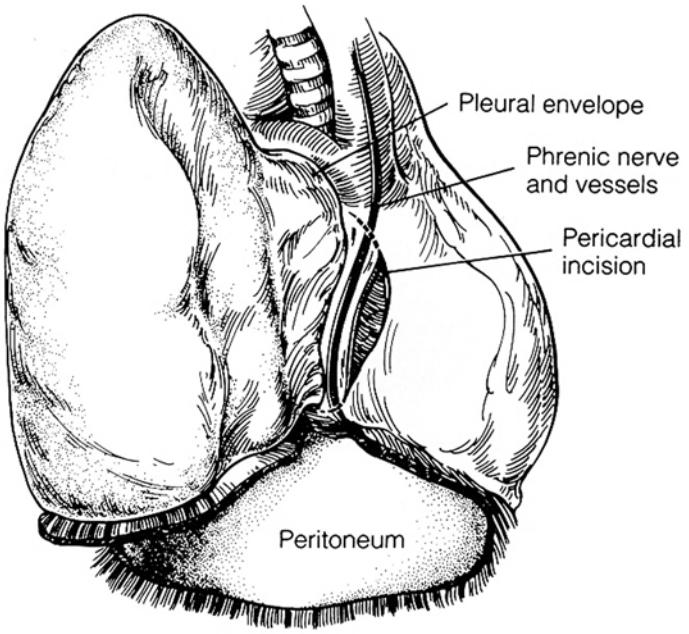


Figure 47.5. Extrapleural pneumonectomy: overall dissection (see text for details).

Diaphragmatic patch rupture has been a vexing problem that requires urgent reoperation as soon as it is recognized. In our earliest reconstruction attempts, we used O-Vicryl sutures anchored in the lateral remnant of the diaphragm muscle to loosely hold down the peritoneum; we placed an impermeable patch only if there was a peritoneal defect (16). The reefing technique was quickly abandoned in favor of patching all patients, with lateral sutures still in the lateral diaphragmatic remnant or around the lower ribs. Sugarbaker, using a leather-working awl that could easily be sterilized, developed a simpler and more reliable lateral anchorage system for the diaphragmatic patch. Loops of suture material passed through the lateral edge of the patch were then brought through the chest wall with the awl, where they were passed through a small postage stamp–sized patch of the same material and a sterile plastic button with the help of two angiocaths. The loop of suture was then tied down to itself onto the button, producing excellent lateral displacement of the patch. We have not recognized a lateral diaphragmatic rupture since adopting this system. Medial ruptures posterior to the pericardial edge and anterior to the thoracic spine have continued to be an infrequent problem. These have been minimized by three techniques: (1) a suture anchoring the patch to the anterior spinal ligament, (2) a tongue of extra patch material folded inferiorly along the lumbar spine in simulation of the diaphragmatic crus, and (3) a composite of two patches of 2-mm Gore-Tex stapled together in the middle with a TA stapler to create a dynamic patch at the center with less tension at the lateral suture lines. This last technique allows the prosthetic patch to "give" without rupture if the patient experiences abdominal distention.

The Division of Thoracic Surgery has extensively used the talent of Marcia Williams as a surgical illustrator, since accurate surgical atlas figures had not been developed for this operation. Illustrations were created from firsthand observation within the operating room. These illustrations have substantially contributed to the understanding of the magnitude of the operation for surgeons as well as nonsurgical care providers. The illustrations in this chapter are examples of her work.

Postoperative Care

The development of the BWH program for surgical care of the pleural mesothelioma patient has benefited from the input of all allied health professionals as well as the thoracic surgeons. Our division has always placed a high priority on a weekly quality assurance meeting attended by all members of the thoracic team. At this regularly scheduled meeting during the regular workweek, nurses, residents, fellows, nutritionists, surgical data managers, pharmacists, social workers, and attending thoracic surgeons discuss patient management issues. It has been our practice to close the operating rooms during this meeting to ensure that all members of the team are in attendance. This quality assurance meeting has significantly contributed to preoperative patient education, postoperative care, and intraoperative management.

Operative mortality following extrapleural pneumonectomy at BWH has consistently declined with increasing experience. This mortality was initially reported as 6% following the first 31 patients (18). This dropped to 5.8% after 52 patients (19), to 5.0% after 120 patients (20), to 3.8% after 183 patients (21), and to 3.4% after 328 patients (Sugarbaker, personal communication). Our nurses and residency staff have become experienced at recognizing complications early and differentiating a normal convalescence from an abnormal convalescence. This recognition of subtle early signs of complications has produced the statistical phenomenon of a simultaneously decreasing perioperative mortality rate with an increasing perioperative morbidity rate. The recognized overall postoperative morbidity rate rose from an initial report of 19% in 1991 (18), to 60.4% in 2003 (personal communication). This suggests that the entire multidisciplinary team had become more adept at recognizing morbidity, and intervening in an aggressive manner to stave off mortality.

The low perioperative mortality rate at BWH for an extrapleural pneumonectomy is dependent on several factors. Technical mastery of the operation has limited the operative time. Equally as important, anesthetic management has progressed sufficiently to anticipate perioperative hemodynamic changes, and reliably extubate the patient soon after emergence from anesthesia. Experience within the nursing staff and surgical residency staff has enabled the identification of postoperative complications early in their course. This latter aspect has been made possible by the close-knit physical location within the hospital of individual perioperative units. The thoracic surgical operating rooms are located within immediate proximity to each other, facilitating the intellectual input of more than one attending surgeon in a given case. We have a dedicated thoracic anesthesia staff and thoracic perioperative nursing staff. The thoracic intensive care unit, intermediate care unit, and postoperative wards are all located on the same floor of the hospital building. Thus, a skilled and experienced perioperative staff with extensive thoracic surgical experience has been developed. For instance, one of the identified perioperative management issues is the need for emergent reopening of a thoracotomy incision and open cardiac massage in the face of sudden cardiac arrest. Closed chest compressions are ineffective in patients who undergo extrapleural pneumonectomy, particularly right-sided resection, since the heart may be displaced away from the thoracic spine and the sternum.

Objective Results of Surgical Advances and Resultant Discoveries

In 1991, Sugarbaker and colleagues (18) published their first case series of patients receiving multimodality therapy for malignant mesothelioma. This retrospective review of 31 patients undergoing extrapleural pneumonectomy and subsequent adjuvant therapy demonstrated that the operation could be performed with acceptable rates of morbidity and mortality (19% and 6%, respectively), which was much improved from earlier series (45% and 31% in 1976, and 24% and 9% in 1986). No meaningful long-term survival assessment could be made.

These promising results of the BWH program reflected not only the refinement of surgical skill and improvement in perioperative care, but also the identification of prognostic variables with a consequent improvement in patient selection. As was recognized in 1976 by Burtchart et al (8), the success of extrapleural pneumonectomy was largely dependent on selection of the most appropriate surgical candidates. Sugarbaker realized the importance of this concept and focused much of his attention on improving staging and defining operative candidates. In the case series published in 1991 (8), the authors noticed trends toward improved survival in the subset of patients with negative histologic margins (Fig. 47.6). Though not statistically significant, this trend was encouraging. The determination of negative histologic margins required sampling at least 14 areas of the pleura in a protocol developed by Joseph Corson of the pathology department.

In 1993, Sugarbaker et al (22) updated their experience after 52 patients had been treated with extrapleural pneumonectomy in a trimodality setting. This analysis demonstrated significantly longer survival in patients with epithelial histology and node negative disease (Fig. 47.7A). Subset analysis of tumor size, gross residual disease, positive margins, and diaphragmatic tumor extension was still not statistically correlated with survival. Based on these findings, a BWH pathologic staging system was proposed.

The original BWH staging system had four stages. Stage I comprised tumors confined within the pleural envelope and without lymph node involvement. Stage II, which would be modified in a few years, also consisted of tumors within the pleural envelope, but with either intraparenchymal (N1) or mediastinal (N2) lymph nodes involved with tumor (22). Stage III disease was made up of locally aggressive and unresectable tumors beyond the pleural envelope that had invaded into the mediastinum or chest wall, or through the diaphragm, or involved contralateral (N3) nodes. Stage IV disease was defined by distant metastases.

The concept of a pathologic staging system quickly led to efforts to accurately stage patients clinically and radiographically prior to

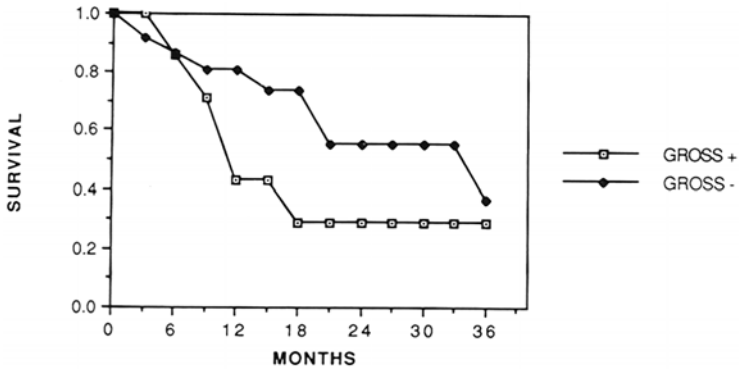


Figure 47.6. Impact of margin status on survival after extrapleural pneumonectomy (EPP) (8).

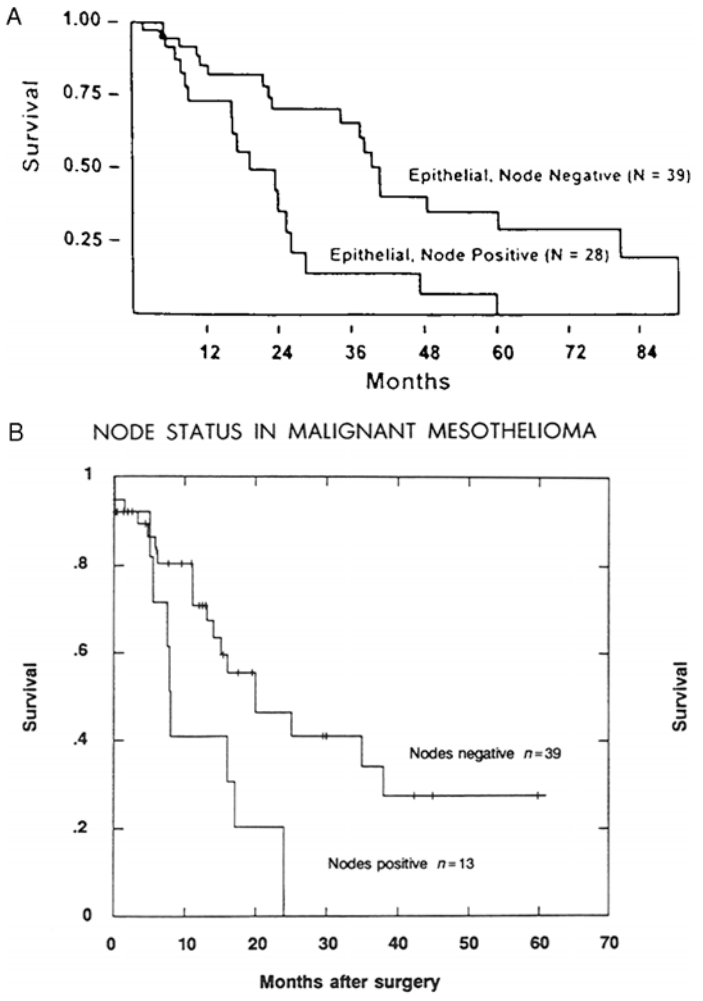


Figure 47.7. Impact of nodal status on survival after EPP in an early (A) (22) and later (B) study (20) from Brigham and Women's Hospital.

attempted surgical resection. A prospective study of chest CT and chest magnetic resonance imaging (MRI) to predict resectability of MPM recruited 34 patients referred for possible extrapleural pneumonectomy (23). All patients underwent both CT and MRI studies preoperatively. At the time of surgery, potential unresectable regions as determined by imaging were explored first and surgery terminated if resection was not possible. Scans that suggested transdiaphragmatic invasion were verified by minimally invasive imaging of the undersurface of the diaphragm in the operating room. Sensitivity for both CT and MRI was above 90% in all regions; MRI was found to be 100% sensitive in predicting unresectability due to diaphragmatic and chest wall involvement, whereas CT was less sensitive (94% and 93%, respectively). For mediastinal invasion, CT was 100% sensitive and MRI had a sensitivy of 92%. Advanced disease precluding extrapleural pneu-

monectomy in referred patients was a vexing problem at this time, as evidenced by the observation that only 24% of these 34 patients were found to be resectable. Since both CT and MRI contributed substantially to avoiding an extended thoracotomy incision in patients who were unresectable, both tests became part of our standard preoperative workup.

Our extrapleural pneumonectomy experience was reanalyzed in 1996, after 120 patients had been treated (20). This report confirmed the favorable prognostic factors of epithelial cell type and lack of nodal disease (Fig. 47.7B). The small numbers available for analysis did not yet allow differentiating a prognostic difference between N1 and N2 nodal disease. In addition, the previously published staging criteria were validated, with survival stratifying according to the BWH pathologic stage. Though there was no direct comparison made to a nonsurgical control group, the data suggested a survival benefit with trimodality therapy resulting in a median survival of 21 months as compared to 4 to 12 months in the untreated population.

Parallel with the ongoing development of surgical expertise, medical oncologists at BWH/DFCI were advancing knowledge and building on their experience. The initial adjuvant chemotherapy program for MPM at the BWH was a combination of 600 mg/m^2 cyclophosphamide, 60 mg/m^2 doxorubicin, and 70 mg/m^2 cisplatin (CAP) chemotherapy. This chemotherapy was planned for every 3 weeks for four to six cycles. It proved to be a difficult adjuvant regimen, however, and for the 88 patients who received this therapy, a median of four cycles was delivered, with a range between one and eight cycles (21).

In 1997, the CAP chemotherapy regimen was changed to a carboplatin and paclitaxel regimen, through the collaborative efforts of Gary Strauss of the medical oncology department, Elizabeth Baldini of radiation oncology, and David Sugarbaker of thoracic surgery. This treatment plan began with extrapleural pneumonectomy. Two cycles of chemotherapy given 3 weeks apart, with two cycles of Taxol (200 mg/m^2 as a 3-hour continuous infusion) and carboplatin [target area under the curve (AUC) 6 mg/mL $\times$ min, IV bolus following Taxol infusion] was started between 4 and 12 weeks postoperatively. Following these two cycles, the patient received thoracic radiation with concurrent weekly Taxol (60 mg/m^2 as a 3-hour continuous IV infusion) given weekly during radiation, for up to 6 weeks. Finally, two additional cycles of Taxol (200 mg/m^2) and carboplatin (target AUC 6 mg/mL $\times$ min) completed the adjuvant therapy (21). This multimodality treatment plan was better tolerated than the previous doxorubicin-based regimen.

Radiation therapy ideally started 3 to 4 weeks following cycle number two of chemotherapy. Radiation was given in five fractions weekly, once per day, to a total dose of 40.5 Gy. This was delivered in 1.5-Gy fractions over $5\frac{1}{2}$ weeks. If a boost dose was delivered to treat a focal positive margin was given, it was administered in 1.8-Gy fractions, yielding a total of boost dose of 14.4 Gy and total cumulative dose of 54.9 Gy. The initial clinical target volume was the entire hemithorax on the involved side. Field borders were defined superiorly by the clearing the first rib.

Laterally, the bony rib cage was cleared by 1.5 cm. Superolaterally the shoulder joint was blocked such that there was a 1.5-cm margin on the bony rib cage. The medial border was 3 cm over the midline to cover the mediastinum. Inferiorly, the field extended at least 1 cm below the diaphragmatic reflection of the pleura, often at the bottom of T12 or L1 vertebrae. A liver block was added at 30 Gy. The liver block extended at least 1 cm above the reconstructed diaphragm. A full bolus of radiation therapy was used to cover the incision as well as any drain or pleuroscopy sites. If a part of the incision or drain site was out of the photon field, that region was treated with a dose of 21 Gy delivered in three fractions (700 cGy × 3) with en face electrons to a depth defined by the thickness of the chest wall as measured by CT or MRI scan.

Although it took from 1980 to 1996 to accumulate 120 patients, 63 additional patients were treated over the next 3 years. The analysis of these 183 patients was published in 1999 (21). Four significant variables of improved survival were identified by log rank test: female sex (p = .03), epithelial cell type (p = .0001), negative resection margins (p = .02), and lack of extrapleural nodal involvement (p = .004). In this analysis, we considered metastases to the extrapleural peridiaphragmatic nodes as N2 disease, since they lay outside the pleural envelope and thus drained directly to the paraesophageal and internal mammary nodes and not through the lung hilum.

These four variables were then entered into a Cox proportional hazards model, which no longer identified gender as a statistically significant variable. The most important predictor of outcome became histologic subtype, followed by N2 nodal disease and positive resection margins (Table 47.1).

Our previously published pathologic staging system was applied to this large group of patients, and survival was significantly stratified by stage (p = .048). Median survival intervals for patients with stage I (n = 66), II (n = 41), and III (n = 69) disease were 25, 20, and 16 months, respectively. The identification of three main influences on survival by the Cox proportional hazards model led us to revise our previous staging system to account for positive margins and extrapleural nodes. In the revised staging system, stage I was unchanged, stage II included tumors limited by the pleural envelope but with tumor involving the resection margins or disease in the N1 intraparenchymal nodes, and stage III tumors either penetrated beyond the pleural envelope or involved the N2 mediastinal nodes (21). These revisions improved the survival stratification of our cohort of 183 patients (p = .0011, Fig. 47.8). This same group of patients was not stratified by the international

Table 47.1. Multivariate analysis of 183 extrapleural pneumonectomy (EPP) resections

Variable	*n*	OR	CI	*p* value
Mixed or sarcomatous cell type	**73**	**3.0**	**2.0–4.5**	**<.0001**
Positive resection margins	**110**	**1.7**	**1.2–2.6**	**.0082**
Metastatic extrapleural nodes	**40**	**2.0**	**1.3–3.2**	**.0026**

n, number of patients; OR, odds ratio; CI, confidence interval.

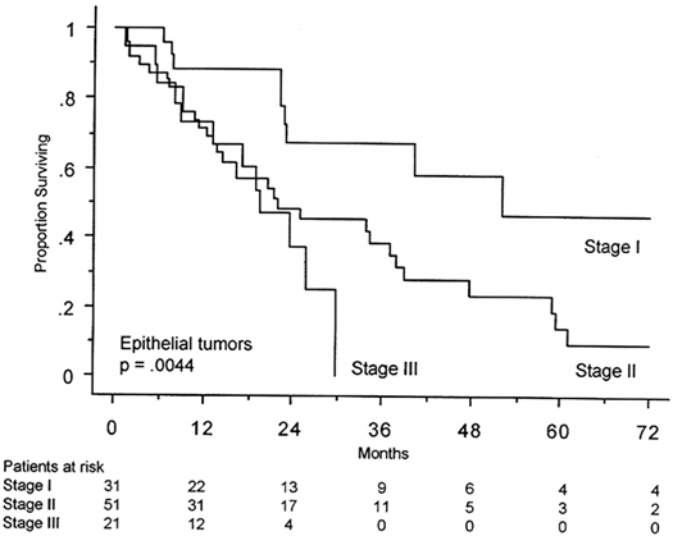


Figure 47.8. Results in malignant mesothelioma patients with epithelial histology having EPP at Brigham and Women's Hospital (21).

tumor, node, metastasis (TNM) staging system for mesothelioms (24) ($p = .31$) or by the Butchart staging system (37) ($p = .09$).

A subset of 31 patients within this group of 183 (17%) had the epithelial subtype, negative resection margins, and negative extrapleural nodal status. These stage I patients (by the revised Brigham staging system) had a 51-month median survival with a 2-year survival of 68% and a 5-year survival of 46%. This was encouraging data after more than a decade of treatment refinement.

The International TNM Staging System (24) and the Butchart staging system (8) failed to stratify survival when applied to our cohort of patients. The TNM staging system placed 8% of our cohort into the stage I category, 11% into stage II, 78% into stage III, and 3% into stage IV. Since the very large majority of patients were categorized as stage III, it becomes difficult to identify patients with different tumor characteristics, which is necessary to stratify survival. In addition, the T descriptor was not a statistically significant predictor of survival on log rank testing, reflecting the inability of this system to describe the biologic behavior of mesothelioma when applied to our patient population.

The staging system proposed by Butchart similarly did not significantly stratify survival in our patients. A small number of patients were categorized as having stage III disease ($n = 5$, 3%). The separate survival implications of pleural envelope penetration and nodal involvement are not taken into account by this early staging system, reflected by the majority of our patients being placed in the stage II category.

The revised Brigham Staging System has proven useful to us. This is an easy-to-use, surgically based staging system, and stratifies patients by ability to completely remove the tumor and involved regional lymph nodes. Observer bias may exist because this staging system orig-

inated at our institution and was based on an earlier cohort. Validation by other institutions is required to judge the utility of this clinical staging system.

Late 1990s: Development of Intraoperative Bicavitary Heated Chemotherapy

Despite these advances in surgical technique and refinement in prognostication and patient selection, the unfortunate fact remained that nearly all patients eventually died of their disease within 10 years of the operation. Recurrences appeared to result by direct extension from the ipsilateral hemithorax. Therefore, in the second half of the 1990s, the BWH group embarked on a new approach to multimodality therapy.

The major treatment plan of the previous 10 years had started with extrapleural pneumonectomy (EPP) because mesothelioma was predominantly a locoregional disease, and much of the early morbidity was from local spread. Since most patients died as a result of the primary cancer invading the diaphragm, chest wall, and mediastinal organs, initial surgical debulking was chosen prior to the initiation of chemotherapy in order to reverse the aggressive natural progression of this disease.

In 1997, Baldini et al (25) published a detailed retrospective review of 49 patients who underwent EPP and some combination of adjuvant chemotherapy and/or radiotherapy with a focus on defining patterns of failure. In this series, overall median survival was 22 months, and 34% attained 3-year survival. Resection margins were microscopically positive in 61% of patients and lymph nodes positive in 29%. Of the 54% of patients with recurrences, 67% percent had the first recurrence within the ipsilateral hemithorax, and 50% had recurrence at some time within the abdomen.

Three potential sources of tumor cells are positive resection margins, free intrathoracic cancer cells that have penetrated the pleura prior to resection, and spillage of tumor at the time of resection. In the abdomen, shed tumor cells have been detected on fluid cytology prior to dissection in 25% of patients amenable to curative resection (26). Surgical dissection causes a dramatic increase in the rate of intraperitoneal cancer cell shedding, up to 60% (27). These free cancer cells were shown to be viable and able to implant (28). These cells can become attached to the cavity surfaces within minutes, and cannot be dislodged with irrigation. They can be entrapped by fibrin accumulations, and their growth stimulated by healing wound growth factors (29). Delayed systemic chemotherapy may have no effect on tumor deposits embedded in fibrin. We considered the use of intraoperative chemotherapy as a potential solution to this problem.

The potential role of intracavitary chemotherapy as a method of improving regional control had been studied previously in a variety of abdominal malignancies. The local application of chemotherapy allows high cytotoxic levels to reach residual tumor cells by diffusion

without the side effects of high-dose systemic chemotherapy. Intracavitary chemotherapy with or without hyperthermia had been favorably reported in the literature.

In 1992, Markman and Kelsen (30) of Memorial Sloan-Kettering Cancer Center, reported the use of intraperitoneal (IP) chemotherapy in the treatment of MPM. Intraperitoneal cisplatin and mitomycin were infused through a peritoneal catheter left in place after surgical debulking. Cisplatin (100 mg/m^2) was given every 28 days and mitomycin (5–10 mg) was given 7 days after each IP cisplatin dose. A maximum of only five courses of cisplatin could be administered because of catheter failure or disease progression. While the median survival for the 19 patients treated in this manner was only nine months, 4 patients (21%) lived for more than 3 years from the initiation of therapy and two patients were clinically disease-free more than 5 years from the start of the intraperitoneal treatment.

Alberts et al (31) published a prospective randomized trial in the *New England Journal of Medicine* in 1996. Intraperitoneal cisplatin was compared to intravenous cisplatin in patients with stage III ovarian cancer following cytoreductive surgery. Among the 654 randomized patients, the estimated median survival was significantly longer in the group receiving intraperitoneal cisplatin (49 months) than in the group receiving intravenous cisplatin (41 months).

The Memorial Sloan-Kettering Cancer Center in New York has completed two studies of intrapleural chemotherapy following radical pleurectomy for MPM (32–34). Intrapleural chemotherapy consisted of cisplatin 100 mg/m^2 and mitomycin 8 mg/m^2. This treatment modality was well tolerated, with only two patients suffering grade 4 renal toxicity out of 28 patients treated. The pharmacokinetics of the drugs were similar to that seen with intraperitoneal chemotherapy. The most common site of recurrence, however, remained the ipsilateral hemithorax in these studies. In our analysis of this work, we felt that pleurectomy would leave more residual tumor than an extrapleural pneumonectomy, and believed that a higher dose of intracavitary cisplatin might be achieved.

The use of hyperthermia as an anticancer treatment stems from observations from about a hundred years ago and from tumor regression after high fever. Studies in the past 40 years have shown that tumor cells have a much higher sensitivity to heat than normal cells (35). Heat increases cell permeability, alters cellular metabolism, and increases membrane transport of drugs.

Stehlin et al (36) used hyperthermic melphalan to perfuse the limbs of patients with melanoma of the extremities. The 5-year survival of the 30 patients treated with hyperthermic melphalin compared favorably to the 27 patients treated with normothermic melphalin (80% vs. 20%). This observation has been confirmed by researchers at the National Cancer Institute of Milan, Italy (37). The 5-year survival of 140 patients with stage IIIA melanoma of the extremity treated with hyperthermic chemotherapy was 51%, compared to 16% for 297 patients with similar stage treated by conventional methods. This suggests a synergistic effect of hyperthermia and chemotherapy.

Van Ruth et al reported using doxorubicin and cisplatin in intraoperative heated chemotherapy protocols for malignant mesothelioma. They found that doxorubicin was able to penetrate into the intercostal muscle specimen. In their hands, intracavitary lavage with heated doxorubicin and cisplatin was a safe procedure with the advantage of high intrathoracic cytostatic drug concentrations, while having limited systemic side effects.

Paul Sugarbaker, the director of surgical oncology of the Washington Cancer Institute at the Washington Hospital Center in Washington, DC, and the older brother of David Sugarbaker, chief of the thoracic surgery division of BWH, had accumulated extensive experience with heated intraperitoneal chemotherapy for peritoneal carcinomatosis of gastrointestinal malignancies as detailed in Chapter 49. His data stimulated the thoracic surgeons in Boston, who saw multiple similarities between peritoneal carcinomatosis and malignant pleural mesothelioma.

The decision to design a phase I dose-escalation trial of heated intraoperative cisplatin at the time of EPP had been supported by BWH/DFCI and the leadership of all professional groups who would be involved in patient care. The obstacles to be overcome were formidable. Protocols to maximize both patient and staff safety were designed by a multidisciplinary "heated chemotherapy" team, which met once a week to develop guidelines for this novel therapy. Ideas were actively sought from surgeons, anesthesiologists, pharmacists, nurses, scrub technicians, medical oncologists, and respiratory therapists to design the method of drug delivery and disposal in the operating room. Safety courses were required for all staff participating in the protocol. Special isolation rooms with chemotherapy precautions were prepared in the intensive care unit (ICU) with guidelines for disposal of patient contact items, which might be contaminated by cisplatin. The institutional review board (IRB)-approved protocol was rewritten to clearly delineate responsibilities for the surgeon, scrub tech, circulating nurse, anesthesiologist, perfusionist, and ICU nurse. Instructions were also written for postoperative cleanup and handling of spills, based on guidelines from the Occupational Safety and Health Administration (OSHA) and the Joint Commission on the Accreditation of Healthcare Organizations (JCAHO).

On September 17, 1998, representatives from the thoracic surgeons, thoracic anesthesia, operating room (OR) nursing and ICU nursing from BWH traveled to Washington Hospital Center to observe patients with peritoneal carcinomatosis treated with heated intraperitoneal chemotherapy infusion. This allowed multidisciplinary interaction with counterparts at the Washington Hospital Center. Technical points were learned, sketches were made, and extensive discussions about the safety and feasibility of this technique were held. This trip proved to be an invaluable source of information in the development of the intrathoracic heated chemotherapy perfusion protocol opened at BWH in the spring of 1999.

In this protocol, patients underwent EPP with the objective of complete cytoreduction of the cancer. Only 1 cm^3 or less residual disease

was permitted. A sodium thiosulfate bolus of $4\,g/m^2$ was given intravenously (IV) followed by a 6-hour infusion of $12\,g/m^2$. This drug is used to bind cisplatin that may have been absorbed into the vascular space to prevent systemic toxicity.

The diaphragm was removed during the EPP. This permitted access to both the abdomen and ipsilateral hemithorax for heated chemotherapy lavage. After the specimen was removed, but prior to reconstruction of the diaphragm and pericardium, a 1-hour lavage of escalating doses of cisplatin heated to 42°C bathed both body cavities. Urine output was maintained at 100 cc per hour during the lavage and for 1 hour afterward.

A temperature probe was placed in the esophagus, abdomen, and chest. A plastic sheet was sewn to the edges of the thoracotomy wound, draped over a retractor (Fig. 47.9). A slit in the plastic cover was made to allow the surgeon's double-gloved hand into the thorax to evenly distribute the perfusate. A perfusion circuit delivered the drug in heated peritoneal dialysate via a catheter in the pelvis, and a drain in the thorax collected the perfusate and returns it to the pump. A heat exchanger maintained the temperature of the lavage for 1 hour. A smoke evacuator was used to pull air from beneath the plastic cover

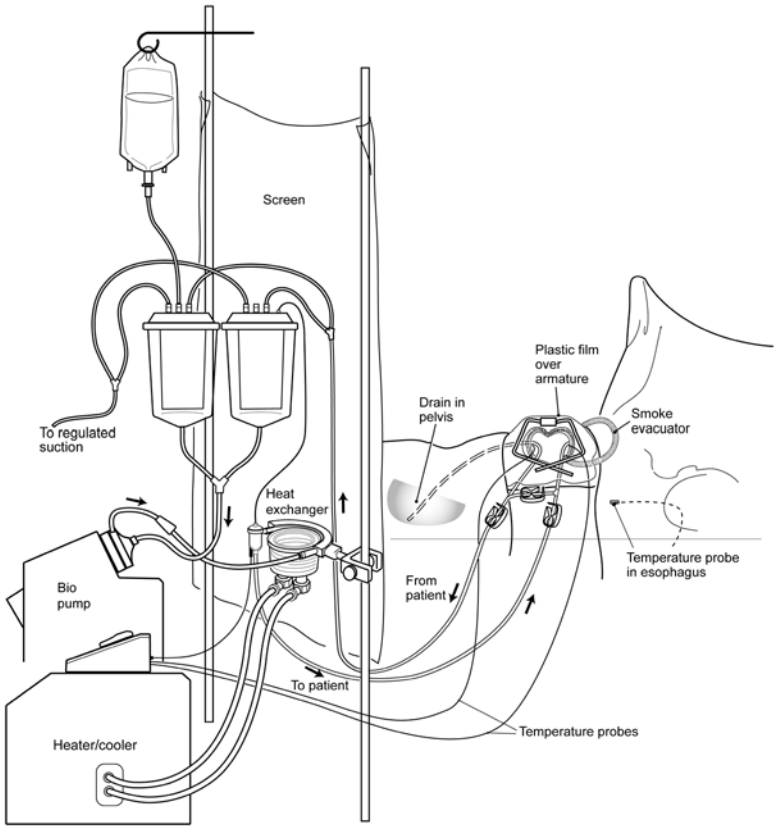


Figure 47.9. Technique of two cavity hyperthermic perfusion for malignant pleural mesothelioma (see text for details).

through activated charcoal, preventing any possible contamination of air in the operating room by chemotherapy aerosols.

Our initial circuit prototype used a roller pump. Overpressurization of the arterial tubing led to drug leakage. At the suggestion of our perfusionist, Daniel Fitzgerald, this circuit was modified to include a centrifugal pumphead.

The initial trial was a dose-escalation trial to determine the maximum tolerated dose (MTD) of heated bicavitary cisplatin. The dose started at 50 mg/m^2, and increased by 50 to 25 mg/m^2 after every three patients until two patients had an irreversible grade 1 renal toxicity. Pharmacokinetic blood and tissue specimens were collected every 10 minutes during the perfusion. Tru-Cut needle biopsies were obtained from the chest wall to determine depth of penetration.

Although final analysis of this protocol has not yet been published, preliminary observations were presented to the American Society of Clinical Oncology (ASCO) meeting in the spring of 2001. Unique aspects of this trial included the magnitude of surgery for cytoreduction (EPP), the bicavitary lavage (both abdomen and thorax), and the heating of the chemotherapy. Of the seventy patients enrolled, 50 completed the protocol. The MTD was significantly higher than in previous trials and the intrathoracic tissue levels of platinum obtained at the MTD were sixfold higher than at the commonly published dose of systemic chemotherapy. Operative mortality was 2%. Constrictive pericarditis was documented in 10% and required reoperation in 8%. Four of 50 patients had a prolonged intubation and 6% suffered grade II renal insufficiency. Grade II and grade III lymphopenia was noted in 33% and 8% of patients, respectively. Of note, 12% had technical complications including bleeding and patch failure. When compared to matched controls, there was an equivalent median length of stay of 9 days. When compared to a matched cohort of patients who underwent EPP alone, the data support the feasibility of the administration of intracavitary heated chemotherapy with comparable morbidity and mortality.

Influenced by Rusch et al (33), Roberts (39), and others, we began to offer radical pleurectomy for patients who were unsuitable for extrapleural pneumonectomy (Fig. 47.3). This included patients with a decline in their functional status and elderly patients. Furthermore, we would perform radical pleurectomy when the original operative intent had been to perform an EPP, but unresectable bulky tumor was found beyond the plane of resection. As a result, we gained experience with this operative alternative over the past decade.

Once our heated chemotherapy trial for EPP patients had been opened, and we had worked out many of the trouble spots, a second trial for pleurectomy patients was written. This also was a dose-escalation trial, but the doses were lowered for fear that there would be more intravascular absorption of the drug through the ipsilateral lung after the visceral pleurectomy.

In the spring of 2003, we presented our initial experience with intraoperative bicavitary hyperthermic cisplatin lavage at the time of radical pleurectomy for pleural mesothelioma at the meeting of the ASCO.

This was a phase I–II study that prospectively enrolled 60 patients with biopsy-proved extrapleural pneumonectomy, not considered to be candidates for extrapleural pneumonectomy. Forty-four of these patients underwent successful radical pleurectomy and a 1-hour lavage of the ipsilateral hemithorax and abdomen with dose-escalated cisplatin at 42°C. Sodium thiosulfate 16 g/m^2 was infused intravenously over 6 hours. The postoperative mortality was five of 44 (11%). The dose-limiting renal toxicity occurred at 250 mg/m^2, establishing the MTD at 225 mg/m^2. Significant cisplatinum was detected in lung and chest wall biopsies obtained at the time of lavage and was linearly related to the perfusate concentration. Interestingly, survival of these patients differed significantly depending on platinum dose. Low-dose patients (50–150 mg/m^2, $n = 9$) had a median survival of 6 months versus a median survival of 9 months for patients with middle doses (175–200 mg/m^2, $n = 8$), and a median survival of 19 months for patients treated at the MTD ($n = 23$, $p = .0017$). Neither age, preoperative forced expiratory volume in 1 second (FEV_1), nor adjuvant therapy accounted for the survival difference, suggesting that intraoperative bicavitary hypothermic cisplatin lavage may have a role to play for those unable to undergo extrapleural pneumonectomy.

The New Millennium and New Frontiers

Folate Antagonists

Despite the combined efforts of researchers throughout the world, the molecular events that ultimately led to the development of MPM still were not well understood by the year 2000. Our basic science laboratories applied screening differential display to explanted tissue samples preserved over the past decade in our tissue bank in an effort to identify how RNA expression in mesothelioma tumor cells differed from that of normal lung and pleura. These tissues were homogenized and the RNA extracted and amplified by the polymerase chain reaction. After electrophoresis, a display of bands of the gene products was displayed with the tissues lying side by side. This allowed the identification of 60 bands that were different between the tissues. One band, with 92% homology to the human α-folate receptor complementary DNA (cDNA), was highly expressed in 45 of 60 mesothelioma tissues studied.

The α-folate receptor is a glycoprotein on the cell membrane that binds folate and brings it within the cell for use in constructing the purines and pyrimidines, basic building blocks of RNA and DNA. Folate is essential for the rapidly dividing cell, and its absence may lead to megaloblastosis and premature cell death.

Other investigators had already noted that methotrexate, which is a chemotherapeutic agent that is a folate analogue and blocks folate metabolism, was one of the few agents that had a significant response to mesothelioma, a notoriously chemotherapy-resistant tumor (40). Two antifolate-based chemotherapy combinations emerged at the start of the new millennium: pemetrexed/cisplatin and raltitrexed/

cisplatin. In a phase I trial of pemetrexed/cisplatin, objective responses occurred in 5 of 11 (45%), and in a phase I trial of pemetrexed/carboplatin, responses occurred in 9 of 29 patients (31%) (40).

A phase III multinational trial randomized 456 patients with MPM to 75 mg/m^2 cisplatin with or without 500 mg/m^2 pemetrexed (41). This was the largest clinical trial ever conducted in the treatment of MPM, and the trial completed accrual in February 2001. Response rates were 41% in the pemetrexed/cisplatin arm, compared to 17% in the cisplatin arm ($p < .0001$). Median survival was significantly better when the antifolate was added (12 months vs. 9 months, $p = .02$). Pemetrexed is now marketed as Alimta by Eli Lilly, and has been approved in combination with cisplatin by the Food and Drug Administration for combination treatment of mesothelioma.

We have had the anecdotal experience of a patient who failed attempted EPP for MPM due to bulky tumor beyond the plane of resection. This patient was then treated in the Alimta/cisplatin trial at DFCI. The radiographic response was dramatic, and the patient successfully underwent EPP afterward. This experience may become a model for a multimodality treatment protocol using neoadjuvant Alimta/cisplatin for patients who appear radiographically unresectable.

Gene Ratios

Investigators at DFCI and in the Thoracic Surgery Division laboratories began to use gene microarrays to analyze mesothelioma tissues. Microarrays are cassettes that can simultaneously test for thousands of genes within a tissue sample. As an applied technique for diagnosis, however, we found it to have limited value in patient care because of the complex computational analysis required, the number of samples needed to draw statistically meaningful conclusions, the inability to independently analyze new samples without reference to additional samples, and the quantity of RNA required for such studies.

In 2002, Gordon et al (42), working within the Division of Thoracic Surgery, overcame these obstacles with the application of gene ratios. When a cell becomes neoplastically transformed (i.e., a tumor cell), changes typically occur in the expression of key genes. In simplistic terms, every cell can be thought of as expressing some benign and some malignant genes. The degree to which these genes are transcribed and subsequently translated into protein (i.e., expressed) can be quite different between normal and cancer cells.

Using a training set of 32 discarded MPM and lung adenocarcinoma samples collected in the tissue bank from 1993 to 2001, five genes were found to be highly expressed in MPM tissues and three genes were found to be highly expressed in lung adenocarcinoma (42). These eight genes can be used to express 15 pairs of ratios with an MPM-associated gene in the numerator and an adenocarcinoma gene in the denominator. Ratios >1 predict MPM and ratios <1 predict adenocarcinoma. These 15 pairs each proved to be between 91% and 98% accurate at predicting the correct histology of an additional 149 test tissue

samples. Accuracy was increased to 95% to 99% by using two or three ratios as a simple test.

The power of a gene ratio test extends beyond the ability to accurately differentiate between two tumor types. This test may be able to predict outcome in patients. To test this hypothesis, Gordon et al (43) defined two outcome groups (good and poor) based on known survival. They then used statistical methodology to correlate gene expression profiling data with survival outcomes to identify gene expression patterns that are markedly different between the two groups. From these data, they developed prognostic expression ratios that proved to be highly accurate in predicting treatment-related outcome in mesothelioma samples. A four-gene expression ratio test accurately predicted treatment-related patient outcome in mesothelioma independent of histology. This test may help stratify patients into treatment groups, which could optimize treatment strategies. It may limit the number of people who undergo radical surgery to those most likely to benefit. Finally, it may suggest a mechanistic pathway by which some tumors act more aggressively, and point the way to new therapies.

References

1. Connelly RR, Spirtas R, Myers MH, Percy CL, Fraumeni JF, Jr. Demographic patterns for mesothelioma in the United States. J Natl Cancer Inst 1987;78:1053–1060.
2. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960; 17:260–271.
3. Selikoff IJ. Air Pollution and Asbestos Carcinogenesis: Investigation of Possible Synergism. IARC Scientific Publication 1977:247–253.
4. Roggli VL, Sharma A, Butnor KJ, Sporn T, Vollmer RT. Malignant mesothelioma and occupational exposure to asbestos: a clinicopathological correlation of 1445 cases. Ultrastruct Pathol 2002;26:55–65.
5. Rom WN, Travis WD, Brody AR. Cellular and molecular basis of the asbestos-related diseases. Am Rev Respir Dis 1991;143:408–422.
6. Antman KH. Current concepts: malignant mesothelioma. N Engl J Med 1980;303:200–202.
7. Worn H. [Chances and results of surgery of malignant mesothelioma of the pleura (author's transl)], Thoraxchir Vask Chir 1974;22:391–393.
8. Butchart EG, Ashcroft T, Barnsley WC, Holden MP. Pleuropneumonectomy in the management of diffuse malignant mesothelioma of the pleura. Experience with 29 patients. Thorax 1976;31:15–24.
9. Baas P. Chemotherapy for malignant mesothelioma: from doxorubicin to vinorelbine. Semin Oncol 2002;29:62–69.
10. Weissmann LB, Antman KH. Incidence, presentation and promising new treatments for malignant mesothelioma. Oncology (Huntington) 1989;3:67–72.
11. Antman KH, Blum RH, Greenberger JS, Flowerdew G, Skarin AT, Canellos GP. Multimodality therapy for malignant mesothelioma based on a study of natural history. Am J Med 1980;68:356–362.
12. Legha SS, Muggia FM. Therapeutic approaches in malignant mesothelioma. Cancer Treat Rev 1977;4:13–23.

13. Cibas ES, Corson JM, Pinkus GS. The distinction of adenocarcinoma from malignant mesothelioma in cell blocks of effusions: the role of routine mucin histochemistry and immunohistochemical assessment of carcinoembryonic antigen, keratin proteins, epithelial membrane antigen, and milk fat globule-derived antigen. Hum Pathol 1987;18:67–74.
14. Montag AG, Pinkus GS, Corson JM. Keratin protein immunoreactivity of sarcomatoid and mixed types of diffuse malignant mesothelioma: an immunoperoxidase study of 30 cases. Hum Pathol 1988;19:336–342.
15. O'Hara CJ, Corson JM, Pinkus GS, Stahel RA. ME1. A monoclonal antibody that distinguishes epithelial-type malignant mesothelioma from pulmonary adenocarcinoma and extrapulmonary malignancies. Am J Pathol 1990;136:421–428.
16. Sugarbaker DJ, Mentzer SJ, Strauss G. Extrapleural pneumonectomy in the treatment of malignant pleural mesothelioma. Ann Thorac Surg 1992;54: 941–946.
17. Byrne JG, Karavas AN, Colson YL, et al. Cardiac decortication (epicardiectomy) for occult constrictive cardiac physiology after left extrapleural pneumonectomy. Chest 2002;122:2256–2259.
18. Sugarbaker DJ, Heher EC, Lee TH, et al. Extrapleural pneumonectomy, chemotherapy, and radiotherapy in the treatment of diffuse malignant pleural mesothelioma. J Thorac Cardiovasc Surg 1991;102:10–14.
19. Sugarbaker DJ, Mentzer SJ, DeCamp M, Lynch TJ Jr, Strauss GM. Extrapleural pneumonectomy in the setting of a multimodality approach to malignant mesothelioma. Chest 1993;103:377S–381S.
20. Sugarbaker DJ, Garcia JP, Richards WG, et al. Extrapleural pneumonectomy in the multimodality therapy of malignant pleural mesothelioma. Results in 120 consecutive patients. Ann Surg 1996;224:288–294.
21. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117:54–63.
22. Sugarbaker DJ, Strauss GM, Lynch TJ, et al. Node status has prognostic significance in the multimodality therapy of diffuse, malignant mesothelioma. J Clin Oncol 1993;11:1172–1178.
23. Patz EF Jr, Shaffer K, Piwnica-Worms DR, et al. Malignant pleural mesothelioma: value of CT and MR imaging in predicting resectability. AJR Am J Roentgenol 1992;159:961–966.
24. Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995;108:1122–1128.
25. Baldini EH, Recht A, Strauss GM, et al. Patterns of failure after trimodality therapy for malignant pleural mesothelioma. Ann Thorac Surg 1997;63: 334–338.
26. Koga S, Kaibara N, Iitsuka Y, Kudo H, Kimura A, Hiraoka H. Prognostic significance of intraperitoneal free cancer cells in gastric cancer patients. J Cancer Res Clin Oncol 1984;108:236–238.
27. Hansen E, Wolff N, Knuechel R, Ruschoff J, Hofstaedter F, Taeger K. Tumor cells in blood shed from the surgical field. Arch Surg 1995;130:387–393.
28. Tanida O, Kaneshima S, Iitsuka Y, Kuda H, Kiyasu Y, Koga S. Viability of intraperitoneal free cancer cells in patients with gastric cancer. Acta Cytol 1982;26:681–687.
29. Sugarbaker PH. Observations concerning cancer spread within the peritoneal cavity and concepts supporting an ordered pathophysiology. Cancer Treat Res 1996;82:79–100.

30. Markman M, Kelsen D. Efficacy of cisplatin-based intraperitoneal chemotherapy as treatment of malignant peritoneal mesothelioma. J Cancer Res Clin Oncol 1992;118:547–550.
31. Alberts DS, Liu PY, Hannigan EV, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 1996;335: 1950–1955.
32. Bains MS, Ginsberg RJ, Jones WG, et al. The clamshell incision: an improved approach to bilateral pulmonary and mediastinal tumor. Ann Thorac Surg 1994;58:30–32.
33. Rusch VW, Niedzwiecki D, Tao Y, et al. Intrapleural cisplatin and mitomycin for malignant mesothelioma following pleurectomy: pharmacokinetic studies. J Clin Oncol 1992;10:1001–1006.
34. Rusch V, Saltz L, Venkatraman E, et al. A phase II trial of pleurectomy/decortication followed by intrapleural and systemic chemotherapy for malignant pleural mesothelioma. J Clin Oncol 1994;12:1156–1163.
35. Strom R, Crifo C, Rossi-Fanelli A, Mondovi B. Biochemical aspects of heat sensitivity of tumour cells. Recent Results Cancer Res 1977:7–35.
36. Stehlin JS Jr, Giovanella BC, Gutierrez AE, de Ipolyi PD, Greeff PJ. 15 years' experience with hyperthermic perfusion for treatment of soft tissue sarcoma and malignant melanoma of the extremities. Front Radiat Ther Oncol 1984;18:177–182.
37. Santinami M, Belli F, Cascinelli N, Rovini D, Vaglini M. Seven years experience with hyperthermic perfusions in extracorporeal circulation for melanoma of the extremities. J Surg Oncol 1989;42:201–208.
38. van Ruth RS, Baas P, Haas RL, Rutgers EJ, Verwaal VJ, Zoetmulder FA. Cytoreductive surgery combined with intraoperative hyperthermic intrathoracic chemotherapy for stage I malignant pleural mesothelioma. Ann Surg Oncol 2003;10:176–182.
39. Roberts JR. Surgical treatment of mesothelioma: pleurectomy. Chest 1999; 116:446S–449S.
40. Fizazi K, John WJ, Vogelzang NJ. The emerging role of antifolates in the treatment of malignant pleural mesothelioma. Semin Oncol 2002;29:77–81.
41. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636–2644.
42. Gordon GJ, Jensen RV, Hsiao LL, et al. Translation of microarray data into clinically relevant cancer diagnostic tests using gene expression ratios in lung cancer and mesothelioma. Cancer Res 2002;62:4963–4967.
43. Gordon GJ, Jensen RV, Hsiao LL, et al. Using gene expression ratios to predict outcome among patients with mesothelioma. J Natl Cancer Inst 2003;95:598–605.

48

Peritoneal Mesothelioma: The Columbia Experience

Jennifer A. Wagmiller, Mary-Louise Keohan, John A. Chabot, Karen Fountain, Mary Hesdorffer, and Robert N. Taub

Malignant peritoneal mesothelioma is a rare malignancy, comprising about one fifth of the 2500 cases of mesothelioma diagnosed in the United States each year (1). Though long-term spontaneous remissions have been reported (2,3), survival without treatment is typically less than 1 year. Treatment regimens employing only single modalities such as systemic chemotherapy, surgery, or intraperitoneal chemotherapy have not improved survival. Over the past 30 years, increasingly aggressive multimodality regimens have been developed that appear to have enhanced survival in selected patients. Given the rarity of this malignancy, the numbers of treated patients are small, and, until recently, prospective clinical trials of consistently used structured treatment regimens had not been carried out.

At the Columbia University Mesothelioma Center, our intent has been to combine as many as possible of the most effective drugs, surgical techniques, and radiotherapy into a structured, highly intense and aggressive multimodality treatment protocol for malignant peritoneal mesothelioma. In two clinical trials that enrolled patients from 1997 to 2002, patients underwent initial cytoreductive surgery followed by normothermic and hyperthermic intraperitoneal chemotherapy, intraperitoneal gamma-interferon, second-look laparotomy, and total abdominal radiation, with encouraging results.

Peritoneal Mesothelioma Background

Peritoneal mesothelioma is associated with documented asbestos exposure, but less frequently than with pleural mesothelioma (15–30% vs. 60–70%) and these patients often have a history of higher asbestos exposure and are younger than those with pleural mesothelioma (4). Apart from asbestos, there is evidence that chronic peritonitis, such as that seen in familial Mediterranean fever (5), and radiation (6) may predispose to peritoneal mesothelioma. Infection with the DNA tumor simian virus 40 (SV40) may as well be a risk factor for the development of mesothelioma (7), though it has been difficult to definitively establish this relationship in laboratory studies.

The tumors arise from the serosal surface of the peritoneal cavity, and, just as in the pleural type (7), may be of epithelial, sarcomatous, or mixed epithelial-sarcomatous histology, sarcomatous having the worst prognosis.

Rationale for Local/Regional Therapy: Debulking, Radiation, and Intraperitoneal Chemotherapy

The natural history of peritoneal mesothelioma suggests that intensive local/regional treatment would be advantageous. Mesothelioma usually stays confined to the abdominal cavity, although it can extend into the pleural space or metastasize to mediastinal or other distant nodes. The epithelial variant ordinarily does not invade solid organs, but can infiltrate into the omentum, causing "caking." Typically there are multiple sites of cancer on the peritoneum at presentation, but the extent of disease can vary. In patients who present with significant ascites, the fluid spreads the mesothelioma cells throughout the peritoneal cavity, causing all surfaces to be exposed (8). In the "pain predominant" clinical presentation (often a manifestation of sarcomatoid mesothelioma), a limited number of sites are common, with organ invasion and nerve infiltration, but distant metastases are rare. In either case, a rationale exists for debulking surgery, and possibly for total abdominal radiation.

Peritoneal mesothelioma is known to be responsive to intracavitary chemotherapy. Intraperitoneal chemotherapy allows much higher levels of drug to be directed to the malignancy, while limiting the amount systemically absorbed and its toxicity. Although intraperitoneal chemotherapy has been administered as a prelude to debulking surgery (1), if massive disease is present it may not be optimal. Studies with several agents, including methotrexate, doxorubicin, 5-fluorouracil, and cisplatin, indicate that cytotoxic levels of drug penetrate tumor nodules to a depth of only 1 to 3 mm (9); thus, tumor nodules larger than 1.5 cm would be reduced in volume by less than one log. This suggests that intraperitoneal chemotherapy might best be applied in patients already debulked by surgery and with only small lesions (0.6 cm or less).

Novel Therapies for Peritoneal Mesothelioma

With refinements in intracavitary chemotherapy for pleural mesothelioma and for gastrointestinal and ovarian abdominal carcinomatosis, techniques used in their treatment have been tried in peritoneal mesothelioma.

Hyperthermic Intraperitoneal Chemotherapy

Since the 1980s, hyperthermic intraperitoneal chemotherapy has been attempted with multiple agents such as doxorubicin, cisplatin, gemcitabine, mitomycin C, and tumor necrosis factor (TNF), in the treat-

ment of ovarian, gastric, and colorectal cancers, sarcoma, and peritoneal mesothelioma. The exact mechanism by which hyperthermic chemotherapy works is not understood, whether by direct effects on cells or by enhancement of drug activity. Such studies are not definitive, but support the use of hyperthermic chemotherapy, on the basis of demonstrated effectiveness and acceptable morbidity and mortality data (10,11).

Immunomodulator Therapy

Intracavitary immunotherapy has been given to pleural mesothelioma patients with early studies suggesting safety and antitumor activity. Eighty-nine patients with early-stage pleural mesothelioma were treated with intrapleural recombinant gamma-interferon (12). Eight patients were demonstrated to have a complete pathologic response and nine to have partial responses. However, in the stage 1 patients, the overall response rate was higher, with 45% of patients showing a response. Toxicities included hyperthermia, liver toxicity, neutropenia, and catheter-related infection (12). Intrapleural interleukin-2 also showed activity and a tolerable side effect profile in a phase II mesothelioma study of 22 patients, 11 with partial response and one with complete response (13).

Multimodality Treatment of Peritoneal Mesothelioma Using Cytoreductive Surgery and Intraperitoneal Chemotherapy

Several U.S. centers have recently published the results of their experience with treatment of malignant peritoneal mesothelioma using cytoreductive surgery with intraperitoneal chemotherapy. In addition to variability in patient selection, surgical technique, and choice and timing of chemotherapy, different combinations of preoperative or postoperative hyperthermic intraperitoneal chemotherapy, systemic chemotherapy, abdominal radiation, and re-laparotomy, were employed.

The earliest published study of aggressive multimodality therapy described six patients with nonbulky peritoneal mesothelioma who were treated with cytoreductive surgery, intraperitoneal doxorubicin, and cisplatin, some of whom were also given systemic chemotherapy followed by whole abdominal radiation (14). In the first report of these patients, all were alive with no evidence of disease at 9 to 34 months. In a later report, 25% of a larger series of patients were alive at 5 years (15), and at least one lived ~20 years when he died of lung adenocarcinoma with no evidence of persistent mesothelioma.

Cytoreductive surgery immediately followed by heated intraperitoneal chemotherapy with mitomycin was used to treat 12 patients between 1992 and 2001, with a reported 33% 5-year survival, and median survival of 34 months (16).

The investigators at the Washington Cancer Institute reported a series of 51 patients treated between 1987 and 2002 (1). They attempted to treat all patients with cytoreductive surgery with hyperthermic intraperitoneal chemotherapy immediately following the debulking. Nine patients with massive ascites had intraperitoneal cisplatin and doxorubicin prior to the initial cytoreductive surgery. The last 18 patients were also eligible to receive a course of normothermic intraperitoneal chemotherapy. In 11 patients, a second-look surgery was done, and in three, a third look. The median survival reported was 50 to 60 months.

While direct comparisons of these regimens are not possible due to study design and length of follow-up, the overall picture is that in these selected patient populations, the aggressive multimodality treatments have extended median survival to at least 19 months. In addition to the survival gains, symptomatic improvement, such as ascites control, was regularly achieved by these aggressive treatments.

In planning our own studies, it remained unclear how to optimally combine these interventions to prolong patient survival while minimizing side effects. One variable among the different series is the timing of the hyperthermic intraperitoneal chemotherapy, which was performed either immediately after the first cytoreductive surgery, or was delayed until second-look surgery/repeat debulking. Another consideration is that recent series did not utilize whole abdominal radiation therapy, although this was integral to the initial Boston multimodality trials. Our philosophy has been to initially maximize intensity of treatment and number of modalities administered in an attempt to improve upon current poor survival figures, with a view toward eliminating unnecessary elements of treatment in successive studies.

The Columbia University Experience

Patients

Forty patients have been enrolled in two consecutive phase I/II studies at Columbia Presbyterian Medical Center between 1997 and 2002. Both protocols were reviewed and approved by the institutional review board. To be eligible, patients must have had histologically confirmed malignant mesothelioma. They were permitted to have had limited chemotherapy and surgery for the disease prior to enrollment (zero to two chemotherapy regimens, but at least 6 weeks must have elapsed since chemotherapy; prior surgical resection preceding disease recurrence was acceptable, but at least 1 week must have elapsed since surgery). Patients who had had prior abdominal or lower chest radiation therapy were ineligible. Pregnant or lactating females were excluded. Patients were required to have a life expectancy of at least 2 months, be over 18 years of age, and have a SWOG performance status of 0 to 2. Patients were required to have adequate hematologic, renal, and hepatic function (white blood cell count of >3000/μL, platelet count of >100,000/μL, blood urea nitrogen (BUN) <1.5× normal, calculated creatinine clearance of ≥45 mL/min, bilirubin <1.5× normal).

Patients with a history of malignancy within the past 5 years other than curatively treated carcinoma in situ of the cervix or skin cancer were ineligible. Additionally, patients could not have a serious medical or psychiatric condition that would prevent intensive treatment.

Treatment

Cytoreduction

All patients underwent exploratory laparotomy through a midline vertical incision, with total omentectomy and excision of all gross peritoneal, retroperitoneal, and pelvic disease. The surgeon attempted to remove all nodules of >1 cm diameter. Peritoneal catheters were placed bilaterally and tunneled through the abdominal wall to prevent leakage of the intraperitoneal chemotherapy. Parenchymal organs were not ordinarily removed as part of the surgery, except for resection of segments of ileum or colon judged to be involved by invasive tumor. If debulking resection was not possible without organ removal, the patient was dropped from the protocol.

Normothermic Intraperitoneal Chemotherapy

Three to 4 weeks following surgery, intraperitoneal therapy was initiated. In the first trial, patients received four courses each of intraperitoneal cisplatin (100 mg/m^2) alternating with doxorubicin (25 mg) weekly, followed by four weekly courses of gamma-interferon (9 million units initial dose, followed by 30 million units weekly for three doses) (17). In the second trial, patients continued to receive doxorubicin (25 mg, weeks 1, 4, 7, 10) but the cisplatin was given in combination with gemcitabine (cisplatin 50 mg/m^2 plus gemcitabine 100 mg/m^2; weeks 2, 5, 8, 11) and followed by gamma-interferon (300 µg week 13, 1000 µg weeks 14, 15, 16).

Second-Look Surgery and Hyperthermic Intraperitoneal Chemotherapy

Two to 4 weeks after the last dose of gamma-interferon, the patients underwent second-look surgical exploration of the abdomen and pelvis. If no gross disease was seen (no nodules >1 cm), multiple biopsies were obtained and hyperthermic intraperitoneal chemotherapy was given. A recirculating perfusion circuit with a roller pump heat exchanger connected to suprahepatic inflow and pelvic outflow catheters was employed to administer the hyperthermic intraperitoneal chemotherapy while the patient was still under general anesthesia (18). In the first protocol, patients received mitomycin 10 mg/m^2 and cisplatin 100 mg/m^2 in 2 L normal saline at 40.5° to 42.5°C infused over 90 minutes. In the second protocol, the dose of cisplatin was reduced to 75 mg/m^2. Cisplatin was not given if the creatinine clearance was less than 45 mL/min.

If technically resectable disease, gross disease (nodules >1 cm) was found, intraoperative hyperthermic chemotherapy was given as described above. Patients with technically unresectable disease were taken off protocol and considered for systemic chemotherapy.

Radiation Therapy

Two to 4 weeks following surgery, all patients except those with unresectable residual disease, began a course of radiation therapy lasting 5 to 7 weeks. All fields were treated each day with blocks over the lower portion of the heart and femoral heads and associated soft tissue of the pelvis. Treatment breaks were given for white blood cell counts of less than 1500 or platelets of less than 75,000, or for intractable nausea and vomiting.

For patients who had not received the full course of intraperitoneal chemotherapy, a 67% transmission block was used to attenuate the dose given to the abdomen above a line drawn through the L5-S1 interspace. They were given daily fractions of 120 cGy in the upper abdomen and 180 cGy in the pelvis (prescribed to the central axis) five times weekly to parallel and opposed anteroposterior (AP) to posteroanterior (PA) portals, utilizing a 6-MV photon beam. After 1800 cGy, full-thickness AP and PA kidney blocks were added. These patients were treated to a total dose of 3000 cGy to the upper abdomen and 4500 cGy to the pelvis.

With patients who did receive the full course of intraperitoneal chemotherapy, patient transmission blocks were not used. The upper abdomen and pelvis were treated uniformly at the rate of 100 to 150 cGy per fraction. Kidney blocks were added after 1400 to 1550 cGy to both the anterior and posterior portals. They were treated to a total dose of 3000 to 3080 cGy to the upper abdomen and 4500 cGy to the pelvis.

Results and Discussion

Twenty-seven patients were enrolled in the first protocol between 1997 and 2000, 23 with the epithelial subtype and four with sarcomatous or mixed (sarcomatous-epithelial) disease (Table 48.1). At this time, median survival for the entire cohort has not been reached (Table 48.2).

Table 48.1. Tobacco and asbestos exposure in 27 patients with peritoneal mesothelioma treated at Columbia-Presbyterian Medical Center

Median age at enrollment	**51 years**
Gender	
Female	**7 patients**
Male	**20 patients**
Asbestos exposure	
Yes	**18 patients**
No	**9 patients**
Tobacco history	
Yes	**17 patients**
No	**10 patients**
Status	
No evidence of disease	**10 patients**
Alive with disease	**6 patients**
Died of disease	**11 patients**

Table 48.2. Outcome of patients treated with trimodal regimen

Subtype of mesothelioma	Number of patients	NED	AWD	DOD
Epithelial	23	10 patients survival 22–61 mo (median 40 mo)	6 patients survival 27–62 mo (median 42 mo)	7 patients survival 9–42 mo (median 20 mo)
Sarcomatous and mixed sarcomatous-epithelial	4	0 patients	0 patients	4 patients survival 1–11 mo (median 4 mo)

NED, no evidence of disease; AWD, alive with disease; DOD, died of disease.

Of the 23 with epithelial disease, 10 patients show no evidence of disease after a median >40 months. Six patients had persistent disease at 27 to 62 months (median >42 months). Seven patients succumbed to progressive disease at 9 to 42 months (median 20 months). The four patients with sarcomatoid or mixed disease did not benefit from multimodal treatment; all progressed and succumbed at 1 to 11 months (median 4 months). The Kaplan-Meier survival curve for the cohort is shown in Figure 48.1. The second trial has enrolled 13 patients and is still accruing; data from that trial are not yet available.

Our experience at the Columbia University Mesothelioma Center, as well as that at other centers, suggests that with aggressive multimodality treatment, peritoneal mesothelioma can be a treatable disease, with some patients achieving long-term disease-free survival. Although the initial patients treated in 1984 all had a minimal disease burden, many of our recent patients had extensive omental disease, or significant nodular disease in the pelvis and colic gutters. This would suggest that our results are due only in part to selection of patients by

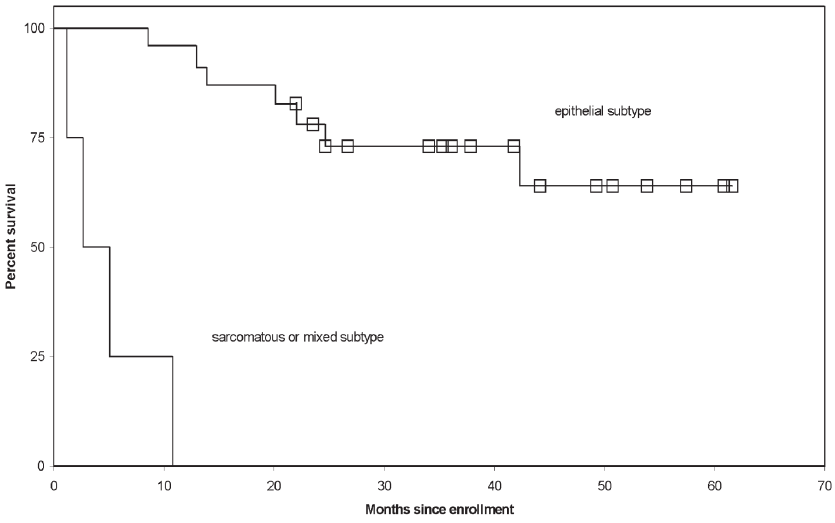


Figure 48.1. Kaplan-Meier survival curve for 27 patients treated with trimodal protocol by subtype. Note that overall median survival has not been reached.

performance status, histology, and operability, and whose likelihood of survival may have been intrinsically better. A recent report of 25 cases of peritoneal mesothelioma in women suggested that prolonged survival after therapy could not be predicted by histology or extent of disease at presentation (19). Certainly the achievement of the disease-free state is almost never achieved without active intervention.

Our results agree with and extend the observation in malignant pleural mesotheliomas (20) of much poorer outcomes for patients with sarcomatous pathology than for those with primary peritoneal disease. We have recently noted that the microarray gene profiles of these patients differ significantly from those with epithelial disease (21). We have noted that our patients with sarcomatous or mixed histology often presented with bulky disease invading solid organs, which persisted or recurred rapidly after surgery. As additional results from genomic studies and from our second phase II trial become available, we hope to better assess which patients should receive aggressive treatment, and whether abdominal radiation contributes to survival.

References

1. Sugarbaker PH, Acherman YI, Gonzalez-Moreno S, et al. Diagnosis and treatment of peritoneal mesothelioma: the Washington Cancer Institute experience. Semin Oncol 2002;29(1):51–61.
2. Schwartz E, Maayan C, Mouallem M, Engelberg S, Friedman E. Malignant peritoneal mesothelioma: long-term spontaneous clinical remission. Med Pediatr Oncol 1991;19(4):325–328.
3. Norman PE, Whitaker D. Nine-year survival in a case of untreated peritoneal mesothelioma. Med J Aust 1989;150(1):43–44.
4. Sebbag G, Sugarbaker PH. Peritoneal mesothelioma proposal for a staging system. Eur J Surg Oncol 2001;27(3):223–224.
5. Gentiloni N, Febbraro S, Barone C, et al. Peritoneal mesothelioma in recurrent familial peritonitis. J Clin Gastroenterol 1997;24(4):276–279.
6. Antman K, Pass H, Schiff P. Management of mesothelioma. In: DeVita V, Rosenberg S, Hellman S, eds. Cancer: Principles and Practice of Oncology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2001.
7. Testa J, Pass H, Carbone M. Molecular biology of mesothelioma. In: DeVita V, Rosenberg S, Hellman S, eds. Cancer: Principles and Practice of Oncology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2001.
8. Sugarbaker PH, Yan H, Grazi RV, Shmookler BM. Early localized peritoneal mesothelioma as an incidental finding at laparoscopy. Report of a case and implications regarding natural history of the disease. Cancer 2000;89(6): 1279–1284.
9. Markman M. Intraperitoneal chemotherapy. Crit Rev Oncol Hematol 1999; 31(3):239–246.
10. Ceelen WP, Hesse U, de Hemptinne B, Pattyn P. Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intra-abdominal cancer. Br J Surg 2000;87(8):1006–1015.
11. Park BJ, Alexander HR, Libutti SK, et al. Treatment of primary peritoneal mesothelioma by continuous hyperthermic peritoneal perfusion (CHPP). Ann Surg Oncol 1999;6(6):582–590.

12. Boutin C, Nussbaum E, Monnet I, et al. Intrapleural treatment with recombinant gamma-interferon in early stage malignant pleural mesothelioma. Cancer 1994;74(9):2460–2467.
13. Astoul P, Picat-Joossen D, Viallat JR, Boutin C. Intrapleural administration of interleukin-2 for the treatment of patients with malignant pleural mesothelioma: a phase II study. Cancer 1998;83(10):2099–2104.
14. Antman KH, Osteen RT, Klegar KL, et al. Early peritoneal mesothelioma: a treatable malignancy. Lancet 1985;2(8462):977–981.
15. Weissman L, Osteen R., Corson J, et al. Combined modality therapy for intraperitoneal mesothelioma. Proc Soc Am Soc Clin Oncol 1988;7:27 (abstract 1063).
16. Loggie BW, Fleming RA, McQuellon RP, Russell GB, Geisinger KR, Levine EA. Prospective trial for the treatment of malignant peritoneal mesothelioma. Am Surg 2001;67(10):999–1003.
17. Keohan ML, Chabot J, Fountain K, et al. A phase II study of trimodal therapy for peritoneal mesothelioma. Proc ASCO 2001;1441.
18. Mongero LB, Beck JR, Kroslowitz RM, Argenziano M, Chabot JA. Treatment of primary peritoneal mesothelioma by hyperthermic intraperitoneal chemotherapy. Perfusion 1999;14(2):141–145.
19. Kerrigan SA, Turnnir RT, Clement PB, Young RH, Churg A. Diffuse malignant epithelial mesotheliomas of the peritoneum in women: a clinicopathologic study of 25 patients. Cancer 2002;94(2):378–385.
20. Sugarbaker DJ, Norberto JJ, Swanson S. Extrapleural pneumonectomy in the setting of multimodality therapy for diffuse malignant pleural mesothelioma. Semin Thorac Cardiovasc Surg 1997;9(4):373–382.
21. Powell C, Borczuk A, Keohan M, Talbot S, Hesdorffer M, Taub R. Malignant peritoneal mesotheliomas of epithelial or biphasic/sarcomatous histology display unique gene expression profiles. Proc ASCO 2003; submitted.

49

Surgery, Hyperthermic Chemoperfusion, and Postoperative Chemotherapy: The National Cancer Institute and Washington Hospital Center Experience

Nancy M. Carroll, Faheez Mohamed, Paul H. Sugarbaker, and H. Richard Alexander

Patients with peritoneal mesothelioma, by virtue of the propensity of the tumor to remain confined to the peritoneal cavity through most, if not all, of the disease course (1), are good candidates for regional intraperitoneal therapy. Regional therapy has the theoretical advantage of exposing tumors to high doses of therapeutic agents while limiting systemic toxicity (2). The barrier effect of the peritoneum provides a pharmacokinetic advantage compared to intravenous administration (2).

The Washington Cancer Institute (WCI) and the National Cancer Institute (NCI) have independently developed protocols utilizing combined therapy consisting of cytoreductive surgery, continuous hyperthermic peritoneal perfusion (CHPP), and postoperative intraperitoneal chemotherapy for the treatment of peritoneal mesothelioma. The largest studies of this combined therapy for peritoneal mesothelioma reported in the literature have been conducted at these two institutions (3–7). This chapter summarizes the experience of the WCI and NCI in treating peritoneal mesothelioma with combined therapy. Though well-controlled random assignment trials comparing combined therapy to alternative therapies have not been conducted, encouraging results in single-arm studies suggest that the consideration of combined therapy as a treatment option for selected patients with peritoneal mesothelioma is warranted.

Presentation

Most patients with peritoneal mesothelioma present with abdominal distention or pain and are found to have diffuse intraperitoneal disease (1). The peritoneal surface is typically studded with nodules, and

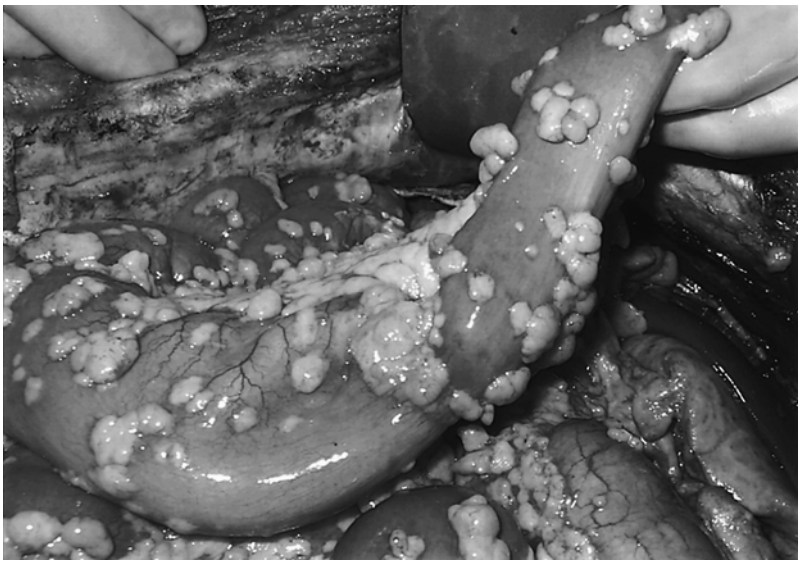


Figure 49.1. Intraoperative photograph of the abdomen of a patient with peritoneal mesothelioma demonstrating the typical studding of the peritoneum with tumor nodules.

dependent peritoneal surfaces may exhibit confluent tumor (Fig. 49.1). The omentum is often extensively involved early in the disease. It is likely that the tumor disseminates throughout the peritoneal cavity by peritoneal fluid flow, gravity, and other factors. The small bowel is often spared early in the disease as, it is thought, peristalsis impedes tumor implantation on the small bowel. However, in advanced conditions, tumor may encase the bowel leading to intestinal obstruction (8). Ascites is present in up to 90% of patients. The peritoneal fluid may be watery or viscous with abundant mucopolysaccharides (8). Often peritoneal fluid cytology does not reveal malignant cells, and the diagnosis is made from specimens obtained at laparoscopy or laparotomy (3).

Early series reported a median survival of patients with peritoneal mesothelioma of approximately 1 year. More recent studies show substantially longer survival. Table 49.1 lists numerous reports of the median survival of peritoneal mesothelioma. Peritoneal mesothelioma shows a wide spectrum of biologic aggressiveness, and a number of histologic variants have been identified. Although some investigators have reported that sarcomatous-type tumors are aggressive and epithelial-type tumors are more indolent (9), it has not been definitively established that histology is associated with outcome (10). Over 80% of peritoneal mesotheliomas are of the epithelial type.

The majority of patients die from complications of intraperitoneal tumor progression, including small bowel obstruction and inanition (1,11). The tumor may spread through the diaphragm to the pleura and pericardium. Metastases can occur, but death is rarely the result of metastatic disease (1). When symptoms from extraperitoneal extension occur, it is usually late in the disease course.

Table 49.1. Median survival of peritoneal mesothelioma

Authors (reference)	Year	*n*	Median survival (months)
Chailleux et al (26)	1988	11/167	10*
Antman et al (27)	1988	37/180	15*
Sridhar et al (28)	1992	13/50	9.5*
Markman et al (29)	1992	19	9
Yates et al (30)	1997	14/272	14*
Neumann et al (31)	1999	74	12 (mean)
Eltabbakh et al (32)	1999	15**	12.5
Park et al (6)	1999	18	26
Loggie et al (33)	2001	12	34
Kerrigan et al (10)	2002	25**	30
Sugarbaker et al	2002	68	67
Alexander et al	2002	49	92

* Combined/pleural.
** All females.

Combined Therapy at the NCI and WCI

For the purposes of this chapter, the term *combined therapy* refers to a treatment strategy consisting of cytoreductive surgery in combination with planned intraoperative delivery of heated chemotherapy and, in some cases, early postoperative intraperitoneal chemotherapy. Surgical cytoreduction consists of systematic resection of tumor with or without involved viscera, omentectomy, peritonectomy, and lysis of adhesions. Subsequently, large-bore catheters are positioned in the abdominal cavity for high-flow delivery of heated chemotherapy, and the abdomen is agitated to ensure even perfusate distribution (4,5,12). This is followed by early postoperative intraperitoneal chemotherapy before adhesions might impair the distribution of chemotherapy. Combined therapy, as practiced at the NCI for patients with mesothelioma, consists of intraoperative administration of cisplatin followed by a single intraperitoneal dwell of 5-fluorouracil (5-FU) and paclitaxel 2 to 10 days after surgery. At the WCI, intraoperative therapy consists of cisplatin and doxorubicin; intraperitoneal paclitaxel is given early postoperatively and may be continued for as long as 6 months. Figure 49.2 outlines the intraoperative and postoperative treatments administered at the WCI and the NCI.

Cytoreduction

It is not surprising that surgery alone has not proven to be sufficient treatment for peritoneal mesothelioma in light of the extent of disease generally found at presentation. Even in patients who can be rendered macroscopically free of disease, there are no studies that suggest that surgery alone affords a survival benefit (8). Similarly, intraperitoneal chemotherapy alone without cytoreduction, has had little success (13), presumably due to limited penetration of drug into tumors. Studies of intraperitoneal drug delivery have shown that direct tumor absorption occurs to a level only a few millimeters from the surface. Dikhoff et al

(14) showed that the concentration of platinum in tumors after intraperitoneal administration remains relatively constant and high up to a distance of 3 mm from the surface of the tumor. However, at a distance of 5 mm from the tumor surface, the concentration was only 20% of that at the tumor surface. Platinum concentrations in tumor following intraperitoneal delivery were severalfold higher than with an equivalent intravenous dose. This suggests that aggressive cytoreduction with the goal of leaving minimal or no gross disease may facilitate delivery of chemotherapy to the entire volume of remaining intraperitoneal tumor, and that patients with a small volume of residual disease following cytoreduction may benefit most from CHPP.

Studies in patients with stage III ovarian cancer, support the notion that microscopic residual disease, as opposed to established tumor, may be the ideal target for intraperitoneal chemotherapy. Patients who could be rendered macroscopically disease free had a greater probability of deriving benefit from intraperitoneal chemotherapy than did those who could not be rendered disease free (15). It remains to be seen whether or not the same holds true for mesothelioma patients.

Because the outcome after combined therapy may be associated with completeness of cytoreduction, scoring systems have been developed to assist in the interpretation of outcome data. The WCI has proposed a scoring system, the peritoneal cancer index, to quantify the extent of disease present at exploration (4). The peritoneal cavity is divided into nine regions by two equally spaced horizontal and vertical lines. An additional four regions are defined as the upper and lower jejunum and the upper and lower ileum. Prior to any cytoreduction, the volume of disease in each of these 13 regions is scored as 0 (no cancer seen), 1

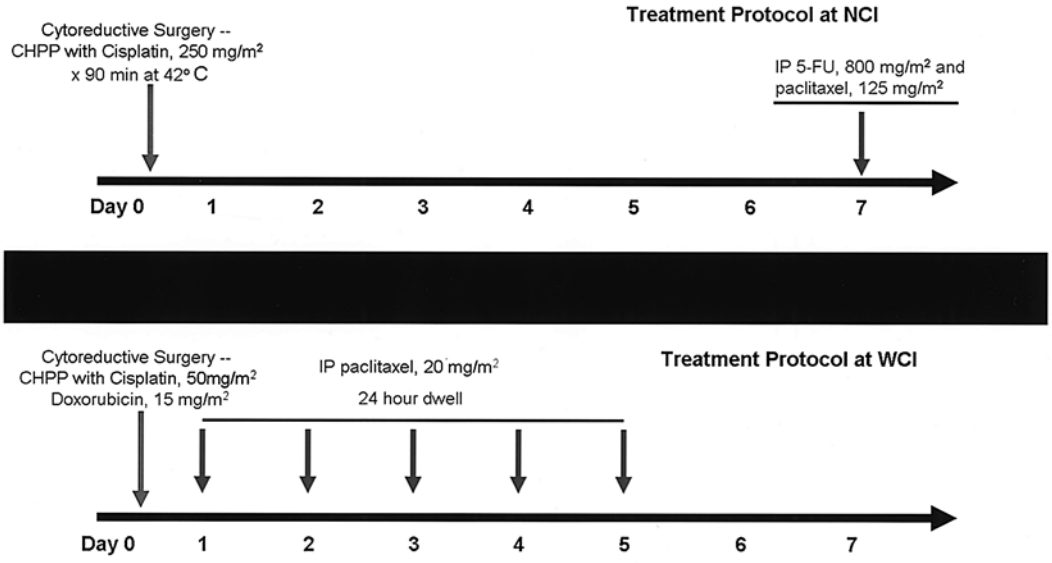


Figure 49.2. Combined therapy for peritoneal mesothelioma as practiced at the National Cancer Institute (NCI) (top) and the Washington Cancer Institute (WCI) (bottom).

(nodules <0.5 cm), 2 (nodules 0.5 to 5 cm), or 3 (nodules >5 cm). The maximum score is 39. Subsequent to cytoreduction, patients are assigned a completeness of cytoreduction score (4): 0, no residual cancer is seen; 1, tumor nodules less than 0.25 cm; 2, tumor nodules 0.25 to 2.5 cm; and 3, tumor nodules greater than 2.5 cm or confluent nodules. At the NCI, after cytoreduction, patients are scored as having minimal (fewer than 100 total lesions, all smaller than 5 mm), intermediate (more than 100 total lesions all smaller than 5 mm), or bulky (residual tumor larger than 5 mm) disease.

Intraoperative Chemotherapy at the NCI

Cisplatin was chosen for the initial CHPP studies at the NCI in light of its cytotoxicity against numerous cell types and extensive history of intraperitoneal use. Phase I studies demonstrated the maximum tolerated dose (MTD) of cisplatin delivered via CHPP to be 250 mg/m^2 and the dose-limiting toxicity was nephrotoxicity (12). Figure 49.3 shows cisplatin concentration in the perfusate and the serum over time during the course of a typical CHPP. Cisplatin concentrations in the perfusate were shown to remain on average 12-fold greater than serum cisplatin concentration throughout the course of the procedure. The estimated clearance of platinum across the peritoneal cavity during CHPP was calculated as approximately 18 mL/min, which compares favorably to the plasma clearance of platinum of 329 mL/min.

Intraoperative Chemotherapy at the WCI

At the WCI, intraoperative doxorubicin is used in addition to cisplatin. Doxorubicin, like cisplatin, has a high ratio of peritoneal to systemic drug exposure following intraperitoneal administration (13). The ratio of the area under the curve for intraperitoneal administration to the area under the curve for intravenous administration is approximately 150:1 in humans (16). In vivo studies indicate that doxorubicin activity is enhanced by cisplatin (17).

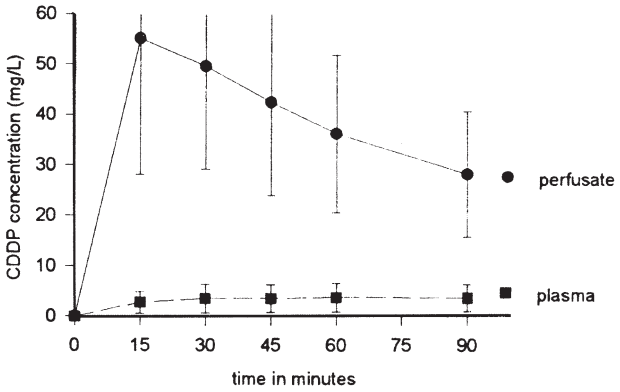


Figure 49.3. Cisplatin (*cis*-diaminedichloroplatinum, CDDP) concentrations in the perfusate and the serum during continuous hyperthermic peritoneal perfusion.

Hyperthermic Chemoperfusion at the NCI and WCI

During CHPP, chemotherapy is delivered at elevated temperatures. Hyperthermia has been shown to enhance chemotherapy penetration into tumors and to work synergistically with chemotherapeutic agents to kill cancer cells (18,19). In vitro studies have demonstrated that cellular uptake of cisplatin and cisplatin cytotoxicity are enhanced by moderate temperature elevation (20). In vivo studies have demonstrated synergy between hyperthermia and cisplatin (20). Zakris et al (21) evaluated the effect of intraperitoneal hyperthermia on cisplatin pharmacokinetics in beagles. Hyperthermia resulted in a longer intraperitoneal drug half-life and a lower area under the concentration versus time curve for free cisplatin in the serum in heated versus unheated dogs. At the WCI, doxorubicin is administered during CHPP along with cisplatin. Animal studies demonstrate that doxorubicin activity is also enhanced by hyperthermia (22).

Hyperthermia alone has been shown to have a lethal effect on neoplastic cells at levels tolerated by normal tissues (23). This effect appears to be mediated through at least two mechanisms. Hyperthermia has a direct cytotoxic effect on many tumor cells, and tumor vasculature seems to be less heat tolerant than normal microcirculation (24). Blood stasis occurs at significantly lower levels of hyperthermia in tumor vessels than in normal vessels. Despite this, hyperthermia alone has shown little efficacy in treating cancer clinically. Hyperthermia may have clinical utility in combination with chemotherapy.

Postoperative Chemotherapy

The NCI and the WCI both administer postoperative intraperitoneal chemotherapy, which results in favorable pharmacokinetics for intraperitoneal tumor exposure to chemotherapy compared to the brief exposure provided at the time of surgery or by systemic delivery. Agents that require mitosis for efficacy may be more effective with prolonged exposure, especially in the case of slowly dividing tumor cells. Paclitaxel is well suited to postoperative intraperitoneal use. Studies show that it is sequestered in the peritoneal cavity even after extensive peritonectomy, resulting in a high peritoneal cavity to serum concentration ratio (3). 5-Fluorouracil also exhibits a high ratio of peritoneal surface exposure to systemic exposure following intraperitoneal administration (13).

Advantages and Disadvantages of Combined Therapy

Nonsurgical intraperitoneal therapies may be limited by incomplete distribution of therapeutic agents to the entire peritoneal surface, especially in the presence of adhesions. Table 49.2 summarizes the advantages and disadvantages of CHPP. In CHPP, chemotherapy is administered following lysis of adhesions promoting optimal distribution of chemotherapy to the peritoneum. Since chemotherapy is delivered after cytoreduction, microscopic or small volume disease is

Table 49.2. Advantages and disadvantages of combined therapy

Advantages
Maximal cytoreduction prior to intraperitoneal (IP) chemotherapy may enhance efficacy of chemotherapy
Intraoperative hyperthermia may enhance efficacy of IP chemotherapy and have an independent tumoricidal effect
Lysis of adhesions facilitates optimal chemotherapy distribution
High regional chemotherapy concentrations are achievable with limited systemic toxicity
Disadvantages
Topically applied chemotherapy has limited tumor penetration
Topically applied chemotherapy may produce local toxicity and have unpredictable systemic effects

treated, which may improve tumor absorption of drugs. In addition, a surgical approach facilitates delivery of intraperitoneal hyperthermia to a degree that is difficult or too toxic to deliver by other means. The peritoneal cavity acts as an efficient heat sink, so a rapid flow of perfusate through the abdominal cavity, beyond that compatible with nonsurgical approaches, is necessary to achieve sustained intraperitoneal hyperthermia.

There are disadvantages to the surgical approach. The CHPP procedure is long and involves significant volume shifts. It is well suited only to patients who can tolerate prolonged general anesthesia and copious intravenous fluid administration. The direct application of chemotherapy and heat to the peritoneal contents theoretically puts patients at risk for regional toxicity, such as ileus, although long-term bowel toxicity has not been observed with the agents used at our institutions. Bowel resection is often performed during cytoreduction and direct application of chemotherapy to the bowel following resection theoretically risks impaired healing.

Technique of Continuous Hyperthermic Peritoneal Perfusion

Continuous hyperthermic peritoneal perfusion (CHPP) begins with cytoreduction through a series of visceral and parietal peritonectomy procedures followed by administration of intraoperative hyperthermic chemotherapy. Surgery is followed by early postoperative chemotherapy. At the WCI, selected patients also receive long-term intraperitoneal paclitaxel, and fit patients who are responding well to therapy often undergo a second-look surgery.

Cytoreductive Surgery Using Peritonectomy Procedures

There are six different peritonectomy procedures that are used to resect cancer on visceral surfaces or to strip cancer from parietal peritoneal surfaces. One or all six of these procedures may be required, depending on the distribution and volume of peritoneal disease.

The standard tool used to dissect tumor on peritoneal surfaces from the normal tissues is a ball-tip electrosurgical handpiece. The ball-tipped instrument is placed at the interface of tumor and normal tissues. The focal point for further dissection is placed on strong traction. The 3-mm ball-tip electrode is used on pure cut at high voltage for dissecting. Using ball-tip electrosurgery on pure cut creates a large volume of plume because of the electroevaporation of tissue. To maintain visualization of the operative field and to preserve a smoke-free atmosphere in the operating theater, a smoke filtration unit is used.

To free the midabdomen of a large volume of tumor, a complete greater omentectomy is performed. The greater omentum is elevated and then separated from the transverse colon. The mound of tumor that covers the spleen is identified. The peritoneum on the anterior surface of the pancreas may need to be elevated from the gland. The greater curvature of the stomach is reflected anteriorly from pylorus to gastroesophageal junction. Greater omentectomy is usually combined with splenectomy to achieve a complete cytoreduction. If the spleen is free of tumor, it is left in situ.

The left upper quadrant peritonectomy involves stripping of all tissue from beneath the left hemidiaphragm to expose diaphragmatic muscle, left adrenal gland, and the cephalad half of the perirenal fat. To achieve a full exposure of the left upper quadrant, the splenic flexure of the colon is released from the left paracolic sulcus and moved medially by dividing tissue along Toldt's line. Numerous blood vessels between diaphragm muscle and its peritoneal surface must be electrocoagulated before their transection or unnecessary bleeding will occur.

The right diaphragmatic peritoneum and epigastric fat pad are stripped away from the right posterior rectus sheath to begin the peritonectomy in the right upper quadrant of the abdomen. Ball-tip electrosurgery on pure cut is used to dissect at the interface of mesothelioma infiltrating the peritoneum and the muscle of the right hemidiaphragm.

The stripping of tumor from the muscular surface of the diaphragm continues until the bare area of the liver is encountered. With both blunt and ball-tip electrosurgical dissection, the tumor is lifted off the dome of the liver by moving through or beneath Glisson's capsule. Isolated patches of tumor on the liver surface are electroevaporated.

Tumor from beneath the right hemidiaphragm, from the right subhepatic space, and from the surface of the liver forms an envelope as it is removed en bloc. The dissection is simplified greatly if the tumor specimen can be maintained intact. The dissection continues laterally on the right to encounter the fat covering the right kidney. Also, the right adrenal gland is visualized as the tumor is stripped from the right subhepatic space. Care is taken not to traumatize the vena cava or to disrupt caudate lobe veins that pass between the vena cava and segment 1 of the liver.

If involved with tumor, the gallbladder is removed in a routine fashion from its fundus toward the cystic artery and cystic duct. The plate of tissue that covers the structures that constitute the porta hepatis usually is infiltrated heavily by tumor. Using strong traction,

the cancerous tissue that covers the structures is stripped from the base of the gallbladder bed toward the duodenum. To continue resection of the lesser omentum, one proceeds along the gastrohepatic fissure that divides liver segments 2, 3, and 4 from segment 1.

As one clears the left part of the caudate liver segment of tumor, the vena cava is visualized directly beneath. To strip the floor of the omental bursa, strong traction is maintained on the tumor, and ball-tip electrosurgery is used to divide the peritoneum joining the caudate lobe of the liver to the vena cava. Division of the phreno-esophageal ligament allows the crus of the right hemidiaphragm to be stripped of peritoneum. Finally, dissection around celiac lymph nodes allows the specimen to be released.

To begin the pelvic peritonectomy, the peritoneum is stripped from the posterior surface of the lower abdominal incision, exposing the rectus muscle. The muscular surface of the bladder is seen as ball-tip electrosurgery strips tumor-bearing peritoneum and preperitoneal fat from this structure. The urachus must be divided and is placed on upward traction as the leading point for dissection of the visceral surface of the bladder. Round ligaments are divided as they enter the internal inguinal ring on both the right and left in the female patient.

The peritoneal incision around the pelvis is completed by dividing the peritoneum along the pelvic brim. Right and left ureters are identified and preserved. In women, the right and left ovarian veins are ligated and divided at the level of the lower portion of the kidney. A linear stapler is used to divide the colon at the junction of sigmoid and descending colon. This allows one to pack all of the viscera, including the proximal descending colon, in the upper abdomen.

In women, the bladder is moved gently off the cervix, and the vagina is entered. The vaginal cuff anterior and posterior to the cervix is divided using ball-tip electrosurgery, and the perirectal fat inferior to the posterior vaginal wall is encountered. Electrosurgery is used to divide the peri-rectal fat beneath the peritoneal reflection. This ensures that all tumor that occupies the cul-de-sac is removed intact with the specimen. The midportion of the rectal musculature is skeletonized and a roticulator stapler is used to staple the rectal stump closed.

Hyperthermic Perfusion at the WCI

After cytoreduction is complete, hyperthermic perfusion is initiated. There are some minor variations in technique between the WCI and the NCI at this point. At the WCI, closed suction drains are placed in the subhepatic space, the left subdiaphragmatic space, and the pelvis. A Tenckhoff catheter is placed through the abdominal wall and positioned in the pelvis and a temperature probe is secured to the end of the Tenckhoff catheter. Bilateral thoracostomy tubes are inserted to prevent chemotherapy accumulation in the chest. A running suture is used to secure the skin edges to the self-retaining retractor. A plastic sheet is incorporated into the sutures to create an open space beneath. This creates a configuration reminiscent of a coliseum and has been

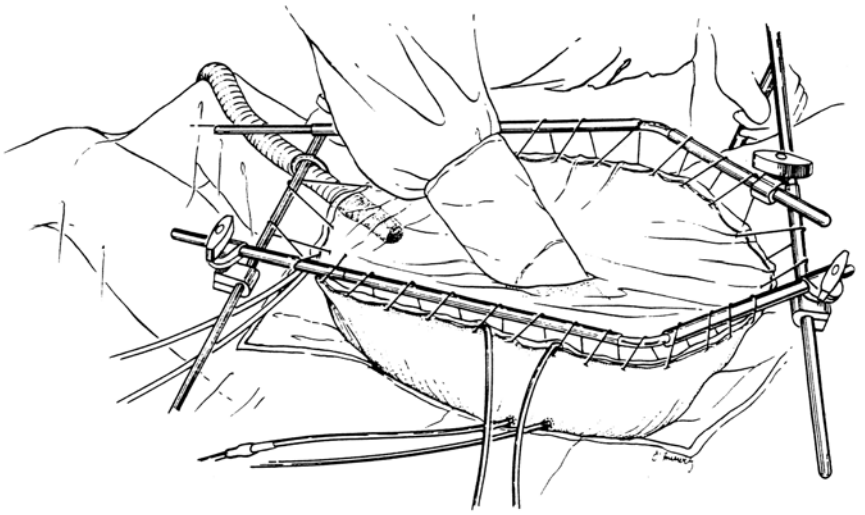


Figure 49.4. Sketch of the coliseum technique of hyperthermic peritoneal perfusion.

termed the coliseum technique. A slit in the plastic allows the surgeon's double-gloved hand access to the abdomen and pelvis. Figure 49.4 shows the abdomen just prior to perfusion. A roller pump forces the chemotherapy solution into the abdomen through the Tenckhoff catheter and pulls it out through the drains. A heat exchanger keeps the fluid being infused at 44° to 46°C so that the intraperitoneal fluid is maintained at 42° to 43°C. The smoke evacuator is used to pull air from beneath the plastic cover, preventing contamination of air in the operating room by chemotherapy aerosols. During the 90 minutes of perfusion, the surgeon vigorously manipulates all viscera to keep adherence of peritoneal surfaces to a minimum, ensuring that all the anatomic structures are uniformly exposed to heat and to chemotherapy. After the perfusion is complete, the abdomen is suctioned dry of fluid and reconstructive surgery is performed.

Hyperthermic Perfusion at the NCI

An inflow catheter is placed over the dome of the liver and an outflow catheter is placed in the pelvis. Multiple abdominal temperature probes are placed and the abdominal fascia is closed. The catheters are attached to a roller pump and heat exchanger, and the abdomen is shaken from its exterior to facilitate even perfusate distribution. Following perfusion, the abdomen is reopened, suctioned dry of fluid, and closed. Anastomoses are constructed prior to perfusion. Colonic anastomoses are protected with a temporary ileostomy that is constructed after perfusion is complete. At the WCI and the NCI, one catheter is left in the abdomen at the end of the procedure for the administration of postoperative intraperitoneal chemotherapy.

Table 49.3. National Cancer Institute (NCI) continuous hyperthermic peritoneal perfusion (CHPP) protocol summary

Protocol	Description	Accrual	Dates	Results
93-C-0048	Phase I trial of escalating cisplatin in patients with peritoneal carcinomatosis	49 patients (13 mesothelioma)	6/93–10/96	MTD = 300 mg/m^2 of cisplatin DLT = renal toxicity effective palliation
94-C-0162	Phase I trial of cisplatin + TNF in patients with peritoneal carcinomatosis	9 patients (5 mesothelioma)	6/94–10/94	MTD = 250 mg/m^2 cisplatin + 0.1 mg TNF DLT = renal toxicity TNF probably not helpful
97-C-0072	Phase I trial of IV taxol followed by CHPP with cisplatin followed by postop 5-FU/ paclitaxel IP dwell	61 patients (15 mesothelioma)	2/97–1/00	MTD = 1100 mg/m^2 5-FU 150 mg/m^2 paclitaxel DLT = 5-FU pancreatitis paclitaxel neutropenia IV taxol too toxic
00-C-0069	Phase II trial of CHPP cisplatin followed by post-op 5-FU/paclitaxel IP dwell	69 patients (28 mesothelioma)	2/00–2/02	Median progression-free survival 17 months Median overall survival 92 months

DLT, 5-FU, 5-fluorouracil; MTD, maximum tolerated dose; TNF, tumor necrosis factor.

NCI Results

Combined therapy, as practiced at the NCI, was developed in three sequential phase I trials using CHPP with increasing doses of cisplatin, tumor necrosis factor (TNF), and early postoperative intraperitoneal paclitaxel and 5-FU. A phase II trial of CHPP with cisplatin followed by postoperative 5-FU and paclitaxel intraperitoneal dwell was recently completed and data analysis is under way. Table 49.3 summarizes the CHPP trials that included patients with mesothelioma conducted at the NCI.

Phase I Trials

A phase I dose-escalation trial of CHPP with cisplatin was initiated for patients with peritoneal malignancies of any histology in 1992. In that trial, dose-limiting renal toxicity was observed at 350 to 400 mg/m^2 of cisplatin. Intravenous sodium thiosulfate was administered prior to CHPP to bind and inactivate cisplatin that was systemically absorbed in an attempt to limit nephrotoxicity (25).

Based on experimental data indicating synergy among TNF, cisplatin, and hyperthermia, and clinical data demonstrating palliation of malignant ascites following TNF intraperitoneal dwell, a phase I trial of escalating doses of TNF administered with a fixed 250 mg/m^2 dose of cisplatin was undertaken. This trial included 27 patients with peritoneal malignancies, four of whom had mesothelioma. The MTD was 0.1 mg/L of perfusate of TNF. The dose-limiting toxicity was nephrotoxicity. Intraperitoneal TNF alone has not been shown to cause renal toxicity. Tumor necrosis factor may enhance the nephrotoxicity of cis-

platin through a number of mechanisms. In light of the dose-limiting nephrotoxicity, it was elected to use cisplatin alone intraoperatively in subsequent clinical trials. Patients in this trial were evaluated at 3 months, 6 months, and every 6 months thereafter with physical exams and computed tomography (CT) scans. Since the patients were cytoreduced to levels that could not be imaged by CT scan, response could not be radiographically assessed. In the four patients with mesothelioma, intraperitoneal recurrences were observed at 3, 5, 24, and 31 months after treatment.

The third phase I trial at the NCI consisted of preoperative intravenous paclitaxel followed by CHPP with cisplatin and early postoperative escalating dose 5-FU and paclitaxel administered as a single intraperitoneal dwell 2 to 10 days after surgery. This protocol was modified to eliminate the preoperative intravenous paclitaxel because of bone marrow suppression. A phase II study of the revised regimen was recently completed.

Between 1993 and 1998, 18 patients with primary peritoneal mesothelioma were treated at the NCI on one of the three previously described phase I protocols. Seventeen of the patients had malignant mesothelioma and one had a multiply recurrent, symptomatic, benign, cystic mesothelioma. Table 49.4 shows the characteristics of the patients. The patients underwent a variety of procedures in an attempt to cytoreduce their tumors including distal pancreatectomy, splenectomy, and bowel resection.

At a median follow-up of 19 months, median progression-free survival was 26 months. Figure 49.5 shows the Kaplan-Meier survival curves constructed for progression-free and overall survival for the patients. Patients were considered to have stable disease until they had radiographic evidence of recurrence. Overall 2-year survival was 80%. The median overall survival had not been reached at the time of the report.

Ten patients had ascites at presentation. Nine of them had resolution of ascites postoperatively (Fig. 49.6). Three patients who developed recurrent ascites at 10, 22, and 24 months after initial treatment received repeat combined therapy. Though re-treatment with intravenous chemotherapy is generally not effective, repeat combined therapy was considered worthwhile because it is unlikely that significant drug resistance would develop after a single intensive exposure with the first CHPP and postoperative chemotherapy dwell. This may, in fact, be the case as all three patients had resolution of their ascites following the

Table 49.4. NCI CHPP protocol summary

Number	**18**
Median age (range)	**47 (15–75)**
Male-to-female ratio	**13:5**
Ascites preoperation	**10**
Prior systemic therapy	**4**
Prior laparotomy	**9**
Prior laparoscopy	**8**

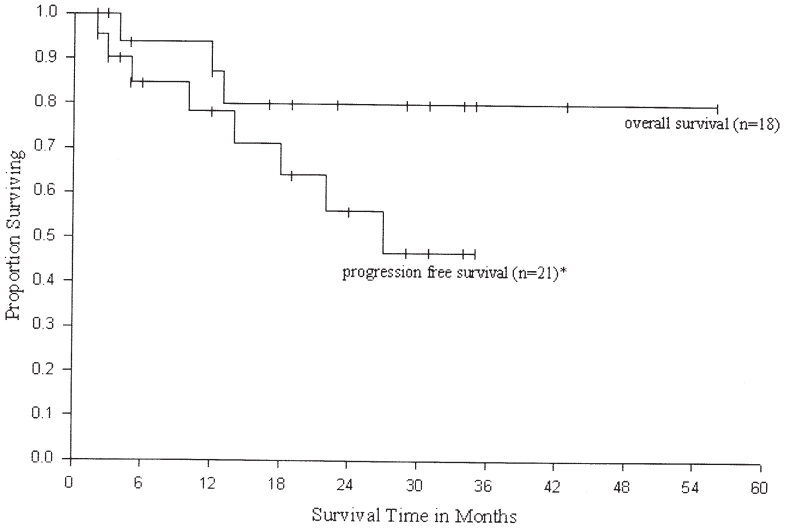


Figure 49.5. Kaplan-Meier survival curves for progression-free and overall survival for patients with peritoneal mesothelioma treated on phase I trials at the NCI between 1993 and 1998. (*The progression-free analysis includes three patients who have been treated twice.)

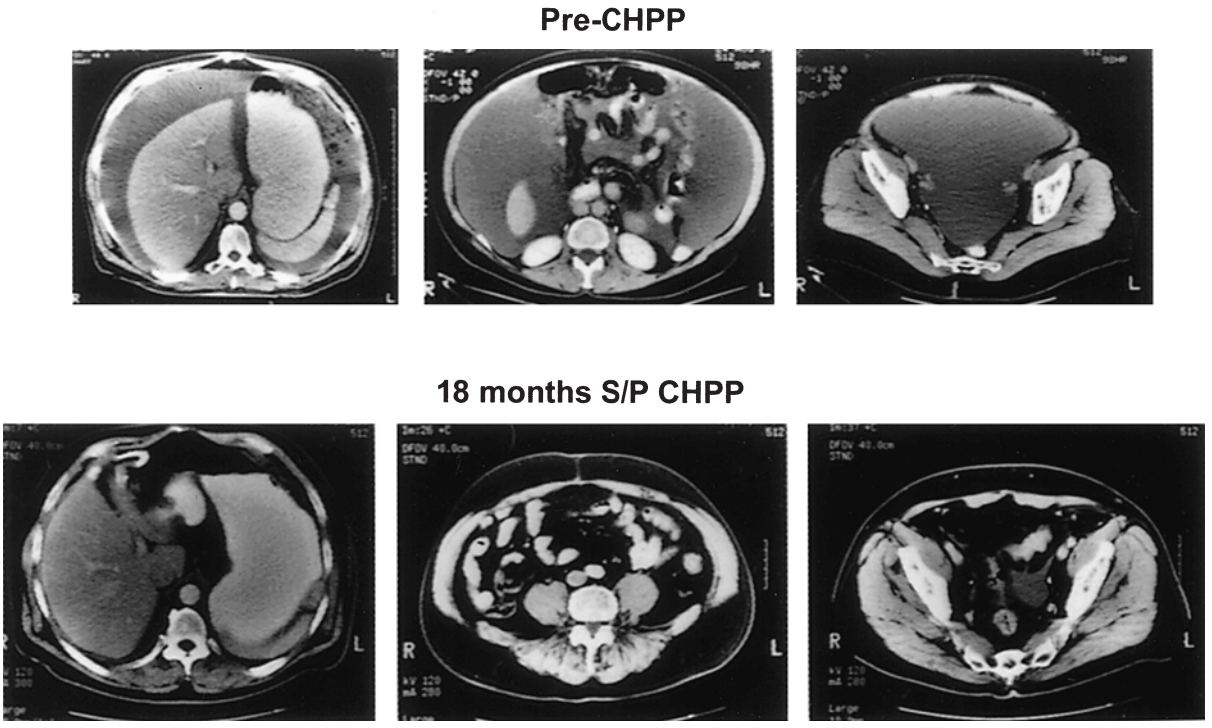


Figure 49.6. Computed tomography (CT) scans of the abdomen of a patient with peritoneal mesothelioma and ascites before (top) and 18 months after (bottom) continuous hyperthermic peritoneal perfusion. Note resolution of ascites.

second treatment, though response to cytoreduction alone cannot be ruled out. The patients did well with ongoing progression-free survival noted at 4, 6, and 24 months after their second perfusion.

Though the number of patients treated is small, the results suggest an association between the extent of residual disease at the end of cytoreduction and outcome. The three patients with bulky residual disease did poorly. They died of disease at 4, 12, and 13 months post-operatively. The four patients with minimal residual disease did well, with no evidence of disease at 29, 31, 34, and 35 months follow-up.

Phase II Trial

A phase II trial of CHPP with cisplatin followed by postoperative 5-FU and paclitaxel intraperitoneal dwell was recently completed at the NCI. Twenty-eight patients who participated in that trial had mesothelioma resulting in a total of 61 mesothelioma patients treated by CHPP on phase I and II studies. The outcomes of 49 of patients with mesothelioma who were treated at the MTD (250 mg/m^2) of cisplatin on all trials have been analyzed. The other 12 patients were not included in the analysis because they were treated with doses of cisplatin above or below the MTD or received TNF, which has been eliminated from the regimen.

The median overall survival of the 49 patients was 92 months and the median progression-free survival was 17 months. Figure 49.7 shows the Kaplan-Meier plot for overall survival. The estimated 3-year overall survival was 59%.

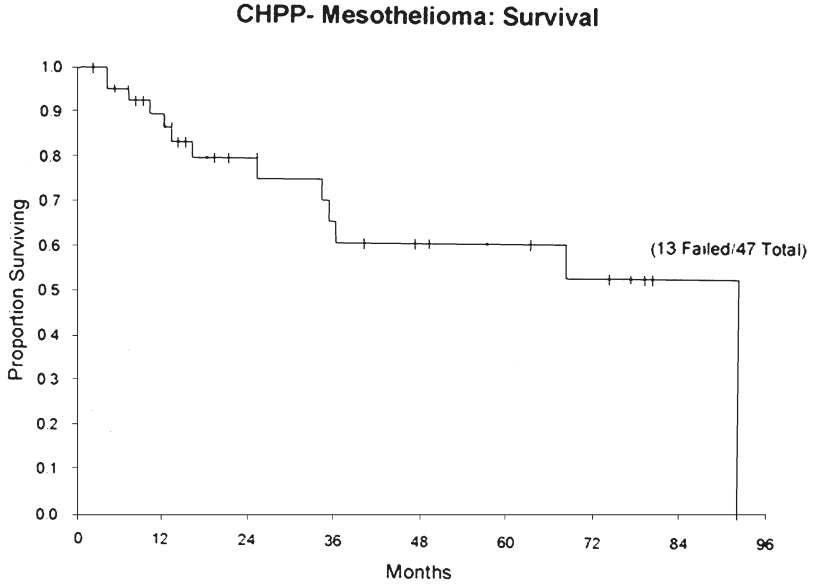


Figure 49.7. Kaplan-Meier plot for overall survival for patients with peritoneal mesothelioma treated on phase I and phase II trials at the NCI.

Cox regression analysis revealed a number of factors associated with improved outcome in this series. Patients who had superficial tumors had better progression-free and overall survival than those with deeply invasive tumors. Patients with little (less than 1 cm nodules) or no residual disease after cytoreduction had better overall and progression-free survival compared to those with residual tumors greater than 1 cm after cytoreduction. Patients who had undergone a cytoreduction procedure prior to undergoing CHPP had improved progression-free and overall survival compared to those who had not undergone prior surgery for mesothelioma. The reason for this is unknown. It is possible that the incidence of indolent disease is higher in the group of patients who had prior surgery and subsequently presented for combined therapy than in the general population of patients with mesothelioma who present to the NCI. Patients with very aggressive tumors are unlikely to recover from an initial cytoreduction and present for a second procedure with the performance status necessary for CHPP. Age less than 60 was a predictor of improved overall survival but did not affect progression-free survival. Gender was not a predictor of outcome in contrast to other studies in which females were found to have better outcomes than males. Histologic subtype of the tumor was also not significant.

WCI Results

Between 1989 and 2003, 90 cytoreductive procedures were performed on 68 patients with peritoneal mesothelioma at the WCI; 68 procedures were a first procedure, 17, a second-look procedure, three, a third-look procedure, and two, a fourth-look procedure. Data on these 68 patients are summarized in Tables 49.5 and 49.6. Fifty-eight patients received perioperative intraperitoneal chemotherapy (CHPP, postoperative treatment, or both), and ten patients did not. Seventeen patients underwent a scheduled second-look cytoreduction 6 to 9 months after the first operation, combined with perioperative intraperitoneal chemotherapy in 15. The median survival was 89 months in the 17 patients who had a second-look surgery compared with 55 months in the other 51 patients. The difference was not significant.

The Kaplan-Meier distribution of 68 patients with peritoneal mesothelioma treated between 1989 and 2003 is shown in Figure 49.8. The overall median survival was 67 months. All patients treated are included without any patient being excluded. The data include all of the patients treated with multiple different regimens, as shown in Table 49.6.

The patient gender had statistically significant prognostic implications in this series. The Kaplan-Meier distribution of 21 female patients treated between 1989 and 2003 is shown in Figure 49.9. At this date the median survival of female patients has not yet been reached. The median survival of male patients was 32.8 months. These results are statistically significant with a p value of .0014.

Table 49.5. Clinical factors with an impact on prognosis in 68 Washington Cancer Institute (WCI) patients with peritoneal mesothelioma

Variables	Number of patients	Median survival (months)	*p* value[a]
Gender			
Male	**47**	**33**	**.0014**
Female	**21**	**NR**	
Age			
>50 years	**34**	**52**	**.0332**
≤50 years	**34**	**NR**	
Pain			
Present	**30**	**55**	**NS**
Absent	**38**	**89**	
Ascites			
Present	**39**	**52**	**NS**
Absent	**29**	**NR**	
Weight loss			
Present	**14**	**26**	**.005**
Absent	**54**	**89**	
Pelvic/Abdominal mass			
Present	**18**	**32**	**NS**
Absent	**50**	**89**	
Incidental findings			
Yes	**13**	**NR**	**.039**
No	**55**	**52**	
Prior surgical score[b]			
0	**25**	**106**	**NS**
1–3	**43**	**52**	
Peritoneal cancer index[c]			
0–28	**43**	**67**	**.046**
29–39	**25**	**26**	
Pathology			
Papillary/epithelial	**54**	**55**	**.002**
Multicystic	**5**	**NR**	
Sarcomatous/biphasic	**9**	**13**	

Survival was measured from time of diagnosis.

NR, median survival not yet been reached; NS, not statistically significant.

[a] Log-rank test.

[b] Describes the procedure done prior to treatment at the WCI: 0, no surgery or biopsy only; 1, exploratory laparotomy or cytoreduction of one region only; 2, moderate cytoreduction (2–5 regions); 3, extensive cytoreduction (>5 regions).

[c] The peritoneal cancer index is determined by dividing the peritoneal cavity into nine regions by two equally spaced horizontal and vertical lines. An additional four regions are defined as the upper and lower jejunum and the upper and lower ileum. The volume of disease in each of these 13 regions is scored as 0 (no cancer seen), 1 (nodules <0.5 cm), 2 (nodules 0.5 to 5 cm), or 3 (nodules >5 cm). The maximum score is 39.

Modified from Sugarbaker PH, Welch L, Mohamed F, Glehen O. A review of peritoneal mesothelioma at the Washington Cancer Institute. In: Sugarbaker PH, ed. Management of Peritoneal Surface Malignancy. Surgical Oncology Clinics of North America (in press).

Table 49.6. Impact of treatment data on 68 WCI patients with peritoneal mesothelioma

Variables	Number of patients	Median survival (months)	*p* value[a]
Completeness of cytoreduction score[b]			
0–2	**41**	**67**	**.003**
3	**27**	**26**	
Perioperative chemotherapy			
Yes	**58**	**55**	**NS**
No	**10**	**NR**	
Metastasis			
Yes	**7**	**15**	**.005**
No	**61**	**55**	
Second-look surgery			
Yes	**17**	**89**	**NS**
No	**51**	**55**	
Status			
No evidence of disease	**22**	**NR**	
Alive with disease	**18**	**NR**	
Dead of disease	**22**	**13**	

Survival was measured from time of diagnosis.
NR, median survival not yet been reached; NS, not statistically significant.
[a] Log-rank test.
[b] 0, no cancer is seen; 1, tumor nodules less than 0.25 cm; 2, tumor nodules 0.25 to 2.5 cm; 3, tumor nodules greater than 2.5 cm or confluent nodules.
Modified from Sugarbaker PH, Welch L, Mohamed F, Glehen O. A review of peritoneal mesothelioma at the Washington Cancer Institute. In: Sugarbaker PH, ed. Management of Peritoneal Surface Malignancy. Surgical Oncology Clinics of North America (in press).

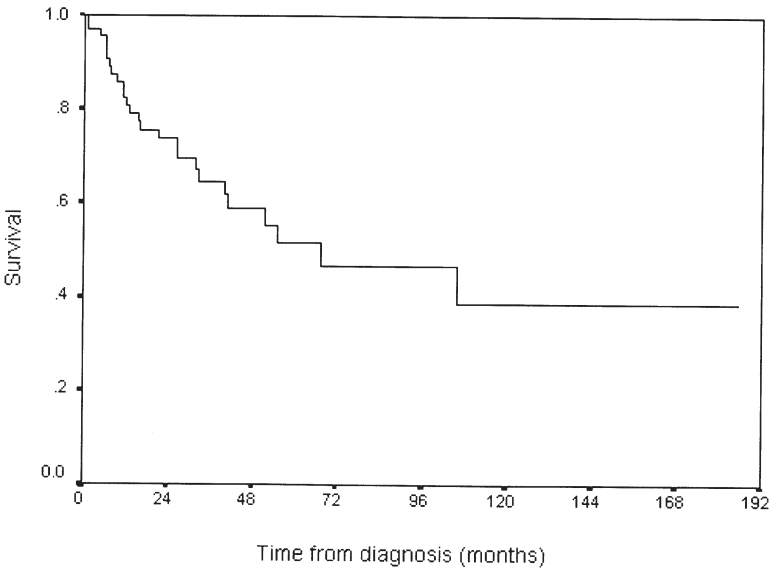


Figure 49.8. Kaplan-Meier survival curve for 68 patients with peritoneal mesothelioma treated at the WCI between 1989 and 2003.

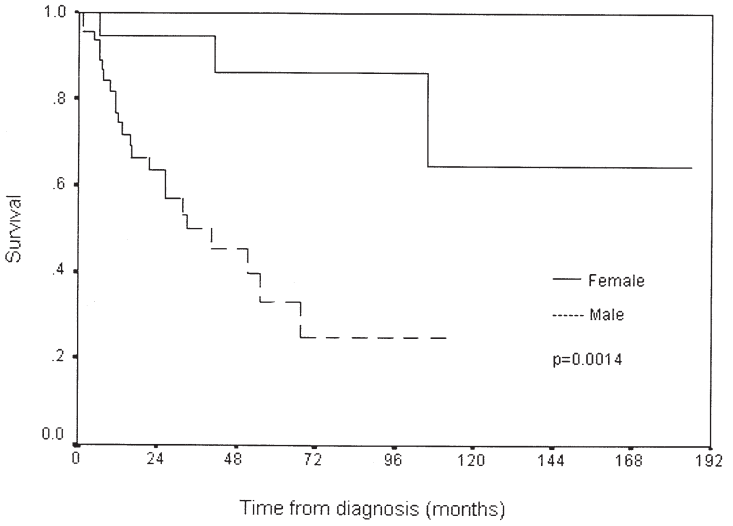


Figure 49.9. Kaplan-Meier survival for 21 female patients with peritoneal mesothelioma treated at the WCI between 1989 and 2003.

The earlier diagnosis in female than in male patients could be one of the explanations of their different prognosis (Table 49.7). In eight of the 21 (38%) female patients, the disease was diagnosed as an incidental finding. A diagnostic laparoscopy led to the biopsy that confirmed peritoneal mesothelioma. In 13 of 57 (23%) male patients, the disease was diagnosed as an incidental finding. None of the female patients presented with weight loss, while 20% of the males presented with weight loss. The mean value of the peritoneal cancer index was 21.1 in female patients, compared to 26.1 in male patients. For males, the mean interval between diagnosis and treatment was 6.9 months compared to 22.1 months for females.

Table 49.7. Differences between male and female peritoneal mesothelioma patients at the WCI

Diagnosis in females is more likely to be made as an incidental finding ($p = .016$)
Females are less likely to have weight loss as a symptom ($p = .003$)
Females have a longer interval between diagnosis and definitive cytoreduction (22 months) than males (7 months), suggesting a more indolent disease process in females ($p = .039$)
Females have a smaller volume of disease observed at the time of definitive cytoreduction as documented by PCI[a] ($p = .046$)
Females have prolonged survival with an identical treatment strategy ($p = .0014$)

[a] The peritoneal cancer index is determined by dividing the peritoneal cavity into nine regions by two equally spaced horizontal and vertical lines. An additional four regions are defined as the upper and lower jejunum and the upper and lower ileum. The volume of disease in each of these 13 regions is scored as 0 (no cancer seen), 1 (nodules <0.5 cm), 2 (nodules 0.5 to 5 cm), or 3 (nodules >5 cm). The maximum score is 39.

Table 49.5 shows the clinical factors with an impact on prognosis. Weight loss was an indicator of poor prognosis in males. The median survival of the patients diagnosed as an incidental finding has not been reached, whereas the median survival of symptomatic patients was significantly shorter at 51 months. The peritoneal cancer index was also a statistically significant predictor of survival. For patients with a peritoneal cancer index of 0 to 28, the median survival duration was 67.4 months compared to only 26.1 months for patients with a score between 29 and 39. The mean peritoneal cancer index was 26.5 in male patients whereas it was 21.1 in female patients ($p = .046$). The completeness of cytoreduction score was also a statistically significant prognostic indicator (Table 49.6). Our data would suggest that female patients with an early diagnosis who are treated in an aggressive fashion have a possibility for cure.

We found no evident difference in pathologic types of peritoneal mesothelioma between male and female patients. Those patients with sarcomatoid, deciduoid, or biphasic histology had a 13-month median survival as compared to a 54-month median survival in the patients with papillary or epithelial histology. In the patients with multicystic pathology, the median survival had not yet been reached. In summary, the gender of the patient, the diagnosis by incidental finding, weight loss, the extent of disease, and its histologic type are important in estimating survival.

There was no statistical indication in our series that intraperitoneal chemotherapy resulted in survival benefit. It is possible that selection factors were important in this evaluation, because patients with a large volume of mesothelioma after cytoreduction were usually not given intraperitoneal chemotherapy. However, it may be unwise to conclude that intraperitoneal chemotherapy may be eliminated from the treatment regimen, especially for its intraoperative or early postoperative administration.

Morbidity and Mortality of Combined Therapy

Sixteen of 68 patients treated at the WCI suffered grade 3 to 4 complications, giving an overall morbidity rate of 23.5%. Eight patients required reoperations, four for persistent bile leak from the liver surface, one for small bowel fistula, and two for late intraabdominal bleeding. At the NCI, the morbidity rate, combining all the phase I data, was 24%. The major toxicity was renal and this was seen in patients treated above what was determined to be the MTD of cisplatin. Patients treated below the MTD of cisplatin had minimal toxicity. Other complications included wound infection, fascial dehiscence, pancreatitis, ileus, atrial fibrillation, line sepsis, and *Clostridium difficile* colitis. At the NCI, hospital stay was not significantly prolonged over what would be expected with aggressive surgical cytoreduction alone, and the mean time to regular diet was approximately 1 week.

Transient elevations in hepatic enzymes and bilirubin have been identified after CHPP, though these are consistent with what is seen

following long operations requiring blood transfusion. A decrease in platelet count is also common and consistent with dilutional and consumptive coagulopathy. This does not appear to be related to cisplatin toxicity.

At the WCI, there were five perioperative deaths (7%), three from sepsis that occurred after surgery and two from pulmonary embolism. At the NCI, there was no operative or treatment-related mortality in this series.

Similarities in Data from NCI and WCI

The amount of tumor present prior to and after cytoreduction was a significant factor in predicting survival at both the NCI and WCI. At WCI the peritoneal cancer index, which quantifies the amount of disease present prior to cytoreduction, was found to be a statistically significant predictor of survival. Patients with a low peritoneal cancer index (localized disease) had an excellent prognosis, whereas patients with a high peritoneal cancer index (diffuse disease) had a poor prognosis. At WCI, the completeness of cytoreduction score quantifies the amount of tumor remaining after cytoreduction. Patients with a low completeness of cytoreduction score (no or minimal remaining tumor) had a significantly higher median overall survival than those with a high completeness of cytoreduction score (greater than 2.5 cm or confluent residual tumor nodules).

At the NCI, patients noted to have superficial disease prior to cytoreduction had a better prognosis than those found to have deeply invasive disease. Similarly, patients with minimal or no residual disease fared better than those with extensive residual tumor after cytoreduction. Patients at the NCI with residual nodules less than 1 cm were found to have better overall survival than patients with larger residual tumors.

In addition to survival, palliation is an important end point in therapy for mesothelioma. Both the WCI and the NCI have reported successful palliation of ascites following combined therapy. All but one of 39 patients with ascites treated at the WCI had no further symptoms from ascites after treatment. Nine of ten patients with ascites treated on phase I trials at the NCI had resolution of their ascites postoperatively. Three patients treated at the NCI who developed recurrent ascites following a response to therapy had resolution of their ascites following a second treatment, suggesting that repeat CHPP and postoperative intraperitoneal chemotherapy may be a useful palliative tool.

Contrasts in Outcome Between the NCI and WCI

Factors were found to be predictive of outcome at one institution but not at the other. Gender was found to be associated with prolonged survival at the WCI but not at the NCI. The WCI data suggest that the superior survival of females may be due, at least in part, to an association between female gender and indolent disease. Though the NCI data did not demonstrate prolonged survival in females, the NCI found

that patients who had undergone prior cytoreduction and subsequently presented for combined therapy, fared better than patients who presented for combined therapy as an initial treatment. It is possible that a history of prior cytoreduction is a marker for indolent disease, since patients with aggressive disease are less likely to be candidates for a second surgery. The role of gender in response to treatment is an important area for research.

The WCI noted that survival correlated with pathologic subtype but no correlation was found at the NCI. Though a large number of patients were treated on these studies, the high incidence of the epithelial subtype makes it difficult to compare survival with other less common subtypes. These differences may become more apparent as more patients are treated.

Summary

Combined therapy appears to be an effective method for the treatment of peritoneal mesothelioma. It can be performed safely and with acceptable morbidity. The WCI has reported a median overall survival of 67 months with a projected 3-year survival rate of 64% in 68 patients treated with various combinations of cytoreduction, CHPP, and intraperitoneal chemotherapy. The NCI has evaluated 49 patients who received combined therapy for peritoneal mesothelioma and found a median overall survival of 92 months with a projected overall 3-year survival of 59%. In both cases the outcomes are remarkably better than those in most previous reports of treatments for peritoneal mesothelioma, though these results need to be interpreted with caution, due to the short follow-up and selected study population.

The essential elements of combined therapy remain to be defined. The individual components of the therapy, including cytoreduction, intraoperative chemotherapy and hyperthermia, postoperative intraperitoneal chemotherapy, and perioperative intravenous chemotherapy, have been studied in various combinations and not in isolation. Further study is needed to define the optimal combination of therapies and to determine which patients may benefit most from the combined therapy approach. Ongoing trials are addressing these questions.

References

1. Antman K, Pomfret F, Aisner J, et al. Peritoneal mesothelioma: natural history and response to chemotherapy. J Clin Oncol 1983;1:386–386.
2. Cho H-K, Lush RM, Bartlett DL, et al. Pharmacokinetics of cisplatin administered by continuous hyperthermic peritoneal perfusion (CHPP) to patients with peritoneal carcinomatosis. J Clin Pharmacol 1999;39:1–8.
3. Sugarbaker PH, Acherman YI, Gonzalez-Moreno S, et al. Diagnosis and treatment of peritoneal mesothelioma: the Washington Cancer Institute experience. Semin Oncol 2002;29(1):51–61.

4. Begossi G, Gonzalez-Moreno S, Ortega-Perez G, Fon LJ, Sugarbaker PH. Cytoreduction and intraperitoneal chemotherapy for the management of peritoneal carcinomatosis, sarcomatosis and mesothelioma. Eur J Surg Oncol 2002;28(1):80–87.
5. Bartlett DL, Buell JF, Libutti SK, et al. A phase I trial of continuous hyperthermic peritoneal perfusion with tumor necrosis factor and cisplatin in the treatment of peritoneal carcinomatosis. Cancer 1998;83:1251–1261.
6. Park BJ, Alexander HR, Libutti SK, et al. Treatment of primary peritoneal mesothelioma by continuous hyperthermic peritoneal perfusion (CHPP). Ann Surg Oncol 1999;6(6):582–590.
7. Ma GY, Bartlett DL, Reed E, et al. Continuous hyperthermic peritoneal perfusion with cisplatin for the treatment of peritoneal mesothelioma. Cancer J Sci Am 1997;3:174–179.
8. Averbach AM, Sugarbaker PH. Peritoneal mesothelioma: treatment approach based on natural history. Cancer Treat Res 1996;81:193–211.
9. Bartlett DL. Peritoneal carcinomatosis. In: DeVita VT, Hellman S, Rosenberg SA, eds. Cancer: Principles and Practice of Oncology. Philadelphia: Lippincott Williams & Wilkins, 2000:1–8.
10. Kerrigan SA, Turnnir RT, Clement PB, Young RH, Churg A. Diffuse malignant epithelial mesotheliomas of the peritoneum in women: a clinicopathologic study of 25 patients. Cancer 2002;94(2):378–385.
11. Antman KH, Blum RH, Greenberger JS, Flowerdew G, Skarin AT, Canellos GP. Multimodality therapy for malignant mesothelioma based on a study of natural history. Am J Med 1980;68:356–362.
12. Alexander HR Jr, Bartlett DL, Libutti SK. National Cancer Institute experience with regional therapy for unresectable primary and metastatic cancer of the liver or peritoneal cavity. In: Markman M, ed. Current Clinical Oncology: Regional Chemotherapy: Clinical Research and Practice. Totowa, NJ: Humana Press, 2000:127–150.
13. Sugarbaker PH. Management of peritoneal-surface malignancy: the surgeon's role. Langenbecks Arch Surg 1999;384:576–587.
14. Dikhoff T, van der Heider J, Dubbelman R, ten Bokkel Huinink WW. Tissue concentration of platinum after intraperitoneal cisplatin administration in patients (PTS). Proc AACR 1985;26:162.
15. Alberts DS, Liu PY, Hannigan EV, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 1996;335(26):1950–1955.
16. Sugarbaker PH, Sweatman TW, Graves T, Cunliffe WJ, Israel MA. Early postoperative intraperitoneal adriamycin. Reg Cancer Treat 1991;4:127–131.
17. Bruckner HW, Cohen CJ, Goldberg JD, et al. Improved chemotherapy for ovarian cancer with cis-diaminedichloroplatinum and Adriamycin. Cancer 1981;47(9):2288–2294.
18. Benoit L, Duvillard C, Rat P, Chauffert B. The effect of intra-abdominal temperature on the tissue and tumor diffusion of intraperitoneal cisplatin in a model of peritoneal carcinomatosis in rats. Chirurgie 1999;124(4):375–379.
19. Herman TS. Temperature dependence of Adriamycin, cis-diaminedichloroplatinum, bleomycin, and 1,3-bis(2-chloroethyl)-1-nitrosourea cytotoxicity in vitro. Cancer Res 1983;43(2):517–520.
20. Takahashi I, Emi Y, Hasuda S, Kakeji Y, Maehara Y, Sugimachi K. Clinical application of hyperthermia combined with anticancer drugs for the treatment of solid tumors. Surgery 2002;131(1 suppl):S78–S84.

21. Zakris EL, Dewhirst MW, Riviere JE, Hoopes PJ, Page RL, Oleson JR. Pharmacokinetics and toxicity of intraperitoneal cisplatin combined with regional hyperthermia. J Clin Oncol 1987;5:1613–1620.
22. Baba H, Maehara Y, Takeuchi H, Inutsuka S, Sugimachi K. Optimal scheduling increases therapeutic gain of Adriamycin combined with hyperthermia. Anticancer Res 1993;13(3):651–654.
23. Giovanella BC, Stehlin JS Jr, Morgan AC. Selective lethal effect of supranormal temperatures on human neoplastic cells. Cancer Res 1976;36:3944–3950.
24. Dudar TE, Jain RK. Differential response of normal and tumor microcirculation to hyperthermia. Cancer Res 1984;44:605–612.
25. Howell SB, Pfeifle CE, Wung WE. Intraperitoneal cisplatin with sodium thiosulfate protection. Ann Intern Med 1982;97:845–851.
26. Chailleux E, Dabouis G, Pioche D, et al. Prognostic factors in diffuse malignant pleural mesothelioma. Chest 1988;93:159–162.
27. Antman K, Shemin R, Ryan L, et al. Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. J Clin Oncol 1988;6:147–153.
28. Sridhar KS, Doria R, Raub WA Jr, Thurer RJ, Saldana M. New strategies are needed in diffuse malignant mesothelioma. Cancer 1992;70(12):2969–2979.
29. Markman M, Kelsen D. Efficacy of cisplatin-based intraperitoneal chemotherapy as treatment of malignant peritoneal mesothelioma. J Cancer Res Clin Oncol 1992;118(7):547–550.
30. Yates DH, Corrin B, Stidolph PN, Browne K. Malignant mesothelioma in South East England: clinicopathological experience of 272 cases. Thorax 1997;52(6):507–512.
31. Neumann V, Muller KM, Fischer M. [Peritoneal mesothelioma—incidence and etiology]. Pathologe 1999;20(3):169–176.
32. Eltabbakh GH, Piver MS, Hempling RE, Recio FO, Intengen ME. Clinical picture, response to therapy, and survival of women with diffuse malignant peritoneal mesothelioma. J Surg Oncol 1999;70(1):6–12.
33. Loggie BW, Fleming RA, McQuellon RP, Russell GB, Geisinger KR, Levine EA. Prospective trial for the treatment of malignant peritoneal mesothelioma. Am Surg 2001;67(10):999–1003.

50

Pericardial and Tunica Vaginalis Mesothelioma

Bruno Pasquotti

The pericardium is a fibroblastic structure lined by mesothelium; the serous visceral layer (epicardium) is adherent to the heart, while the fibrous parietal layer is free. The pericardium extends nearly to the middle of the ascending aorta, on the pulmonary artery, and to the bifurcation. The majority of neoplastic lesions are metastatic tumors arising from breast and lung cancer, melanoma, acute leukemia, and lymphoma. Metastatic lesions may involve the whole pericardium or present as isolated nodules. Pleural mesothelioma often extends into the pericardium, and when this occurs at an early stage, it is difficult to determine where the tumor originated. A very rare lesion is the multilocular mesothelial inclusion cyst (MMIC)—so-called benign multicystic mesothelioma (1). It is a neoplasm to be placed between benign adenomatoid mesothelioma and malignant epithelial mesothelioma; the differential diagnosis includes lymphangioma and malignant cystic mesothelioma (2). Another rare neoplasm of the pericardium is the solitary fibrous tumor (localized fibrous mesothelioma) classified by Klemperer and Rabin (3) in 1931.

Pericardial malignant mesothelioma is the most common primary neoplasms of the pericardium (50% of all primary pericardial tumors). It is characterized by difficult and late diagnosis, local aggressiveness, and poor survival. This is quite a rare neoplasm as demonstrated by the data of one large autopsy study where the incidence of primitive pericardial mesothelioma neoplasm rarely reaches 0.0022% (4); for Grebenc et al (5), it represents less than 1% of all mesotheliomatous tumor, while for Murai (6), its incidence is about 6%. According to McDonald et al's (7) estimates based on the Canadian population, the incidence of the disease would be as low as one case in 30,000,000 people. At present, fewer than 150 cases have been presented in the medical literature. Many cases are found only at autopsy, and in less than 30% of these it has been possible to advance a histologic diagnosis in a living patient and usually during emergency thoracotomy (8). The mechanisms of pathogenesis are unknown. Contrary to pleural and peritoneal mesothelioma, it is suggested that this condition does not show a clear correlation to exposure to asbestos, despite some of

the data reported in the literature (9,10); it can also be produced by therapeutic radiation exposure (11). A link between simian virus 40 (SV40) and pericardial mesothelioma is not noted. This neoplasm affects mainly male patients (2:1 male-to-female ratio) in their fourth to seventh decade of life (12).

Diffuse pericardial mesothelioma is classified into epithelial, biphasic, and fibrosarcomatous; two thirds are mixed and one third epithelioid. It may be confined to the pericardium either as a localized mass or as a diffuse one with a massive fibrous reaction encasing the heart, and it may give rise to constrictive pericarditis (13). In the literature, there are only four cases of localized pericardial mesothelioma (14). On the cut surface the tumor may be yellowish-white or gray-white, solid with focal necrosis; pericardial effusion or hemorrhagic fluid is present due to the erosion of the cardiac chambers or of the intrapericardial blood vessels, thus causing acute pericardial distension and sudden, fatal cardiac tamponade. Local infiltration into the cardiac muscle and mediastinal tissue is reported occasionally and distant metastases even more rarely.

Primary pericardial mesothelioma is a diagnostic and therapeutic dilemma because signs and symptoms are nonspecific, and because of the small number of cases and the early occurrence of cardiac complications. The diagnostic profile includes the following investigations: history, clinical investigations, chest x-ray, transthoracic ultrasound, computed tomography (CT) scan, magnetic resonance imaging (MRI), and ultrasonographic supported puncture. The clinical situation is usually correlated to the cardiac function damage that manifests itself in the presence of effusive pericarditis, constrictive pericarditis, and sometimes effusive-constrictive pericarditis with cardiac tamponade but without coronary/myocardial cause. Physiopathologically, the diastolic filling of the ventricles is hindered, thus causing reduction of the flow and systemic hypertension.

Patients present a history of cough, orthopnea, progressive dyspnea, nonradiating substernal pain, pathologic murmurs, fatigue, and arrhythmia. Physical examination may reveal shortness of breath and distended jugular veins; Kussmaul's sign and accentuated paradoxis pulse may be present. Sometimes we observe hepatomegaly, increased abdominal girth, and peripheral edema. In older patients, the upper torso and the arms can show evidence of muscular deterioration. Electrocardiology shows low-voltage complexes (QRS) with nonspecific precordial ST segment changes reflecting the absence of an inflammatory element in the disease, atrial fibrillation, and atrial flutter; sometimes anomalies of the left atrium can be observed and these are represented by the mitral P. The chest x-ray can show either normal or increased cardiac shadow; the increase may only be apparent due to the concurrent pericardial effusion or to an enormously thickened pericardium. The upper right part of the mediastinum may be prominent due to the distention of the superior vena cava. The presence of calcifications characterizes less than 50% of the patients, but is not diagnostic for constriction. The majority of patients also presents with pleural effusion. Two-dimensional echocardiography shows a dilated

pericardial sac, but it may be difficult to distinguish pericardial effusion from the tumor or pericardial thickening. Pericardial thickening presents as constant echo-poor districts. The investigation can show a still strongly echogenous pericardium, an interventricular sect protrusion into the left ventricle during inhalation, a severe protodiastolic filling, and distention of the hepatic veins as well as of the inferior vena cava.

Postcontrast CT provides information regarding the presence and the extension of the tumor; CT is complementary to echocardiography in the diagnosis and management of pericardial effusion and thickening. In cases of primary tumor, MRI may show a mass of the pericardial layer, lack of continuity of the normal pericardium, and pericardial thickening and effusion diagnostic for effusive-constrictive or constrictive pericarditis. The CT scan should be used initially. The MRI could be used to complement CT, or can be employed to distinguish between constrictive pericarditis and restrictive cardiomyopathy as normal pericardial thickness excludes most cases of constrictive pericarditis.

The differential diagnosis between pericardial mesothelioma and other primary (solitary fibrous tumor, synovial sarcoma, epithelioid angiosarcoma, adenomatoid tumor) or metastatic tumors is very difficult; the imaging features cannot be differentiated because any neoplastic conditions show diffuse plaques, or multicentric nodules. Another difficult differential diagnosis is that involving constrictive pericarditis of either tubercular or inflammatory nature when diffuse thickening of pericardium and fluid collection are also present (15,16). Magnetic resonance imaging is emerging as the best modality for demonstrating the extent and nature of the constrictive process and hence, its resectability (17). No studies have used fluorodeoxyglucose positron emission tomography (FDG-PET) imaging.

Early diagnosis is mandatory and the limitation of the diagnostic tools is very important. Echocardiography and CT are available in assisting in fluid aspiration or biopsy of the pericardium. The cytologic analysis of pericardium fluid is often inconclusive (18), due to the difficulty of formulating a differential diagnosis in the presence of metastatic lesions caused by adenocarcinoma (lung, breast, gastrointestinal tract), mesothelial cell hyperplasia, or reactive cells (19). Precise distinction requires histopathologic examination. Comprehensive analysis including gross appearance, histology, histochemistry, immunohistochemistry, and electron microscopy is recommended. Although it is now unlikely, the diagnosis of tubercular pericarditis has to be excluded in the presence of bloody effusion.

Therapeutic decisions are dependent on the general status of the patient, on the presence or absence of clinical signs of cardiac danger, and on the available treatment for each specific case. In extreme cases, patients have no chance of benefiting from surgical treatment; in this situation diagnostic procedures should be as brief and noninvasive as possible, intervening only to alleviate the symptoms and improve the quality of life. In these patients with a short survival time, in the presence of pericardial fluid, it is possible to perform or repeat a pericar-

diocentesis and instillation of chemotherapeutics (cisplatin) with a low frequency of complications, while another noninvasive alternative to surgery is percutaneous balloon pericardiotomy with a success rate of 90% (20); subxiphoid drainage is suitable in performing a pericardioscopy and a pericardial biopsy (21).

When the prognosis for a patient is better, and survival is assessed as being more than 12 months, there are aggressive treatments available such as total pericardiectomy or pericardial resection through a median sternotomy, left lateral thoracotomy, or video-assisted thoracic surgery (VATS). Surgery is the best choice of treatment if the tumor is localized to the pericardium but remains palliative. Mesothelioma of the pericardium is resistant to radiation therapy alone or in combination with chemotherapy. Except for gemcitabine, with a response rate up to 40%, chemotherapy with doxorubicin, cyclophosphamide, cisplatinum, mitomycin, paclitaxel, or carboplatin as single agent or in combination, has no significant activity in this disease and does not improve the outcome of these patents (22–25). A multimodality approach is encouraging. Clinical deterioration with subsequent death within 4 to 12 months is the natural story of these patients; the prognosis is poor due to its late presentation, difficulty in removing the neoplasm surgically, and poor response to radio- and chemotherapy (5). Usually patients die from cardiac complications.

Tunica vaginalis testis is a peritoneal sac located anterolateral to the testis. It has two layers: visceral and parietal. The visceral layer lies on the anterolateral surface of the testis and epididymis; the parietal layer lines the inner surface of the scrotal sac. Malignant mesothelioma of the tunica vaginalis is an uncommon paratesticular tumor and since the first cases described by Bailey et al (26) in 1955 and Barbera and Rubino (27) in 1957, fewer than 100 cases have been reported in the literature (28,29). It represents less than 5% of all mesotheliomas and is highly aggressive. The testicular malignant mesothelioma has an age distribution with peak onset in the sixth decade, although some cases (10%) have been described in adolescents (30,31). In contrast to pleural mesothelioma, the association between asbestos and malignant mesothelioma of the tunica vaginalis has not been well described. Jones et al (30) reported only 1 of 11 cases (9%) with a history of asbestos exposure, while Antman et al (31) described the link in 11 of 27 cases, (41%) with a latent period of up to 40 years. Trauma and previous herniorrhaphy have also been proposed by Chen et al (32) in 1982 and Plas et al (29) in 1998 as etiologic factors.

Malignant mesothelioma of the tunica vaginalis consists of multiple, firm white-yellow nodules or papillary excrescences that stud the serosal surface of the tunica and that may eventually encase the scrotal contents; the tunica vaginalis is thickened. Cystic spaces may be seen in the tumor, which may be intraparenchymal with invasion of the testis (33). The tumor may extend to the surrounding structures (34). Inguinal, pelvic, retroperitoneal and supraclavicular lymph nodes may be involved (26) in approximately 15% to 25% of cases (35). The neoplasm spreads less commonly to the lung, liver, and bone. A case has been reported by Poggi et al (36) with involvement of the pleura and

peritoneum. McDonald et al (37) and Menut et al (38) reported a bilateral malignant mesothelioma in two patients. Histologically the pattern is usually an epithelial mesothelioma with a papillary, tubulopapillary, or solid pattern; sometimes a biphasic pattern is produced (39).

The differential diagnosis includes mesothelial hyperplasia either spontaneous or secondary to inflammatory processes, adenomatoid tumor, embryonal carcinoma, well-differentiated papillary mesothelioma, and metastatic tumor from prostatic and lung carcinoma. Histology, immunohistochemistry with currently available markers, and electron microscopy examinations may confirm the diagnosis of malignant mesothelioma. The most common symptoms are related to a swelling of the scrotum, or to a progressive or rapid enlargement of the scrotum due to hydrocele with or without a paratesticular or testicular mass lesion; the tumor may invade the testis or the skin, and spread to the locoregional lymph nodes.

Diagnosis is not easy due to the nonspecific symptoms, and lack of tumor markers (40). To date, only five patients (3% of published cases) have obtained a preoperative correct diagnosis by cytology of the hydrocele fluid (41,42) or suspected nodule (28). An accurate preoperative diagnosis consists of physical examination, sonographic examination using an ultrasound scanner, ultrasound-guided fine-needle aspiration (FNA) of fluid or a nodule, cytologic examination, and a CT scan of the chest, abdomen, and pelvis for staging. At times ultrasonography can be difficult due to the small size of the neoplastic nodules (diameter less than 0.5 cm) and to the presence of papillary, exophitic lesions or rather to the infiltrative aspect of the neoplasm. The presence of a simple or complex hydrocele can either be associated or not with multiple endoscrotal nodular lesions of varying diameter, meaning a lesion often starting from the scrotal wall. The cytologic investigation of the endoscrotal fluid or nodular lesions is not always diagnostic; the diagnosis will then most often be obtained intraoperatively. The rapid reaccumulation of fluid within a short time should hint at the neoplastic nature of the lesions. In a small surgical biopsy, reactive mesothelial hyperplasia may mimic the neoplasm (23).

There are no data in the literature to suggest whether or not to proceed with a color Doppler investigation. Wolanske and Nino-Murcia (40) describe a decreased vascularity in the tumor compared with testicular parenchyma. Because of the rarity of the neoplasm and its inauspicious diagnosis, a correct therapeutic protocol is still under discussion. There is no role for local preserving excision (transcrotal procedures) or resection of the hydrocele wall because the tumor has the potential for local invasion and recurrence. When there is suspicion of a mesothelioma of the tunica vaginalis, considering the general condition of the patient, the pathologic stage, and the disease stage, it is correct to proceed with radical inguinal orchiectomy (31,43) in association with an inguinal lymphadenectomy aimed at obtaining the correct diagnosis and local control of the disease. Huncharek et al (44) report the case of a patient with a tumor located exclusively in the tunica vaginalis, surviving over 10 years after radical surgery.

The role of inguinal or pelvic lymphadenectomy has not yet been decided because of the low incidence of lymph node metastasis, which doesn't reach 10% of cases at diagnosis. Less than 9% of patients present with a dissemination to the retroperitoneal lymph nodes, which are the first station for all neoplasms of the testis. Pelvic node dissection or a retroperitoneal lymph node dissection cannot be employed as a staging modality but only as treatment (45). According to Carp et al (43), a retroperitoneal lymph node dissection should be performed only if the pelvic nodes are positive or if CT scan shows an enlargement of the paraaortic nodes. When there is scrotal involvement, hemiscrotectomy should be considered. Surgery also is indicated for recurrent tumor.

Just as in pericardial mesothelioma, the role of adjuvant chemotherapy (doxorubicin, cisplatin, or cyclophosphamide), radiotherapy, and immunotherapy in combination or alone still has to be defined. After radical surgery, radiation therapy to the pelvis and groin may prevent local recurrence, which manifests itself in not less than 60% of cases in the first 2 years of treatment and therefore, requires an instrumental follow-up every 3 months for at least 2 years (clinical examination, CT scan of abdomen and pelvis every 6 months, and chest radiology) (29,40), and annual examination thereafter. A revision of the data reported in the literature identifies two important diagnostic parameters: the age of the patient (age less than 60 shows a better prognosis) and the degree of metastatis or local extent of tumor. There is no evidence that conservative or radical surgery influences survival. After radical or palliative treatment prognosis is poor and the median survival is less than 20 months.

References

1. Drut R, Quijano G. Multilocular mesothelial inclusion cysts (so-called benign multicystic mesothelioma) of pericardium. Histopathology 1999;34: 472–474.
2. Weiss SW, Tavassoli FA. Multicystic mesothelioma—an analysis of pathologic findings and biological behavior in 37 cases. Am J Surg Pathol 1988; 12(10):737–746.
3. Klemperer P, Rabin CB. Primary neoplasms of the pleura: a report of five cases. Arch Pathol 1931;11:385–412.
4. Cohen JL. Neoplastic pericarditis. Cardiovasc Clin 1976;7:257–268.
5. Grebenc ML, Rosado de Christenson M, Burke A, Green CE, Galvin J. Primary cardiac and pericardial neoplasms: radiologic-pathologic correlation. Radiographics 2000;20:1073–1110.
6. Murai Y. Malignant mesothelioma in Japan: analysis of registered autopsy cases. Arch Environ Health 2001;56(1):84–88.
7. McDonald AD, Harper A, El Attar GA, McDonald JC. Epidemiology of primary malignant mesothelial tumors in Canada. Cancer 1970;26:914–919.
8. Hillerdal G. Malignant mesothelioma 1982: review of 4710 published cases. Br J Dis Chest 1983;77:321–343.

9. Churg A, Warnock ML, Bersch KG. Malignant mesothelioma arising after direct application of asbestos and fiberglass to the pericardium. Am Rev Respir Dis 1978;118:419–424.
10. Burke A, Virmani R. Tumors of the Cardiovascular System. Atlas of Tumor. Pathology, 3rd series, fascicle 2. Washington, DC Armed Forces Institute of Pathology.
11. VelissarisTJ, Tang ATM, Millward-Sadler GH, Morgan JM, Tsang GM. Pericardial mesothelioma following mantle field radiotherapy. J Cardiovasc Surg 2001;42:425–427.
12. Kahn EI. Primary pericardial mesothelioma following exposure to asbestos. Environ Res 1980;23:270–281.
13. Bohn U, Gonzalez JL, Martin LM, et al. Meningeal and brain metastases in primary malignant pericardial mesothelioma. Ann Oncol 1994;5:660–661.
14. Val-Bernal JF, Figols J, Gomez-Romn JJ. Incidental localized (solitary) epithelial mesothelioma of the pericardium: case report and literature review. Cardiovasc Pathol 2002;11(3):181–185.
15. Moncada R, et al. Diagnostic role of computed tomography in pericardial disease: congenital defects, thickening, neoplasms, and effusions. Am Heart J 1982;103:263–268.
16. Delille J, et al. Maximal thickness of the normal human pericardium assessed by electron beam computed tomography. Eur Radiol 1999;9:1183–1189.
17. Ohnishi J, Shiotani H, Ueno H, Fujita N, Matsunaga K. Primary pericardial mesothelioma demonstrated by magnetic resonance imaging. Jpn Circ J 1996;60:898–900.
18. Thomason R, Schlegel W, Lucca M, Cummings S, Lee S. Primary malignant mesothelioma of the pericardium: case report and literature review. Tex Heart Inst J 1994;21(4):32.
19. Kaul TK, Fields BL, Kahn DR. Primary malignant pericardial mesothelioma: a case report and review. J Cardiovasc Surg 1994;35:261–267.
20. Palacios I, Tuzcu E, Ziskind A, Younger J, Block P. Percutaneous balloon pericardial window with malignant pericardial effusion and tamponade. Cathet Cardiovasc Diag 1991;22:244–249.
21. Huang-Joe Wang, et al. Technical and prognostic outcomes of double-balloon pericardiotomy for large malignancy-related pericardial effusions. Chest 2002;122:893–899.
22. Hollins J, Lowman A, Assey ME. Primary pericardial mesothelioma: diagnostic and therapeutic challenges in management. South Med J 1988; 81:537–538.
23. Van Meerback JP, Baas P, Debruyne C, et al. A phase II study of gemcitabine in patients with malignant pleural mesothelioma. Cancer 1999;85:2577–2582.
24. Nambiar CA, Tareif R, Ravindran J, Banerjee A. Primary pericardial mesothelioma one-year event-free survival. Am Heart J 1992;124:802–803.
25. Chahinian AP, Antman K, Aisner J, et al. A randomized phase II trial of cisplatin with mitomycin or doxorubicin for malignant mesothelioma. J Clin Oncol 1993;11:1559–1565.
26. Bailey GN, Willi RA, Wilson JV. A case of adenocarcinoma of the appendix testis. J Pathol Bacteriol 1955;69:326–328.
27. Barbara V, Rubino M. Papillary mesothelioma of the tunica vaginalis. Cancer 1957;10:183–184.
28. Bruno C, Minnit S, Procacci C. Diagnosis of malignant mesothelioma of the tunica vaginalis testis by ultrasound-guided fine needle aspiration. J Clin Ultrasound 2002;30:181–183.

29. Plas E, Riedl CR, Pflueger H. Malignant mesothelioma of the tunica vaginalis testis. Cancer 1998;83:2437.
30. Jones MA, Young H, Scully RE. Malignant mesothelioma of the tunica vaginalis. Am J Surg Pathol 1995;19:815–825.
31. Antman K, Cohen S, Dimitrov NV, et al. Malignant mesothelioma of the tunica vaginalis testis. J Clin Oncol 1984;2:447–451.
32. Chen KTK, Arhelger RB, Flam MS, Hanson JH. Malignant mesothelioma of the tunica vaginalis testis. Urology 1982;20:316.
33. Attanoos RL, Gibbs AR. Primary malignant gonadal mesotheliomas and asbestos. Histopathology 2000;37:150–159.
34. Van der Rhee HJ, Van Vloten WA, Scheffer E, Zwartendijk J. Cutaneous manifestation of malignant mesothelioma of the tunica vaginalis testis. J Cutan Pathol 1983;10:213–216.
35. Mathew BS, Jyothirmaryi R, Nair MK. Case report: malignant mesothelioma of the tunica vaginalis testis presenting with spinal metastasis—report of two cases. Br J Radiol 1996;69:1067–1068.
36. Poggi A, Longo F, et al. A case of mesothelioma of the tunica vaginalis testis, with involvement of the pleura and peritoneum. Tumori 2000;86:256–257.
37. McDonald RE, Sago AL, Novicki DE, Bagnall JW. Paratesticular mesotheliomas. J Urol 1983;130:60–361.
38. Menut P, Herve JM, Barbagelata M, Botto H. Bilateral malignant mesothelioma of the tunica vaginalis. Apropos of a case. Prog Urol 1996;6:587–589.
39. Eimoto T, Inoue I. Malignant fibrous mesothelioma of the tunica vaginalis. Cancer 1977;39:2059–2066.
40. Wolanske K, Nino-Murcia N. Malignant mesothelioma of the tunica vaginalis testis. Atypical sonographic appearance. J Ultrasound Med 2001;20: 69–72.
41. Japtko L, Horta A, Schreiber K, et al. Malignant mesothelioma of the tunica vaginalis testis: report of the first case with preoperative diagnosis. Cancer 1982;49:119–127.
42. Gupta SC, Gupta AK, Misra V, Singh PA. Pre-operative diagnosis of malignant mesothelioma of tunica vaginalis testis by hydrocele fluid cytology. Eur J Surg Oncol 1998;24(2):153–154.
43. Carp N, Petersen RO, Kusiak JF, Greenberg RE. Malignant mesothelioma of the tunica vaginalis testis. J Urol 1990;144:1475–1478.
44. Huncharek M, Klassen M, Christiani D. Mesothelioma of the tunica vaginalis testis with possible occupational asbestos exposure. Br J Urol 1995;75: 679–681.
45. Smith JJ, Malone MJ, Geffin J, Silverman ML, Libertino JA. Retroperitoneal lymph node dissection in malignant mesothelioma of tunica vaginalis testis. J Urol 1990;144:1242–1243.

51

New Target Therapies for Malignant Mesothelioma

Camillo Porta and Luciano Mutti

The Gompertzian-like growth kinetic of the vast majority of human solid malignancies, together with their genetic instability, which leads to the development of drug resistance, contributes to the limited therapeutic efficacy of common antineoplastic drugs. Indeed, with traditional cytotoxic agents, we cannot reach the several logs of cell kill that are required to completely eradicate an established human tumor; furthermore, these drugs are not specific and kill all replicating cells, including a large number of nonneoplastic ones, causing often severe toxicity.

However, newer cancer therapies directed at specific and common molecular alterations in signaling pathways of cancer cells, sustaining tumor cell growth, invasion, and metastasis, and preventing apoptosis, are now rapidly leaving the laboratory bench and reaching patients' bedside; this wave of so-called smart drugs holds promise for a radical change in the way we treat a number of solid as well as hematologic malignancies, and this is especially true for those neoplasms that are refractory, or became resistant, to standard treatment options.

Malignant mesothelioma (MM) is one of these aggressive malignancies, whose incidence is expected to dramatically rise in the next decade in Europe (1); rarely suitable for radical surgical resection and usually resistant to both radiotherapy and chemotherapy, MM has a very poor prognosis, so that a considerable therapeutic skepticism usually surrounds patients affected with this tumor. Thus, effective systemic treatment options for this tumor are desperately needed.

Growth factors and growth factor receptors are ideal targets for novel therapeutic approaches to human cancer, since they have been shown to play a significant role in the development and progression of a number of tumors, including MM (2); furthermore, transformed cells have a reduced requirement for exogenously supplied growth factors, being able to produce high levels of peptide growth factors as well as to simultaneously express their receptors (autocrine loop). The cellular ligands of the above growth factors mainly belong to the family of tyrosine kinases, a family of transmembrane proteins that, upon acti-

vation, transmit the signal downstream, leading to tumor cell proliferation, growth, spread, and survival (3).

This chapter reviews both theoretical issues and the preclinical and clinical data regarding the potential therapeutic role in MM of a number of new drugs directed against specific tumor targets, including growth factor receptors, as well as other complex molecular mechanisms supporting tumor angiogenesis and preventing cancer cell apoptosis.

Targeting the Epidermal Growth Factor Receptor Pathway

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein belonging to the ErbB family of tyrosine kinase proteins. This family includes four members: the EGFR (also known as ErbB1/HER1), ErbB2/neu/HER2, ErbB3/HER3, and ErbB4/HER4. Each of these proteins possesses three different domains: the *extracellular domain*, which is involved in recognizing and binding the ligands that are able to activate the receptor; the *membrane spanning sequence*, which is involved in the interaction between receptors; and the *intracellular domain*, in which resides the enzymatic activity of the tyrosine kinase that is able to phosphorylate tyrosine residues on different intracellular adaptor proteins, and the receptor itself (4).

Upon binding with ligands such as the epidermal growth factor (EGF) itself, the transforming growth factor-α (TGF-α), and others, receptor dimerization, which is essential for the subsequent generation of intracellular signal, is rapidly induced; receptor pairings can consist of two molecules of the same type (homodimers) or two molecules of different types (heterodimers); receptor dimerization is followed by activation of intrinsic protein tyrosine kinase activity, tyrosine phosphorylation, and activation of intracellular signal transduction pathways, such as the PI3K/AKT and the ras/raf/MEK/MAPK pathways. Ultimately, the activation of the EGFR mediates several tumor cell functions, including proliferation, survival, induction of angiogenesis, invasion, and metastasis, as well as resistance to both chemotherapy and radiotherapy (4).

The EGFR is broadly expressed in a variety of cells of ectodermal or mesodermal origin, and its overexpression commonly occurs in a number of human tumors, especially of epithelial origin. This expression has been often correlated with a poor prognosis (5).

Several different anti-EGFR therapies have been developed in the past two decades, including murine or humanized anti-EGFR antibodies (6), and, more recently, EGFR-tyrosine kinase inhibitors (EGFR-TKIs) (7), which mainly act directly, inhibiting tyrosine kinase phosphorylation by physical interaction with either the adenosine triphosphate (ATP) or the enzyme-substrate binding sites (8). However, since response to anti–EGFR-TKIs could be observed also in patients with very low levels of expression of the target receptor, i.e., EGFR, more complex mechanisms of action have also been hypothesized (reviewed in ref. 7).

Several TKIs have shown antitumor activity in vitro and in vivo in preclinical models (9,10); among them, two orally active quinazoline derivatives, ZD1839 (Iressa, Astra Zeneca) and OSI 774 (Tarceva, OSI Pharmaceuticals/Genentech/Roche) are in an advanced stage of development, large phase III studies with these drugs being in progress worldwide for different indications.

Among a wide range of human tumors, including lung, breast, stomach, colon, pancreas, prostate, kidney, ovary, head and neck, and testicular cancer, MM also has been demonstrated to overexpress EGFR; indeed, in the early 1990s, EGFR expression was reported in 68% of paraffin-embedded MM specimens (11), as well as in four of four MM cell lines (12); more recently, Govindan et al (13) showed by immunohistochemistry that 11 of 13 epithelioid, two of four biphasic, and one of seven sarcomatoid MMs overexpressed EGFR. The latter observations, along with a more thorough understanding of EGFR-TKI's antitumor activity and the demonstration of their activity in vitro against MM cell lines (14), led to the launch of clinical trials of both ZD1839 and OSI 774 in MM patients.

The Cancer and Leukemia Group B (CALGB) has recently completed a phase II trial of ZD1839 in patients with MM (15), but the final results have not been published yet. The South West Oncology Group (SWOG), under the auspices of the National Cancer Institute (NCI), has recently activated another phase II study (protocol SWOG-S0218) using OSI 774. Thus, despite the lack of mature data from ongoing clinical trials, the targeted inhibition of the EGFR signaling pathway represents a promising strategy to treat MM patients.

Targeting the Platelet-Derived Growth Factor Receptor Pathway

The various platelet-derived growth factor (PDGF) and PDGF receptor (PDGFR) isoforms compose a family of ligands and receptors; PDGF is a 30-kd protein consisting of disulfide-bonded homodimers or heterodimers of A and B chains. All three combinations of subunits may occur: AA, AB, and BB. The recently identified PDGF C isoform seems to occur only as a homodimer (CC), since it is not yet known whether it can also form heterodimers with other PDGF chains (16).

The PDGFRs occur as α and β homodimers or α/β heterodimers and, again, belong to the protein tyrosine kinase family of receptors. The extracellular portion of these proteins are characterized by the presence of five immunoglobulin-like domains, created by regularly spaced disulfide bonds; the intracellular portions of each receptor contains a conserved tyrosine kinase domain into which an interrupting sequence of approximately 100 amino acids is inserted. A similar structure is found also in the PDGFR closely related CSF-1 and c-Kit receptors (17). The α receptor can bind to all dimeric PDGF isoforms (AA, BB, AB, CC), whereas the β receptor chain preferentially binds to the B isoform.

After binding of the dimeric ligand to the extracellular portion of the two PDGFR chains, a receptor homodimer or heterodimer is formed, allowing autophosphorylation of receptor tyrosine residues, the re-

cruitment of transduction molecules, their binding to phosphorylated receptors, and, ultimately, the downstream transmission of the signal that drives cell growth, cell morphology changes, and prevention of apoptosis (16).

Whereas normal PDGF function is critical for normal embryonic development and adult homeostasis, overactivity of the PDGF/PDGFR axis has been implicated in several disorders characterized by excessive cell growth, including certain malignancies (18). Since glial cells, fibroblasts, and smooth muscle cells are the normal physiologic targets of PDGF, tumors derived from these cells have been first analyzed for autocrine stimulation through the above pathway; however, coexpression of PDGF and PDGFRs, suggestive of autocrine growth stimulation, has also been described in a number of other types of human tumors, including meningiomas, melanomas, neuroendocrine tumors, and ovarian, pancreatic, gastric, lung, and prostate cancer (16,18). Recent advances support a key role for PDGF/PDGFR's autocrine loop also in the growth and spread of MM (19,20). Indeed, overexpression of PDGF-β receptors has been demonstrated in MM cell lines, while normal mesothelial cells predominantly express PDGF-α receptors (21), as well as in MM xenografts in nude mice and in MM patient specimens (15).

Furthermore, one of the most common genetic abnormalities observed in MM involves chromosome 22q13, which codes for the β-chain of PDGF; transduction of a hammerhead ribozyme against PDGF-β messenger RNA (mRNA) into MM cell lines led to a significant reduction of cell growth (22); similarly, blocking PDGF-α resulted in MM growth inhibition (23).

Among several inhibitors able to block the PDGF/PDGF-R autocrine loop, SU101, GFB-111, and STI-571 are exciting new drugs that are just entering clinical development in humans. SU101 (or leflunomide, Sugen Inc.) has been shown to be able to inhibit different tyrosine kinase, including PDGF-R, thus negatively affecting tumor growth in vitro and in vivo (24), leading to several phase I–II studies in solid tumors. Even though no data on the efficacy of SU101 on MM are available so far, the capability of this drug to inhibit also cyclooxygenase-2 (COX-2) activity, directly involved in MM proliferation (25), reinforces the rationale for testing SU101 also in MM in the future.

GFB-111 is another recently discovered drug that selectively binds PDGF and, to a lesser extent, vascular endothelial growth factor (VEGF), blocking PDGF-induced receptor autophosphorylation, and consequently cell growth, in glioblastoma and lung adenocarcinoma (26). Testing its efficacy against MM could be intriguing because of the capability of this molecule to target two growth factors, PDGF and VEGF, heavily involved in MM growth and spread.

Finally, STI-571 Imatinib mesylate, Gleevec (Novartis Pharma), is a highly selective inhibitor of the bcr/abl mutated tyrosime kinase, as well as of both c-kit and PDGF-Rs; due to its documented activity against chronic myeloid leukemia and gastrointestinal stromal tumors, it is the first PDGF inhibitor that was approved by the Food and Drug Administration (FDA) for use in humans (27). In vitro experiments

from our group demonstrated that STI-571 can cause MM apoptosis and death via inhibition of the AKT/PIK3 pathway, and that it can also enhance MM chemotherapy sensitivity (manuscript submitted). Several groups, including ours, have started to use Gleevec in MM patients, even though it is too early even for preliminary data on drug efficacy.

Targeting Vascular Endothelial Growth Factor Signaling Pathway

The growth of new capillary blood vessels, i.e., angiogenesis, is required for both local tumor growth as well as the process of invasion and metastasis; indeed, besides allowing oxygen and other nutrients to reach the growing tumor, newly formed vessels also represent a way for tumor cells to reach the circulation and use it for colonizing distant sites (28).

The angiogenic phenotype of tumors is regulated by local balance in the activities of antiangiogenetic and proangiogenetic factors; VEGF is one of the main proangiogenetic factors known, and represents one of the main targets for antiangiogenetic therapy, a rational and promising new approach to cancer treatment.

Vascular endothelial growth factor signaling through its receptors plays a key role in the process of both angiogenesis and lymphangiogenesis, and in tumor growth in a number of solid tumors, including MM. Indeed, VEGF (29–33), VEGF-C (30), and their receptors (30,32) have been found to be overexpressed both in MM cells and in pleural effusion from MM patients, with respect to nonmalignant mesothelial specimens and effusions. Furthermore, neutralizing antibodies against the VEGF receptors Flt-1 or Flk/KDR significantly reduced MM growth in vitro (32), while, in a nude mice model, pretreatment with an anti-VEGFR antibody was able to reduce malignant pleural effusion in vivo (31). Moreover, VEGF levels, as well as microvascular density, which significantly correlated with both VEGF and VEGF-C, proved to be negative prognostic factors in MM patients (30,32).

Finally, a tight relationship exists among simian virus 40 (SV40), VEGF, and MM; indeed, we recently demonstrated that SV40 can cause VEGF release in SV40-positive MM cells in vitro, thus leading to human umbilical vein endothelial cell (HUVEC) proliferation, and that the entire viral genome is required for this effect (34); finally, the SV40-related Tag protein has been demonstrated to be able to enhance nonneoplastic human mesothelial cell proliferation in $p53^{wt}$ MM cells through the induction of VEGF expression (35).

As far as anticancer treatments targeting angiogenesis are concerned, at present, three angiogenesis inhibitors, SU5416, bevacizumab, and thalidomide, are being assessed within clinical trials in patients with MM.

SU5416 (Sugen Co.) inhibits the tyrosine kinase activity of the VEGF receptor flk-1, thus stopping the downstream transduction of the signal (36). In a recent phase II study performed at the University of Chicago,

SU5416 has been administered at the dose of 145 mg/m^2 i.v. over 1 hour, twice weekly, every 4 weeks for a minimum of two courses, obtaining 36% minor objective responses and 28% disease stabilizations, with an acceptable toxicity profile, in both pretreated and naive unresectable MM patients (37). These results prompted the researchers to start to combine this VEGF inhibitor with a cisplatin/gemcitabine combination chemotherapy in their subsequent trial, which is in progress.

Bevacizumab (Avastin, Genentech) is a recombinant human anti-VEGF monoclonal antibody that blocks the binding of VEGF to its receptors. Since the combination of anti-VEGF antibodies and cisplatin produces a striking inhibitory effect in the animal model (38), investigators in the United States are evaluating bevacizumab with cisplatin/gemcitabine chemotherapy in a double-blind, placebo-controlled, randomized phase II trial in patients with MM.

Thalidomide (Grünenthal) is a glutamic acid derivative initially introduced as a sedative hypnotic nearly 40 years ago; it was withdrawn following numerous reports linking it to a characteristic pattern of congenital abnormalities in babies born to mothers who used this drug. It has been gradually reintroduced into clinical practice, albeit under strict regulation, since it was found to be useful to treat erythema nodosum leprosum and HIV wasting syndrome. Recognition of its antiangiogenetic properties led to its evaluation in the treatment of a number of malignancies, starting with multiple myeloma (39). Even though the complex anticancer mechanisms of action of thalidomide are not yet fully understood, a number of biologic actions have been documented: the suppression of bFGF- and VEGF-driven angiogenesis, the inhibition of the production of tumor necrosis factor-α (TNF-α), which has been reported to be angiogenic, and a direct antiproliferative effect, at least against myeloma cells (40).

At present, two trials of thalidomide in MM are ongoing, one in the United States and one in the Netherlands, but in both cases results are not mature enough to draw conclusions on the effectiveness of this agent in MM.

Targeting the Hepatocyte Growth Factor Signaling Pathway

Hepatocyte growth factor/scatter factor (HGF/SF) is a multifunctional growth factor that can induce diverse biologic events. In vitro, these include scattering, invasion, proliferation, and morphogenesis. In vivo, HGF/SF is responsible for many processes during embryonic development and a variety of activities in adults, and many of these normal activities have been implicated in its role in tumorigenesis and metastasis. The c-Met receptor tyrosine kinase is the only known receptor for HGF/SF and it mediates all HGF/SF-induced biologic activities. Upon HGF/SF stimulation, the c-Met receptor is tyrosine-phosphorylated, which is followed by the recruitment of a group of signaling molecules or adaptor proteins to its cytoplasmic domain and its multiple docking sites. This action leads to the activation of several different signaling

cascades that form a complete network of intra- and extracellular responses (41).

c-Met gene amplification can be found in only a few primary carcinomas but in a significant proportion of derived metastasis; plasma HGF levels increase in patients with a number of malignancies, including esophageal, gastric, colorectal, lung, and breast cancer, as well as hepatocellular carcinoma; moreover, the occurrence of an HGF/Met autocrine loop has been reported in a number of cancers, e.g., ovarian, pancreas, colorectal, and thyroid carcinoma. Finally, both HGF plasma levels and tissue expression have been found to be negative prognostic factors in a variety of human cancers (42).

An HGF/SF/c-Met autocrine loop has been demonstrated both in MM cell lines and in MM tissue samples (43–46), and their overexpression has been associated with an increased microvessel density, as well as with increased matrix metalloproteinases expression, thus supporting the additional role of this factor in both tumor angiogenesis and invasion (47,48).

We have also demonstrated that when SV40 infects human mesothelial cells, it induces Met receptor activation via an autocrine loop; furthermore, SV40 replicates in and infects the adjacent human mesothelial cells, inducing an HGF-dependent Met activation and cell-cycle progression into S-phase, thus explaining how a limited number of SV40-positive cells may be sufficient to direct noninfected human mesothelial cells toward malignant transformation (49).

Also, HGF/SF plays a pivotal role in SV40/asbestos-dependent carcinogenesis, conferring progressive resistance of human mesothelial cells to genotoxic agents, i.e., asbestos, and allowing DNA-damaged cells to survive and undergo transformation via PI3K/AKT signaling activation; analogously, SV40-positive MM (with PI3K/AKT activation) demonstrates significant lower chemosensitivity compared to the SV40-negative ones (manuscript submitted).

Unfortunately, so far, no specific Met activity inhibitor has been identified, although the antiangiogenic drug SU5416 (see above), at relatively high concentrations, may also inhibit Met tyrosine kinase. Despite this issue, the HGF/SF/c-Met signaling pathway also may be inhibited downstream by interfering with the PI3K/AKT pathway (see below).

Targeting the Downstream Signaling Pathway

In addition to tyrosine kinase receptors, several downstream cytoplasmic kinases also are known to play a role in regulating cell cycle, gene transcription, motility, survival, and metabolism in cancer cells. For MM, the PI3K/AKT pathway is downstream of many of the above receptor activation-mediated signaling pathways and thus is another potential target for cancer treatment.

CCI779, a compound currently under clinical investigation in several cancers, has the nuclear protein mTOR, directly involved in the trans-

mission of the AKT-dependent proliferative signal, as its target; thus, its use in MM seems potentially interesting and worthwhile.

Targeting the Proteasome/Ubiquitin Pathway

The rapid, efficient, and irreversible elimination of damaged or obsolete proteins is a key mechanism for controlling the activation or repression of a number of cellular processes, including cell-cycle progression and apoptosis. The proteasome/ubiquitin complex represents the primary component of the protein degradation pathway of the cell, 80% of all cellular proteins being processed by this enzyme.

The proteasome is a large, multiprotein particle present in both the cytoplasm and the nucleus of all eukaryotic cells. It is composed of two functional components: a 20S core catalytic complex and a 19S regulatory subunit. The proteins to be degraded are marked with ubiquitin chains, which bind to a receptor on the 19S complex. Once recognized by the regulatory complex, the ubiquitin chain is removed and the protein denatured in preparation for degradation. The protease activity resides in a channel at the center of the 20S complex, which is formed from four stacked, multiprotein rings. The outer α subunit rings form a narrow channel that allows only denatured proteins to enter the catalytic chamber formed by the central β subunit rings; inside the catalytic chamber, proteins are surrounded by six protease-active sites (three on each β subunit ring) that complete the degradation process (50). Proteolysis by the proteasome/ubiquitin pathway is a key metabolic process, and complete abrogation of proteasome activity results in cell death; in particular, cyclins, cyclin-dependent kinase inhibitors, and tumor suppressor genes, e.g., *cyclin B1*, $p21^{Waf1/Cip1}$, *p27*, and *p53*, are all processed through this pathway, and the inhibition of their degradation sensitizes cells to apoptosis (51).

The nuclear factor (NF)-κB signaling pathway is another critical target for proteasome inhibitors. Indeed, NF-κB is a cellular survival factor, whose transcription is prevented in quiescent cells through binding of a specific inhibitor, IκB, which sequesters the NF-κB p50/p65 heterodimer in the cytoplasm; this repression is released in response to cellular stresses that cause targeted, ubiquitin/proteasome-mediated degradation of IκB (52); free NF-κB then translocates to the nucleus to activate genes that protect the cell from apoptosis and promote cell growth and differentiation, as well as the synthesis of growth factors and angiogenesis factors (53). Dysregulation of the NF-κB signaling is an important feature of some malignancies (54,56), and activation of this pathway can stimulate proliferation or reduce the effectiveness of chemotherapy or ionizing radiations (57,58).

For all the above reasons, and considering the fact that transformed cells are more sensitive to proteasome inhibition due to the loss of protective checkpoints mechanisms (59), targeting the proteasome/ubiquitin pathway has emerged as a promising novel therapeutic approach in oncology. PS-341 (Velcade, Millennium Pharmaceuticals) is a small molecule that is a potent and selective inhibitor of the proteasome; in

vitro and mouse xenograft studies have clearly shown that this molecule possesses antitumor activity in a variety of human cancers (60); preclinical and phase I studies have recently assessed the best treatment schedule, and a number of phase II studies are ongoing.

Even though, at present, no published data in MM, either in vitro or in vivo, are available, targeting the NF-κB signaling is particularly attractive in MM; indeed, asbestos fibers, i.e., the main recognized cause of MM, has been demonstrated to be able to cause the translocation of NF-κB p65 subunit into the nucleus, and to increase NF-κB DNA binding activity in rat lung epithelial and pleural mesothelial cells (61); moreover, NF-κB also has been implicated in the pathogenesis of benign pleural inflammation and exudation in at least one mouse model (62). Furthermore, the cyclin-dependent kinase inhibitor p27 is degraded by the proteasome (63), and the decreased protein levels observed in some malignancies are achieved by upregulation of proteasome-mediated p27 degradation; interestingly, when fibroblasts transformed by the oncogenic virus SV40, another key character in the process of MM tumorigenesis, are treated with a proteasome inhibitor, p27 levels increase and apoptosis ensues (64). Finally, recent data from our laboratories suggest that PS-341 could potentiate the antitumor activity of other agents against MM, both in vitro and in vivo (65).

References

1. Peto J, De Carli A, La Vecchia C, Levi F, Negri E. The European mesothelioma epidemic. Br J Cancer 1999;79:666–672.
2. Fitzpatrick PR, Peroni DJ, Bielefeldt-Ohmann H. The role of growth factors and cytokines in the tumorigenesis and immunobiology of malignant mesothelioma. Am J Respir Cell Mol Biol 1995;12:455–460.
3. Levitzki A. Tyrosine kinase as targets for cancer therapy. Eur J Cancer 2002;38(suppl 5):S11–18.
4. Normanno N, Bianco C, De Luca A, Salomon DS. The role of EGF-related peptides in tumor growth. Front Biosci 2001;6:d685–707.
5. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37(suppl 4):S9–15.
6. Mendelsohn J. The epidermal growth factor receptor as a target for cancer therapy. Endocr Relat Cancer 2001;8:3–9.
7. Normanno N, Maiello MR, De Luca A. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs): simple drugs with a complex mechanism of action? J Cell Physiol 2002;194:13–19.
8. Baselga J. New technologies in epidermal growth factor receptor-targeted cancer therapy. Signal 2000;1:12–21.
9. Ciardiello F, Tortora G. A novel approach to the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res 2001;7:2958–2970.
10. Shawver LK, Slamon D, Ullrich A. Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell 2002;1:117–123.
11. Dazzi H, Haselton PS, Thatcher N, et al. Malignant pleural mesothelioma and epidermal growth factor receptor (EGF-R). Relationship of EGF-R with histology and survival using fixed paraffin embedded tissue and the F4 monoclonal antibody. Br J Cancer 1990;61:924–926.

12. Morocz IA, Schmitter D, Lauber B, et al. Autocrine stimulation of a human lung mesothelioma cell line is mediated through the transforming growth factor alpha/epidermal growth factor receptor mitogenic pathway. Br J Cancer 1994;70:850–856.
13. Govindan R, Ritter J, Suppiah R. EGFR and HER2 overexpression in malignant mesothelioma. Proc Am Soc Clin Oncol 2001;20:3106(abstract).
14. Jänne PA, Taffaro ML, Salgia R, Johnson BE. Inhibition of epidermal growth factor receptor signaling in malignant pleural mesothelioma. Cancer Res 2002;62:5242–5247.
15. Nowak AK, Lake RA, Kindler HL, Robinson BWS. New approaches for mesothelioma: biologics, vaccines, gene therapy, and other novel agents. Semin Oncol 2002;29:82–96.
16. George D. Platelet-derived growth factor receptors: a therapeutic target in solid tumors. Semin Oncol 2001;28(suppl 17):27–33.
17. Yarden Y, Escobedo JA, Kuang WJ, et al. Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature 1986;323:226–232.
18. Ostman A, Heldin CH. Involvement of platelet-derived growth factor in disease: development of specific antagonists. Adv Cancer Res 2001;80: 1–38.
19. Langerak AW, De Laat PA, Van der Linden-Van Beurden CA, et al. Expression of platelet-derived growth factor (PDGF) and PDGF receptors in human malignant mesothelioma in vitro and in vivo. J Pathol 1996;178: 151–160.
20. Gerwin BI, Lechner JF, Reddel RR, et al. Comparison of production of transforming growth factor-beta and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. Cancer Res 1987;47:6180–6184.
21. Versnel MA, Claesson-Welsh L, Hammacher A, et al. Human malignant mesothelioma cell lines express PDGF-beta receptors whereas cultured normal mesothelial cells express predominantly PDGF-alpha receptors. Oncogene 1991;6:2005–2011.
22. Dorai T, Kobayashi H, Holland JF, Ohnuma T. Modulation of platelet-derived growth factor-beta mRNA expression and cell growth in a human mesothelioma cell line by a hammerhead ribozyme. Mol Pharmacol 1994; 46:437–444.
23. Garlepp MJ, Leong CC. Biological and immunological aspects of malignant mesothelioma. Eur Respir J 1995;8:643–650.
24. Xu X, Shen J, Mall JW, et al. In vitro and in vivo antitumor activity of a novel immunomodulatory drug, leflunomide: mechanisms of action. Biochem Pharmacol 1999;58:1405–1413.
25. Marrogi A, Pass HI, Khan M, Metheny-Barlow LJ, Harris CC, Gerwin BI. Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2): in vitro antiproliferative effects of a COX-2 inhibitor. Cancer Res 2000;60:3696–3700.
26. Blaskovich MA, Lin Q, Delarue FL, et al. Design of GFB-111, a platelet-derived growth factor binding molecule with antiangiogenic and anticancer activity against human tumors in mice. Nat Biotechnol 2000;18: 1065–1070.
27. Pindolia VK, Zarowitz BJ. Imatinib mesylate, the first molecularly targeted gene suppressor. Pharmacotherapy 2002;22:1249–1265.
28. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002;29(suppl 16):15–18.

29. Konig JE, Tolnay E, Wiethege T, Muller KM. Expression of vascular endothelial growth factor in diffuse malignant pleural mesothelioma. Virchows Arch 1999;435:8–12.
30. Kumar-Singh S, Weyler J, Martin MJ, Vermeulen PB, Van Marck E. Angiogenic cytokines in mesothelioma: a study of VEGF, FGF-1 and -2, and TGF-beta expression. J Pathol 1999;189:72–78.
31. Ohta Y, Shrindhar V, Bright RK, et al. VEGF, VEGF type C, and their receptors play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumors. Br J Cancer 1999;81:54–61.
32. Zebrowski BK, Yano S, Liu W, et al. Vascular endothelial growth factor levels and induction of permeability in malignant pleural effusions. Clin Cancer Res 1999;5:3364–3368.
33. Strizzi L, Catalano A, Vianale G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol 2001;193:468–475.
34. Caciotti P, Strizzi L, Vianale G, et al. The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. Am J Respir Cell Mol Biol 2002;26:189–193.
35. Catalano A, Romano M, Martinotti S, Procopio A. Enhanced expression of vascular endothelial growth factor (VEGF) plays a critical role in the tumor progression potential induced by simian virus 40 large T antigen. Oncogene 2002;21:2896–2900.
36. Fong TAT, Shawver LK, Sun L, et al. SU 5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. Cancer Res 1999;59:99–106.
37. Kindler HL, Vogelzang NJ, Chien K, et al. SU 5416 in malignant mesothelioma: a University of Chicago phase II consortium study. Proc Am Assoc Clin Oncol 2001;20:341(abstract).
38. Kabbinavar FF, Wong JT, Ayala RE, et al. The effect of antibody to vascular endothelial growth factor and cisplatin on the growth of lung tumors in nude mice. Proc Am Assoc Cancer Res 1995;36:488(abstract).
39. Kumar S, Witzig TE, Rajkumar SV. Thalidomide as an anti-cancer agent. J Cell Mol Med 2002;6:160–174.
40. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nature Rev Cancer 2002;2:727–739.
41. Zhang YW, Vande Woude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. J Cell Biochem 2003;88:408–417.
42. Funakoshi H, Nakamura T. Hepatocyte growth factor: from diagnosis to clinical applications. Clin Chim Acta 2003;327:1–23.
43. Harvey P, Warn A, Newman P, et al. Immunoreactivity for hepatocyte growth factor/scatter factor and its receptor met, in human lung carcinomas and malignant mesotheliomas. J Pathol 1996;180:389–394.
44. Klominek J, Baskin B, Liu Z, Hauzenberger D. Hepatocyte growth factor/scatter factor stimulates chemotaxis and growth of malignant mesothelioma cells through c-met receptor. Int J Cancer 1998;76:240–249.
45. Harvey P, Warn A, Dobbin S, et al. Expression of HGF/SF in mesothelioma cell lines and its effects on cell motility, proliferation and morphology. Br J Cancer 1998;77:1052–1059.
46. Thirkettle I, Harvey P, Hasleton PS, Ball RY, Warn RM. Immunoreactivity for cadherins, HGF/SF, met, and erbB-2 in pleural malignant mesothelioma. Histopathology 2000;36:522–528.

47. Tolnay E, Kuhnen C, Wiethege T, Konig JE, Voss B, Muller KM. Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma. J Cancer Res Clin Oncol 1998;124:291–296.
48. Harvey P, Clark IM, Jaurand MC, Warn RM, Edwards DR. Hepatocyte growth factor/scatter factor enhances the invasion of mesothelioma cell lines and the expression of matrix metalloproteinases. Br J Cancer 2000; 83:1147–1153.
49. Cacciotti P, Libener R, Betta P, et al. SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. Proc Natl Acad Sci USA 2001;98:12032–12037.
50. Stock D, Ditzel L, Baumeister W, et al. Catalytic mechanism of the 20S proteasome of *Thermoplasma acidophilum* revealed by x-ray crystallography. Cold Spring Harb Symp Quant Biol 1995;60:525–532.
51. An WG, Hwang SG, Trepel JB, et al. Protease inhibitor-induced apoptosis: accumulation of wt p53, $p21^{Waf1/Cip1}$, and induction of apoptosis are independent markers of proteasome inhibition. Leukemia 2000;14:1276–1283.
52. Roff M, Thompson J, Rodriguez MS, et al. Role of IκBα ubiquitination in signal-induced activation of NF-κB in vivo. J Biol Chem 1996;271:7844–7850.
53. Baldwin AS. Control of oncogenesis and cancer therapy resistance by transcription factor NF-κB. J Clin Invest 2001;107:241–246.
54. Kordes U, Krappmann D, Heissmeyer V, et al. Transcription factor NF-κB is constitutively activated in acute lymphoblastic leukemia cells. Leukemia 2000;14:399–402.
55. Tricot G. New insights into the role of microenvironment in multiple myeloma. Lancet 2000;355:248–250.
56. Patel NM, Nozaki S, Shortle NH, et al. Paclitaxel sensitivity of breast cancer cells with constitutively active NF-κB is enhanced by IκBα super-repressor and parthenolide. Oncogene 2000;19:4159–4169.
57. Cusack JC, Liu R, Baldwin AS. NF-κB and chemoresistance: potentiation of cancer drugs via inhibition of NF-κB. Drug Resist Update 1999;2:271–273.
58. Russo SM, Tepper JE, Baldwin AS, et al. Enhancement of radiosensitivity by proteasome inhibition: implication for a role of NF-κB. Int J Radiat Oncol Biol Phys 2001;50:183–193.
59. Drexler HC. Activation of the cell death program by inhibition of proteasome function. Proc Natl Acad Sci USA 1997;94:855–860.
60. Adams J. Development of the proteasome inhibitor PS-341. Oncologist 2002;7:9–16.
61. Janssen YM, Driscoll KE, Howard B, et al. Asbestos causes translocation of p65 protein and increases NF-κB DNA binding activity in rat lung epithelial and plural mesothelial cells. Am J Pathol 1997;151:389–401.
62. Frode-Saleh TS, Calixto JB. Synergistic anti-inflammatory effect of NF-κB inhibitors and steroidal or nonsteroidal anti-inflammatory drugs in the pleural inflammation induced by carrageenan in mice. Inflamm Res 2000; 49:330–337.
63. Pagano M, Tan SW, Theodoras AM, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. Science 1995;269:682–685.
64. An B, Goldfarb RH, Siman R, et al. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-

dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death Differ 1998;5:1062–1075.

65. Sartore-Bianchi A, Nici L, Porta C, Chatterjee D, Mutti L, Calabresi P. The combination of the novel camptothecin analogue Gimatecan (ST1481) plus the proteasome inhibitor PS341 produces an enhanced pro-apoptotic effect in a malignant mesothelioma cell line. Proc Am Assoc Cancer Res 2003; 44:742(abstract R729).

52

Gene Therapy for Malignant Pleural Mesothelioma

Steven M. Albelda, Larry R. Kaiser, and Daniel H. Sterman

Given the current limitations in therapy for malignant mesothelioma, new approaches are needed. Malignant pleural mesothelioma has several characteristics that make it an attractive target for gene therapy: (1) there is no curative therapy, although some slowing of tumors has been reported with pemetrexid/cisplatin; (2) the pleural space is accessible for biopsy, local (as opposed to systemic) vector delivery, and analysis of treatment effects; and (3) morbidity and mortality are often related to local extension of disease, rather than distant metastases. Therefore, unlike other tumors that metastasize earlier in their course, relatively small increments of improvement in local control in mesothelioma could result in significant survival benefit.

Gene Therapy: Principles and Vectors

Gene therapy is the transfer of genetic material, including complementary DNA (cDNA), full-length genes, RNA, or oligonucleotides into cancer or host cells. The mechanism for transfer of this genetic material is termed the "vector." Although conceived as a treatment for inherited recessive disorders in which transfer of a normal copy of a defective gene could prevent disease onset or reverse phenotypic expression, it soon became clear that one of the most important targets for gene therapy would be cancer.

A prerequisite for successful gene therapy is efficient gene transfer. A variety of viral and nonviral gene transfer vectors are currently available, which range from replicating and nonreplicating viruses, to bacteria, to liposomes (for reviews see refs. 1 and 2). As summarized in Table 52.1, each of these vectors has certain advantages with regard to DNA carrying capacity, types of cells targeted, in vivo gene transfer efficiency, duration of expression, and induction of inflammation.

Since most of the gene therapy trials for mesothelioma, in both animals and humans, have involved the use of replication incompetent adenovirus, this chapter concentrates on modifications of this vector.

Table 52.1. Characteristics of gene therapy vectors

Vector	DNA-carrying capacity (kb)	Cell range	In vivo gene delivery efficiency	Duration of expression	Co-transfer viral gene elements	Inflammatory response
Retrovirus	<8	Replicating cells only	Low	Stable	Yes	Low
Adenovirus	7–8	Most cells	Moderate	Transient	Yes	High
Adeno-associated virus	<5	Primarily muscle, liver, and brain	Low	Stable	Minimal	Low
Lentivirus	<8	Many nondividing cells	Low	Stable	Yes	Low
Liposome	>10	Most cells	Low	Transient	No	Moderate

The interested reader is referred elsewhere for details regarding other vector systems (3–6).

Recombinant adenovirus vectors have been derived by genomic deletion of viral gene functions involved in replication (i.e., the E1A/B regions) and provision of these functions *in trans* via a packaging cell line (7). The deleted gene regions can then be replaced with expression cassettes containing the desired gene allowing high-efficiency transduction in a wide range of target cells (including nondividing cells) and high expression levels of the delivered transgene (8). This vector system offers a number of advantages including good in vivo transduction efficiency, permitting direct gene delivery to many tissue sites, including the pleural space. Although the two primary disadvantages of adenoviruses, transient gene expression and prominent locally and systemic inflammatory responses elicited by virions, are an issue for long-term replacement gene therapy, these inflammatory responses, which include an early "innate" immune response resulting in cytokine secretion, may actually be advantageous for cancer gene therapy.

Several different cancer gene therapy approaches are currently being explored for malignant pleural mesothelioma including the use of "suicide" genes, delivery of tumor suppressor genes, and transfer of immunomodulatory genes (Table 52.2). Several of these have been applied in phase I clinical trials of malignant pleural mesothelioma utilizing a variety of vector systems including recombinant adenovirus, vaccinia virus, and modified ovarian carcinoma cells (9–11). Others remain in the preclinical stage, but with plans for clinical trials in the near future (Table 52.2).

"Suicide" Gene Therapy

One prominent approach in cancer gene therapeutics is so-called suicide gene therapy. This method involves the transduction of tumor cells with cDNA encoding an enzyme that converts a benign prodrug to a toxic metabolite (12). Administration of the prodrug thus results in selective accumulation of toxin in the tumor cells and cell death. The

Table 52.2. Gene therapy approaches for mesothelioma

Strategy	Vector	Therapeutic Gene	Molecular Mechanism	Location
Suicide gene	Recombinant, replication deficient, adenovirus	Herpes simplex thymidine kinase	Delivery of enzyme capable of generating toxic metabolite after exposure to ganciclovir	University of Pennsylvania Medical Center, Philadelphia, PA
Genetic immunopotentiation	Replication-restricted vaccinia virus	Human IL-2	Augmentation of immune response to tumor	Queen Elizabeth II Medical Center, Perth, Australia
	Vaccinia virus	Modified SV40 T-antigen	Stimulation of immune response against SV40+ mesothelioma cells	Wayne State University Medical Center, Detroit, MI
	Replication deficient, adenovirus	Interferon-beta	Induction of antitumor immune response	University of Pennsylvania Medical Center (pending)
	Cationic liposome	Prokaryotic DNA	Nonspecific induction of innate and acquired immunity	No known clinical protocols
Combination suicide gene/tumor vaccine	Irradiated, allogeneic ovarian carcinoma cells	Herpes simplex thymidine kinase	Generation of toxic metabolite and antitumor immune responses	LSU Medical Center, New Orleans, LA
Mutation compensation	Oligonucleotides	Antisense SV40 Tag	Inhibition of dominant oncogenes	No known clinical protocols
	Adenovirus	Wild-type p14(ARF)/p16	Restoration of tumor suppressors	No known clinical protocols
	Adenovirus	Wild-type p53/Bak	Induction of apoptosis	No known clinical protocols
Replication-competent viral lytic therapy	Replication-restricted adenovirus: ONYX-015	None	Tumor-restricted viral replication and cytotoxicity	No known clinical protocols

IL-2, interleukin 2; SV40, simian virus 40.

enzymes encoded by the suicide gene are often of nonhuman origin, e.g., the herpes simplex virus-1 thymidine kinase (HSV*tk*) gene, which limits toxicity in normal tissue (13). For instance, HSV*tk* differs enough from mammalian thymidine kinases that transfected malignant cells, but not normal tissue, convert the nucleoside analogue ganciclovir (GCV) to its toxic metabolite. After enzymatic conversion to GCV-*mono*phosphate (GCV-MP) by HSV*tk*, it is rapidly metabolized to GCV-*tri*phosphate (GCV-TP) by endogenous mammalian kinases. Intracellular production of these GCV metabolites causes tumor cell death, or "suicide" (12,14).

Bystander Effects of HSV*tk* Suicide Gene Therapy

Although relatively efficient, adenoviral gene transfer is not possible to every tumor cell. Thus the presence of a "bystander effect," whereby untransfected cells are killed by an indirect mechanism, is extremely important (15). Such a bystander effect has been observed in the HSV*tk*/GCV system (15–19). The nature of this bystander effect is complex and appears to involve passage of toxic GCV metabolites from transduced to nontransduced cells via gap junctions or apoptotic vesicles (20,21), and induction of antitumor immune responses capable of killing tumor cells not expressing the HSV*tk* transgene (15).

HSVtk/GCV Gene Therapy for Malignant Pleural Mesothelioma

A number of studies showed that an adenoviral vector expressing HSV*tk* (Ad.HSV*tk*), combined with ganciclovir therapy, could be used to kill mesothelioma cells in vitro and in animal models (22–27). Based on these efficacy data and on preclinical toxicity studies showing minimal toxicity (28), a phase I clinical trial for patients with pleural mesothelioma began in November 1995 at the University of Pennsylvania Medical Center. The goals of this trial were to determine the toxicity, gene transfer efficacy, and immune responses generated in response to the intrapleural instillation of Ad.HSV*tk*. Mesothelioma patients who met inclusion criteria (including patent pleural cavities) underwent intrapleural administration of a single dose of Ad.HSV*tk* vector followed by 2 weeks of intravenous GCV (9,29,30). The adenoviral vector used was a so-called first-generation replication-incompetent virus, deleted in the early gene regions E1 and E3 with the HSV*tk* gene inserted in the E1 region.

Twenty-six patients (21 male, five female), ranging in age from 37 to 81, were enrolled in the study between November 1995 and November 1997 (Table 52.3) (29). Intratumoral HSV*tk* gene transfer was documented by immunohistochemistry (IHC) utilizing a murine monoclonal antibody directed against HSV*tk* in all patients treated at dose levels of 3.2×10^{11} plaque-forming units (pfu) or greater (29). Ad.HSV*tk*/GCV gene therapy was well tolerated, and a maximum tolerated dose (MTD) was not achieved. Strong antiadenoviral humoral and cellular immune responses were noted, including the generation of high serum and pleural fluid titers of antiadenoviral neutralizing antibodies, the generation of serum antibodies against adenoviral

Table 52.3. Results of University of Pennsylvania phase I clinical trials of Ad.tk/GCV gene therapy for mesothelioma

Patient age/sex	Stage/ cell type	Vector dose (pfu)	Post-Rx survival[a]	Gene transfer	Tumor response
1 62/M	IA/E[b]	1×10^9	72 months	—	SD × 2 yrs
2 56/M	III/E	1×10^9	8 months	—	—
3 69/M	III/B	1×10^9	20 months	+	—
4 66/M	II/E	3.2×10^9	11 months	—	—
5 71/M	IA/E	3.2×10^9	58 months	—	SD × 3 yrs
6 71/M	II/B	1×10^{10}	4 months	+	—
7 70/M	II/E	1×10^{10}	6 months	—	—
8 60/M	II/E	1×10^{10}	27 months	+	—
9 74/M	II/B	3.2×10^{10}	2 months	NP	—
10 60/M	III/E	3.2×10^{10}	9 months	—	—
11 37/F	IV/E	1×10^{11}	16 months	—	—
12 37/M	III	1×10^{11}	2 months	—	—
13 65/F	III/E	1×10^{11}	10 months	+	—
14 66/F	IA/E	3.2×10^{11}	50 months	+	SD × 2 yrs
15 60/M	IV/B	3.2×10^{11}	5 months	+	—
16 69/M	IB/E	3.2×10^{11}	8 months	+	—
17 70/F	IB/E	3.2×10^{11}	15 months	+	—
18 69/F	IB/E	3.2×10^{11}	14 months	+	—
19 75/M[c]	II/E	3.2×10^{11}	8 months	+	—
20 68/M[c]	IV/B	3.2×10^{11}	1 month	+	—
21 71/M[c]	IB/E	3.2×10^{11}	41 months	+	—
22 76/M[c]	IB/E	3.2×10^{11}	33 months	+	—
23 81/M[c]	II/E	3.2×10^{11}	25 months	+	—
24 71/M	II/E	1×10^{12}	21 months	+	—
25 65/M	II/E	1×10^{12}	5 months	+	—
26 67/M	IA/E	1×10^{12}	22 months	+	PR (CT)
27 67/M[d]	III/B	1×10^{11}	7 months	+	—
28 53/M[d]	III/E	1×10^{11}	13 months	+	—
29 30/F[d]	I/E	5×10^{11}	37 months	+	SD
30 56/F[d]	IA/E	5×10^{11}	37 months	+	SD
31 66/M[d]	II/E	5×10^{11}	9 months	+	—
32 74/M[b,d]	I/E	5×10^{11}	19 months	+	PR (PET)
33 64/M[b,d]	I/E	5×10^{11}	10 months	+	—
34 69/M[b,d]	II/E	5×10^{11}	26 months	NP	—

E, epithelioid; B, biphasic; NP, test not performed; SD, stable disease (CT +/− PET); PR, partial response.

[a] All patients deceased except patients 1, 29, 30, and 34.

[b] Received 15 mg/kg/day of GCV × 14 days.

[c] Received adjuvant corticosteroids.

[d] Received third-generation E1-/E4-deleted adenoviral vector.

structural proteins, and increased peripheral blood mononuclear cell proliferative responses to adenoviral proteins (30).

In a small pilot study, five patients (patients 19 to 23) received intravenous corticosteroids around the time of vector instillation. This trial was designed to preliminarily assess the effects of immunosuppression on the degree of intratumoral gene transfer and antiadenoviral immune responses. Decreased fever and hypoxemia were noted in the corticosteroid-treated cohort, but there was also an increased incidence of reversible mental status changes (31). No diminution in antiadenoviral immune responses was demonstrated in the group receiving

corticosteroids, nor were there any appreciable differences in the degree of intratumoral gene transfer.

Of the 26 patients enrolled in the initial phase I trial, 25 have since died, with a median posttreatment survival of approximately 11 months (Table 52.3). Several patients with stage IA/IB epithelioid mesothelioma had posttreatment survivals of greater than 3 years, with one patient surviving over 4 years. Of the trial participants who are deceased, all had progressive mesothelioma as their primary cause of death, typically with invasion of the mediastinum and the contralateral hemithorax, and transdiaphragmatic extension, as well as with widespread metastatic disease, a fairly common finding in advanced mesothelioma.

Additional Phase I Trials of Ad.HSV*tk* Gene Therapy for Mesothelioma

Based on these results, two additional phase I trials were initiated. In the first trial, a second-generation Ad.HSV*tk* vector containing deletions in the E1 and E4 regions with preservation of the E3 region was used. Based on animal studies, this vector was chosen for diminished cytopathic effects and reduced cellular immune responses (32) and, since two replication-necessary genes are deleted, simple recombination could not produce replication competent virus in the vector production process.

Five patients were treated under this protocol, starting at a dose one log lower than the highest dose used with the E1/E3-deleted Ad vector (1.5×10^{13} viral particles). Dose-related gene transfer was detected in all patients at both dose levels via immunohistochemistry using an anti-HSV*tk* monoclonal antibody. Of the five patients treated in this second phase I trial, there are two surviving (patients 29 and 30), both of them treated at the higher dose level of 5.0×10^{13} particles of Ad.HSV*tk* (Table 52.3). Each of the patients had stage I epithelioid mesothelioma at diagnosis. Both have had clinically and radiographically stable disease without other antitumor therapy for 54 months after treatment. Patient 29, a 34-year-old woman, has demonstrated diminished tumor metabolic activity on serial follow-up 18-fluorodeoxyglucose positron emission tomography (18-FDG-PET) scans (Fig. 52.1). This delayed decrease in tumor metabolic activity several months after completion of the gene therapy protocol suggests the induction of a secondary immune bystander effect induced by Ad.HSV*tk*/GCV (33).

The third trial involved gradual dose escalation of ganciclovir in combination with intrapleural delivery of the E1/E4-deleted Ad vector. One cohort of three patients was treated with 3.0×10^{13} particles of Ad.HSV*tk* (E1/E4-deleted) and 7.5 mg/kg ganciclovir IV b.i.d. (15 mg/kg/day). All three patients tolerated the treatment well. One of three patients is still alive, albeit with evidence of significant tumor progression, now 26 months after treatment (Table 52.3). No durable clinical responses were noted in any of the three patients treated in this protocol.

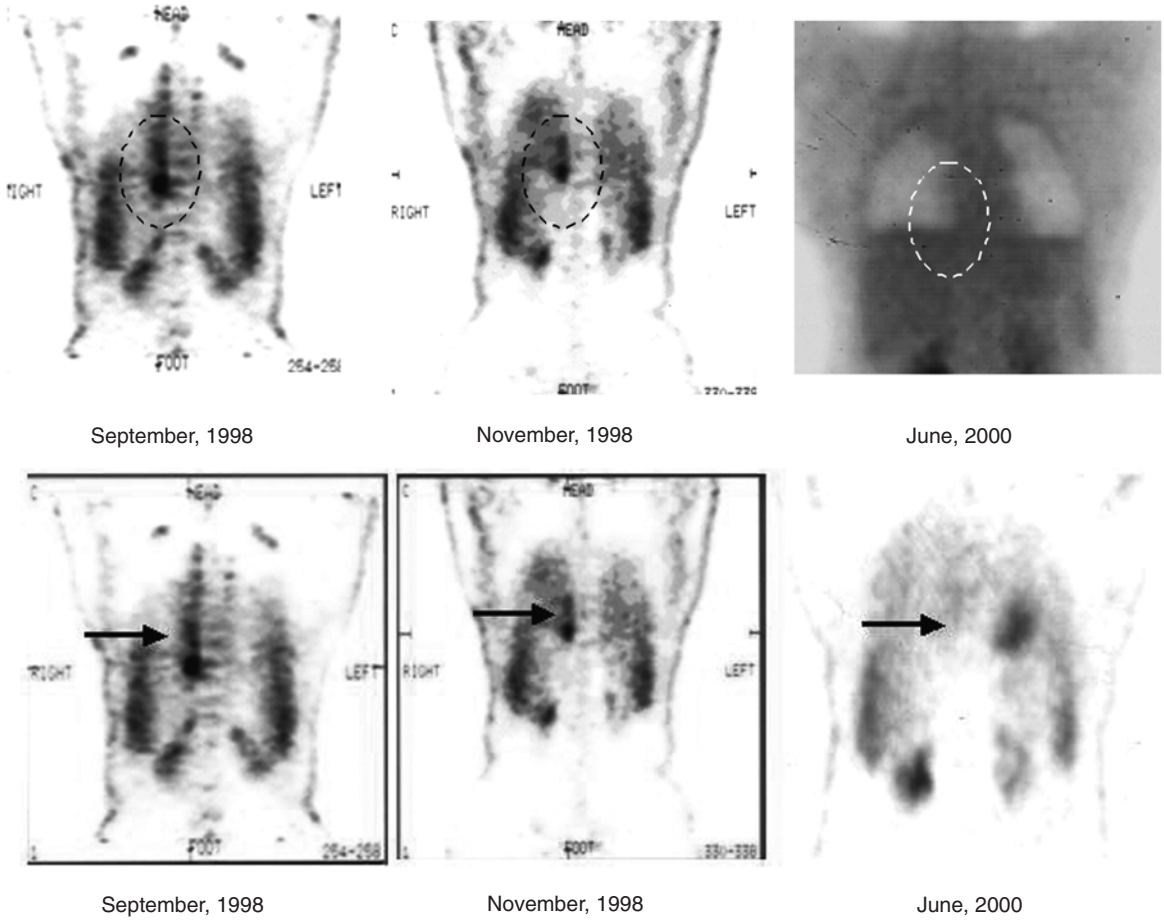


Figure 52.1. One of the eight patients enrolled in E1/E4-deleted vector protocols, patient 29 had objective evidence of tumor response on pre– and post–gene therapy 18-fluorodeoxyglucose positron emission tomography (FDG-PET) imaging, with near-complete absence of FDG uptake on an 18-FDG-PET scan performed 18 months after completion of the protocol (see text). This objective metabolic response correlated with her excellent clinical status and stability on serial chest computed tomography (CT) scans. The patient has had no other antineoplastic therapy other than our gene therapy protocol.

Lessons Learned

Based on our clinical trial experience, the Ad.HSV*tk*/GCV suicide gene therapy approach showed some potential for the treatment of malignant mesothelioma, as well as other localized malignancies. Unfortunately, these phase I trials were halted because of the death of a participant in a gene therapy trial for ornithine transcarbamylase (OTC) deficiency at the University of Pennsylvania Medical Center utilizing a similar adenoviral vector backbone (34). Nonetheless, one of the most valuable aspects of our trial has been the identification of specific challenges that need to be addressed, such as gene transfer efficiency.

Using the current strategy, therapeutic efficacy could only be expected in patients with relatively small tumor burdens (small

nodules or diffuse, "thin" tumors). An alternative treatment schema maximizing the vector-to-tumor cell ratio would involve surgical "debulking" to minimize tumor mass, followed by adjuvant administration of Ad.HSV*tk*/GCV. Another method of improving intratumoral gene transfer would be repeated administration of vector and GCV (i.e., three doses over a 3-week period). Completed studies in immunocompetent mice with established peritoneal tumors by our group (35) and others (36) showed marked increases in efficacy after multiple intraperitoneal injections of Ad.HSV*tk*, each followed by a course of GCV. Importantly, data from our initial clinical trials suggest that gene transfer is possible even in patients with titers of anti-Ad neutralizing antibodies of up to 1:500, as would be expected with repeated administration of Ad vector.

Another approach to the gene transfer problem is to maximize the efficacy of the expressed HSV*tk* enzyme. "Molecular remodeling" of the HSV*tk* enzyme has allowed increasing specificity for GCV and a cyclovir (ACV) and concomitantly decreased thymidine utilization (37). These HSV*tk* mutants show increased ACV- and GCV-mediated cytotoxicity, and enhanced bystander effects in mixing experiments (38,39). We have produced adenoviral vectors containing the mutated HSV*tk*s and demonstrated enhanced cell killing and augmented bystander effect in in vitro and in vivo tumor models (40).

Suicide Gene Vaccines

A growing body of evidence supports the hypothesis that in most models tested, treatment with HSV*tk*/GCV results in an immunologic bystander effect that enhances antitumor cytotoxicity both at the site of vector delivery as well as at distant, nontransduced tumor sites (15,19,41–43). We believe that we have seen evidence of this immune bystander effect in our mesothelioma phase I clinical trials with the progressive decline in tumor metabolic activity seen on PET scan in patient 29 over 36 months posttreatment (Fig. 52.1). This putative antitumor immune reaction may result from *non*apoptotic HSV*tk*/GCV-mediated tumor necrosis, a type of cell death that releases so-called danger signals that then activate significant cellular immune responses (43,44). Generation of these danger signals may be enhanced by transduction of tumor cells with the HSV*tk* gene *plus* a cytokine gene, such as the gene for interleukin-2 (IL-2). Augmented tumor cytotoxicty has been reported with HSV*tk* plus IL-2 in a number of tumor models (45).

This method of causing mesothelioma tumor destruction via the immunologic bystander effects of HSV*tk*/GCV gene therapy, a presumptive suicide gene vaccine, was studied in a phase I clinical trial conducted by Schwarzenberger and colleagues (10,46) at the Louisiana State University (LSU) Medical Center in New Orleans (Table 52.2). The protocol designed by the LSU investigators consisted of the intrapleural instillation via an indwelling pleural catheter of an irradiated ovarian carcinoma cell line retrovirally transfected with HSV*tk* (PA1-STK cells), followed by systemic administration of GCV (10).

Schwarzenberger and colleagues hypothesized that the the PA1-STK cells would migrate to areas of intrapleural tumor after instillation, undergo necrotic cell death after exposure to GCV, and generate immune responses that would facilitate killing of adjacent mesothelioma cells. Antimesothelioma immune responses in this system are related to the local generation of proinflammatory cytokines, which, in turn, summon an influx of cytotoxic lymphocytes to the area producing hemorrhagic tumor necrosis (10,46). In the patients treated to date, minimal side effects have been seen, while preliminary findings showed significant posttreatment increases in the percentage of CD8 T lymphocytes in pleural fluid (46).

Cytokine Gene Therapy for Mesothelioma

There has been significant interest in the delivery of genes encoding for proinflammatory cytokines to the pleural space of patients with malignant mesothelioma. One of the rationales for cytokine gene therapy is that exogenous cytokines are known to have direct antiproliferative effects on mesothelioma cells, as well as the ability to activate intrapleural and intratumoral immune effector cells in vivo. Expression of cytokine genes by tumor cells generates a high level of intratumoral cytokines in paracrine fashion, inducing powerful local cytokine effects without significant systemic toxicity. Prolonged local cytokine expression can induce activation of tumor-associated dendritic cells (DCs) to express major histocompatibility complex (MHC) tumor antigen complexes in conjunction with co-stimulatory molecules. These activated DCs can then migrate to regional lymph nodes where they stimulate proliferation of tumor-specific CD8 and CD4 lymphocytes, inducing antitumor cytotoxicty at distant sites of tumor. In addition, some proinflammatory cytokines, such as IL-2, have the capability of direct intratumoral activation of $CD8^+$ tumor infiltrating lymphocytes, overcoming tolerance signals to produce tumor-specific cytotoxic T lymphocytes (CTLs). Increased intratumoral IL-2 may also activate natural killer (NK) cells and lymphokine-activated killer cells (LAKs). Animal experiments have shown that injection of IL-2–transduced tumor cells increases specific antitumor activity, generates systemic responses to the parental tumor, augments the immune response against autologous tumor, and causes rejection of rechallenged tumor cells (47,48).

Several published phase I and phase II clinical trials have documented mesothelioma tumor responses to intrapleural infusion of IL-2, type I interferons [interferon-α and interferon-β (IFN-β)], and type II interferons [interferon-γ (IFN-γ)] (49–55). In particular, Boutin and colleagues (52,53) at the Hôpital de la Conçeption in Marseilles, France, demonstrated significant response rates in pleural mesothelioma after intrapleural instillation of IFN-γ, including several complete pathologic responses in patients with stage IA disease (tumor limited to the parietal and diaphragmatic pleual surfaces) (52,53).

The first human clinical trial of direct intratumoral delivery of cytokine genes in malignant pleural mesothelioma using this method of in vivo genetic immunotherapy was conducted by investigators at Queen Elizabeth II Hospital in Perth, Australia, using a recombinant vaccinia virus (VV) expressing the human IL-2 gene (Table 52.2). A vaccinia vector was chosen because of its large genome, proven safety in human vaccines, and availability of anti-VV antibodies for evaluation of vector-induced immune responses. In addition, insertion of the IL-2 gene into the thymidine kinase region of the VV rendered it partially replication-restricted, allowing for relatively more expression in tumor cells. The VV–IL-2 vector at a dose of 1×10^7 pfu was serially injected into palpable chest wall lesions of six patients with advanced malignant mesothelioma. Toxicities were minimal, and there was no clinical or serologic evidence of spread of vaccinia virus to patient contacts. No significant tumor regression was seen in any of the patients, and only modest intratumoral T-cell infiltration was detected. VV–IL-2 messenger RNA (mRNA) was detected by reverse-transcriptase polymerase chain reaction (PCR) in serial tumor biopsies for up to 6 days after injection, but declined to low levels by day 8. The prolonged nature of IL-2 gene expression in this trial was remarkable, considering the fact that significant serum titers of anti-VV neutralizing antibodies were generated in all patients (56).

The Future of Genetic Immunotherapy for Mesothelioma

Several other candidate cytokine genes are being evaluated for therapeutic effectiveness in animal models of mesothelioma. Caminschi and colleagues at (57) Queen Elizabeth II Medical Center in Perth, have investigated genetic alteration of murine mesothelioma cell lines with the gene for interleukin-12 (IL-12), one of the most active immunomodulatory cytokines. This same group previously demonstrated that systemic administration of exogenous IL-12 induced strong antitumor immune responses in mice bearing syngeneic mesothelioma tumors. The Perth group showed that injection of murine mesothelioma cells transfected with the IL-12 gene (AB1–IL-12) did not produce tumors in immune-competent mice, but did so in athymic nude mice, implicating a T-cell–dependent mechanism of IL-12 activity. Immune-competent mice challenged with AB1–IL-12 were protected from subsequent challenge with parental tumor not expressing IL-12, demonstrating induction of long-term immunity. In addition, AB1–IL-12 injection reduced the incidence of tumor development from parental cell challenge at a distant site (58).

Innate and adaptive antitumor immune responses can also be elicited by delivery of nonspecific immunostimulatory genes. As an example of this paradigm, Lukacs and colleagues (59) transferred mycobacterial heat shock protein gene *(HSP-65)* via a cationic liposome into the abdominal cavities of mice bearing intraperitoneal sarcomas

resulting in a significant antitumor response. Interestingly, Lanuti and colleagues (60) in our laboratory, found that the antitumor effects of heat shock protein gene transfer via cationic liposomes could be reproduced in a syngeneic murine model of mesothelioma, but appeared to be related to nonspecific effects of lipid-plasmid DNA (pDNA) complexes. Rudginsky and colleagues (61) at Genzyme Corp. (Framingham, MA) further explored the potential of prokaryotic DNA induction in mesothelioma cells. They conducted a series of experiments confirming antitumor responses and increased survival with liposomal delivery of fragments of bacterial plasmid DNA, genomic *Escherichia coli* DNA, and synthetic CpG oligonucleotides. No increased survival or tumor reductions were seen with liposomal delivery of eukaryotic DNA or with methylated bacterial DNA. Therefore, the unmethylated CpG motifs of prokaryotic DNA play a crucial role in the development of innate and adaptive antitumor immune responses. Based on these studies, therefore, a case could then be made for a straightforward clinical trial of intrapleural delivery of nonspecific lipid-pDNA in patients with mesothelioma (Table 52.2).

As previously mentioned, the type I (α, β) and type II (γ) interferons have been shown to have clinical antitumor activity when administered exogenously to patients with pleural mesothelioma. Interferon-β, for example, has potent antiproliferative in vitro effects on mesothelioma cells, and strong immunostimulatory actions in animal models, but is limited in clinical use by toxicity of systemic administration (62). Odaka and colleagues (63,64) at the University of Pennsylvania Medical Center, therefore investigated the effects of IFN-β gene therapy in murine models of mesothelioma. The Penn investigators showed that a single intraperitoneal (i.p.) injection of a recombinant adenovirus, engineered to express the murine IFN-β gene (Ad.muIFN-β), can eradicate small syngeneic murine mesothelioma tumors in >90% of animals tested. Intraperitoneal Ad.muIFN-β gene therapy resulted in significant reduction of subcutaneous tumors at a distant site, as well. These effects of Ad.muIFN-β were clearly shown in several experiments to be mediated by $CD8^+$ T lymphocytes. Additional studies have shown that the combination of intratumoral treatment with Ad.IFN-β, followed (in 3 days) by surgical debulking, led to a high cure rate in very large tumors. Based on these promising preclinical studies and a toxicology trial performed in mice showing a good safety profile, we have initiated a phase I clinical trial of intrapleural delivery of Ad.muIFN-β for the treatment of mesothelioma (Table 52.1). If the phase I trial shows safety, a phase II "neoadjuvant" immunotherapy/surgical approach will be proposed.

Induction of Apoptosis

One of the primary approaches to cancer gene therapy research over the past decade has been mutation compensation—the replacement of absent or mutated tumor suppressor genes responsible, at least in part, for the malignant phenotype of the cancer cell. Intratumoral delivery

of the wild-type *p53* gene, for example, has been the most frequent method of experimental gene therapy of solid tumors, as mutations in the *p53* tumor suppressor gene account for the majority of genetic abnormalities in solid tumors. Most mesotheliomas, however, contain wild-type *p53* and a normal copy of the cell cycle regulator pRb. The most common molecular abnormality found in pleural mesotheliomas is absent expression of the cyclin-dependent kinase (CDK) inhibitor, $p16^{INK4a}$. This mutation can lead to unmitigated progression through the cell cycle despite the presence of normal pRb expression and wild-type *p53*, and therefore, the development of a neoplastic phenotype (65).

Kratzke and colleagues at the University of Minnesota School of Medicine have demonstrated that reexpression of $p16^{INK4a}$ in mesothelioma cells in vitro and in vivo results in cell cycle arrest, cell growth inhibition, apoptosis, and tumor reduction (65). In addition, the Minnesota investigators have recently shown that repeated administration of an adenoviral vector expressing wild-type $p16^{INK4a}$ into established human mesothelioma xenografts in athymic nude mice resulted in prolongation of survival compared with controls receiving saline or an Ad vector expressing the marker gene *lacZ* (66). Successful application of this technology to human clinical trials is dependent on the development of more efficient means of tumor cell transduction.

Investigators at the Thoracic Oncology Laboratory, University of California at San Francisco (UCSF) Cancer Center, are targeting another common mutation in mesotheliomas for mutation compensation gene therapeutic approaches. Jablons and colleagues at UCSF have demonstrated that homozygous deletion of the INK4a/ARF locus is common in human mesotheliomas (67). The p14(ARF) protein encoded by the INK4a/ARF locus promotes degradation of the p53 binding protein called MDM2, which functions to bind p53 and inactivate it. Thus, production of the ARF protein prevents MDM2-mediated neutralization of p53 and favors p53-mediated cell cyle arrest. Deletion of the INK4a/ARF locus abrogates p14(ARF) protein expression, which leads to higher levels of MDM2. MDM2 binds p53 and inactivates it, leading to unchecked progression through the cell cycle. The UCSF group transfected human mesothelioma cell lines with an adenoviral vector encoding for human p14(ARF) cDNA (Ad.p14). Overexpression of p14(ARF) within the mesothelioma cells led to increased amounts of p53 and p21, and dephosphorylation of pRb. In addition, Ad.p14 inhibited mesothelioma cell growth via induction G(1)-phase cell cycle arrest and apoptotic cell death (67).

Despite the fact that most mesotheliomas have wild-type p53 (wt-p53), the function of p53 in mesothelioma cells may be abnormal secondary to binding of p53 by inhibitor proteins such as MDM2 and simian virus 40 (SV40) large-T antigen. Therefore, there may be a rationale for gene therapy of mesothelioma via overexpression of wt-p53 within the cell. Giuliano and colleagues (68) in Chieti, Italy, performed a series of experiments in which they transfected human mesothelioma cells with a replication-deficient adenoviral vector carrying the *wt-p53* gene. They demonstrated greater than 80% inhibition of tumor cell growth in vitro at a multiplicity of infection (MOI) of 25 with docu-

mentation of induction of apoptosis in the dying tumor cells. In addition, Giuliano and colleagues showed that ex vivo transfer of the *wt-p53* gene to mesothelioma cells inhibited growth of tumor implants in nude mice. In immunodeficient mice with established human mesothelioma xenografts, intratumoral injection of the *wt-p53* gene inhibited tumor growth and prolonged survival. It is not inconceivable, therefore, to consider human clinical trials of Ad *wt-p53* gene therapy in mesothelioma akin to those conducted in lung cancer, head and neck cancer, and metastatic colon cancer (Table 52.2).

An alternate method of inhibiting mesothelioma cells is the introduction of "downstream" promoters of apoptosis such as the proapoptotic Bcl-2 family member Bak. Pataer and colleagues (69) at M.D. Anderson Cancer Center in Houston co-delivered binary adenoviral-Bak/GV-16 vectors into *wt-p53* positive and mutated *p53* mesothelioma cell lines in vitro, along with binary Ad.*lacZ*/GV-16 control vectors. The M.D. Anderson group demonstrated marked induction of apoptosis and decreased cellular viability in both *p53* "sensitive" and "resistant" cell lines with *Bak* gene transfer, but not with *lacZ* delivery. Thus, gene transfer in vivo with proapoptotic Bcl-2 family members would be a reasonable strategy for future mesothelioma gene therapy clinical trials. Alternatively, inhibition of endogenous inhibitors of apoptosis is a possible approach. Xia et al (70) have recently shown widespread expression of the inhibitor of apoptosis, survivin, in mesothelioma. Interestingly, antisense oligonucleotides to survivin, induced apoptosis in mesothelioma cell lines overexpressing survivin.

SV40: Is There a Role in Therapy for Mesothelioma?

One of the most remarkable developments in mesothelioma research over the past several years has been the discovery of simian virus 40 (SV40) sequences in mesothelioma tumor specimens from the United States and several European countries. SV40, a nonhuman polyomavirus that was a contaminant of some polio vaccines in the 1950s and 1960s, carries the ability to transform normal cells via the oncogenic properties of its large-T antigen (Tag), and can induce the formation of mesotheliomas in hamsters after injection into the pleural space or peritoneal cavity (71). Laboratory analysis of a subset of human mesotheliomas has demonstrated coimmunoprecipitation of SV40 Tag with tumor suppressor gene products, such as the p53 and pRB proteins (72). The presence of SV40 Tag within tumor cells binding and inactivating wild-type p53 and pRB may explain the unusually high rate of wild-type p53 and pRb within mesotheliomas, unlike most other solid tumors.

The potential role for SV40 as a causative factor in mesothelioma oncogenesis and proliferation has inspired several new experimental gene therapy approaches. Schrump and Waheed (73), at the thoracic oncology branch of the National Cancer Institute, have shown that antisense oligonucleotides designed to abrogate SV40 Tag expression induce apoptosis and enhance sensitivity to chemotherapeutic agents

in SV40 (+) mesothelioma cells in vitro. Another strategy has been advocated by Imperiale and colleagues (74) at the University of Michigan and Wayne State University Medical Centers, who are developing a genetically engineered vaccine to SV40 Tag. SV40 is an excellent candidate for antigen-specific immunotherapy, because Tag is a viral antigen that should not induce immune tolerance, unlike most other tumor antigens. The Michigan group has created a recombinant, truncated version of Tag (mTag), modified to exclude the domains involved in oncogenic function: the J domain and the p53 and pRB binding domains. They have cloned the mTag gene into a vaccinia vector (vac-mTag), and have demonstrated significant antitumor immune responses in Balb/c mice carrying Tag(+) tumors. A phase I dose-escalation safety and toxicity trial in patients with Tag-expressing mesotheliomas is planned (Table 52.2) (74).

Replicating Viruses

An additional mechanism of maximizing intratumoral gene transfer would be to produce adenoviral vectors capable of selective replication in mesothelioma cells. In this approach, tumor killing could occur via two mechanisms: direct tumor lysis due to viral replication or augmentation of transgene delivery, such as HSV*tk*. Widespread dissemination would likely be precluded by the intact host immune response (75).

One approach to make such tumor selective virus is to substitute the adenoviral E1 promoter with promoters for mesothelioma-related proteins, such as manganese-superoxide dismutase (MnSOD), calretinin, and mesothelin (76–79). Work by Kinnula's group (76) in Finland, has shown that MnSOD is very highly expressed in human malignant mesothelioma tissues and cell lines. Calretinin is a 29-kd calcium-binding protein that is expressed primarily in the nervous system, but high levels of expression have also been noted in cells of mesothelial origin (77,78). Mesothelin is a 40-kd surface protein of unknown function that is expressed only on the tissues forming the pleural, pericardial, and peritoneal membranes (79). Another approach is to use more general tumor-selective promoters, such as promoters responsive to the transcription factor E2F (80) or the survivin gene (81). Our group has recently generated such an E2F-driven virus and showed that it selectively replicated in and lysed tumor cells, compared with non-transformed cells (80).

ONYX-015 is a conditionally replication competent adenovirus lacking the E1b 55-kd gene, and therefore can only replicate in tumor cells lacking functional p53. One of the functions of E1b 55-kd is to bind and inactivate wild-type p53. Clinical trials of ONYX-015 in patients with cancers of the head and neck and lung have shown evidence of tumor reduction with minimal toxicity. As described above, in mesothelioma, unlike many other solid tumors, genetic alterations in p53 are uncommon, but functional inhibition of p53 can be achieved via deletions in the INK4a/ARF locus. The UCSF group demonstrated

in vitro cytotoxicity of ONYX-015 on mesothelioma cell lines lacking p14(ARF), and increased resistance of these same cell lines to ONYX-015 after transfection of the tumor cells with Ad.p14 (82).

To date, replicating adenoviruses have proven safe in phase I clinical trials, but have had limited efficacy. With further refinements or with combinations with other treatments (such as chemo- or radiation therapy), this approach could prove useful in the future.

Summary

Gene therapy for mesothelioma is in its infancy, yet the results of recent phase I clinical trials and ongoing preclinical studies offer significant promise for the future. Intrapleural and intratumoral injections of viral and nonviral vectors encoding therapeutic genes have proved safe in humans with evidence of intratumoral gene transfer and expression of therapeutic proteins. Anecdotal tumor responses have been seen in suicide gene therapy trials, either as a result of direct cytotoxicity or via induction of bystander immunologic phenomena. Our group is vigorously pursuing an immuno-gene therapy approach with an adenovirus expressing interferon-β. Expanding knowledge of the cellular and molecular abnormalities responsible for the carcinogenesis of mesothelioma has led to the development of new gene therapy approaches targeting oncoproteins and mutant tumor suppressor genes. Implementation of these experimental modalities on a routine basis for mesothelioma patients remains several years in the future. Nevertheless, the lack of significant benefit from standard anticancer treatments in the disease argues strongly for patient enrollment in clinical studies of various gene therapy approaches to determine safety, toxicity, and efficacy, as well as to guide future laboratory investigation.

References

1. Curiel DT, Pilewsky JM, Albelda SM. Gene therapy approaches for inherited and acquired diseases of the lung. Am J Respir Cell Mol Biol 1996; 14:1–18.
2. Wivel NA, Wilson JM. Methods of gene delivery. Hematol Oncol Clin North Am 1998;12:483–501.
3. Buchschacher GL Jr, Wong-Stall F. Development of lentiviral vectors for gene therapy for human diseases. Blood 2000;95:2499–2504.
4. Monahan PE, Samulski RJ. AAV vectors: is clinical success on the horizon. Gene Ther 2000;7:24–30.
5. Ponnazhagan S, Curiel DT, Shaw DR, Alvarez RD, Siegal GP. Adeno-associated virus for cancer gene therapy. Cancer Res 2001;61:6313–6321.
6. Chesnoy S, Huang L. Structure and function of lipid-DNA complexes for gene delivery. Annu Rev Biophys Biomol Struct 2000;29:27–47.
7. Zhang WW. Development and application of adenoviral vectors for gene therapy of cancer. Cancer Gene Ther 1999;6:113–138.

8. Yeh P, Perricaudet M. Advances in adenoviral vectors: from genetic engineering to their biology. FASEB J 1997;11:615–623.
9. Treat J, Kaiser LR, Sterman DH, et al. Treatment of advanced mesothelioma with the recombinant adenovirus H5.010RSVTK: a phase 1 trial (BB-IND 6274). Hum Gene Ther 1996;7:2047–2057.
10. Schwarzenberger P, Harrison L, Weinacker A, et al. Gene therapy for malignant mesothelioma: a novel approach for an incurable cancer with increased incidence in Louisiana. J La State Med Soc 1998;150:168–174.
11. Robinson BW, Mukherjee SA, Davidson A, et al. Cytokine gene therapy or infusion as treatment for solid human cancer. J Immunother 1998;21:211–217.
12. Tiberghien P. Use of suicide genes in gene therapy. J Leukoc Biol 1994;56: 203–209.
13. Hoganson DK, Batra RK, Olsen JC, Boucher RC. Comparison of the effects of three different toxin genes and their levels of expression on cell growth and bystander effect in lung adenocarcinoma. Cancer Res 1996;56:1315–1323.
14. Matthews T, Boehme R. Antiviral activity and mechanism of action of ganciclovir. Rev Infect Dis 1988;10:S494.
15. Pope IM, Poston GJ, Kinsella AR. The role of the bystander effect in suicide gene therapy. Eur J Cancer 1997;33:1005–1016.
16. Ram Z, Culver KW, Walbridge B, Blaese RM, Oldfield EH. In situ retroviral-mediated gene transfer for the treatment of brain tumors in rats. Cancer Res 1993;53:83–88.
17. Freeman SM, Abboud CN, Whartenby KA, et al. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993;53:5274–5283.
18. Hasegawa Y, Emi N, Shimokata K, et al. Gene transfer of herpes simplex virus type I thymidine kinase gene as a drug sensitivity gene into human lung cancer lines using retroviral vectors. Am J Respir Cell Mol Biol 1993; 8:655–661.
19. Caruso M, Panis Y, Gagandeep S, Houssin D, Salzmann JL, Klatzmann D. Regression of established macroscopic liver metastases after in situ transduction of a suicide gene. Proc Natl Acad Sci USA 1993;90:7024–7028.
20. Elshami AA, Saavedra A, Zhang HB, et al. Gap junctions play a role in the bystander effect of the herpes simplex virus thymidine kinase/ganciclovir system in vitro. Gene Ther 1996;3:85–92.
21. Mesnil M, Yamasaki H. Bystander effect in herpes simplex virus-thymidine kinase/ganciclovir cancer gene therapy: role of gap-junctional intercellular communication. Cancer Res 2000;60:3989–3999.
22. Smythe WR, Hwang HC, Amin KM, et al. Use of recombinant adenovirus to transfer the herpes simplex virus thymidine kinase (HSVtk) gene to thoracic neoplasms: an effective in vitro drug sensitization system. Cancer Res 1994;54:2055–2059.
23. Smythe WR, Kaiser LR, Amin KM, et al. Successful adenovirus-mediated gene transfer in an in vivo model of human malignant mesothelioma. Ann Thorac Surg 1994;57:1395–1401.
24. Smythe WR, Hwang HC, Elshami AA, et al. Successful treatment of experimental human mesothelioma using adenovirus transfer of the herpes simplex-thymidine kinase gene. Ann Surg 1995;222:78–86.
25. Hwang HC, Smythe WR, Elshami AA, et al. Gene therapy using adenovirus carrying the herpes simplex thymidine kinase gene to treat in vitro models of human malignant mesothelioma and lung cancer. Am J Respir Cell Mol Biol 1995;13:7–16.

26. Elshami A, Kucharczuk J, Zhang H, et al. Treatment of pleural mesothelioma in an immunocompetent rat model utilizing adenoviral transfer of the HSV-thymidine kinase gene. Hum Gene Ther 1996;7:141–148.
27. Esandi MC, van Someren GD, Vincent AJ, et al. Gene therapy of experimental malignant mesothelioma using adenovirus vectors encoding the HSV*tk* gene. Gene Ther 1997;4:280–287.
28. Kucharczuk JC, Raper S, Elshami AA, et al. Safety of adenoviral-mediated transfer of the herpes simplex thymidine kinase cDNA to the pleural cavity of rats and non-human primates. Hum Gene Ther 1996;7:2225–2233.
29. Sterman DH, Treat J, Litzky LA, et al. Adenovirus-mediated herpes simplex virus thymidine kinase gene delivery in patients with localized malignancy: results of a phase 1 clinical trial in malignant mesothelioma. Hum Gene Ther 1998;9:1083–1092.
30. Molnar-Kimber KL, Sterman DH, Chang M, et al. Humoral and cellular immune responses induced by adenoviral-based gene therapy for localized malignancy: results of a phase 1 clinical trial for malignant mesothelioma. Hum Gene Ther 1998;9:2121–2133.
31. Sterman DH, Molnar-Kimber K, Iyengar T, et al. A pilot study of systemic corticosteroid administration in conjunction with intrapleural adenoviral vector administration in patients with malignant pleural mesothelioma. Cancer Gene Ther 2000;7:1511–1518.
32. Gao GP, Yang Y, Wilson JM. Biology of adenovirus vectors with E1 and E4 deletions for liver-directed gene therapy. J Virol 1996;70:8934–8943.
33. Sterman DH, Recio A, Molnar-Kimber K, et al. Herpes simplex virus thymidine kinase (HSV*tk*) gene therapy utilizing an E1/E4-deleted adenoviral vector: preliminary results of a phase I clinical trial for pleural mesothelioma. Am J Respir Crit Care Med 1999;159:A237.
34. Carmen IH. A death in the laboratory: the politics of the Gelsinger aftermath. Mol Ther J Am Soc Gene Ther 2001;3:425–428.
35. Lambright ES, Force SD, Lanuti M, et al. Efficacy of repeated adenoviral suicide gene therapy in a localized murine tumor model. Ann Thorac Surg 2000;70:1856–1870.
36. Al-Hendry A, Magliocco AM, Al-Tweigeri T, et al. Ovarian cancer gene therapy: repeated treatment with thymidine kinase in an adenovirus vector and ganciclovir improves survival in a novel immunocompetent murine model. Am J Obstet Gynecol 2000;182:553–559.
37. Black ME, Newcomb TG, Wilson HMP, Loeb LA. Creation of drug-specific herpes simplex virus type 1 thymidine kinase mutants for gene therapy. Proc Natl Acad Sci USA 1996;93:3525–3529.
38. Qiao HJ, Black ME, Caruso M. Enhanced ganciclovir killing and bystander effect of human tumor cells transduced with retroviral vector carrying a herpes simplex thymidine kinase gene mutant. Hum Gene Ther 2000;11:1569–1576.
39. Black ME, Kokoris MS, Sabo P. Herpes simplex virus-1 thymidine kinase mutants created by semi-random sequence mutagenesis improve prodrug-mediated tumor cell killing. Cancer Res 2001;61:3022–3026.
40. Wiewrodt R, Amin K, Keifer M, et al. Adenovirus-mediated gene transfer of enhanced herpes simplex thymidine kinase mutants improves prodrug-mediated tumor cell killing. Cancer Gene Ther, in press.
41. Hall SJ, Sanford MA, Atkinson G, Chen SH. Induction of potent antitumor natural killer cell activity by herpes simplex virus-thymidine kinase and ganciclovir therapy in an orthotopic mouse model of prostate cancer. Cancer Res 1998;58:3221–3225.

42. Freeman SM, Ramesh R, Marogi AJ. Immune system in suicide gene therapy. Lancet 1997;349:2–3.
43. Vile RG, Castleden S, Marshall J, Camplejohn R, Upton C, Chong H. Generation of an anti-tumor immune response in a non-immunogenic tumour: HSV*tk* killing in vivo stimulates a mononuclear cell infiltrate and a Th1-like profile of intratumoural cytokine expression. Int J Cancer 1997; 71:267–274.
44. Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile R. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nat Med 1998;4:581–587.
45. Chen SH, Li Chen XH, Wang Y, et al. Combination gene therapy for liver metastasis of colon carcinoma in vivo. Proc Natl Acad Sci USA 1995;92: 2577–2581.
46. Kolls J, Freeman S, Ramesh R, et al. The treatment of malignant pleural mesothelioma with gene modified cancer cells: a phase I study. Am J Respir Crit Care Med 1998;157:A563.
47. Leong CC, Marley JV, Loh S, Robinson BWS, Garlepp MJ. The induction of immune responses to murine mesothelioma by IL-2 gene transfer. Immunol Cell Biol 1997;75:356–359.
48. Fakharai H, Shawler D, Gjerset R, et al. Cytokine gene therapy with interleukin-2-transduced fibroblasts: effects of IL-2 dose on anti-tumor immunity. Hum Gen Ther 1995;6:591–601.
49. Christmas T, Manning LS, Garlepp MJ, Mush AW, Robinson BW. Effect of Interferon-alpha 2a on malignant mesothelioma. Interferon Res 1993;13:9–12.
50. Astoul P, Viallat JR, Laurent JC, Brandley M, Boutin C. Intrapleural IL-2 in passive immunotherapy for malignant pleural effusion. Chest 1993;103: 209–213.
51. Astoul P, Picat-Joossen D, Viallat J, Boutin C. Intrapleural administration of interleuken-2 for the treatment of patients with malignant pleural mesothelioma: a phase II study. Cancer 1998;83:2099–2104.
52. Boutin C, Viallat J, VanZandwijk N, et al. Activity of intrapleural recombinant gamma-interferon in malignant mesothelioma. Cancer 1991;67:2033–2037.
53. Boutin C, Nussbaum E, Monnet I, et al. Intrapleural treatment with recombinant gamma-interferon in early stage malignant pleural mesothelioma. Cancer 1994;74:2460–2467.
54. Robinson B, Bowman R, Manning L, Musk A, Van Hazel G. Interleukin-2 and lymphokine-activated killer cells in malignant mesothelioma. Eur Respir Rev 1993;3:220–222.
55. Goey SH, Eggermont AM, Punt CJ, et al. Intrapleural administration of interleukin-2 in pleural mesothelioma: a Phase I-II study. Br J Cancer 1995; 72:1283–1288.
56. Mukherjee S, Haenel T, Himbeck R, et al. Replication-restricted vaccinia as a cytokine gene therapy vector in cancer: persistent transgene expression despite antibody generation. Cancer Gene Ther 2000;7:663–670.
57. Caminschi I, Venetsanakos E, Leong CC, Garlepp MJ, Scott B, Robinson BWS. Interleukin-12 induces an effective antitumor response in malignant mesothelioma. Am J Respir Cell Mol Biol 1998;19:738–746.
58. Caminschi I, Venetsanakos E, Leong CC, Garlepp MJ, Robinson BW, Scott B. Cytokine gene therapy of mesothelioma. Immune and antitumor effects of transfected interleukin-12. Am J Respir Cell Mol Biol 1999;21: 347–356.

59. Lukacs KV, Nakakes A, Atkins, CJ, Lowrie DB, Colston MJ. In vivo gene therapy of malignant tumors with heat shock protein-65 gene. Gene Ther 1997;4:345–350.
60. Lanuti M, Rudginsky S, Force S, et al. Cationic lipid:bacterial DNA complexes elicit anti-tumor effects and adaptive immunity in murine intraperitoneal tumor models. Cancer Res 2000;60:2955–2963.
61. Rudginsky S, Siders W, Ingram L, Marshall J, Scheule R, Kaplan J. Antitumor activity of cationic lipid complexed with immunostimulatory DNA. Mol Ther J Am Soc Gene Ther 2001;4:347–355.
62. Rosso R, Rimoldi R, Salvati F, et al. Intrapleural natural beta interferon in the treatment of malignant pleural effusions. Oncology 1988;45:253–256.
63. Odaka M, Sterman DH, Wiewrodt, et al. Eradication of intraperitoneal and distant tumor by adenovirus-mediated interferon-β gene therapy is attributable to induction of systemic immunity. Cancer Res 2001;61:6201–6212.
64. Odaka M, Wiewrodt R, DeLong PA, et al. Analysis of the immunological response generated by Ad.IFN-γ during successful peritoneal tumor gene therapy. Mol Ther 2002;6:210–218.
65. Frizelle SP, Grim J, Zhou JX, et al. Re-expression of $p16^{INK4a}$ in mesothelioma cells results in cell cycle arrest, cell death, tumor suppression, and tumor regression. Oncogene 1998;16:3087–3095.
66. Frizelle SP, Rubins JB, Zhou JX, Curiel DT, Kratzke RA. Gene therapy of established mesothelioma xenografts with recombinant $p16^{INK4a}$ adenovirus. Cancer Gene Ther 2000;7:1421–1425.
67. Yang C, You L, Yeh C, et al. Cell cycle arrest and induction of apoptotic death in mesothelioma cells by the adenovirus-mediated $p14^{ARF}$ expression. J Natl Cancer Inst 2000;92:636–641.
68. Giuliano M, Catalano A, Strizzi L, Vianale G, Capogrossi M, Procopio A. Adenovirus-mediated wild-type p53 overexpression reverts tumourigenicity of human mesothelioma cells. Int J Mol Med 2000;5:591–596.
69. Pataer A, Smythe WR, Yu R, et al. Adenovirus-mediated Bak gene transfer induces apoptosis in mesothelioma cell lines. J Thorac Cardiovasc Surg 2001;121:61–67.
70. Xia C, Xyu Z, Yuan X, et al. Induction of apoptosis in mesothelioma cells by antisurvivin oligonucleotides. Mol Cancer Ther 2002;9:687–694.
71. Cicala C, Pompetti F, Carbone M. SV40 induces mesothelioma in hamsters. Am J Pathol 1993;142:1524–1533.
72. Carbone M, Rizzo P, Grimley PM, et al. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. Nat Med 1997;3:908–912.
73. Schrump DS, Waheed I. Strategies to circumvent SV40 oncoprotein expression in malignant pleural mesothelioma. Semin Cancer Biol 2001;11:73.
74. Imperiale MJ, Pass HI, Sanda MG. Prospects for an SV40 vaccine. Semin Cancer Biol 2001;11:81–85.
75. Alemany R, Balague C, Curiel DT. Replicative adenoviruses for cancer therapy. Nat Biotechnol 2000;18:723–727.
76. Kahlos K, Anttila S, Asikainen T, et al. Manganese superoxide dismutase in healthy human pleural mesothelium and in malignant pleural mesothelioma. Am J Respir Cell Mol Biol 1998;18:579–580.
77. Doglioni C, Dei Tos AP, Laurino L, et al. Calretinin: a novel immunocytochemical marker for mesothelioma. Am J Surg Pathol 1996;20:1037–1046.
78. Gotzos V, Vogt P, Celio M. The calcium binding protein calretinin is a selective marker for malignant pleural mesotheliomas of the epithelial type. Pathol Res Pract 1996;192:137–147.
79. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci USA 1996;93:136–140.

80. Tsukuda K, Wiewrodt R, Molnar-Kimber K, Jovanovic V, Amin K. An E2F-responsive replication-selective adenovirus targeted to the defective cell cycle in cancer cells: potent antitumoral efficacy but no toxicity to normal cells. Cancer Res 2002;62:3438–3447.
81. Ambrosini G, Adid C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997;3:917–921.
82. Yang C, You L, Yeh C, et al. $p14^{ARF}$ modulates the cytolytic effect of ONYX-015 in mesothelioma cells with wild-type p53. Cancer Res 2001;61:5959–5963.

53

Immunotherapeutic Approaches and Vaccination Strategies

Anna K. Nowak, Richard A. Lake, and Bruce W.S. Robinson

Malignant mesothelioma is increasing in incidence and is invariably fatal. Patients commonly present with advanced disease, making treatments, such as surgery and radiotherapy anatomically and technically difficult and often unsuccessful in this group of patients. Furthermore, chemotherapy has historically been ineffective, although in recent years newer regimens with higher response rates have been reported (1–3), but are not curative. Hence, there is a need for new treatments. New therapies, such as immunotherapy and vaccine therapy, therefore, can be readily tested in this disease. This chapter discusses the theoretical and preclinical bases of these experimental strategies, and provides an overview of the clinical trials performed in this area. Although this chapter discusses immunotherapy, vaccine therapy, and combination chemoimmunotherapy separately, these areas are linked and such separations are artificial but necessary.

The Immune Response to a Solid Tumor

To understand how novel therapies might alter the immune response to tumor, it is important to first understand how the immune system responds to cancer under normal circumstances. The antitumor immune response consists of an innate or nonspecific component and an adaptive or specific component. The innate and adaptive immune systems do not act in isolation, each producing soluble factors, such as interferon-γ (IFN-γ) and interleukins, which may have a stimulatory or inhibitory role for cells from the other arm of the immune response, and there may also be crosstalk between cell populations from the innate and adaptive immune systems, adding further complexity to this interaction.

An effective immune response to tumor requires both recognition of tumor by the immune system and the subsequent development of an adequate immune response with the ability to infiltrate the tumor and kill tumor cells. The immune system must recognize tumor antigens; they must be taken up, processed, and presented to $CD4^+$ T cells by

dendritic cells, and directly or cross-presented to $CD8^+$ T cells in the context of appropriate co-stimulation. $CD8^+$ T cells must be capable of proliferating, entering the tumor milieu, and then effectively killing tumor or activating other local cells to do so. The magnitude and duration of the cellular response must be adequate to eradicate tumor. Important points in the development of antitumor immunity will be discussed.

The Innate Immune Response

The innate immune system originally evolved to produce a rapid, first-line defense against pathogens. It has not evolved to recognize antigens, and thus has a less important role in antitumor immunity than the adaptive immune system. However, natural killer (NK) cells may have an antitumor role as mediators of antibody-dependent cellular cytotoxicity (ADCC), killing tumor cells coated with antibody via their receptor for immunoglobulin G (IgG). Furthermore, they interact with the adaptive immune system by secreting IFN-γ and other cytokines, such as interleukin-1 (IL-1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) when activated. They may also have a role in surveillance against malignant transformation. Neutrophils play an important role in acute inflammation and the immediate response against infection, but their role in antitumor immunity has received little attention, despite the fact that neutrophil infiltration of tumors corresponds with a favorable prognosis in some studies, and that most cytokine treatments of experimental tumors show a strong neutrophil infiltrate in responding sites (4). Once recruited into the tumor, neutrophils can produce IL-1β, tumor necrosis factor-α (TNF-α), and interferons, and can also kill tumor cells by ADCC (5). Tumor macrophages infiltrates are often heavy, and these cells may have a role in antigen presentation and tumor cytotoxicity, e.g., via production of nitrous oxide (NO).

The Adaptive Immune Response

The adaptive immune response entails the interaction between B and T lymphocytes and antigens. The two arms of the adaptive immune system consist of the cellular or T-lymphocyte arm, and the humoral or B-lymphocyte/antibody arm. Although the adaptive immune system responds more slowly than the innate immune system, its advantage lies in its increased specificity. While early stages of lymphocyte development are not dependent on the presence of antigen, their subsequent survival, differentiation, and proliferation become antigen-dependent, underlying the specificity of adaptive immunity.

Tumor antigens are usually proteins, expressed by the tumor cell, which can be recognized by the adaptive immune system. They may result from somatic mutations in normal gene products, mutated oncogenes, viral proteins, normal gene products with a restricted tissue distribution, such as the cancer-testis antigens, and normal tissue-specific gene products. It now seems clear that the failure to elicit an effective immune response is not due to a lack of tumor antigen expression or

to a lack of host recognition of tumor antigens. The failure to control or eradicate tumor appears to occur in the priming or licensing of the cell-mediated effector arm. Tumor antigens, including mesothelioma antigens, are constitutively presented in lymph nodes draining tumors and can stimulate a T-cell proliferative response, regardless of where in the cell the tumor antigens are expressed (6–8).

The cellular arm of the adaptive immune system is predominantly composed of T lymphocytes, and there is abundant evidence that these cells are important in antitumor immunity. $CD4^+$ and $CD8^+$ T lymphocytes can recognize antigen presented to them by antigen presenting cells (APCs) in the appropriate major histocompatibility complex (MHC) context and with the correct expression of co-stimulatory molecules. Activated T cells can then proliferate and move from the lymph node to the periphery as functional immune effector cells. Both $CD4^+$ and $CD8^+$ T cells are important for optimal antitumor immunity. T-cell-subset depletion experiments show that both cell types are required for rejection of tumor in vaccination studies (9). $CD4^+$ lymphocytes have an important role at numerous points along the pathway of initiating, maintaining, and directing the activation of $CD8^+$ T lymphocytes, but it is the $CD8^+$ T cells that, when appropriately activated, differentiate to become cytotoxic effector cells, with the ability to target and kill tumor.

The humoral arm of the adaptive immune response consists of B lymphocytes and their differentiation into antibody-secreting mature plasma cells and antibodies. They may have a role as potential APCs, but they are not necessary for an efficient immune response, and may in some circumstances be detrimental to T-cell activation and tumor recognition. Although antibody responses do not generally correlate with response against tumor, in some experimental situations immune sera have been shown to enhance tumor growth, probably by blocking access of tumor-specific lymphocytes to their target (10). In addition, B-cell–deficient mice in some systems control tumor growth more readily than their normal littermates, and the presence of B cells in the priming phase may result in disabled $CD4^+$ T cell help for $CD8^+$ cytotoxic T lymphocyte (CTL) (11).

How Do Tumors Escape Immune Destruction?

Early theories of antitumor immunity suggested that there was constant immune surveillance for tumors, which arose frequently but were then eliminated as tumor antigens were recognized as foreign or nonself. Tumors that did grow were felt to have escaped immune surveillance. However, this theory was challenged by the observation that immunodeficient mice did not have a higher incidence of tumor occurrence compared with immunocompetent mice, although recent data have again supported this notion. There are several explanations as to why the immune system fails to eradicate many tumors, even those expressing foreign antigens. First, MHC class I molecule expression is necessary for antigen presentation to $CD8^+$ T lymphocytes. While most cells, including tumor cells, express MHC class I molecules, some

tumors can downregulate class I expression. The progressive loss of class I antigens may have a role in tumor progression and the development of metastatic potential. This supports a "surveillance" mechanism because selection pressure by $CD8^+$ T cells must be present for class I loss variants to occur. Second, even when tumor cells express MHC class I molecules, they do not generally express co-stimulatory molecules of the B7 family and are therefore unable to provide signal two, which may lead to anergy and consequent lack of proliferation and IL-2 production if the $CD8^+$ T-cell receptor (TCR) becomes engaged (12). Third, even if $CD8^+$ lymphocytes are appropriately stimulated, the tumor may provide an inhospitable environment for effector lymphocytes. They may be large and poorly vascularized, and the endothelium and extracellular matrix may prevent T-cell infiltration. The tumor environment can contain numerous immunosuppressive cytokines, including transforming growth factor-β (TGF-β), IL-10, and vascular endothelial growth factor (VEGF), which may enhance the ability of the tumor to evade host immune responses. The expression of Fas ligand on tumor cells may induce tumor-reactive CTLs to enter the apoptotic pathway on contact with tumor. Finally, even if tumors stimulate an effective immune response, they may be too large for effective immunologic destruction when this response matures. It is possible that tumor must reach a certain critical mass to activate naive cells, trigger expansion, and induce effector function. They may be ignored early in their development because antigen-presentation levels remain below a certain threshold. Necrosis and apoptosis are more likely to occur in larger tumors, with tissue damage activating host dendritic cells (DCs) to acquire antigen and present it to tumor-specific T cells in the draining lymph node. However, by the time an effective antitumor response has been stimulated, it may be too late. It is likely that some or all of these factors contribute in varying degrees to the ability of individual tumors to escape immune destruction, and the problem is almost certainly multifactorial.

Immunotherapy in Malignant Mesothelioma

Why Might Immunotherapy be Effective in Malignant Mesothelioma?

Although malignant mesothelioma does not belong to the group of classically immunogenic tumors, such as malignant melanoma and renal cell carcinoma, in which spontaneous regression is sometimes reported, there is good evidence that the immune system can recognize this tumor. About 40% of patients with this tumor demonstrate high levels (titers >1/100) of antibodies reactive with mesothelioma cell line lysates in Western blot (13). There is no evidence that these antibodies affect the course of the disease. There is a relationship between tumor infiltrating lymphocytes and prognosis, which suggests that improving the immune response to this tumor may improve patient outcome (14). Furthermore, spontaneous regression has been reported in this disease, associated with evidence of immune responsiveness (15)

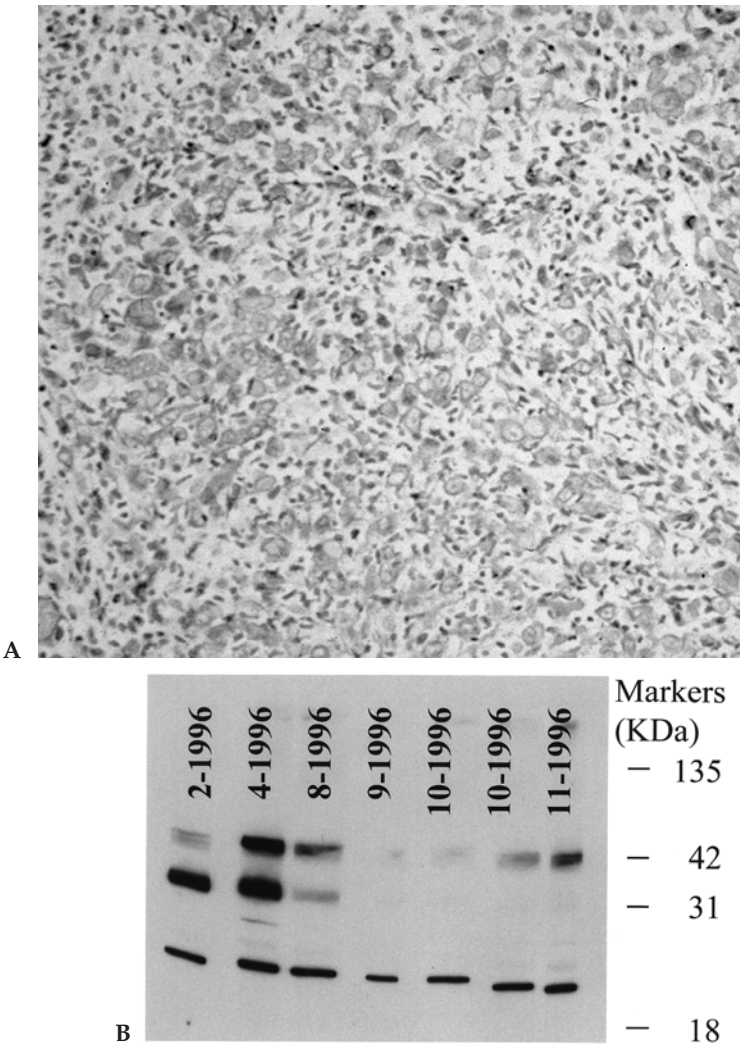


Figure 53.1. A: Tumor biopsy from a patient who underwent a spontaneous computed tomography (CT) scan confirmed spontaneous regression. Review of the initial mass showing mesothelioma tumor cells with a pronounced mononuclear cell infiltrate, clearly distinguishable from the tumor cells that are stained with an anticytokeratin stain (stained brown) from the mononuclear infiltrate. B: Western blot of serial serum samples from this patient showing changes in the antimalignant mesothelioma specific humoral immune response over time.

(Fig. 53.1). The impetus to pursue immunotherapy in mesothelioma arose because of a lack of effective conventional therapies, coupled with the fact that mesothelioma patients, due to their otherwise poor prognosis, are often motivated and willing participants in clinical trial programs. As mesothelioma is often localized to the pleural space, which is readily accessible, local treatments can be tried. Similarly, it is not difficult to obtain tumor tissue to study surrogate biomarkers of treatment efficacy, such as expression of transduced genes and lym-

phocytic infiltration of tumor. Further support for this treatment strategy comes from evidence that both mesothelioma patients and asbestos-exposed persons without mesothelioma have some immune dysfunction, and in vitro studies also support the detrimental effects of asbestos on immune cells. Although patients with mesothelioma maintain normal white cell counts, immunoglobulin levels, and total serum proteins, they show decreased mitogen responsiveness and lymphokine-activated killer (LAK) cell activity against mesothelioma tumor targets (16–18). Both humoral and cell-mediated antibody-dependent cellular toxicity is abnormal (19); NK cell activity is decreased, and $CD4^+$ lymphocyte numbers are reduced. T cells infiltrating murine mesotheliomas also show low surface expression of CD3, a feature of T-cell downregulation. Furthermore, asbestos itself suppresses NK and LAK cell function in vitro (17,20,21). Thus, it may be reasonable to explore treatments that can improve the immune response to this disease.

Tumor Antigens in Malignant Mesothelioma

For the immune system to mount a response against tumor, the tumor must express antigens that distinguish them from the surrounding normal tissue. Nevertheless, despite the presence and effective presentation of antigens, many tumors still evade immunologic destruction. Thoracic malignancies, including mesothelioma, have long been considered nonimmunogenic and not amenable to immunotherapy. However, the lack of an effective immune response is unlikely to be due to the lack of antigen expression, with several tumor antigens being recently identified in malignant mesothelioma. The cancer-testis antigens are normal gene products, but usually have a restricted tissue distribution. These include the MAGE and GAGE antigen groups. Malignant mesothelioma cells have been found to express numerous cancer-testis antigens, with individual cells expressing up to four concurrently. These included MAGE-1, -2, and -3, GAGE 1–2, GAGE 1–6, SSX-2, and SSX 1–5 (22). We have recently shown that up to 80% of patients express at least one GAGE isoform. The SEREX technique [serologic analysis of recombinant complementary DNA (cDNA) expression libraries] identifies antitumor antibody responses by using sera from the patients to probe tumor cDNA libraries and identify antigens generating a humoral response. Antigens discovered by SEREX techniques generally also elicit a cellular response (23), and the majority of tumor antigens recognized by this analysis are self antigens, suggesting that reactions occur to immunogenic proteins to which the host is not fully tolerant, as a form of autoimmunity (24). We have identified several tumor antigens in mesothelioma using SEREX (13). Antibodies to topoisomerase IIb were found in 13 of 14 patients, and thus there may be shared tumor antigens in mesothelioma. Although none of the antibodies found in this study were unique to mesothelioma, this finding merely supports the idea that immunogenic tumors more commonly overexpress self antigens rather than novel tumor antigens. As mesotheliomas express tumor antigens, and these antigens can be rec-

ognized by the host, antigen expression and recognition are unlikely to be the limiting factors in the antitumor immune response.

Antigen Presentation and Co-Stimulation

Tumor antigens are presented to $CD8^+$ T cells in the context of the class I MHC molecule. The expression of MHC antigens by tumors may affect antigen presentation to $CD8^+$ T cells and APCs, and influence the subsequent immune response. Although human malignant mesothelioma cell lines express high levels of class I MHC molecules, the tumor cells are poor APCs (25). Antigen-presenting cells, such as DCs, upregulate their expression of co-stimulatory molecules like B7-1 and MHC class II molecules after receiving signals from chemokines and cytokines (GM-CSF, IL-4, TNF-α, IL-1β) and bacterial products (lipopolysaccharide, LPS). Final maturation can then occur after DCs interact with $CD4^+$ T-cell surface molecules (CD40–CD40 ligand interaction) and cytokines, such as IFN-γ, and further co-stimulatory molecules are expressed. While tumors express MHC class I molecules, they rarely express co-stimulatory molecules or secrete cytokines, and thus cannot present antigen in the right context to stimulate a cytotoxic response. The provision of co-stimulation by transfecting B7-1 into tumor has been tried in mesothelioma animal models. Transfecting B7-1 into nonimmunogenic mesothelioma cell lines inhibited tumor growth, and mice also rejected the parent cell line, although rejection was not $CD4^+$ independent. Cytotoxic T cells were effectively generated. In the same system, transfection of tumor with B7-2 did not affect the development of tumors in vivo (26). The murine mesothelioma cell lines used in these experiments were asbestos-induced and are similar in many respects to human malignant mesothelioma (27). These results suggest that transfecting tumor cells with B7 may allow them to present antigen better to effector T cells, probably by increasing antigen delivery via host APCs. This approach could be used clinically in cancer vaccination by transfecting allogeneic tumors. There have been no clinical trials of this approach as yet in mesothelioma.

Nonspecific Immunostimulants

The history of immunotherapy in mesothelioma mirrors its development in other tumor types. In the 1970s, bacillus Calmette-Guérin (BCG) was widely tested as an immunotherapy in many tumor types, including mesothelioma (28). Later, NK cells were discovered, and interleukins, which could stimulate the development of LAK cells, became available as purified recombinants. In the following decade, NK and LAK cell therapy, together with cytokine therapy, became a major focus of research in many malignancies. Natural killer cells are cytotoxic to both virally infected cells and tumor cells. They are able to mediate direct lytic effects on virally infected cells and can also mediate ADCC. Lymphokine-activated killer cells are generated by culturing leukocytes, together with high doses of IL-2 for several days, and IL-2 and the interferons increase the cytotoxicity of this population. They can lyse not only those cells that are susceptible to NK cell lysis, but

also fresh tumor cells that are normally resistant to NK lysis (29). Both fresh mesothelioma cells and cultured cell lines are sensitive to lysis by IL-2–activated LAK cells (30), although they are not destroyed by NK cells. Despite these interesting preclinical observations, LAK cell therapy was toxic in clinical practice and tested only in small numbers of patients with mesothelioma.

Cytokine Therapy

Since the large-scale production of cytokines and interferons became possible, interest in the therapeutic possibilities of these proteins has risen. Following antigen presentation, the cytokine environment is responsible for promoting an optimal Th1-type response or, instead, a less effective Th2-type humoral response. A Th1 response produces IL-2, TNF-α, and IFN-γ, which mediate local inflammation and cytotoxicity, inducing a strong cellular immune response. A Th2 response produces IL-4, IL-5, IL-6, and IL-10, which stimulate B-cell proliferation and subsequent antibody production. Hence, if the tumor cytokine milieu can be changed to promote a Th1 response, antitumor immunity may be generated more effectively. In mesothelioma, the pleural space is accessible to treatment strategies attempting to instill or induce high cytokine levels in the local tumor area, and this tumor rarely metastasizes. Hence, this approach has been exploited in both animal models and patients. A further benefit of intrapleural cytokine administration in this disease is the decrease in systemic side effects of cytokines, which can be severe and even cause mortality. High intratumoral cytokine concentrations can be achieved by either transducing tumor cells to express high cytokine levels using a viral vector (31), or by injecting or infusing the cytokine directly into the tumor (32). The first strategy is discussed later with other methods of gene therapy.

The interferons are cytokines with antiviral activity that have both direct antiproliferative effects and a role in regulation of gene expression. Interferon-α (IFN-α) induces increased cell-surface expression of MHC molecules on both normal and malignant cells, which may increase the susceptibility of malignant cells to immune destruction. Preclinically, the effects of IFN-α on malignant mesothelioma cell lines are variable (33,34), and both human and murine malignant mesothelioma cell lines can be inhibited by IFN-α in animal models, independent of the presence of T cells (35,36). Following these preclinical observations, IFN-α was tried in patients. One of 13 patients treated with systemic IFN-α showed an objective tumor response (37), and intracavitary treatment for malignant pleural effusion from mesothelioma did not give any objective responses (38). In our center, a response rate of 13% was observed from 25 patients treated with systemic therapy (39). Some patients exhibited dramatic responses that lasted from 5 to 13 years, but side effects limit widespread use. Although IFN-α shows additive or synergistic tumor inhibition both in vitro and in vivo when combined with TNF-α (34), IFN-γ, and the cytotoxic drug methotrexate (40), this approach has been difficult in the clinical situation. A combination of doxorubicin and IFN-α

achieved a 16% partial response (PR) rate, but was too toxic for further development (41).

Interferon-γ also shows antiproliferative activity in vitro, alone and in combination with TNF-α (33,34). In vivo effects may be partially mediated through upregulation of MHC expression on mesothelioma cells, improving antigen presentation and hence, acting as a better target for lytic T cells (25). While intrapleural IFN-γ has been tested in the phase II setting, it has shown any significant efficacy only in early-stage disease. Of 12 patients with stage I disease, there were four complete and one partial response. However, only one of 10 patients with stage II disease attained a partial response (42). The combination of IFN-γ and TNF-α was toxic in a phase II study of patients with solid tumors, although one patient with mesothelioma was noted to clear his ascites of malignant cells (43). Interferon-β also inhibits human mesothelioma cell growth in vitro (44). However, it has not produced any responses in patients (45), and was highly toxic. Single-agent interferons have not been developed further for mesothelioma, in part, due to their high toxicity. All patients develop fever, and intrapleural therapy has been complicated by empyema.

Interleukin-2 is an autocrine T-cell growth factor released by these cells after antigen recognition. It is required for survival and proliferation of activated T cells, and production is augmented by CD28 co-stimulation. Without IL-2 and co-stimulation, the T cell may become anergic or die. In addition, in some situations IL-2 can have a direct antiproliferative effect on human malignant mesothelioma cells in vitro, independent of its effects on the cellular immune response (46). The ability of IL-2 to induce activation and expansion of LAK cells led to interest in clinical trials of this cytokine. While in vivo murine mesothelioma growth is inhibited by combination therapy using IL-2 and LAK cells (Manning et al, unpublished data), no responses were seen in a pilot study of intrapleural IL-2/LAK cells in five patients with this disease, and the toxicity of the combination was excessive, even when IL-2 was given intrapleurally (47). There have been several further clinical trials of IL-2 in mesothelioma. A 5-day continuous infusion of intrapleural IL-2 was reported to give an objective response in seven of 15 patients in a phase I study (48), and a follow-up to this study, giving 21×10^6 IU IL-2 daily for 5 days, reported 12 objective responses in 22 patients (55%) and a survival benefit in responding patients (28 months vs. 8 months) (49). Despite this short, high-dose course, little toxicity was reported. Low-dose, daily intrapleural administration of IL-2 (14 days in a 4-week cycle) was not as effective, with four of 21 evaluable patients with stage I/II disease achieving a partial response (50). Intrapleural IL-2 levels were up to 6000-fold greater than systemic levels in this trial, and LAK activity was seen in intrapleural mononuclear cells from all patients. The dose was limited by fever, flu-like symptoms, and catheter infection.

Another interleukin, IL-12, has also been studied in murine and human mesothelioma. Systemic IL-12 could prevent tumor growth in some mice in a murine mesothelioma model, and tumor regression and growth inhibition occurred following intralesional IL-12 injection. This

correlated with an increased intratumoral infiltrate of $CD4^+$ and $CD8^+$ T cells (51). When a murine mesothelioma cell line was transfected with the IL-12 gene, tumor did not grow, and furthermore, paracrine secretion of IL-12 slowed growth of distally implanted tumor. Interleukin 12 has not been tested clinically in this disease.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has numerous actions that may be beneficial to antitumor immunity, including increasing lymphocyte activation, augmenting antigen presentation, stimulating antigen presentation by dendritic cells and macrophages, and enhancing monocyte antitumor activity in vitro. GM-CSF has been transduced into murine tumors, and can give durable, specific antitumor immunity (52,53). In a murine mesothelioma model, transfection of GM-CSF gives a dose-dependent antitumor effect (Davidson JA et al, unpublished observations). Clinically, six patients with stage II disease received intralesional rhGM-CSF infused for 8 weeks via catheter in a pilot clinical trial (32). A lymphocytic tumor infiltrate was seen in one patient who had a partial response, and tumor necrosis occurred in one patient, but only at the catheter site. The technical demands and toxicity of this approach precluded further study.

Immunomodulatory Gene Therapy

As an alternative to giving local or systemic treatment with cytokines, tumors can be transfected with genes for cytokines to induce antitumor immunity through paracrine secretion. Tumors producing IL-2 are heavily infiltrated by lymphocytic cells, whereas IL-4–producing tumors stimulate a massive infiltrate of macrophages and eosinophils but few T cells (54–56). Hence, genes for both these cytokines have been transfected into tumors in an attempt to activate $CD4^+$ T cells or give direct stimulation to $CD8^+$ T cells (reviewed in ref. 57). In animal models, transfection of IL-4 into a tumor cell line both mediates rejection of the transfected cell line and can generate immunity sufficient to reject the untransfected parent cell line, a phenomenon primarily mediated by $CD8^+$ T cells (58). Tumor cells transfected to secrete IL-2 also produced an antitumor response protecting against subsequent challenge with the parental cell line, an effect not requiring $CD4^+$ T cells (59). Other cytokines that have been similarly studied in vivo include IFN-γ, IFN-α, TNF-α, GM-CSF, IL-6, and IL-1β (60; reviewed in ref. 57). Our group has transfected a series of cytokine genes into malignant mesothelioma cell lines. Tumors transfected with IL-2, IL-4, IL-12, or GM-CSF were all rejected in vivo in a murine mesothelioma model (61,62). Although all transfected cell lines were rejected, rejection of the parental cell line was not seen for any of these cell lines (62), suggesting that the clinical utility of this approach may be limited. However, the combination of debulking surgery and cytokine gene therapy can reduce the rate of tumor growth in an experimental untransfected metastasis (63). Those transfectants expressing B7.1 or producing high GM-CSF levels were most effective at reducing tumor growth rate, while those producing IL-2 and IL-4 did not show much

growth delay. This combination of debulking surgery and cytokine gene therapy has not yet been tested clinically.

In patients, gene therapy has been explored as a way of delivering cytokines in order to circumvent some of the problems of infusional cytokine treatment; it may enable the cytokine to be continuously produced, in high levels, and in the correct location. In reality, however, the ideal vector to fulfill this promise has not yet been identified. Our group has studied vaccinia virus (VV) as a vector for cytokine gene transfer in malignant mesothelioma (31). The IL-2 gene was inserted into the TK region of the vaccinia virus, which then became replication deficient. Six patients received one to three weekly intratumoral injections of vaccinia virus–IL-2 (VV–IL-2), receiving up to 10^7 plaque-forming units (pfu) per injection. Previous studies suggested that vaccinia virus was immunogenic and hence, unlikely to maintain long-term expression of the transfected cytokine. The VV IgG titers did increase over the treatment course; however, tumor biopsies showed prolonged, although low level, intratumoral expression of VV–IL-2 mRNA. Virus was neither excreted nor transmitted to contacts. Although there were no tumor responses, 50% of injection-site biopsies showed T-cell infiltration. We are continuing to investigate cytokine gene transfection in animal models of mesothelioma. Nevertheless, it is debatable whether continuous cytokine production, as occurs with successful delivery of gene therapy, is the ideal schedule for cytokine administration. Data from animal models have shown that continuous infusion of IL-2 is less effective in producing tumor remission than intermittent injections (van Bruggen et al, unpublished data).

Mesothelioma Vaccines

Cancer vaccines aim to stimulate a specific immune response in the tumor host. For a vaccine to have any degree of success, the patient must have an intact cell-mediated immune system, and the tumor must bear antigens that are recognizable and can generate an immune response. This therapeutic strategy is most likely to succeed when the tumor burden is low (adjuvant therapy following chemotherapy or surgery) or as a low-toxicity prophylaxis in high-risk populations, such as those exposed to high levels of asbestos. The tumor antigen does not need to be identified in order to use vaccination as a treatment strategy. When the tumor does not express known antigens, autologous whole tumor cells or tumor lysates may be used or, less attractive, allogeneic cells. Production of tumor lysates can expose intracellular proteins, which may encourage a more immunogenic mode of presentation. Autologous tumor can be surgically obtained and then inactivated by enzymatic digestion, freezing, thawing, and then irradiation. Allogeneic tumor can be obtained from one or several cell lines that can be mixed and irradiated. The success of allogeneic tumor vaccines requires shared tumor antigens between the autologous tumor and the patient's tumor. If shared tumor antigens have already been identified, antigenic peptides or proteins can be manufactured, or APCs transfected with genes for tumor antigens. An example of the

identification and use of a possible shared tumor antigen as a vaccine in mesothelioma is the use of vaccinia virus transfected with a modified simian virus 40 (SV40) T antigen (64). This vaccine has been tested in animal models and found effective against tumor challenge and also established tumors. It has not been tested clinically in this disease.

The availability of the right antigen is important, but without the appropriate context of antigen presentation, anergy, ignorance, or clonal deletion may occur in the specific T-cell population. The "danger theory" suggests that signals from inflammation or tissue destruction may be necessary for appropriate antigen presentation (65). The absence of danger may be why some antigen-bearing tumors do not stimulate an immune response. A successful vaccine must be able to either break tolerance or activate T cells of different specificities, such as to low affinity or cryptic antigens. One method of increasing the immunogenicity of vaccines is to use an "adjuvant," which may be nonspecific [BCG, Detox (Ribi Immunochem Research, Hamilton, MT), or the glycoside QS-21]. Lipopolysaccharide and CpGs, which bind toll-like receptors, are also excellent adjuvants (66). Immunostimulatory cytokines, such as GM-CSF, are effective adjuvants (67), and the genes for GM-CSF or other cytokines and co-stimulatory molecules can be transduced into tumor cells prior to vaccination (56), although this approach is not really technically feasible for autologous vaccination. Autologous tumor may be more readily mixed with microspheres containing cytokines or with inert bystander cells previously transduced with the gene of interest (68). In murine models, tumors transfected with the gene for GM-CSF can protect from tumor development and further challenge with the untransfected parent cell line (53). The use of adjuvant GM-CSF has been tried clinically in colon and prostate cancer and melanoma. We are currently conducting a pilot study of autologous vaccination in malignant mesothelioma. Tumor is removed by resection or thoracoscopically, and then inactivated by freeze-thawing and subsequent irradiation. Tumor cells are mixed with soluble GM-CSF, and injected subcutaneously, fortnightly for 3 months. There is an initial lead-in period of 9 weeks in order to monitor baseline antitumor immune responses. Although tumor response and survival will be monitored, the primary aim of this pilot study is to demonstrate an immune response. This is assessed using Western blotting and enzyme-linked immunosorbent assay (ELISA) of serial serum samples, together with delayed-type hypersensitivity (DTH) skin testing using lysed irradiated autologous tumor cells. To date, eight patients have completed the trial. Two patients had a positive initial DTH skin test, and two further patients have subsequently developed positive DTH tests. One patient had a positive initial Western blot, and one patient has developed a positive Western blot after vaccination. Toxicity has been acceptable, with no hospital admission related to vaccination treatment. Median survival from diagnosis to date is 12 months, with four of eight patients remaining alive. The longest surviving patient is alive 25 months postdiagnosis, and this patient had an initial positive DTH skin test, which has remained positive throughout the vaccination program. The

development of tumor-specific immunity in some patients is encouraging, and the trial is ongoing.

Tumor Vaccine with Suicide Gene Therapy

A further approach currently under investigation is the use of allogeneic irradiated ovarian cancer cells that have been transduced with the gene for herpes simplex virus-1 thymidine kinase (HSV*tk*) (PA-1-STK cells). "Vaccination" with these cells is followed by systemic treatment with ganciclovir. In vitro, these cells are capable of killing both murine and human mesothelioma cell lines, and when used in vivo, improved survival in a murine model of mesothelioma (69). In human trials, radiolabeled PA-1-STK cells have been instilled into the pleural cavity of mesothelioma patients, and can travel to an intrapleural tumor (70). This system is now being tried in a phase I setting in conjunction with ganciclovir treatment. It is hoped that a "bystander" effect will kill neighboring mesothelioma cells (71). The concomitant production of proinflammatory cytokines such as IL-2 and TNF-α, may also induce a more immunostimulatory tumor microenvironment (71). A clinical trial has been reported, with six patients treated with up to 1×10^8 PA-1-STK cells alone, and a further three patients receiving intrapleural PA-1-STK cells, followed by ganciclovir treatment. In the results reported to date, increased CD8 T lymphocytes have been shown to enter the pleural fluid after this treatment, and no alloreactivity has been reported (71).

Chemoimmunotherapy

Until recently, there has been no "gold standard" for chemotherapy in the treatment of mesothelioma. This has opened possibilities for investigational treatments combining chemotherapy and immunotherapy. Much of this work has been tried directly in patients with little preclinical exploration of the combination in models of mesothelioma. It has been hypothesized that the inflammation induced by an antecedent tumor vaccine may disrupt tumor architecture, thus possibly increasing the effect of cytotoxic drugs. However, there have also been theoretical concerns that chemotherapy may be detrimental to antigen-specific antitumor immunity, due to its toxic effects on dividing lymphocytes.

We have been examining the effects of chemotherapy on antigen-specific antitumor immunity and combination chemoimmunotherapy in a murine model of mesothelioma. The cell line used was generated from asbestos inoculation into the peritoneal cavity of mice, and has subsequently been transfected with the hemagglutinin antigen (HA) as a tumor "neoantigen" (72). The immune response can then be followed in vivo using, among other tools, T-cell receptor transgenic mice with $CD4^+$ and $CD8^+$ T cells with specificity for this antigen. We have shown that gemcitabine chemotherapy decreases humoral immunity and inhibits B-cell proliferation (73). However, gemcitabine appears to have a priming effect on cell-mediated immunity, increasing cross-

presentation, T-cell proliferation to specific tumor antigens, and tumor lymphocyte infiltration (74). Immunotherapy with a virus expressing HA is more effective when given after a full course of gemcitabine chemotherapy than when given alone. Furthermore, treatment of established murine mesothelioma using gemcitabine, followed by immunotherapy with an activating anti-CD40 antibody, results in cures of between 40% and 80% of mice. Alternative schedules using immunotherapy prior to or during chemotherapy are not effective (75). CD40 activation alone has not been studied in mesothelioma; however, this strategy was ineffective in clinical trials in other cancers (76) as well as alone in murine mesothelioma (75). Nevertheless, it would be interesting to perform a clinical trial of this combination in mesothelioma, particularly in a setting of minimal residual disease, such as the post-surgical period.

In clinical trials, combining chemotherapy with cytokines has been most widely explored. A trial showing some minor activity of IFN-α alone (39) was followed by a trial of IFN-α together with doxorubicin (41). Four of 25 patients treated (16%) had a partial response to treatment, a response rate no different from that to either agent alone. However, the regimen was unacceptably toxic. Interferon-α was also combined with mitomycin C and cisplatin, following encouraging preclinical data (77,78). This was a nonrandomized two-arm study, comparing 28-day cycles of IV cisplatin and mitomycin and subcutaneous IFN-α with a group receiving best supportive care. There was no difference in survival between the two groups, and six of 43 patients (14%) had a partial or complete response. Interferon-α and cisplatin have been tested together in a combination that was toxic but gave partial responses in 10 of 25 patients (40%) (79). A subsequent study by the same investigators gave a 50% higher dose of IFN-α, which was not more efficacious (PR 26%) and was too toxic to pursue further (80). The response rate was not further improved by the addition of mitomycin C or IL-2 (81). The group performing these trials has now abandoned this approach in mesothelioma in favor of combination raltitrexed and oxaliplatin. Finally, IFN-α and carboplatin have been tried together with a partial response rate of 7%, no different from that of carboplatin alone (82).

A trial of chemoimmunotherapy has used a different approach. SRL172 is a suspension of heat-killed *Mycobacterium vaccae*, which has been used in combination with mitomycin C, vinblastine, and cisplatin (83). Theoretically, when chemotherapy kills tumor cells, tumor-specific or tumor-associated antigens may be released, and this chemotherapy effect, in combination with the nonspecific stimulant of SRL172, could lead to improved antigen recognition. Nine trial patients had mesothelioma, and of these, two of four patients in the combination therapy group had a partial response, compared with one of five in the chemotherapy-only group. Newer, more active chemotherapy combinations, together with appropriate scheduling of chemotherapy and immunotherapy on the basis of preclinical information, should lead to improved efficacy of chemoimmunotherapy combinations.

Conclusions and Future Directions

There has been much recent progress in the understanding of the immunology of malignant mesothelioma, particularly in identification of shared tumor antigens and in tumor models of the disease expressing neoantigens. However, gene therapy, immunotherapy, and cancer vaccination are yet to be established as conventional therapies. As more effective chemotherapy is becoming available (1–3), we may no longer be able to treat patients on clinical trials of immunotherapies as first-line treatment, although as yet there is no evidence that early chemotherapy is more beneficial for survival than delayed chemotherapy. Patients will be presenting for experimental therapies in the context of chemotherapy failure, together with chemotherapy, or in a postsurgical setting. Traditional markers of response may be obscured, emphasizing the need to develop alternative biomarkers of efficacy that can be used at an earlier stage of disease. Techniques, such as microarray analysis, may help us to predict which patients are likely to respond to treatments such as chemotherapy or immunotherapy. The principles of advances in immunotherapy in other malignancies can be applied to mesothelioma, at least to generate hypotheses and suggest new therapies for trial in animal models. It seems clear that single modalities of conventional therapies will not be successful alone, and we must look at combinations of treatments that include novel agents.

Acknowledgments

The authors would like to thank our colleagues in the Tumor Immunology Group, University Department of Medicine, University of Western Australia, for providing us with their unpublished data. A.K.N. is the recipient of an Eva K.A. Nelson scholarship from the University of Western Australia.

References

1. Byrne MJ, Davidson JA, Musk AW, et al. Cisplatin and gemcitabine treatment for malignant mesothelioma: a phase II study. J Clin Oncol 1999;17(1): 25–30.
2. Nowak AK, Byrne MJ, Williamson R, et al. A multicentre phase II study of cisplatin and gemcitabine for malignant mesothelioma. Br J Cancer 2002; 87(5):491–496.
3. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2002;21(14):2636–2644.
4. Di Carlo E, Forni G, Lollini P, Colombo MP, Modesti A, Musiani P. The intriguing role of polymorphonuclear neutrophils in antitumor reactions. Blood 2001;97(2):339–345.
5. Kindzelskii AL, Petty HR. Early membrane rupture events during neutrophil-mediated antibody-dependent tumor cell cytolysis. J Immunol 1999;162(6):3188–3192.

6. Robinson BW, Lake RA, Nelson DJ, Scott BA, Marzo AL. Cross-presentation of tumour antigens: evaluation of threshold, duration, distribution and regulation. Immunology Cell Biol 1999;77(6):552–558.
7. Marzo AL, Lake RA, Lo D, et al. Tumor antigens are constitutively presented in the draining lymph nodes. J Immunol 1999;162(10):5838–5845.
8. Creaney J, McLaren B, Stevenson S, et al. P53 autoantibodies in patients with malignant mesothelioma: stability through disease progression. Br J Cancer 2001;84:52–56.
9. Pardoll DM, Topalian SL. The role of $CD4^+$ T cell responses in antitumor immunity. Curr Opin Immunol 1998;10(5):588–594.
10. Hellstrom KE, Hellstrom I. Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. Adv Immunol 1974;18:209–277.
11. Qin Z, Richter G, Schuler T, Ibe S, Cao X, Blankenstein T. B cells inhibit induction of T cell-dependent tumor immunity. Nature Med 1998;4(5):627–630.
12. DeSilva DR, Urdahl KB, Jenkins MK. Clonal anergy is induced in vitro by T cell receptor occupancy in the absence of proliferation. J Immunol 1991;147(10):3261–3267.
13. Robinson C, Callow M, Stevenson S, Scott B, Robinson BW, Lake RA. Serologic responses in patients with malignant mesothelioma: evidence for both public and private specificities. Am J Respir Cell Mol Biol 2000;22(5):550–556.
14. Leigh RA, Webster I. Lymphocytic infiltration of pleural mesothelioma and its significance for survival. South Afr Med J 1982;61(26):1007–1009.
15. Robinson BW, Robinson C, Lake RA. Localized spontaneous mesothelioma regression—possible immunological mechanism. Lung Cancer 2001.
16. Haslam PL, Lukoszek A, Merchant JA, Turner-Warwick M. Lymphocyte responses to phytohaemagglutinin in patients with asbestosis and pleural mesothelioma. Clin Exp Immunol 1978;31(2):178–188.
17. Manning LS, Davis MR, Robinson BW. Asbestos fibres inhibit the in vitro activity of lymphokine-activated killer (LAK) cells from healthy individuals and patients with malignant mesothelioma. Clin Exp Immunol 1991;83(1):85–91.
18. Garlepp M, et al. Mesothelioma: recent studies of growth regulation. In: Sourcebook on Asbestos Diseases: Asbestos Medical Research. New York: Garland Publishing, 1992.
19. Lew F, Tsang P, Holland JF, Warner N, Selikoff IJ, Bekesi JG. High frequency of immune dysfunctions in asbestos workers and in patients with malignant mesothelioma. J Clin Immunol 1986;6(3):225–233.
20. Robinson B. Asbestos and cancer: human natural killer cell activity is suppressed by asbestos fibres but can be restored by recombinant interleukin-2. Am Rev Respir Dis 1989;139:897–901.
21. Bielefeldt-Ohmann H, Jarnicki AG, Fitzpatrick DR. Molecular pathobiology and immunology of malignant mesothelioma. J Pathol 1996;178(4):369–378.
22. Sigalotti L, Coral S, Altomonte M, et al. Cancer testis antigens expression in mesothelioma: role of DNA methylation and bioimmunotherapeutic implications. Br J Cancer 2002;86(6):979–982.
23. Jager E, Chen YT, Drijfhout JW, et al. Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J Exp Med 1998;187(2):265–270.
24. Naftzger C, Takechi Y, Kohda H, Hara I, Vijayasaradhi S, Houghton AN. Immune response to a differentiation antigen induced by altered antigen:

a study of tumor rejection and autoimmunity. Proc Natl Acad Sci USA 1996;93(25):14809–14814.
25. Christmas TI, Manning LS, Davis MR, Robinson BW, Garlepp MJ. HLA antigen expression and malignant mesothelioma. Am J Respir Cell Mol Biol 1991;5(3):213–220.
26. Leong CC, Marley JV, Loh S, Milech N, Robinson BW, Garlepp MJ. Transfection of the gene for B7-1 but not B7-2 can induce immunity to murine malignant mesothelioma. Int J Cancer 1997;71(3):476–482.
27. Davis MR, Manning LS, Whitaker D, Garlepp MJ, Robinson BW. Establishment of a murine model of malignant mesothelioma. Int J Cancer 1992; 52(6):881–886.
28. Webster I, Cochrane JW, Burkhardt KR. Immunotherapy with BCG vaccine in 30 cases of mesothelioma. South Afr Med J 1982;61(8):277–278.
29. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med 1982;155(6):1823–1841.
30. Manning LS, Bowman RV, Darby SB, Robinson BW. Lysis of human malignant mesothelioma cells by natural killer (NK) and lymphokine-activated killer (LAK) cells. Am Rev Respir Dis 1989;139(6):1369–1374.
31. Mukherjee S, Haenel T, Himbeck R, et al. Replication-restricted vaccinia as a cytokine gene therapy vector in cancer: persistent transgene expression despite antibody generation. Cancer Gene Therapy 2000;7(5):663–670.
32. Davidson JA, Musk AW, Wood BR, et al. Intralesional cytokine therapy in cancer: a pilot study of GM-CSF infusion in mesothelioma. J Immunother 1998;21(5):389–398.
33. Bowman RV, Manning LS, Davis MR, Robinson BW. Chemosensitivity and cytokine sensitivity of malignant mesothelioma. Cancer Chemother Pharmacol 1991;28(6):420–426.
34. Hand AM, Husgafvel-Pursiainen K, Tammilehto L, Mattson K, Linnainmaa K. Malignant mesothelioma: the antiproliferative effect of cytokine combinations on three human mesothelioma cell lines. Cancer Lett 1991;58(3): 205–210.
35. Suzuki Y, Chahinian AP, Ohnuma T. Comparative studies of human malignant mesothelioma in vivo, in xenografts in nude mice, and in vitro. Cell origin of malignant mesothelioma. Cancer 1987;60(3):334–344.
36. Bielefeldt-Ohmann H, Fitzpatrick DR, Marzo AL, Jarnicki AG, Musk AW, Robinson BW. Potential for interferon-alpha-based therapy in mesothelioma: assessment in a murine model. J Interferon Cytokine Res 1995;15(3): 213–223.
37. Ardizzoni A, Pennucci MC, Castagneto B, et al. Recombinant interferon alpha-2b in the treatment of diffuse malignant pleural mesothelioma. Am J Clin Oncol 1994;17(1):80–82.
38. Lissoni P, Barni S, Ardizzoia A, Paolorossi F, Tisi E, Crispino S, Tancini G. Intracavitary administration of interleukin-2 as palliative therapy for neoplastic effusions. Tumori 1992;78(2):118–120.
39. Christmas TI, Manning LS, Garlepp MJ, Musk AW, Robinson BW. Effect of interferon-alpha 2a on malignant mesothelioma. J Interferon Res 1993;13(1): 9–12.
40. Hand A, Pelin K, Mattson K, Linnainmaa K. Interferon (IFN)-alpha and IFN-gamma in combination with methotrexate: in vitro sensitivity studies in four human mesothelioma cell lines. Anticancer Drugs 1995;6(1):77–82.
41. Upham JW, Musk AW, van HG, Byrne M, Robinson BW. Interferon alpha and doxorubicin in malignant mesothelioma: a phase II study. Aust N Z J Med 1993;23(6):683–687.

42. Boutin C, Viallat JR, Van ZN, et al. Activity of intrapleural recombinant gamma-interferon in malignant mesothelioma. Cancer 1991;67(8):2033–2037.
43. Smith JW, Urba WJ, Clark JW, et al. Phase I evaluation of recombinant tumor necrosis factor given in combination with recombinant interferon-gamma. J Immunother 1991;10(5):355–362.
44. Von Hoff DD, Huong AM. Effect of recombinant interferon-beta ser on primary human tumor colony-forming units. J Interferon Res 1988;8(6): 813–820.
45. Von Hoff D, Metch B, Lucas JG, Balcerzak SP, Grunberg SM, Rivkin SE. Phase II evaluation of recombinant interferon-beta (IFN-beta ser) in patients with diffuse mesothelioma: a Southwest Oncology Group study. J Interferon Res 1990;10(5):531–534.
46. Porta C, Danova M, Orengo AM, et al. Interleukin-2 induces cell cycle perturbations leading to cell growth inhibition and death in malignant mesothelioma cells in vitro. J Cell Physiol 2000;185(1):126–134.
47. Robinson B, Bowman R, Manning L, Musk A, Van Hazel G. Interleukin-2 and lymphokine activated killer cells in malignant mesothelioma. Eur Respir Rev 1993;3(11):220–222.
48. Astoul P, Viallat JR, Laurent JC, Brandely M, Boutin C. Intrapleural recombinant IL-2 in passive immunotherapy for malignant pleural effusion. Chest 1993;103(1):209–213.
49. Astoul P, Picat-Joossen D, Viallat JR, Boutin C. Intrapleural administration of interleukin-2 for the treatment of patients with malignant pleural mesothelioma: a phase II study. Cancer 1999;83(10):2099–2104.
50. Goey SH, Eggermont AM, Punt CJ, et al. Intrapleural administration of interleukin 2 in pleural mesothelioma: a phase I–II study. Br J Cancer 1995; 72(5):1283–1288.
51. Caminschi I, Venetsanakos E, Leong CC, Garlepp MJ, Scott B, Robinson BW. Interleukin-12 induces an effective antitumor response in malignant mesothelioma. Am J Respir Cell Mol Biol 1998;19(5):738–746.
52. Colombo MP, Ferrari G, Stoppacciaro A, et al. Granulocyte colony-stimulating factor gene transfer suppresses tumorigenicity of a murine adenocarcinoma in vivo. J Exp Med 1991;173(4):889–897.
53. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 1993;90(8):3539–3543.
54. Tepper RI, Coffman RL, Leder P. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. Science 1992;257(5069):548–551.
55. Tepper RI, Pattengale PK, Leder P. Murine interleukin-4 displays potent anti-tumor activity in vivo. Cell 1989;57(3):503–512.
56. Pardoll DM. Paracrine cytokine adjuvants in cancer immunotherapy [review]. Ann Rev Immunol 1995;13:399–415.
57. Parmiani G, Colombo MP, Melani C, Arienti F. Cytokine gene transduction in the immunotherapy of cancer [review]. Adv Pharmacol (New York) 1997; 40:259–307.
58. Golumbek PT, Lazenby AJ, Levitsky HI, et al. Treatment of established renal cancer by tumor cells engineered to secrete interleukin-4. Science 1991;254(5032):713–716.
59. Fearon ER, Pardoll DM, Itaya T, et al. Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. Cell 1990;60(3):397–403.
60. Gansbacher B, Bannerji R, Daniels B, Zier K, Cronin K, Gilboa E. Retroviral vector-mediated gamma-interferon gene transfer into tumor cells gen-

erates potent and long lasting antitumor immunity. Cancer Res 1990;50(24): 7820–7825.
61. Leong CC, Marley JV, Loh S, Robinson BW, Garlepp MJ. The induction of immune responses to murine malignant mesothelioma by IL-2 gene transfer. Immunol Cell Biol 1997;75(4):356–359.
62. Caminschi I, Venetsanakos E, Leong CC, Garlepp MJ, Robinson BW, Scott B. Cytokine gene therapy of mesothelioma. Immune and antitumor effects of transfected interleukin-12. Am J Respir Cell Mol Biol 1999;21(3):347–356.
63. Mukherjee S, Nelson D, Loh S, et al. The immune anti-tumor effects of GM-CSF and B7-1 gene transfection are enhanced by surgical debulking of tumor. Cancer Gene Ther 2001;8(8):580–588.
64. Imperiale MJ, Pass HI, Sanda MG. Prospects for an SV40 vaccine. Semin Cancer Biol 2001;11(1):81–85.
65. Matzinger P. An innate sense of danger. Semin Immunol 1998;10(5):399–415.
66. Medzhitov R, Janeway C. The Toll receptor family and microbial recognition [review]. Trends Microbiol 2000;8(10):452–456.
67. Simons JW, Mikhak B, Chang JF, et al. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. Cancer Res 1999;59(20):5160–5168.
68. Pardoll DM. Cancer vaccines [review]. Nature Med 1998;4(5:suppl):31.
69. Schwarzenberger P, Lei D, Freeman SM, et al. Antitumor activity with the HSV-tk-gene-modified cell line PA-1-STK in malignant mesothelioma. Am J Respir Cell Mol Biol 1998;19(2):333–337.
70. Harrison LH Jr, Schwarzenberger PO, Byrne PS, Marrogi AJ, Kolls JK, McCarthy KE. Gene-modified PA1-STK cells home to tumor sites in patients with malignant pleural mesothelioma. Ann Thorac Surg 2000; 70(2):407–411.
71. Schwarzenberger P, Harrison L, Weinacker A, et al. The treatment of malignant mesothelioma with a gene modified cancer cell line: a phase I study. Human Gene Ther 1998;9(17):2641–2649.
72. Marzo AL, Lake RA, Robinson BW, Scott B. T-cell receptor transgenic analysis of tumor-specific CD8 and CD4 responses in the eradication of solid tumors. Cancer Res 1999;59(5):1071–1079.
73. Nowak AK, Robinson BW, Lake RA. Gemcitabine exerts a selective effect on the humoral immune response: implications for combination chemo-immunotherapy. Cancer Res 2002;62(8):2353–2358.
74. Nowak AK, Lake RA, Marzo AL, et al. Induction of tumor cell apoptosis in vivo increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T-cells. J Immunol 2003; 170(10):4905–4913.
75. Nowak AK, Robinson BWS, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003;63(15):4490–4496.
76. Vonderheide RH, Dutcher JP, Anderson JE, et al. Phase I study of recombinant human CD40 ligand in cancer patients. J Clin Oncol 2001;19(13): 3280–3287.
77. Sklarin NT, Chahinian AP, Feuer EJ, Lahman LA, Szrajer L, Holland JF. Augmentation of activity of cis-diaminedichloroplatinum(II) and mitomycin C by interferon in human malignant mesothelioma xenografts in nude mice. Cancer Res 1988;48(1):64–67.

78. Metintas M, Ozdemir N, Ucgun I, et al. Cisplatin, mitomycin, and interferon-alpha2a combination chemoimmunotherapy in the treatment of diffuse malignant pleural mesothelioma. Chest 1999;116(2):391–398.
79. Soulie P, Ruffie P, Trandafir L, et al. Combined systemic chemoimmunotherapy in advanced diffuse malignant mesothelioma. Report of a phase I–II study of weekly cisplatin/interferon alfa-2a. J Clin Oncol 1996; 14(3):878–885.
80. Trandafir L, Ruffie P, Borel C, et al. Higher doses of alpha-interferon do not increase the activity of the weekly cisplatin-interferon combination in advanced malignant mesothelioma. Eur J Cancer 1997;33(11):1900–1902.
81. Fizazi K, Caliandro R, Soulie P, et al. Combination raltitrexed (Tomudex(R))-oxaliplatin: a step forward in the struggle against mesothelioma? The Institut Gustave Roussy experience with chemotherapy and chemo-immunotherapy in mesothelioma. Eur J Cancer 2000;36(12):1514–1521.
82. O'Reilly EM, Ilson DH, Saltz LB, Heelan R, Martin L, Kelsen DP. A phase II trial of interferon alpha-2a and carboplatin in patients with advanced malignant mesothelioma. Cancer Invest 1999;17(3):195–200.
83. O'Brien ME, Saini A, Smith IE, et al. A randomized phase II study of SRL172 (Mycobacterium vaccae) combined with chemotherapy in patients with advanced inoperable non-small-cell lung cancer and mesothelioma. Br J Cancer 2000;83(7):853–857.

54

Economic Aspects of Mesothelioma

Joyce A. Lagnese

The economic aspects of mesothelioma may be summed up in one word: enormous. These impacts over the past few decades and into the foreseeable future may be measured in many billions of dollars, which represents a major transfer of wealth in our economy. These economic aspects have resulted in the creation and destruction of entire industries, and the impact has been felt by many people and throughout wide sectors of society. The most immediate impact is on the victims and their families, who have been forced to cope with the devastating effects of the disease. The best documented impact is the costs associated with asbestos litigation. Asbestos has been the most litigated mass tort in American history. It has been variously referred to as an "elephantine mess" (1) and a "disaster of major proportions to both the victims and producers of asbestos products" (2, p. 2). The abatement of asbestos in buildings has also had a major impact on American society. The economic dislocation caused by bankruptcy has also been significant. Although mesothelioma affects people worldwide, this chapter focuses on its impact in the United States.

Relationship of Mesothelioma to Asbestos in General

The economic aspects of mesothelioma are often difficult to separate from the economic aspects of asbestos in general. Mesothelioma has conventionally been thought of in the law as a "signature" disease caused exclusively by exposure to asbestos (3). Yet other diseases, such as asbestosis and certain forms of other cancers, are also recognized as resulting from or associated with asbestos exposure. This makes it difficult to utilize conventional measuring techniques to isolate economic aspects unique to mesothelioma. Where this isolation is possible, the information is presented below. In general, however, the economic impact of mesothelioma must often be roughly extrapolated from information relating to asbestos generally.

Economic Value of Asbestos Litigation

Asbestos litigation involves huge amounts of money. So far, defendant companies have paid approximately $54 billion in claims and related costs, and estimates for future liability have ranged from $145 billion to $210 billion (4, p. vi). Some of the more extreme examples of verdicts in asbestos cases include the following:

$55 million in one case (later reduced to $2.75 million) (5)
$150 million to five workers, including $60 million in punitive damages (4, p. 59)
$150 million to six workers with asbestos claims (the local media reported that none of the six plaintiffs had actually developed asbestosis or mesothelioma) (4, p. 59)

Although the percentage of mesothelioma cases is small (approximately 3%) compared to the total of asbestos cases, mesothelioma cases play a very prominent role in asbestos litigation and represent roughly 17% of the allocation of compensation. A mesothelioma case is generally regarded as of very high economic value. The average plaintiff in a mesothelioma case currently receives a verdict in excess of $6 million. When this is multiplied by the number of pending mesothelioma cases, the amount is phenomenal. Compensation for mesothelioma claims has also been rising sharply in recent years. The values of mesothelioma cases are often the "drivers" for increasing the economic impact of asbestos litigation in general. This phenomenon has a ripple effect throughout the various features of asbestos litigation.

History of Asbestos Litigation

Asbestos litigation began in the late 1960s and early 1970s in the wake of the seminal studies of Selikoff of Mount Sinai Hospital in New York City, establishing a link between asbestos exposure and disease. Most of the early claimants were insulators and other asbestos workers who brought product liability suits against asbestos product manufacturers. These suits were aided by documents suggesting that certain defendants suppressed information relating to the dangers of asbestos. Perhaps the most prominent defendant in the early years was the Johns-Manville Corp., a leading manufacturer of insulation materials. The litigation continued throughout the next three decades involving an ever-expanding network of plaintiffs and defendants. Damages awards became higher. Many defendants could no longer stand the financial strain. Johns-Manville declared bankruptcy in 1982. By 2002, it was joined in bankruptcy by over 59 other defendants (4, p. 71). The list of corporate bankruptcies includes such well-known names as W.R. Grace & Co., Babcox & Wilcox, Pittsburgh Corning Corp., Owens Corning Corp., and Armstrong World Industries. Some commentators had predicted that the pool of plaintiffs would decrease at the turn of

the century since most asbestos-containing products were removed from the market in the 1970s. Yet the litigation proceeds unabated. No accurate prognosis or end is in sight.

Sources of Compensation

Every dollar attributable to mesothelioma travels a very tortuous path. Substantial payments for asbestos disease come from various private or governmental benefits programs. The most documented asbestos payments are those that come from personal injury claims. When these claims first began to accumulate and were submitted for insurance coverage, a series of disputes arose between policyholders and insurers over the extent of such coverage. One of the major issues involved the time period when coverage was "triggered": when the disease was created or when it was manifested? Given the long latency periods, this question put many years of coverage in controversy, with enormous economic consequences. These types of issues spawned a decade of litigation. The cases were decided mostly in favor of coverage in the 1980s and early 1990s. Most of the financing for mesothelioma and other asbestos liability have thus come from primary, excess, and reinsurance assets. This has had a significant impact on the insurance industry, both in the United States and abroad. United States insurance companies had spent about $21.6 billion on asbestos claims through 2000 (4, p. 54). The crisis in the Lloyds insurance market in London in the late 1980s has been substantially attributed to American asbestos liabilities.

When insurance is unavailable, the assets for recovery come from the individual corporate resources of the defendants. When defendants declare bankruptcy, a separate mechanism can be created for the financing of asbestos liability depending on the particular circumstances of the defendant. A good example is the so-called Manville Trust, which was created out of the assets of the company and exists for the sole purpose of providing compensation. There has been some controversy over the extent to which the purposes of some corporate restructuring has been to avoid asbestos liability (6). Occasionally, companies have emerged in some form from bankruptcy to rejoin the list of defendants.

The Manville Personal Injury Settlement Trust was the first example of this latter process. The trust was established in 1986, four years after Johns-Manville Corp. filed a petition for reorganization under Chapter 11 of the bankruptcy code (7). In November 1989, the United States Bankruptcy Court for the Southern District of New York approved the Manville Trust. The trust was established and organized in a fashion that would provide settlements to those injured by their exposure to asbestos, while limiting the need for litigation through aggressive settlement tactics. To date, the trust has paid out over $2.9 billion to approximately 500,000 claimants. There are currently a number of firms attempting to follow the lead of Johns-Manville in emergence from bankruptcy.

Measures of Damages

In a personal injury asbestos case, the conventional measures of damages normally include medical expenses (so-called special damages), pain and suffering, the value of loss of life, loss of consortium of the victim's spouse, and punitive damages when the defendant's actions are found to be egregious. More controversial damages questions involve compensation for fear of cancer, for the risk that one might become ill in the future, or for medical monitoring. In some cases, the medical bills may be paid through the medical insurance coverage of the plaintiff and there are other sources of compensation, such as government benefits, workers' compensation, and the personal resources of the victims. Frequently, however, these items of damages become issues in litigation. In the case of an abatement question, the issue of economics normally arises in the form of a governmental abatement directive sometimes followed by property damage litigation.

Volume of Cases

Perhaps the single most influential feature of asbestos litigation is the large volume of cases. From the 1970s to the present day, an estimated 600,000 cases have been filed in the courts of the United States. According to testimony in Congress, there were an estimated 200,000 cases pending in 1999, with 20,000 to 50,000 new cases filed every year (8). Between 1993 and 1999, the number of pending cases nationwide has doubled. Filings have increased to more than 90,000 in 2001, compared with 20,000 in the early part of the decade. Predictions for the filing of future claims have ranged from a few hundred thousand to over 2 million (4, p. 46).

The number of mesothelioma cases has also been on the rise. However, the number of such cases has not grown as rapidly as other asbestos cases, and mesothelioma cases are decreasing as a percentage of the whole (4, p. 46). In the early 1970s, mesothelioma cases accounted for roughly 10% of all claims. This number had fallen to about 5% by the late 1970s, remaining at about that level through the 1980s. Beginning in the late 1980s, the percentage of mesothelioma cases fell even further. Through the 1990s and continuing today, mesothelioma cases account for only 3% to 4% of all asbestos-related claims. As noted above, compensation received by mesothelioma claimants is typically higher than that received by those with other asbestos-related claims. While the mean award for asbestosis increased nearly fivefold from $1 million in 1999 to $5 million in 2001, the mean award for mesothelioma claims rose dramatically as well. In 1998, the mean mesothelioma verdict was $2 million. This number increased to over $6 million in 2001. Even though some of the staggeringly large awards may have been reduced by remittitur or on appeal, they reverberate through the litigation generally and raise the overall costs of mesothelioma dramatically.

Asbestos was so widely used in society that its effects were felt by hundreds of thousands of people, who have relatively easy access to the civil justice system. It is common for lawyers working in tandem with unions and certain physicians to organize mass lung screenings of workers for the purpose of collecting large numbers of plaintiffs. Plaintiffs are also actively solicited through advertising and other means. The Internet is filled with advertisements for mesothelioma lawyers. Claimants are attracted to the system by the prospect of substantial recoveries. This phenomenon has been felt unevenly throughout the country. As might be expected, large concentrations of cases have occurred in areas where asbestos usage was more common, such as in shipyards, power plants, and refineries. Concentration of cases has also been affected by political, economic, and cultural factors. It has been reported that the largest concentrations have occurred in the states of California, Pennsylvania, New Jersey, and Illinois before 1988 and Mississippi, New York, West Virginia, Ohio, and Texas after 1998 (4, pp. 26, 36). Every case that is filed comes with numerous contestable issues. Examples of such issues include whether the plaintiff was exposed to the product of a particular defendant (the typical asbestos plaintiff names an average of 60 defendants), whether the plaintiff's condition was specifically caused by that product, and whether the disease of the plaintiff was caused by asbestos rather than some other substance, such as tobacco. When the number of plaintiffs and defendants is multiplied in any particular set of cases, the potential for disputes is magnified exponentially. This gives rise to gridlock in the judicial system. The courts do not have enough time or resources to deal with every such issue in every case.

The crowding of dockets has given rise to various negotiating strategies on the part of the participants in this process—strategies that are usually managed by experienced law firms. It is a typical strategy of the plaintiffs to collect as many cases as possible and to present the system with demands for settlement in which individual analysis of cases is subordinated to the imperative of the need for massive compensation. Mesothelioma cases play an important role in this process, since they are generally perceived as the more serious and thus economically valuable cases. In cases where settlement does not occur and the cases go to trial, it is typically the strategy of plaintiffs to load the mix with serious cases with a view toward increasing the overall jury award. It is also the strategy of the plaintiffs to focus on the reprehensibility of corporate behavior in order to inflame the jury and inflate the award. Plaintiffs often press for easy access to the system and for trial. The typical strategy of the defendants, by contrast, is to focus on the factors of the cases that are individual rather than on the factors that are common. Defendants have tended to resist large consolidations or class actions (especially for trial as distinguished from settlement) and to be interested in a specific case-by-case inquiry into whether a particular asbestos-containing product caused a particular injury in a given plaintiff. Defendants are also resistant to easy access by the plaintiffs to the system and less anxious to try corporate behavior especially in the context of punitive damages. The willingness of defendants to

settle cases is often influenced by economic considerations, such as the availability of insurance, predictability, and the general capacity to "manage the flow."

Transactional Costs

The contentious nature of the asbestos litigation system creates enormous transactional costs. These include attorneys fees, expert witness expenses, governmental and insurance resources, and many other hidden costs. While estimates vary, it is generally agreed that over 50% of the money in the system is consumed in transactional costs. The most commonly cited statistic has been that of a RAND Corp. study in 1984 that estimated that 61% of monies paid to resolve asbestos claims were spent on legal fees and expenses. The more recent 2002 RAND report confirms that over 50% of all money spent on asbestos claims is consumed by transactional costs (4, p. 60). The 1991 report of the Judicial Conference Ad Hoc Committee on Asbestos Litigation concluded, "The transaction costs associated with asbestos litigation are an unconscionable burden on the victims of asbestos disease" (2, p. 13).

Judicial Management Techniques

Given the above dynamics, the form in which the judicial system chooses to manage asbestos cases becomes very important. There has been substantial controversy over this subject. In the early years of the litigation, most cases were handled individually or in small groups. As this became increasingly impossible, judges began to experiment with various aggregative approaches. Cases grouped for trial became larger and larger. Class action questions became more common. As the numbers increased, efficiencies of scale were perceived in trying specific issues collectively rather than each individual case one by one. Thus, for example, a court might try the issue of corporate responsibility or general causation generically over a large number of individual cases. Some courts employed systems of bifurcation in which liability was tried separately from damages. Many variations in these styles of piecemeal litigation were developed in response to the dynamics of litigation in particular jurisdictions or the perceived advantages of moving the greatest numbers of cases through the system with the minimal of transactional costs. Frequently a judicial management technique intended to streamline the system had the opposite effect by encouraging the filing of more cases. As stated by one commentator, "If you build a superhighway, there will be a traffic jam" (9).

Notwithstanding these efforts, cases continued to overwhelm the system. In 1991, the Judicial Conference Ad Hoc Committee on Asbestos Litigation, chaired by Chief Justice William H. Rehnquist, called for a legislative solution. In the event Congress did not act, the Rehnquist committee suggested a backup plan of greater aggregative approaches. In 1991, the Federal Judicial Panel on Multidistrict Litiga-

tion consolidated all federal asbestos cases into one jurisdiction (Philadelphia) for pretrial and some settlement proceedings (10). The effect of this federal action has been somewhat offset by the resulting practice of many plaintiffs in filing their cases in state courts where there has been a greater perceived ability to obtain higher recoveries and punitive damage awards. Some state courts have also ordered massive consolidations or class actions. The movement toward greater aggregation has been limited, however, by jurisprudential perceptions of the system as requiring the trial of individual issues. For example, in what was perhaps one of the most experimental aggregative approaches of them all, a federal trial court in Texas consolidated several thousand cases, tried what was perceived to be a representative sample, and extrapolated the results from the sample to the remaining cases. This approach was rejected on appeal as inconsistent with the nature of judicial power (11). Several United States Supreme Court decisions rejected on technical grounds attempts to settle large categories of cases (12,13). These disputes over the form of the litigation process, rather than the facts of individual cases, have added to the transactional costs. Calls for federal legislative action have so far gone unheard. Congressional reluctance to enter this fray may perhaps be partially explained on grounds of fear of a federally financed bailout (14). Lobbying efforts have increased in the wake of ever-increasing number of bankruptcies and the spread of asbestos liability to mainstream American companies, but no comprehensive legislative solution seems imminent. The Senate Judiciary Committee held its most recent hearing on asbestos litigation reform on September 25, 2002. However, as of this writing, no action has been taken by the committee.

Medical Expert Witnesses

Medical expert witnesses play an important role in asbestos litigation. As might be expected in an adversary system of litigation, lawyers tend to seek out experts who are perceived to be helpful to their own side in a particular case. Excessive partisanship, however, is normally somewhat restrained by the opportunity for cross-examination and the desire to appear credible before judges and juries. Physicians who appear frequently as expert witnesses are well known to experienced attorneys. They are tracked by various publications and databases, and the transcripts of testimony given in previous cases are readily available.

Notwithstanding these governing devices, there have been problems experienced in the use of expert witnesses. Physicians have been instrumental in the process of assembling large numbers of cases of individuals asserted to be suffering from asbestos-related disease. There have been abuses in situations where the physician's compensation was related to the number of plaintiffs produced. A judicial experiment utilizing court-appointed rather than privately retained experts resulted in a dramatic decline in the incidence of findings of asbestos-related disease (15). More generally, there has been a reaction in the

courts against the excessive use of experts utilizing dubious scientific methodology or "junk science"—a reaction epitomized in the U.S. Supreme Court case of *Daubert v. Merrel Dow Pharmaceuticals* (16). The former laissez-faire attitude of the courts has given way to what has been termed a "gatekeeping" role in which judges are often quite active in screening out medical expert testimony deemed to be unscientific.

Punitive Damages

Punitive damages are one of the most controversial economic aspects of asbestos litigation. Punitive damages are awarded to a plaintiff in addition to compensatory damages. Their purpose is punishment and deterrence. Given the well-developed evidence of corporate misconduct on the part of some defendants in the earlier years, it is not difficult to argue a punitive damages case to a jury, and such arguments often have great populist appeal (e.g., "send a message" to the corporate boardroom). Such damage awards can be quite high. The effect of such awards far transcends their imposition in a single case. The fear of punitive damages induces defendants to settle cases at a premium. A single mass consolidation accompanied by punitive damages can be a "bet the company" type of case and can create enormous pressure to settle.

Another problem is the multiple imposition of punitive damages for a single course of conduct. The punitive damage–inducing conduct on the part of certain corporate defendants occurred generations ago and took place only once, although its effect was felt by many people. When those many people file lawsuits years later, each claims the same entitlement to punitive damages. To the extent these claims are successful, the defendant may be ordered to pay many times over for the same conduct. Numerous courts and commentators have expressed frustration with this phenomenon on the grounds that it prematurely exhausts resources that would otherwise be available for the satisfaction of future compensatory damages awards (17). So far, however, there has been mixed success at curtailment of these practices. Some courts have finessed the problem by indefinitely deferring claims for punitive damages until all compensatory damage obligations are satisfied.

Abatement of Asbestos in Buildings

Economic impacts are also felt in various measures of asbestos abatement and the resulting property damage litigation. When the dangers of asbestos became known, many building authorities required abatement measures of various kinds. Buildings, such as the World Trade Center and many of the nation's public schools, were forced to take abatement measures. Sometimes there were disputes over the appro-

priateness of certain abatement measures or whether the conditions of certain "sick buildings" were due to asbestos or other contaminants. As in the case of personal injury, the defendants facing such liabilities turned to their insurance carriers with predictable disputes. There were also significant transactional costs, albeit less than in the area of personal injury.

The Creation of Industries

The activity involved in handling these disputes has spawned a virtual industry associated with asbestos litigation. At the apex of this industry are the plaintiffs' attorneys. While thousands of attorneys handle asbestos litigation, there are a relatively small number of influential lawyers and law firms that have been very successful and have developed national or regional reputations. Asbestos cases are generally handled on a contingency-fee basis with such fees constituting a substantial part of the recovery. These fees can become extremely high when spread over a major recovery in thousands of cases. For example, it has been estimated that in one mass consolidation of asbestos cases in Baltimore, the attorneys' fees from settlements alone were $120 million to $125 million and that the total was expected to rise to $300 million after trial (18). One commentator has estimated that plaintiffs' lawyers' effective rates of return expressed on an hourly basis in asbestos cases range from $1000 to $5000 per hour, and in cases of mass consolidations, hourly rates have been $50,000 per hour, with total fees ranging from $200 million to $500 million or more (19). The assets accumulated by some lawyers in these cases have allowed them to exercise considerable political influence and to finance expansion into other mass tort cases such as tobacco litigation. Defense attorneys are also a significant part of the asbestos litigation industry. While they are typically paid by the hour rather than on a contingency fee basis, the massive time and effort required to handle the litigation has generated significant law firm revenues. Asbestos cases also require expert witnesses, typically members of the medical profession. It is not unusual for an experienced expert to command fees of thousands of dollars per day for expert testimony or consultation. Other experts include economists, industrial hygienists, historians, epidemiologists, and an almost infinite variety of other specialties, depending on the peculiarities of a given case. Behind the front lines of the litigated cases, there are many other people whose role is instrumental in the handling of the asbestos problem and who are thus significant factors in its overall economic impact. These include the management of corporate defendants and insurance companies, the administrators of claims-paying agencies, such as the Manville Trust, and various members of the judicial branch and other governmental entities. There is also a small publishing and educational seminar industry that is devoted exclusively to reporting on asbestos matters. Asbestos litigation has also given rise to a substantial body of scholarly literature.

The Destruction of Industries

The present and foreseeable future costs of asbestos litigation have led a large number of companies to file for bankruptcy protection (4, p. 71). The wave of bankruptcies began in the 1980s, with 16 firms filing for protection. The number remained virtually the same through the 1990s, with 18 bankruptcies reported through the decade. However, the number of bankruptcies related to asbestos has recently accelerated. There have been more filings since 2000, at least 22 through July 2002, than there were in the 1970s and 1980s combined.

The recent RAND report estimated the amount of corporate investment and economic growth lost due to asbestos litigation (4, p. 74). It determined that if the costs of asbestos litigation reach the predicted $200 billion level, there will have been a $33 billion reduction in corporate investment. This reduction in the investment level of large companies has already resulted in the loss of an estimated 138,000 jobs and will likely result in the loss of an additional 290,000 jobs in the future. This loss of jobs has and will continue to have a major impact on the economy as a whole.

The New Wave of Asbestos Litigation

One of the more interesting economic effects of the system in recent years is the so-called new wave of asbestos litigation involving unimpaired plaintiffs and peripheral defendants. Since most asbestos-containing products were banned from the marketplace a generation ago, it had been predicted that the incidence of asbestos-related disease would decrease and that the claims would accordingly decline. These predictions have not come to pass. The volume of new asbestos cases today is actually increasing (4). To some degree, this is probably a product of lawyer solicitation. It is also, however, a product of the fact that many individuals have radiographic markers of asbestos exposure but may never become symptomatic or impaired. According to Congressional testimony in 1999, experts have projected that 50% to 80% of the current claims are filed by individuals with no impairment (20). In some jurisdictions, the claims of these individuals are deferred. In others, however, they are encouraged by statutes of limitations and policies that recognize their compensability or that permit claims for fear of cancer or for medical monitoring.

As the number of claims are holding steady or increasing, the number of traditional defendants left standing who have escaped bankruptcy has dramatically declined. This has forced plaintiffs' representatives to expand the universe of potentially responsible defendants and to bring actions against entities whose connection to asbestos has been peripheral. Over 6000 companies have been named as defendants in asbestos cases (4, p. vi). Examples include companies in the textile, pulp and paper, food, automotive, and energy industries. Well-known corporate defendant names include Chiquita Brands, General Electric, Sears & Roebuck, Georgia Pacific, Dow Chemical, Ford,

General Motors, and Daimler Chrysler. According to the recent RAND study, more than 1000 American corporations have been made asbestos defendants, and these companies are scattered across 75 of the 83 industrial categories used by the Department of Commerce (4, p. 50). The employees, retirees, and shareholders of these companies are affected by this asbestos liability. The economic impact of this new wave of asbestos litigation has yet to be fully felt.

Conclusion

Asbestos litigation is the longest running mass tort in United States history. Mesothelioma is its most conspicuous type of case. The economic aspects of these phenomena have had a major effect on the American economy.

References

1. Ortiz v. Fibreboard, 527 U.S. 815, 821 (1999).
2. Report of the Judicial Conference Ad Hoc Committee on Asbestos Litigation, chaired by Chief Justice Rehnquist of the U.S. Supreme Court.
3. In re Joint Eastern and Southern Dist. Asbestos Lit., 827 F. Supp. 1014, 1026 (S.D.N.Y. 1993) reversed on other grounds, 52 F.3d 1124 (2d Cir, 1995).
4. Carroll S, Hensler D, Abrahamse A, et al. Asbestos litigation costs and compensation: an interim report. RAND, 2002.
5. Faulk RO. Asbestos litigation crisis requires policymakers attention. Washington Legal Foundation, February 22, 2002.
6. Schmoll v. AC&S, Inc., 703 F. Supp. 868 (D. Or. 1988) affirmed 977 F.2d 499 (9th Cir, 1992) [Transfer of assets from Raymark to Raytech designed to improperly escape asbestos liability].
7. Statement of David T. Austin before the United States Senate Committee of the Judiciary, Sept. 25, 2002.
8. Finding solutions to the asbestos litigation problem: the fairness in Asbestos Compensation Act of 1999, hearing before the Subcommittee on Administrative Oversight of the Courts of the Committee on the Judiciary, October 5, 1999.
9. McGovern F. The defensive use of federal class actions in mass torts. Ariz Law Rev 1997;39:606.
10. In re Asbestos Products Liability Litigation (VI), 771 F. Supp. 415 (J.P.M.L. 1991).
11. Cimino v. Raymark Industries, 151 F.3d 297 (5th Cir. 1998).
12. Amchem Products v. Windsor, 521 U.S. 591 (1997).
13. Ortiz v. Fibreboard, 527 U.S. 815 (1999).
14. Report of the hearings on the Fairness in Asbestos Compensation Act of 1999 before the Subcommittee on Judicial Oversight of the Courts of the Senate Committee on the Judiciary on October 5, 1999. The act under consideration involved a proposal for a nationwide administrative claims resolution process. One opponent (Rep. Scott of Virginia) referred to the pending bill as a "bailout for an industry responsibility for the disability and death of millions of Americans." The bill failed and its principal sponsor went into bankruptcy.

15. Rubin CB, Ringenbach L. The use of court experts in asbestos litigation. 137 F.R.D. 35 (1991).
16. Daubert v. Merrel Dow Pharmaceuticals, 506 U.S. 914 (1983).
17. In re Collins, 233 F.3d 809, 812 (3d Cir. 2000) cert. den. 121 S. Ct. 2216 (2001).
18. Blum A. Megafees in Baltimore Megacase. National Law J August 29, 1992:2.
19. Brickman L. On the relevance of the admissibility of scientific evidence: tort system outcomes are principally determined by lawyers' rates of return. Cardozo Law Rev 1994;15: 1773.
20. October 5, 1999 Hearings on the Fairness in Asbestos Compensation Act of 1999, Testimony of Congressman Moran, p. 6.

Index