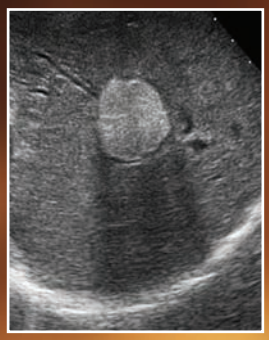
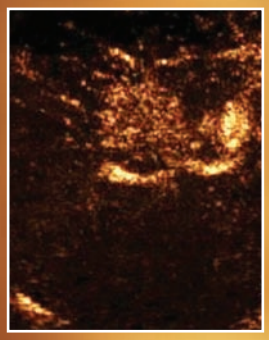
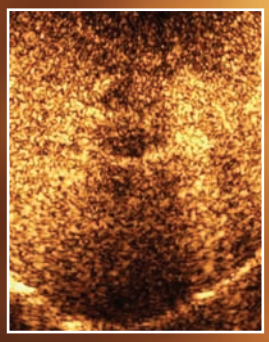
Hepatocellular Carcinoma

A Practical Approach







Edited by

Bandar Al Knawy

K Rajender Reddy

Luigi Bolondi

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Edited by

Bandar Al Knawy MD FRCPC

Division of Gastroenterology and Hepatology, King Abdulaziz Medical City, Saudi Arabia

K Rajender Reddy MD

Professor of Medicine and Surgery, Director, Hepatology and Medical Director, Liver Transplantation, Hospital of the University of Pennsylvania, Philadelphia, USA

Luigi Bolondi MD

Professor of Internal Medicine, Chairman, Department of Digestive Diseases and Internal Medicine, University of Bologna, Italy

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Website: www.informahealthcare.com

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Contents

Contributors

Dania Cioni Division of Diagnostic Imaging and Intervention, Department of Liver Transplantation, Hepatology and Infectious Diseases, University of Pisa, Lisanello Hospital, Pisa, Italy

Massimo Colombo 1st Division of Gastroenterology, Department of Medicine, A.M. and A. Migliavacca Center for Liver Disease, Fondazione IRCCS Maggiore Hospital Mangiagalli and Regina Elena, University of Milan, Milan, Italy

Laura Crocetti Division of Diagnostic Imaging and Intervention, Department of Liver Transplantation, Hepatology and Infectious Diseases, University of Pisa, Lisanello Hospital, Pisa, Italy

Jiangao Fan Department of Gastroenterology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Geoffrey C. Farrell Australian National University Medical School at The Canberra Hospital, Canberra, Australian Capital Territory, Australia

Richard B. Freeman Division of Transplantation, Tufts Medical Center, Boston, Massachusetts, U.S.A.

Gregory J. Gores Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

Francesca Guerrieri Laboratory of Gene Expression, Fondazione Andrea Cesalpino and Laboratoire Associe' INSERM UNIT 785, Villejuif, France and Rome, Italy

Kiyoshi Hasegawa Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Saad M. Ibrahim Section of Interventional Radiology, Department of Radiology, Northwestern Memorial Hospital, Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois, U.S.A.

Donald M. Jensen Department of Medicine, Center for Liver Diseases, The University of Chicago, Chicago, Illinois, U.S.A.

Ahmed O. Kaseb Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, U.S.A.

Norihiro Kokudo Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Laura M. Kulik Department of Hepatology, Northwestern University, Chicago, Illinois, U.S.A.

Riccardo Lencioni Division of Diagnostic Imaging and Intervention, Department of Liver Transplantation, Hepatology and Infectious Diseases, University of Pisa, Lisanello Hospital, Pisa, Italy

Massimo Levrero Laboratory of Gene Expression, Fondazione Andrea Cesalpino; Rome Oncogenomic Center, Regina Elena Cancer Institute; Laboratoire Associe' INSERM UNIT 785, Villejuif and Dept of Internal Medicine, University of Rome La Sapienza, Rome, Italy

Robert J. Lewandowski Section of Interventional Radiology, Department of Radiology, Northwestern Memorial Hospital, Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois, U.S.A.

Masatoshi Makuuchi Department of Digestive Surgery, Japanese Red Cross Medical Center, Tokyo, Japan

Ryota Masuzaki Department of Gastroenterology, University of Tokyo, Tokyo, Japan

Smruti R. Mohanty Department of Medicine, Center for Liver Diseases, The University of Chicago, Chicago, Illinois, U.S.A.

Mary F. Mulcahy Division of Hematology and Oncology, Department of Medicine, Robert H. Lurie Comprehensive Cancer Center, Northwestern Memorial Hospital, Chicago, Illinois, U.S.A.

Masao Omata Department of Gastroenterology, University of Tokyo, Tokyo, Japan

Emanuele Palescandolo Laboratory of Gene Expression, Fondazione Andrea Cesalpino, Rome, Italy

Natalia Pediconi Laboratory of Gene Expression, Fondazione Aandrea Cesalpino and Rome Oncogenomic Center, Regina Elena Cancer Institute, Rome, Italy

M. Clotilde Della Pina Division of Diagnostic and Interventional Radiology, Department of Oncology, Transplants and Advanced Technologies in Medicine, University of Pisa, Pisa, Italy

Ahsun Riaz Section of Interventional Radiology, Department of Radiology, Northwestern Memorial Hospital, Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois, U.S.A.

Riad Salem Section of Interventional Radiology, Department of Radiology, Northwestern Memorial Hospital, Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois, U.S.A.

William Sanchez Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

Angelo Sangiovanni 1st Division of Gastroenterology, Department of Medicine, A.M. and A. Migliavacca Center for Liver Disease, Fondazione IRCCS Maggiore Hospital Mangiagalli and Regina Elena, University of Milan, Milan, Italy

Valeria Schinzari Laboratory of Gene Expression, Fondazione Andrea Cesalpino and Laboratoire Associe' INSERM UNIT 785, Villejuif, France and Rome, Italy

Shuichiro Shiina Department of Gastroenterology, University of Tokyo, Tokyo, Japan

Melanie Thomas Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, U.S.A.

Stefania Vossio Laboratory of Gene Expression, Fondazione Andrea Cesalpino and Laboratoire Associe' INSERM UNIT 785, Villejuif, France and Rome, Italy

Preface

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer-related mortality after lung and stomach cancers. It is associated with a poor prognosis when diagnosis is made when patients reach a symptomatic state. The lack of sensitive and specific tumor markers has further hampered our ability to effectively screen patients at risk for HCC and diagnose this malignancy at an early stage. Yet simple and widely available tool of ultrasonography, when done well, had allowed for early diagnosis of HCC. Currently, the only curative therapies for HCC are surgical resection and liver transplantation. Such modalities, particularly liver transplantation, are not widely available. HCC management, from screening patients with chronic liver disease through diagnosis and therapy, now forms a major part of hepatology practice that necessitates a multidisciplinary approach that involves surgery, hepatology, interventional radiology, oncology, and pathology.

Nonsurgical therapies such as ablative therapy serve to treat this malignancy effectively, while the underlying chronic liver disease serves as major limitation in aggressive therapy, which in turn contributes to liver disease–related morbidity and mortality. Effective therapies are urgently needed to improve survival in patients with advanced HCC. Better understanding of the role of hepatitis viruses and the molecular mechanisms involved in hepatocarcinogenesis are leading to newer and effective systemic therapies, which will complement the more established locoregional treatments. Recently, targeted agents have shown encouraging efficacy in clinical trials in patients with advanced HCC. Agents under investigation are directed toward molecular mechanisms underlying the pathology of HCC and include inhibitors of angiogenesis and epidermal growth factor receptor–mediated signaling.

With *Hepatocellular Carcinoma: A Practical Approach*, the editors present the most recent developments on the diagnosis, screening, and comprehensive medical and surgical management of HCC. It aims to benefit clinicians who deal with its daily issues, especially gastroenterologists, hepatologists, radiologists, liver surgeons, and oncologists. This concise textbook deals with all aspects of HCC in a practical, evidence-based approach and will provide step-by-step management guidelines, applicable to all clinical scenarios. International faculty will illustrate current trends in incidence of HCC, adequacy and effectiveness of screening high-risk populations, means of early diagnosis, and optimal approaches to management and treatment including local ablation therapy, chemoembolization, liver resection, transplantation, and chemotherapeutic agents.

We hope you enjoy reading and adding *Hepatocellular Carcinoma: A Practical Approach* to your collection of textbooks.

Bandar Al Knawy, K. Rajender Reddy, Luigi Bolondi

1 Epidemiology of the Hepatocellular Carcinoma

Angelo Sangiovanni and Massimo Colombo

1st Division of Gastroenterology, Department of Medicine, A.M. and A. Migliavacca Center for Liver Disease, Fondazione IRCCS Maggiore Hospital Mangiagalli and Regina Elena, University of Milan, Milan, Italy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality, with an estimated worldwide prevalence of 632,000 cases (1). Accounting for about 90% of all primary liver cancers, HCC is unique in that it develops in the background of well-recognized risk factors that are responsible for marked variations of the tumor prevalence and clinical presentation worldwide. Sequential scrutiny of the cancer registries has demonstrated important variations in the temporal trends of HCC incidence, with some evidence for an increase in incidence in resource-rich countries. In this region, molecular epidemiology investigations have predicted a further rise in HCC incidence and mortality rates in the next decades due to the accumulation of patients with chronic liver diseases who are expected to develop HCC.

THE CHANGING PATTERN OF EPIDEMIOLOGY

The fact that HCC incidence is not equally distributed throughout the world reflects the heterogeneous geographical distribution of the relevant environmental risk factors. The vast majority (>80%) of the cases of HCC occurs in the Far East and in sub-Saharan Africa, mostly as a consequence of chronic infection with hepatitis B virus (HBV), where the age-standardized incidence rates range between 28.5 and 48.8 per 100,000 males and 11.6 and 14.6 per 100,000 females (2). Half of the world cases of HCC occur in China, corresponding to an incidence rate of 35.2 per 100,000 males and 13.3 per 100,000 females. Southern European countries, like Spain, Greece, and Italy, have medium-rate incidence rates, ranging between 7.5 and 13.5 per 100,000 males and between 2.4 and 4.6 per 100,000 females compared with low-rate areas like North and South America, Northern Europe, and Oceania, where the incidences range between 2.0 and 3.6 per 100,000 males and 1.1 and 2.2 per 100,000 females (2). The comparative analysis of the cancer registries during different time points indicated HCC epidemiology to have changed in the last decades. A rise in HCC incidence has been documented between 1978 and 1982 and 1993 and 1997 in several low-rate areas, including the United Kingdom, Australia, and the United States (Fig. 1) (3,4), probably related to the spread of hepatitis C virus (HCV) infection, through infected blood, medical devices, and injection of illicit drugs. In the same time frame, HCC incidence decreased among selected populations like Chinese in Singapore and Shanghai and in a resource-rich country like Spain. Though the reasons for a decreased incidence of HCC in high-rate areas are unclear, many clues point to a reduced exposure of the population to dietary hepatocarcinogen aflatoxin B1 (AFB1) as a consequence of specific health policy and campaigns of mass vaccination of newborns against the HBV (5,6). The fact that age-adjusted mortality rates of HCC mirror the incidence rates worldwide indicates that the survival of HCC patients is poor and no greater than one year, on average. In the referral centers of resource-rich countries, up to 50% of all patients with an HCC will be diagnosed or treated with a Tumor node metastases stage I (TNMI) or Tumor node metastases stage II (TNMI II) tumor, compared with 20 years ago when patients with an early-stage tumor represented a tiny minority of the incident cases. These changes in the epidemiology of HCC will impact on health policy due to the increased financial resources needed for staging and treatment, including an increased need for donated livers (7).

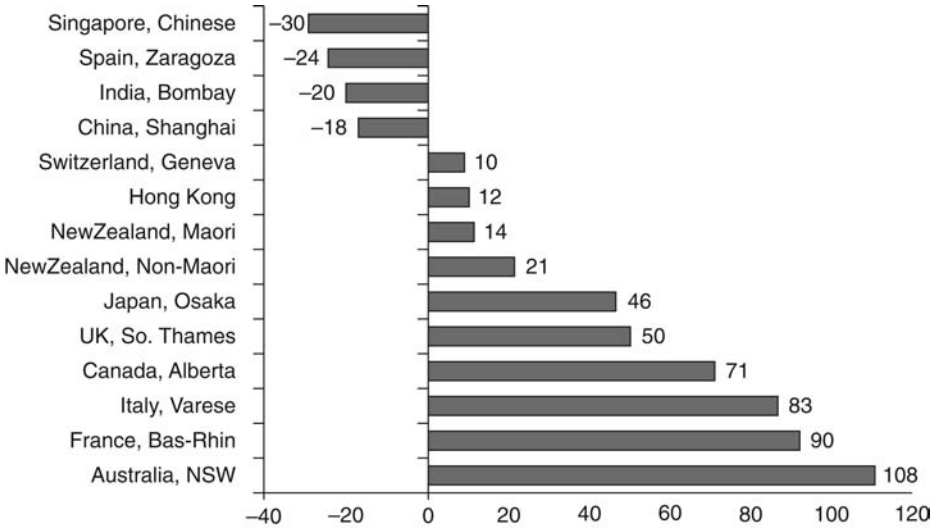

Figure 1 Changes in the incidence of hepatocellular carcinoma (3). *Abbreviation*: HCC, hepatocellular carcinoma.

CONSTITUTIONAL RISK FACTORS

The observation that HCC incidence varies among different ethnics living in the same geographical region bended support to the hypothesis that race and ethnicity influence HCC risk. In the United States, where the age-adjusted incidence of HCC, at all ages and among both sexes, was repeatedly found to be higher in Asians and African Americans than in Caucasians, discrepancies between Caucasians and ethnics progressively diluted off in descendants living in the same area, pointing to the dominant role of environmental risk factors over constitutional risk factors (8). The variability of HCC incidence among different ethnics may also depend on differences in the prevalence and acquisition time of major risk factors like HBV and HCV. In any geographical area and whatever the changes in incidence rates of HCC, males are more often affected by HCC than females, with male to female ratio ranging between 2:1 and 4:1. The higher-risk ratios for HCC are found in medium-risk European countries like Switzerland (4.1:1), Italy (5.1:1), and France (5.0:1), whereas the lowest sex ratios are reported in endemic regions like Colombia (1.2:1) and Costa Rica (1.6:1). Higher rates of liver cancer in males have been long interpreted as reflecting sex-related differences in exposure to risk factors since men are more likely to be chronically infected with the hepatitis viruses, drink excess alcohol, smoke more cigarettes, and have increased iron stores than females. However, males have higher body mass index and circulate more androgenic hormones than females, both having the potential of increasing HCC risk (9,10). In the diethylnitrosamine mouse model, HCC developed more frequently in males than in females, paralleling an increase in serum interleukin-6 (IL-6). Interestingly, gender differences in mice hepatocarcinogenesis were abolished by ablation of the IL-6 gene, suggesting that estrogen-mediated inhibition of IL-6 production by Kupffer cells protects females from liver cancer (11).

While the highest age-specific rates occur among persons aged 75 years and older in low-risk populations, it peaks between ages 60 and 65 years in high-risk African populations. Different patterns of age-specific incidence of HCC reflect differences in the etiology and dominant modalities of hepatitis virus transmission in the population (vertical vs. horizontal transmission). In Far East and Africa, the dietary exposure to aflatoxin-contaminated foodstuffs likely accelerates the natural history of HBV-related HCC. In the United States, the incidence of HCC has increased steadily in the last two decades among the general population because of increased rates of HCC among young whites exposed to HCV, as documented by the analysis of hospitalization certificates in several sentinel countries (12–15). The finding that the changes in tumor incidence in the resource-rich areas of the world

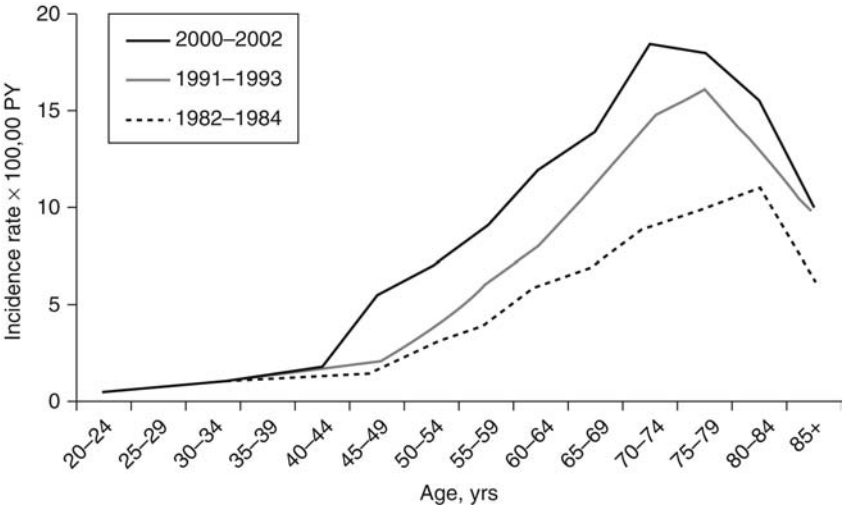

Figure 2 The United States temporal trends in the age distribution of new cases of HCC in men (13). *Abbreviation*: HCC, hepatocellular carcinoma.

are associated with a shift toward younger patients (Fig. 2) is having practical clinical implications, since HCV-related HCC is a dominant indication for liver transplantation in these regions (2,16).

ENVIRONMENTAL RISK FACTORS

Chronic infection with the hepatitis viruses and alcohol abuse are the most important environmental risk factors for HCC, since these are the relevant etiologic factors for cirrhosis too (Table 1) (17). Cirrhosis, in fact, is the main risk factor for HCC, independently on the etiology and the geographical area being present in more than 80% of the patients with HCC. In patients with cirrhosis, the yearly rate of conversion to HCC ranges between 1.4% and 6.9% (18).

In Asia and Africa, the dominant risk factor of HCC is chronic HBV infection compared with other low-risk areas like North America and Europe, where HCV infection accounts for the large proportion of cases. In Asia, HBV infection is largely acquired by maternal to child transmission (vertical route), while consumption of AFB1-contaminated foodstuffs is thought to boost the carcinogenetic risk due to HBV. HCV is the dominant hepatitis virus in Japan, where the virus began to circulate after World War II as a consequence of transfusion with infected blood and use of contaminated needles (19). In low-rate HCC areas, the increasing number of HCC is related to increased number of persons living with cirrhosis attributed to HCV and, to a lesser extent, to HBV infection, coupled with an improvement in survival among cirrhotic patients. Molecular clock analysis indicates that the epidemic of HCV spread in Japan in the

Table 1 Risk Factors of HCC: Estimates of The Percentage of Attributable Fraction According to Geographical Areas (17)

Risk factors	Europe/United States	Japan	Africa/Asia
HBV	22 (4–58)	20 (18–44)	60 (40–90)
HCV	60 (12–72)	63 (48–94)	20 (9–56)
Alcohol	45 (8–57)	20 (15–33)	(11–41)
Tobacco	12 (0–14)	40 (9–51)	22
Aflatoxin exposure	Limited	Limited	High
Other	<5	–	<5

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.

twenties, in Central Europe after World War II, and in the United States in the sixties, the latter mainly as a result of intravenous drug use first and contaminated blood transfusions later (20). The HCV-related HCC in low-rate countries is estimated to peak around 2010 (21).

HBV

The estimated 300 million persons with chronic infection worldwide have a 5- to 15-fold increased risk of HCC compared with the general population (2). While the risk of HCC is highest in patients with cirrhosis, HBV causes HCC in up to 40% of patients prior to development of cirrhosis. In low–HCC incidence areas, HBV is being transmitted mainly through sexual and parenteral routes (horizontal transmission), while the vertical route of transmission is implicated in most cases in the high incidence areas.

Sex, race, family history of HCC, exposure to aflatoxin, alcohol, tobacco, and coinfection with other hepatic viruses, such as hepatitis D virus (HDV) and/or HCV, increase the risk of HCC in HBV carriers. Large cohort studies in Asia indicate high levels of HBV replication to be associated to increased HCC risk, providing the rationale for early treatment of patients with high viremia, with the aim of reducing the HCC risk. Indeed, either spontaneous or treatment-induced seroconversion from hepatitis B e antigen (HBeAg) to anti-hepatitis B e antigen (anti-HBe) is associated with improved clinical outcomes and a reduced HCC risk, particularly in patients with limited hepatic fibrosis (22). A worrisome aspect of HBV is its ability to generate occult infections in the liver of persons who are immune to HBV, with the HBV DNA persisting in hepatocytes maintaining intact carcinogenic potential. The etiologic role of HBV in causing HCC is well documented by the decrement of HCC incidence among adolescents who participated in the programs of mass vaccination against HBV in Taiwan (23).

HCV

Chronic HCV infection is a leading risk factor in the majority of the resource-rich countries (24). A meta-analysis of 21 case-control studies showed a 17-fold increased risk of HCC in HCV-infected patients compared with HCV-negative controls (95% CI, 14–22) (25), though the risk of HCC in HCV patients greatly varied in accordance with sex, age of infection, and cofactors like insulin resistance, alcohol abuse, and duration of infection. The relationship between duration of infection and HCC risk is difficult to extrapolate, since the majority of the infected patients have silently acquired HCV in the community. HCV increases HCC risk not only by both promoting cirrhosis but also by causing specific genetic lesions to the infected liver cells.

The genotype 1b of HCV has been associated to a higher risk of HCC in some studies of the natural history of HCV-related cirrhosis (26–29), but not in others (18,30). There is no evidence that such viral factors as genotype and viral load are important in modulating progression to cirrhosis or HCC.

Alcohol

Alcohol has no direct carcinogenic effects on the liver, but chronic consumption of more than 50 g alcohol per day is associated with an increased risk of cirrhosis in both sexes. The hepatic carcinogenic effect of alcohol is potentiated by coinfection with HCV and HBV and by increased iron stores in the presence of genetic markers of hemochromatosis (25,31). The likely carcinogenic mechanism of alcohol is the oxidative stress, which causes damage to DNA, of liver cell proliferating during cirrhosis. Debated issues are whether the risk of HCC can be attenuated by alcohol withdrawal in patients with established cirrhosis and whether nutrition plays any protective role against HCC in alcoholics.

Aflatoxin

Aflatoxin, a mycotoxin produced by the *Aspergillus flavus* contaminating the foodstuffs stored in warm, dump condition, is a relevant contributor of HCC of regional importance, being associated with increased risk of HCC in HBV-infected persons in parts of Africa and Asia (32). Aflatoxin is hepatocarcinogenetic in animal models, being metabolized after ingestion to AFB1-*exo*-8,9-epoxide, an active intermediate, which affects cell DNA by inducing mutations in the p53 tumor-suppressor gene in 30% to 60% of HCC patients living in aflatoxin-endemic areas (33,34). The urinary excretion of aflatoxin metabolites was associated with a fourfold increase in HCC,

and a positive interaction between aflatoxin exposure and chronic HBV infection was reported in studies from China (35). Studies in populations are in place in China to evaluate whether inhibitors of aflatoxin metabolism protect the general population from HCC.

EMERGING RISK FACTORS

Nonalcoholic Fatty Liver Disease

A relevant proportion of patients with an HCC and cryptogenic cirrhosis have demographic and clinical features of nonalcoholic steatohepatitis (NASH). The association between NASH and HCC is strongly supported by case-control studies (36–38), but not by prospective studies (39–41). However, clinical and epidemiological investigations, which link obesity and diabetes to HCC, support the role of NASH in HCC too. The likely mechanism leading to HCC in patients with NASH is oxidative stress coupled with liver cell proliferation during development of cirrhosis.

Obesity

In a large prospective cohort study evaluating more than 900,000 individuals in the United States followed for 16 years, liver cancer mortality rates were exceedingly higher in men with greater than 35 baseline body mass index than in persons with a normal body mass index (42). Though the increased rates of mortality in obese patients could also reflect difficulties in the treatment of this set of patients, an increased risk of HCC was also reported in overweight and obese persons in Sweden and Denmark, independently from mortality rates (43,44). The pathogenetic link between overweight, obesity, and HCC likely is hepatic necroinflammatory activity (39,45) and fibrosis, which prevail in the livers with excess steatosis (46–52).

Diabetes Mellitus

Diabetes mellitus is one of the main etiologic factors of nonalcoholic fatty liver disease (NAFLD)/NASH. A significant positive association between diabetes and HCC was reported in eight studies, though difficulties to determine the temporal relationship between diabetes and HCC might have biased the results of these investigations. Cohort studies prospectively evaluating patients for extended time periods showed diabetes to be a significant risk factor for HCC and not vice versa (53–55), and to parallel duration of follow-up (53). Despite the proportion of patients with diabetes who developed HCC is small, the large number of diabetics worldwide grants for a large number of HCC cases due to diabetes that is expected to occur worldwide (56).

Tobacco

Cigarette smoking positively correlates with HCC risk in specific subgroups of patients like those chronically infected with HBV or HCV. The effect of smoking on HCC risk likely is weak, limited to a subset of the general population and confounded by alcohol due to the overlap of exposure to these two risk factors.

Oral Contraceptives

The role of estrogen and progesterone as risk factor for HCC is unclear. In fact, nuclear estrogen receptors are increased in HCC, and estrogen and progesterone induce and promote liver tumors in animal models (57). Estrogens might cause liver neoplasia by increasing proliferation rates, thus increasing rates of spontaneous mutations (58). Several studies, including small sample sizes, concluded either for increase in risk of HCC or no association. A review of 12 case-control studies reported a nonsignificant pooled estimate with an OR of 1.6. However, there is an indication of a weak association between long-term use of oral contraceptives and increased HCC risk (59).

Hemochromatosis

Population studies showed a 1.7-fold increase in the incidence rates of HCC among individuals with hereditary hemochromatosis confirming preliminary observations in smaller studies in related population (60–63). In these studies, however, cirrhosis was difficult to dissect from iron overload as a potential cause of HCC. Experimental and clinical studies differ as far as the

carcinogenic role of iron is concerned. Iron overload is a well-recognized carcinogen in the former studies, whereas it is associated with an increased risk of HCC in patients with hemochromatosis, only. Recently, an association emerged between increased risk of HCC in alcoholics presenting liver iron overload and C282Y mutation for hemochromatosis (31), further stressing the importance of oxidative stress in alcohol-related hepatocarcinogenesis.

Genetic Epidemiology

The role of genetic heterogeneity as a risk for HCC in humans is debated. Genetic epidemiology studies evaluating different polymorphisms have largely provided equivocal results, reporting both positive associations with HCC, association only within a limited subset of the population, no association at all, or negative associations. The lack of reproducibility of studies of genetic epidemiology reflect inadequate sample sizes of the studies, to reliably detect the likely small effects of individual genes on risk within a background of strong environmental risk factors and polygenic influences on the development of disease (64,65). Furthermore, virtually all of these studies were inadequate to assess interactions with other environmental risk factors, since thousand cases and controls are required to adequately assess the effects of gene-gene or gene-environment interactions.

Vinyl Chloride

Professional exposure to vinyl chloride is a well-established risk factor for angiosarcoma of the liver (66). Studies of the association between occupational exposure to vinyl chloride and cancer mortality have indicated a small but significant increase of standardized mortality ratio for liver cancers other than angiosarcoma of 1.3 (66). The association of HCC and vinyl chloride is hotly debated, since it stems from pooling data for HCC and other epithelial tumors of the liver.

CONCLUSIONS

An increase in HCC incidence is expected in many areas of the world, including Europe and North America, as a consequence of the accumulation of patients with cirrhosis due to virus hepatitis. The shift from older to younger patients with a virus-related HCC will have practical influence: overall it will increase indications to orthotopic liver transplantation. In the resource-rich countries, the epidemiological pattern of HCC has been changing in terms of tumor stage at presentation, mainly as a consequence of the worldwide diffusion of surveillance programs for early detection. In the referral centers, up to 50% of all patients with an HCC are now being diagnosed or treated with a TNMI or TNMII tumor compared with 20 years ago when few incident patients with an early cancer represented 10% of all patients treated. These changes in the epidemiology of HCC will impact on health policy due to the increased financial resources needed for therapy.

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2 Molecular Pathogenesis

Massimo Levrero

Laboratory of Gene Expression, Fondazione Andrea Cesalpino; Rome Oncogenomic Center, Regina Elena Cancer Institute; Laboratoire Associe' INSERM UNIT 785, Villejuif and Dept of Internal Medicine, University of Rome La Sapienza, Rome, Italy

Natalia Pediconi

Laboratory of Gene Expression, Fondazione Aandrea Cesalpino and Rome Oncogenomic Center, Regina Elena Cancer Institute, Rome, Italy

Stefania Vossio, Valeria Schinzari, and Francesca Guerrieri

Laboratory of Gene Expression, Fondazione Andrea Cesalpino and Laboratoire Associe' INSERM UNIT 785, Villejuif, France and Rome, Italy

Emanuele Palescandolo

Laboratory of Gene Expression, Fondazione Andrea Cesalpino, Rome, Italy

Hepatocellular carcinoma (HCC) is one of the most frequent solid tumors worldwide and represents the third cause of mortality among deaths from cancer (1). HCC frequency is particularly high in Asia and Africa because of the high frequency of viral hepatitis infections and aflatoxin B1 (AFB1) exposure. The increased incidence of HCC in the United States, United Kingdom, and France over the last 10 years (2,3) is thought to reflect the increase of viral hepatitis C infections.

Chronic hepatitis B and C infections represent major risk factors for HCC development, being implicated in more than 70% of HCC cases worldwide. Additional etiological factors, which often represent cofactors of an underlying Hepatitis B Virus (HBV)- or Hepatitis C Virus (HCV)-related chronic liver disease, include toxins and drugs (e.g., alcohol, aflatoxins, microcystin, anabolic steroids, vinyl chloride), metabolic liver diseases (e.g., hereditary hemochromatosis, a1-antitrypsin deficiency), steatosis, nonalcoholic fatty liver diseases, and diabetes (4,5). In most HCC cases, one or more of these risk factors can be identified, either alone or in combination (6), and the presence of each risk factor among patients varies according to their geographical origin (7). Similarly to other solid tumors, several genetic and epigenetic alterations of genes that control the cell cycle and cell proliferation accumulate during the multistep process of hepatocytes transformation and HCC development (Fig. 1). However, the large number of different risk factors that are associated with HCC is reflected in the natural clinical diversity of HCCs as well as in the broad variety of accumulated genetic and epigenetic alterations. However, most HCCs develop in cirrhotic livers, and cirrhosis of any origin as well as dysplastic nodules have long been considered to be the likely precursors of HCC because of their frequent association with the HCC occurrence (8).

Recently, the combination of genome-wide assessment of genetic and epigenetic alterations, together with transcriptome and systematic pathway analyses, has not only confirmed the molecular diversity but also allowed the molecular classification of HCCs and, hopefully, in the near future, will be used to identify subgroups of tumors likely to be efficiently targeted by specific drugs. In this chapter, the available evidence for a direct oncogenic potential of HBV and HCV, the major carcinogenetic pathways found to be altered in HCC and how they are affected by hepatitis viruses, and, finally, how genetic and molecular classifications might predict the efficiency of the new targeted drugs will be reviewed.

MECHANISMS OF LIVER ONCOGENESIS

The exponential relation between HCC incidence and age suggests that, as in other human cancers, multiple steps are required, probably involving independent genetic lesions. The long latency period between HBV or HCV infection and HCC has often been used to support the

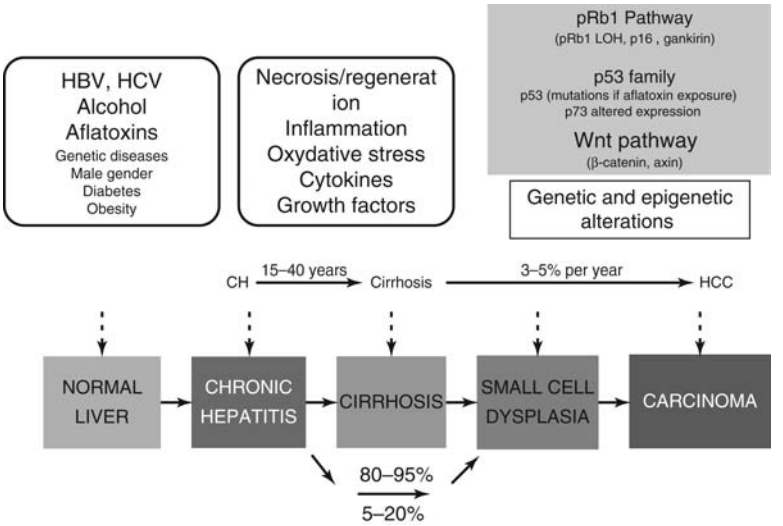


Figure 1 Molecular pathogenesis of human HCC: a multistep process driven by chronic inflammation. Hepatocarcinogenesis is a multistep process that may last for decades and involves the progressive accumulation of different genetic alterations, ultimately leading to malignant transformation. Dysplastic nodules and macroregenerative nodules are considered preneoplastic lesions. The detailed analysis of HCC development in experimental animals and the comparison of the results with HCC in humans have identified a variety of genetic and epigenetic alterations in fully developed HCC and, to a lesser extent, in morphologically defined preneoplastic precursor lesions. *Abbreviation*: HCC, hepatocellular carcinoma.

concept of an indirect action of these viruses. It is generally considered that long-term liver damage due to the immune response against infected hepatocytes triggers chronic inflammation, oxidative DNA damage, continuous cell death, and consequent cell proliferation, and potentiates the action of exogenous carcinogenic factors, such as aflatoxins and alcohol. Increasing experimental evidence suggests, however, that HBV and HCV contribute to HCC by directly modulating pathways that promote the malignant transformation of hepatocytes (4,5,9) (Fig. 2).

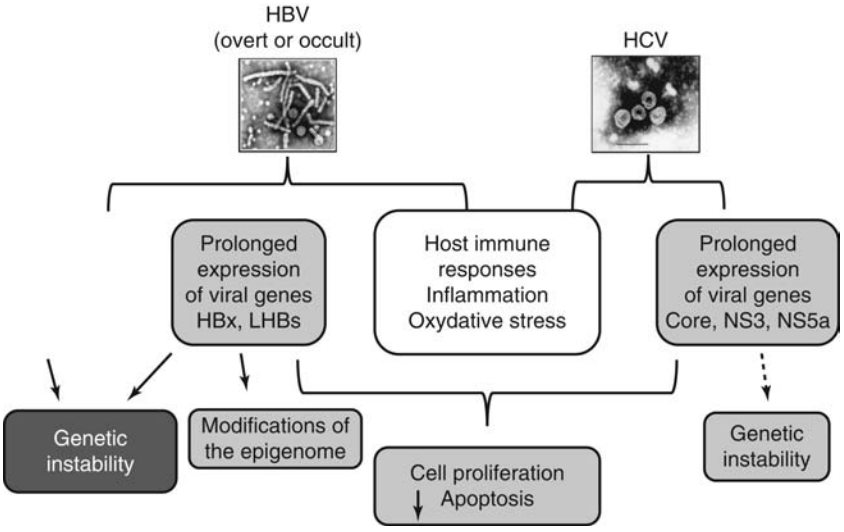


Figure 2 Direct and indirect roles of hepatitis viruses in HCC pathogenesis. Increasing experimental evidence suggests that HBV and HCV contribute to HCC both by directly modulating pathways that promote the malignant transformation of hepatocytes and indirectly by promoting long-term liver damage, chronic inflammation, cell death, regeneration, and oxidative DNA damage. *Abbreviation*: HCC, hepatocellular carcinoma.

Genomic Instability

The majority of HCC cells display a high incidence of chromosome instability that is already evident in cirrhotic liver tissues and has been found to increase during the hepatocarcinogenesis process (10). Chromosome instability measured by the fractional allelic loss has been shown to be an independent prognostic marker of prognosis and recurrence in resected HCC (11). HCC tissues associated with HCV infection show significant losses of heterozygosity (LOH), although the rate of LOH is higher in HBV-associated tissues (10) and both HBV HBx- and HCV NS5a-encoded proteins induce chromosomal instability by affecting the mitotic checkpoints (12,13).

Aging, Telomeres, and HCC

Hepatocytes in cirrhotic livers display decreased proliferation rates with a dominant replicative senescence phenotype characterized by critically shortened telomeres and permanent cell cycle arrest (14). Indeed, the length of telomeres progressively shortens from normal liver to chronic liver disease and reaches the shortest levels in HCC (15,16). The low or absent telomerase activity in cirrhotic liver suggests that the repeated proliferation cycles of hepatocytes in the precirrhotic stages of liver disease leads to the progressive loss of telomere sequences and senescence arrest. The emergence of malignant hepatocytes in the context of senescent cirrhotic tissue requires that transformed cells bypass senescence and survive despite critically shortened telomeres. Many studies have showed that 80% to 90% of HCCs display a high telomerase activity (17). The integration of HBV DNA sequences into TERT gene provides evidence for a virus-induced deregulation of TERT expression (18,19). HBx and PreS2 proteins upregulate TERT expression (20). How TERT expression is reactivated in HCC cells is, however, only partially clarified. The TERT gene promoter displays binding sites for many transcription factors, including the estrogen receptor, Sp1, Myc, and ER81 acting positively, and vitamin D receptor, MZF-2, WT1, Mad, E2F1, and SMAD-interacting protein-1 (SIP1, also called ZEB-2 or ZFHX1B) acting negatively (21). Despite TERT activation, telomers remain very short in HCC cells, and this may predispose to occasional telomere instability leading to increased rate of chromosomal instability and polyploidy that is quite frequent in these tumors (20).

Hepatic Progenitor Cells and HCC

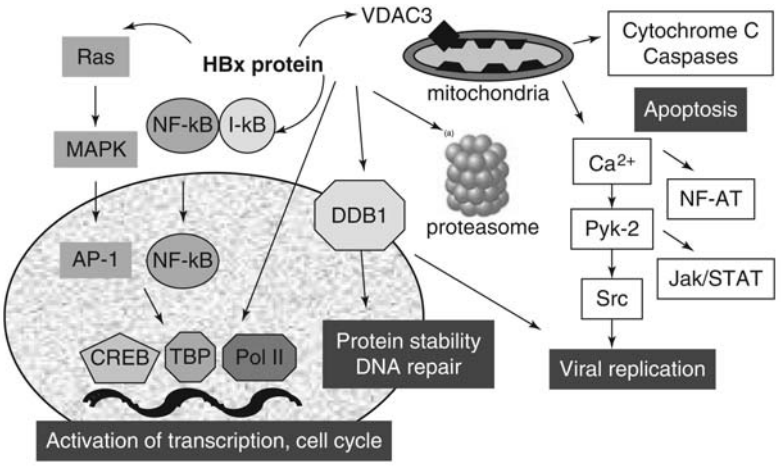
The concept of cancer arising from few stem cells, or more committed progenitors that maintain/reacquire full self-renewal capacity, undergoing an aberrant and poorly regulated process of organogenesis, is well established for leukemias (22). Increasing evidences support similar process in skin cancers, liver cancers, breast cancer, various neoplasias in the gut, gliomas, and medulloblastomas. Stem cells and cancer cells share the ability of self-renewing. Stem cells can be divided into two functional classes: first, stem cells that are responsible for tissue renewal are found in bone marrow, skin, and intestine and are continuously active at a slow rate to replace terminally differentiated cells that are physiologically lost; second, stem cells that are inactive until required in response to environmental factors such as satellite cells in muscles and hepatic progenitor cells (HPCs) in the liver. The concept of cancer stem cells being the targets of transformation and the source of proliferating cancer cells in the fully developed tumors has many critical implication in understanding, managing, and treating cancers. Cancer stem cells might explain the "field cancerization" : even a single phenotypically silent mutation in stem/progenitor cells could generate a cancer-prone field, leading to apparently independent tumors arising from nearby sites. This phenomenon would fit easily with situation observed in liver cancers. Tissue environment and the rate of turnover of tissue stem/progenitor cells in the field will determine the probability of developing a fully transformed cell. Whether stem cells themselves accumulate mutations to generate neoplasis or whether they establish a clone of cancer-prone cells, they make attractive therapeutic targets. If tumor growth and metastasis are driven by a small population of cancer stem cells, this may explain the failure in developing therapies that are consistently able to eradicate solid tumors. Indeed, either cancer stem cells are less sensitive to therapies, thus remaining viable after therapy to reestablish the primary and/or the metastatic tumor, or cancer stem cells may

give raise to new population of drug-resistant cells. HPCs (also called oval cells in rodent models of carcinogenesis) are small epithelial cells, which can differentiate toward both hepatocytes and cholangiocytes and are located in the smallest branches of the biliary tree (canal of Hering and/or the ductular compartment). Additional HPCs can be also found in periductular locations, and some evidence suggests that these particular HPCs may be of bone marrow origin. In animal models, liver cancers can originate from hepatocytes as well as from immature progenitor cells (23). Progenitor cells are activated when the mature cell compartments are damaged and, especially, when these mature cell compartments are inhibited in their replication (23). About 20% to 40% of HCCs display phenotypic markers of HPCs (24) and share a common transcriptional signature with normal HPCs in cDNA microarray-based analysis (25). HCCs expressing progenitor cell features have a worse prognosis and higher recurrence after treatment compared with HCCs that are negative for these markers (25).

VIRUSES AND CANCER

A role for viral proteins in HCC oncogenesis might be sensitization of liver cells to mutagens. HBV infection can promote carcinogenesis by three different mechanisms (reviewed in Ref. 9). First, integration of the viral DNA into the host genome can induce chromosome instability (26). HBV DNA integration in host chromosomes, although dispensable for viral replication, is detected in about 80% of HCCs. Second, classic retrovirus-like insertional mutagenesis can occur with HBV integration at specific sites providing a growth advantage to a clonal cell population in which additional mutations accumulate. Evidence was first provided in two independent HCCs, with retinoic acid receptor β (RARβ) and cyclin A as target genes. Recently, 15 new genes were found to be targeted by HBV integration in tumors, including recurrent HBV DNA integration into the hTERT gene encoding the catalytic subunit of telomerase, suggesting that viral integration in the vicinity of genes controlling cell proliferation, viability, and differentiation is frequently involved in HBV hepatocarcinogenesis (19,27,28). The third mechanism of HBV carcinogenesis linked to HBV infection is based on the ability of viral protein, in particular HBx, to modulate cell proliferation and cell viability and to sensitize liver cells to mutagens. In transgenic mouse models, unregulated expression of the HBV X and S proteins are associated with hepatocarcinogenesis (29,30). The HBx protein (Fig. 3) behaves as a promiscuous transactivator of cellular genes such as oncogenes, growth factors, and cytokines; it binds and inactivates p53 and interacts with the DNA repair protein DDB1, which may affect repair functions and allow the accumulation of genetic changes (9). HBx activates calcium-dependent signaling events that might account for induction of apoptosis (9). HBx also interacts with the peptidyl–prolyl cis–trans isomerase Pin1, and this interaction leads to HBx stabilization, enhanced HBx-mediated transactivation of target genes, and increased cellular proliferation (31). Finally, HBx has been shown to modulate chromatin dynamics in HCC cells and tissues. HBx increases enhance total DNA methyltransferase (DNMT) activity by the upregulation of DNMT1, DNMT3A1 and DNMT3A2, and selectively facilitate the regional hypermethylation of the promoters of certain tumor suppressor genes, such as IGFBP3 (32). HBx also binds several nuclear proteins involved in the regulation of transcription, including component of the basal transcriptional machinery (RPB5, TFIIB, TBP, TFIIH), coactivators (CBP, p300, and PCAF), and transcription factors (ATF/CREB, ATF3, c/EBP, NF-IL-6, Ets, Egr, SMAD4, Oct1, RXR receptor, p53). Recently, it has been shown that HBx is bound in vivo to the promoters of a number of CREB-regulated genes and favors transcription by increasing the amount of CBP and p300 recruited on the same promoters (33). Rearrangement of integrated HBV sequences in HCC may also lead to abnormal expression of the S gene protein. Specific activation of cRaf-1/Erk2 signaling by the truncated pre-S2S protein results in an increased proliferation rate of hepatocytes (34).

In the case of HCV the virus-induced chronic inflammation and the effects of cytokines in the development of fibrosis and liver cell proliferation are considered major pathogenic mechanisms. Increasing experimental evidence suggests that also HCV contributes to HCC by



HBx interacting proteins

Apoptosis	p53
Mitochondrial associated proteins	HVDAC3
Heat shock proteins	Hsp60, Hsp70
Signal transduction pathways	14-3-3
Proteasome complex	PSMA7, PSMC1
DNA Repair	UV-DDB1
Chromatin Remodeling	PCAF, CBP, p300, HDAC1, DNMT3a
Basal transcription	RPB5, TFIIB, TBP, TFIIH (ERCC2, ERCC3)
Transcription Factors	RXR, RMP, Egr-1, NFkB
bZip transactivators	CREB, ICER IIg, Gadd143/Chop10, ATF2, ATF3, NF-IL6
Peptidyl-prolyl-isomerase	Pin1
Unknown	HBXIP, PA28

Figure 3 Multiple cellular targets of the regulatory protein HBx. The regulatory protein HBx, in addition to being required for viral replication, contributes to hepatocytes transformation by multiple mechanisms mediated by the interaction with a large number of cellular proteins that modulate cell proliferation, cell death, transcription, and DNA repair.

directly modulating pathways that promoting the malignant transformation of hepatocytes (reviewed in Ref. 5). HCV is an RNA virus that does not integrate into the host genome, but HCV proteins interact with many host cell factors well beyond their roles in the viral life cycle and are involved in a wide range of activities, including cell signaling, transcription, cell proliferation, apoptosis, membrane rearrangements, vesicular trafficking, and translational regulation. At least four of the HCV gene products, namely, HCV core, NS3, NS4B, and NS5A, have been shown to exhibit transformation potential in tissue culture, and several potentially oncogenic pathways have been shown to be altered by the expression HCV proteins (Fig. 4). Transgenic mice carrying the complete HCV genome or the HCV core develop liver steatosis and have high rates of HCC (35), perhaps through interaction with cellular proteins required for the control of cell growth. The HCV core protein induces oxidative stress in transgenic mice (5). The NS5A protein of HCV can sequester p53 in the cytoplasm, downregulate p21, activate STAT3, and inhibit TNFα-mediated apoptosis as well as alter intracellular calcium levels and induce oxidative stress. Both HCV core and NS5A induce the accumulation of wild-type β-catenin (36,37).

Thus, persistent stimulation of cellular stress responses by accumulation of viral proteins within hepatocytes predisposes the cell to genetic alterations and plays an important role in HCC. Whether HCC once established becomes independent of viral genes expression is unknown.

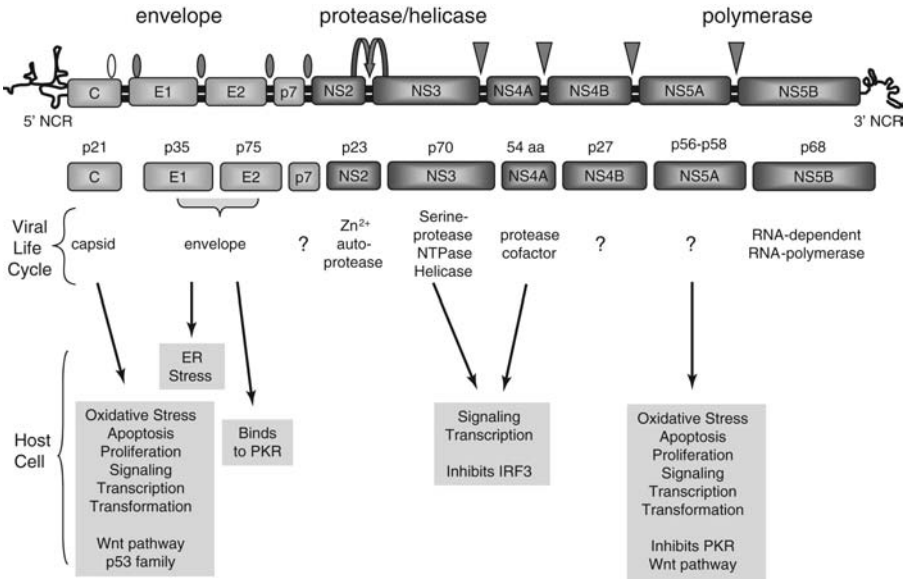


Figure 4 HCV proteins. Besides their roles in the viral life cycle, HCV proteins interact with many host cell factors and impact on a wide range of cellular activities, including cell signaling, transcriptional modulation, transformation, apoptosis, membrane rearrangements, vesicular trafficking, and translational regulation.

GENETIC AND EPIGENETIC ALTERATIONS IN HCCs

Different genetic alterations have been described in human HCC, including gain and loss of chromosomal DNA, allelic losses (LOH) on several chromosomal regions, and mutations of oncogenes and tumor-suppressor genes, yielding a very heterogeneous profile of alterations. A number of recurrent chromosomal alterations that do not appear to be linked to specific HCC risk factors have been detected by microsatellite allelotypes and comparative genomic hybridization (CGH). Allelic losses occur more frequently on chromosomes lp, 4q, 6q, 8p, 9p, 13q, 16p, 16q, and 17q, whereas DNA gains are found on chromosomes 1q, 7q, 8q, and 17q (reviewed in Ref. 38). These observations suggest that these parts of the human genome contain important genes involved in hepatocellular carcinogenesis, and targeted tumor-suppressor genes have been identified on chromosomes 17p, 13q, 16p, 9p, and 6q, corresponding to inactivation of TP53, RB1 (retinoblastoma 1), axin1 (axis inhibition protein 1), CDKN2A (cyclin-dependent kinase inhibitor 2A, also named p16), and IGF2R (insulin-like growth factor 2 receptor), respectively. Accumulation of large-scale chromosomal alterations probably reflects the fact that control mechanisms that safeguard chromosomal integrity are abrogated. In this respect, it is important to point out that, although all HCCs display a relevant grade of genomic instability, HBV-related tumors generally harbor a higher rate of chromosomal abnormalities than tumors linked to other risk factors (39,40). As already mentioned, it has been proposed that HBV infection might directly generate genomic instability, either through viral DNA integration (26) or through the activity of the X protein (12). In addition to genetic mutations, epigenetic mechanisms such as hypermethylation of promoters containing CpG island have been shown to frequently modify gene expression patterns in HCC. A number of tumor suppressor genes, including pl6INK4A, SOCS-1, APC, RASSF1A, GSTP1, and E-cadherin, are silenced by DNA methylation in a large proportion of liver tumors, and this process often starts at preneoplastic (cirrhotic) stages (41). In some reports, a higher rate of promoter methylation for specific genes such as pl6INK4A and E-cadherin has been observed in HBV-related tumors compared with non- viral tumors, but the mechanisms are unknown (42–44). Recently, it has been shown that the extent of genome-wide hypomethylation and regional CpG hypermethylation of tumor suppressor genes correlate with the biological features and clinical outcome of HCC patients (45). Activation of Ras and downstream Ras effectors due to epigenetic silencing of inhibitors of the

Ras pathway was very frequent in HCC. Further, the selective inactivation of SPRY1 and SPRY2, DAB2, and SOCS4 and SOCS5 genes and inhibitors of angiogenesis (BNIP3, BNIP3L, IGFBP3, and EGLN2) was associated with poor prognosis. Importantly, epigenetic silencing of many putative tumor suppressor genes found in HCC was also detected in nontumorous liver and may play a role in predisposing to liver transformation.

A number of genetic alterations that are characteristic of specific carcinogens or transformation mechanism have also been described. After exposure to the mycotoxin AFB1 and the conversion of AFB1 to its active metabolites, specific DNA adducts bearing GC > TA transversion are found in the liver. In particular, a G > T transversion at codon 249 of the tumor suppressor protein p53 (TP53) leading to the arginine to serine amino acid substitution R249S can be found in 50% to 90% of HCCs from regions where AFB1 exposure level is high (46). AFB1 exposure has also been related to specific chromosomal deletions (16q23) in tumors and to the deletion of a chromosomal fragile site located in the WWOX tumor-suppressor gene. Chromosome 4q deletions differ in HCCs related to HBV infection compared with those related to alcohol intake (47). KRAS mutations have been observed in 33% of 18 vinyl chloride–associated HCCs, and three mutations were found in adjacent non-neoplastic liver tissue (48). Vinyl chloride is a carcinogen associated with the development of liver angiosarcomas and rarely with HCC. Inactivation due to biallelic mutations of the transcription factor (TCF) 1 gene, coding for hepatocyte nuclear factor (HNF) 1, is present in 60% of the liver cell adenoma cases (49) and may be rare early step in the development of some HCCs occurring in noncirrhotic liver.

CARCINOGENETIC PATHWAYS ALTERED IN HCCs

Altogether, the genetic and epigenetic alterations accumulated in HCC are quite numerous, with over 20 different genes and at least five principal signaling pathways involved. In addition, components of the same carcinogenetic pathways may also be functionally deregulated in the absence of direct mutations or epigenetic changes or targeted by viral proteins (Fig. 5).

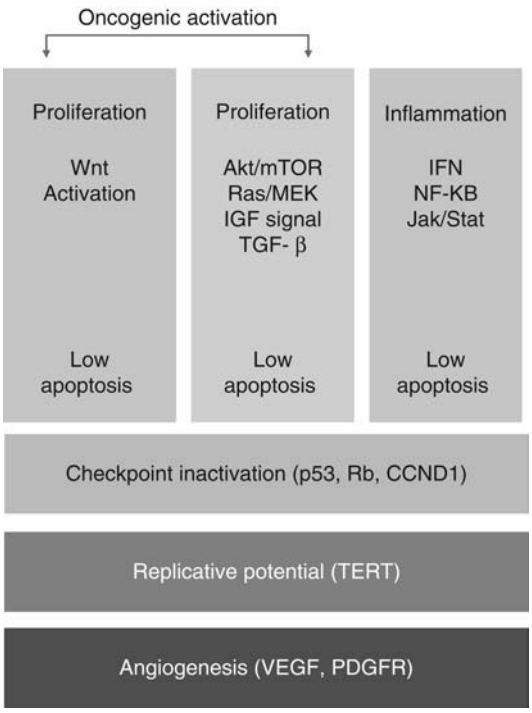


Figure 5 Carcinogenetic pathways altered in HCCs. The genetic and epigenetic alterations accumulated in HCC are quite numerous, with over 20 different genes and at least 5 principal signaling pathways involved. In addition, components of these as well as additional carcinogenetic pathways are functionally deregulated in the absence of direct mutations or epigenetic changes or targeted by viral proteins. As a result, HCC display a loss of the cell cycle and genome integrity checkpoints controlled by pRb and p53, an increased replicative potential due to TERT activation and an important neo-angiogenesis. *Abbreviation*: HCC, hepatocellular carcinoma.

The tumor suppressor protein p53 protects cells from accumulating genetic damage. p53 is stabilized and activated in response to DNA damaging signals and induces growth arrest (until DNA can be repaired) or apoptosis. Inactivation of p53, either by mutation or functional interactions, allows cells with compromised genome integrity to survive inappropriately and to accumulating multiple genetic lesions that eventually lead to transformation. Mutations in p53 are the most common in all human cancers. In HCCs the genetic inactivation of TP53 is observed in 10% to 50% of the cases (50). HBx also binds to the p53 protein and interferes with its proapoptotic activity by sequestering it in the cytoplasm, by directly interfering with its DNA transcriptional activity, or by disrupting protein–protein interactions between p53 and other cellular proteins (9). The "hot spot" mutation of codon 249 of p53 tumor suppressor gene, leading to the expression of a DNA binding defective p53 mutant protein (51), is found in up to 70% of HCCs in patients from southern Africa and the Qidong area in China and it is associated, as already mentioned, with high food contamination with the AFB1 mycotoxin (52). In other regions, where aflatoxin levels in food are low or undetectable, p53 mutations are rarely observed (<4% of HCCs) and no specific gene mutation pattern can be detected, whereas the p53 paralog and tumor suppressor p73 is often deregulated. Although p73 point mutations are rare in human cancers (53,54), it has been recently shown that mice that are p63+/− or p73+/− develop malignant tumors at high frequency. In addition, p53+/−, p63+/− and p53+/−, and p73+/− mice develop a more severe phenotype (higher tumor burden and metastases). These results indicate that p73 and the entire p53 family have an important role in tumor suppression (55). p73 gene gives rise to multiple protein isoforms because of alternative promoter utilization and alternative mRNA splicing. The different isoforms can be classified in two main classes: those containing the TA domain (TAp73) and those lacking it (DNp73). TA proteins mimic p53 function in cell culture, including transactivating many p53 target genes and inducing apoptosis, whereas DNp73 isoforms do not activate transcription but instead they acts as a dominant negative inhibitors of its own TA isoforms and of p53 (56), and they may have oncogenic effects (54).

p73 gene expression is transcriptionally regulated by E2F-1 during the S phase of the cell cycle, and TAp73 proteins accumulate in S phase cells (57). In many tumors, pRb inactivation and E2F1 deregulation lead to the overexpression of wild-type TAp73 as well as of other pro-apoptotic E2F1 target genes, including APAF1, caspase 7, and caspase 3. This results in a higher sensitivity of transformed and highly proliferating cells to apoptotic stimuli that are counteracted during tumor progression by mutation or inactivation of apoptosis effector genes and the activation of anti-apoptotic genes and pathways. The P1p73 promoter is activated by E2F1 in response to DNA damage to elevate TAp73 protein levels in the cell (58), and the E2F1–TAp73 pathway is an important component of the cell response to DNA damage and a major determinant of tumor chemosensitivity, in particular when p53 is inactivated (59,60). The activity of E2F1 on the P1p73 promoter in cells exposed to DNA damaging agents is positively regulated by the PCAF acetyltransferase and the WW-domain protein YAP and down-regulated by the histone deacetylases hSirt1 and HDAC1 (58,61). The selective abrogation of PCAF, p73, or YAP expression by specific siRNAs greatly reduces p53-independent apoptosis in cells exposed to DNA-damaging drugs (58,61). Interestingly, YAP ability to coactivate E2F1 and to induce apoptosis in response to DNA-damaging drugs is abrogated by its retention in the cytoplasm mediated by the AKT kinase and potentiated by drugs that inhibit AKT (61). Conversely abrogation of hSirt1 expression or inhibition of its enzymatic activity results in a strong increase of drug-induced cell apoptotic cell death. More recently, we have identified the functional interaction between PCAF and the class III deacetylase hSirt1 as an important determinant for P1p73 activity and E2F1-dependent p73-mediated apoptotic responses in HCC cell lines (62). DNp73s is expressed from an alternative P2p73 promoter (56). DN-p73 proteins accumulate progressively in chronic hepatitis, cirrhosis, and HCC and confer to HCC cells a chemoresistant phenotype (63,64). The P2p73 promoter is controlled in hepatocytes, in addition to NFkB, p53, and p73, by β-catenin (64). Abrogation of β-catenin expression leads to decreased expression of DNp73 and strong potentiation of apoptosis in response to DNA-damaging agents. These results link β-catenin activation with p53 and TAp73 functional inactivation that is mediated by DNp73 overexpression and does not require the selection of p53 gene-inactivating mutations.

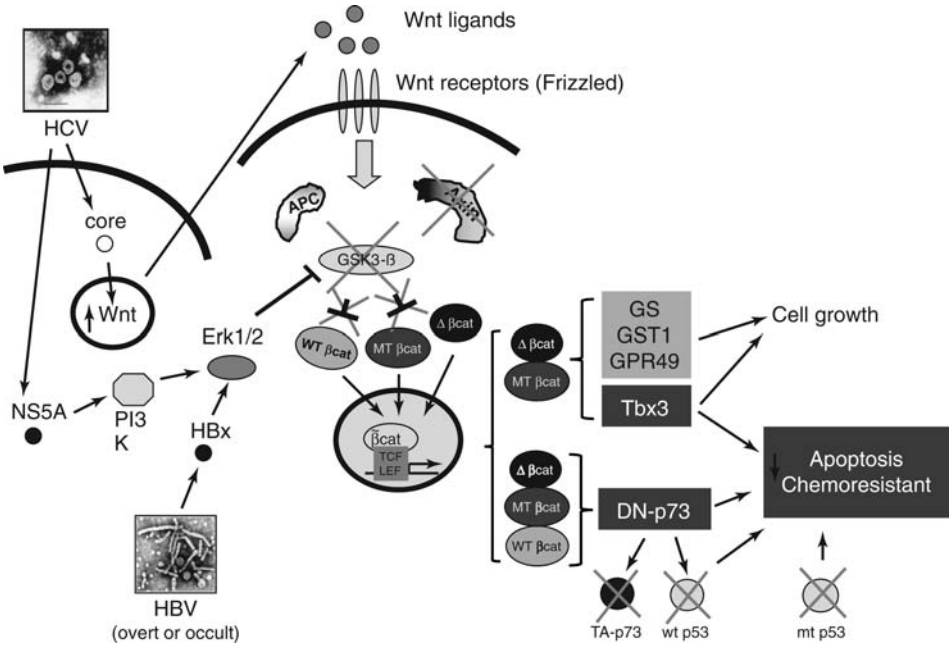


Figure 6 The Wnt pathway is targeted by genetic and epigenetic events in HCV-related HCCs. β-Catenin accumulation in human HCC may be triggered by multiple mechanisms. Activating mutations of β-catenin and axin1 gene mutations occur in a substantial proportion of HCC patients but do not account for all HCC cases with β-catenin nuclear accumulation. Fzd-7 is overexpressed in many human HCCs and induces wild-type β-catenin stabilization/activation. HCV core protein upregulates transcription of several growth-related genes, including Wnt-1 that is secrete and activates signaling through the frizzled receptors. NS5A activates the PI3K (65), which in turn phosphorylates and inactivates the GSK-3, leading to accumulation of β-catenin and stimulation of β-catenin-dependent transcription. The HBV-encoded protein HBx also activates the β-catenin pathway through an Erk-mediated inactivation of GSK-3. In HCCs, β-catenin activates a specific transcriptional program that includes glutamine synthetase, ornithine aminotransferase, glutamate transporter 1, the cytochromes CYP1A2 and CYP2E1, GPR49, TBX3, and DNp73, but not the well-known Wnt downstream cell cycle target genes cMyc and yclinD1. β-Catenin-dependent overexpression of DNp73 leads to p53 and TAp73 functional inactivation without requiring the selection of p53 gene-inactivating mutations. *Abbreviations*: HCC, hepatocellular carcinoma; GSK-3, glycogen synthase kinase-3; Fzd-7, frizzled-type 7 receptor.

The activation of the cell proliferation and pro-survival Wnt/wingless pathway is activated in a large proportion of human HCCs as a result of β-catenin-activating mutations and axin1-inactivating mutations, or functional inactivation of GSKβ3 kinase by HCV and HBV oncoproteins, or overexpression of Wnt ligands and frizzled receptors (Fzds) (51) (Fig. 6). The Wnt/frizzled/β-catenin signaling is mediated by a complex interaction between a Wnt ligand (Wnt) and an Fzd, mostly in cooperation with the low-density lipoprotein receptor (LDLR)-related protein (LRP-5 or LRP-6) coreceptors. The Wnt/β-catenin pathway is involved in developmental control, cell adhesion, and cell proliferation. Cellular levels of β-catenin are tightly regulated by proteasome-dependent degradation, which is in turn controlled by the activity of the Adenomatosis Polyposis Coli (APC) and axin1 proteins and the GSK3β kinase. Accumulation of nuclear β-catenin-containing complexes leads the unrestricted transcription of β-catenin/TCF downstream target genes, including several cell cycle control genes. Interestingly, the expression of miR21, a micro RNA (miRNA) overexpressed in HCC (see below), is regulated by β-catenin (66). Importantly, aberrant Wnt/β-catenin signaling has been shown to induce chromosomal instability in colon cancer (67), but the potential role of Wnt deregulation in chromosomal instability in HCCs remains to be investigated. Differently from colon carcinoma, however, somatic APC gene mutations appear to be rare in HCC, whereas activating mutations of β-catenin were reported in 15% to 40% of HCCs (51), and axin1 gene mutations, with β-catenin accumulation in the absence of mutation of the β-catenin gene, have been found in 9% of HCCs (68). Frizzled-type 7 receptor (Fzd-7) is overexpressed in

about 90% of HCV-related HCCs and in around 75% of the corresponding peritumorous/precancerous liver parenchyma as compared with normal livers leading to wild-type β-catenin stabilization/activation (69). Recently, it has also been reported that the HCV core and NS5A and the HBV HBx proteins induce the accumulation of wild-type β-catenin through the functional inactivation of the GSK3β kinase (36,37,70). Altogether, the Wnt/β-catenin pathway emerges as a common target for HCV and HBV in human HCCs, also independently from axin/β-catenin gene mutations. Both in the normal adult liver and HCCs, β-catenin activates a specific transcriptional program that includes the liver-specific enzymes glutamine synthetase (GS), ornithine aminotransferase, glutamate transporter (GLT-1), the cytochrome P450 enzymes CYP1A2 and CYP2E1, the G-protein-coupled receptor GPR49, the T-box gene family transcriptional repressor TBX3, and the dominant negative p53-paralog DNp73 oncogene, but not the well-known Wnt downstream cell cycle target genes cMyc and cyclinD1 (64,71,72). Recently, activating β-catenin mutations and loss of function mutation in axin1 have been found to direct the activation of different subsets of β-catenin target genes in liver cancers (71). Both the tumor-derived gain-of-function β-catenin G34V mutant and the deleted β-catenin expressed in HCCs activate the liver-specific GS, GPR49 genes, and the anti-apoptotic genes Tbx3 and DNp73, whereas wild-type β-catenin activates Tbx3 and DNp73 expression and only to a much lesser extent the GPR49 and GS promoters (71). Thus, DNp73 and Tbx3 are direct target genes of β-catenin irrespective of its activating mechanisms, and β-catenin overexpression contributes to HCC chemoresistance and p53/TAp73 functional inactivation, mediated by Tbx3 and DNp73 overexpression.

Other pathways have been found to be frequently activated in HCCs either by genetic and epigenetic events or by functional interactions. Inactivation of the tumor suppressor retinoblastoma pathway occurs in up to 50% of HCCs through RB1 and CDKN2A promoter methylation (73,74) and the rare gankirin gene mutation (75). The proliferative and pro-survival IGF pathway is activated through either IGF2 overexpression or rare IGF2R-inactivating mutations (76). The extracellular signal-related kinase (ERK) pathway [also known as the mitogen-activated protein kinase (MAPK)] pathway is a mitotic and pro-survival signal transduction cascade that is involved in a large number of cell functions. Blocking ERK signaling induces growth arrest and apoptosis in human HCC cell lines. The small GTP protein Ras and the serine/threonine kinase Raf are important molecular signal transducers in this pathway. Persistent overexpression of cRaf is involved in carcinogenesis and, phosphorylated cRaf has been found to be overexpressed in almost all HCC and liver cirrhosis specimens examined (77). Downstream cRaf signaling is mediated by MAPK/ERK kinase (MEK), also known as MAPK kinase. MEK in turn phosphorylates and activates the final downstream signaling molecules ERK1 and ERK2. ERKs are activated in 93% of HCC and 53% of liver cirrhosis samples (77). The phosphatidylinositol-3 kinase (PI3K) anti-apoptotic and pro-survival signaling pathway is also affected in HCCs. PI3K is regulated in part by action of PTEN, the product of the phosphatase, and tensin homolog gene, a tumor suppressor gene that is mutated in many human cancers. The hepatocytes from PTEN knockout mice are hyperproliferative, resistant to apoptosis, and display an abnormal activation of the PI3K downstream kinase AKT and MAPKs (78). Cumulatively, deletion of the PTEN gene or, more often, reduced expression of its protein product due to promoter methylation is found in nearly half of patients with HCC (79–81). Decreased PTEN is an independent prognostic factor for worse overall survival and is significantly correlated with higher-grade tumors and advanced disease stage (79). As already mentioned, both HBV- and HCV-encoded proteins alter the PI3K-signaling pathway, and HBx protein directly interacts with both PI3K and AKT (37,82). A crucial biological event for HCC tumor progression is the development of new blood vessels that starts in the pre-neoplastic and in the early neoplastic lesions. Normal angiogenesis is controlled by a balance between proangiogenic and antiangiogenic factors that are disrupted during HCC development and progression because of the increased secretion of angiogenic factors [vascular endothelial growth factors (VEGFs), platelet-derived growth factors (PDGFs), placental growth factor (PlGF), transforming growth factor-α and transforming growth factor-β (TGF-α and TGF-β), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), angiopoietins (ANGs), and interleukins (IL-4 and IL-8)] by tumor cells, tumor-infiltrating inflammatory cells, and

surrounding normal liver cells (83). A crucial role is played in the process by the receptors for these angiogenic factors and in particular the receptor tyrosine kinases (RTKs) VEGFR-1 (or FLT-1), VEGFR-2 (or KDR or FLK-1), and VEGFR-3, the PDGFRs, the bFGFRs, and Tie-2, the receptor for angiopoietins. Development of new blood vessels requires interaction among multiple cell types, including endothelial cells, pericytes (stromal smooth muscle-like cells that surround capillaries), and tumor cells. Endothelial cells secrete PDGF, stimulating and recruiting other endothelial cells and pericytes. Pericytes secrete VEGF and angiopoietins, which in turn stimulate and recruit endothelial cells. HCC tumor cells secrete VEGF, bFGF, PDGF, and TGFs- that support the recruitment and stimulation of blood vessel cell types. VEGFs are the best-investigated growth factors implicated in HCC. Serum concentrations of VEGF increase during disease progression and have been used as a prognostic marker in HCC. HBV may also contribute directly to HCC tumor progression by stimulating angiogenesis. The HBx protein activates the expression of several hypoxia-induced angiogenic factors that are secreted in response to the low oxygen level (hypoxia) in the center of growing HCCs (84).

MICRO RNA AND HCC

miRNAs are short noncoding RNAs that regulate gene expression by binding complementary sequences in the 3′UTRs of their target mRNAs and inducing their degradation or translational repression. Hybridization-based microarrays and qRT-PCR have both been used to investigate miRNAs expression profiles in human HCCs and have identified nonoverlapping signatures of miRNAs up- and downregulated in human HCCs as compared with the paired peritumoral cirrhotic tissues or cirrhotic livers without HCC (85–89). We have recently confirmed the upregulation (HCC vs. paired NT tissues) of miR-18a, miR-21, miR-34a, miR-221, and miR-224. miR-199a was downregulated in tumor samples as compared with both the paired NT tissues and normal livers. Importantly, the combined evaluation of three miRNAs (miR-21 and miR-221 or miR-224 upregulation and miR-199a downregulation) allowed the correct classification of all tumor samples (66). Specific targets of miRNA modulated in HCC have also been recently identified. Thus, miR-221 controls p57 and p27 CDKNs (90), miR-122 targets cyclinG1, and its downregulation might contribute to genomic instability in HCCs (91), whereas miR-21 targets the tumor suppression gene PTEN, leading to the activation of AKT pathway (92). Much less is known on the pathways and transcription factors that regulate miRNA's expression in the liver. Sequence analysis and binding sites scanning of miR-21, miR-221, miR-224, and miR-34b pre-miRNA promoters revealed the presence of multiple E2F1, NFkB, TCF/β-catenin, and p53 responsive elements. ChIP analysis showed that p65/NFkB was recruited on both the miR-21 and miR-224 promoters but not on the miR-221 promoter. β-Catenin was also recruited onto the miR-21 promoter. Both miR-21 promoter occupancy and transcription of the endogenous miR-21 were strongly stimulated by LiCl-induced β-catenin stabilization that mimics GSKβ3 functional inactivation and axin1 loss of function. miR-34b is regulated at the transcriptional level by the p53 family members. Identification of deregulated miRNAs not only contributes to the understanding of molecular pathways and circuits involved in human HCCs and, potentially, to the identification of new therapeutic targets but can also provide strong diagnostic signatures.

GLOBAL STUDY OF THE MOLECULAR ALTERATIONS IN HCC

Genome-wide analysis of genetic alterations in HCC has showed that genetic alterations are not randomly distributed in tumors but are closely associated in clusters. These studies have also enabled the definition of two main mechanisms of hepatocarcinogenesis, the classification of HCCs in subsets of genetically and molecularly homogeneous tumors, and have identified a number of biomarkers potentially useful for diagnosis, classification, and prognostication. Laurent-Puig and coworkers (40) have showed, in a series of 137 HCC tumors from France, that HBV infection is closely related with a higher chromosome instability together with TP53 and axin1 mutations. A second hepatocarcinogenesis pathway, defined by the presence of β-catenin mutation associated with chromosome 8p deletion in a context of chromosome

stability is associated with the absence of HBV infection (40). More recently, the same group has performed a genome-wide transcriptomic analysis of 60 HCC tumors, well annotated for all the known relevant genetic and clinical parameters (65) (Fig. 7A). The unsupervised transcriptomic analysis identified six subgroups of HCC (termed "G1–G6") associated with specific clinical and genetic characteristics. Tumors from groups G1 to G3 were highly chromosome unstable, whereas tumors from G4 to G5 were more chromosome stable (G4–G5). G1 and G2 tumors were both related to HBV infection and displayed frequent activation of the PI3K/AKT pathway but differed for the overexpression of genes expressed in fetal liver and controlled by parental imprinting (G1) and the frequent mutation of the PIK3CA and TP53 genes (G2). G3 tumors showed overexpression of genes controlling the cell cycle associated with either TP53 mutations or P16 methylation. G4 was more heterogeneous subgroup of HCCs that included the rare HCCs with TCF1 mutations. G5 and G6 were strongly related to β-catenin mutations leading to Wnt pathway activation, with the G6 tumors displaying a more aggressive phenotype with satellite nodules and E-cadherin underexpression. A small signature of 16 genes allows the correct classification of all analyzed HCC to one of these subgroups. Lee and coworkers (41) have analyzed the expression profile of 91 primary HCCs and 60 matched non-tumor-surrounding samples from patients from China and revealed two distinct clusters (A and B) of differentially expressed genes that predicted difference in survival. The gene expression signature of the low-survival HCC group included cytokeratin 19, an intermediate filament cytoskeletal protein that is also a marker of hepatic progenitor cells, genes involved in cell proliferation, and anti-apoptosis. Overall, the cluster A signature significantly overlapped with the genes expressed in the G1/G2 HCC groups described by Boyault and colleagues (65). The overexpression and the prognostic role of CK19 in HCCs have been confirmed by several studies and by proteomic analysis (93). Wurmbach and coworkers (94) have used a similar transcriptomic analysis to identify several genes that define the transition from cirrhosis to dysplasia, early and advanced HCCs related to HCV infection (Fig. 7B). They found that in cirrhotic tissues JAG1 (a ligand of notch receptors), STAT1 and the toll-like receptors CXCL9-11 are overexpressed. In dysplastic nodules, the EPO, EPOR, and CISH genes are induced together with genes of the Jak/STAT pathway (STAT3 and SOCS3) and the GADD45B, GADD45G, and GADD45A genes involved in cell cycle control. In early HCC, there is a decreased expression of genes of the toll-like receptor, Jak/STAT and TGF-β pathways (TLR4, CD14, IFNAR1, FOS, EPO, EPOR, SPRY2, SOCS2, BMPR2, ID2, THBS1, and DCN), whereas a number of genes of the β-catenin and hedgehog pathways (DKK1, Fzd6, Fzd7, PLCB1, LEF1, and PTCH) are overexpressed. In advanced HCCs, the activation of cell cycle and mitosis-controlling genes (PRIM1, PRIM2, ASPM, PTTG1, CCNB1, CDKN2C, CDKN2A) is the prevalent alteration. In a second paper, Llovet and colleagues also show how the evaluation by real-time qPCR of only five genes (3 upregulated: TERT, glypican 3, and survivin; 2 downregulated: LYVE-1, E-cadherin) allows to discriminate dysplasia from early HCC in fine needle biopsy samples from patients (95) (Fig. 7B). Real-time RT-PCR has been also used to develop a molecular diagnostic index for HCC (96). Among more than 200 candidate genes, 13 were found to be overexpressed in HCCs relative to premalignant or normal liver tissue and were validated for their diagnostic value when combined. These genes included the RTKs PDGFRa and Tie-2, IGF2, the adhesion or gap junction components connexin 26 and thrombospondin 1, and the DNA-modulating enzymes topoisomerase II and TERT (96).

FROM MOLECULAR SIGNATURES TO TARGETED THERAPIES

Although molecular signatures were mostly designed to discriminate among HCC, cirrhosis, and normal liver tissue for diagnostic purposes and to classify tumors according to their prognosis, they may also be used to determine the presence of the alteration of specific pathways and to predict putative response to targeted drugs. Many targeted anticancer agents that have been clinically approved recently or are under development focus on components of the signaling networks that are altered in HCCs. Recently, one of these targeted drugs sorafenib, an oral multikinase inhibitor that targets both the tumor cell and tumor vasculature, has been approved by the U.S. FDA and the EC EMEA on the basis of results of the large phase III SHARP

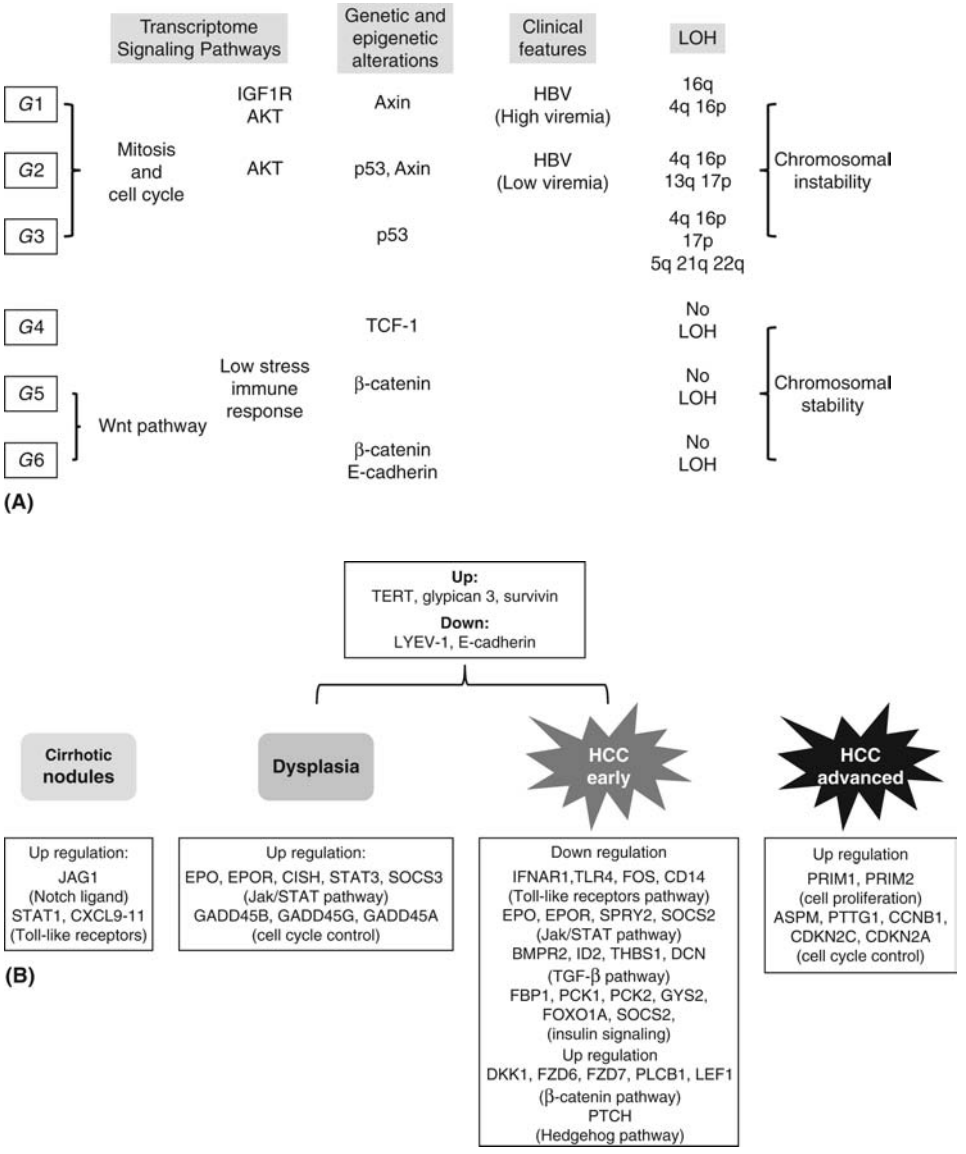


Figure 7 Genome-wide studies and transcriptomic signatures in HCC. The combination of genome-wide assessment of genetic and epigenetic alterations, together with transcriptome and systematic pathway analyses, has confirmed the molecular diversity of HCCs and also allowed the molecular classification of HCCs. **(A)** The genome-wide transcriptomic analysis performed by Zucman-Rossi and coworkers (65) identifies six homogeneous subgroups of HCCs (termed "G1–G6") associated with specific clinical and genetic characteristic. **(B)** Specific sets of genes define the transition from cirrhosis to dysplasia, early and advanced HCCs related to HCV infection (94). *Abbreviation*: HCC, hepatocellular carcinoma.

study for the treatment of patients with advanced HCC and conserved liver function (97). Sorafenib blocks tumor-cell proliferation by targeting Raf/MEK/ERK signaling at the level of Raf kinase and exerts an antiangiogenic effect by targeting VEGF receptors (VEGFR-2, VEGFR-3) as well as several receptor tyrosine kinases, including vascular PDGF receptor (PDGFR), FLT3, Ret, and cKit. Interestingly, in the phase II study of sorafenib in patients with advanced HCC that preceded the SHARP trial (98), patients with tumors expressing a higher baseline of phosphorylated ERK levels had a longer time to progression following treatment with sorafenib compared with patients with lower pERK-expressing tumors.

CONCLUSIONS

Extensive evidence indicates that HCC is an extremely complex tumor at the molecular and genetic levels. Major risk factors for developing HCC include chronic HBV or HCV infection and the exposure to environmental or ingested toxins. These lead in most cases to liver cirrhosis and the accumulation of genetic and epigenetic changes, such as activation of oncogenes and inactivation of tumor suppressor genes. Abnormal hepatocyte proliferation and survival result from a complex network of interacting signaling pathways that include increased growth factor/growth factor receptor signaling (e.g., EGFR, IGFR), constitutive mitogenic intracellular signaling (e.g., Raf/MEK/ERK, PI3K/AKT, and Wnt/β-catenin pathways), inactivation of pro-apoptotic and anti-proliferative tumor suppressors (e.g., p53 and pRb), increased anti-apoptotic signaling (e.g., PTEN/AKT, NFkB). The initial steps of carcinogenesis are followed by tumor angiogenesis, mediated by tumor cell/stromal cell interactions and the activation of multiple angiogenic factors. These cellular, molecular, and genetic abnormalities—and others that are being identified through gene expression profiling—represent potential therapeutic targets to inhibit the initiation and progression of HCC. Molecular signatures and biomarkers identified through transcriptomic and proteomic approaches and global classification of HCC will be of pivotal importance to define homogeneous subgroups of tumors with similar genotypes and signaling pathways alterations.

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3 HCC Screening and Surveillance

Ryota Masuzaki and Masao Omata
Department of Gastroenterology, University of Tokyo, Tokyo, Japan

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide (1–5). The majority of cases with HCC have a chronic liver disease in the background liver, especially chronic hepatitis because of a hepatitis C virus (HCV) or hepatitis B virus (HBV) infection (6,7). Thus, at least some of the high-risk patients for HCC can be readily demarcated. Indeed, regular HCC surveillance is commonly performed as part of standard clinical practice for chronic viral hepatitis patients (8).

To conduct surveillance of HCC in patients with chronic liver diseases, ultrasonography and tumor marker tests play important roles and are being widely used. However, there is insufficient evidence to suggest that surveillance by ultrasonography or tumor marker tests improves the prognosis of patients with HCC or increases the chances of local therapies, such as resection and local ablation therapy, or indeed radical treatments such as liver transplantation. Similarly, usefulness of computed tomography (CT) or magnetic resonance imaging (MRI) in the surveillance of HCC remains unclear.

The primary objective of screening and surveillance for HCC should be to reduce mortality as much as possible in patients who actually develop the cancer, in an acceptably cost-effective fashion. To attain this objective two distinct issues deserve meticulous consideration: target population and the mode of surveillance.

TARGET POPULATION

HCC is a type of cancer that has been observed to show significant regional clustering (9). HBV, HCV, and other environmental factors may play important roles in the development of HCC, with the relative importance of individual factors varying widely in each geographic area (7,10–12). In Japan, HCV infection is responsible for about 80% cases of HCC, whereas HBV infection is responsible for 10% and alcohol for about 5% (4,13). These values may differ substantially in other countries. For example in China, where the prevalence of HBV infection is much higher, HBV infection is by far the predominant etiology of HCC. In the United States, nonalcoholic steatohepatitis (NASH) is reportedly a major background of the cancer. Surveillance is not recommended for the general population, given the low incidence of HCC among population with no risk factors. A commonly accepted rate that requires surveillance is greater than 0.2% per year. Thus, the first step in the screening for HCC should be the screening for patients at a risk of HCC development. Since chronic viral hepatitis, due either to HBV or HCV, may be asymptomatic, mass screening for hepatitis virus infection of either HBV or HCV, will be justified if the prevalence of infection is reasonably high in the region. Indeed, mass screening for HBV and HCV infection has been performed among general population over 40 years of age in Japan since 2002. The cost-effectiveness of this program is, however, yet to be evaluated.

Persistent infection with HBV is a major risk factor for HCC. HBV carriers have a 223-fold higher risk of developing the cancer than noncarriers (14). Of the HBV carriers, HBe antigen positive patients are at a higher risk of HCC than HBe antigen negative ones (relative risk, 6.3 times) (15,16). Recently the results of a large-scale, long-term cohort study conducted in Taiwan has been reported, showing that the serum level of HBV DNA is the strongest risk factor for both the progression to cirrhosis and the development of HCC among HBV-positive patients, independently of serum HBe antigen/antibody status or alanine aminotransferase (ALT) levels (17). Together with the advent of reliable quantitative assays, the determination of

HBV DNA levels may replace the determination of HBe antigen/antibody status as a risk indicator of HCC.

While the prevalence of chronic HBV infection has been high in limited geographic areas, such as east and southeast Asia and sub-Saharan Africa, the prevalence of chronic HCV infection has been increased recently in several developed countries, including Japan, south European countries, and the United States. With chronic hepatitis C, the risk of HCC development increases with the progression of liver fibrosis (6,18). Chronic hepatitis C patients with cirrhosis stand at a very high risk of HCC (19). In Japan, HCV infection spread nationally mainly in the 1950s and 1960s and is currently, after a few decades required for progression to cirrhosis, by far the most predominant cause of HCC. The peak of viral spread in the United States took place a couple of decades later, and the incidence of HCV-related HCC is now rapidly increasing (20,21). In addition to the degree of liver fibrosis, male gender, older age, and heavy alcohol consumption are also known risk factors for HCV-related HCC. Human immunodeficiency virus (HIV) coinfection is an important risk factor of rapid progression of liver fibrosis, which constitutes a serious clinical problem especially in United States in the present.

Cirrhosis due to etiologies other than chronic viral hepatitis also represents risk of HCC development. Major etiologies include alcoholic liver disease and NASH (22–24), whose relative importance may differ geographically. Schoniger-Hekele et al. reported that alcoholic liver disease accounted for 32% of all HCCs in an Austrian cohort (25). In the United States, the approximate hospitalization rate for HCC related to alcoholic cirrhosis is 8 to 9/100,000/yr compared with about 7/100,000/yr for hepatitis C (26). NASH is a chronic liver disease that is gaining increasing significance because of its high prevalence worldwide and its potential progression to cirrhosis, HCC, and liver failure. Although NASH has been described in cohorts of patients with HCC (27,28), incidence of HCC in cirrhosis due to NASH is not well known. Aflatoxin may play a role in certain areas.

In brief, the evaluation of degree of liver fibrosis is of paramount importance in assessing the risk of HCC development in patients with chronic liver diseases of any etiology. Histologic evaluation of liver biopsy samples has been considered as the gold standard for the assessment of liver fibrosis. However, the invasiveness accompanying liver biopsy poses a considerable problem with its clinical feasibility. In clinical practice, repeated assessment of liver fibrosis will be often required because once noncirrhotic liver may change into cirrhosis over time, sometimes rather rapidly. Consequently, noninvasive evaluation of liver fibrosis is one of the main themes of current hepatology.

Recently developed transient elastography well correlates with histological liver fibrosis stage (29–31). Cut-off value for the diagnosis of histological cirrhosis is reported to be 12.5 to 14.9 kPa (29,31). Higher value of liver stiffness may need proper attention for decompensation and HCC development. Fibrotest is based on the age and gender of patients combined with five biochemical markers (total bilirubin, haptoglobin, γ-glutamyl transpeptidase, α-2 macroglobulin, and apolipoprotein A1) (32). An index of 0 to 0.10 had a 100% negative predictive value, while an index of 0.60 to 1.00 had a greater than 90% positive predictive value for a Metavir score of F2 to F4. APRI is the aspartate aminotransferase level/upper limit of normal divided by the platelet count (10^9/L) multiplied by 100 (33). For a hypothetical patient with AST 90 IU/L (upper limit of normal, 45) and platelet count 100 ($\times 10^9$/L), the APRI is 2.0, which means the patient has a 41% likelihood of advanced fibrosis and 5% chance of having minimal or no fibrosis. Applicability of these methods for surveillance should be determined in future prospective studies.

Those patients who are considered to be at a nonnegligible risk of HCC development should be subjected to surveillance program, which will be discussed later. Possible exceptions may be those patients with severe liver dysfunction who could not receive any treatment if diagnosed with HCC, or those with other life-threatening conditions.

SURVEILLANCE METHODOLOGY

Traditionally two methodologies have been employed in the surveillance for HCC in high-risk patients: tumor marker determination and diagnostic imaging. Serum α-fetoprotein (AFP) concentration is the representative of the former and liver ultrasonography, of the latter. The

usefulness of a surveillance program should have been evaluated on the basis of beneficial effects on the outcome of patients with HCC thus diagnosed relative to the cost. However, few prospective randomized trials have been performed comparing the outcome of HCC patients on or out of a surveillance program. Thus, currently available evidence regarding the effects of surveillance on decreasing overall or disease-specific mortality has come mostly from retrospective or case-control studies.

α-Fetoprotein

AFP is a glycoprotein with a molecular weight of 72 kDa. The main physiologic function of AFP appears to be the regulation of fatty acids in fetal as well as in proliferating adult liver cells (34). Since 1968, AFP has been used as a serum marker for human HCC (35). AFP as a marker reportedly has a sensitivity of 39% to 65%, a specificity of 76% to 94%, and a positive predictive value of 9% to 50% (Table 1) (36–42). Studies assessing the usefulness of AFP in HCC screening have varied widely in their design and in the characteristics of targeted patients in terms of etiology, severity of background liver diseases, and so forth. Moreover, specificity and sensitivity inevitably vary depending on the cut-off level chosen for the diagnosis.

An intrinsic disadvantage of AFP as a tumor marker is the fact that serum level of AFP can increase in patients without HCC when hepatitis is active, partly because of accelerated cellular proliferation in regeneration. The value of 20 ng/mL is often adopted as the upper limit of normal range of AFP because serum AFP level rarely exceeds this level in healthy subjects. However, values slightly above this level are hardly diagnostic of HCC among patients with chronic hepatitis, and adopting a low cut-off value would result in an inappropriately low specificity. AFP levels above 400 ng/mL can be considered almost definitely diagnostic of HCC but sensitivity would inevitably become lower with higher cut-off levels. There is an additional disadvantage when AFP is to be used in HCC surveillance. Small HCC tumors, the detection of which is the primary objective of surveillance, are less likely to be AFP-producing, and serum AFP level of AFP may not be high even if they are AFP-producing.

It has been claimed that AFP determination can be dismissed as a screening test for HCC, except when ultrasonography is either not available or of such poor quality that lesions smaller than 2 cm in diameter will not be detected. One such case is the HCC screening in Alaskan hepatitis B careers, among whom AFP testing allowed detection of tumors at an earlier, treatable stage (43). Although screened subjects showed an increased survival rate compared with historic controls, this must have been affected by lead-time and length-time bias inherent in retrospective studies on screening.

Ultrasonography

Ultrasonography became available for identifying intrahepatic lesions in the early 1980s (44). This imaging modality is appealing because it is almost completely noninvasive. The ribs and the air in lung and gastrointestinal tract surround the liver and they may hinder ultrasound imaging, but the imaging of liver has been much facilitated by the improvements in devices and techniques. The reported sensitivity of ultrasound imaging in detecting HCC nodules has been highly variable, ranging from 35% to 84% (45), depending on the expertise of the operator as well as on the ultrasound equipment used. Indeed, later, more sophisticated ultrasound instruments can produce images with much better resolution, improving the detectability of small intrahepatic lesions. It should be noted, however, that ultrasound diagnosis is heavily operator dependent. A high level of skill and experience is required to record high-quality images and make accurate diagnosis. In addition, ultrasound diagnosis may be hardly possible due to patients' physical conditions such as excessive obesity.

A previous study reported the sensitivity of ultrasonography for HCC detection to be as low as 20.5% (46), as compared with the pathology of explanted livers that were removed from patients who underwent liver transplantation. Small HCC nodules less than or equal to 2 cm in diameter constituted 85% of the lesions that had not been detected by ultrasonography (47). Ultrasound detectability of HCC nodules depends on tumor size: nodules of >5.0 cm, 3.1 to 5.0 cm, 2.1 to 3.0 cm, and 1.0 to 2.0 cm in diameter showed a detection rate of 92%, 75%, 20%, and 13.6%, respectively (46).

Table 1 Surveillance Studies for Hepatocellular Carcinoma

						AFP				Ultrasonography		
Author Year	Number screened	Incidence of HCC (%/yr)	%HCV	%HBV	%Alcohol	Interval (mo)	Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	Interval (mo)	Sensitivity (%)	Specificity (%)
Oka 1990	140	6.5		20	19	2	500	25	91.0	3	85	
Pateron 1994	118	5.8		4.2	69.5	6	100	21	93	6	78	93
Sherman 1995	1069	0.47	0	100		6	20	64.3	91.4	6	78.8	93.8
Bolondi 2001	313	4.1	64.2	17.3		6	20	41.0	82			

Although these data are rather disappointing, other reports indicated that the detectability of ultrasonography for intrahepatic nodules is almost comparable to that of CT (48–51). In a study on nodules that were 2 cm or smaller in diameter in patients with chronic hepatitis, the detection capability of ultrasonography was better than that of CT or MRI for the nodular lesions and that ultrasonography was superior for the detection of all cases with adenomatous hyperplasia and well differentiated HCC (52). After all, ultrasonography is indispensable in the screening of HCC for its noninvasiveness and less expensiveness. Definite diagnosis of HCC depends on the evaluation of its vascularity, of which conventional ultrasonography is not capable. CT or MRI with contrast enhancement usually follows ultrasonography when HCC is suspected by ultrasonography.

Ultrasonography, when conducted by less experienced operators, has blind spots. Moreover, the resolution may not be satisfactory in cirrhotic patients with rough echo patterns in the background liver. Thus, it may be expected that detection capability of HCC would improved with use of CT or MRI in combination with ultrasonography. However, there are few reports on HCC surveillance that actually employed CT or MRI, and its cost-benefit also remains unclear.

Recently several contrast enhancement materials have been developed for ultrasonography. They are very useful in the differential diagnosis of intrahepatic nodules or the demarcation of intrahepatic lesions before percutaneous ablation. However, their role in HCC screening is yet to be defined.

Combination of α-fetoprotein and Ultrasonography

In the screening of HCC, serum AFP measurement is less sensitive than ultrasonography but specificity may be comparable when using appropriate cutoffs. Screening by the combination of ultrasonography and AFP may lead to improved detection but previous reports were generally negative (37,53–55). However, in a nonrandomized study conducted on patients with cirrhosis, the sensitivity of detection was reported to be increased when both ultrasonography and AFP measurements were conducted as compared with either was conducted alone (53).

Recently a randomized trial evaluated HCC screening with AFP and ultrasound every six months compared with no screening in over 18,000 Chinese patients with HBV infection. The results indicated that more cases of HCC were diagnosed in the screened group than in the nonscreened group (86 vs. 67) and the overall survival was better in the former group: 65.9%, 52.6%, and 46.4% at 1, 3, and 5 years, respectively, compared with 31.2%, 7.2%, and 0%.

A retrospective study assessed HCC screening in 367 patients of 70 years or older, with AFP measurements and ultrasonography every 6 or 12 months. The screening allowed more frequent diagnosis of HCC at an early stage, increased the proportion of patients who could receive a curative treatment, and improved their prognosis as compared with patients not screened. The apparent benefit on survival was restricted to the first three years after detection of HCC, probably because of a shorter life expectancy of older people (56).

New Serum Markers and New Methods

Recent developments in gene-expression microarrays, proteomics, and tumor immunology permit thousands of genes and proteins to be screened simultaneously. New biomarkers are expected to be established in the next decade for cancer screening, including HCC. To establish a formal framework to guide biomarker evaluation and development, a five-phase program is adopted by the Early Detection Research Network (EDRN) of the National Cancer Institute (57). Several newly markers, including des-γ-carboxyprothrombin (DCP), AFP-L3, glypican-3, insulin like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF) currently appear promising. They are to be evaluated further in phase 2 studies to determine the ability to detect early stage HCC, followed by phase 3 studies that will retrospectively determine whether they can detect preclinical diseases. If results hold up, there should follow the phase 4 studies to assess prospectively their ability to detect early HCC, and the phase 5 studies to confirm that surveillance using these markers can reduce morbidity and mortality from HCC.

The detection sensitivity of dynamic CT and that of dynamic MRI are both high for hypervascular HCC. Considering that patients with HCC undergo repeated imaging

examinations and that the diagnostic capabilities of dynamic CT and MRI are almost the same, dynamic MRI, which does not involve exposure to X rays, may be more advantageous. However, MRI systems that allow high-quality dynamic studies are not yet as widely used as high-speed CT systems, therefore, not all institutions can perform dynamic MRI. They would then have no other choice but to perform high-speed dynamic CT, such as helical CT, or even more advanced systems such as multidetector CT (MDCT). The development of MDCT has dramatically accelerated scan acquisition in liver CT (58). With MDCT, high-speed volume coverage of the entire liver is possible in 4 to 10 seconds, which allows the acquisition of two separate series of scans in the arterial phase, termed early arterial and late arterial phase scans (59,60).

In ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), FDG is taken up by tumor cells with active glucose metabolism, and specifically accumulates there, blocking the metabolic pathway. In a study evaluating the diagnosis of HCC using a quantitative standardized uptake value (SUV), the SUV for HCC was lower than that of metastatic liver cancer (61). In general, FEG-PET is not recommended for the diagnosis of HCC because it is expensive and not superior to conventional diagnostic imaging techniques, such as CT and MRI.

STANDARDIZED RECALL PROCEDURES

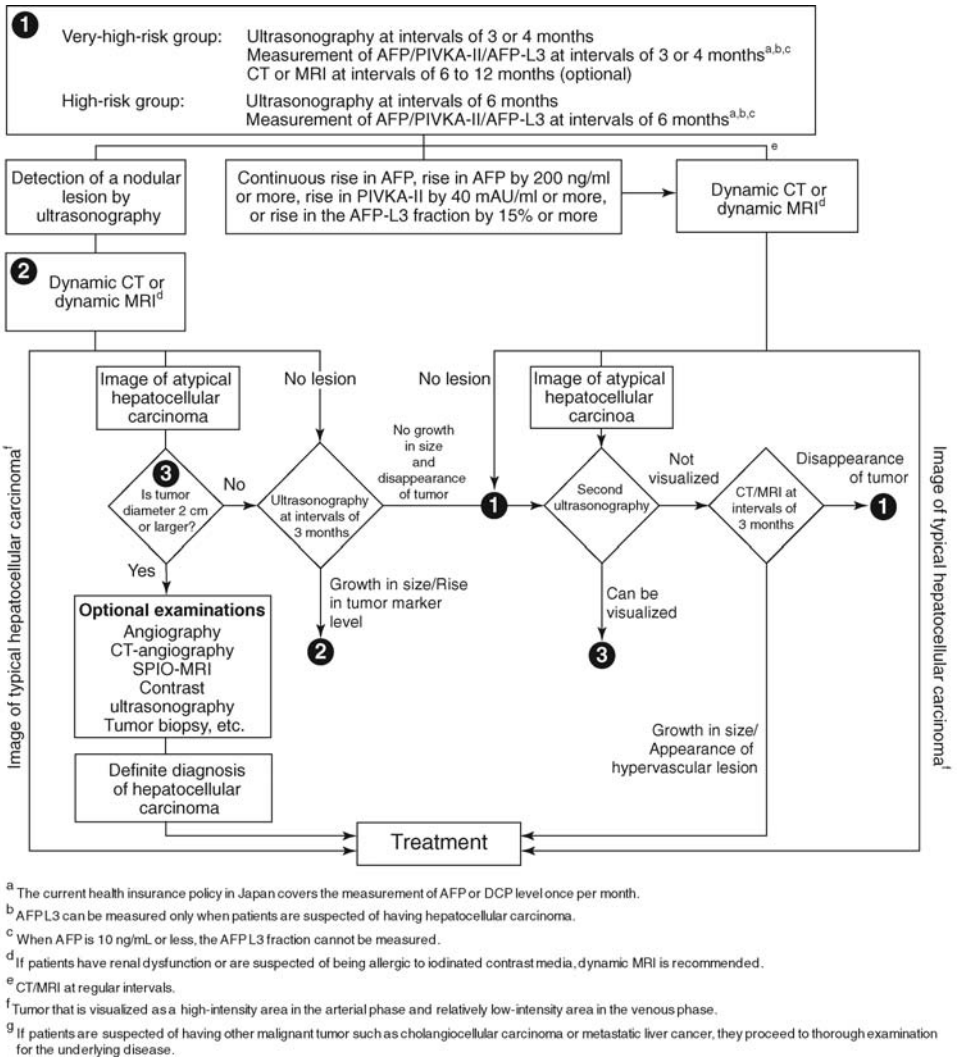
Once patients are found to have an abnormal surveillance test, they need to be recalled for subsequent evaluation. However, in spite of various recall algorithms described in the literature, none has been tested in a prospective fashion. Furthermore, recall procedures should differ for abnormal AFP values and for abnormal ultrasonography findings. Increases in serum AFP level need to be interpreted against background liver diseases. Reactivated chronic hepatitis B is often accompanied by an increase in AFP levels. Pregnancy may cause temporary elevation in AFP levels, sometimes together with an increase in the proportion of its L3 fraction. Thus, patients showing an increase in serum AFP levels require a detailed clinical evaluation to determine the cause for the increase.

When a low-echoic lesion is newly detected with ultrasonography in the liver of a patient at risk of HCC, a complete evaluation is strongly recommended. Typically, this involves further imaging by CT or MRI with contrast enhancement and a presence of hyperattenuation in the arterial phase with washout in the late phase can be considered as a definite sign of HCC (62). In ambiguous cases needle tumor biopsy under ultrasound guidance is recommended. However, it is controversial whether all suspicious nodules should be subjected to liver tumor biopsy because of concerns about possible tumor seeding.

SCREENING INTERVAL

Since the risk of HCC development does not usually diminish spontaneously in patients who are a target of HCC screening, a surveillance program for HCC should consist of repeating screenings at a determined interval. Ultrasonography is superior to CT in this setting for its noninvasiveness and cost-effectiveness. The guideline of American Association for the Study of Liver Diseases (AASLD) proposes ultrasound surveillance to patients at high risk of HCC at an interval of six months. The guideline explicitly indicates that the surveillance interval should depend not on the size of risk of HCC but exclusively on tumor doubling times, to detect cancer nodules while they are small enough for curative treatments.

However, in Japan, ultrasound surveillance with a shorter interval of three to four months is encouraged for extremely high-risk patients while an interval of six months is recommended for high-risk ones (Fig. 1) (63). In Japan, chronic hepatitis C patients with cirrhosis show HCC incidence rates of 6% to 8% per year, constituting an extremely high-risk group. Theoretically, shorter surveillance intervals lead to tumor detection at smaller sizes. However, it is not known whether the difference in detected tumor size, if any, is large enough to affect prognosis in a cost-effective fashion. Although there is no prospective comparison of different schedules, one retrospective study in cirrhotic patients and a mathematic model



[a] The current health insurance policy in Japan covers the measurement of AFP or DCP level once per month.
[b] AFP L3 can be measured only when patients are suspected of having hepatocellular carcinoma.
[c] When AFP is 10 ng/mL or less, the AFP L3 fraction cannot be measured.
[d] If patients have renal dysfunction or are suspected of being allergic to iodinated contrast media, dynamic MRI is recommended.
[e] CT/MRI at regular intervals.
[f] Tumor that is visualized as a high-intensity area in the arterial phase and relatively low-intensity area in the venous phase.
[g] If patients are suspected of having other malignant tumor such as cholangiocellular carcinoma or metastatic liver cancer, they proceed to thorough examination for the underlying disease.

Figure 1 Surveillance algorithm for hepatocellular carcinoma in Japan. *Abbreviations*: AFP, α-fetoprotein; PIVKA-II, prothrombin induced by vitamin K absence-II; DCP, des-γ-carboxyprothrombin; CT, computed tomography; MRI, magnetic resonance imaging; SPIO, superparamagentic iron oxide.

applied to hepatitis B virus careers suggested that a longer screening interval is as effective as the six-month intervals in terms of survival.

It is controversial whether AFP determination is to be included in surveillance program for HCC. However, if AFP is to be measured, it should be measured repeatedly, and an abnormal level of AFP must be interpreted not by simple comparison with a given cut-off value but in the context of time series. An abrupt elevation of serum AFP levels in the absence of exacerbation of hepatitis may indicate development of HCC even if ultrasonography is apparently negative, and further evaluation with contrast-enhanced CT or MRI should be considered.

COST EFFECTIVENESS

According to a decision-analysis model, the cost-effectiveness ratio of screening European patients with only Child–Pugh class A ranged between $48,000 and $284,000 for each additional life-year gained (64). However, this study did not take into account liver

transplantation as a treatment option. In a group of patients who could anticipate excellent survival, the cost-effectiveness ratio ranged between $26,000 and $55,000. In another study among 313 Italian patients with cirrhosis undergoing serum AFP and liver ultrasonography every six months, the cost per one case of treatable HCC was $17,934, and the cost per year of life saved was $112,993 (40). In the United States, the cost for each quality-adjusted life-year (QALY) gained through surveillance was estimated to range from $35,000 to $45,000 (64). HCC screening in patients waiting for liver transplantation has been associated with a cost per year of life saved ranging from $60,000 to $100,000, depending on the screening modality used (65).

It must be emphasized that the cost effectiveness of HCC screening has been assessed by retrospective analysis or by using decision models. Although retrospective studies have a selection bias, decision-analysis models are based on simulation of costs and health outcomes and, therefore, their results may vary greatly according to different assumptions, such as the incidence of HCC in the screening population, the screening interval, the modality of diagnosis, the type of treatment after diagnosis, the doubling time of tumors, and the tumor recurrence rate. In particular, there should be a feasible treatment that can favorably affect prognosis of patients if a screening is to be cost effective at all.

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4 Prevention of Hepatocellular Carcinoma

Geoffrey C. Farrell
Australian National University Medical School at The Canberra Hospital, Canberra, Australian Capital Territory, Australia

Jiangao Fan
Department of Gastroenterology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, the third most common cause of cancer mortality in men, and the sixth most common in women (1–7). As described in earlier chapters (chaps. 1 and 3), the geographic distribution of HCC varies considerably. Eighty percent of new cases occur in sub-Saharan Africa or Eastern Asia; the Pacific rim and southern Europe are other high-incidence zones. In low-rate countries, such as Australia and the United States, the incidence and mortality of HCC have increased at least twofold in recent decades, largely because of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (1–8). Despite efforts to improve early diagnosis and treatment, therapeutic options for HCC have not impacted on the mortality. The global health burden of HCC can only be reduced substantially by widespread application of effective prevention programs (9–12).

Primary prevention of HCC is possible because in more than 90% of cases, this cancer occurs in the context of known risk factors, most of which are theoretically preventable (9–12). These risk factors have been discussed in earlier chapters and are summarized in Table 1. The most important are chronic hepatitis B (CHB) and chronic hepatitis C (CHC), one or both of which are implicated in at least 80% of HCC cases worldwide (5–17). While HBV and HCV may have some oncogenic properties, in the vast majority of cases, hepatocarcinogenesis involves continuing hepatitis activity and development of cirrhosis. It follows that interruption of disease progression by timely and effective antiviral treatment offers another level of HCC prevention (Table 2) (10–19).

Other causes of cirrhosis that can lead to HCC include alcohol liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), hereditary hemochromatosis (HH), and, less commonly, immune-mediated or metabolic liver diseases, such as primary biliary cirrhosis (PBC) and α-1 antitrypsin deficiency. With some of these conditions, appropriate public health measures (e.g., to reduce alcohol consumption) or early therapeutic intervention for "at-risk" individuals before development of cirrhosis (e.g., hemochromatosis) could prevent HCC (8,10,13,17). Additional risk factors account for some regional or individual variability in HCC risk. Other host and environmental factors can therefore influence the development of HCC, even though they may not be necessary or sufficient for hepatocarcinogenesis when present alone. Some host factors include age and male gender, excessive alcohol consumption, cigarette smoking, and diabetes. Familial clustering of HCC cases has also been described, but the role of genetic susceptibility factors that cannot be readily explained by HBV infection is not well characterized (10,13,17). Food- and water-borne carcinogens, such as aflatoxin B1 and fumonasin from contaminated crops, have contributed to unusually high rates of HCC in parts of China and sub-Saharan Africa (9,11,20). Clean water and uncontaminated food supplies offer an additional basic condition to prevent HCC.

In this chapter, we will focus particularly on primary prevention of HCC by preventing contraction of liver disease and development of its complications. The roles of hepatitis B vaccination, which is already reducing incidence of HCC in some areas, and prevention of HCV infection will be discussed. For those already infected, a newer aspect is prevention of HCC afforded by more effective antiviral treatment of chronic HBV and HCV infection, and early

Table 1 Causes and Risk Factors for Hepatocellular Carcinoma, and General Approaches to Prevention

Etiology or risk factors	Major approaches to prevention
Major causes	
Chronic hepatitis B virus infection	Vaccination; antiviral therapy
Chronic hepatitis C virus infection	Screening of blood; antiviral therapy
Alcoholism and related liver disease	Abstinence or reduction of alcohol intake
Minor causes	
Hereditary hemochromatosis	Family screening; iron depletion
Nonalcoholic fatty liver disease	[a]Treating metabolic risk factors
Primary biliary cirrhosis	[a]Using ursodeoxycholic acid
Risk factors	
Dietary pollution	Avoiding exposure to aflatoxins
Overweight/obesity	[a]Diet and aerobic exercise
Type 2 diabetes	[a]Optimal control of blood glucose
Tobacco smoking	[a]Controlling tobacco use

[a]These approaches have not yet been shown to be effective.

Table 2 Levels of Prevention Against Development of Hepatocellular Carcinoma

Classification	Description	Example
Primary prevention	A. Prevent liver disease	Screen donor blood for HBV, hepatitis C virus, and human immunodeficiency virus HBV vaccination Universal precautions to prevent blood contamination in health care settings Avoid exposure to aflatoxin Reduce intake of alcohol Prevent overweight/obesity by dietary modification and increased physical activity Tobacco awareness
	B. Measures to slow progression to cirrhosis, and alter susceptibility to HCC with chronic liver disease	Antiviral treatment of chronic hepatitis B. Antiviral treatment of hepatitis C Early detection (family screening) for hereditary hemochromatosis Treatment of other liver diseases
Secondary prevention	Measures to prevent tumor recurrence after curative treatment	Antiviral therapy; interferon α and β Chemoprevention (retinoids and vitamin K analogs)^{131}Iodine-labeled lipiodol transarterial chemoembolization
Tertiary prevention	Early detection to improve treatment outcomes	HCC screening: hepatic ultrasonography, α-fetoprotein, and other serological tests

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

intervention in HH (9,11,18). Secondary prevention is the protection against HCC recurrence conferred by treatment of underlying liver disease after resection or ablation of an initial HCC (10,11). Theoretically, HCC should also be amenable to chemoprevention, pharmacological or nutritional interventions that reduce HCC incidence among those at high risk of the disease, but recent developments in this area are few. In practical terms, the distinction between primary and secondary prevention is sometimes blurred, as summarized in Table 2 (9,11,18,19).

PRIMARY PREVENTION OF HCC

Prevention and Control of Hepatitis B

Prevention of HBV Infection

About 360 million people worldwide have chronic HBV infection. At least 40% of these will die from HCC or cirrhosis (21–24). The primary goal of hepatitis B prevention programs is to reduce new cases of HBV infection and thereby prevent its sequelae (24–29). Safe and effective

Table 3 Immunization Strategies for Preventing Hepatitis B

Routine screening of all pregnant women for serum HBsAg
Appropriate immunoprophylaxis of infants born to HBsAg-positive women
Routine vaccination of infants with first dose administered within 24 hours of birth
Routine vaccination of all adolescents who have not been previously vaccinated
Routine vaccination of adults at high risk of infection, who have not been previously vaccinated

Note that this applies to both high- and low-risk countries.
Abbreviation: HBsAg, hepatitis B virus surface antigen.

vaccines have been available for more than 20 years (Table 3), and universal hepatitis B immunization is recommended by the World Health Organization (WHO) (25,30–35). There remains a need for "catch-up programs," but more widespread hepatitis B vaccination should ultimately reduce the prevalence of chronic HBV infection, thereby reducing onset of chronic hepatitis B (CHB) and its attendant risks of cirrhosis and HCC (31,34–36).

Active immunoprophylaxis. Effective hepatitis B vaccines became available in the mid-1980s (24,25). Both plasma-derived and recombinant DNA vaccines are highly immunogenic. Following intramuscular injection (deltoid) of prescribed doses in a three-injection schedule (Table 4), they confer antibody against hepatitis B surface antigen (anti-HBs) seroconversion rates of ~95% (25,30–35). Rates are slightly lower in elderly and obese subjects, and in renal failure or with immunosuppression (25,34). When the endpoint of a rise in anti-HBs titer to 100 IU/mL is achieved, long-term protection against HBV infection is obtained (25,34).

Taiwan became the first jurisdiction to introduce a countrywide infant hepatitis B vaccination program in 1984 (22,37). Since then rates of chronic HBV infection have been reduced in children and adolescents from over 10% to less than 1% (37). By 1997, this had produced a reduction of HCC in children and adolescents, showing the benefits of HBV vaccination policies for prevention of long-term outcomes like HCC (38,39). Mathematical modeling to extrapolate such community benefits to adults has been used to estimate that routine infant hepatitis B vaccination, with 90% coverage and first dose administered at birth, can prevent 84% of global HBV-related deaths (40). Similar reductions would be expected in new cases of HBV-related HCC.

China accounts for more than 50% of the world's cases of HCC (1–7). Infant hepatitis B vaccination was added to the Chinese National Immunization Program in 2002, and hepatitis B vaccination starting at birth increased from 64% in 2004 to 81% in 2006; coverage with the complete hepatitis B vaccination series increased from 52% in 2001 to 92% in 2006 (41–43). According to a recent national survey, hepatitis B virus surface antigen (HbsAg) carrier rates (chronic HBV infection) have already decreased, from 9.8% in 1992 to below 7.2% in 2006 (44).

Hepatitis B vaccination was first recommended for all infants and children by WHO in 1992 (45). Up to 2005, 154 (80%) of 192 WHO member states reported having integrated hepatitis B vaccines into their routine, extended infant vaccination schedules. Global coverage with three-dose hepatitis B vaccines increased from 32% in 2001 to 55% in 2005. However, as

Table 4 Recommended Doses and Schedules for Hepatitis B Vaccination

Brand names	Subjects (age)	Doses (μg)	Volume (mL)	Schedule (mo)
[a]Engerix-B®	<20 yr	10	0.5	0,1,6
	≥20 yr	20	1.0	0,1,6
	[b]Dialysis	40	2.0	0,1,2,6
[c]Recombivax HB®	<20 yr	5	0.5	0,1,6
	≥20 yr	10	1.0	0,1,6
	[b]Predialysis/dialysis	40	1.0	0,1,6

[a]Marketed by GlaxoSmithKline, North Carolina, U.S.A.
[b]Hepatitis B vaccination is less effective in patients with renal failure.
[c]Marketed by Merk & Co., Inc., New Jersey, U.S.A.

Table 5 HBV Vaccination Coverage in Infants Across WHO Major Geographical Regions in 2005

Region	Coverage with three-dose hepatitis B vaccines (%)
Southeast Asia	27
Africa	39
Eastern Mediterranean	74
Europe	76
American	85
Western Pacific	87

Abbreviations: HBV, hepatitis B virus; WHO, World Health Organization.
Source: From Ref. 46.

shown in Table 5, coverage varies across regions, and there is need for more effective coverage rates in some areas of highest risk (46).

The WHO Western Pacific Region has committed to reducing prevalence of chronic HBV infection in children aged less than five years to less than 2% by 2012 (34,40,45). Achieving this goal will require continued commitment to increase vaccination coverage in impoverished regions, ensuring that infants born at home are vaccinated within 24 hours of birth, and taking efforts to vaccinate adolescents/young adults who are at risk for HBV by sexual transmission. Additional benefits could come from development of novel hepatitis B vaccines, including those with pre-S determinants that may be even more immunogenic than current vaccines directed solely at the surface domain of HBV (30,31). In addition, development of plant-derived vaccines could minimize costs by incorporating the vaccine into a form that is effective orally (47). Outstanding issues of hepatitis B vaccination include the suboptimal uptake rates for adults, including health care workers, and the need (or otherwise) for booster immunization at adolescence or in the case of suboptimal titers of anti-HBs. These matters have been discussed elsewhere (22,25,27) and are beyond the scope of the present review.

Passive immunoprophylaxis. Hepatitis B immunoglobulin (HBIG) contains high titers of anti-HBs prepared from pooled plasma. It is indicated in certain high-risk postexposure settings (25,33). However, HBIG confers effect immunity against HBV infection for only three to six months, so active immunization should be started at the same time as passive immunization (25,33).

Globally, the primary mode of transmission of HBV is from mother to newborn baby (vertical transmission). Immunoprophylaxis of newborns to HBsAg-positive women is required, particularly those with high levels of infectivity (acute hepatitis B, hepatitis B e antigen (HbeAg)-positive CHB, high-titer serum HBV DNA). Dosing recommendations are 0.13 mL of HBIG per kg body weight immediately after delivery or up to 12 hours after birth, in combination with hepatitis B vaccination. Delivery of HBIG does not reduce efficacy of hepatitis B vaccines, and the combination results in greater than 90% protection against perinatal transmission of HBV (vs. ~80% with either HBIG or vaccination alone) (25,33). Failure of immunoprophylaxis may be due to in utero transmission, perinatal transmission related to high virus inoculum, or the presence of HBsAg gene escape mutants (25,48). In an attempt to reduce the viral road and risk of vertical transmission for mothers with high-titer serum HBV DNA, lamivudine can be administered in the last trimester of pregnancy (49). The safety of lamivudine administration in late pregnancy has been established for women with human immunodeficiency virus (HIV) and HBV infection, and small-scale studies have demonstrated preliminary evidence of efficacy against perinatal HBV infection. Other HBV antivirals (entecavir, tenofovir, telaprovir, adefovir) are not known to be safe in pregnancy and should not be used to prevent HBV infection of the neonate (49).

In persons at risk for HBV infection after needlestick or sexual exposure, administration of HBIG (0.06 mL/kg) within 48 hours and no more than seven days following exposure, in combination with active hepatitis B immunization, is recommended (25,33). A second dose of

HBIG 30 days later may decrease the risk of transmission of HBV (25,33). In many countries, limited availability of HBIG will restrict its use, and hepatitis B vaccination alone will be recommended; postexposure prophylaxis has been demonstrated for hepatitis B vaccination following sexual exposure to HBV (50).

Liver transplantation is conceptually the ideal curative treatment for HCC because it eliminates both the cancer and the underlying, premalignant cirrhotic liver (51). HBV recurrence after liver transplantation has been dramatically reduced, and the natural history substantially improved, by long-term use of HBIG (>6 months) together with lamivudine or other antiviral therapy (adefovir add-on if lamivudine resistance has occurred) before and after liver transplantation (52–56). In addition, successful hepatitis B vaccination using three double doses of vaccine (with novel adjuvant), followed by withdrawal of HBIG, has been reported to prevent HBV recurrence post transplant, but this requires further study (57).

Other prevention measures. Similar to HCV (see below), HBV infection can also be prevented by screening blood, plasma, organ, tissue, and semen donors; virus inactivation of plasma-derived products; risk reduction counseling and services, such as needle exchange programs for injection drug users; changes in sexual practices (use of condoms); and implementation and maintenance of infection control practices (25–30). In developed countries, posttransfusion hepatitis B has decreased since the mid-1980s when blood donor screening for viral hepatitis became available. Use of medical disposables has further decreased nosocomial transmission, and the risk of HBV infection is limited to sexual activity (heterosexual or human with human), injection drug use (IDU), and possibly dental therapy, acupuncture, body piercing, and tattooing. In developing countries, almost half of blood units are not screened for viral hepatitis, medical contamination of injectables and medical devices remains possible, and safe disposal of sanitary materials contaminated with blood is often not available. Public education, in parallel with public health control measures to counteract HBV transmission by health care practices, is required to reduce HBV incidence in these populations.

Treatment of Chronic Hepatitis B

Hepatocarcinogenesis is a multistep process in which clones of altered hepatocytes survive because of selective advantages in cell proliferation or survival (see chap. 3). As an etiological agent, chronic HBV infection may operate at multiple "levels" in such development. HBV may be directly oncogenesic via random insertions into host DNA (insertional mutagenesis) and viral transactivation of oncogenes, such as hepatitis B x protein (HBX)-mediated activation of *c-myc*. Nonetheless, the most important role of the virus is from incitement of hepatic injury and inflammation that are part of mechanism of CHB. In CHB, the cytokine products of the hepatic inflammatory response mediate injury and cell death to hepatocytes, as well as cause DNA damage as a result of oxidative stress. Cytokines and the microenvironment of altered matrix and blood flow in active cirrhosis may promote proliferation of surviving altered hepatocytes or accentuate cell death of remaining normal hepatocytes (7,18). In this way, cirrhosis comprises a "growth pressure" that could contribute to the proliferative response of altered (dysplastic) liver cells into premalignant clones and, ultimately, HCC (7,13,15,18).

This concept about the importance of liver disease for hepatocarcinogenesis is supported by the clinical observations that ~80% of HBV-associated HCC occur with CHB at the stage of cirrhosis (7,13,15,18). However, the role of the virus is pivotal for inciting such "premalignant" liver disease, as shown in the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/cancer HBV study (REVEAL-HBV study) of persons aged 30 years or older in Taiwan (58,59). Both the risk of cirrhosis and that of HCC developing in the ensuing decade of life were directly related to serum HBV DNA levels at entry (58) or any subsequent time (59). This indicates that either the virus itself or a host response that occurs at higher levels of viral replication (HBV DNA >10,000 IU/mL) is responsible for causing liver cancer (58,59). Additional evidence comes from studies employing carefully constructed genetic and reconstitution models of chronic HBV infection in transgenic mice; the results show that hepatocarcinogenesis requires a Th1 inflammatory response to viral epitopes (60).

The proposal that HBV viral replication is required for hepatocarcinogenesis, as well as *continued hepatitis* and development of cirrhosis, infers that effective antiviral therapy of CHB

should prevent development of HCC (7,18). Until recently there were few data to support this concept. In two retrospective studies from Taiwan, patients with CHB-related cirrhosis who had been treated with interferon exhibited a lower rate of subsequent HCC development than those who had not been treated (61,62). An earlier European study, as well as other interferon trials on non-cirrhotic individuals, had not always shown a positive effect of interferon (Table 6) (63–66).

More recently, a large Asia-Pacific multicenter, double-blind, randomized controlled trial examined the effects of lamivudine versus placebo for histologically advanced stage CHB Cirrhosis Asian Lamivudine Multicenter study (CALM study) (67). Endpoints were defined by the combination of clinical, laboratory, and imaging data. The study was stopped after 30 months because of the clear superiority of lamivudine in preventing disease progression. The incidence of new cases of HCC in the antiviral treatment group was $\sim 50\%$ of that of placebo-treated controls ($P < 0.05$) (Table 6), although significance was lost ($P = 0.052$) when tumors detected in the first year of the study were excluded.

The CALM study has been criticized for its relatively small numbers of cancers and short duration of follow-up. However, further, near identical results are evident from a large retrospective audit of prolonged lamivudine therapy from Japan (68). It should also be noted that the rate of lamivudine drug resistance [YMDD mutations (Tyr-Met-Asp-Asp mutations)] at 30 weeks in the CALM study was 49% (67). This effectively means that antiviral efficacy was suboptimal in half the cases. Newer drugs (tenofovir, entecavir, telbivudine) are more potent HBV antivirals than lamivudine (69–71). It is therefore speculated that the capacity of HBV antiviral therapy to protect against liver cancer is greater than that indicated by the CALM study. This conclusion is supported by the numerous studies demonstrating that effective antiviral therapy of CHB substantially reduces hepatic necroinflammatory activity and arrests (or reverses) fibrotic progression toward cirrhosis (66–69). Long-term observations are now required to establish the extent to which such protection can be afforded when treatment is started *before* development of cirrhosis and when optimal levels of antiviral efficacy are obtained.

Prevention and Control of Hepatitis C

Chronic HCV infection is the second major risk factor for HCC (19,25,72–74). There are at least 180 million persons chronically infected worldwide, making HCV approximately one-third as prevalent as HBV (14,15,19). The contribution of HCV infection to incidence of HCC is supported by strong lines of evidence, as discussed in earlier chapters.

Prevention of HCV Infection

Transmission of HCV, while broadly similar to that of other blood-borne viral infections like HBV and HIV, is predominantly by direct inoculation of infected blood (25,75–77). Unlike HBV, sexual and vertical transmissions play only very minor roles. Instead, IDU and medical contamination of blood, particularly transfusion of blood products, account for the vast majority of infections (25,75–77).

To date, attempts to develop a protective HCV vaccine have been unsuccessful due to relatively low immunogenicity, need to include multiple viral epitopes into a polyvalent vaccine, and rapid mutation rate of HCV, which often exists as a diverse population of minor variants or "quasispecies" (78,79). Until an effective pre- or postexposure immunoprophylactic measure becomes available, prevention of HCV infection rests on curbing the social and medical factors involved with blood contamination (Table 7) (75,77–86).

Injection drug use. In North America, Western Europe, Australia, and New Zealand, more than 90% (arguably >99%) of new HCV infections arise from behaviors associated with IDU. In parts of Asia as well, IDU has become an important mode of spread (25,71–75,77,83,86–88). While shared needles and blood-contaminated syringes are the most obvious vehicles, use of communal articles to prepare injectables, contaminated fingers, swabs, and dressings is another way in which, even with sterile needles and syringes, the act of intravenous drug injection can transmit HCV (76,84,87). In numerous studies, the prevalence of HCV infection among those who inject drugs ranges from 40% to 90%, depending largely on duration of IDU. Infection

Table 6 Results of Randomized Controlled Trials of Antiviral Therapy on Incidence of HCC Among Patients with CHB

Author (year) (reference number)	*N*	Treatment	Mean age (yr)	Cirrhosis at entry	Mean follow-up (yr)	Number of patients (%) with HCC			Other therapeutic outcomes
						Treated group	Control group	*P* value	
Mazzella (1996) (63)	62	Interferon	48	62 (100%)	4	2/34 (5.9%)	4/28 (14%)	>0.05	NS
Lin (1999) (61)	101	Interferon	32	12 (12%)	7	1/67 (1.5%)	4/34 (12%)	<0.05	Increased cumulative survival
Lin (2004) 62	210	Interferon	32	23 (11%)	7	5/176 (2.8%)	5/34 (15%)	<0.05	Increased cumulative survival
Liaw[a] (2004) (67)	651	Lamivudine	43	217 (33%)[b]	2.7	17/436 (3.9%)	16/215 (7.4%)	<0.05	Reduced incidence of hepatic decompensation

[a]The principal investigators participating in the Cirrhosis Asian Lamivudine Multicentre Study.
[b]All had advanced hepatic fibrosis, Ishak fibrosis scores 4, 5, or 6.
Abbreviations: CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; NS, not stated.

Table 7 Risk Factors for HCV Infection, and Opportunities for Prevention

Risk factor	Preventive strategy
Injection drug use	Provide access to disposable needles and syringes.
Contaminated blood and related products	Screen blood, organ, and tissue donors
All types of health care settings	Implementation and maintenance of infection control practices ("universal precautions"), directed at avoiding blood contamination[a].
Persons with HCV infection	Identify infected persons for counseling and possible treatment.
Persons with multiple sex partners	Follow safer sex practices and use barriers.

[a]Key examples are nonuse of multiuse vials for injection medication and use of disposable devices for invasive procedures and gloving.
Abbreviation: HCV, hepatitis C virus.
Source: From Ref. 86.

is most likely in the first year of IDU, with rates up to 30% to 40% in some studies (88), and by three years of regular drug use, the prevalence of HCV infection typically exceeds 60% (25,71–75,85,88).

Concomitant HIV infection is also frequent among HCV-infected IDUs, ranging from less than 2% in Australia to more than 50% in South China and Spain (75). The lower frequency in Australia is attributable to the lower prevalence of HIV in Australia in the early 1980s, when the incidence of new HCV infections was rising appreciably, and the introduction of public health measures that included "safe sex" messages and disposable needle and syringe programs (25,75).

Prevention of HCV infection by IDU is partly thwarted by sociopolitical factors that prevent more widespread introduction of "safe using" or other "harm reduction" strategies for those who use illicit drugs (25,71–75,85). Attempts to prevent IDU by educational measures and public awareness campaigns have not been conspicuously successful (76). On the other hand, community-based needle and syringe exchange programs in countries like Australia while demonstrably preventing HIV, HBV, and hepatitis D virus (HDV) infections have had little impact on the incidence of new HCV infections (89). The main reason for this is thought to be the high prevalence rate in the target subpopulation (those who use drugs) at the time of introduction of such programs, other breeches of "safe injecting technique" mentioned earlier (88), and the chaotic nature of IDU as a human behavior, particularly at its inception. Other attempts to prevent IDU-transmitted HCV include availability of needle and syringe exchange facilities in areas of highest transmission of HCV, such as incarceration facilities, more widespread and comprehensive education on prevention to young people, and possibly safe injection areas for those unable or unwilling to enter rehabilitation programs for narcotic and other addictions.

Medical transmission.

Contaminated blood products Identification of the hepatitis C virus in 1989 was followed by rapid development of serum diagnostic tests (anti-HCV). By 1991, second-generation anti-HCV tests had been developed, which conferred high sensitivity and specificity for chronic HCV infection. More recent (third- and fourth-) generation anti-HCV tests, together with molecular assays (HCV RNA), have incrementally improved safety of blood products for transfusion (25,82–84). Introduction of donor testing for HCV has been slow in less affluent countries; it was introduced in Bangladesh in 2002, and for some it still remains a public health imperative (25,73,83). Soon after the development of diagnostic tests for HCV, it was shown that several cases of posttransfusion hepatitis C had terminated in HCC, usually in older subjects after two decades or more of HCV infection. Thus, while there are no controlled studies to show that screening of blood products to prevent HCV infection reduces risk of HCC, this follows logically from the importance of chronic HCV infection, a cause of cirrhosis and HCC (19,76,81).

Among blood products that have transmitted HCV, immunoglobulin preparations and clotting factor concentrates are particularly important (25). Prospective study of large cohorts of young Irish women who were inoculated with HCV-contaminated batches of anti-D

immunoglobulin (to prevent Rhesus blood group incompatibility) has provided powerful epidemiological evidence about the role of chronic HCV infection in development of cirrhosis. As opposed to older patients infected by blood transfusion, rates of cirrhosis among these women have been low, only 2% after 17 years of follow-up, and none had HCC (90,91). Conversely, patients with hemophilia who became infected with HCV in the 1970s and 1980s often develop cirrhosis (92,93). Of these patients, a high proportion (~60%) are coinfected with HIV (92,93), and among this important subgroup, rates of cirrhosis and HCC are high. Prevention now centers both on screening donors and on heat inactivation of clotting factor preparations.

Contaminated injection medicines, medical devices, health care worker transmission, and other nosocomial spread Prevention of HCV transmission in hospitals and other medical settings is vital (25,83,86). Measures include adoption of "universal precautions" against infection in the health care setting, worker training and education, abolition of reuse or multiuse vials of injectables (anesthetics, analgesics, antibiotics), extensive use of disposable equipment, and use of gloves whenever handling wounds or other blood-contaminated sites, such as the mouth. Details of these matters, and the manifold evidence that they have not yet been implemented with 100% reliability even in well-resourced countries, have recently been reviewed (86). They are also the subject of guidelines promulgated by several bodies (77,80,84). Unfortunately, the associated increase in health care costs may not be possible in some countries.

Needlestick injury. This is a particular hazard of health care workers, but may also occur in society. Hollow needles used for IDU may harbor infectious blood for at least 24 hours. The risk of HCV infection approximates 7% (25,72,75,77,80). Close monitoring of the affected person for earliest features of HCV infection is warranted, ideally with polymerase chain reaction (PCR) for HCV RNA, but if the expense prohibits this, at least with serum alanine aminotransferase (ALT) and anti-HCV. Treatment of HCV infection during the first six to nine months substantially reduces the risk of chronicity, with its attendant risks of CHC, cirrhosis, and HCC, as reviewed elsewhere (77,80,83,84).

Body piercing, cocaine snorting. Instances of HCV transmission by tattooing (social/cosmetic, traditional/religious) have been documented, and tattoo parlors should be regulated for safe practices that avoid blood contamination (25). Body piercing and cocaine snorting with a straw (that could injure nasal mucosa) have occasionally been shown to transmit HCV but play a negligible role overall (25,75,86).

Sexual and vertical transmission. Sexual transmission of HCV is rare if the index case has chronic HCV infection (25,75). However, for patients with acute hepatitis C, the risk of viral transmission appears higher. They should be advised to refrain from sex or at least to use barrier precautions (25).

Vertical transmission of HCV may account for some instances of familial clustering, but other factors (shared IDU, body piercing, unsafe medical practices) are more important. Risk factors include concomitant HIV infection and high-titer HCV RNA levels. Mode of infant delivery neither increases the risk nor protects against it (77). Monitoring at-risk newborns should not be performed until 18 months after birth, as recommended elsewhere (77), because transmission of maternal immunoglobulin confounds diagnostic interpretation of a positive anti-HCV test. Positive HCV RNA results may also not indicate onset of established chronic HCV infection, and in any event, hepatitis C in children is mild and HCV infection may resolve spontaneously.

Treatment of Chronic Hepatitis C

The weight of accumulated evidence indicates that effective antiviral therapy of CHC substantially reduces risk of HCC (Tables 8 and 9) (63,94–108). Optimal response to antiviral therapy of CHC is defined as *sustained antiviral response* (SVR), PCR undetectable by a sensitive serum assay (<50 IU/mL) six months after completing therapy. Other responses include

Table 8 Results of Randomized Controlled Trials of Interferon Treatment on Incidence of HCC Among Cirrhotic Patients with Chronic Hepatitis C

Author (year) (reference number)	*N*	Mean age (yr)	Mean follow-up (yr)	Number of patients (%) with HCC			Other therapeutic outcomes
				Treated group	Control group	*P* value	
Nishiguchi (1995, 2001) (94,95)	90	56	4.5	2/45 (4%)	17/45 (38%)	<0.05	Improved survival
Mazzella (1996) (63)	284	53	2.7	5/193 (2.6%)	9/92 (9.8%)	<0.05	Slowed disease progression
Valla (1999) (96)	99	57	3.1	5/47 (11%)	9/52 (17%)	>0.05	No improvement
Bernardinello (1999) (97)	61	NS	5	2/38 (5.3%)	1/23 (4.3%)	>0.05	No improvement
Azzaroli (2004) (98)	60	56	5	0/30 (0%)	9/30 (30%)	<0.05	Improved survival
Soga[a] (2005) (99)	133	NS	5	5/103 (4.9%)	7/30 (23.3%)	<0.05	NS

[a]No details about whether it is cirrhosis of the treated patients.
Abbreviations: HCC, hepatocellular carcinoma; NS, not stated.

end-of-treatment response followed by relapse (*response relapse*), or *nonresponse,* in which HCV RNA in serum remains detectable during therapy. In addition to these definitions based on the antiviral response, a distinction is sometimes made between a biochemical response (serum ALT returns to normal) and nonresponse.

Between 1989 and 1998, monotherapy with short-acting (conventional) interferon α products was the usual treatment, and SVR rates were poor: ~15% for genotype 1, ~35% for genotypes 2 and 3, but apparently higher in Japan where larger doses of interferon were used. Response was lower if cirrhosis was established (19,80–84). In the late 1990s, interferon/ribavirin combination became the standard of care, substantially improving SVR. During the last five years, pegylated interferon α plus ribavirin has been recommended as the optimal therapy in Europe, North America, and Asia-Pacific regions (77,80–84). SVR rates vary according to genotype, presence of cirrhosis, viral load, and host factors, from ~90% with genotype 2 or 3 with no cirrhosis to 30–40% among Afro-Americans, genotype 1, high viral load, and cirrhosis. This has been reviewed elsewhere (82,83).

With CHC, HCC almost invariably (>90% cases) develops at the stage of cirrhosis, and cases in which cirrhosis was not present on the liver biopsy taken before diagnosis of HCC have typically shown stage 3 fibrosis (19,109). Few controlled trials of the effect of antiviral therapy (interferon monotherapy) on HCC incidence in patients with CHC have been published (Table 8) (63,94–99). The overall impression is that interferon therapy substantially reduced HCC incidence, from over 20% during the subsequent 5 to 10 years to less than 5%. Since then at least 10 studies have reported on rates of HCC according to treatment outcome and pretreatment histological severity of hepatitis C. Some of the larger or better described studies are summarized in Table 9 (100–108). It should be noted that few of these studies, mostly older ones, found any effect of interferon treatment versus no treatment on HCC risk, but almost all of them observed that rates of HCC were reduced in those with response to antiviral therapy. The most impressive results are among those with SVR (Table 9).

The conclusion from published studies of antiviral therapy of CHC is that protection against HCC conferred by SVR is at least 90% (98–108). In Japanese studies, which report thousands of cases, rates of HCC are lowest in those with both SVR and biochemical response and intermediate in biochemical response to interferon without SVR (100–104). Further, the accumulated world experience of HCC developing in patients after SVR is small, possibly no more than a few hundred cases (109), which compares favorably with the high rate of 1% to 3% per annum in untreated CHC-cirrhosis or nonresponders to antiviral therapy (Tables 8 and 9) (19). However, analysis of the characteristics of those who have developed HCC after SVR may give insights into risk factors for hepatocarcinogenesis that have implications for prevention (109). Such cases are virtually confined to men aged 65 years or older and seem more common in Japan than elsewhere, and patients are more likely to have had cirrhosis at the time of

Table 9 Effect of Viral Response to Interferon Treatment on Incidence of HCC Among Patients with Chronic Hepatitis C

Author (year) (reference number)	Treated number of patients studied	Mean follow-up (yr)	Number of patients (%) with HCC			
			Sustained viral response	Response relapse	Nonresponse	Not treated
Imai (1998) (100)	419	4	1/151 (0.7%)	7/120 (5.8%)	20/148 (14%)	19/144 (12%)
Kasahara (1998) (101)	1022	7	5/313 (1.6%)	9/304 (3%)	32/405 (7.9%)	NS
Okanoue (1999) (102)	1148	2.7	3/316 (1%)	8/264 (3%)	41/568 (7%)	NS
Takimoto (2002) (103)	652	4.5	0.29% (201)[a]	0.75% (95)[a]	2.3% (356)[a]	NS
Kashiwagi (2003) (104)	351	5.7	1/66 (1.5%)	NS	16/194 (8.2%)	NS
Coverdale (2004) (105)	384	9.4	1/50 (2%)	5/136 (4%)	18/157 (11%)	7/71 (10%)
Yu (2005) (106)	214	6	1/87 (1.1%)	NS	12/113 (11%)	NS
Hung (2006) (107)	132	3	5/73 (6.8%)	NS	11/43 (26%)	NS
Yu (2006) (108)	1057	5	12/715 (1.7%)	NS	39/342 (11%)	54/562 (9.6%)

[a]Annual incidence of HCC by the Kaplan-Meier method.
Abbreviations: HCC, hepatocellular carcinoma; NS, not stated.

treatment, continuing biochemical abnormalities (indicating continuing hepatic inflammation and liver injury), and a history of alcohol abuse or some other potential cause of liver disease (63,94–109). The latter includes some cases with serum positive for antibody against hepatitis B core antigen (anti-HBc) and negative for HBsAg, indicative of possible "occult" HBV infection, diabetes, and obesity (7,19,109). The implication for prevention of HCC is that SVR to antiviral therapy should not be regarded as 100% protective against development of HCC; continued screening for early onset of HCC (tertiary prevention) is indicated, particularly among older men with other risk factors (109).

Most of the data summarized in Tables 8 and 9 are for interferon monotherapy. By comparison, combination interferon/ribavirin, particularly pegylated interferon/ribavirin, produces SVR more often, and responses have been at least as durable as with interferon monotherapy (110). Further, reversal of hepatic fibrosis with combination antiviral therapy appears to be more rapid than with interferon monotherapy (111). It is therefore likely that the protection against development of HCC afforded by SVR to contemporary antiviral therapy may be even greater than the results promulgated for conventional interferon monotherapy (19,112). Further follow-up data are required to substantiate this hypothesis. On the other hand, attempts to reduce incidence of HCC among those with non-SVR to interferon-based therapy with long-term low-dose pegylated interferon have been unsuccessful (113).

Prevention of Other Virus Infections, HIV and HDV

In general, combined virus infections (HBV + HCV, HBV + HDV, HBV + HCV + HDV, HIV + HBV, HIV + HCV) greatly increase the risks of cirrhosis and HCC (114–116). HDV, often referred to as δ hepatitis, requires the presence of HBsAg to enter host hepatocytes (114). The two viruses may be acquired simultaneously, particularly from IDU, or HDV may occur as a superinfection in someone with chronic HBV infection. HDV was common in Southern Europe and South America but rare in Asia and North America. In western countries, the almost unique risk factor has been IDU. For reasons that are not entirely clear, the incidence and prevalence of HDV infection have decreased remarkably worldwide (75), although a recent renaissance may be evident in Europe (117). The obligatory need of HDV for HBsAg means that vaccination against HBV also protects against HDV. Likewise, adoption of the measures to prevent blood contamination in society and medical settings, discussed earlier, will also prevent HDV infection (75,114).

HIV does not directly cause chronic liver disease or HCC but increases the severity of both CHB and CHC (115,118,119). Men coinfected with HIV and HCV have a higher risk of HCC, which occurs approximately a decade earlier in life compared with uncomplicated CHC (119). Similar observations have been reported for HIV and HBV coinfection (118). Implications for prevention of HCC include measures to prevent spread of HIV, whether by IDU (as discussed earlier) or by sexual transmission (practising safe sex, use of condoms, circumcision, treatment and sexual counseling of viremic individuals) (118). It can also be anticipated that earlier and more effective treatment of HIV/HBV and HIV/HCV coinfections should reduce the risk of HCC by preventing development of cirrhosis and reduction of hepatitis activity (118,119). Details of treatment for HIV/hepatitis virus coinfections are beyond the scope of this chapter and have recently been reviewed elsewhere (118,119).

Avoiding Exposure to Aflatoxins and Other Toxins

Aflatoxins

The aspergillus fungal toxin, aflatoxin B1, particularly its epoxide metabolite, is a potent mutagen (20). Aflatoxin contamination of the food supply occurs in tropical and subtropical humid areas, which coincide with high prevalence for HBV (7,18,20,119). The importance of aflatoxin arises because it acts synergistically with HBV infection to amplify risk for HCC (120). The mechanism involves mutation of tumor suppressor genes, particularly p53. So-called p53 "mutation hotspot" found at codon 249 of exon 7 provides a molecular signature for aflatoxin exposure (120). From a public health perspective, the combination of hepatitis B vaccination programs and efforts to reduce aflatoxin exposure or to pharmacologically attenuate the toxicological consequences of unavoidable fungal exposure should have a major impact on the incidence of HCC (9,121–123).

Several inexpensive agricultural strategies can be adopted to reduce the quantity of aflatoxin in food (9,121,122). These include genetic modification of crops to enhance fungal resistance and biocontrol of aspergillus by flooding fields with non-toxigenic fungi. Much of the aflatoxin contamination of food occurs after harvesting and during food storage. Methods to reduce humidity can limit fungal growth. Drying the crop in the sun on a mat, discarding visibly moldy kernels or nuts (such as peanuts) before storage, use of natural fiber sacks for storage, and placing these on wooden pallets to keep the crop dry are all very effective. In addition, shifting the staple diet from corn to rice limits exposure to aflatoxin in highly prone areas. The decrease in aflatoxin contamination in the food supply as a result of economic development, coupled to an increase in standard of living, has likely contributed, at least in part, to the decreasing burden of HCC in Singapore and Shanghai (China) over the past two decades (20).

Because aflatoxin contamination in food is not completely avoidable, prevention of aflatoxin-related HCC can also be attempted by the use of agents able to modulate the metabolism of aflatoxin once ingested (124). This introduces the concept of chemoprevention of HCC, which will be canvassed later. Randomized clinical trials (RCTs) with oltipraz and chlorophyllin have been conducted in HBsAg carriers exposed to dietary aflatoxin. Both agents modulate levels of aflatoxin biomarkers in the study participants (125,126). However, confirmation of the ultimate objective of preventing liver cancer would require a large expenditure of resources that may not be economically feasible.

Other Fungal and Algal Toxins, and Water Purity

Additional bio-contamination of food and water could contribute to high rates of HCC in some areas, most of them also endemic for HBV. In sub-Saharan Africa and China, *fumonisin* is another fungal toxin that has been linked epidemiologically and mechanistically with liver cancer (127,128). Control measures are fundamentally similar to those for aflatoxin, particularly avoidance of moldy corn.

In Taiwan, consumption of pond or surface water, particularly that visibly contaminated by algal blooms (green water), was a risk factor for HCC in older studies (20). Provision of clean and uncontaminated water is a health priority for the world's poorest communities. Prevention of HCC can be added to the multiple reasons to strive for this basic health objective.

Nutritional Modification

While there is no proven influence of specific nutrients on risk of HCC, micronutrients may play a role (129–135). Selenium is an essential micronutrient, required for selenium-containing antioxidant enzymes such as superoxide dismutase (129). Dietary selenium and retinoic acid are important "natural" antioxidants that inhibit development of liver tumors in animals (129). In epidemiological studies of people with chronic HBV infection from China, selenium deficiency in the water supply and low baseline serum levels of retinol and selenium were associated with increased risk of HCC, and restoration of these trace elements may be effective in chemopreventive strategies (129–135). In addition, diets high in fruits and vegetables or fiber appeared protective to some cancers (20,130–134). However, RCTs of antioxidant-rich dietary supplements, such as selenium, vitamin A, vitamin C, and vitamin E, on the incidence of HCC in high-risk populations are now needed.

The consistent inverse relationship between coffee drinking and HCC is interesting (136–142). Levels of coffee consumption have been associated with reduced risk of cirrhosis irrespective of etiology of liver disease, but it is unclear whether consumption of coffee actually protects against liver injury to reduce risk of HCC (136–142). Other explanations are possible. For example, dyspepsia as a side effect of prolonged high blood caffeine levels may lead to dose reductions, since high blood levels are a likely result from the known impairment of caffeine metabolism, which occurs in cirrhosis (143). Consumption of green tea is another possible protective factor against HCC (129,144,145). Currently, there is insufficient evidence to recommend increased consumption of green tea and coffee as preventive measures against HCC.

Prevention and Control of Alcoholism and Related Liver Disease

Chronic alcohol use of greater than 80 g/day for more than 10 years is an independent and strong risk factor for HCC, as shown by case-control studies (146–150). In the United States and Italy, excessive alcohol use remains the most common cause of HCC, although the risk of developing HCC with alcoholic cirrhosis may be somewhat less than for CHB or CHC-related cirrhosis (146–149). In addition to the primary role that ALD plays as a cause of cirrhosis, excessive alcohol consumption has deleterious effects on the liver in the presence of comorbid conditions, such as chronic HBV and/or HCV infections, HH, type 2 diabetes, and obesity (17,109,150). For example, previous or continued excessive alcohol ingestion contributes importantly to a continuing risk of HCC following SVR to treatment of CHC, as discussed (sect. "Treatment of Chronic Hepatitis C").

Reduction of Alcohol Consumption

Rates of alcoholism are increasing in parts of Asia and Africa where chronic viral hepatitis is endemic, indicating that alcohol abuse will continue to be an important primary or contributing cause of HCC on a world scale (151,152). Recognition that hazardous drinking is linked to liver injury and risk of liver cancer should motivate individual counseling on health maintenance, as well as rational public health policies. A principal objective in the community is to reduce per capita alcohol consumption. Historically, prevention strategies have involved limiting availability, enforcement of stricter laws pertaining to alcohol use, education to improve community standards, and increasing the cost of alcoholic beverages through taxation. Measures that address the economic and physical accessibility of alcohol, particularly making it more expensive, appear to be the most effective (151,152).

Primary care providers can be asked to screen for, and to treat alcohol dependency and abuse. Office screening tools in medical practice, combined with relatively brief interventions, can be powerful methods to assist those numerous individuals at risk for and those with ALD. Sadly, despite its undoubted importance as a risk factor, there is limited evidence to support the effectiveness of alcohol reduction interventions at reducing the incidence of HCC (151,152).

Treatment of Alcoholic Liver Disease

Continued abstinence from alcohol consumption and correction of nutritional deficiencies are critical aspects for the long-term management of patients with ALD (153). A survival benefit from abstinence has been established, but it is unclear the extent to which this reflects protection from liver failure and complications of portal hypertension and infection versus any protection from earlier carcinogenic effects of alcohol (151,153). There are few studies that have addressed whether continued alcohol ingestion is relevant to onset of HCC, or whether the risk decreases in those who achieve and remain abstinent (146–149). In one study, the risk appeared to be highest during the first 10 years after stopping excessive alcohol consumption (147). Further studies are required to establish this, and to identify whether individual risk factors additional to the undoubted importance of age and male gender are important, particularly those that might be amenable to therapeutic intervention.

The treatment of alcoholic cirrhosis remains symptomatic, except for liver transplantation in advanced cases in which abstention from alcohol has been sustained at least six months (154). The risk for HCC in decompensated alcoholic cirrhosis approaches 1% per year, a risk that may not decrease with abstinence (146). Therefore, patients with end-stage ALD should be considered for liver transplantation once abstinent; the results are similar to those in nonalcoholic patients (153,154).

Early Detection and Treatment of Iron Overload Disorders

At the stage of cirrhosis, HCC is the most common cause of death from HH (155,156). Timely and regular phlebotomy therapy protects against development of cirrhosis, and thereby prevents onset of HCC (155,156). It is therefore important to identify persons with hemochromatosis gene defects before excessive iron stores cause tissue damage (155,157–161). This is accomplished by family screening after discovery of an affected proband (158,159).

In European (especially Anglo-Celtic) populations, more than 80% of cases of HH are caused by homozygosity of C282Y, which is readily detected by a simple molecular assay based on restriction fragment length polymorphisms (155).

Disease penetrance for homozygous C282Y individuals varies from 10% to 60% (155,157). Phlebotomy should be performed when there is evidence of iron accumulation, such as sustained elevation of serum ferritin and increased transferrin iron saturation (155–159). Those younger than 40 years with serum ferritin levels below 1000 ng/mL and normal ALT do not have advanced hepatic fibrosis or cirrhosis (155). Liver biopsy is therefore unnecessary, phlebotomy should be performed to restitute normal body iron stores, and screening for HCC is not required (156). In other circumstances, liver biopsy is indicated to establish the state of hepatic fibrosis and the corresponding risk of HCC. Once cirrhosis is established, the risk of HCC is *not* abolished by phlebotomy (155,156,159). Screening for HCC, and other preventive measures (curbing excessive alcohol intake, cessation of cigarette smoking, correction of obesity and treatment of diabetes, increasing vegetable consumption) should be introduced (155–160).

In cases of secondary iron overload (due to multiple blood transfusions, for example, for thalassemia major) unable to tolerate phlebotomy (160,162), iron chelators, including desferrioxamine or newer oral iron chelators such as deferiprone and deferasirox may be useful (161,163). However, the effects, if any, on HCC prevention have not yet been documented.

Treatment of Primary Biliary Cirrhosis

Development of HCC in patients with stage IV PBC (cirrhosis) is rare. The risk is higher in men (164). Ursodeoxycholic acid (UDCA) is the only medication so far demonstrated to slow disease progression to death or liver transplantation (165–168), but there is no evidence that UDCA reduces HCC risk in PBC (165,168).

Control of Obesity and Related Liver Disease

Obesity and resultant insulin resistance are the critical risk factors for NAFLD and are pathogenically related to type 2 diabetes, cardiovascular disease and some cancers (169–172). Diabetes appears to be an independent risk factor for HCC, irrespective of the primary cause (173). It is therefore possible that the current pandemic of obesity and diabetes has contributed to the doubling of HCC incidence in the United States and Australia during the past two decades (172). It follows that proper management of overweight/obesity could decrease cancer incidence (174,175).

Treatment of Overweight and Obesity

Currently available weight loss treatments include dietary intervention, particularly coupled to increased physical activity, behavior modification, pharmacotherapy, and bariatric surgery (174,175). Common behaviors among patients with obesity who have achieved successful long-term weight loss without bariatric surgery are a guide to cost-effective interventions (174). Such include: (*i*) consume a diet low in calories and fat; (*ii*) regular engage in moderate physical activity; (*iii*) check body weight and abdominal circumference regularly (174). At the community level, efforts are needed to promote healthy food choices, to restrict overeating at times of diminishing energy requirements (e.g., retirement, increasing age), and to adopt strategies such as improved urban planning that promote more physical activity, including organized exercise programs. Reversal of the obesity epidemic requires a multipronged approach: education, time planning, recreational facilities and possible legislation to improve food choices. These concepts are relevant to primary prevention of obesity-related liver injury and HCC, as well as other common cancers (colon, breast, pancreas), for which lifestyle aspects may likewise play primary roles (175).

Treatment of NAFLD and Cryptogenic Cirrhosis

NAFLD is a spectrum of fatty liver disease that includes the necroinflammatory fibrosing disorder of nonalcoholic steatohepatitis (NASH) and, most likely, many cases of cryptogenic

cirrhosis occurring in association with obesity and diabetes (176). HCC can arise from NASH and related cirrhosis, although it appears to do so less frequently than for HCV-related cirrhosis (176). As for other disorders, older men may be at higher risk (177–180). The worrying rise in the global prevalence of NAFLD and NASH and the slow progress in identifying useful pharmacological therapy highlight the importance of effective management for underlying obesity and diabetes (172).

Lifestyle modification that includes dietary restriction and exercise to achieve judicious weight loss, particularly that required to correct central obesity, coupled to appropriate management of diabetes and dyslipidemia, is recommended as the first approach (181–183). Those with morbid obesity [body mass index (BMI) > 35 kg/m^2 in Europeans; possibly >30 kg/m^2 in Asians] refractory to medical and lifestyle approaches should be considered for bariatric surgery (181,183). Other potential therapeutic modalities against NASH include insulin-sensitizing agents (metformin, rosiglitazone, and piaglitazone), antioxidants, cytoprotective agents, and lipid-lowering drugs. However, these remain investigational and are currently not recommended for routine clinical practice (181–183).

Tobacco Smoking Awareness

Tobacco use, the most important preventable cause for many cancers, is only weakly linked to risk for HCC (17,184–186). A Greek study found an interaction between alcohol drinking and tobacco smoking for HCC risk, after controlling for HBV and HCV infections (17). However, in the presence of stronger risk factors, any possible carcinogenic effect of tobacco is easily masked (184–186). Public health and education policies to discourage active and passive smoking are important measures to reduce the incidence of lung and other cancers (187,188), but any impact on the risk of HCC is likely to be negligible.

Prevention of Sex Steroid–Related Liver Tumors

Estrogens and androgenic steroids have both been related to development of liver tumors, mostly benign. This topic has been reviewed elsewhere, and there are few recent developments (189,190). Long-term high dose estrogens-containing oral contraceptive steroids increase the risk of HCC, particularly the fibrolamellar variant that occurs in non-cirrhotic liver. Avoidance of continued oral contraceptive use without interruption, particularly with higher estrogen–containing preparations is now advised for a variety of health reasons, particularly thromboembolic and other vascular complications. Regular physical examination to detect liver enlargement or liver tumors, or annual ultrasound screening would be reasonable precautions if medical indications are such that prolonged exposure to high-dose estrogens is unavoidable. Similar arguments can be mounted for androgenic steroids. However, the effects, if any, of oral contraceptive steroids on HCC risk in HBV-endemic countries could not be detected in epidemiological studies (191).

SECONDARY PREVENTION

Secondary prevention is a treatment aimed at prevention of tumor recurrence after effective local control of an initial liver cancer with surgery or ablative therapy. It is an important issue as recurrence of HCC, including formation of new tumors, is extremely high after apparently effective local treatment by hepatic resection or percutaneous ablation (192). Recurrence within two years is likely to represent microscopic metastases from the original tumor, while tumors occurring after more than two years are usually new HCCs arising as the result of the underlying premalignant liver disease, typically, cirrhosis associated with CHB or CHC (9,192).

Apart from liver transplantation to remove premalignant cirrhosis, tertiary preventive strategies against HCC include systemic and local chemotherapy (9,192). The latter includes transarterial chemoembolization (TACE) with ^{131}iodine-labeled lipiodol, administration of antiviral therapies (such as interferon) (192), and oral administration of retinoid and vitamin K analogs. Each of these approaches has been evaluated by RCT (Table 10), and the results have been promising (192–208). These include extended disease-free interval and improvement of

Table 10 RCT of Agents Used to Prevent Tumor Recurrence After Resection or Ablation of Hepatocellular Carcinoma

Author (year) (reference number)	Prevention strategy	Number of treated vs. untreated	Etiology of hepatitis (*n*)	Median follow-up (yr)	Outcomes
Muto (1996) (193,194)	Polyprenoic acid 600 mg/d for 12 mo	44 vs. 45	HCV (89)	3	Reduced late recurrence
Kakizaki (2007) (195)	Menatetrenone 45 mg/d for 36 mo	30 vs. 30	HCV (60)	3	Reduced late recurrence, not survival
Mizuta (2006) (196)	Menatetrenone 45 mg/d for 36 mo	32 vs. 29	HCV (61)	3	Reduce late recurrence and improved survival
Mazzaferro (2006) (197)	IFNα 3 MIU 3/wk for 48 wk	76 vs. 74	HCV (80) HBV + HCV (70)	4	Reduced late recurrence with HCV alone
Sun (2006) (198)	IFNα 5 MIU 3/wk for 18 mo	118 vs. 118	HBV (236)	3	Reduced early recurrence and improved survival
Nishiguchi (2005) (199)	IFNα 6 MIU/d for 2 wk, then 2–3/wk for 88 wk	15 vs. 15	HCV 30	2	Reduced recurrence and improved survival
Lin (2004) (200)	IFNα 3 MIU 3/wk for 24 mo, or 3 MIU/d for 10 d every mo for 6 mo, then 3 MIU/d for 10 d every 3 mo for 18 mo	20 vs. 10	HBV (16) HCV (13) HBV + HCV (1)	2	Reduced 1- and 4-year recurrence
Shiratori (2003) (201)	IFNα 6 MIU 3/wk for 48 wk	49 vs. 25	HCV 74	7	Improved long-term survival; no effect on first recurrence
Miyaguchi (2002) (202)	IFNα 3 MIU 3/wk for 4 mo	22 vs. 24	HCV (46)	1	Reduced recurrence and improved survival
Kubo (2001, 2002) (203,204)	IFNα 6 MIU/d for 2 wk, 3/wk for 14 wk, and 2/wk for 88 wk	15 vs. 15	HCV (30)	2	Reduced late recurrence and improved survival
Ikeda (2000) (205)	IFNβ 6 MIU 2/wk for 36 mo	10 vs. 10	HCV (20)	2	Reduced early recurrence
Lau (1999, 2008) (206,207)	^{131}Iodine- lipiodol, 1850-MBq dose intra-arterial infusion	21 vs. 22	HBV (43)	10	Improved 8-year disease-free and overall survival
Lo (2007) (208)	IFNα 10 MIU/m^2 3/ wk for 16 wk	40 vs. 40	HBV (77), HCV (2), HBV + HCV (1)	5	Reduced early recurrence and improved 5-year survival

Note: '*n*' means 'number of patients with specific etiology'.
Abbreviations: RCT, randomized control trial; IFN, interferon; MIU, Million International Units; HBV, hepatitis B virus; HCV, hepatitis C virus.

overall survival after liver resection or ablation for HCC. However, benefit appears confined to late recurrence (formation of new tumor), and to date there is no effective chemotherapy to prevent early recurrent HCC (eradication of microscope metastases).

Polyprenoic acid (an acyclic derivative of retinoic acid) and menatetrenone (vitamin K2) have both demonstrated efficacy for preventing late recurrence of HCC after curative therapy in single studies (193–196). It is unclear from the available literature whether there are any possible safety issues to be resolved (209,210). Further, the apparently beneficial findings must be confirmed in larger RCTs before any tertiary prevention strategy enters routine clinical practice.

An important exception is to treat CHB and CHC with currently available agents. Several RCTs have shown beneficial effects of adjuvant therapy with either interferon α or β, either for the entire study population or for defined subpopulations, after hepatic resection or ablation for HCC (192,197–208). In terms of early recurrence, the effect seems particularly striking for patients with CHB, but interferon consistently reduces late recurrence after local control of HCC in patients with CHC (Table 10). Eradication of HCV infection appears to be essential for such prevention of HCC recurrence and improvement of survival. These encouraging data should stimulate the search for more effective strategies, for example, use of pegylated interferon in combination with ribavirin. Alternatively, pegylated interferon or more potent nucleoside analogs with a high threshold for drug resistance should be used in the case of CHB.

The third approach is irradiation of the liver using intra-arterial ^{131}iodine-labeled lipiodol after HCC resection, with the aim of eradicating neoplastic foci. In a small RCT, ^{131}iodine-labeled lipiodol reduced recurrence rate and increased disease-free survival compared with controls (206). The beneficial effect appeared to last for up to eight years after randomization (207). In addition, postoperative adjuvant intra-portal chemotherapy alone or TACE combined with portal vein chemotherapy may improve survival of HCC patients provided there are no contraindications, such as portal vein occlusion (210–212).

CONCLUSIONS AND PERSPECTIVE

HCC is unusual among common human cancers in that one or more causative agents can be identified in most cases. Further, although a slight oversimplification, HCC can generally be viewed as a complication of cirrhosis, and the incidence is highest when the factors causing liver injury and inflammation (hepatitis) continue to operate. On a world scale, chronic HBV infection is the commonest cause of HCC. This is a readily preventable infection. Effective vaccines have been available for 25 years, and their community application in infant vaccination programs has already been shown to reduce childhood cases of HCC. Introduction of hepatitis B vaccination into areas of greatest need, including the most populous and most economically challenged regions of the world, remains incomplete and is an important issue to address at the world health level.

Meanwhile, the full impact of this crucial preventive strategy on HCC incidence is unlikely to be appreciable for another 30 to 40 years. Therefore, the most exciting development of the last 3 to 5 years is the discovery that effective antiviral therapy of CHB carries considerable potential to prevent development of HCC, within the first 3 years for cases at advanced fibrotic stage or cirrhosis. Further observations on whether earlier (before cirrhosis) and more effective (more potent, higher–resistance threshold) antiviral therapy reduces HCC risk by more than 50%, and the full long-term benefits of suppressing viral load by antiviral therapy are awaited with interest.

HH is readily detectable by a genetic test, and tissue injury reversible before complications of cirrhosis and HCC ensue. Other liver diseases leading to HCC are more difficult to prevent. Transmission of HCV, the second major cause of HCC, by medical contamination can be, and now should be, fully prevented by screening of donor blood and universal precautions against nosocomial transmission of blood-borne viruses. In most countries, however, IDU has become the most common, virtually exclusive, route of HCV transmission. To date, public health and community education projects appear to have made

little impact on the current high incidence of new HCV infections. There is a great need for development of a hepatitis C vaccine, because prevention of primary infection would be by far the most effective preventive strategy against CHC and resultant cirrhosis and HCC.

Meanwhile, effective treatment of hepatitis C can reduce the risk of HCC substantially even at the stage of cirrhosis. Cases of CHC among those older than 40 years must therefore be carefully assessed for fibrotic severity to inform decisions about antiviral therapy. Alcohol contributes to HCC as a complication of cirrhotic ALD but also in those infected with HCV, HBV, and HH, and possibly those with obesity and diabetes. Community-based programs to discourage and deal with excessive alcohol intake, as well as those aimed at reducing the pandemic of obesity and diabetes, are therefore vital for the attempts to interrupt the rising tide of HCC in countries where until recently it has not been a common cause of cancer death.

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5 Tumor Markers and Molecular Biology

Smruti R. Mohanty and Donald M. Jensen
Department of Medicine, Center for Liver Diseases, The University of Chicago, Chicago, Illinois, U.S.A.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Currently, the rate of HCC is rising throughout the world, including the United States. The vast majority of HCC is diagnosed in a late stage, leading to poor prognosis. Surgical resection, including liver transplantation, is the only potentially curative therapy if HCC is diagnosed in an early stage. However, a significant number of patients develop recurrent HCC despite receiving surgical resection or liver transplantation. Therefore, screening for HCC in patients with cirrhosis and the ability to predict individual recurrence risk, including assessing prognosis are important in guiding therapy. Current screening criteria for HCC include the combination of serum α-fetoprotein (AFP) and an ultrasound of liver at six-month intervals in patients with cirrhosis. However, AFP has a poor sensitivity and specificity for the early diagnosis of HCC. Therefore, additional tumor or molecular markers need to be studied for their utility for screening and assessing prognosis of HCC in future studies. Several tumor markers including AFP-L3, des-γ-carboxyprothrombin (DCP), Golgi protein 73 (GP73), hepatoma-specific γ-glutamyl transferase (HS-GGT), human cervical cancer oncogene (HCCR), and telomerase have been identified as potential tumor markers for screening for HCC in patients with cirrhosis. Furthermore, recent advancements in the understanding of hepatic carcinogenesis have also resulted in investigations of several tumor markers and molecular biomarkers for their screening potential and prognostic significance in terms of angiogenesis, invasion, and metastasis in patients with HCC. In addition, proteomics analysis might be expected to generate new HCC-specific markers in the future. Recently, several potential molecular markers including Bcl-2, heat shock protein (HSP), vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), angiopoietins, and matrix metaloproteinases have shown promise in clinical studies as prognostic markers following resection of HCC for recurrence and/or overall survival in patients with HCC. This chapter reviews a number of tumor markers, including molecular biomarkers, with potential as tools for early identification of HCC and prognostic indication.

HCC is the third leading cause of death and is the fifth most common cancer worldwide (1). Cirrhosis, from variety of causes including hepatitis B and C, is the leading risk factor for HCC. Even though the prevalence of HCC is the highest in Asia and Africa, the incidence of HCC in western countries, including the United States, is rising because of the increasing rate of hepatitis C virus (HCV) infection.

For the last two decades, the major focus has been on early diagnosis, management, and prevention of HCC. The incidence of HCC, however, continues to rise. Therefore, patients who are at high risk for HCC should be screened regularly using serum AFP and a liver ultrasound every six months. Unfortunately, the sensitivity and specificity of AFP for early diagnosis of HCC is extremely poor, and it varies with cutoff value, etiology of liver disease, and tumor stage (2). Despite the possibility of early diagnosis of HCC, a significant number of patients present with advanced disease. Currently, curative therapies for HCC, including surgery or liver transplantation, are most effective when HCC is diagnosed in an early stage. However, the long-term prognosis of patients undergoing potential curative resection for HCC is still poor because of a lower reported five-year survival rates ranging from 17% to 53% (3,4). Furthermore, a significant number of patients are diagnosed at an advanced stage. Even though liver transplantation is a good option for early HCC associated with cirrhosis, its utility is limited by a shortage of liver grafts and limitations in the size and number of tumors suitable for treatment. Finally, for patients who have advanced or metastatic HCC, there is no effective

chemotherapy. Because treatment is most effective in the early stages of the disease, there is a need for effective biomarkers for the early diagnosis and surveillance of HCC, including monitoring for postoperative recurrence. In this chapter, we will describe currently available tumor markers and discuss some new markers, including molecular markers that have shown promising results in clinical studies for early diagnosis, monitoring metastasis, and postoperative recurrence of HCC.

SIGNIFICANCE OF TUMOR MARKERS

In general, tumor markers are used to aid in early diagnosis, assessment of stage, and prognosis of cancer. A tumor marker is a substance created by a tumor or by the host in response to a tumor and is detectible in biological fluids or tissues. Typically, a tumor marker is used to differentiate between benign and malignant tumors. In the case of HCC, a tumor marker should differentiate HCC from regenerative nodules and benign neoplasms. Tumor markers can also be used to localize tumors, monitor effectiveness of therapy, and detect recurrence of a tumor. Finally, tumor markers can be used to screen general as well as at-risk populations. It is important that tumor markers be easily measurable, minimally invasive, relatively inexpensive, and provide reproducible results (5,6). Tumor markers, also referred to as biomarkers, include enzymes, isoenzymes, hormones, oncofetal antigens, carbohydrate epitopes, oncogene products, and genetic alterations. Unfortunately, none of the currently available tumor markers qualifies as ideal for HCC. Currently, the Early Detection Research Network (EDRN) of the National Cancer Institute, United States, is evaluating several biomarkers for cancer surveillance, including biomarkers for HCC and their prognostic significance. The biomarkers must be validated by a five-phase program, which includes preclinical exploratory study, clinical assay and validation, retrospective longitudinal assessment, prospective screening assessment, and cancer control, as outlined by the EDRN (7).

CURRENTLY USED TUMOR MARKERS

Tumor markers may be considered indicators of a premalignant state or precursors for HCC, and hence may be particularly important for the early diagnosis of HCC. Since the prognosis of advanced HCC is poor, early diagnosis of small tumors at an early stage is critical for successful treatment, including surgical resection, liver transplantation, and transarterial chemoembolization (TACE)/radiofrequency ablation (RFA). Early identification and treatment may result in lower rates of recurrent HCC, better prognosis, and prolonged survival (8–10). Therefore, a cancer-screening program has been recommended by a consensus conference for early diagnosis of HCC in patients with cirrhosis, which uses serum AFP and ultrasonography (US) (2). Even though screening for the early diagnosis of HCC has been shown to be cost-effective, increased survival is still an unresolved issue. It has been noted that screening cost can be attributed largely to the cost of treatment of HCC rather than to screening per se (11). This conclusion is mainly due to the fact that the currently available diagnostic tests are not very useful for early diagnosis of HCC, which allows the HCC to progress to advanced stages and results in greater treatment costs. Thus, an acceptable balance between early diagnosis of HCC in patients with cirrhosis and cost-effective survival benefits has not been found. Therefore, new serum tumor markers, including oncofetal antigens, glycoprotein antigens, enzymes, and isoenzymes, are being studied for early diagnosis and prognostic indication (Table 1).

SERUM TUMOR MARKERS

Oncofetal Antigens and Glycoprotein Antigens

α-Fetoprotein and α-Fetoprotein-L3

AFP is an oncofetal glycoprotein that is present at increased levels in sera of patients with cirrhosis and HCC. It is a fetal-specific antigen, primarily produced in fetal liver. Its serum concentration falls rapidly after birth and synthesis decreases in adult life. However, a

Table 1 Currently Used and Investigational Serum Tumor Markers

Markers	Characteristics	Cutoff level	Sensitivity (%)	Specificity (%)	Comments
AFP (12–20)	Oncofetal glycoprotein	10–16 ng/dL 20 ng/dL	60–80 39–66	70–90 76–97	Poor marker alone
AFP-L3 (21–28)	AFP variant (subtype)	10%	39.9	93.4	Useful in combination markers
		15%	36.1–96	92–99.5	
GP73 (29–32)	Golgi-specific membrane protein	10 relative units	69	86	Promising marker
GPC3 (33–39)	Oncofetal glycoprotein	2 ng/dL	51	90	Limited utility as a marker
DCP (40–50)	Abnormal prothrombin	40 mAU/mL	48–62	81–98	Useful in combination markers
HS-GGT (51–53)	Membrane-bound protein	5.5 IU/mL	43.8–74	Not available	Nonspecific marker
AFU (55–58)	Lysomal enzyme	870 nmol/mL/hr	82	71	Lower specificity and poor marker

Note: AFP has high false positive and false negative rates with poor performance as a tumor maker. It may be a useful maker in combination with DCP or GP73. AFP-L3 is superior and more specific for HCC than to AFP. It may be also useful as a prognostic marker. GP73 is a superior screening marker compared with AFP and requires further validation in disease-specific HCC. DCP has lower sensitivity and has limited sensitivity for small HCC ($<$3 cm) compared with AFP. However, it has been noted to have higher sensitivity and specificity in combination with other markers.
Abbreviations: AFP, α-fetoprotein; GP73, Golgi protein 73; GPC3, glypican-3; HS-GGT, hepatoma-specific γ-glutamyl transferase; DCP, des-γ-carboxyprothrombin; HCC, hepatocellular carcinoma.

significant number of patients with HCC may have a high concentration of AFP due to tumor expression. Unfortunately, the sensitivity and specificity of AFP for screening and surveillance of HCC vary according to study design, test thresholds, and study population. Overall, sensitivity and specificity range from 39% to 65% and 76% to 97%, respectively (12–15). In two studies, when the AFP cutoff value was increased from 10 ng/dL to 16 ng/dL, sensitivity and specificity changed to 60% to 80% and 70% to 90%, respectively (16,17). Thus, the high variability of sensitivity and specificity is mainly due to different cutoff values used in different studies that are retrospective in nature. Although AFP values above 400 ng/dL are considered diagnostic for HCC, the percentage of patients with such a high level of AFP is small (15). On the other hand, patients with high AFP (above 400 ng/dL) tend to have larger tumor size, multilobar or diffuse involvement, portal vein thrombosis, and a lower survival rate (18,19). It has also been noted that the utility of AFP in differentiating HCC from benign liver diseases is limited with high false positive and false negative rates (20). Although AFP is considered a gold standard as a serum marker of HCC, its utility as a screening test for HCC is of questionable value because of its poor performance.

To improve the performance of AFP as a HCC serum marker, the role of variant forms of AFP has been investigated. Three different AFP variants, AFP-L1, AFP-L2, and AFP-L3, have been studied. Each variant has a different sugar chain with a differential affinity for lectins, such as *Lens culinaris* agglutinin. AFP-L3 (*Lens culinaris*-reactive AFP) is superior and more specific for HCC than is total AFP (21,22). The assay for AFP-L3 can simultaneously measure total serum AFP and AFP-L3 and was developed by Wako Pure Chemical Industries, Hyogo, Japan (23,24).

The U.S. Food and Drug Administration (FDA) recently approved AFP-L3 as a screening marker for HCC. The percentage of AFP-L3 is calculated as a ratio of AFP-L3 to total AFP. Initial investigation demonstrated a sensitivity and specificity of 55.3% and 93.9%, respectively, when a 15% AFP-L3 cutoff value was used (25). However, a more recent study, using

the same cutoff value showed sensitivity and specificity of 96% and 92%, respectively (26). Another study using 10% to 15% AFP-L3 cutoff values exhibited a lower sensitivity (30.9–36.1%) but comparable specificity (93.4–99.5%) (27). The diagnostic accuracy of AFP-L3 versus total AFP in differentiating patients with cirrhosis and regenerating nodules from patients with HCC, however, has not been examined. Interestingly, several studies have reported that AFP-L3 may be a useful prognostic marker for HCC, and higher percentage values are associated with large tumor size, poorly differentiated HCC, vascular invasion, and metastasis (26–28). Therefore, it appears that because of inconsistency of its sensitivity and specificity data for predicting HCC occurrence, AFP-L3 is still unreliable, even though it is more specific than total AFP. AFP-L3 may not be very useful for surveillance even though it may prove to be a useful prognostic marker in patients with known HCC.

Golgi Protein 73

GP73 is a Golgi-specific membrane protein that is upregulated in virus-infected hepatocytes in patients with cirrhosis and HCC (29,30). However, the function of GP73 in normal liver cells, including its upregulation and release into blood in patients with HCC, is still not well known. GP73 is measured by immunoblot assay with densitometric analysis. It is superior to AFP in detecting HCC in cirrhotic patients and it is elevated in 57% of patients with HCC who have a normal AFP (31). Its sensitivity and specificity are 69% and 86%, respectively, compared with 30% and 96% using AFP at the optimal cutoff value (31,32). Even though it is a promising marker for early diagnosis of HCC, further studies are required to validate the aforementioned data and confirm GP73's role.

Glypican-3

Glypican-3 (GPC3) is a cell surface oncofetal glycoprotein that is normally involved in the regulation of cell proliferation during embryo development and acts as a tumor suppressor (33,34). It is highly expressed in patients with HCC cells but absent in hepatocytes of healthy people or those with hepatitis (35,36).

Increased serum levels of GPC3 fragments have been noted in HCC patients in recent studies. The C-terminal fragment of GPC3 was found in the serum of 53% of patients with HCC compared with none in healthy control subjects (37). Furthermore, the fragments were present in only 1 of 20 cirrhotic patients, suggesting a high specificity. Similar findings were also reported by Nakatsura et al. (38). In comparison, Hippo et al. reported that the N-terminal fragment of GPC3 was predominant in the serum of HCC patients and reported a sensitivity and specificity (51% and 90%, cutoff: 2.0 ng/dL) comparable to that of AFP (55% and 90%, cutoff: 20 ng/dL) (39). In addition, this study also showed that the soluble form of GPC3 was more sensitive than AFP in detecting well-differentiated and moderately differentiated HCC. However, GPC3 has no higher sensitivity or specificity compared with AFP as a tumor marker. Therefore, its clinical utility as a tumor maker for HCC is limited if it is used alone. However, the utility of GPC3 combined with two or more additional markers, including AFP, should be tested to compare sensitivity and specificity with currently approved tumor makers.

Enzymes and Isoenzymes

Des-γ-Carboxyprothrombin

DCP is an abnormal prothrombin, lacking γ-carboxylase activity, which results in a reduced carboxylation of glutamic acid residues at the N-terminus region. This reduced activity occurs because of an acquired posttranslational defect of the prothrombin precursor in HCC cells (40). Liebman et al. described DCP as a serum tumor marker for HCC and reported higher levels of DCP in patients with HCC and recurrent HCC after surgical resection (41). DCP is found in 50% to 60% of patients with HCC. A DCP level of 40 mAU/mL is considered the cutoff value. However, the sensitivity and specificity of this value vary widely, ranging from 48% to 62% and 81% to 98%, respectively (42–48). DCP has not been shown to have a superior sensitivity

and specificity compared with AFP. The sensitivity of DCP was limited for small HCC (<3 cm) (41,49,50). In addition, there was no correlation between AFP and DCP results. DCP, however, is more specific for HCC compared with AFP since AFP can be elevated in patients with chronic liver disease without HCC. Furthermore, studies have indicated that DCP-positive HCC cells have a higher rate of intrahepatic spread, portal vein invasion, and liver capsule infiltration.

Since AFP (and AFP-L3) and DCP levels do not correlate in patients with HCC, researchers have suggested studying the utility of these two markers together to improve early diagnosis of HCC. One study reported a higher sensitivity and specificity with these combined markers (45). Consequently, DCP has been included in the list of HCC markers recommended for study of early diagnosis of HCC by the EDRN network. In summary, it appears that DCP is a more reliable prognostic marker than AFP in patients with HCC rather than a marker for early diagnosis. In addition, determination of AFP, AFP-L3, and DCP levels may be useful to monitor tumor progression and efficacy of treatment in patients with HCC.

γ-Glutamyl Transferase

γ-Glutamyl transferase (GGT) is a membrane-bound enzyme that exhibits a tissue-specific expression and is influenced by various physiological and pathological conditions, including fetal liver development and hepatic carcinogenesis (51,52). Its concentration is high in embryonic liver and decreases after birth. In general, total GGT activity increases with chronic liver disease, HCC, and extrahepatic tumors. There are several isoforms of GGT, which are divided into several subfractions on the basis of the gel electrophoresis mobility. HS-GGT and (I′, II, and II′) have been detected in the sera of patients with HCC. It has been reported that HS-GGT activity significantly increased in HCC, with 86% of patients above 5.5 IU/mL compared with less than 3% of patients with other diseases (53). GGT has been noted to be useful as a specific HCC marker, and its sensitivity has been reported to be 74% in detecting any size of HCC and 43.8% in detecting small HCC (54). Thus, it appears that HS-GGT is a valuable tumor marker for detecting HCC and may be a good supplementary marker to AFP and AFP-L3.

α-L-Fucosidase

α-L-fucosidase (AFU) is a lysosomal enzyme whose activity is elevated in the sera of patients with HCC compared with both healthy subjects and patients with chronic liver disease (55,56). Its sensitivity and specificity at a cutoff value of 870 nmol/mL/hr are 82% and 71%, respectively. In addition, in 85% of patients who later developed HCC, increased levels of AFU above 700 nmol/mL/hr were noted at least six months before detection by ultrasound (56). Several small studies reported that AFU could be useful as a complementary marker in conjunction with AFP for detecting early HCC (57,58). However, specificity of AFU is limited for early detection because other diseases including diabetes, pancreatitis, and hypothyroidism are associated with increased AFU. In addition, its serum activities are affected by ethnicity and prolonged storage of sera, which results in a significant increase in enzyme activities. Therefore, the potential for AFU as HCC marker is unclear.

SIGNIFICANCE OF MOLECULAR MARKERS

Because of recent advances in the understanding of tumor biology, focus has shifted toward molecular cancer biomarkers, in terms of both their prognostic significance and therapeutic targets. Molecular biomarkers also have been shown to play a significant role in hepatocellular carcinogenesis. Therefore, the examination of current knowledge with respect to the prognostic value of molecular markers in HCC is very important. Several molecular biomarkers are discussed below, including tumor suppression genes, oncogenes, cell cycle regulators, apoptotic regulators, growth factors and receptors, proliferation indices, telomerase, and markers of angiogenesis, invasion, metastasis, and genomic instability (Table 2).

Table 2 Novel Molecular Biomarkers for HCC Screening and Prognosis

Molecular markers	Mechanism of action	Expression characteristic	Type of marker	Comments
p53 gene (62)	Tumor suppressor gene	Reduced disease-free and overall survival after resection	Prognostic marker	Requires further studies
Phosphatase and tensin homologue (63–66)	Tumor suppressor gene	Reduced overall survival	Prognostic marker	Requires further studies
C-met and c-myc oncogenes (67–70)	Oncogene	Early recurrence, reduced disease-free and overall survival after resection	Prognostic markers	Requires further studies
Human cervical cancer oncogene (71–73)	Cervical oncogene	Expressed in various cancers	Screening marker	Requires further studies
Cyclin A and D1 (74–78)	Cell cycle regulators	Reduced disease-free and overall survival after resection	Prognostic markers	Conflicting results
INK4 (79)	Cell cycle regulators	Increased recurrence after resection	Prognostic marker	Requires further studies
p27 and p57 (62)	Cell cycle regulators	Correlated with disease-free and overall survival after resection	Prognostic markers	Requires further studies
Bcl-xL and Bax (80,81)	Apoptosis mediator	Correlated with disease-free and overall survival after resection	Prognostic markers	Requires further studies
Survivin (82–84)	Apoptosis mediator	Correlated with recurrence and disease-specific survival after resection	Prognostic marker	Requires further studies
Heat shock protein and glucose-regulated protein (85–88)	Apoptosis mediator and tumor-immune response	Correlated with carcinogenesis and vascular invasion	Prognostic markers	Requires more studies in disease-specific HCC
Microvessel density markers (89–94)	Angiogenesis	Correlated with disease-free and overall survival after resection	Prognostic markers	Variable results
Vascular endothelial growth factor (94–104)	Angiogenesis	Early recurrence, reduced disease-free and overall survival after resection	Prognostic markers	Variable results
Hypoxia-inducible factor-1 class (98,99,104)	Angiogenesis and expressed in hypoxia	Reduced disease-free and overall survival after resection	Prognostic markers	Requires further studies in large samples
Inducible nitric oxide synthase (83,105)	Angiogenesis	Reduced disease-free and overall survival after resection	Prognostic markers	Variable results
Basic endothelial growth factor (102,106)	Angiogenesis	Reduced disease-free survival	Prognostic marker	Variable results
Platelet-derived-endothelial growth factor (107,108)	Angiogenesis	Reduced disease-free survival and recurrence after resection	Prognostic marker	Requires further studies
Tissue factor (109,110)	Angiogenesis	Reduced disease-free survival	Prognostic marker	Requires further studies
Endostatin/collagen XVIII (111)	Angiogenesis inhibitors	Reduced disease-free and overall survival after resection	Prognostic marker	Requires further studies

(*Continued*)

Table 2 Novel Molecular Biomarkers for HCC Screening and Prognosis (*Continued*)

Molecular markers	Mechanism of action	Expression characteristic	Type of marker	Comments
Interleukin-8 (112,113)	Angiogenesis	Correlated with disease-free and overall survival	Prognostic marker	Requires further studies
Angiopoietins (99,114)	Endothelial cell growth factors	Correlated with disease-free survival	Prognostic markers	Requires further studies
Matrix metaloproteases (115–118)	Degradation of extracellular matrix	Correlated with invasion and metastasis	Prognostic markers	Requires further studies
Urokinase plasminogen activator and urokinase plasminogen activator receptor (119,120)	Serine protease	Reduced disease-free and overall survival after resection	Prognostic markers	Requires further studies
E-cadherin (77,121–124)	Anti-apoptosis and angiogenesis	Early recurrence and reduced overall survival after resection	Prognostic markers	Conflicting results and requires further studies
Transforming growth factors-β (125–129)	Cell growth and angiogenesis	Decreased survival in inoperable HCC	Prognostic markers	Lack of disease specificity
Hepatocyte growth factor (130,131)	Cell growth factor	Increased level in HCC compared with hepatitis or cirrhosis	Screening marker	Lack of disease specificity
IGF (130,132–137)	Anti-apoptosis and carcinogenesis	Correlated in patients with HCC	Screening marker	Lower sensitivity and specificity
Insulin-like growth factor binding protein-2 (138,139)	IGF-signaling pathway	Increased level in HCC	Screening marker	Requires studies in large samples
Leptin (92,140)	Cell growth and angiogenesis	Increased overall survival after resection	Prognostic marker	Requires further studies
hTERT mRNA (141,142)	Cell growth and carcinogenesis	Increased expression of hTERT mRNA in HCC	Screening marker	Requires further studies

Abbreviations: INK, inhibitor of cyclin-dependent kinase; Bcl-xL, B-cell lymphoma-extra large; Bax, Bcl-2 associated X; IGF, insulin-like growth factor; hTERT, human telomerase reverse transcriptase; HCC, hepatocellular carcinoma.

MOLECULAR MARKERS

Tumor Suppressor Genes

p53 Gene

The p53 gene is a tumor suppressor gene that is responsible for the cell cycle and induction of apoptosis in response to severe damage to cellular DNA. Mutations of p53 result in unregulated replications of damaged DNA, including genomic instability leading to cancer. Multiple studies have reported the mutation of p53 in 24% to 69% of patients with HCC (59–61). However, the results of immunohistochemical expression of the p53 gene in HCC show conflicting results because of differences in methodologies, variations in antibody staining, and subjective analysis of staining (62). Multiple studies have reported that p53 mutation is a marker for poor prognosis suggesting shorter disease-free and overall survival in patients with HCC (62). The limitation of the prognostic data in p53 mutation studies is due to cohorts of patients with mixed etiological factors for HCC, which results in poor correlation of disease-free and overall survival with p53 mutation. Therefore, p53 mutation analysis cannot be used as a prognostic marker for HCC until more studies are conducted, which investigate specific HCC etiologies.

Other Tumor Suppressor Genes

The prognostic values of positive expression of the phosphatase and tensin homologue (PTEN) and loss of expression of the non-metastic (nm) protein tumor suppressor genes have been evaluated for overall survival following resection of HCC. These conditions have been shown to be related with decreased overall survival (63–66). However, more studies are needed to confirm the clinical application of these tumor suppression genes as prognostic indicators.

Oncogenes

C-met and C-myc Oncogenes

C-met and c-myc oncogenes have been evaluated as prognostic markers in patients with HCC. C-met is overexpressed in HCC, and its expression is associated with decreased overall survival (67,68). Similarly, increased c-myc expression has been associated with early recurrence of HCC and reduced disease-free and overall survival following HCC resection (69,70). The utility of these oncogenes as prognostic markers for HCC is still unclear and requires further investigation.

Human Cervical Cancer Oncogene

HCCR is a recently identified cervical oncogene (71). Increased levels of HCCR have been reported in various cancer tissues including breast, kidney, ovary, stomach, and colon. Two HCCR isoforms (HCCR-1 and HCCR-2) have been identified, although the functions of these isoforms are unknown (72). HCCR has been found in HCC cells without expression in the surrounding cirrhotic tissue, including normal hepatocytes (73). In addition, the ELISA assay used for the detection of c-terminus domains of HCCR-1 and HCCR-2 in sera showed that HCCR was 20% to 40% higher in HCC patients compared with cirrhotic controls without showing any difference between normal subjects and non-cirrhotic patients with chronic hepatitis. HCCR was also more sensitive (78% vs. 46%) than AFP but had similar specificity. In addition, a trend toward increased sensitivity (69% vs. 46%) for the detection of small HCC (<2 cm) was reported with HCCR compared with AFP, but the finding was not statistically significant. Further studies will be required to evaluate the clinical utility of HCCR as markers for HCC detection.

Cell Cycle Regulators

Cyclin and Cyclin-Dependent Kinases

Cyclin/cyclin-dependent kinases are important for cell cycle progression and have been investigated as prognostic markers following resection of HCC. Overexpression of cyclin A and cyclin D1 has been shown to be associated with decreased disease-free survival and

overall survival following resection of HCC (74–76). However, other studies showed no significant association of cyclin A and D1 as prognostic markers (77,78). Thus, the prognostic significance of cyclin A and cyclin D1 in patients with HCC is not well established.

Cyclin-Dependent Kinase Inhibitors

Cylin-dependent kinase inhibitors are negative regulators of cell cycle and inhibit the G1/S transition. Two families of cyclin-dependent kinase inhibitors including inhibitor of cyclin-dependent kinase (INK)4 and KIP/CIP have been evaluated as prognostic markers of HCC. In the case of the INK4 family, methylations of promoter regions of p15 and p16 leading to gene silencing have been reported in patients with HCC and these patients have been shown to have greater recurrence of HCC following resection (79). In the KIP/CIP family, p27 and p57 have been studied extensively with regard to their expression as it correlates with disease-free and overall survival following HCC resection (62). Expression of p27 has been associated with increased disease-free and overall survival, whereas loss of p57 expression has been shown to independently predict reduced overall and disease-free survival following hepatic resection in HCC patients. However, it is not clear whether cyclin-dependent kinase inhibitors can be used as prognostic indicators in patients who had resection of HCC in clinical settings.

Apoptosis-Mediated Factors

Bcl-2 Family

The Bcl-2 family of proteins including B-cell lymphoma-extra large (Bcl-xL) and Bcl-2 associated X (Bax) mediate apoptosis either by promoting or inhibiting apoptosis. Several studies have reported the prognostic significance of Bcl-2 family of apoptotic mediators in patients following resection of HCC. Bcl-xL overexpression independently predicts decreased disease-free and overall survival following resection of HCC (80,81). On the other hand, expression of Bax has been reported to independently predict increased overall survival following resection of HCC (80).

Survivin

Survivin is an anti-apoptotic protein, which inhibits apoptosis by targeting the terminal effectors caspase-3 and caspase-7 in the apoptotic protease cascade. Survivin expression has been associated with poor survival following resection of HCC. Nuclear survivin expression has been reported to be associated with decreased disease-free survival (82). In addition, patients with tumors expressing survivin mRNA were reported to have higher rates of recurrence and poor disease-specific survival rates than patients whose tumors did not express survivin mRNA following resection of HCC (83,84).

Heat Shock Protein

HSP is a highly conserved protein and is produced in response to heat and other physical and chemical stressors (85). HSP belongs to a family of stress-induced proteins called HSP and glucose-regulated proteins (GRPs). It participates in a variety of complex protein functions, including folding, extension, assembly, and protein transport between organelles. In neoplasms, expression of HSP has been associated with regulation of apoptosis and tumor-immune response. Expression of HSP27, HSP70, HSP90, GRP78, and GRP94 increased in a stepwise HCC development process from a dysplastic nodule to early HCC and then to advanced HCC in hepatitis B virus (HBV)-infected patients (86). However, a strong correlation of expression was only found with GRP78 regarding the stepwise HCC development process toward advanced HCC. In addition, GRP78, GRP94, and HSP 90 were each significantly associated with vascular invasion and intrahepatic metastasis. Another study using immunohistochemical analysis showed that 90% of hepatitis B DNA-positive HCC patients strongly expressed GRP94 compared with 46% of HBV DNA-negative patients (87). Additionally, expression of HSP70 and HSP27 might play an important role in hepatic carcinogenesis. HSP70 was found to be corelated with large tumor size, presence of portal vein6invasion, and high tumor stage (88). Similar correlations were reported with HSP27 in HBV-associated HCCs.

Markers of Angiogenesis

Microvessel Density

Microvessel density (MVD) utilizes immunohistochemical staining of endothelial cell markers and is used as an index of tumor angiogenesis. Commonly used MVD markers including CD34, CD31, and von Willebrand factor (vWF) have been used as prognostic markers after resection of HCC. MVD using CD34 has been shown to decrease disease-free survival (89,90) and overall survival (91). On the other hand, two recent studies using CD31 and CD34 markers did not show prognostic significance in patients who had resection of HCC (92,93). MVD using vWF independently predicted decreased disease-free survival (94). However, vWF did not show any prognostic value in a larger study (90). The differing results from studies of MVD may reflect small sample size and selection bias. Therefore, the utility of endothelial MVD markers as prognostic indicators remains unclear in patients who had HCC resection.

Vascular Endothelial Growth Factor

VEGF is an angiogenic stimulator and regulates tumor neovascularization. It has been reported on extensively for its prognostic significance in HCC. VEGF expression in HCC tumors is associated with an increased risk of early recurrence of HCC (95) and a greater risk of metastatic recurrence (96). However, other studies using VEGF assessed by immunohistochemistry showed variable results regarding its prognostic role for survival following resection of HCC (94,97–99). It also has been reported that serum levels of VEGF are correlated with tumor VEGF expression (100). Elevated serum VEGF levels prior to resection have been shown to independently predict decreased disease-free and overall survival following resection of HCC in multivariate analysis (101,102). Furthermore, the expression of VEGF16S isoform mRNA in resected HCC specimens has been reported to independently predict recurrence of HCC and recurrence-related mortality in patients (103). In addition, preoperative serum levels of VEGF16S isoform mRNA have been noted to be an independent prognostic marker for HCC recurrence and recurrence-related mortality (104). Therefore, preoperative serum levels of VEGF and VEGF16S isoform mRNA in patients with HCC may be used to assess prognosis preoperatively.

Hypoxia-Inducible Factor

Hypoxia-inducible factor-1 (HIF-1) plays an important role in angiogenesis. HIF-1α is an active subunit, which is expressed in response to hypoxic conditions. However, it is rapidly degraded in normal conditions. HIF-1α activates transcription of several genes, including erythropoietin-inducible nitric oxide synthase (iNOS), platelet-derived endothelial growth factor (PD-EGF), and VEGF (62). HIF-1α has been reported to be expressed in several cancers and is associated with resistance to chemotherapy and poor prognosis. Several studies reported that expression of HIF-1α in HCC tumor cells was associated with reduced disease-free and overall survival following resection of HCC (98,99,104). However, the results of these studies did not show a statistical significance in survival analysis.

Nitric Oxide Synthase

Nitric oxide synthase (NOS) has been reported to be responsible for tumor angiogenesis, and it exists in three isoforms: endothelial NOS, neuronal NOS, and iNOS. Overexpression of iNOS has been reported in HCC tumor cells and has been associated with aggressive tumor growth, increased tumor recurrence, and poor prognosis (83). On the contrary, another study did not find any prognostic significance of iNOS (105). However, when iNOS expression is taken together with COX-2 expression, the combined negative expression of these two factors independently predicted decreases in disease-free and overall survival following liver resection in a multivariate analysis.

Basic Fibroblast Growth Factor

The prognostic significance of the serum level of basic fibroblast growth factor (bFGF), a heparin-binding protein that has a mitogenic effect in endothelial cells, has been evaluated in patients following HCC resection. One study showed that a bFGF level above 10.8 pg/mL

independently predicted reduced disease-free survival in a multivariate analysis (106). However, another study using a lower cutoff value (>2.1 pg/mL) of serum bFGF did not show any prognostic significance (102).

Platelet-Derived Endothelial Growth Factor
PD-EGF is an angiogenic factor and plays a role in tumorogenesis. It has been reported that increased PD-EGF activity in the normal tissue adjacent to HCC is associated with reduced disease-free survival (107) and increased frequency of tumor recurrence more than 24 months after HCC resection (108).

Tissue Factor
Tissue factor (TF) is a glycoprotein involved in the extrinsic coagulation pathway by binding factor VII. TF expression has been reported to independently predict decreased diseased-free and overall survival after HCC resection (109,110).

Endostatin/Collagen XVIII
Angiogenesis inhibitors such as endostatin/collagen XVIII inhibit the proliferation and migration of endothelial cells. Higher expression of endostatin/collagen XVIII in adjacent non-tumor tissue has been reported to be significantly associated with shorter overall and disease-free survival following tumor resection and was found to independently predict tumor recurrence (111).

Interleukin-8
IL-8 is a multifunctional chemokine that has been reported to affect neutrophil activity, including chemotaxis, enzyme release, and expression of surface adhesion molecules. In addition, it induces tumor angiogenesis including vascular endothelial cell proliferation. It is expressed in both tumor cells and sera of patients with HCC (112). Low preoperative levels of IL-8 when compared with patients with high preoperative levels have been reported to be associated with greater disease-free and overall survival (113). In addition, IL-8 has been noted to be an independent prognostic factor for overall survival on multivariate analysis (113).

Angiopoietins
Angiopoietins are endothelial cell growth factors. Angiopoietin-1 (Ang-1) promotes vascular maintenance and maturation, while angiopoietin-2 (Ang-2) antagonizes Ang-1 with induction of angiogenesis in the presence of VEGF. Overexpression of Ang-2 is associated with overall poor prognosis in HCC patients. High Ang-2 has been associated with decreased disease-free survival following resection of HCC (99) and the ratio of Ang-2/Ang-1 is found to be an independent prognostic factor for overall survival on multivariate analysis (114).

Markers Associated with Invasion and Metastasis

Matrix Metaloproteases
The matrix metaloproteases (MMPs) are group of protein-degrading enzymes with an ability to degrade the extracellular matrix. These enzymes play an important role in HCC invasion, including metastasis. Elevated levels of MMP-2 have been associated with post-resection recurrence of HCC (115,116). Both MMP-2 and MMP-7 overexpression were reported to be associated with first postoperative year recurrence (117). On the other hand, human macrophage MMP-12 expression was found to independently predict improved overall survival on a multivariate analysis (118). Therefore, it appears that MMP may be used as prognostic markers for HCC.

Urokinase Plasminogen Activator
The serine protease, urokinase plasminogen activator (uPA), converts plasminogen to plasmin, which later degrades the extracellular matrix with activation of MMP. Expression of both uPA and its receptor, uPAR, is associated with growth and invasion of HCC. In one study,

increased expression of uPA, assessed by ELISA, was associated with decreased disease-free survival after resection of HCC (119). In addition, the combined expression of uPA, uPAR, and plasmin activator inhibitor-1 (PAI-1) in resected HCC specimens was associated with reduced survival (120).

The Cadherin/Catenin Complex

E-cadherin is a membrane glycoprotein that mediates cell-to-cell attachment. β-catenin mediates attachment of E-cadherin to α-catenin that directly connects to the actin filaments of the cell. β-catenin also acts both as an adhesion molecule and as an effector for the Wnt pathway within cells. Activation of the pathway leads to nuclear translocation and stimulation of cell proliferation, anti-apoptotic and pro-angiogenesis genes. Loss of E-cadherin expression or its mRNA has been reported to be associated with early recurrent HCC (121) and reduced overall survival (122) following resection. However, there was no association of E-cadherin expression and overall survival following resection of HCC smaller than 3 cm in another study (77). Several studies have evaluated β-catenin expression in patients with HCC and reported conflicting results with nuclear expression of β-catenin and its correlation with survival in multivariate analysis (77,123,124). The possible explanation of these discrepancies might be due to the analysis of differing antibodies and areas of tumor tissue. Therefore, the cadherin/catenin complex requires further study of its application as a prognostic marker for HCC.

Growth Factors and Receptors

Transforming Growth Factor-β1

Transforming growth factors-β (TGF-βs) are a class of polypeptides that regulate cell growth, differentiation, angiogenesis, tumor invasion, and immune function. TGF-β1 is the predominant form in humans that promotes angiogenesis and suppresses immune function. Even though the TGF-β1 expression following resection of HCC did not show a survival correlation (125), higher expression of TGF-β1 was reported to be an independent prognostic factor of decreased survival in patients with inoperable HCC (126). Elevated serum levels of TGF-β1 in patients with HCC have been found compared with healthy individuals and patients with chronic nonmalignant diseases (127,128). At the cutoff value of 800 pg/mL, greater than 95% sensitivity and 68% specificity for the detection of HCC was found, suggesting its superior efficacy compared with that of AFP (127). Furthermore, elevated serum TGF-β1 levels were reported in 25% of HCC patients with normal AFP (128). Elevated urinary TGF-β1 has also been reported to be associated with decreased survival in patients with HCC, although not all patients underwent resection of HCC (129). Therefore, TGF-β1 may be a good complement to AFP for the diagnosis of HCC. Serum TGF-β1 levels, however, might increase in HCC patients with decompensated cirrhosis because of its decreased clearance. Hence, its usefulness in diagnosis of HCC might be limited considering patients with advanced liver diseases. In addition, because of a lack of disease-specific TGF-β1 expression, TGF-β1 might be upregulated in patients with wound healing, angiogenesis, fibrosis, and extrahepatic tumors, suggesting a further limitation of its clinical use in patients with HCC (127).

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF) is a multifunctional factor with various biological activities. The relationship between HGF and liver diseases including chronic hepatitis, fulminant hepatitis, and HCC has been extensively studied (130). The serum level of HGF is measured by enzyme-linked immunosorbent assays. In patients with HCV-related chronic liver disease with HCC, the serum concentrations of HGF were reported to be significantly higher compared with that in patients with chronic hepatitis or cirrhosis without HCC (131). In addition, all patients with HGF greater than 0.6 ng/mL were diagnosed with HCC irrespective of AFP or DCP level. However, the sensitivity and specificity of HGF were not reported in this study. Increased serum levels of HGF have also been reported in extrahepatic malignancies including squamous cell carcinoma of esophagus, lymphoma, and several nonmalignant conditions. Hence, additional studies are required to determine whether inflammatory changes rather than hepatic carcinogenesis can explain increased HGF levels in patients with chronic hepatitis and HCC.

Insulin-Like Growth Factors

Insulin-like growth factors (IGFs) are polypeptides that play an important role for hepatic carcinogenesis. IGF-I and IGF-II are highly expressed during hepatic carcinogenesis. IGF-I is a potent mitogenic factor with anti-apoptotic effects in many cell systems (130). In a study of 114 patients with HCV-related cirrhosis with a long-term follow-up of HCC development, it was found that IGF-I levels in the serum were significantly lower in patients with HCC compared with patients without HCC, indicating 70% sensitivity of IGF-I for diagnosis of HCC (132). In addition, the decrease in serum IGF-I levels were noted 6 to 12 months before the diagnosis of HCC. On the other hand, increased IGF-II levels have been reported in persons with HCC compared with patients with benign tumors and cirrhosis (133). IGF-II mediates the neovascularization of HCC by increasing both VEGF mRNA and VEGF levels in human HCC cells (134,135).

Two studies examining IGF-II as a marker for HCC have compared the serum levels of AFP and IGF-II in patients with HCC or cirrhosis and normal control subjects (136,137). IGF-II was measured by immunoradiometry and its level was expressed as the ratio of IGF-II to prealbumin, taking into account the effect of nutritional status on serum IGF-II levels. IGF-II/prealbumin ratios were increased in the HCC group compared with cirrhotics and normal control subjects. However, the sensitivity and specificity of IGF-II were 42% and 96%, respectively, suggesting inferior performance compared with AFP as a tumor marker. Therefore, IGF-II is not a strong candidate as a tumor marker.

Insulin-like Growth Factor–Binding Protein-2

Insulin-like growth factor–binding protein-2 (IGFBP-2) is involved in the IGF signaling pathway (138). Ranke et al. reported that serum levels of IGFBP-2 were found to be increased above the age-adjusted normal value in 37 out of 50 patients with HCC (139). This increase was not due to tumor-induced weight loss, since it did not correlate with patients' body mass index. However, sensitivity and specificity of IGFBP-2 have not been reported. Its potential as a tumor marker for HCC remains to be determined in future studies.

Leptin Receptor

Leptin is a circulating hormone secreted by adipocytes, and it mediates cell growth, differentiation, and angiogenesis. It has been associated with several malignancies. Increased expression of leptin and its receptor, Ob-R, have been reported to be independent prognostic factors for increased overall survival in HCC patients following resection in multivariate analysis (92,140). However, the role of leptin and its receptors needs further evaluation in clinical studies regarding their application as prognostic markers in patients with HCC.

Telomerase Reverse Transcriptase mRNA

Human telomerase reverse transcriptase (hTERT) mRNA is a ribonuclear protein, and it is a novel marker for HCC. It has been reported that the HCC patients have significantly higher expression of hTERT mRNA in serum compared with healthy adults or patients with chronic liver disease without HCC (141,142). It is measured by real-time quantitative reverse transcription polymerase chain reaction, and its sensitivity and specificity have been reported to be 88.2% and 70.0%, respectively, in detecting HCC, which is better than current tumor markers, such as AFP and DCP (141). Therefore, hTERT may be an excellent candidate as a HCC tumor marker for the diagnosis of HCC.

CONCLUSION

HCC is a heterogeneous cancer with no promising therapy in advanced stages. It is a very aggressive tumor worldwide with poor survival outcomes. Therefore, early detection and resection of HCC may provide an excellent opportunity to improve long-term survival. Unfortunately, current HCC screening and diagnostic methods identify only a small number of patients at an early stage of HCC who qualify for resection or liver transplantation. Currently,

AFP and its variant AFP-L3 are the only serological tumor markers that are used for screening of HCC in clinical practice. However, AFP has major limitations including low sensitivity and specificity. In addition, more than 20% of patients with HCC do not have elevated AFP levels, and AFP levels may not discriminate between benign liver diseases and HCC. Therefore, there is an urgent need to identify additional serum tumor markers or diagnostic biomarkers that can assist in early diagnosis of HCC, including predicting prognosis and survival following resection of HCC. In addition, there is a need for a standardized approach to identify and assess biomarkers, including validation of those biomarkers in large cohorts of patients to assess their application in early diagnosis and accurate prediction of disease progression and prognosis. Research advances in molecular biology of hepatocarcinogenesis have identified a variety of biomarkers as described above. These markers have been studied for both early HCC detection and prognosis. They may be used as individual markers or as supplements to AFP for early diagnosis, tumor recurrence following resection, metastasis, tumor-free survival, and overall prognosis. However, because these studies have used different assays, cutoff values, and study populations, it is very hard to compare their utilities in clinical settings for screening and prognosis of HCC.

Currently, HCC tumor markers or molecular biomarkers show promise as a new generation of candidate markers for clinical studies and may replace or supplement AFP for screening and surveillance of HCC. Novel approaches involving proteomics technology may result in the identification of new tumor or biomarker candidates in the near future. These biomarkers should be evaluated in well-designed studies, alone or in combination, to determine their diagnostic accuracy, sensitivity, and specificity for the early detection of small HCC by using the strict EDRN criteria, which will provide an objective assessment for their clinical utility.

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6 Imaging of Hepatocellular Carcinoma

Riccardo Lencioni, Laura Crocetti, and Dania Cioni

Division of Diagnostic Imaging and Intervention, Department of Liver Transplantation, Hepatology and Infectious Diseases, University of Pisa, Lisanello Hospital, Pisa, Italy

M. Clotilde Della Pina

Division of Diagnostic and Interventional Radiology, Department of Oncology, Transplants and Advanced Technologies in Medicine, University of Pisa, Pisa, Italy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most frequent cancer in the world and the third most common cause of cancer-related mortality (1). HCC mostly develops in patients with chronic liver disease caused by viral hepatitis or alcohol abuse. More than 80% of all HCC tumors worldwide occur when the underlying chronic liver disease has reached the stage of cirrhosis (2). Carcinogenesis in liver cirrhosis is a complex multistep process characterized by the development of nonmalignant hepatocellular lesions that eventually progress into frank HCC (3). Imaging patients with cirrhosis for early detection of HCC, therefore, is a challenging issue. It is accepted that dynamic contrast-enhanced imaging techniques [including contrast ultrasound (US), multidetector computed tomography (CT), and magnetic resonance imaging (MRI)] can establish the diagnosis of HCC in nodular lesions larger than 1 cm demonstrating arterial hypervascularization with venous washout. Two different approaches can be currently used to detect and characterize nodular lesions in cirrhosis: (*i*) the vascular approach, which is aimed at showing the different vascular supply of HCC and nonmalignant hepatocellular lesions by using dynamic imaging techniques (including contrast-enhanced US, multidetector computed tomography (CT), and magnetic resonance imaging (MRI) (3), and (*ii*) the cellular approach, which relies on the ability of MRI in combination with liver-specific contrast agents (including hepatocyte-targeted agents and reticuloendothelial system–targeted agents) to show the changes in hepatobiliary function or Kupffer cell content as associated with malignancy (4).

IMAGING NODULAR LESIONS IN CIRRHOSIS: THE VASCULAR APPROACH

Carcinogenesis in liver cirrhosis is morphologically associated with the presence of distinct nodular lesions in the liver. These hepatocellular nodules include hyperplastic lesions such as a large regenerative nodule (LRN) and a neoplastic lesion such as low-grade and high-grade dysplastic nodules (DNs) (5). While LRN is thought to carry a malignant potential not greater than that of the cirrhosis, DNs are considered as precancerous lesions (6). Clinical follow-up studies have revealed that a considerable proportion of high-grade DNs progress into HCC within a few years (7,8). A recent study has shown that the risk of developing HCC is fourfold higher in patients with high-grade DN; in contrast, the risk of malignant transformation of a low-grade DN is low (9).

Low-grade DN is characterized by preserved hepatic architecture and mild cytological atypia. Portal areas are present, and sometimes an increase in the number of unpaired arterioles can be detected (5). High-grade DN is a neoplastic lesion with incipient malignancy. A number of architectural abnormalities can be documented, and portal areas may be present or absent in the nodule. An increased number of unpaired arteries is usually detectable (10,11). High-grade DN has a vascular profile that partially overlaps with that of HCC, and distinction from well-differentiated tumor may be very difficult (10,11). In well-differentiated HCC, architectural abnormalities are

associated with an abnormally high number of capillarized vessels, muscularized unpaired arterioles, and infiltration of portal tract/fibrous septa/veins by single hepatocytes (12).

Sinusoidal capillarization and increase in number of nontriadal arteries give to HCC the typical arterial hypervascularization that can be depicted by different imaging modalities and is presently considered a fundamental criterion for the diagnosis of HCC in cirrhosis (13,14).

Contrast-Enhanced Ultrasound

Ultrasound is the imaging technique most commonly used worldwide for early detection of HCC in surveillance programs (13). The introduction of microbubble contrast agents and the development of contrast-specific scanning techniques have opened new prospects in liver US (15). Contrast-specific techniques produce images on the basis of nonlinear acoustic effects of microbubbles and display enhancement with high contrast and spatial resolution. The advent of second-generation agents and low mechanical index real-time scanning techniques has been instrumental in improving the easiness and the reproducibility of the examination (15–20). Typical HCC shows strong intratumoral enhancement in the arterial phase (i.e., within 20–35 seconds after the start of contrast injection) followed by rapid washout in the portal venous and delayed phases (19,20) (Fig. 1). In contrast, LRN and DN usually do not show any early contrast uptake and resemble the enhancement pattern of liver parenchyma. In two recent series, selective arterial enhancement at contrast US was observed in 91% to 96% of HCC lesions, confirming that contrast US may be a tool to show arterial neoangiogenesis of HCC, even in tiny lesions (18,19) (Fig. 2). Assuming findings on spiral CT as the standard of reference, the sensitivity of contrast US in the detection of arterial hypervascularity was 97% in lesions larger than 3 cm, 92% in lesions ranging from 2 to 3 cm, 87% in lesions ranging from 1 to 2 cm, and 67% in lesions smaller than 1 cm (19).

Multidetector Computed Tomography

With the introduction of spiral scanners, the role of CT in liver imaging has changed dramatically. Owing to the advantage of scanning the whole liver during a single breath-hold, a comprehensive evaluation of the hepatic parenchyma during the different phases of contrast enhancement has become feasible. The standard spiral CT examination protocol for detection and characterization of HCC should include unenhanced and contrast-enhanced images obtained in the arterial phase (scanning initiated at about 25–30 seconds after the start of contrast injection), the portal venous phase (scanning initiated at about 70–80 seconds after the start of contrast injection), and the delayed phase (scanning initiated at about 180–210 seconds after the start of contrast injection). Proper timing of arterial-phase imaging is crucial to identify hypervascular nodules and requires the use of a test dose injection or a bolus track system to initiate the scanning at an optimal phase of opacification. The recent coupling of multidetector row scan technology with spiral image acquisition has further enhanced the performance of CT in liver imaging. Multidetector spiral CT offers a marked reduction in the time required for thin-section imaging of the entire liver relative to standard single-detector spiral CT. In addition, the increased temporal resolution permits hepatic imaging during two distinct arterial phases: the early arterial phase and the late arterial phase, acquired during the same breath-hold. Doubling the classic arterial phase of single-detector spiral CT may offer advantages. The early arterial phase, in fact, is a true CT arteriography and can be used to assess vascular anatomy.

At spiral CT, typical HCC lesions show clear-cut enhancement in the arterial phase and rapid washout in the portal venous and delayed phases (Fig. 3). In contrast, LRNs and DNs usually fail to exhibit this feature and appear isoattenuating or hypoattenuating to surrounding liver parenchyma (21,22). Identification of morphological features of HCC may support the diagnosis of HCC in questionable cases. Tumor capsule appears as a peripheral rim that is hypoattenuating on unenhanced and arterial-phase images and hyperattenuating on delayed-phase images. The CT detection rate of the capsule is strongly dependent on lesion size and is

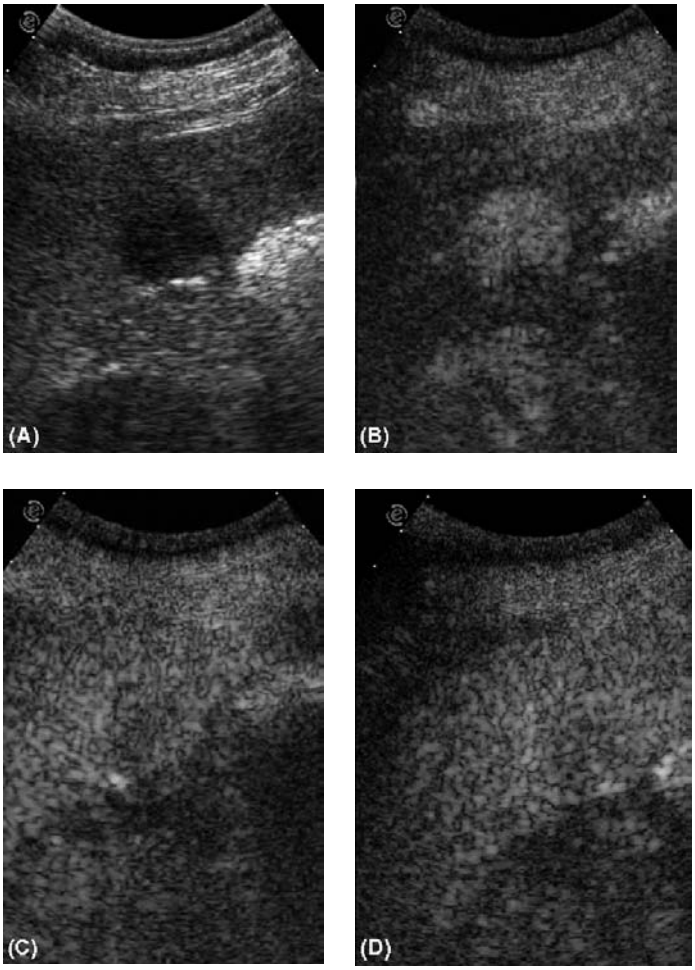

Figure 1 Contrast-enhanced US of hepatocellular carcinoma. At baseline US examination (**A**), the lesion appears as a hypoechoic nodule. At contrast-enhanced US study, the lesion shows early enhancement in the arterial phase (**B**) with washout in the portal venous (**C**) and delayed (**D**) phases. *Abbreviation*: US, ultrasound.

low in small tumors because the capsule itself is thin and poorly developed (23). Internal mosaic architecture, with components showing various attenuation indexes on CT images, is another typical feature of HCC that, however, is usually detected in larger nodular lesions (Fig. 4). Invasion of portal vein branches, with partial or complete neoplastic thrombosis, is quite frequent in advanced tumors and is best shown on portal venous phase images (24).

In a recent meta-analysis evaluating the accuracy of different imaging modalities in the diagnosis of HCC, spiral CT had a sensitivity of 67.5% (95% CI 55–80%) and a specificity of 92.5% (95% CI 89–96%), abstracting and pooling the data coming from 10 studies using histopathology of the explanted liver as standard of reference (25). In five series that reported careful lesion-by-lesion imaging-pathological correlation in explanted livers, the sensitivity of spiral CT in detection of HCC lesions ranged from 52% to 79% (26–30) (Table 1). Of interest, 89% to 100% of HCC lesions greater than 2 cm were detected, while only 10% to 47% of lesions smaller than 1 cm and 44% to 67% of lesions of 1 to 2 cm were identified. In a recent series in which multidetector spiral CT was used, the sensitivity in diagnosing HCC of 1 to 2 cm was of

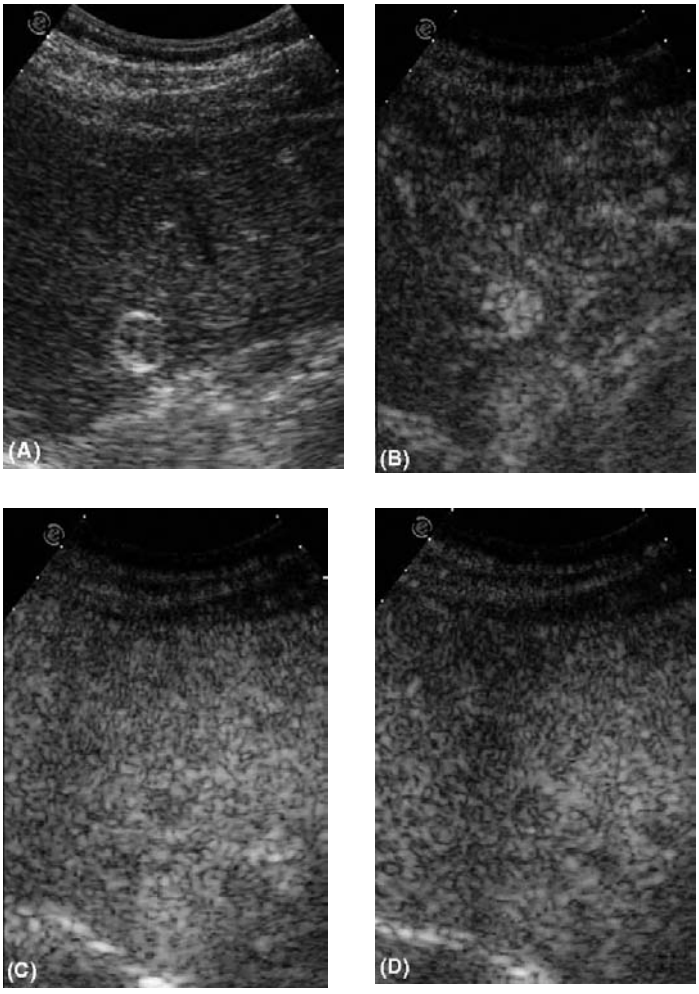

Figure 2 Contrast-enhanced US of HCC. The tiny lesion is hypo-hyperechoic at baseline US (**A**). Hypervascularization in the arterial phase (**B**) with washout in the portal venous (**C**) and delayed (**D**) phases is consistent with HCC. *Abbreviations*: US, ultrasound; HCC, hepatocellular carcinoma.

67%, with a positive predictive value of 61% (30). False-positive interpretations are usually caused by small lesions or pseudolesions (30–33). It is well known that hyperattenuating nodules may correspond to nonmalignant hepatocellular lesions, such as high-grade DN (28,30). Moreover, small (less than1.5 cm) flash-filling hemangiomas may enhance homogeneously in the arterial phase and be misinterpreted as small HCC (32). However, these entities usually do not exhibit contrast washout and show attenuation equivalent to that of the aorta during portal venous and delayed-phase CT imaging (33). Nontumorous arterioportal shunts can also be a cause of pseudolesion, although in most cases they have a typical wedge-shaped and homogeneous appearance (with or without internal linear branching structures representing early opacification of portal veins during the arterial phase) and are isoattenuating or slightly hyperattenuating during the portal venous phase (34).

Dynamic Magnetic Resonance Imaging

Over the past few years, MRI of the liver has progressed significantly. Technical advances in hardware and software have allowed the acquisition of images with excellent anatomical

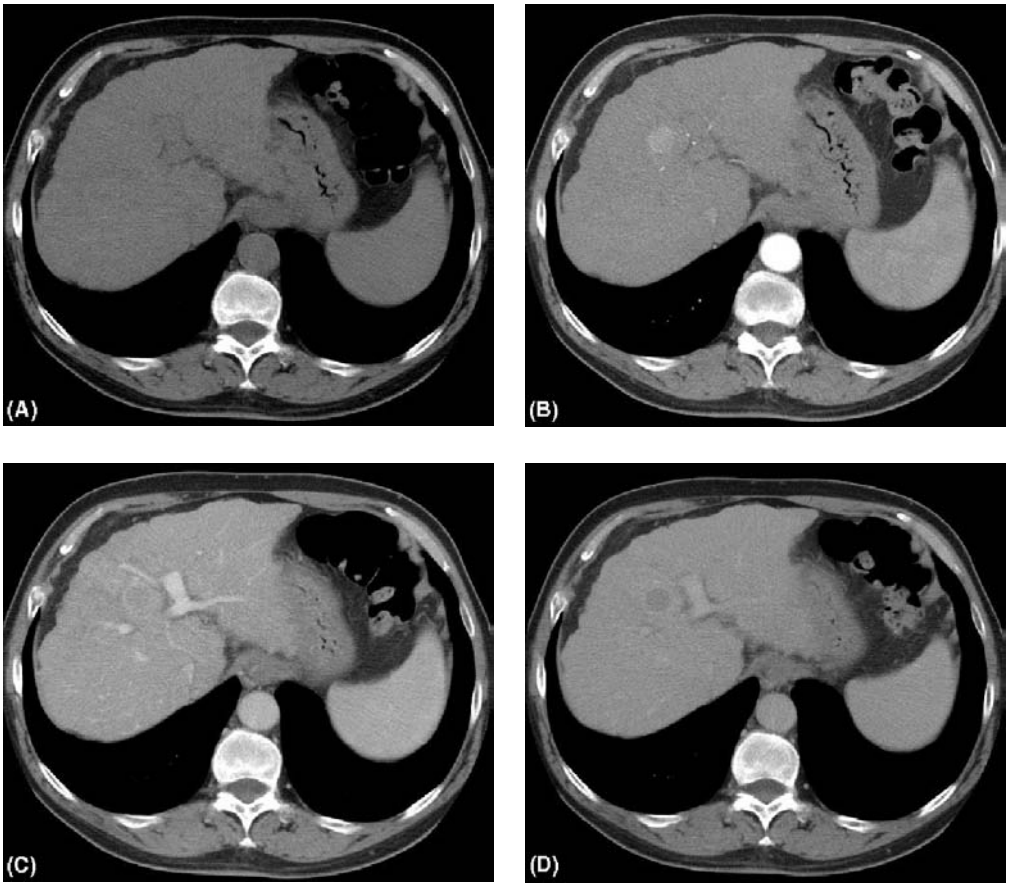

Figure 3 Multidetector computed tomography of hepatocellular carcinoma. At baseline scan (**A**), the lesion is isoattenuating with respect to liver parenchyma and thus undetectable. At contrast-enhanced study, the lesion shows early enhancement in the arterial phase (**B**) with washout in the portal venous (**C**) and delayed (**D**) phases.

detail, largely free of artifacts secondary to respiratory motion. Fast sequences have reduced image acquisition time, thereby improving patient acceptance and allowing more efficient utilization of machine time. New volumetric sequences have enabled three-dimensional serial dynamic imaging of the liver with a very high spatial and temporal resolution, reducing section misregistration and motion artifacts while improving multiplanar reformations. The standard examination protocol for the detection and characterization of HCC includes T1-weighted fast spoiled gradient-echo sequences with fat suppression, respiratory-triggered or breath-hold T2-weighted fast spin-echo sequences with fat suppression, and serial dynamic T1-weighted fast spoiled gradient-echo sequences after bolus injection of a gadolinium chelate. Additional sequences, such as out-of-phase spoiled gradient-echo T1-weighted sequences, may be performed to provide comprehensive information or to solve specific diagnostic issues.

Dynamic MRI well demonstrates the typical vascular features of overt HCC, i.e., arterial-phase enhancement with portal venous and/or delayed-phase washout (Fig. 5). This feature enables differentiation of frank HCC from LRN or DN, which are usually not hypervascular (14). Nevertheless, as discussed for CT, nonmalignant hepatocellular lesions—especially high-grade DN—may show increased arterial blood supply and be indistinguishable from a small HCC. In addition, nontumorous arterioportal shunts may cause false-positive interpretations (35). In one study, the majority (93%) of hepatic arterial phase–enhancing lesions less than 2 cm that were

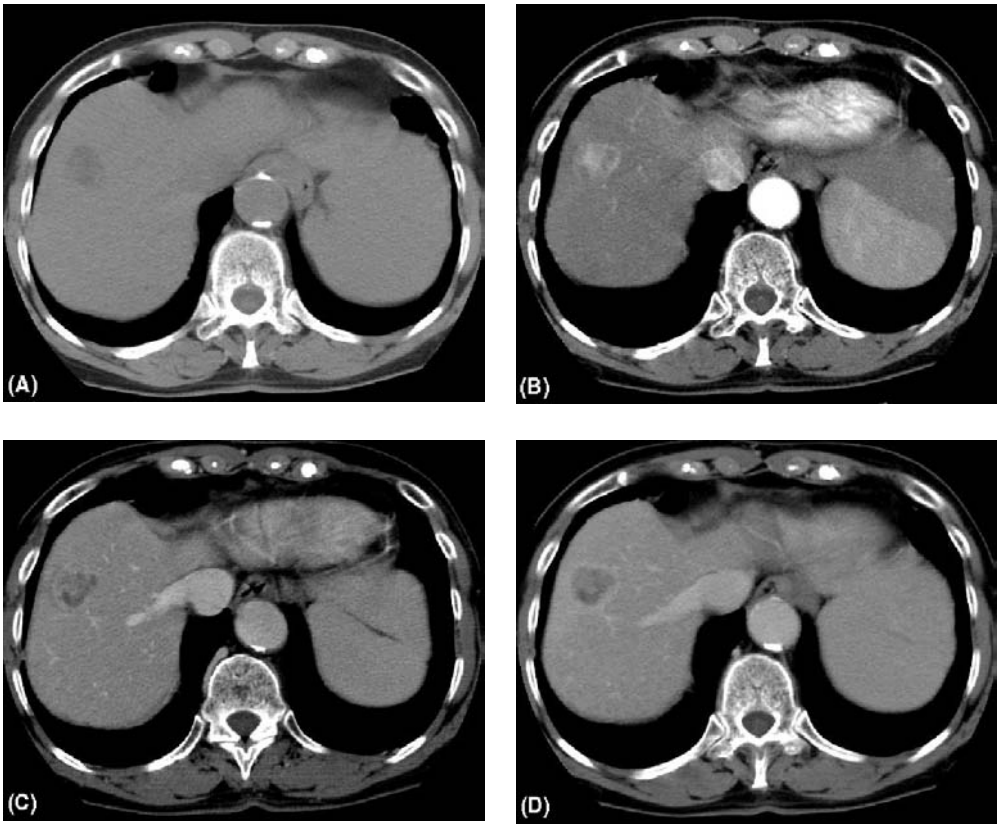

Figure 4 Multidetector computed tomography of hepatocellular carcinoma. At baseline scan (**A**), the lesion appears mostly hypoattenuating with respect to liver parenchyma. At contrast-enhanced study, internal inhomogeneity with areas of various attenuation showing different levels of enhancement in the arterial (**B**), portal venous (**C**), and delayed (**D**) phases is observed.

Table 1 Sensitivity and Positive Predictive Value of Spiral CT in the Detection of HCC in Series with Lesion-by-Lesion Imaging-Pathological Correlation in Explanted Livers and Subgroup Analysis According to Lesion Size

Author, year (reference number)	Number of patients/ number of lesions	Overall lesion sensitivity (percentage)	Sensitivity for lesions <1 cm (percentage)	Sensitivity for lesions 1–2 cm (percentage)	Sensitivity for lesions >2 cm (percentage)	Positive predictive value (percentage)
Rode et al., 2001 (26)	43/13	7/13 (54%)	3/7 (43%)	3/5 (60%)	1/1 (100%)	N/A
de Lédinghen et al., 2002 (27)	34/54	28/54 (52%)	2/8 (25%)	15/34 (44%)	11/12 (92%)	28/37 (76%)
Burrel et al., 2003 (28)	26/70	43/70 (61%)	2/20 (10%)	17/26 (65%)	24/24 (100%)	43/49 (87%)
Valls et al., 2004 (29)	85/85	67/85 (79%)	–	23/38 (61%)	44/47 (94%)	67/76 (88%)
Ronzoni et al., 2007 (30)	88/139	89/139 (64%)	28/60 (47%)	28/42 (67%)	33/37 (89%)	89/133 (67%)

Abbreviations: CT, computed tomography; HCC, hepatocellular carcinoma; N/A, not available.

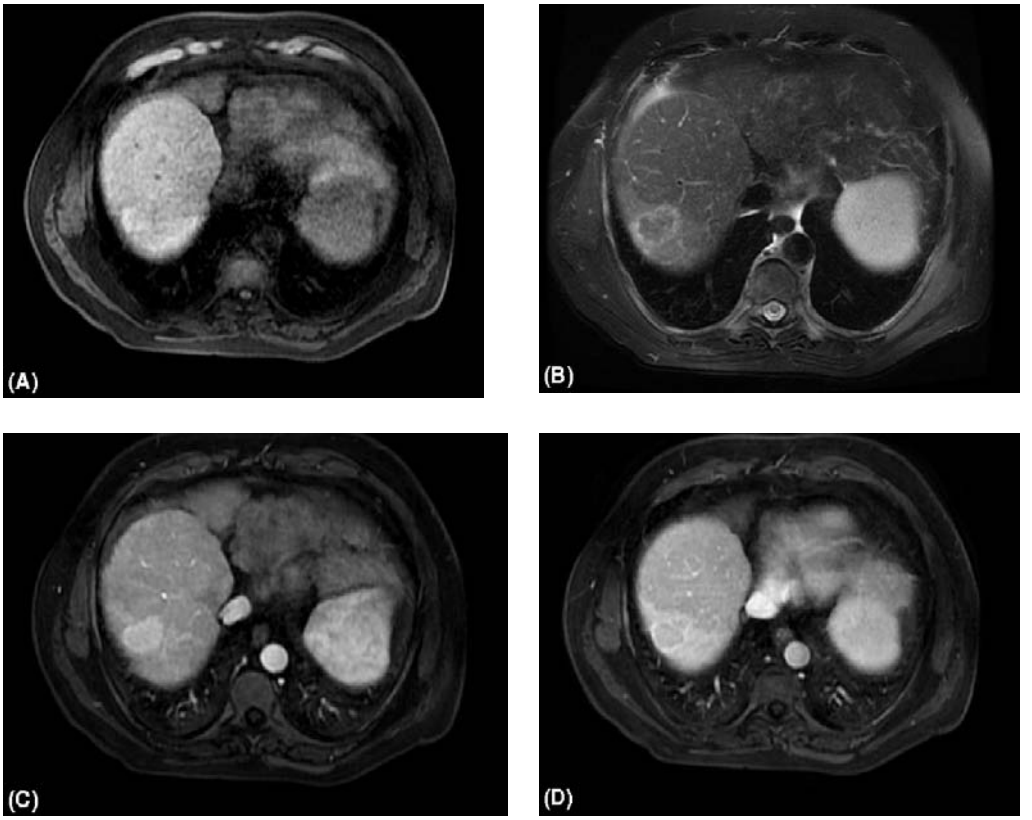

Figure 5 Dynamic magnetic resonance imaging of hepatocellular carcinoma. At baseline, the lesion appears iso-hyperintense with respect to liver parenchyma on the T1-weighted image (**A**) and isointense with hyperintense rim on the T2-weighted image (**B**). At contrast-enhanced study, early enhancement is observed in the arterial phase (**C**), with washout in the portal venous phase (**D**).

occult at T2-weighted and portal- and/or equilibrium-phase MRI had no correlative pathological finding, even in patients with pathologically proved HCC (36).

In the above mentioned meta-analysis evaluating the accuracy of different imaging modalities in the diagnosis of HCC, dynamic MRI had a sensitivity of 80.6% (95% CI 70–91%) and a specificity of 84.8% (95% CI 77–93%), abstracting and pooling the data coming from nine studies using histopathology of the explanted liver as the standard of reference (25). In five series with histopathological correlation with the explanted liver and careful subgroup analysis according to lesion size, lesion-by-lesion analysis revealed a sensitivity of 33% to 78%, with positive predictive values ranging from 54% to 92% (26–28,37,38) (Table 2). In particular, 100% of HCC lesions greater than 2 cm were detected, while 4% to 71% of lesions smaller than 1 cm and 52% to 89% of lesions of 1 to 2 cm were identified. In one comparative study with explant correlation, the sensitivity for the identification of HCC lesions ranging from 1 to 2 cm was significantly higher for MRI than for spiral CT (28).

IMAGING NODULAR LESIONS IN CIRRHOSIS: THE CELLULAR APPROACH

HCC shows a variety of MRI features, which reflect the variable characteristics of this malignancy in tumor architecture, grading, stromal component, as well as intracellular content of certain substances, such as fat, glycogen, or metal ions, that greatly affect the appearance of the lesion on baseline T1-weighted and T2-weighted magnetic resonance (MR) images (14).

Table 2 Sensitivity and Positive Predictive Value of Dynamic MRI in the Detection of HCC in Series with Lesion-by-Lesion Imaging-Pathological Correlation in Explanted Livers and Subgroup Analysis According to Lesion Size

Author, year (reference number)	Number of patients/ number of lesions	Overall lesion sensitivity (percentage)	Sensitivity for lesions <1 cm (percentage)	Sensitivity for lesions 1–2 cm (percentage)	Sensitivity for lesions >2 cm (percentage)	Positive predictive value (percentage)
Rode et al., 2001 (26)	43/13	10/13 (77%)	5/7 (71%)	4/5 (80%)	1/1 (100%)	N/A
de Lédinghen et al., 2002 (27)	34/54	33/54 (61%)	2/8 (25%)	19/34 (56%)	12/12 (100%)	33/37 (89%)
Krinsky et al., 2002 (37)	24/118	39/118 (33%)	3/72 (4%)	11/21 (52%)	25/25 (100%)	39/45 (87%)
Burrel et al., 2003 (28)	29/76	58/76 (76%)	8/23 (34%)	25/28 (89%)	25/25 (100%)	58/64 (90%)
Lauestein et al., 2007 (38)	115/36	28/36 (78%)	10/18 (56%)		18/18 (100%)	28/30 (93%)

Abbreviations: MRI, magnetic resonance imaging; HCC, hepatocellular carcinoma; N/A, not available.

MRI with use of liver-specific contrast agents (including hepatocyte-targeted agents and reticuloendothelial system-targeted agents) may be useful to clarify questionable cases because of the ability to show the changes in hepatobiliary function or Kupffer cell content associated with malignancy (39).

Magnetic Resonance Imaging with Hepatocyte-Targeted Agents

During the carcinogenetic pathway, progressive loss of biliary polarization of the hepatocyte and derangement of the microscopic secretory structure are observed. While in low-grade DN, the biliary domain of the cells is preserved and bile ducts are present in portal areas (12), in high-grade DN, the biliary function can be partially impaired and bile ducts can be missing. In well-differentiated HCC, bile canaliculi are nearly always present between cells and bile pigment may be found in tumor cells or in dilated canaliculi. However, the organization of portal areas is completely lost, and bile ducts are absent. Biliary function is lost by tumoral cells, and bile is rarely present in poorly differentiated HCC (40).

Biliary function of hepatocellular nodule can be investigated by hepatocyte-targeted contrast agents. These agents are paramagnetic compounds that are partially taken up by the hepatocytes and excreted in the biliary tract (41). In the hepatobiliary phase, these agents produce sustained enhancement of liver parenchyma on T1-weighted images (41). HCC demonstrates variable uptake of hepatobiliary contrast agents (42–44). While moderately or poorly differentiated HCC fail to take up the contrast agent and stand out as hypointense lesions on T1-weighted images, well-differentiated HCC may preserve, to some extent, hepatocyte-like function and take up the contrast agent, appearing hyperintense or isointense to surrounding liver (42–44). Hyperintensity is the result of quicker clearance of the contrast agent by liver parenchyma than by the nodule (42). Benign hepatocellular lesions, including DNs, typically have enhancement as well (42–45). Nevertheless, hepatobiliary-enhanced MRI may be helpful in ruling out some false-positive diagnoses of dynamic studies, such as those caused by hyperperfusion abnormalities (46).

Magnetic Resonance Imaging with Reticuloendothelial System–Targeted Agents

It has been shown by histopathological studies that dysplastic lesions in cirrhosis possess an almost identical or sometimes slightly increased number of Kupffer cells when compared with cirrhotic parenchyma. The number of Kupffer cells in cancerous tissues decreases as tumor size increases and as grading of the tumor increases (47). Reticuloendothelial system–targeted

contrast agents are superparamagnetic particles of iron oxide, which produce distortions of local magnetic field resulting in signal loss on T2-weighted images. Once injected intravenously, these agents are rapidly removed from the circulation by the reticuloendothelial system (48). Kupffer cells in the liver play a dominant role in this process, taking up more than 80% of circulating particles. DNs—that contain nearly the same number of Kupffer cells as the surrounding cirrhotic hepatic parenchyma—are isointense or even hypointense to liver on post-contrast T2-weighted MR images. In contrast, moderately or poorly differentiated HCC are hyperintense as a result of the drop in signal intensity of surrounding liver parenchyma. Unfortunately, in small, well-differentiated HCC, Kupffer cells may be present, as endothelial cells morphologically resemble normal sinusoidal endothelial cells, giving an environment similar to that of normal hepatic parenchyma (47). Thus, the features of well-differentiated HCC may overlap with those of DN (47–52).

GUIDELINES FOR DIAGNOSTIC WORK-UP

Current guidelines for imaging cirrhotic patients are based on the more established vascular approach. Since the prevalence of HCC among nodules detected during US surveillance is strongly related to the size of the lesion, the work-up depends on the size of the lesion. Lesions smaller than 1 cm in diameter have less likelihood of being HCC. However, minute hepatic nodules detected by US may become malignant over time. Therefore, these nodules need to be followed up to detect growth suggestive of malignant transformation. A reasonable protocol is to repeat US every three months until the lesion grows to more than 1 cm, at which point additional diagnostic techniques are applied. It has to be emphasized, however, that the absence of growth during the follow-up period does not rule out the malignant nature of the nodule because even an early HCC may take more than one year to increase in size. When the nodule exceeds 1 cm in size, the lesion is more likely to be HCC, and diagnostic confirmation should be pursued. It is accepted that the diagnosis of HCC in cirrhosis can be made without biopsy in a nodule larger than 1 cm that shows characteristic vascular features of HCC—i.e., arterial hypervascularization with washout in the portal venous or delayed phase—even in patients with normal α-fetoprotein value. Such lesions should be treated as HCC, since the positive predictive value of the clinical and radiological findings is as high as 100% provided that examinations are conducted by using state-of-the-art equipment and interpreted by radiologists with extensive expertise in liver imaging (53). For lesions ranging from 1 to 2 cm, current guidelines require typical imaging findings to be confirmed by two coincident dynamic imaging modalities to allow a noninvasive diagnosis (Table 3). For nodules above 2 cm, a single imaging technique—out of contrast-enhanced US, multidetector CT, and dynamic MRI—showing the characteristic vascular profile of HCC mentioned above—may confidently establish the diagnosis (Table 3). It has to be pointed out, however, that if the diagnosis is made by using contrast-enhanced US, additional investigation with multidetector CT or dynamic MRI is required to provide a comprehensive assessment of the liver parenchyma and rule out additional tumor foci. Moreover, it has to be stressed that noninvasive criteria based on imaging findings can be applied only in patients with established cirrhosis. For nodules

Table 3 Diagnostic Criteria for HCC

- Cytohistological criteria
- Noninvasive criteria (restricted to cirrhotic patients)
  1. One imaging technique[a]
     Focal lesion >2 cm with arterial hypervascularization and venous washout
  2. Two coincident imaging techniques[a]
     Focal lesion 1–2 cm with arterial hypervascularization and venous washout

[a]Three techniques considered: contrast US, multidetector computed tomography, and dynamic magnetic resonance imaging.
Abbreviations: US, ultrasound; HCC, hepatocellular carcinoma.
Source: From Ref. 13.

detected in noncirrhotic livers, as well as for those showing atypical vascular patterns, biopsy is still recommended.

CONCLUSION

The correct labeling of any hepatic nodule detected in a patient with liver cirrhosis is essential for the clinical management, particularly when orthotopic liver transplantation, surgical resection, or percutaneous ablation can be suitable treatment options. While the correct diagnosis of tumors larger than 2 cm can be confidently established in most instances, characterization of small nodules remains a challenging issue despite the many technological advances. In fact, the imaging features of the different entities that take part in the multistep process of hepatocarcinogenesis do overlap. In addition, imaging techniques remain relatively insensitive for the detection of small satellite lesions associated with the main tumor. Presently, there is no evidence that MRI performed with liver-specific contrast agent can replace dynamic contrast-enhanced techniques, which should be considered the standard approach for imaging cirrhotic patients.

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7 Staging

William Sanchez and Gregory J. Gores
Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major global health problem. In the year 2000, HCC was the third leading cause of cancer-related death worldwide, accounting for over half a million deaths (1). The majority of patients with HCC are asymptomatic until the tumor is at an advanced stage. Surveillance programs for patients at risk for HCC can identify individuals with early-stage tumors who, in turn, should be evaluated for curative surgical procedures (e.g., hepatic resection or liver transplantation). Palliative therapies, such as hepatic artery chemoembolization, may be appropriate for patients with advanced disease. Therefore, accurate tumor staging to determine the burden of disease is critical for the management of patients with HCC.

CLINICAL EPIDEMIOLOGY OF HCC

Chronic viral hepatitis is the major risk factor for HCC and is present in 75% to 80% of cases (1). There is a marked global geographic variability in the incidence of HCC, largely due to the endemic nature of chronic hepatitis B infection in Asia and sub-Saharan Africa. In regions with a high prevalence of hepatitis B infection, HCC may develop in patients without cirrhosis. Conversely, in Western Europe, North America, and Japan HCC typically develops in the setting of cirrhosis, most often due to chronic hepatitis C, which further complicates its management. The observed incidence of HCC in the United States doubled between 1985 and 1998 and is projected to continue rising (2,3). HCC-related mortality and health care expenditures have increased in conjunction with the increasing incidence of HCC in the United States (4).

CLINICAL STAGING OF HCC

Tumor staging for HCC requires a careful assessment of the degree of tumor burden within the liver and diagnostic tests to assess for extrahepatic spread. While hepatic function and a patient's performance status play important roles in determining the prognosis of patients with HCC, they are not incorporated in the traditional tumor node metastasis (TNM) staging system for HCC and are incorporated in some but not all other tumor staging systems. Ultimately, the ability to accurately diagnose and quantify tumor burden within the liver relies on the use of dynamic, contrast-enhanced cross-sectional imaging such as computed tomography or magnetic resonance imaging. A comprehensive review of imaging techniques for HCC is beyond the scope of this chapter and is addressed elsewhere in this text.

It is important to assess for extrahepatic spread of HCC early in the evaluation of a patient with newly diagnosed HCC. Given that patients with distant spread of disease are considered to be at an advanced stage regardless of tumor burden within the liver, identifying metastatic disease has significant implications on prognosis and therapy. Currently, the extrahepatic staging of HCC is based solely upon imaging studies. Common sites for remote spread include the pulmonary parenchyma and osseous metastases. Imaging of the chest with standard chest radiographs or computed tomography is recommended. The use of bone scans for the detection of asymptomatic bony metastases varies by institution and is most commonly used in the context of evaluating potential candidates for liver transplantation. Standard positron emission tomography (PET) imaging currently has insufficient negative predictive value to be used routinely for staging extrahepatic HCC.

HCC STAGING SYSTEMS

Staging classifications for solid organ malignancies are used to accurately define cancer burden in the site of origin as well as local and distant spread. Accurate tumor staging is necessary to inform treatment decisions such as whether surgical, local-regional, or systemic therapies would be most appropriate. Accurate tumor staging also provides patients with important information about their prognosis. Furthermore, a clear tumor staging schema plays a vital role in the evaluation of new therapies for cancer, providing investigators and clinicians a common language for the accurate comparison of treatment trials (5,6).

Determining prognosis and selecting among treatment options for patients with HCC are complicated by the fact that HCC frequently develops in the setting of cirrhosis. The natural history of HCC is influenced by several factors, including: tumor characteristics such as size, vascular invasion, and multicentricity; the presence or absence of metastatic disease; the severity of underlying hepatic dysfunction; the presence of comorbid illnesses; and the efficacy of treatments used (7). Traditionally, solid organ malignancies have been staged on the basis of resected specimens using the TNM system. Given that the degree of hepatic dysfunction present directly impacts upon prognosis and therapeutic options, several staging systems for HCC incorporate measures of liver function in addition to measures of tumor burden. A number of additional staging systems have been proposed for use in HCC, including Barcelona Clinic Liver Cancer (BCLC), Cancer of the Liver Italian Program (CLIP), Chinese University Prognostic Index (CUPI), Groupe D'etude de Traitment du Carcinoma Hepatocellulair (GRETCH), Japanese Integrated System (JIS), and Okuda staging systems.

Currently, no international consensus has been reached on the most appropriate staging classification for HCC, and this remains a topic of active study and debate (7). Furthermore, international medical and surgical societies have endorsed the use of different staging systems for HCC (7–10). Multiple studies comparing a variety of staging systems in differing cohorts of patients have been published—including a recent study comparing 12 staging systems in patients with unresectable HCC (11)! Comparative studies of staging systems for HCC are limited by heterogeneity in diagnostic criteria for HCC, including the frequency of biopsy for histologic confirmation of diagnosis, heterogeneity in imaging studies used to diagnose HCC and their ability to resolve small lesions, and differing rates of viral hepatitis B and C in the study populations.

TNM Staging

The TNM staging system is in wide use for a variety of solid organ cancers, not only for HCC. The TNM classification is widespread and is in common use globally. The current TNM staging for HCC has been revised by the American Joint Commission on Cancer (AJCC) in 2002 (see Table 1) (12). Despite the most recent AJCC revision, concern regarding insufficient discrimination of early-stage disease exists because of the lowest tumor classification being for

Table 1 AJCC TNM Staging

T classification	Definition		
T1	Single tumor <5 cm without vascular invasion		
T2	Single tumor with vascular invasion or multiple tumors all <5 cm		
T3	Multiple tumors, any tumor >5 cm or any tumor involving a major branch of the portal or hepatic veins		
Staging	T	N	M
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	Any	N1	M0
Stage IV	Any	Any	M1

Abbreviations: AJCC, American Joint Commission on Cancer; TNM, tumor node metastasis.
Source: From Ref. 12.

Table 2 UNOS TNM Staging

T Classification	Definition		
T1	Single tumor <2 cm		
T2	Single tumor 2–5 cm or up to 3 tumors, all <3 cm		
T3	Single tumor >5 cm or up to 3 tumors, any >3 cm		
T4a	>3 tumors, any size		
T4b	Any tumor involving a major branch of the portal or hepatic veins		
Staging	T	N	M
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage IVA1	T4a	N0	M0
Stage IVA2	T4b	N0	M0
Stage IVB	Any	Any	Any

Abbreviations: TNM, tumor node metastasis; UNOS, United Network for Organ Sharing.
Source: From Refs. 13 and 14.

tumors less than 5 cm in diameter. The TNM classification has been modified by the American Liver Tumor Study Group and the Liver Cancer Study Group of Japan to further stratify tumor classification to include tumors as small as 2 cm in diameter (see Table 2) (10,13).

TNM staging benefits from widespread use and acceptance. Currently, United Network for Organ Sharing, the organ allocation administration in the United States, allocates donor organs for liver transplantation for the treatment of HCC based on the revised TNM classification (14). The fundamental concern regarding the TNM staging system is that it does not incorporate any measure of liver functional reserve, which is of critical importance in HCC. Unlike other solid organ malignancies such as breast and colon adenocarcinoma, prognosis for HCC is impacted less by distant metastases than by local spread and hepatic dysfunction. TNM classification has also traditionally been based on resection histology. Currently, cross-sectional imaging may inaccurately stage HCC based on tumor classification by underestimating or overestimating tumor size.

Okuda Staging Classification

Initially published in 1985, the Okuda classification was derived from a retrospective analysis of a cohort of 850 patients with HCC. The Okuda classification incorporates gross measures of hepatic functional reserve as well as tumor size. Hepatic function is scored by dichotomous assessments of ascites, serum albumin, and serum total bilirubin. Tumor size is only assessed as being less than or greater than 50% of liver area on cross-sectional imaging. The Okuda stage is then based on the sum of the individual scores (Table 3) (15).

Table 3 Okuda Staging System

Parameter	Value	Score
Tumor size	<50%	1
	>50%	0
Ascites	Present	1
	Absent	0
Serum albumin	<3 g/dL	1
	>3 g/dL	0
Serum total bilirubin	>3 mg/dL	1
	<3 mg/dL	0

Score	Okuda stage
0 points	A
1–2 points	2
3–4 points	3

Source: From Ref. 15.

Table 4 CLIP Score

	Score		
Parameter	0	1	2
Tumor morphology	Single lesion, <50% of liver area involved	Multiple lesions, <50% of liver area involved	>50% of liver area involved
CTP class	A	B	C
AFP (ng/mL)	<400	≥400	
Portal vein thrombosis	Absent	Present	

Abbreviations: CLIP, Cancer of the Liver Italian Program; AFP, α-fetoprotein; CTP, Child–Turcotte–Pugh.
Source: From Ref. 22.

The Okuda classification is limited to identifying patients with advanced disease (Okuda stage III), many of whom are already symptomatic. Patients with Okuda stage III HCC are not appropriate for enrollment into clinical trials due to their poor prognosis, and so palliative therapy is recommended (6). The Okuda classification does not adequately segregate patients with early- and intermediate-stage HCC, in whom curative therapies may be appropriate. Multiple studies of contemporary staging systems have demonstrated improved discriminative ability compared with the Okuda stage in large cohorts of Western and Japanese patients, and therefore Okuda classification has largely been supplanted by newer staging systems (16–21).

CLIP Score

In an effort to improve the discrimination of early- and intermediate-stage HCC over the Okuda system, investigators from Italy developed the CLIP score. The CLIP score incorporates measures of tumor size, vascular invasion, α-fetoprotein (AFP) level, and hepatic function as measured by Child–Turcotte–Pugh (CTP) class. Individual measures are assigned a score (0, 1, or 2 points), and patients are classified on a seven-point scale based on the sum of scores (Table 4). Patients with a score of 4 or greater are considered to have advanced disease and carry similar poor prognosis.

The CLIP score was initially derived from a retrospective cohort of 435 patients with HCC from 16 Italian medical centers (22). The CLIP score has been subsequently externally validated in several prospective cohorts from Europe (18,23) and larger retrospective cohorts from North America and Japan (17,21). The CLIP score was observed to have improved discriminatory ability compared with Okuda, TNM, and CTP classification, particularly in earlier stages of disease.

The strengths of the CLIP score include its simplicity in calculation from readily available clinical data as well as the fact that it has been externally validated. Liver functional reserve is assessed using the widely recognized CTP score. The CLIP score also incorporates an assessment of portal venous invasion, an important prognostic factor. In a joint consensus statement the American Hepato-Pancreato-Biliary Association and the American Joint Committee on Cancer endorsed CLIP for use as the clinical staging system of choice for HCC (9).

There are several limitations of the CLIP score. Foremost, the lowest categorization for tumor burden is less than 50% hepatic involvement, an exceedingly broad range, which includes patients with small tumor to those with clinically advanced disease. CLIP also includes the use of serum AFP level, which is an imperfect tumor marker. At a cutoff of 400 ng/mL, AFP has a positive predictive value of only 60%, and in a cohort of U.S. patients with HCC, 38% had AFP values of less than 20 ng/mL (8,20,24). While the CLIP score has been observed to be superior to Okuda and TNM staging, subsequent studies have suggested that newer staging systems such as JIS and BCLC may have performance characteristics equivalent or superior to CLIP (16,19,20,25).

GRETCH Staging Classification

The GRETCH classification (also known as the French classification) was constructed from a multivariate analysis of 761 patients who presented with HCC at 24 centers in Europe and Canada. A weighted score is generated using the Karnofsky index (a measure of functional

Table 5 GRETCH Staging System

	Score			
Parameter	0	1	2	3
Karnofsky index	≥80%			<80%
Bilirubin (μmol/L)	<50			≥50
Alkaline phosphatase	<2x ULN		≥2x ULN	
AFP (μg/L)	<35		≥35	
Portal vein thrombosis	Absent	Present		

Score	GRETCH stage
0 points	A
1–5 points	B
≥6 points	C

Abbreviations: ULN, upper limits of normal; AFP, α-fetoprotein; GRETCH, Groupe D'etude de Traitment du Carcinoma Hepatocellulair.
Source: From Ref. 26.

status), the presence of portal invasion, and serum measures of bilirubin, alkaline phosphatase, and AFP. Patients are stratified into three stages (A, B, and C) based on the total score (Table 5) (26).

The main advantages of the GRETCH classification over the Okuda classification are the use of a weighted scoring system and the inclusion of a measure of functional status, which plays an important role in clinical decision making. However, the GRETCH classification uses alkaline phosphatase as a measure of hepatic function, a variable that has not consistently been identified as a significant prognostic factor in other multivariate analyses. The GRETCH classification also incorporates serum AFP level, which has significant limitations (see above).

While the GRETCH classification was internally validated in a cohort of 255 patients, the one-year patient survival in stages B and C were 20% and 3%, respectively (26). This suggests that a large proportion of the study cohort had advanced disease and limits the ability of the GRETCH classification to discriminate patients with early- and intermediate-stage HCC. Furthermore, a subsequent study observed that while GRETCH improved prognostication of one-year survival compared with the Okuda classification, it was not able to better discriminate three-year survival, again suggesting insufficient discrimination of early-stage HCC (19).

CUPI Score

The CLIP and GRETCH classifications were constructed using cohorts of Western patients in whom HCC typically complicates cirrhosis. Given the significant disease burden from hepatitis B virus (HBV)–related HCC in Asia, with half of global cases occurring in China, investigators from Hong Kong constructed the CUPI for HCC. The CUPI score was derived from a large single-center cohort of 926 patients with HCC, of which 79% were HBV surface antigen positive and 18% were non-cirrhotic (27).

Investigators based the CUPI score on TNM staging as a measure of tumor burden and added weighted scores for symptoms, ascites, and serum values of bilirubin, AFP, and alkaline phosphatase. The CUPI score is then calculated from the sum of the six individual weighted scores (Table 6). Compared with TNM staging and Okuda staging, CUPI was found to have better discrimination for survival at three months (27).

Factors in favor of the CUPI score include a more weighted stratification of tumor burden than the Okuda and CLIP classifications. While no measure of performance status is included in the model, the presence of symptomatic HCC is heavily weighted. The variables, which comprise the CUPI score, are all clinically readily available; however, given the variable weighting of individual factors, CUPI is somewhat more cumbersome to use in clinical practice compared with the CLIP score.

The major limitation of the CUPI score is its derivation from a cohort of patients with predominantly HBV-related HCC. This limits applicability of the scoring system in Western populations where HBV is less common. Furthermore, the CUPI score has not been validated

Table 6 CUPI Score

Parameter		Score
TNM stage	I and II	–3
	III	–1
	IV	0
Asymptomatic disease		–4
Presence of ascites		3
AFP $\geq$500 ng/mL		2
Bilirubin	$<$2 mg/dL	0
	2–3 mg/dL	3
	$>$3 mg/dL	4
Alkaline phosphatase $\geq$200 IU/L		3

Score	Category
$\leq$1 point	Low risk
2–7 points	Intermediate risk
$\geq$8 points	High risk

Abbreviations: AFP, α-fetoprotein; TNM, tumor node metastasis.
Source: From Ref. 27.

prospectively. As with other staging systems, the CUPI score may not segregate patients with early-stage disease adequately. In the study cohort, patients identified as low-risk by CUPI (score of 1 or less) had a one-year survival less than 50%, suggesting insufficient discrimination among patients with earlier stages of disease (27).

JIS Score

Compared with guidelines from the American Association for the Study of Liver Disease and European Association for the Study of the Liver, the Japanese Society of Hepatology endorses a more aggressive surveillance program for patients at risk for HCC in Japan. An expert panel of the Japanese Society of Hepatology recommended a protocol of ultrasonography and three serum tumor markers every three to four months with dynamic computed tomography or magnetic resonance imaging every 6 to 12 months in patients at the highest risk for HCC (including patients with cirrhosis from chronic hepatitis B or C) (10). Consequently, a larger proportion of patients with HCC are diagnosed at early stages of disease and undergo treatment with curative intent. Given the limitations of the CLIP score in discriminating patients with early-stage disease (i.e., all unifocal tumors occupying $<$50% of the hepatic volume are scored equally), investigators in Japan proposed and subsequently validated the JIS score (25,28).

The JIS score combines the CTP classification (0, 1, and 2 points for CTP class A, B and C, respectively) with TNM staging by the Liver Cancer Study Group of Japan criteria (0–3 points for TNM stages I–IV, respectively) (Table 7). Seven-hundred twenty-two patients in Japan presenting with HCC over a 10-year time frame were evaluated by CLIP and JIS scores. The JIS classification was observed to perform better and was better able to discriminate among patients with early disease compared with CLIP. This was demonstrated by the fact that the 10-year survival rate for patients in the best prognostic group differed significantly between CLIP and JIS (23% and 65%, respectively) (28). The JIS has subsequently been validated in a large cohort of over 4500 patients from five centers in Japan (25).

While several large studies have observed that JIS is superior to CLIP or BCLC among patients with HCC in Japan (25,29), JIS has only been validated in patients from Japan in whom the majority had early-stage disease and underwent surgical therapy. Additionally, comparisons between JIS and other staging systems are negatively impacted by the inclusion of patients diagnosed as early as 1976, which limits the reliability of the assessment of tumor burden. Ultimately, whether the JIS staging system is applicable to Western patients with HCC is uncertain. Several comparative studies in North American and European cohorts suggest that JIS may be less suitable among cohorts where more patients have advanced disease (20,30).

Table 7 JIS Score

Parameter		Score
CTP class	A	0
	B	1
	C	2
TNM stage	I	0
	II	1
	III	2
	IV	3

Abbreviations: CTP, Child–Turcotte–Pugh; TNM, tumor node metastasis; JIS, Japanese Integrated System.
Source: From Refs. 25 and 28.

Table 8 BCLC Staging System

BCLC stage	Performance status	Tumor morphology	CTP class
0 Very early HCC	0	Single lesion <2 cm	No portal hypertension and normal bilirubin
A1 Early HCC	0	Single lesion <5 cm	No portal hypertension and normal bilirubin
A2 Early HCC	0	Single lesion <5 cm	Portal hypertension and normal bilirubin
A3 Early HCC	0	Single lesion <5 cm	Portal hypertension and elevated bilirubin
A4 Early HCC	0	2 or 3 lesions, all <3 cm	CTP class A–B
B Intermediate HCC	0	Large or multicentric tumor	CTP class A–B
C Advanced HCC	1–2	Portal vein invasion, or nodal or distant spread	CTP class A–B
D End stage HCC	3–4	Any of the above	CTP class C

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; CTP, Child–Turcotte–Pugh.
Source: From Refs. 31–33.

BCLC Staging System

The BCLC staging system was derived from data from multiple cohorts of patients with HCC from the BCLC center. One of the advantages of the cohort is that the data used in constructing the BCLC staging system are taken from a wide spectrum of patients and include surgical outcomes among patients with early disease and natural history studies of untreated HCC. The BCLC stage stratifies patients into five stages (very early, early, intermediate, advanced, and terminal) according to performance status, CTP class, and tumor factors including size, multifocality, and vascular invasion (Table 8). Additionally, the BCLC staging system is linked to treatment recommendations based on the cancer stage (31–33).

The BCLC system has several strengths, which include the use of a measure of performance status, assessment of liver function using the widely accepted CTP class, and discrimination of tumor burden into several categories, which include early stages of disease. The BCLC has been validated in cohorts of patients from Europe and the United States (20,34). In comparison to prior staging systems, the BCLC classification has improved discrimination of patients with earlier stages of disease. Among European patients undergoing both surgical and nonsurgical therapies, retrospective analyses found that BCLC was able to predict survival more accurately than Okuda, TNM, JIS, CUPI, CLIP, and GRETCH scores (16,30,35). Subsequently, BCLC was prospectively analyzed in a cohort of 195 Italian patients and found to be a better independent predictor of survival than TNM, Okuda, CLIP, and JIS scores (34). In a cohort of 239 U.S. patients with HCC, BCLC was found to have the highest discrimination between groups and the most homogeneity with groups compared with Okuda, GRETCH, CLIP, JIS, CUPI, and TNM staging (20). Because of these strengths, the

BCLC has been endorsed by both the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases (7,8).

Some researchers raised concerns regarding the linkage of BCLC to suggested therapies with treatment-related variables potentially impacting outcomes more than tumor stage (10,36). Also the BCLC staging system was constructed from a cohort of European patients with HCC, and its applicability to cohorts of patients from Asia and with chronic hepatitis B remains less certain. Various investigators in Japan have compared BCLC staging with JIS and found that in cohorts of Japanese patients, JIS has greater prognostic power (29,37).

FUTURE DIRECTIONS

An ideal staging system for HCC would effectively discriminate patients with early, advanced, and end-stage tumor, accurately measure performance status as well as hepatic functional reserve, incorporate important clinical factors such as tumor invasion in a weighted fashion, be equally applicable in patient populations from across the globe, and be readily calculated at the bedside by the clinician. Given the heterogenous nature of HCC, no such ideal system exists. Refinements to existing scoring systems and better understanding of the molecular biology of HCC will allow more accurate staging in the future.

The majority of staging systems incorporate some measure of liver functional reserve, often the use of the CTP score as in the BCLC, CLIP, and JIS scores. The model for end-stage liver disease (MELD) score has been widely recognized to accurately predict liver-related mortality and is in fact used to prioritize organ allocation for patients with chronic liver disease awaiting transplantation in the United States (38,39). Recently, investigators from Taiwan have prospectively evaluated 430 patients with HCC using BCLC, CLIP, and JIS scores both as originally described and modified to use MELD score (categorized as <10, 10–14, and >14) in the place of CTP classification. Modified versions of CLIP and JIS had improved homogeneity and discriminative ability, while the modified BCLC showed no significant improvement (40). The present study suggests that more accurate assessment of liver functional status can improve staging systems. Ideally, the use of MELD as a continuous variable, as opposed to three MELD categories, may further improve discrimination for patients with HCC. This hypothesis requires further prospective investigation.

Improvements in imaging technology will continue to impact the accuracy of staging of HCC. As imaging resolution improves, the ability to detect small metastases and satellite tumor nodules will increase. Currently, contrast-enhanced computed tomography or magnetic resonance imaging remains the mainstay for noninvasive staging of HCC. The experience with use of PET using standard fluorodeoxyglucose (FDG) has been unsatisfactory in staging HCC due to a high false-negative rate. The use of (11)C-acetate as a radiotracer in addition to (18)F-Flurodeoxy glucose (F-FDG) has been reported to significantly improve the sensitivity of PET for HCC metastases (41,42). A larger, multicenter trial will be needed before (11)C-acetate PET can be widely recommended as a staging procedure for HCC.

Ultimately, advances in understanding the molecular biology of HCC will play a significant role in the ability to accurately stage the disease. Currently, no biologic or genetic markers, which predict the behavior of HCC, have been identified (6). Molecular testing of tumor samples to identify genetic markers of aggressive disease is needed as are molecular techniques to distinguish between de novo multicentric diseases and intrahepatic metastases, which are likely to behave differently.

CONCLUSIONS

Staging for HCC requires the assessment of the degree of intrahepatic disease burden as well as the presence of extrahepatic spread. Prognosis for this common cancer is also significantly impacted by the degree of liver dysfunction present, as HCC commonly arises in the setting of cirrhosis. Given the multiple potential treatment options, including radical therapies with curative intent, accurate staging is critical.

Currently, there are multiple different staging systems for HCC that have been published and debated in the medical literature. While each has strengths, none is ideal, and no global consensus exists regarding which system to use. The characteristics of the population at risk for HCC significantly impacts on the performance of various staging systems, and it may be that for clinical practice different staging systems may provide the best prognostic information in different regions. Currently, the BCLC staging system has been endorsed for use by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver and seems well suited for use in patients from the Western hemisphere. Ultimately, a staging system with performance characteristics that provide equal discrimination for tumor stage in varying global settings is needed to guide advances in the therapy of HCC.

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8 Local Ablation Therapy

Shuichiro Shiina
Department of Gastroenterology, University of Tokyo, Tokyo, Japan

INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most common malignant neoplasms in the world (1) and is on the increase in western countries (2,3). Unlike other solid tumors, surgery plays a limited role in the treatment of HCC (4–6). Only 20% to 30% of patients can be candidates for hepatectomy because of underlying cirrhosis or multiple lesions. Furthermore, this cancer frequently recurs even after apparently curative resection (7) because of latent metastasis or metachronous multicentric carcinogenesis. Liver transplantation may be effective in some cases (8), but its feasibility is restricted by organ donor shortage.

Consequently, various nonsurgical therapies have been introduced. Among them, image-guided local ablation therapies, such as percutaneous ethanol injection (9–11), microwave coagulation (12), and radiofrequency ablation (13–15), have been playing important roles in the treatment of small HCCs (Table 1). They are potentially curative, minimally invasive, and easily repeatable. At our institute, we have treated 90% of previously untreated patients with HCC by local ablation therapies. We have performed ethanol injection on a total of 2000 patients since 1985 and microwave coagulation on 200 since 1995, with satisfactory long-term results. However, since the introduction of radiofrequency ablation into clinical practice in 1999, there has been a drastic shift from ethanol injection and microwave coagulation to radiofrequency ablation (Fig. 1) (15). A randomized controlled trial by us (16) and that by others (17,18) proved that radiofrequency ablation is superior to ethanol injection in the treatment of HCC.

In this chapter, ethanol injection and microwave coagulation will first be described briefly and then radiofrequency ablation in more detail.

PERCUTANEOUS ETHANOL INJECTION

In percutaneous ethanol injection, absolute ethanol is injected directly into lesions through 21- to 22-gauge needles that are inserted under ultrasound guidance (19). It can destroy a considerably large volume of tissue in one ablation. Ethanol injection was introduced into clinical practice in early 1980s (9–11). It has enabled us to treat HCC potentially curatively by nonsurgical measures. Ethanol injection has been widely performed as a standard therapy for small HCCs, such as those 3 cm or less in diameter (19–20).

Histopathological examinations after the therapy have revealed that ethanol injection can destroy the tumor completely when it is performed properly (21). Some investigators have reported that its long-term survival may be similar to that of surgery (22–25). According to the report of the 17th nationwide follow-up survey of the Liver Cancer Study Group of Japan, the 1-, 2-, 3-, 4-, 5-, 7-, and 10-year survival rates of all 14,726 patients treated by ethanol injection were 91.3%, 77.5%, 63.0%, 50.2%, 39.4%, 24.4%, and 12.3%, respectively (26). At our institute, the cumulative survival rates of 685 patients on whom ethanol injection was performed as the initial treatment were 91.0%, 80.5%, 67.6%, 57.0%, 49.0%, 34.5%, and 17.4% at 1, 2, 3, 4, 5, 7, and 10 years, respectively (27). A recent randomized controlled trial showed that there is no statistical significance for recurrence and survival between ethanol injection and surgical resection (28).

Its efficacy is not very reliable, however, because spread of injected ethanol is largely restricted by the capsule or septa of the lesion (Fig. 2) (21). Thus, the number of patients treated by ethanol injection has sharply decreased since the recent introduction of radiofrequency

Table 1 List of Local Ablation Therapy for Hepatocellular Carcinoma

A.	Percutaneous injection
	1. Ethanol
	2. Acetic acid
	3. Hot saline
	4. Chemotherapeutic agents
B.	Percutaneous application of energy source
	1. Radiofrequency
	2. Microwave
	3. Interstitial laser
	4. Cryoablation

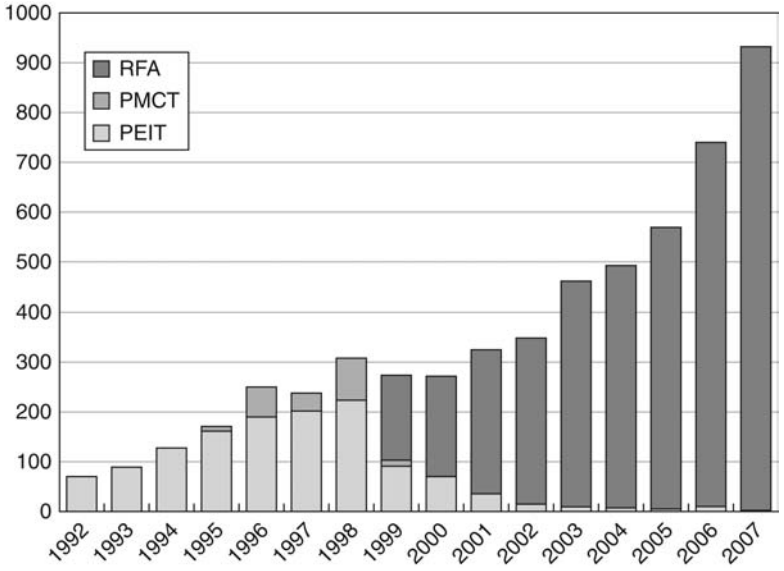


Figure 1 Transition of local ablation therapies for liver tumors at the Department of Gastroenterology, University of Tokyo. We have performed image-guided local ablation therapies on 90% of previously untreated patients with hepatocellular carcinoma. Since the introduction of radiofrequency ablation into clinical practice in 1999, there has been a drastic shift from ethanol injection and microwave coagulation to radiofrequency ablation. In 2007, we performed radiofrequency ablation on a total of 929 patients with liver tumors while we did ethanol injection on two. *Abbreviations*: PEIT, percutaneous ethanol injection therapy; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation.

ablation (15). A randomized controlled trial by us (16) and that by others (17,18) showed that radiofrequency ablation is superior to ethanol injection for small HCCs from the viewpoint of not only treatment response but also recurrence and survival.

Nowadays, ethanol injection is a treatment of choice only in cases in which radiofrequency ablation cannot be performed safely, such as those in which enteric-biliary reflux is observed so that radiofrequency ablation may develop liver abscess, or those in which adhesion exists between the lesion and the gastrointestinal tract, so that radiofrequency ablation may cause the gastrointestinal tract perforation or penetration even after artificial ascites technique.

PERCUTANEOUS MICROWAVE COAGULATION

In percutaneous microwave coagulation, the cancer tissue is ablated by dielectric heat produced by microwave energy emitted from the inserted monopolar-type electrode (16 gauge) (12). Microwave coagulation has been used in liver surgery to control bleeding from

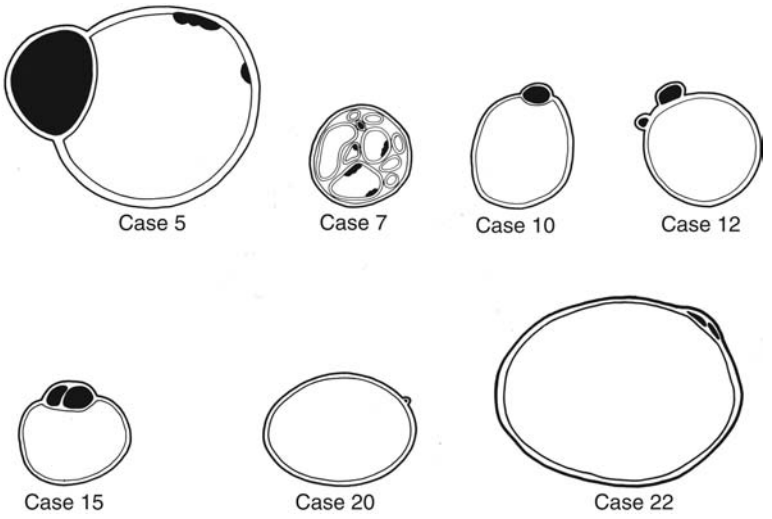


Figure 2 Schematic diagram of cases of incomplete necrosis treated by ethanol injection. In an early period of our study, we histopathologically examined 24 lesions of hepatocellular carcinoma treated by ethanol injection and found that the lesion was completely necrotic in 17 cases, 90% necrotic in six, and 70% necrotic in the remaining. Undestroyed cancer tissue remained in small nodules around the main lesion, along the edge of the lesion, or in portions isolated by septa. This is because spread of injected ethanol is largely restricted by the capsule or septa of the lesion, which results in less reliable efficacy in ethanol injection than thermal ablation.

liver transaction planes during resection. Heat may be conducted considerably homogeneously in all directions; the capsule or septa of the lesion may not prevent the conduction very much. It can surely destroy a certain amount of tissue, although its necrotic area is smaller (2 cm in diameter and 2.5 cm in length) compared with that by ethanol injection. Microwave coagulation became popular in Japan in late 1990s. However, since the spread of radiofrequency ablation, microwave coagulation has rarely been performed (15).

According to the report of the 15th nationwide follow-up survey of the Liver Cancer Study Group of Japan, the 1-, 2-, 3-, 4-, and 5-year survival rates of all 828 patients treated by microwave coagulation were 93.8%, 85.6%, 77.1%, 67.3%, and 57.2%, respectively (29).

RADIOFREQUENCY ABLATION

In radiofrequency ablation, the electrode is inserted into the tumor under image guidance. Then radiofrequency energy is emitted from the exposed portion of the electrode, which is converted into heat and causes necrosis of the tumor. Radiofrequency ablation can ablate a tissue of up to 3 cm in diameter or more as expected. Thus, advantage of this therapy with ethanol injection is that it can ablate a large volume of tissue in one ablation and that with microwave coagulation is that it can surely destroy a certain size of tissue.

Radiofrequency ablation is mainly performed percutaneously under ultrasound guidance, while it can also be used under laparotomy (30), laparoscopy (31), or thoracoscopy (32).

Several types of radiofrequency ablation systems are commercially available (33). In RITA (Mountain View, California, U.S.) and Boston Scientific (Natick, Massachusetts, U.S.) systems, expandable-type electrodes are used; multiple thin curved monopolar electrodes extend from the central cannula (18–14 gauge) of the electrode. Radiofrequency emanates from each of these hooks, resulting in increased coagulation. In Valley Lab (Boulder, Colorado, U.S.) system, cooled-tip electrodes (17 gauge) are used. These electrodes have two hollow lumens that permit continuous internal cooling of the tip with a chilled perfusate. As a result, heating of tissues nearest to the electrode is reduced, which allows for greater current deposition without tissue charring, or impedance rises. We use the cooled-tip electrodes at our institute because some lesions can be ablated with these electrodes, but not with the expandable-type

ones (34). In Japan, more than 1400 institutes have introduced radiofrequency ablation in the treatment of liver tumors, and the system with the cooled-tip electrodes has an 80% share of the market, while in other countries, the systems with expandable-type electrodes are more widely used.

There have been a few randomized controlled trials to compare radiofrequency ablation with ethanol injection. In a study by Lin et al., 157 patients with 186 HCCs 4 cm or less were randomly assigned to radiofrequency ablation, conventional ethanol injection, and higher-dose ethanol injection (18). Radiofrequency ablation was superior to conventional ethanol injection and higher-dose ethanol injection from the viewpoint of the local tumor progression, overall survival, and cancer-free survival. In the other study by Lin et al., 187 patients with HCC were assigned to radiofrequency ablation, ethanol injection, or acetic acid injection (35). Radiofrequency ablation was superior to ethanol injection and acetic acid injection with respect to local recurrence, overall survival, and cancer-free survival rates, but radiofrequency ablation also caused more major complications.

In our study, 232 patients with HCC who had three or fewer lesions, each 3 cm or less in diameter, and liver function of Child-Pugh class A or class B were entered onto a randomized controlled trial (16). The primary endpoint was survival, and the secondary endpoints were overall recurrence and local tumor progression. Radiofrequency ablation had a 46% smaller risk of death, a 43% smaller risk of overall recurrence, and an 88% smaller risk of local tumor progression than that by ethanol injection. The incidence of adverse events was not different between the two therapies.

Recently, a randomized controlled trials to compare radiofrequency ablation with surgical resection reported that there was no difference between these two treatments from the viewpoints of overall survival and disease-free survival, while posttreatment complications were more often and severe after surgery (36).

Patient Selections

The general requirements for radiofrequency ablation are as follows:

- Histopathologically confirmed HCC or characteristic imaging features of HCC
- Unresectable lesions or refusal of surgery
- Absence of apparent vascular or biliary invasion
- Absence of refractory ascites
- Absence of marked bleeding tendency (prothrombin times should be 50% or more; platelet transfusion must be used if a patient has a platelet count of less than $50{,}000/mm^3$)
- Serum bilirubin level of less than 3.0 mg/dL
- Lesions located in portions where the electrode can be inserted and held safely
- Informed consent

With regard to the size of the lesions, radiofrequency ablation is usually performed for small lesions up to 3 cm in diameter, since the size of necrosis achieved by each radiofrequency ablation process is limited. Larger tumors can be treated by radiofrequency ablation, however, with overlapping of ablated areas. Combination of transcatheter arterial chemoembolization and radiofrequency ablation is often useful for large tumors.

With regard to the number of the lesions, most investigators have performed radiofrequency ablation on patients with three or fewer lesions. It is impractical to treat very many lesions with radiofrequency ablation because of the number of necessary treatment sessions. In addition, it is very likely that there are also small undetectable metastases in cases of many lesions, and therefore, even if all detected lesions are treated by radiofrequency ablation, complete cure cannot be expected in those cases. In cases of more than three lesions, in which complete cure cannot be expected, radiofrequency ablation may still be performed with a combination of transcatheter arterial chemoembolization. Chemoembolization is first performed to treat all lesions, and then radiofrequency ablation may be performed against main lesions.

Anatomical locations of the tumor may have potential influence on the efficacy and complication of radiofrequency ablation, although we have put no restrictions on lesion

location and have successfully performed radiofrequency ablation on more than 99% of patients at our institute (34). It is risky to perform ablation for lesions adjacent to the Glisson's capsule. It frequently causes biliary injury, which results in biliary stricture or biloma. It may also damage the hepatic artery and the portal vein, which results in hepatic infarction. "Heat-sink" effect due to the blood flow may cause incomplete necrosis of lesions contiguous with large vessels. It is also risky to do ablation for other near organs, such as the gallbladder and the heart. Lesions near the gastrointestinal tract may be treated safely if the artificial ascites technique can separate the lesion from the tract (37). Subcapsular lesions are reported to be at a high risk of malignant cell seeding (38), and thus, if possible, the lesion should be punctured through the nontumorous liver. Lesions beneath the diaphragm can be ablated successfully if one has the artificial pleural effusion technique (39).

Radiofrequency ablation can be used even in cases in which complete cure cannot be expected, since it is not very invasive and it definitely reduces the tumor mass. In those cases, only main lesions may be treated by radiofrequency ablation to reduce the tumor burden, and some lesions may be treated by other therapies.

Technique

Planning ultrasound should be performed carefully to select the optimal approach. In Japan radiofrequency ablation is performed on an inpatient basis (15). The patients should fast at least four hours before the treatment and should be given premedications for analgesia and sedation.

In radiofrequency ablation, grounding is achieved by attaching two pads to the patient's thighs. A 17-gauge, cooled-tip electrode with a 2- or 3-cm exposed tip is attached to a radiofrequency generator (CC-1 Cosman Coagulator, Valley Lab, Boulder, Colorado, U.S.). After local anesthesia, the electrode is inserted under ultrasound guidance (Fig. 3). During ablation, the temperature is measured with a thermocouple in the electrode. Tissue impedance is also monitored by circuitry incorporated into the generator. A peristaltic pump infused 0°C saline into the electrode lumen to maintain the tip temperature below 20°C . Radiofrequency energy is usually delivered for 6 to 12 minutes for each application as follows: after measurement of baseline impedance, generator output is gradually increased up to 1400 mA. This level is maintained until the impedance increased over 10 ohms from the baseline. Then the current is temporarily reduced until impedance becomes stabilized. Output is decreased and increased repeatedly for the remainder of the session to avoid tissue charring. Twelve-minute ablation using a 3-cm exposed-tip electrode produces a quasi-spherical necrotic volume 3 cm in diameter. For large lesions, the electrode is repeatedly inserted into different sites, so that the entire lesion can be enveloped by assumed necrotic volumes. Following the procedure, the patients should remain in bed until the next morning.

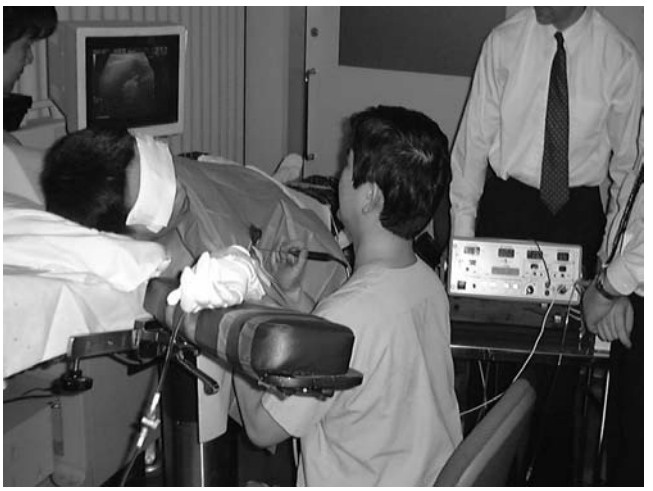
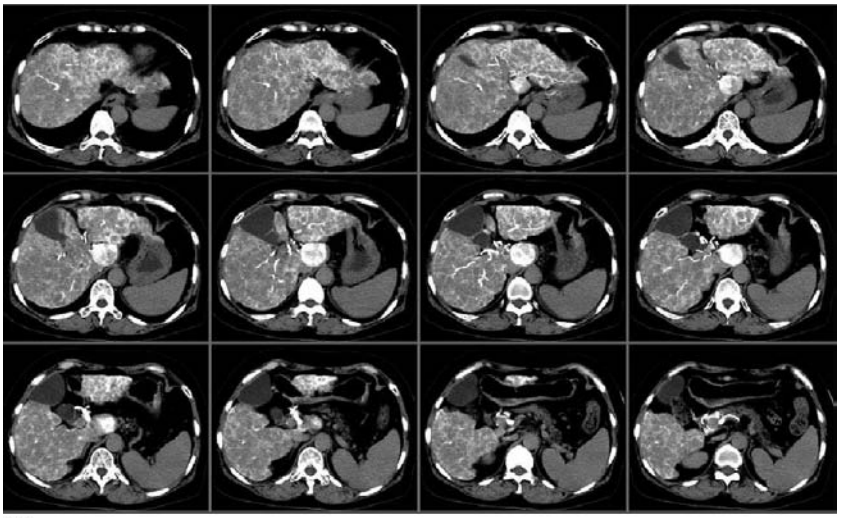
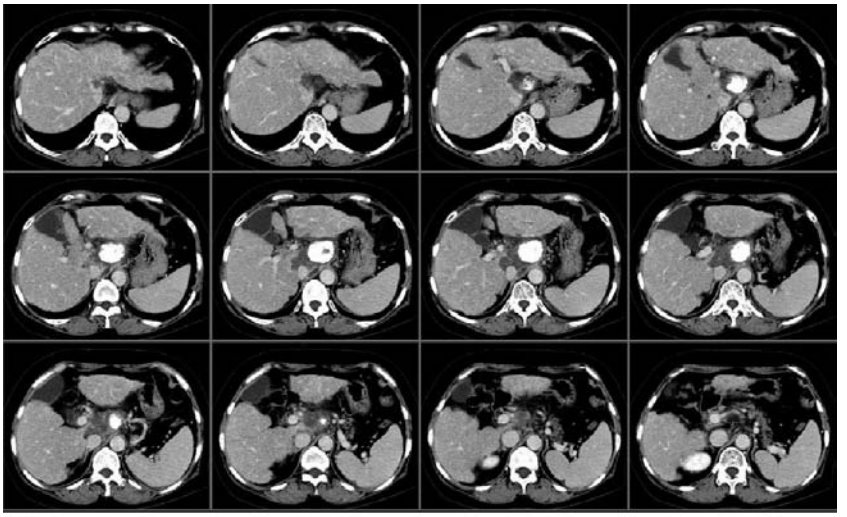


Figure 3 A view of percutaneous radiofrequency ablation. An electrode is inserted into the lesion under ultrasound-guidance.



(A)



(B)

Figure 4 (**A**) CT scan taken before radiofrequency ablation. A 68-year old woman had hepatocellular carcinoma of 5 cm in the Spiegel lobe. (**B**) CT scan taken after the second procedures of radiofrequency ablation. Transcatheter arterial embolization had been performed before radiofrequency ablation. Thus, there was a deposit of Lipiodol in the tumor. The deposit of Lipiodol is surrounded with a non-enhanced zone, which represent ablative margin. Five years have passed since the treatment. However, there has been no local tumor progression.

If the entire lesion is assumed to have become necrotic, enhanced CT is performed (Fig. 4). When any possible undestroyed portions remain, the therapy is repeated until CT demonstrates the entire tumor necrosis.

Local Tumor Progression and Distant Recurrence

Although many investigators reported that radiofrequency ablation could achieve complete tumor necrosis in most cases on CT, local tumor progression is not infrequent; local tumor progression rate at three years was reported to be 1.7% to 20.4% (16,18,40,41). The most important factor associated with failure of the local tumor control is tumor size (40–42). It is not easy to obtain a certain amount of safety margin all around the large tumor in three dimensions. Although various new radiofrequency ablation devices to increase the ablation

Table 2 Studies Reporting Long-Term Survival Outcomes of Radiofrequency Ablation

Authors and year	Conditions of the cases	Number of cases	Survival rates		
			1 yr	3 yr	5 yr
Rossi et al., 1996	1–2 HCCs < 3 cm	39	94	68	40
Buscarini et al., 2001	Child A–B, 1–3 HCCs < 3.5 cm	82	89	62	33
Lencioni et al., 2005	Child A–B, 1 HCC < 5 cm or 3 HCCs < 3 cm	187	97	71	48
Machi et al., 2005	Unresectable or recurrent HCC	65	75	50	40
Tateishi et al., 2005	Naïve patients	319	95	78	54
	Nonnative patients	345	92	62	38
Cabassa et al., 2006	Child A–B, 1 HCC < 5 cm or 3 HCCs < 3 cm	59	94	65	43

Abbreviation: HCC, hepatocellular carcinoma.

volume have been introduced, a large tumor of 3 cm or more still requires multiple overlapping ablations.

While local tumor progression is related to incomplete tumor ablation, distant intra- and extrahepatic recurrence is mainly determined by the biological characteristics and natural history of HCC. The incidence of distant intra- and extrahepatic recurrence ranges from 41% to 73%.

Survival

According to the report of the 17th nationwide follow-up survey of the Liver Cancer Study Group of Japan, the 1-, 2-, 3-, 4-, and 5-year survival rates of all 5478 patients treated by radiofrequency ablation were 94.9%, 85.7%, 76.7%, 67.2%, and 57.3%, respectively (26). At our institute, the cumulative survival rates of 909 patients on whom radiofrequency ablation was performed as the initial treatment were 96.5%, 88.6%, 81.8%, 71.1%, 60.6%, and 34.1% at 1, 2, 3, 4, 5, and 8 years, respectively (27). In other studies, survival rates of patients treated by radiofrequency ablation range from 75% to 97% at 1 year, from 50% to 78% at 3 years, and from 33% to 54% at 5 years (Table 2) (43–48). Survival depends on not only tumor factors but also liver function.

Adverse Effects and Complications

Knowledge of the broad spectrum of adverse effects and complications and relevant management is mandatory to perform radiofrequency ablation safely, although its mortality and morbidity rates are much lower than those reported for surgery. Common adverse effects of radiofrequency ablation were pain, fever, nausea, and asymptomatic right pleural effusion. Mulier et al. reviewed 82 articles and reported that the mortality and morbidity rates of 3670 patients treated by radiofrequency ablation were 0.5% and 8.9%, respectively (49). There were 20 deaths reported as a result of sepsis ($n = 7$), liver failure ($n = 7$), cardiac complications ($n = 4$), peritoneal hemorrhage ($n = 1$), and bile duct stricture ($n = 1$). Major complications were abdominal bleeding (1.6%), abdominal infection (1.1%), bile tract damage (1.0%), liver failure (0.8%), dispersive pad skin burn (0.6%), hepatic vascular damage (0.6%), visceral damage (0.5%), cardiac complications (0.4%), myoglobinemia or myoglobinuria (0.2%), tumor seeding (0.2%), coagulopathy (0.2%), and others. The complication rate was similar for the percutaneous (7.2%), laparoscopic (9.5%), and simple laparotomic (9.9%) approach, while the laparotomic combined with cryotherapy or hepatic or extrahepatic resection had a morbidity rate of 31.8%.

A multicenter study in Italy reported that 6 deaths (0.3%) were noted among 2320 patients treated with the cooled-tip electrode, including multi-organ failure following intestinal perforation ($n = 2$), septic shock following *Staphylococcus aureus*-caused peritonitis ($n = 1$), massive hemorrhage following tumor rupture ($n = 1$), liver failure following stenosis of right bile duct ($n = 1$), and sudden death of unknown cause three days after the procedure ($n = 1$) (50). Fifty patients (2.2%) had additional major complications. Common complications were

peritoneal hemorrhage (0.5%), neoplastic seeding (0.5%), intrahepatic abscess (0.3%), and intestinal perforation (0.2%). An increased number of treatment sessions were related to a higher rate of major complications.

A multicenter survey in Korea revealed one procedure-related death (0.09%) due to peritoneal hemorrhage and 37 major complications (2.4%) among 1139 patients in 11 centers. Reported complications were hepatic abscess (0.7%), peritoneal hemorrhage (0.5%), biloma (0.2%), ground pad burn (0.2%), pneumothorax (0.2%), vasovagal reflex (0.1%), and others (51).

CONCLUSIONS

In the treatment of HCC, image-guided percutaneous local ablation therapies have been playing more and more important roles. Among various local ablation therapies, radiofrequency ablation has been replacing ethanol injection as a standard therapy for patients who have unresectable HCC or who do not want surgery. Further investigations would be necessary to determine whether radiofrequency ablation can be a replacer of surgery for resectable HCC. In those trials, the primary endpoint must be overall survival. Recurrence-free survival cannot be a surrogate endpoint in HCC.

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9 Chemotherapy and Novel Systemic Therapies

Ahmed O. Kaseb and Melanie Thomas

Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, U.S.A.

INTRODUCTION

The emergence of chemotherapy in the 1950s has led to the availability of systemic therapies for patients with hematologic malignancies and advanced solid tumors. These advances proved the concept of using chemotherapy to indeed cure cancer, in case of hematologic malignancies, and provided the rationale for integrating chemotherapy into combined modality approaches for solid tumors. Skipper et al. (1) has defined the invariable inverse relation between cell number and curability in a leukemia model. Therefore, when treatment failed in sensitive cell lines, it was thought to be attributed to the initial high tumor burden, which was too high for even potentially curative doses of chemotherapy to eradicate cancer cells. This relationship could be applied to other hematologic malignancies and solid tumors. However, recent advances in our understanding of the molecular pathways by which chemotherapy exerts its cytotoxic activity, and by which genetic change can result in resistance to drug therapy, has provided a basis for our understanding of chemoresistance and the development of innovative therapeutic strategies.

RESISTANCE TO SYSTEMIC CHEMOTHERAPY IN HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is potentially curable by surgical resection and liver transplantation. However, the majority of patients present with advanced stage disease, which is most commonly accompanied by severe background liver disease. Hence, surgery is feasible for only a small fraction of patients with localized disease, and liver transplantation is severely limited by the availability of liver donors. Systemic cytotoxic therapies have demonstrated a very limited impact on the natural history of advanced HCC (Table 1). A 1997 meta-analysis evaluating the results of 37 randomized clinical trials of systemic and regional chemotherapy in 2803 HCC patients concluded that nonsurgical therapies were ineffective or minimally effective at best (22). In addition, molecular characterization of HCC has led to the recognition of defined aberrant signaling pathways, which helped in subsequent development of targeted agents as potential choices for the treatment of this chemoresistant disease. In HCC, several crucial intracellular signaling pathways such as the Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) pathway and phosphoinositide-3 kinase/protein kinase B/mammalian target of rapamycin (PI3k/Akt/mTOR) pathway, in addition to several growth and angiogenic factors/receptors such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and vascular endothelial growth factor receptor (VEGFR) have been recognized. Subsequently, targeted agents have entered clinical trials in HCC patients. This is of particular significance for HCC in light of the lack of existing effective systemic therapy for this cancer. Furthermore, the assessment of systemic treatment response has changed over the years. It is now well recognized that the conventional markers of radiographic response [World Health Organization (WHO) or Response Evaluation Criteria In Solid Tumors (RECIST) criteria] are poorly related to tumor cell kill in liver tumors and that end points other than radiographic tumor shrinkage, such as time to tumor progression, progression-free survival (PFS), and certainly overall survival, are more meaningful measures of therapeutic benefit (23). Finally, Large HCCs commonly develop areas of central necrosis, which may interfere with drug delivery to the growing tumor. Topoisomerase IIa

Table 1 Summary of Selected Clinical Trials in Patients with Advanced Hepatocellular Carcinoma

Study (reference number)	Regimen	Phase	Sample size	Response rate (%)	Median survival
Cytotoxic chemotherapy					
Yeo et al. (2)	PIAF vs. adriamycin	III	94/94	20.9 vs. 10.5	8.6 vs. 6.83 mo
Mok et al. (3)	Nolatrexed vs. doxorubicin	II	37/17	0	4.9 vs. 3.7 mo
Posey et al. (4)	TI38067 vs. adriamycin	II/III	169/170	NA	5.7 vs. 5.6 mo
Gish et al. (5)	Nolatrexed vs. doxorubicin	III	222/222	1.4 vs. 4.0	5.5 vs. 8 mo ($p = 0.0068$)
Patt et al. (6)	Thalidomide	II	37	6	6.8 mo
Pastorelli et al. (7)	Pegylated doxorubicin + gemcitabine	II	35	23	8.8 mo
Immunotherapy/hormonal therapy					
Barbare et al. (8)	Tamoxifen vs. BSC	II	210/210	NA	4.8 vs. 4.0 mo
Lee et al. (9)	Dendritic cells	II	31	12.9	1-yr survival 40%
Targeted biologic therapy					
Zhu et al. (10)	Cetuximab	II	30	0	9.6 mo
Llovet et al. (11)	Sorafenib vs. placebo	III	299/303	2.3	10.7 vs. 7.9 mo ($p = 0.00058$)
Abou-Alfa et al. (12)	Sorafenib	II	137	2.2	9.3 mo
Philip et al. (13)	Erlotinib	II	38	9	13 mo
Thomas et al. (14)	Erlotinib	II	40	0	10.75 mo
Thomas et al. (15)	Bevacizumab + erlotinib	II	34	20.6	19 mo (PFS 9)
Ramanathan et al. (16)	Lapatinib	II	30	5	6.2 mo
Gruenwald et al. (17)	Cetuximab	II	32	0	NR
O'Dwyer et al. (18)	Gefitinib	II	31	3	PFS 2.8 mo; OS 6.5 mo
Combination cytotoxic + biologic therapy					
Sun et al. (19)	Capecitabine, oxaliplatin, bevacizumab	II	30	11	PFS 5.4 mo
Louafi et al. (20)	GEMOX + cetuximab	II	43	23	9.2 mo
Zhu et al. (21)	GEMOX + bevacizumab	II	33	20	9.6 mo

Abbreviations: PIAF, cisplatinum, interferon, doxorubicin, and 5-fluorouracil; OS, overall survival; GEMOX, gemcitabine, oxaliplatin; PFS, progression-free survival; TTTF, time to treatment failure; NR, not reported; NA, not applicable; BSC, best supportive care.

encodes an enzyme that is the target for anticancer chemotherapeutic agents such as doxorubicin, and mutations are associated with resistance (24). There is upregulation of topoisomerase IIa in doxorubicin-resistant HCC cell lines, and its expression is associated with an aggressive tumor phenotype (25). Cancer cells, including HCC cells, often have intrinsic drug resistance mediated via enhanced cellular drug efflux of several cytotoxic agents. This phenomenon is associated with increase in a drug transporter family, the adenosine triphosphate–binding cassette proteins that include multi drug resistant (MDR1), p-glycoprotein (p-gp), and the multidrug resistance protein (MRP) (26,27). Both these are upregulated in HCC (27,28). Upregulation of MDR1 accompanied by a decrease in doxorubicin accumulation levels has been reported in certain HCC cell lines (29). The *H19* gene is thought to induce p-gp expression and MDR1-associated drug resistance in HCC cells through regulating MDR1 promoter methylation (29). Besides, co-expression of p53 and p-gp may contribute to HCC chemoresistance in HCC cell lines (30). In addition, recent evidence suggests that hypoxia, MDR1 expression, and an angiogenic HCC phenotype may be interacting (31,32). Thus, to improve the outcome for patients with advanced HCC, alternatives to traditional cytotoxic chemotherapy agents are clearly an unmet need.

MOLECULAR THERAPEUTIC TARGETS IN HEPATOCELLULAR CARCINOMA

The growth of HCC depends on stimulatory effects of various growth factors, which bind to tyrosine kinase receptors and hence activate various intracellular signaling pathways, which subsequently lead to tumor cell proliferation, survival, migration, and metastasis. Both the

extracellular growth factors and the intracellular signaling pathways represent potential molecular targets for therapy of HCC. However, the antiangiogenic pathway looks more promising in HCC.

Targeting Growth Factors in Hepatocellular Carcinoma

The EGFR is frequently expressed in human hepatoma cells, and epidermal growth factor (EGF) may be one of the mitogens needed for the growth of hepatoma cells (33,34). Several strategies have been tested in HCC; one is through using neutralizing monoclonal antibody such as cetuximab, and another is through using small molecule tyrosine kinase inhibitor such as gefitinib and erlotinib, as demonstrated in HCC cell cultures (35,36). Erlotinib is an orally active and selective inhibitor of the EGFR/HER1-related tyrosine kinase enzyme. EGFR/HER1 expression was detected in 88% of the patients in a phase II study of erlotinib (14). In two phase II studies of this agent, the response rate was less than 10%, but the disease control rate was more than 50%, and median survival times were 10.75 and 13 months, respectively (13,14). Other studies of anti-EGFR agents in HCC are summarized in Table 2.

Table 2 Ongoing Clinical Trials of Systemic Antiangiogenic Therapy in HCC

Treatment	Phase	Patients	Trial number/sponsors
AZD-2171 (Cediranib)	II	Unresectable or metastatic HCC	NCT00238394 NCI
AZD-2171 (Cediranib)	II	Unresectable, locally advanced, or metastatic HCC	NCT00427973[a] Massachusetts General Hospital/NCI
Bevacizumab	II	Unresectable or metastatic HCC	NCT00162669 Institute Gusatve Roussy
Bevacizumab + erlotinib	II	Unresectable HCC	NCT00242502 M. D. Anderson Cancer Center
Bevacizumab + erlotinib	II	Unresectable or metastatic HCC	NCT00287222 University of Arkansas/Genentech
Bevacizumab + erlotinib	II	Advanced unresectable HCC	NCT00365391 Mayo Clinic/NCI
Bevacizumab + sirolimus	I	Unresectable, locally advanced, or metastatic HCC	NCT00467194 NCI
BMS-582664 vs. doxorubicin	II	Unresectable, locally advanced, or metastatic HCC	NCT00355238 Bristol-Myers Squibb
BMS-582664	I	Advanced HCC with varying levels of hepatic impairment	NCT00437424[a] Bristol-Myers Squibb
Sorafenib + tegafur/uracil (UFUR)	II	Unresectable, locally advanced, or metastatic HCC	NCT00464919 National Taiwan University Hospital
Doxorubicin +/− sorafenib	II	Unresectable or metastatic HCC	NCT00108953[b] Bayer
Pazopanib	I	HCC	NCT00370513 GlaxoSmithKline
Sunitinib	II	Unresectable, locally advanced, or metastatic HCC	NCT00361309 Massachusetts General Hospital
Sunitinib	II	Unresectable HCC	NCT00247676 Pfizer
Thalidomide	III	Locally advanced or metastatic HCC	NCT00225290 TTY Biopharm
Thalidomide + tegafur/uracil (UFUR)	II	Unresectable, locally advanced, or metastatic HCC	NCT00384800 Far Eastern Memorial Hospital

[a]Not open yet.
[b]No longer recruiting patients.
Abbreviation: HCC, hepatocellular carcinoma; BMS, Bristol-Myers Squibb.

Rationale for Antiangiogenic Therapy of Hepatocellular Carcinoma

The cancer cell has been the only target of anticancer therapy for more than 50 years. However, the cancer cell is genetically unstable, and mutations accumulate. On the other hand, antiangiogenic therapy targets endothelial cells, which are genetically stable. The genetic stability of endothelial cells may make them less susceptible to acquired drug resistance. As a result, angiogenesis inhibitors are emerging as a new class of therapeutic agents.

Angiogenesis' role in the initial progression from a premalignant tumor to a cancer prompted investigators to study the role of vascular endothelial growth factor (VEGF) in the natural history of HCC (37). In 1999, a group of researchers suggested that the degree of VEGF tissue expression increased according to the stepwise development of HCC (38). In addition, studies have shown that the VEGF was frequently expressed in HCC. In a quantitative analysis study, VEGF expression was demonstrated in 63.9% of encapsulated HCC and 78.3% of non-encapsulated HCC (39). Another study reported VEGF tissue expression in 88.8% of HCC (40). Notably, studies have suggested a correlation between the degree of VEGF tissue expression and the intensity of both the magnetic resonance signal and the computed tomographic enhancement of the hepatic artery, which represent radiologic vascular signals (41–43). Hence, the hypervascular nature of HCC has led to increasing interest in exploring the potential of antiangiogenic therapy in this disease.

Clinical Trials of Antiangiogenic Agents in Hepatocellular Carcinoma

Several vascular-targeted agents including thalidomide, sunitinib, sorafenib, and bevacizumab have been tested in advanced HCC (Tables 1 and 2).

Thalidomide's mechanism was not clearly known but was thought to be partly based on its antiangiogenic effects (44–46). Nevertheless, several clinical trials of thalidomide alone or in combination with epirubicin or interferon showed rare responses ranging from 0% to 6.3% (6,47–52). A recent report in abstract form of 19 patients treated with oral thalidomide 200 mg/day showed a continuous six-month PFS of 41% (53).

Sunitinib, an oral multikinase inhibitor, exerts an antiangiogenic effect by targeting VEGFR and PDGFR tyrosine kinases. A phase II study of sunitinib alone in 19 patients with unresectable or metastatic HCC reported a partial response in one patient (54).

Sorafenib, an oral multikinase inhibitor, exerts an antiangiogenic effect by targeting VEGFR and PDGFR tyrosine kinases. It exerts its effect through targeting Raf/MEK/ERK signaling at the level of Raf kinase and exerts an antiangiogenic effect by targeting VEGFR-2/3. A phase II study of sorafenib alone in advanced HCC showed 2.2% partial and 5.8% minor response (12). Recently, a randomized, placebo-controlled phase III trial of sorafenib in advanced HCC reported a 2.8-month improvement in median overall survival (HR 0.69, 95% CI 0.55–0.87%, $p = 0.0006$) along with increased time to progression and disease control rate (55**)**.

Bevacizumab is a recombinant, humanized monoclonal antibody that targets VEGF and may augment chemotherapy administration by making tumor vasculature less permeable and decreasing the elevated tumor interstitial pressure (56,57). Two recent phase II trials of bevacizumab alone in advanced HCC suggested an improved disease control, with one reporting response rate of 12.5% (all PR) with disease control rate (PR + SD) of 67% (51,58). Bevacizumab was also combined with gemcitabine and oxaliplatin in a phase II trial of advanced HCC, with overall response rate of 20% (21). A phase II trial of bevacizumab + capecitabine as a first-line treatment for advanced HCC recently reported response rate (CR + PR) of 16% (95% CI 4.5–36.1%) and disease control rate (CR + PR + SD) of 60% (95% CI 38.7–78.9%), with a median overall survival of 10.7 months (95% CI 5.3–14.7) and median PFS of 4.1 months (59). In addition, a phase II study of advanced or unresectable metastatic HCC treating 30 patients with bevacizumab, oxaliplatin, and capecitabine reported an 11% response rate, with mean PFS of 5.4 months (19). An ongoing phase II, single-arm, open-label trial of bevacizumab and erlotinib in 29 patients with unresectable HCC recently showed a 22% response rate (CR + PR) (15).

Moreover, there are a number of ongoing clinical trials of different vascular-targeted agents in HCC, as shown in Table 2. Preclinical studies of agents that target other foci that interact with VEGF, such as HIF-1α and a proliferation-inducing ligand (APRIL), have shown promising results with reduced VEGF expression in HCC cell cultures (60,61).

Furthermore, minor or low response rates from thalidomide (6,62) and megestrol (63,64) were reported.

ADDITIONAL MOLECULAR PATHWAYS TARGETED IN HEPATOCELLULAR CARCINOMA CLINICAL TRIALS

Mitogen-Activated Protein Kinase Pathway

The mitogen-activated protein kinase pathway (MAPK) pathway includes a cascade of phosphorylation of four major cellular kinases, ras, raf, MAPK, and ERK, which are responsible mainly for cellular differentiation and proliferation signal transduction. These intermediates are found to be high in both HCC cell lines and human specimens (65–68). Notably, for Ras proteins to be competent for signal transduction, posttranslational modification by incorporation of farnesyl and geranylgeranyl groups is required. One of the inhibitors of farnesyl transferase has been developed to prevent prenylation of Ras proteins; ABT-100, which has been shown to prevent the development of chemically induced HCC in rats (69). Raf kinase inhibitor Bay-439006 (sorafenib) is a therapeutic agent that targets this pathway and is currently the most promising molecular targeting drug for HCC that has undergone phase I, II, and III trials. It targets both raf and VEGFR. A phase II trial of sorafenib demonstrated antitumor activity in advanced HCC patients. This study did not meet its primary end point of response on the basis of WHO criteria, with a response rate of 2.2%. However, 33.6% of patients had stable disease for at least four months, with many showing central tumor necrosis (12). On the basis of the encouraging overall survival of 9.2 months reported in the phase II trial, a placebo-controlled international trial was conducted in HCC patients with Childs-Pugh A cirrhosis. Preliminary data presented in abstract form from the phase III trial showed better survival in the sorafenib arm (10.7 months) compared with placebo (7.9 months) (70). These results concluded that this agent offers a survival advantage compared with placebo and with several cytotoxic agents (based on historical controls), but this may be comparable to survival observed with other biologic agents (Table 2).

Phosphoinositide-3 Kinase/Protein Kinase B/Mammalian Target of Rapamycin Pathway

Activation of PI3k subsequently activates Akt kinase that phosphorylates and inactivates several pro-apoptotic proteins. This in turn leads to a number of downstream events that are responsible for cellular proliferation and apoptosis, and is closely linked to cell cycle (71,72). This pathway is known to be upregulated in a subset of HCC patients. Furthermore, mTOR is a downstream of Akt, which regulates cellular translational activities through the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and the 40s ribosomal protein S6 kinase (p70s6k). They are linked with the translation of mRNAs of genes regulating cell proliferation and angiogenesis, such as c-myc, cyclin D1, and HIF-1α (73). Rapamycin is an antibiotic that inhibits mTOR and is clinically used as an immunosuppressive drug to prevent graft rejection. It was shown to possess activity against HCC cell lines (74,75).

Epigenetic Changes

During the process of hepatic carcinogenesis, epigenetic modifications, namely hypermethylation and histone deacetylation, are accumulated in chronically injured liver. These events lead to inactivation of a number of tumor suppressor genes in HCC. Recent advances in epigenetic therapy (such as 5-aza-2′-deoxycytidine and SAHA) had been achieved in both hematologic malignancies and solid tumors. HCC cell lines were shown to be chemosensitized by epigenetic therapy (76,77). A novel histone deacetylase inhibitor, PXD-101A, is currently undergoing a multicenter phase I/II evaluation in Hong Kong.

Immunotherapy for Hepatocellular Carcinoma

Cancer cells are among harmful challenges to the body that include viruses, bacteria, and unicellular and multicellular pathogens. The immune system composes active defense strategies to counteract these challenges and is potentially capable of interacting, directly or

indirectly, with nearly every cell in the body. Increasing evidence suggests that immune responses have a role in controlling cancer, suggesting investigating their candidacy targets in cancer therapy. Cases of HCC spontaneous regression were reported, and suggested a possible immune mechanism involvement. Furthermore, tumor-associated proteins such as α-fetoprotein (AFP) could act as targets for immune-mediated attack (78,79). Notably, HCCs are often infiltrated with lymphocytes, and patients with higher levels of tumor-infiltrating lymphocytes have been shown to have a better prognosis after resection and transplantation (78). A randomized, controlled clinical trial has reported improved disease-free survival after HCC resection by infusion of lymphocytes activated by anti-CD3 and interleukin 2, possibly through T-cell adoptive immunotherapy (80).

Humans have a complicated immune system that functions to clear invading organisms and abnormal cells. However, cancers are able to arise despite this mechanism. Vaccines have the potential of benefiting cancer patients by stimulating an immune response against tumor-associated antigens. Dendritic cells (DCs) are one of the most efficient methods of stimulating immune responses. The concept in HCC was reported in a study showing that DC transduced with adenovirus encoding AFP were able to delay the growth of an AFP-producing tumor cell line in mice, an effect that was found to be associated with the appearance of AFP-specific cytotoxic T lymphocytes (81). Another strategy, through using fusions of DCs and syngeneic hepatoma cells, reported delaying the growth of implanted hepatoma cells and prevented local recurrence after surgical resection in rats (82). Several techniques have been recently reported, one study through using DC loaded with autologous tumor or hepatoma cell line lysates (10) and another through DC direct injection into tumors (83) with limited clinical responses. Furthermore, another group developed an HCC vaccine consisting of autologous formalin-fixed tumor tissue fragments, biodegradable microparticles containing human granulocyte macrophage colony-stimulating factor and human interleukin 2, and tuberculin. In a phase I/II clinical trial, the vaccine caused few adverse effects and significantly improved the recurrence-free survival of patients who had undergone liver resection for HCC compared with controls (84). Similar encouraging results were reported in another trial (85).

OTHER AGENTS TESTED IN HEPATOCELLULAR CARCINOMA

Interferons comprise a group of related proteins whose effects include antiviral activity, growth-regulatory properties, inhibition of angiogenesis, regulation of cell differentiation, enhancement of major histocompatibility complex antigen expression, and a wide variety of immunomodulatory activities. In HCC, the effects of systemic interferon therapy have generally been poor (86,87). However, the combination of chemotherapy and interferon has led to improved response rates but with no effects on survival. Moreover, there are indications that interferon may be useful in reducing recurrence rates after more definitive treatment (88).

Hormonal agents were also investigated in HCC. Growth of HCC has been proposed to be modulated by estrogens, which justified evaluating the efficacy of estrogen receptor blockage by tamoxifen. However, results have been conflicting (8,89). Lack of tamoxifen efficacy could be attributed either to a low expression of estrogen receptors in HCC (90) or to the expression of mutated estrogen receptors, which is known to be associated with male sex, unfavorable prognosis, and tumor progression (91). Furthermore, results of antiandrogen protocol trial were negative (92). In addition, marginal results have been found for octreotide (93,94). These agents seemed attractive because of minimal toxicities, but so far, responses have been disappointing.

Vitamin K has been assessed for its HCC-inhibitory actions. This idea is based on the characteristic biochemical defect in HCC of elevated plasma levels of immature prothrombin (DCP or PIVKA-2), due to a defect in the activity of prothrombin carboxylase, which is a vitamin K–dependent enzyme. Addition of vitamin K to HCC cell lines in vitro results in a correction of this defect and growth inhibition (95,96). Even massive vitamin K doses appear to be free of any human toxicity. This approach appears to be encouraging, especially because several long-term survivors are now accumulating in these studies.

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10 Chemoembolization, Radioembolization, and Other Novel Intra-arterial Therapies

Saad M. Ibrahim, Ahsun Riaz, Robert J. Lewandowski, and Riad Salem

Section of Interventional Radiology, Department of Radiology, Northwestern Memorial Hospital, Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois, U.S.A.

Laura M. Kulik

Department of Hepatology, Northwestern University, Chicago, Illinois, U.S.A.

Mary F. Mulcahy

Division of Hematology and Oncology, Department of Medicine, Robert H. Lurie Comprehensive Cancer Center, Northwestern Memorial Hospital, Chicago, Illinois, U.S.A.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignancy that arises from aberrant hepatocytes and commonly occurs in the setting of underlying liver disease. The overwhelming majority of patients are precluded from surgical interventions; most patients are either unresectable/non-transplantable by current criteria or inoperable secondary to underlying comorbidities. For patients fortunate enough to undergo operative cure, exceptionally high five-year recurrence rates have been reported (1–4). Without treatment, the prognosis remains dismal and the outcome is uniformly fatal. Although survival depends on many factors, size and stage of tumor at presentation appear to be significant prognosticators of outcome.

Given the increasing incidence of this disease and the lack of a global consensus for treating the unresectable patient, novel liver-directed therapies have been investigated with promising results. Within the last 5 to 10 years, image-guided endovascular therapies have revolutionized the treatment approach for patients with HCC. The guiding principle for targeting tumor through arterial channels is that cancer cells are principally perfused from an arterial source, whereas normal liver tissue is primarily supplied by the portal vein (5–7). This disparity has been successfully exploited by percutaneous transcatheter therapies and is the basis for selectively targeting hepatic neoplasm. Numerous investigators have shown survival benefit with the use of transarterial chemoembolization (TACE), transarterial embolization (TAE), radioembolization (RE), and drug-eluting beads (DEB). Common to all of these modalities is the induced cytotoxic insult to the tumor while concomitantly sparing normal hepatic parenchyma.

TECHNIQUE

Patients are selected for liver-directed therapy following a thorough medical examination. This evaluation includes a complete history and physical, assessment of laboratory values, and determination of baseline performance status. Patients must have adequate liver and renal function and a predicted life expectancy greater than three months for consideration. The overall therapeutic benefit to the patient is then ascertained and patients are either deemed appropriate candidates or excluded from therapy.

All transarterial therapies target liver malignancies through the arterial channels that perfuse them. As a result, angiographic studies are a necessary component of these therapies. The angiographic evaluation is conducted to assess visceral anatomy, identify anatomic variants, isolate the hepatic circulation, and determine the location of tumor(s) for therapeutic infusion(s) (8).

The angiographic evaluation is carried out in the following order: (*i*) abdominal aortogram, (*ii*) superior mesenteric arteriogram, and (*iii*) celiac angiogram. The abdominal aortogram is performed to assess the patency of the superior mesenteric and celiac arteries as well as assess for aortic tortuosity. The superior mesenteric arteriogram is performed to assess for variant arterial anatomy and the patency of the portal vein. A celiac angiogram is performed to evaluate the hepatic arterial vessels, identify additional vascular variants, and determine the tumor's blood supply.

To help mitigate the complications of nontarget delivery of the therapeutic, it may be necessary to prophylactically embolize vessels to isolate the hepatic arterial supply. This prevents the inadvertent deposition of the therapeutic to areas other than the neoplasm. The severe adverse events associated with unplanned administrations have been previously published (9–15).

Following the anatomic vascular assessment, tumor is targeted by whole-liver, lobar, or segmental approaches. Following therapy, patients are assessed for treatment-related complications. Depending on the device, patients are discharged the day of treatment (radioembolization) or admitted for overnight observation (TACE, TAE, DEB) and managed for post-embolization syndrome (PES). With the exception of RE, PES is a common manifestation of therapies that occlude tumor-feeding arteries (TACE, TAE, DEB).

TRANSARTERIAL CHEMOEMBOLIZATION

Introduction

Using percutaneous endovascular techniques, transarterial chemoembolization (TACE) is used to deliver potent anticancer drug(s) directly into tumor-feeding arteries. As a result, tumors are exposed to very high drug concentrations, while systemic exposure is minimized. Following chemotherapeutic infusion, embolizing agents are injected into target arteries to prevent drug washout and induce ischemic tumor necrosis. Commonly used embolizing agents include gelatin sponge (16), polyvinyl alcohol (PVA) (17), or autologous blood clots (18,19).

The chemotherapeutic agent(s) of choice includes doxorubicin alone or in combination with mitomycin C and cisplatin. Generally, the chemotherapy is emulsified in lipiodol to increase the intra-tumoral retention of the drug (20–22). Lipiodol is used as a vehicle to carry and localize the anticancer drugs within the tumor.

Patient Selection

TACE is generally contraindicated in patients with PVT, encephalopathy, or biliary obstruction. Relative contraindications include serum bilirubin (>2 mg/dL), lactate dehydrogenase (>425 U/L), aspartate aminotransferase (>100 U/L), tumor burden exceeding $>50\%$ of the liver, ascites, bleeding varices, thrombocytopenia, or cardiac or renal insufficiency.

Toxicities

Common treatment-related side effects include a well-characterized PES consisting of transient right upper quadrant abdominal pain, nausea, vomiting, fever, and elevated transaminases. This is usually self-limiting and resolves within 7 to 10 days. Less common, but severe, treatment-related toxicities have been reported with TACE. These include acute liver failure, liver abscess, acute cholecystitis, bile duct injury, renal dysfunction, gastrointestinal bleeding, and cardiac toxicity (23,24). The results from 37 studies and 2858 patients showed the overall treatment-related 30-day mortality was a median of 2.4% (0–9.5%) (16). Death was generally attributed to liver failure, acute renal failure, GI bleeding, tumor rupture, and sepsis. Treatment generally necessitates one to two days of in-house observation, although certain experienced centers have treated patients on an outpatient basis.

Investigations

Two landmark studies published in 2002 demonstrated a statistically significant survival advantage for patients treated with TACE versus less optimal therapeutic interventions (25,26). Llovet et al. reported on the survival outcomes in patients treated with fixed interval

(intention-to-treat) chemoembolization, particle embolization, and conservative measures (26). Survival outcomes for the three arms showed a survival benefit in stringently selected patients treated with chemoembolization and embolization compared with those treated conservatively. Within the same year, Lo et al. reported on a group of patients with unresectable HCC treated with TACE or best supportive care (25). The authors of this study concluded that TACE significantly improved survival in select patients with unresectable HCC. A recent meta-analysis of seven published randomized controlled trials concluded that TACE was an effective palliative treatment modality for unresectable HCC (27).

In a phase III trial enrolling 291 unresectable HCC patients, Cheng et al. randomized patients to TACE ($n = 95$), RFA ($n = 100$), and TACE/RFA ($n = 96$). Patients were required to meet very stringent inclusion criteria: (*i*) unresectable; (*ii*) <3 tumors, all between 3.5 to 7.0 cm in longest diameter; (*iii*) lesions > 0.5 cm away from the hepatic hilum or gallbladder; (*iv*) no previous treatment for HCC. Exclusion criteria included Child-Pugh C disease, >75 years, PVT, extrahepatic metastases, infiltrative tumor(s), gastrointestinal bleeding within the past month, refractory ascites, encephalopathy, renal failure, arterial access contraindications (platelet count $<60 \times 10^3/\mu L$, prothrombin <60%), allergic reactions to platinum, creatinine $\geq$ 1.36 mg/dL, or end-stage tumor disease. Toxicities common to all three groups were (*i*) fever, pain, and vomiting; (*ii*) serum elevations of AST, ALT, and bilirubin; and (*iii*) ascites, gastrointestinal hemorrhage, encephalopathy, pleural effusion, liver abscess, and spontaneous bacterial peritonitis. Both cholecystitis and inguinal hematoma were observed in the TACE and TACE/RFA groups, whereas tumor seeding and skin burns were observed in the RFA and TACE/RFA groups. Disease recurrence was observed in 80%, 81%, and 59% of patients in the TACE, RFA, and TACE/RFA groups. Median survival for TACE, RFA, and TACE/RFA were 24, 22, and 35.8 months, respectively. Survival rates were significantly higher in the TACE/RFA group compared with either TACE or RFA individually.

DRUG-ELUTING BEADS

Introduction

Drug-eluting beads (DEB) represent an innovative and novel method of enhancing the delivery of potent anticancer agents at the site of tumor using transarterial techniques. The unique properties of beads allow for fixed dosing and the capability to release the anticancer drugs in a sustained and controlled manner. Significant reductions in peak plasma concentrations have been reported with DEB when compared with conventional TACE. Investigators have shown a greater amount of the anticancer agent being sequestered by the tumor versus distributing in the systemic circulation (28).

The microspheres are composed of PVA polymers modified with sulfonate groups. By an ion exchange process the sulfonate groups actively sequester doxorubicin from solution (29). The reported plasma concentration of the drug remains at a level that is both steady and lower than traditional TACE (28).

Investigations

Varela et al. assessed the applicability, safety, and efficacy of DEB in a study consisting of 27 patients (30). PES was observed in 41% and 18% of patients following the first and second treatment, respectively. Severe treatment-related adverse events included liver abscesses in two patients, one of whom expired. An objective tumor response of 67% was reported using the EASL (European Association for the Study of the Liver) and AASLD (American Association for the Study of Liver Diseases) guidelines.

Investigators reported the early results of 71 patients prospectively enrolled and treated segmentally with doxorubicin-loaded beads (31). All patients had underlying cirrhosis and Child-Pugh A or B disease. Complete and partial responses at 24 months were 16% and 66% using EASL necrosis guidelines, respectively. Survival at 30 months was 88%. All patients reported varying degrees of PES. Severe adverse events included liver abscess, cholecystitis, and pleural effusion. The authors concluded that DEB was a safe and effective treatment option in patients not eligible for curative surgical treatments with high rates of response.

The use of DEB over traditional TACE represents a new paradigm shift. The published data shows both increased intra-tumoral retention and decreased systemic bioavailability of drug in patients treated with DEB when compared with TACE. Although this technique is relatively new, the efficacy of this therapy appears promising (30,32,33). Further confirmatory studies and randomized controlled trials are necessary to fully elucidate the safety and efficacy of this new and evolving therapy.

TRANSARTERIAL EMBOLIZATION

Introduction

Transarterial embolization (TAE), commonly referred to as "particle" or "bland" embolization, is the administration of microparticles with the intent of completely occluding tumor-feeding arterioles. The concept of inciting tumor necrosis and cell death by severing its blood supply was first introduced in the 1950s (34). Although several embolizing agents exist, gelatin sponge particles, PVA, and acrylic copolymer gelatin particles are the commonly used occluding agents.

Patient Selection

Patients with Child-Pugh C disease, significant biliary obstruction, and a tumor burden exceeding 50% of the hepatic volume are generally excluded from therapy. Additional exclusionary criteria include uncontrolled liver disease (gastrointestinal bleeding, encephalopathy), liver compromise, bilirubin values > 2.0 mg/dL, and arterial access contraindications (platelet count $<70{,}000/\text{mm}^3$, prothrombin $<50\%$) (35,36).

Toxicities

Varying degrees of PES occurs in nearly all patients following TAE. The extent of PES is generally related to the size of the particles and extent of the liver embolized. Other treatment-related complications have included arterial dissection, bacteremia, cardiac arrhythmia, myocardial infarction, pulmonary edema, and hepatic decompensation (35,37). Nontarget embolization has resulted in cholecystitis, pancreatitis, and skin changes.

Investigations

Marelli and colleagues compared the outcomes of TAE versus TACE from three randomized controlled trials (16). The meta-analysis failed to demonstrate a significant survival difference between the two modalities in all studies, suggesting the observed tumor response and survival benefit observed with TACE may have been the result of ischemia rather than the effects of chemotherapy (26,38,39).

Forty-five patients that had recurrence of disease following resection were treated with TAE (40). The median number of embolizations per patient was 3.2. The median length of hospital stay was 3.2 days following therapy. Median follow-up was 34 months in patients that were alive. Median overall survival was 46 months with one-, two-, and five-year actuarial survivals of 86%, 74%, and 47%, respectively. Complications included a myocardial infarction culminating in death. Five grade 3 complications were reported following therapy: duodenitis ($n = 2$), pancreatitis ($n = 1$), and external iliac dissection ($n = 2$). The authors concluded that TAE following disease recurrence was an effective method of salvage therapy for patients with good liver function.

A retrospective chart review of 46 patients treated with PVA was conducted by Brown et al. (41). The heterogeneous cohort underwent 86 TAE sessions in which 81% developed PES post-procedurally. Three severe adverse events were reported: one patient had a splenic infarct and two others had transient hepatic failure. Overall actuarial survivals were 50% at one year and 33% at two years. The authors concluded that treatment with TAE was well tolerated with favorable survival outcomes.

In a retrospective study, investigators reported on 36 patients with unresectable HCC that were treated with cyanoacrylate and lipiodol (35). The investigators reported PES consisting of fever, nausea, and/or abdominal pain in all treated patients. One patient had

treatment-related hepatic failure and was successfully resuscitated from a ventricular arrhythmia. One patient reportedly had a cerebral infarction. One patient with end-stage disease and segmental PVT died within 30 days of therapy from hepatic failure. Median survival was 26 months from the time of diagnosis. The authors concluded that these agents were feasible in treating unresectable patients with beneficial effects on survival.

In a study that included 40 patients with solitary lesions up to 7 cm, Maluccio et al. compared the survival outcomes of resection versus TAE/ablation (42). Patient baseline demographics were not significantly different between the two groups. There were, however, more Okuda II patients in the TAE/ablation group ($p < 0.001$). A median recurrence-free survival was longer in the surgical group. The median follow-up was 23 months. The one-, three-, and five-year actuarial survival rates for the TAE/ablation group were 97%, 77%, and 56% versus 81%, 70%, and 58% for the surgical group. The difference in survival between the two studies did not reach statistical significance ($p = 0.200$). The authors concluded that combination modality (TAE/ablation) was as effective as resection in treating focal lesions up to 7 cm.

Over an eight-year period, the therapeutic outcomes from 322 TAE-treated patients were reported by Maluccio et al. (37). All patients were treated using a standardized method with the intent of occluding distal tumor–feeding arteries. Repeat treatments were carried out for lesions demonstrating continued viability following treatment and for newly developed tumors. There was a 2.5% 30-day peri-procedural mortality. Grade 3 to 4 complications included vascular dissections ($n = 3$), myocardial infarctions ($n = 3$), and contrast-induced nephropathies ($n = 9$). Toxicities related to nontarget embolization included cholecystitis ($n = 3$), pancreatitis ($n = 3$), and liver decompensation ($n = 9$). The median follow-up was 20 months. The median survival for the entire cohort was 21 months from the time of first treatment. The one-, two-, and three-year survivals were 66%, 46%, and 33%, respectively. The authors concluded that embolization with particles was effective in treating unresectable HCC and that particles alone may be the critical component of intra-arterial embolotherapy.

RADIOEMBOLIZATION

Introduction

External beam radiation has historically played an inconsequential role in patients with liver malignancies as a result of normal hepatic tissue demonstrating radiosensitivity (43). With radiation doses exceeding 35 Gy, a syndrome characterized by anicteric ascites, hepatomegaly, and elevated liver enzymes has been shown to develop weeks to months following therapy (43,44). As a result of this limitation and the need for tumoricidal doses, radiation therapy has been successfully delivered via an arterial route directly to the site of the tumor. Radiation doses as high as 150 Gy have been delivered without the complications seen with external beam therapy (45–48).

Radioembolization (yttrium-90, rhenium-188) refers to the administration of radioactive microspheres directly into the tumor-feeding arteries. Although this was first introduced in the early 1960s (49), only within the last decade has this therapy gained widespread awareness and usage (50).

Unlike the other intra-arterial modalities, RE necessitates a pretreatment nuclear scan with a radioactive tracer: technetium-99-labeled macroaggregated albumin. This scan helps determine the percentage of hepatopulmonary shunting and gastrointestinal flow in a given patient. In the event a shunt to the gastrointestinal tract is identified, coil embolization of the vessel mitigates the risks associated with nontarget radiation.

Following the nuclear scan, computed tomography (CT) or magnetic resonance imaging (MRI) is used to calculate the dosage required for treatment. Microsphere administration varies according to the type, size, and number of microspheres delivered per treatment.

Patient Selection

RE is contraindicated in patients with exaggerated hepatopulmonary shunting and/or non-correctable gastrointestinal flow. Patients are generally excluded from therapy if a single

treatment would result in ≥30 Gy of radiation to the lungs or >50 Gy over multiple sessions (51,52). Additionally, a patient is contraindicated from treatment if nontarget gastric radiation is an inevitable consequence of therapy that cannot be corrected by coil embolization. As with all other intra-arterial therapies, relative contraindications include compromised pulmonary function, inadequate liver reserve, serum creatinine >2.0 mg/dL, and a platelet count $<70{,}000/mm^3$.

Toxicities

Common clinical toxicities associated with the use of Y90 include a post-radioembolic syndrome consisting of vague abdominal pain, fever, and fatigue (53–55). Adverse events that have been reported with Y90 include cholecystitis, pleural effusion, pancreatitis, gastroduodenitis, gastric ulceration, radiation pneumonitis, radiation hepatitis, and hepatic fibrosis and portal hypertension (54,56–62).

Investigations

Carr et al. reported on 65 patients with biopsy-proven HCC and treated with a median dose of 134 Gy of Y90 microspheres (63). Toxicities included vague abdominal pain, cholecystitis, and elevated liver enzymes in 9, 2, and 25 patients, respectively. Seventy-five percent of patients demonstrated lymphopenia without clinical sequelae. Median survivals were 21.6 and 10.1 months for Okuda I (65%) and Okuda II patients (35%), respectively.

In a small study, investigators reported on the safety of Y90 in 15 patients with imaging proven PVT (64). There were no serious adverse events related to treatment. Two patients continued to progress despite treatment. Eight patients had stable disease or improved liver function. This was the first study to demonstrate the safety and efficacy of Y90 for treating patients with PVT.

Investigators reported on the safety and efficacy of Y90 in which 37 of 108 patients had documented PVT (65). Adverse events included elevations in serum bilirubin (40%), ascites (18%), and hepatic encephalopathy (4%) in the majority who had both main PVT and cirrhosis. Median survival for patients without PVT was 27.1 months. Patients with cirrhosis, PVT, and both PVT and cirrhosis had median survivals of 12.8, 4.5, and 3.4 months, respectively. The authors concluded that PVT and/or cirrhosis did not increase the risk of liver failure in HCC patients treated with Y90.

Geschwind et al. reported on 80 patients treated with Y90 microspheres using a segmental, regional, and whole liver approach (66). Patients were stratified according to Child-Pugh, Okuda, and Clip scoring systems. Median survivals for Okuda I (68%) and Okuda II (38%) were 20.1 and 10.8 months, respectively.

In a retrospective review, Goin et al. reported on 121 patients, with advanced disease, treated with Y90 (67). The cohort consisted of 57%, 39%, and 23% Okuda I, II, and III patients, respectively. Liver-related toxicities were observed in 15 patients. Adverse events included radiation pneumonitis ($n = 1$) and gastrointestinal bleeding ($n = 1$).

Salem et al. reported on the safety, tumor response, and survival outcomes of 43 consecutive patients treated with Y90 (53). An objective tumor response was observed in 47%. Median survivals for low- and high-risk patients were 20.8 and 11.1 months, respectively. The authors reported no procedure-related life threatening events.

Sangro et al. reported on 24 Child-Pugh A patients treated with Y90 RE. The authors observed tumor reduction in 19 patients (68). There were no cases of PES and all patients were discharged within 24 hours of treatment. The authors reported two fatal events attributable to therapy. None of the patients had progressed at 12.5 months of therapy.

Kulik et al. recently described a 35-patient cohort consisting of patients who were not candidates for transplantation, resection, or RFA that were treated with Y90 RE (69). Sixty-six percent of these patients were successfully downstaged to transplantation, resection, or RFA. Eight patients were transplanted and one patient underwent resection. Five of seven explants in the transplanted patients demonstrated complete necrosis by pathologic examination. Tumor partial response rate was 50% by WHO criteria. Median time to partial response was 75 days, while median time to maximum response was 120 days. One-, two-, and three-year survivals were 84%, 54%, and 27%, respectively. Median survival for the entire cohort was 800 days.

Investigators also studied the use of Rhenium-188 (Re-188) for patients with inoperable HCC (70). A multicenter clinical trial was completed looking at Re-188 lipiodol delivered in a transarterial fashion. Ninety-three patients were successfully treated with a mean age of 53 years. Mean cumulative dose was 7.8 GBq. Forty percent of patients had more than three lesions and in 50% of patients, tumor was either unilateral, occupying 50% or more of the liver, or bilateral. There was portal vein thrombosis in 38% of patients, Child-Pugh B disease in 37% of patients, and Okuda stage II or III disease in 50% of patients. Treatment was well tolerated. Median survival for the entire cohort was 11.9 months. The authors concluded that this was a safe, effective, and promising therapy in patients with HCC.

Numerous phase II studies have demonstrated the safety and efficacy of RE for the treatment of unresectable HCC. There exists, however, a strong need to carry out controlled investigations (phase III) to validate the results of these and other radioembolic studies.

CONCLUSION

Treatment of HCC is a challenge, given that most tumors occur in the setting of underlying liver disease. Even in patients with varying degrees of liver dysfunction, intra-arterial therapies have gained widespread recognition as promising therapeutic tools in treating this otherwise uniformly fatal disease. The unique aspects of these therapies are the minimal toxicity profiles and highly effective tumor responses compared with conventional systemic therapies.

As the delivered agent becomes more efficacious (drugs, radiation, ischemia), the anticipation is that this will result in higher treatment responses and survival benefits. The advent and rapid adoption of cytostatic-targeted therapies (e.g., Raf kinase inhibitors) represent a new and novel method of treating the unresectable patient. Clinical investigations into combining the effects of these cytostatic therapies with the cytotoxic effects of intra-arterial therapies are currently underway and the results from these studies may provide important clinical data that may translate into enhanced clinical outcomes and overall survivals.

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11 Liver Resection

Kiyoshi Hasegawa and Norihiro Kokudo

Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Masatoshi Makuuchi

Department of Digestive Surgery, Japanese Red Cross Medical Center, Tokyo, Japan

INTRODUCTION

Although remarkable progress has been made in the treatment of hepatocellular carcinoma (HCC), local control is still the most important consideration. Liver resection is now established as the first-line therapeutic modality for local control of a primary HCC, including cases with intrahepatic metastasis. Liver transplantation, percutaneous ablation [ethanol injection, microwave coagulation, and radiofrequency ablation (RFA)], and transcatheter arterial chemoembolization (TACE) are the second-line choices of treatment for HCC, but liver function is well preserved; liver resection should be considered first. In this chapter, we summarize current role of liver resection in treatment for HCC.

INDICATIONS

The indications for liver resection and the selection of procedure are primarily governed by liver function. Accurate preoperative evaluation of liver function is of utmost importance to preventing postoperative liver failure, the most critical complication following liver resection. The Child-Pugh score and classification are popular in the West, but they provide only a rough method of evaluating liver function. In the Far East, the indocyanine green retention rate at 15 min (ICG R15) is regarded as an important method for accurate evaluation (1,2). Makuuchi's criteria for deciding indications for liver resection and selecting surgical procedure, consisting of the ICG R15 value, ascites, and jaundice, are widely accepted, especially in Japan (Fig. 1) (3). The maximal volume of the liver that can be resected is estimated, and the surgical procedure is chosen based on the volume.

SHORT-TERM OUTCOME

The safety of liver resection is now established. The 17th report of the Liver Cancer Study Group of Japan states that the operative mortality of patients who underwent liver resection for HCC was 0.8% (4,5). Some high-volume centers from the Far East have reported zero mortality (6,7). The following four points are important to achieve safe liver resection: (*i*) accurate preoperative evaluation of liver function, as mentioned above, (*ii*) accurate and appropriate setting of a division plane based on the findings of intraoperative ultrasonography (IOUS), (*iii*) intermittent inflow occlusion to minimize blood loss, and (*iv*) accurate preoperative estimation of liver volume to be resected.

IOUS was first introduced in the field of liver surgery in the late 1970s by Makuuchi (8,9), and enables demonstration of the location of tumors and anatomic structures that could not be visualized before the introduction of IOUS. IOUS has markedly improved the safety of liver resection, because it allows the surgeon to avoid injuring major vessels. IOUS has also enabled new liver resection procedures, such as inferior right hepatic vein-preserving hepatectomy (10) and the ultrasonically guided subsegmentectomy (11). IOUS has been and will continue to be indispensable to liver surgery.

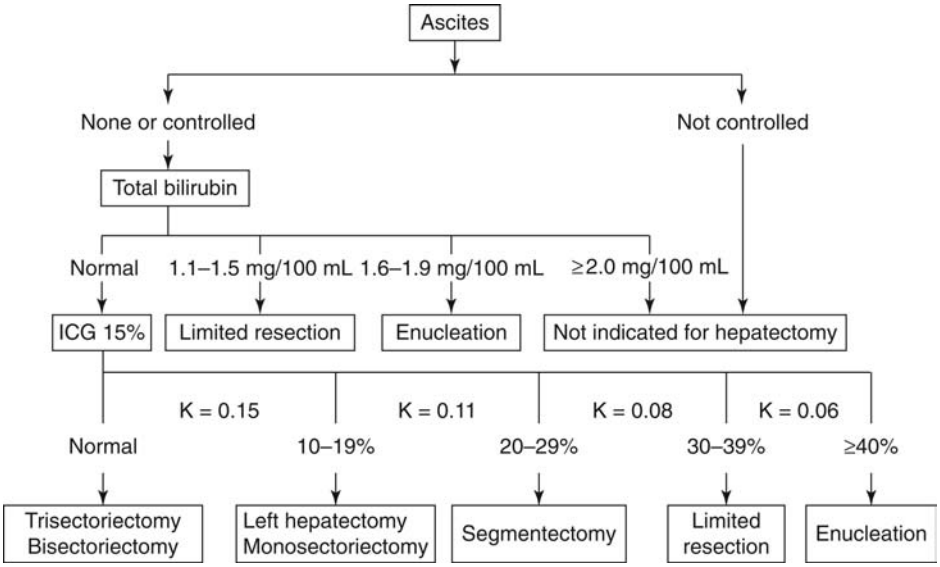

Figure 1 A decision tree for selection of operative procedures in patients with hepatocellular carcinoma. *Source*: From Ref. 3.

Because ischemia reperfusion injury was earlier regarded as critical in a damaged liver, liver parenchyma was divided with neither inflow nor outflow occlusion. Thus, liver resection was inevitably associated with loss of a large volume of blood, which sometimes led to liver failure and death. In the early 1980s, the hemi-hepatic vascular occlusion method (12) was devised, and the total inflow occlusion (Pringle's maneuver) (13) came to be widely applied. These inflow occlusion methods contributed to a marked reduction in blood loss. Total vascular exclusion was performed for a time (14–16), but because of the complicated procedure and postoperative liver dysfunction, it is rarely used today (17). Clavien et al. found that 10 minutes of ischemic preconditioning by inflow occlusion significantly improves postoperative liver function (18). Imamura et al. showed that a combination of preconditioning and intermittent inflow occlusion lowered serum transaminase levels, even during recovery of liver grafts in living-donor liver transplantation (LDLT) (19). The superiority of intermittent inflow occlusion over continuous or total occlusion is now widely accepted.

Resection of a liver volume greater than the estimated permissible volume increases the risk of postoperative liver failure. Thus, accurate preoperative evaluation of liver volume to be resected and preserved is also important, and volumetry by computed tomography is a useful method of making the estimates (20). If the liver volume to be resected exceeds a permissible volume determined by the Makuuchi's criteria (Fig. 1) (3), portal vein embolization should be considered as a preparatory procedure to induce hypertrophy of the future remnant liver (21,22). In HCC patients, this procedure is usually performed after TACE to promote the hypertrophy process and to prevent tumor growth while waiting for hypertrophy to occur (23). This portal vein embolization technique has contributed to the safety and the expansion of the indications of liver resection for HCC.

DEVELOPMENT OF NEW SURGICAL TECHNIQUES AND DEVICES

The continuous attempts and endeavors of liver surgeons have led to the development of the various surgical techniques and devices described below that have improved the short- and long-term results of liver resection for HCC.

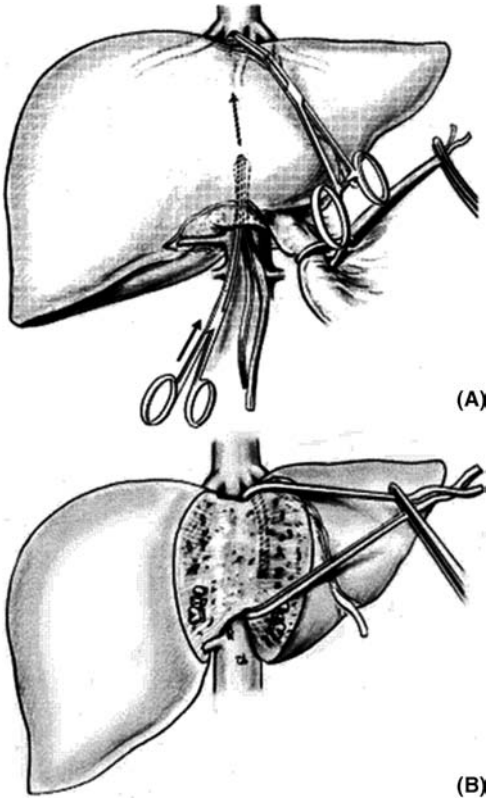

Figure 2 Procedures of the hanging maneuver. **(A)** The forceps are inserted in front of the inferior vena cava from the caudal side of the liver. **(B)** The tape placed between the liver and the inferior vena cava is lifted up during liver parenchymal transection. *Source*: From Ref. 24.

Hanging Maneuver

In the hanging maneuver proposed by Belghiti (24), surgical tape is inserted between the liver and the anterior surface of the inferior vena cava (Fig. 2A), and lifting the tape allows the liver to be suspended during division of the liver parenchyma (Fig. 2B). Compression of the liver parenchyma reduces blood loss, and makes it easy to determine the proper direction of division of the liver parenchyma. The hanging maneuver can omit mobilization procedure of the right liver during right hemi-hepatectomy, especially for a huge liver tumor. This maneuver is useful, in spite of the risk of injuring the short hepatic veins, and it has been applied to liver transection for left hepatectomy (25), left caudate lobectomy (26), and graft recovery in LDLT (27).

Laparoscopic Liver Resection

Laparoscopy currently plays a major role in the field of abdominal surgery as a means of achieving minimal invasiveness. Although laparoscopic liver resection is associated with the risk of blood loss and air embolism, it has been performed aggressively (28–30). The laparoscopic approach is safe and clinically useful for a small tumor (<3 cm in diameter) located in the periphery of the liver (28,30), e.g., in the left or right lateral sector (31).

New Surgical Devices

Various surgical devices for liver parenchyma division have been developed, e.g., an ultrasonic dissector, water jet, dissecting sealer, and vessel-sealing system, to reduce intraoperative blood loss during liver resection. In a historical control study, Fan et al. found that introduction of the ultrasonic dissector markedly reduced blood loss (32), whereas in a randomized controlled trial (RCT) Takayama et al. showed that the quality of the operation by the conventional clamp

crushing method was superior to the quality of the operation performed with the ultrasonic dissector, in spite of similar blood loss (33). Both positive and negative opinions have been advanced regarding the dissecting sealer (34,35). LigaSure® vessel-sealing system has been reported to be useful for decreasing blood loss and operation time (36,37), but the results of the latest RCT indicated little clinical benefit (38).

Hepatic Venous Reconstruction

The clinical significance of the volume of liver congestion has recently been assiduously investigated, especially in operations of a donor and recipient in LDLT (39), and criteria for judging whether congestion is present have been established (40). Because the congested portion of the liver will atrophy and become nonfunctional at some stage (39), adequate reconstruction of the hepatic vein should be performed to guarantee maximal functional liver volume after LDLT. Various techniques of reconstruction using vein auto- and allografts have been proposed (41,42), and hepatic venous reconstruction is also recommended if the functional volume of the residual liver after liver resection for a malignant tumor is inadequate. In most patients with an HCC that is likely to undergo expansive growth, it is easy to detach the HCC from the hepatic vein, even when they are tightly adhered to each other. However, if the HCC is the mixed or sclerosed type, detachment may be difficult because of tumor invasion. Reconstruction techniques that use an autologous vein graft obtained from the resected liver specimen (Fig. 3) (43) are useful in such cases, as reported for metastatic liver tumors (44).

Preoperative Simulation in Liver Resection

Three-dimensional (3-D) virtual hepatectomy simulation software has recently been developed, and it enables accurate preoperative recognition of the anatomic relationships between tumors and vessels in the liver (45,46). Three-dimensional simulation based on data obtained by multidetector row computed tomography is useful for planning appropriate procedures of liver resection (Fig. 4) (46,47) and graft recovery in LDLT (Fig. 5) (48). It is also effective for preoperative estimation of the volume to be resected and preserved (49).

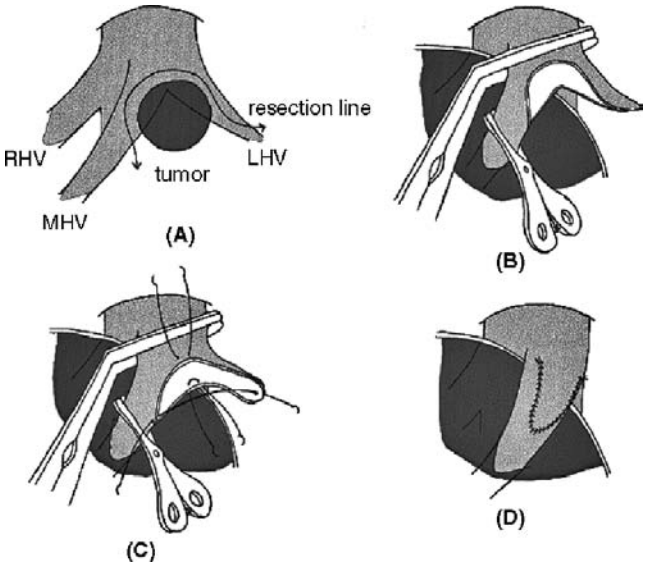

Figure 3 Rotating left hepatic vein flap technique. **(A)** The tumor has invaded the middle and left hepatic veins. The resection line of the hepatic veins is determined after transection of the liver parenchyma and exposure of the venous wall. **(B)** En bloc resection of the tumor and the hepatic venous wall is accomplished. **(C,D)** The venous wall is closed using a continuous suture. *Source*: From Ref. 43.

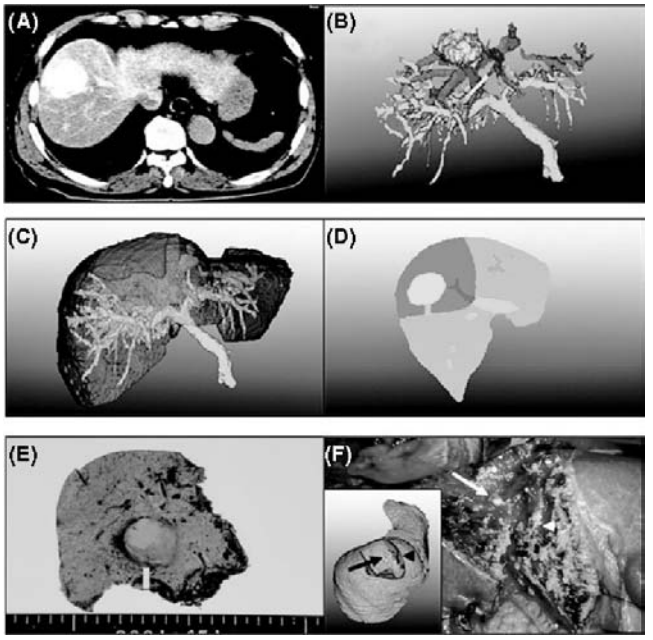

Figure 4 Preoperative hepatectomy simulation in HCC in Couinaud's segment 8. **(A)** Axial CT arteriography. **(B)** Coronal view of an integrated 3-D image. The segment 8 branch (P8) was identified as a tumor-bearing portal branch. Note the clipping point (*bar*) at the proximal origin of P8. **(C)** Clipping of P8 prompted volumetric calculation of the corresponding portal perfusion area for segment 8 resection. **(D)** Predicted coronal section showing estimated resection margin (*bar*). The liver resection area is shown. **(E)** Resected specimen showing actual margin (*bar*). **(F)** Operative findings. The liver cut surface after segment 8 resection showed the P8 stump (*white arrow*) and middle hepatic vein (*white arrowhead*). Inset: The predicted cut surface showed the corresponding P8 stump (*black arrow*) and middle hepatic vein (*black arrowhead*). *Source*: From Ref. 46.

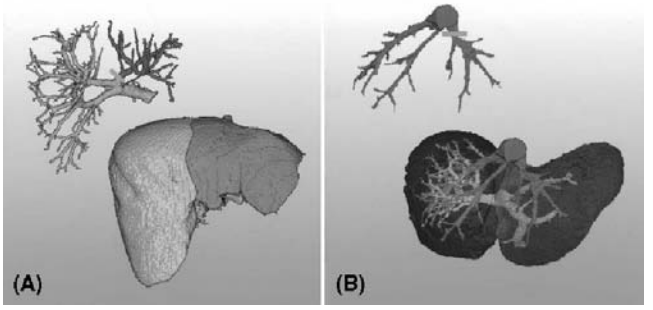

Figure 5 Concept of preoperative simulation based on hepatic circulation. **(A)** Simulation for left lobe graft is shown as an example. Clipping of the left portal branch prompted volumetric calculation of the proposed liver graft. **(B)** The drainage area of the left hepatic vein was calculated by clipping of the corresponding vein at its origin. *Source*: From Ref. 48.

LONG-TERM OUTCOMES

Overall, 3-, 5-, and 10-year survival rates after liver resection for HCC of 70.5%, 54.6%, and 28.9%, respectively, have been reported (4,5), and they are regarded as acceptable, but the high incidence of recurrence is a serious problem. Even after curative resection, the incidence of recurrence at 3 and 5 years has been shown to be about 50% to 60% and 70% to 100%, respectively (50–55), and in 80% of the cases the recurrence is in the liver. HCC is characterized by intrahepatic dissemination of cancer cells through the portal venous system (Fig. 6A) (11). Anatomic resection, in which the entire segment containing the tumor-bearing portal branches is completely and systematically removed (Fig. 6B) (11), is desirable to prevent recurrence via the above-mentioned pathway. Recent retrospective studies (55–59) of HCC have shown that anatomic resection is superior to nonanatomic resection for a single tumor (Fig. 7) (57). As long as liver function is adequate, anatomic resection is strongly recommended as the surgical procedure for HCC.

How to cope with intrahepatic recurrences is another important consideration in the treatment of HCC. The choices for treating recurrence are the same as for primary HCC, namely, liver resection, transplantation, percutaneous ablation, and TACE. Because the

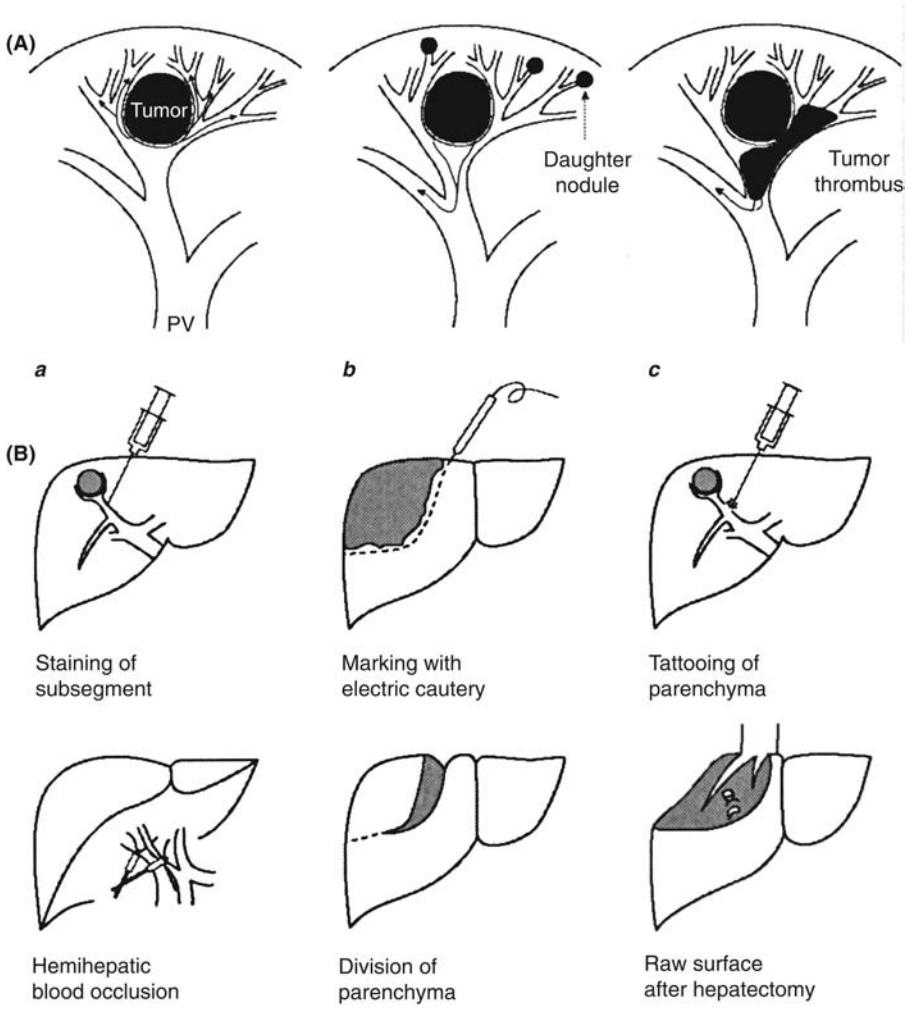

Figure 6 Anatomic resection for HCC. **(A)** Schema of intrahepatic spread of HCC. (*a*) An HCC has invaded the nearby portal vein branches, and tumor cells are delivered to the periphery. (*b*) Tumor cells form microscopic tumor thrombi then metastasize intrahepatically. (*c*) Tumor thrombi become a source of wider disease dissemination. **(B)** Procedures of anatomic resection for HCC. *Abbreviations*: HCC, hepatocellular carcinoma; PV, portal vein. *Source*: From Ref. 11.

three-year survival rate after repeat resection has been shown to be acceptable (about 80%), liver resection remains the first-line therapy (54,55). The indications for repeat liver resection and the surgical procedure to select are based on the same criteria as for the initial operation, i.e., assessment of liver function and tumor-related factors.

ATTEMPTS TO TREAT ADVANCED HCC

Liver resection is the only hope of cure for advanced HCC, such as HCC associated with vascular invasion and large tumors. Vascular invasion, including portal vein tumor thrombus (PVTT), is the most unfavorable prognostic factor in patients with HCC. Neither liver transplantation nor percutaneous ablation is indicated in cases of advanced HCC associated with PVTT. However, if liver function is well preserved (ICG R15 $< 20\%$), a combination of TACE and subsequent liver resection has provided a satisfactory survival of 42% at 5 years (60).

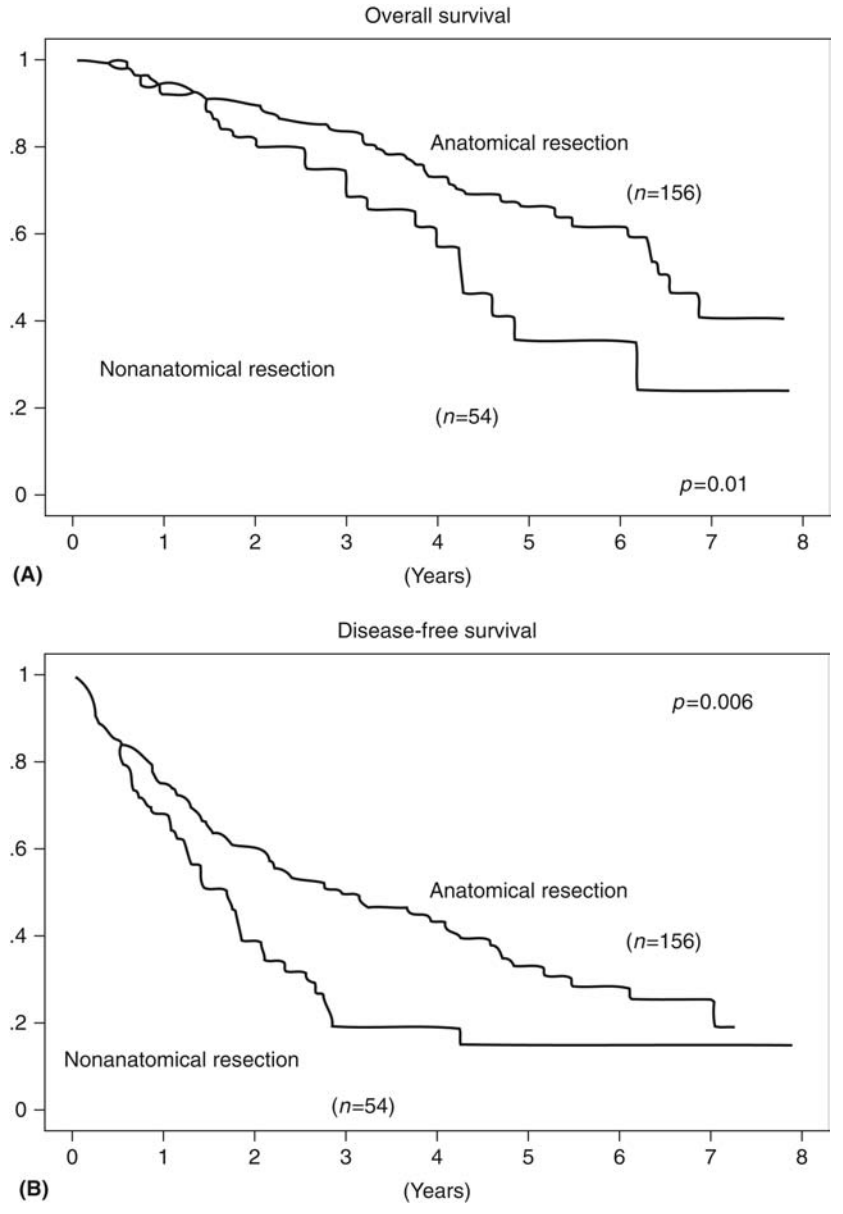

Figure 7 Long-term results after anatomic and nonanatomic resections for hepatocellular carcinoma. **(A)** Overall survival rates of the patients undergoing anatomic and nonanatomic resections for a solitary HCC. The survival rate after the anatomic resection group is significantly better than the nonanatomic group (p = 0.01). **(B)** Recurrence-free survival rates of the patients undergoing anatomic and nonanatomic resections for a solitary HCC. The survival rate after the anatomic resection group is significantly better than the nonanatomic group (p = 0.006). *Source*: From Ref. 57.

Even if the PVTT extends into the main and/or contralateral portal vein, hemi-hepatectomy with removal of the PVTT may improve the prognosis (61).

Liver surgeons have also challenged to treat for large HCCs (62–66). Especially for large HCCs greater than 10 cm in diameter without vascular invasion, 3- and 5-year survival rates of 51.1% and 38.2%, respectively, have been reported after liver resection (67). According to the report of Pandey et al., in the absence of vascular invasion, cirrhosis, or multiplicity, the 5-year survival rate was 58% in large HCC cases, which was acceptable (68). Liver resection should be aggressively pursued in cases of advanced HCC.

ADJUVANT THERAPY

Several adjuvant therapies have been advocated to reduce the high rate of recurrence after liver resection. Because intrahepatic metastasis and second primary carcinogenesis are in theory regarded as the major pathways of HCC recurrence (53), adjuvant therapy for HCC should target one or the other or both of them.

Adoptive immunotherapy (69) and TACE with ^{131}I-labeled iodized oil (70) have been confirmed by RCTs as effective means of preventing intrahepatic metastasis. However, they have not been widely adopted, perhaps because of the difficulty of applying them clinically. Anti-cancer drugs are another possible choice for adjuvant therapy to prevent recurrence, but oral uracil-tegafur has been confirmed to have negative impact on overall survival rather than to be ineffective in preventing recurrence (71). Until now, no clinically useful adjuvant therapy for HCC is established. Sorafenib, which is effective for an advanced HCC (72), is now expected as a hopeful adjuvant.

Interferon therapy (73) and retinoid therapy (74) have also been confirmed by RCTs as effective in preventing second primary carcinogenesis. Although they are not used routinely, recent RCTs have suggested that interferon may be effective as adjuvant therapy for HCC associated with infection by HBV (75) and HCV (76).

COMPARISON WITH OTHER TREATMENTS

It is still difficult to select the best therapy for HCC according to various tumor and liver function–related factors. The results of a nationwide survey conducted by the Liver Cancer Study Group of Japan indicated that liver resection is superior to percutaneous ablation and TACE (4,5,77). Although its superiority has not been confirmed by an RCT, if the liver function is well preserved, liver resection should be considered as the treatment for HCC first. Patients whose liver function is insufficient to undergo liver resection and whose carcinoma is not so advanced (i.e., within the Milan criteria) (78) may be good candidates for liver transplantation, unless older than 60 years. Percutaneous ablation (ethanol injection, microwave coagulation,

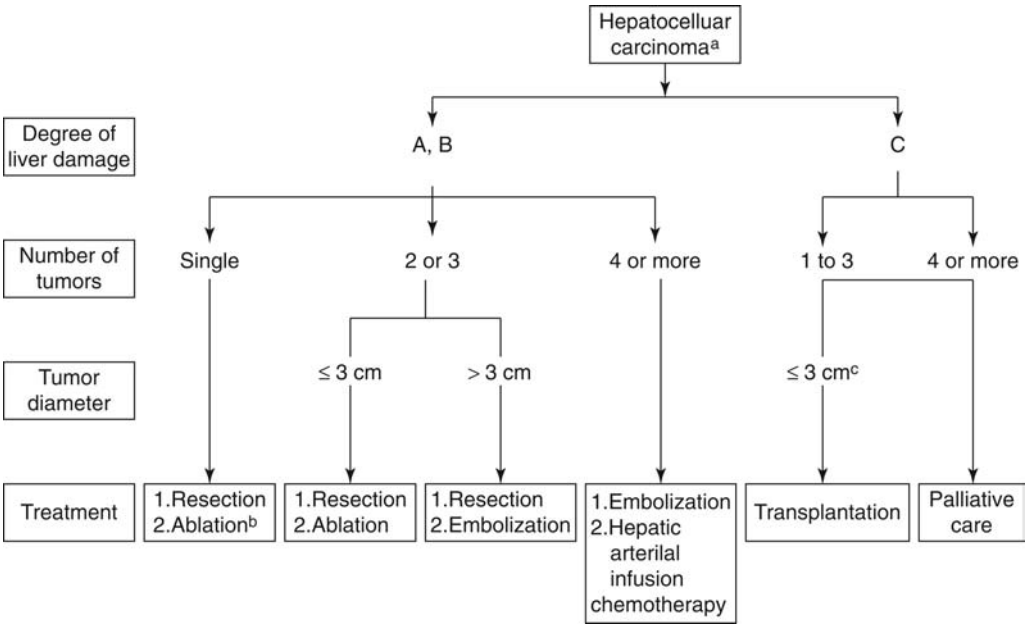

Figure 8 Treatment algorithm for hepatocellular carcinoma. [a]Presence of vascular invasion or extrahepatic metastasis to be indicated separately. [b]Selected when the severity of liver damage is class B and tumor diameter is $\leq$2 cm. [c]Tumor diameter is $\leq$ 5 cm when there is only one tumor. *Source*: From Refs. 83 and 84.

and radiofrequency ablation) may be considered in HCC patients who are suitable for neither resection nor transplantation. Radiofrequency ablation is regarded as the ablation procedure of first choice on the basis of the results of the latest RCTs (79). Although TACE is inferior to resection and ablation for local control of HCC, it has the great advantage that it can be performed in patients with poor liver function and/or multiple tumors in the both liver lobes. The results of both RCTs that compared surgery and ablation (80,81) suggested the similar clinical efficacy; however, there were critical defects in the study designs of both RCTs (82). No suggestions are ever definite.

Several different treatment algorithms are available, perhaps because of differences in social conditions, medical levels, and systems in different countries. The algorithm (Fig. 8) (83,84) from Japan recommends liver resection as the first-line treatment for HCC with three or fewer nodules, regardless of tumor size, if the degree of liver damage is grade A or grade B. The guidelines of the European and American associations for the study of the liver, on the other hand, recommend liver resection only for a single HCC without portal hypertension (85,86). However, a recent report from Japan showed that the overall survival rate of 58% after liver resection for HCC with multiple tumors and/or portal hypertension was satisfactory, if the liver function was classified as Child-Pugh A (87). The indication of liver resection for HCC can be extended over the recommendation by the European and American guidelines on the basis of recent improvements in surgical techniques and outcomes.

CONCLUSION

Liver resection has played and will continue to play a central role in the treatment of HCC. Surgeons will constantly endeavor to achieve further improvement in the short- and long-term outcome of surgery for HCC in the future.

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12 Liver Transplantation as Treatment for HCC

Richard B. Freeman
Division of Transplantation, Tufts Medical Center, Boston, Massachusetts, U.S.A.

INTRODUCTION

Surgery has remained the primary treatment for hepatocellular cancer (HCC) for over 50 years. However, since underlying cirrhosis, particularly cirrhosis caused by viral disease, is frequently associated with HCC, surgical resection is often limited by a lack of hepatic reserve and complications of portal hypertension. Thus, in most cases surgery can be applied to fewer than 25% of cases (1). It is for this reason that pioneering liver transplant surgeons began exploring the use of liver transplantation (LT) as the treatment for HCC in the late 1960s. The transplant option had several theoretical advantages because (*i*) it offered the potential to completely excise large tumors with adequate margin without being limited by tumor proximity to vital vascular or biliary structures, (*ii*) it replaced the diseased liver, thereby eliminating the need to leave adequate hepatic reserve, and (*iii*) it removed the entire liver, which otherwise would remain at risk for malignant transformation after partial hepatic resections. However, early results reported by groups from Germany (2), France (3), and the United States (4) and others were not encouraging as recurrence rates were very high. These initial dismal results discouraged transplant programs from pursuing LT for HCC for several years.

Enthusiasm was rekindled though, with the publication of reports from the Milan group indicating that the recurrence rates were quite low and post-transplant graft and patient survival were excellent when patients with early-stage HCC were selected for LT (5). The selection criteria employed by the Milan investigators, one tumor less than 5 cm or no more than three tumors with the largest one no more than 3 cm in diameter, have since become widely recognized as the Milan criteria. Since the publication of these results, numerous other centers (6,7) have reported similar outcomes around the world. With this widespread validation, HCC has become an accepted indication for LT with the more recent focus on determining which patients with HCC disease beyond the Milan criteria will also have acceptable results after LT. In this chapter, we will focus on selection of patients with HCC for LT and the impact of this selection on other LT candidates, the purpose and results of neo-adjuvant systemic and local therapies before LT, whether the technique of LT (e.g., living donor or deceased donor LT) affects the results for HCC recipients, and the overall outcomes of LT for HCC.

SELECTION OF CANDIDATES FOR LT

The profound mismatch between the number of available organs and the number of patients potentially treatable with LT imposes more than just simple risk benefit evaluations for selecting candidates for treatment of their HCC with LT. On average, LT recipients without a malignant indication for LT enjoy more than a 65% chance at five-year survival (8). This fact must be considered when selecting patients with HCC for LT. Data from Mount Sinai (9) and Germany (2) indicate that five-year survival of 20% to 30% can be achieved for very advanced HCC with LT, which, given the alternative, would justify LT as a standard oncologic treatment option if there were no donor resource constraints. However, these oncologically acceptable survival rates are not acceptable for the vast majority of patients waiting for LT. So pure individual patient benefit cannot be the sole criterion for selection of HCC candidates for transplantation.

Mazzaferro's Milan criteria identified a set of tumor characteristics that has subsequently been shown in many studies to carry acceptably low rates of tumor recurrence after transplant. Numerous investigators have attempted to define the optimal HCC predictive factors that will select candidates at acceptably low risk for recurrent disease after transplantation (6,10–15). All of these papers have used morphological criteria that are based on either preoperative data such

as scan images, α-feto-protein (AFP) levels (14) and, in some cases, on biopsy data or they have been based on histologic criteria derived from the explanted liver after transplantation. The prognostic factors derived from explanted livers may not be useful however in selecting candidates for LT, since these data are only available after the decision to transplant has been made. Preoperative imaging is notoriously unreliable especially for smaller lesions (16) with the overall accuracy no better than 50% in recent series (15,17–19). Consistently, tumor number and tumor size have been identified as predictors, largely because they are surrogates for more aggressive tumors that invade the vasculature (20,21) with vascular invasion cited most frequently as the most important predictor of HCC recurrence after LT (Tables 1 and 2) (Fig. 1). In fact, in a recent study using the Organ Procurement and Transplantation Network (OPTN)

Table 1 Staging Systems for HCC

References	Variables included in staging system
Okuda (23)	Liver replacement by tumor (> or <50%), ascites, albumin, bilirubin
CLIP (24)	Child–Pugh score, tumor distribution, AFP, and portal vein thrombosis
BCLC (25)	Tumor size and number, portal hypertension, bilirubin
ACC TNM (26)	Tumor size and number
CUPI (27)	TNM, symptoms, ascites, AFP, bilirubin, alkaline phospatase
French (28)	Karnofsky score, bilirubin, alkaline phosphatase, AFP, portal obstruction
JIS (29)	TNM, Child–Pugh

Abbreviations: AFP, α-feto-protein; CLIP, Cancer of the Liver Italian Program; BCLC, Barcelona Clinic Liver Cancer; ACC TNM, American Joint Committee on Cancer Tumor Node Metastasis; CUPI, Chinese University Prognostic Index; JIS, Japanese Integrated Staging Score.

Table 2 Factors Associated with HCC Recurrence After LT

Reference	*n*	Predictors of recurrence
Schlitt (11)	69	Tumor size, number, vascular invasion, stage IVA
Roayaie (30)	311	Tumor size, grade, AFP, liver disease
Mazzaferro (5)	49	Tumor size, number, vascular invasion
Jonas (6)	120	Tumor size, grade, vascular invasion
Yao (31)	70	Tumor size, number, combined diameter
Marsh (32)	407	Vascular invasion
Marsh (34)	107	Fractional allelic loss

Abbreviations: HCC, hepatocellular cancer; LT, liver transplantation; AFP, α-feto-protein.

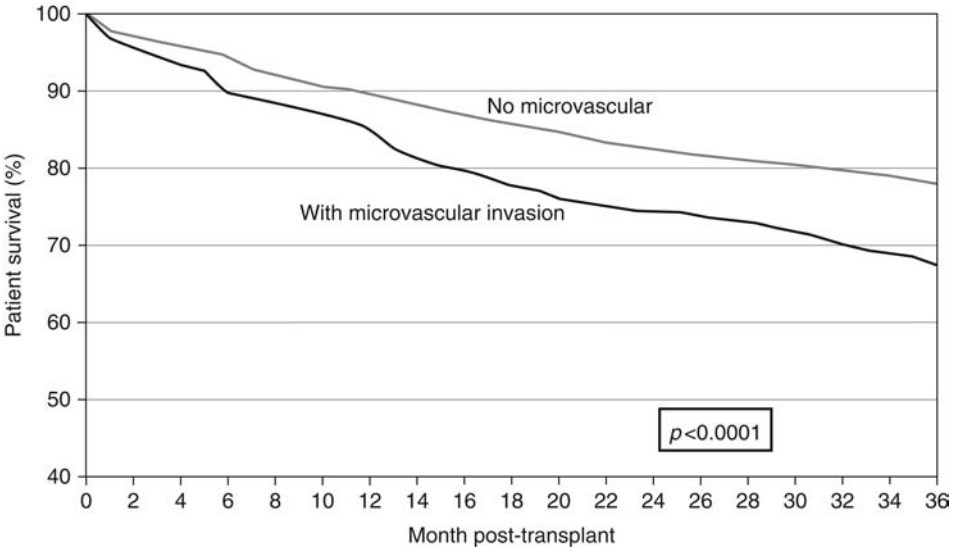

Figure 1 Post transplant survival with and without microvascular invasion, *n* = 2848. Kaplan–Meier technique.

database, Ioannou and colleagues found that tumor characteristics and serum AFP were the only HCC factors predictive of survival after LT. They found that patients with tumors 3 to 5 cm in size but still within the Milan criteria had poorer survival (22).

Some have argued that preoperative biopsy can be useful for assessing tumor grade, another variable found to be predictive of recurrence of HCC after LT (33), or that these specimens can be analyzed for molecular patterns that have been associated with more aggressive tumors that are more likely to recur (34). But biopsy has a high false-negative rate (35), even when repeated (36), and in addition to the well-documented bleeding risks (37) for cirrhotic patients, there are reports of tumor seeding in the biopsy needle tracts (38,39) that make this approach indicated only in certain circumstances.

Despite these limitations, investigators around the world have proposed morphologic criteria that go beyond Milan criteria that seem to predict favorable outcome, at least in some studies with limited sample sizes. Yao et al., from the University of California at San Francisco (UCSF), initially described a set of criteria based on pathological review of a single tumor up to 6.5 cm in size or three lesions, the largest of which must be no greater than 4.5 cm in size and a total tumor diameter of less than 8 cm (31). More recently, this group has verified that these pathological criteria also are reasonably accurate for selecting low–recurrence risk cases when selection is governed by preoperative imaging (40). However, a much larger French study (41) of 479 HCC LT recipients found that patients meeting the UCSF criteria defined by preoperative measures had a much poorer five-year survival rate compared with patients with tumors meeting Milan criteria (48% 5-year survival within UCSF criteria but $>$ Milan criteria vs. 60% 5-year survival within Milan). These investigators suggested that, because the waiting time of patients in their series was relatively short and therefore rapidly progressing tumors could not be identified in the short time frame, patients were included in their UCSF criteria cohort who had aggressive tumors that would have been eliminated by the UCSF group because of the much longer waiting time in California. These results suggest that waiting time may play a critical role for selecting candidates with HCC for LT because it may allow transplant programs the time to assess the progression of the HCC lesions and identify tumors that have a higher risk of recurrence by observing their progression preoperatively. This more rapid progression to unfavorable stages while waiting has been termed "dropping out" from the waiting list.

Much debate has centered on expansion of selection criteria beyond Milan. Many investigators have identified tumor characteristics, largely based on pathologic criteria that are predictive of patient and disease-free survival that approximates results achieved with application of the Milan criteria. Investigators from the University of Pittsburgh have demonstrated that genotypic changes in microsatellite alleles in HCC lesions can select patients with a low likelihood of recurrence (34). However, recent Markov models comparing the survival benefit of LT for patients with HCC $>$ Milan with the harm caused to other patients on the waiting list have questioned the utility of broadening the selection criteria for HCC beyond Milan. In the base-case analysis cited in this study, the strategy of transplanting the patient with $>$ Milan HCC resulted in a 44% increased risk of death and a utility loss of three quality-adjusted years of life across the pre- and post-transplant periods for a representative cohort of patients. This harm outweighed the benefit of transplantation for a patient with $>$ Milan HCC having a five-year post-transplant survival of less than 61%. This survival threshold was most sensitive to geographic variations in organ shortage, with the threshold varying from 25% (region 3) to $>$ 72% (regions 1, 5, 7, and 9). The authors concluded that expansion of the Milan criteria will require demonstrating survival rates of approximately 61% at five years after transplantation for HCC candidates outside of Milan criteria to offset the diversion of organs away from other candidates without HCC. In regions with less severe organ shortage, a more aggressive approach to transplanting these patients may be justified (42).

NEO-ADJUVANT TREATMENTS WHILE WAITING

Clinicians caring for patients with HCC who are waiting for LT have employed several loco-regional treatments for HCC that do not require surgical approaches for their application. These so-called ablative treatments (ATs) have been advocated for LT candidates with HCC for

three reasons: (*i*) to reduce the risk of progression beyond favorable stages and thereby prevent dropping out from the waiting list, (*ii*) improve post-LT patient survival by reducing HCC recurrence afterward, and (*iii*) downstage more advanced tumors to less advanced stages where more favorable LT results may be achievable. While there are single-center reports advocating AT for each of these reasons, very few have sufficient sample sizes to justify widespread application of these AT, and more systematic analyses of these reports have concluded that there is no evidence to date that they are of any benefit in any of the three areas mentioned above (43).

Recent studies have concluded that only when the waiting time for LT is greater than one year does AT become cost effective for reducing this dropout rate (44). In one report U.S. investigators using the national OPTN database found that AT treatment was not associated with the dropout rate, likely because the overall waiting time for LT under the prevailing LT prioritization rules was only three months on average (45). Other investigators from Spain found that percutaneous ethanol injection (PEI) of HCC lesions for waiting LT candidates was cost effective only if the projected LT waiting time was going to be more than 12 months (44). Since PEI is relatively inexpensive, it is unlikely that more expensive (and more invasive) AT will be cost effective for patients waiting for less than one year for LT.

Well-designed, randomized trials of pre-LT AT comparing various AT modalities for their effect on post-LT patient or disease-free survival are lacking. AT methods including PEI (46–48), transarterial chemoembolization (TACE) (49–52), and thermal ablation, most frequently practiced as radio frequency ablation (RFA) (53), have been reported. However, many of these treatments have been applied to patients with advanced-stage HCC making assessment of their utility for earlier stages difficult. While several investigators have assessed the outcome of LT for patients with HCC given various AT, most series have included very small sample sizes making stage-specific stratifications impossible. They rarely include comparison groups, and follow-up of more than a handful of patients beyond three years after transplant is not reported. In one randomized trial of 86 patients, RFA was found to be superior to PEI for inducing tumor necrosis (48), but no post-LT survival data was included. This relatively small trial did not incorporate histologic confirmation of tumor necrosis, but the findings have led many centers to abandon PEI in favor of RFA (54). In another recent report summarizing multiple forms of AT given to 116 HCC patients before LT, the investigators found no overall benefit for AT. However, median follow-up was only 892 days, and the various forms of AT and other risk factors were not accounted for because of the small sample size (55). Nonetheless, TACE and RFA have become the preferred neo-adjuvant treatment of HCC before LT.

RFA, as a treatment for liver transplant candidates with HCC, has been evaluated in several studies. In one report of 33 cases, 15 went on to transplant with three-year actuarial survival reported, but there was no control group included (56). In another study of 50 patients treated with RFA before LT, 55% of patients achieved a complete response histologically. Three-year post-LT actuarial survival rates were acceptable (83%), but median follow-up was only 22 months, and no untreated control group with which to compare results was mentioned in this report (57). In one additional study, 52 consecutive HCC cases were treated with RFA with 41 receiving transplantation (58). Median follow-up was only 9.4 months, and several of the included patients also had additional types of AT administered before transplantation. Again no untreated control group was included. There are other studies comprising more mixed treatments, fewer patients, and less rigorous follow-up than those cited above. On the basis of the available literature, it is difficult to offer any evidence-based recommendations regarding the effect of RFA on LT candidates with HCC.

The evidence for TACE inducing HCC necrosis and affecting LT outcomes for HCC patients is equally soft. In the study of 116 patients, cited above, only 7% of treated nodules were completely necrosed, and 42% had no necrosis visible at all (33). A recent systematic review of the literature assessing TACE as a neo-adjuvant treatment before LT concluded that TACE does not have any impact on post-LT survival nor does it provide consistent results for downstaging tumors (43). These authors summarized 12 different trials of TACE before LT and noted that, except for one multicenter trial from France, there were no control groups and also noted very heterogeneous study designs in these reports. In the case-controlled study from

14 French LT centers, 100 patients treated with a median of 1 TACE procedure, a mean of 4.2 months before LT, demonstrated no significant difference in actuarial overall or disease-free survival at five years compared with HCC patients who were not treated before LT (59). The French investigators did not observe a stage-specific effect for TACE either, but their largest subgroup consisted of only 60 patients. They observed a trend toward improved five-year survival for the 30 patients in whom significant necrosis was observed in the TACE-treated explanted liver, but these differences did not reach statistical significance (63% 1-year patient survival in TACE treated vs. 54% 1-year survival in the no treatment group, $p = 0.9$), and these survival rates are much lower than those generally reported for HCC patients given LT in the United States (86% 1-year survival). These data were accrued from patients transplanted between 1985 and 1998, and considerable historical biases may be prevalent. Three other studies, each describing less than 50 patients each, where case, or historical, control groups are used for comparison, have found no significant improvement in post-LT survival for patients given TACE, but none of these studies had power calculations to address type 2 statistical errors (60–62).

Recently, U.S. investigators reported an analysis of the OTPN data in which selection bias for pre-LT AT was accounted for by using propensity adjustments (63). In this analysis the propensity adjustment indicated that there may be a small amount of selection bias in applying AT to LT candidates with HCC, but even when this is accounted for, patients given AT before LT for HCC seem to have a reduced relative risk of graft failure compared with those who received no AT (Fig. 2).

Downstaging of HCC lesion by AT preoperatively has yielded mixed results. One earlier report of 35 HCC liver transplant candidates treated with TACE before transplantation showed a significant downstaging effect (22), but a more recent report suggests that candidates with greater than stage II disease who are downstaged do not have results equivalent to candidates who presented with stage II disease (64). These findings have been further confirmed recently where patients within Milan criteria who responded to TACE had improved post-LT outcomes, but those outside of Milan criteria before TACE had inferior post-LT survival regardless of their response to TACE (65). In this study, very limited subgroup analysis was done, and no other factors predictive of outcome other than initial tumor stage were assessed, likely due to the relatively small sample size of 106 patients who received transplantation. In contrast, another recent report from UCSF indicated that patients with stage IIIA disease who were successfully downstaged to stage II or less HCC with a variety and heterogeneous array of AT treatments had excellent post-LT outcomes, but there were only 16 patients in this group with more than 1-year follow-up (66), and no untreated controls were included. RFA applied

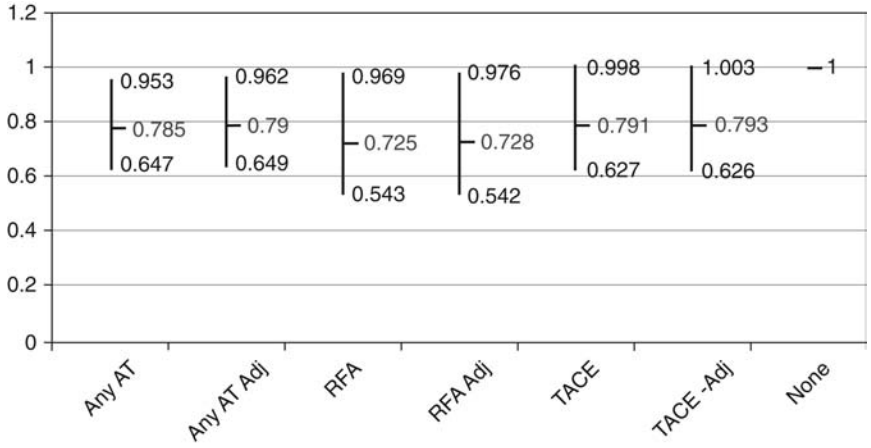

Figure 2 Relative risk of graft loss (*red*) and 95% confidence intervals (*blue*) after LT for HCC depending on ablation. Reference is no ablation. *Abbreviations*: AT, ablative treatment; adj, adjusted with propensity analyis; RFA, radio frequency ablation; TACE, transarterial chemoembolization; HCC, hepatocellular cancer; LT, liver transplantation.

to single HCC lesions before LT also has been examined in 50 patients in whom 55% of the treated HCC lesions were successfully downstaged, but no control group was included making the efficacy of this treatment difficult to assess (20). A more recent report from Europe found that HCC LT recipients who responded to repeated TACE treatments had superior survival compared with patients who had no, or transient, responses before LT regardless of the pretreatment stage (67). However, except for response to treatment, this report offered no other analysis of potentially confounding or contributing factors to their results. There were only seven HCC recurrent events reported in this analysis, which severely limited statistical evaluation of subgroups or risk factors.

Another approach aimed at delaying LT for some patients with HCC has been to perform liver resection for early stage HCC (68) and then proceed with LT if there is unfavorable histologic characteristics (69) or if HCC recurs after the resection. Proponents of this so-called "salvage transplant for HCC" protocol suggest that some of these candidates will be cured by resection alone, thereby lessening the pressure on the donor pool (70). Initial reports suggested that resected patients who subsequently came to transplant were not disadvantaged in terms or morbidity due to previous surgery or overall survival in deceased donor (70) or living donor programs (71), but a more recent study comparing 17 patients treated with salvage transplantation with 197 patients receiving primary LT for HCC found that salvage transplantation after resection was associated with increased morbidity, mortality, and tumor recurrence compared with the patients treated with primary LT (72). In another small series comparing primary liver resection with LT for early-stage HCC where salvage LT was offered to 5 of the 23 resected patients, overall five-year survival rates were 35% compared with 60% after primary LT (73). Recurrence-free survival at five years was similarly different, and almost half of the resection patients experienced a recurrence. Salvage transplant was performed for five of the patients with small, liver-confined recurrences with 100% disease-free survival at 18 months.

LIVING DONOR LT FOR HCC CANDIDATES

The donor resource is even more severely constrained in Asia where HCC is highly prevalent, and the majority of LT are performed with living donors. Selection of candidates for living donor liver transplantation (LDLT) in Asia (74) and in the U.S. (75) has been broadened somewhat because there is no concern for maximizing the results for the limited deceased donor pool. The major concern in the LDLT scenario is minimizing donor risk and informing the donor about the likelihood of success for the recipient. In the LDLT situation then the degree of success is not necessarily required to be in the range of success achievable for other LT indications but only has to be in a range acceptable for the donor and the recipient (75). However, concern has been raised that the relative availability of LDLT that avoids prolonged waiting time may lead to transplantation of patients with more aggressive tumors because waiting for a deceased donor allows programs to observe tumor progression and select more favorable candidates (76). Nonetheless, decision analyses have suggested that LDLT is worthwhile when waiting time for deceased donor LT is greater than six months (77).

The largest series of LDLT for HCC comes from Japan. Todo reported results for 316 patients with HCC who received LDLT in Japan. The one- and three-year recurrence-free survival rates were 72.7% and 64.7%, respectively, with about one-half of these patients beyond Milan criteria. For patients within the Milan criteria, patient survival and disease-free survival at three years were 78.7% and 79.1%, respectively, compared with 60.4% and 52.6%, respectively, for patients beyond Milan criteria (78). There are concerns that small-for-size syndrome that can occur with LDLT may predispose recipients to a higher risk of recurrence (79). A series from Taiwan in which all HCC recipients transplanted with LDLT were within Milan criteria reported excellent five-year overall survival rates of 90%. These investigators noted that patients outside of Milan criteria who were downstaged with preoperative treatments to within Milan criteria experienced similar results (80). In this highly selected series, there did not seem to be a penalty for the relatively rapid time to carry out transplantation for these patients. Other investigators have also demonstrated good results with LDLT for patients beyond Milan criteria in small series (75).

MOST RECENT U.S. RESULTS

In the most recent OPTN/Scientific Registry of Transplant Recipients (SRTR) annual report (81), three-year survival for adult recipients with HCC is slightly but significantly inferior to adult recipients without HCC when adjusted for MELD score and numerous other potentially confounding variables (Fig. 3). However, HCC recipients who receive AT before transplantation have improved outcomes compared with patients who do not receive AT (Fig. 4). Centers that are more apt to apply AT before LT for HCC patients appear to have better outcome for LT for HCC recipients than centers where AT is applied less frequently (Fig. 5). Thus, with longer follow-up now available in the U.S. LT transplant system, recurrence of HCC maybe contributing to slightly poorer outcomes compared with patients with nonmalignant LT indications. There is a suggestion that these slightly inferior results may be ameliorated by AT application before LT, although more analyses preferably with prospective randomized trials is required.

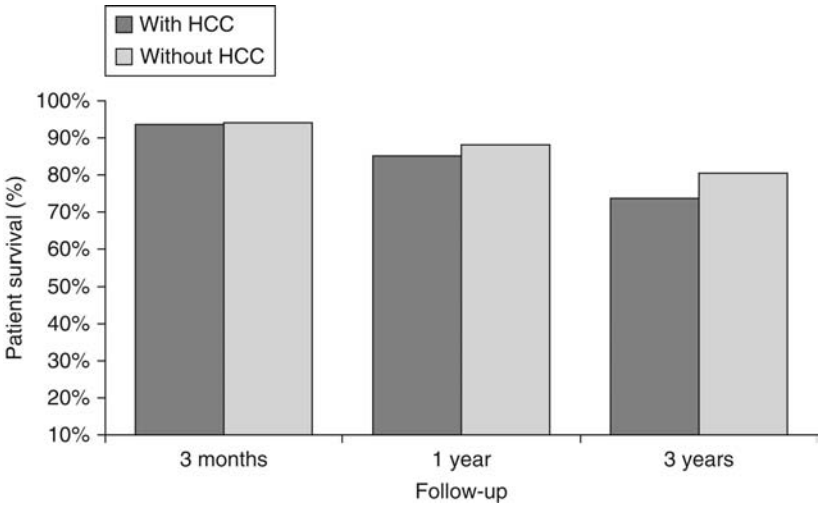

Figure 3 Adjusted patient survival for adult recipients with HCC and no HCC. *Abbreviation*: HCC, hepatocellular cancer.

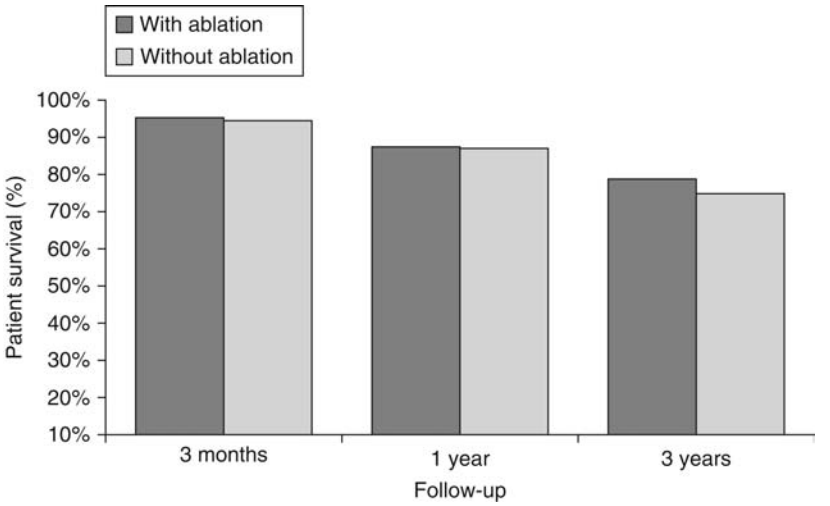

Figure 4 Adjusted patient survival for patients with and without AT before LT. *Abbreviations*: LT, liver transplantation; AT, ablative treatment.

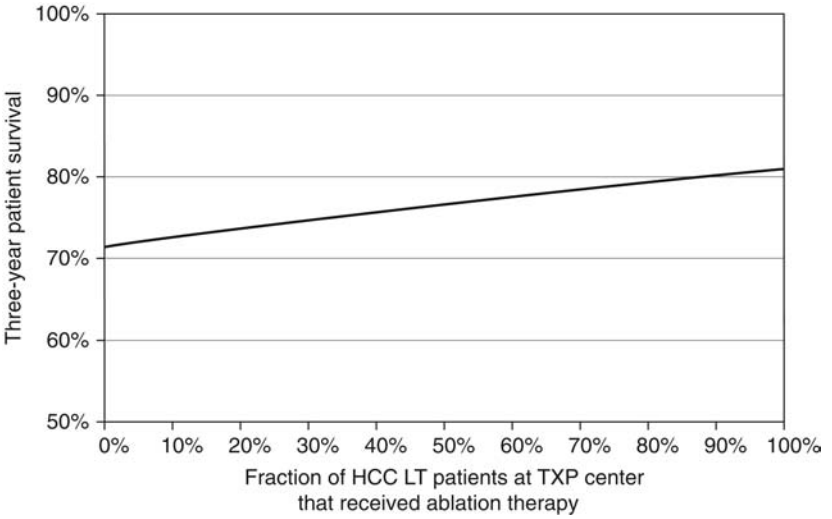

Figure 5 Three-year patient survival by transplant center level of AT. *Abbreviation*: AT, ablative treatment.

CONCLUSIONS

LT for HCC, especially for patients with early-stage HCC, remains the best treatment in terms of maximizing patient and disease-free survival. Even for more advanced cases, LT offers a small chance at cure that no other therapy can achieve. However, other patients who do not have malignancies often have better long-term prognoses with LT compared with candidates with more advanced HCC disease. Thus, expansion of the HCC criteria for LT must be justified, not only in terms of survival benefit for the potential recipients but also in terms of the effects on all candidates needing access to the deceased donor pool. AT may offer downstaging effects and some control of HCC that results in improved long-term survival for HCC recipients, but better designed trials are required to provide more solid evidence to support the preliminary conclusions derived from the retrospective observational trials published to date. The donor pool limitations do not necessarily apply in the case of LDLT and thus, with the consent of donor and recipient, it is acceptable to consider LDLT for more advanced HCC than would otherwise be considered for deceased donor LT.

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