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REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY

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Hepatocellular Carcinoma: Epidemiology and Molecular Carcinogenesis

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Primary liver cancer, which consists predominantly of hepatocellular carcinoma (HCC), is the fifth most common cancer worldwide and the third most common cause of cancer mortality. HCC has several interesting epidemiologic features including dynamic temporal trends; marked variations among geographic regions, racial and ethnic groups, and between men and women; and the presence of several well-documented environmental potentially preventable risk factors. Moreover, there is a growing understanding on the molecular mechanisms inducing hepatocarcinogenesis, which almost never occurs in healthy liver, but the cancer risk increases sharply in response to chronic liver injury at the cirrhosis stage. A detailed understanding of epidemiologic factors and molecular mechanisms associated with HCC ultimately could improve our current concepts for screening and treatment of this disease.

Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality.[1] Hepatocellular carcinoma (HCC) accounts for between 85% and 90% of primary liver cancers. HCC has several interesting epidemiologic features including dynamic temporal trends; marked variations among geographic regions, racial and ethnic groups, and between men and women; and the presence of several well-documented environmental potentially preventable risk factors. Moreover, there is a growing understanding of the molecular mechanisms that induce hepatocarcinogenesis, which almost never occurs in the healthy liver but the cancer risk increases sharply in response to chronic liver injury at the cirrhosis stage. In this review we summarize epidemiologic factors, disease-specific factors, molecular mechanisms, and current concepts for screening, diagnosis, and treatment of HCC.

Global Incidence of HCC

Distribution

Liver cancer burden is not distributed evenly throughout the world (Figure 1). Most HCC cases (>80%) occur in either sub-Saharan Africa or in Eastern Asia. China alone accounts for more than 50% of the world's cases (age-standardized incidence rate: men, 35.2/100,000; women, 13.3/100,000). Other high-rate (>20/100,000) areas include Senegal (men, 28.47/100,000; female, 12.2/100,000), Gambia (male, 39.67/100,000; female, 14.6/100,000), and South Korea (male, 48.8/100,000; female, 11.6/100,000).

North and South America, Northern Europe, and Oceania are low-rate (<5.0/100,000) areas for liver cancer among most populations. Typical low incidence rates are those of Canada (male, 3.2/100,000; female, 1.1/100,000), Colombia (male, 2.2/100,000; female, 2.0/100,000), United Kingdom (male, 2.2/100,000; female, 1.1/100,000), and Australia (male, 3.6/100,000; female, 1.0/100,000). Southern European countries, typified by rates in Spain (male, 7.5/100,000; female, 2.4/100,000), Italy (male, 13.5/100,000; female, 4.6/100,000), and Greece (male, 12.1/100,000; female, 4.6/100,000) are medium-rate (5.0–20.0/100,000).[2]

An exception to the predominance of HCC among primary liver cancer is the Khon Kaen region of Thailand, which has one of the world's highest rates of liver cancer (annual age-standardized incidence rate during 1993 to 1997 men, 88.0/100,000; women, 35.4/100,000).[2] However, because of endemic infestation with liver flukes, the major type of liver cancer in this region is intrahepatic cholangiocarcinoma rather than HCC.[3]

Encouraging trends in liver cancer incidence have been seen in some of these high-rate areas.[4] Between 1978 and 1982 and 1993 and 1997, decreases in incidence were reported among Chinese populations in Hong Kong, Shanghai, and Singapore.[2] In addition to these areas,

Abbreviations used in this paper: AFB_1, aflatoxin B_1; CI, confidence interval; GST, glutathione S-transferase; IGF2R, insulin-like growth factor 2 receptor; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

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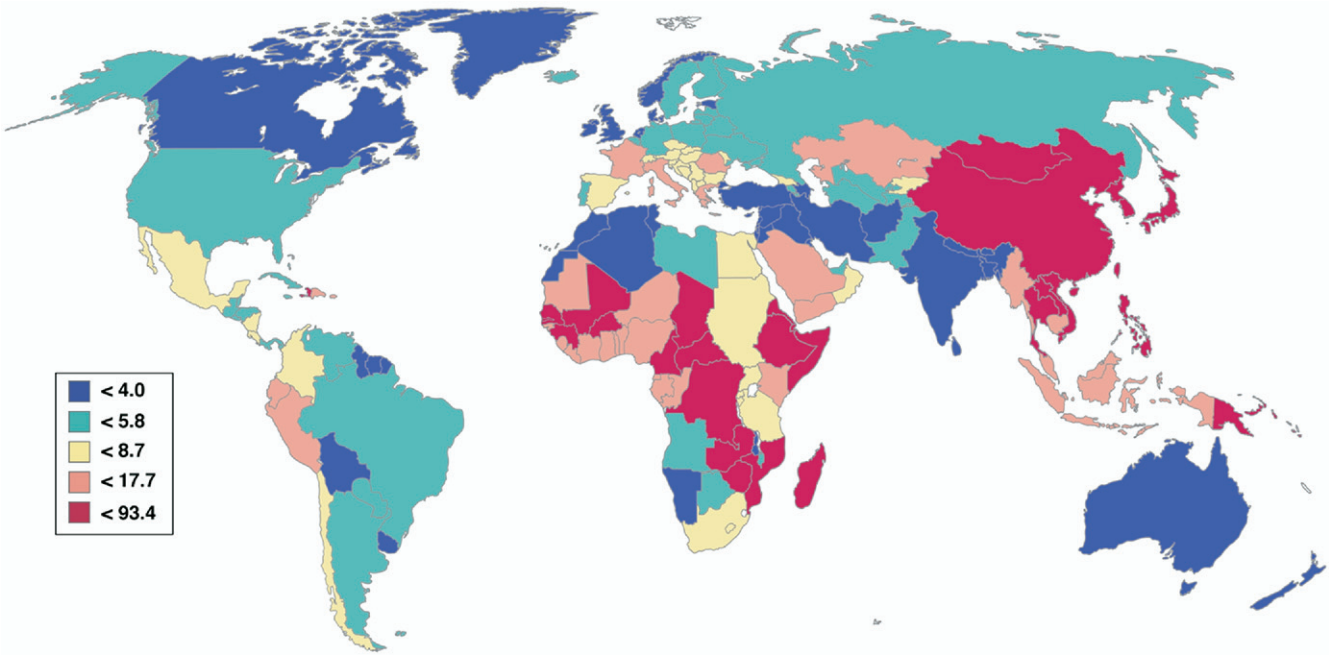

Figure 1. Regional variations in the mortality rates of HCC categorized by age-adjusted mortality rates. The rates are reported per 100,000 persons.

Japan also began to experience declines in incidence rates among males for the first time between 1993 and 1997.

Many high-rate Asian countries now vaccinate all newborns against hepatitis B virus (HBV) and the effect on HCC rates already has become apparent.[5] A Chinese government program started in the late 1980s to shift the staple diet of the Jiangsu Province from corn to rice may have limited exposure to known hepatocarcinogen aflatoxin B_1 (AFB_1) in this area.[6]

In contrast, registries in a number of low-rate areas reported increases in HCC incidence between 1978 and 1982 and 1993 and 1997. Included among these registries are those in the United States, United Kingdom, and Australia.[4] Reasons for both the decreased incidence in high-rate areas and the increased incidence in low-rate areas are not yet clear, suggesting that each area will be an important case study. It has been hypothesized, however, that increased incidence in low-rate areas may be related to a greater prevalence of hepatitis C virus (HCV) infection within these areas.

Race/Ethnicity

HCC incidence rates also vary greatly among different populations living in the same region. For example, ethnic Indian, Chinese, and Malay populations of Singapore had age-adjusted rates ranging from 21.21/100,000 among Chinese males to 7.86/100,000 among Indian males between 1993 and 1997.[2] The comparable rates for females were 5.13/100,000 among ethnic Chinese and 1.77/100,000 among ethnic Indians. Another example is the United States where, at all ages and among both sexes, HCC rates are 2 times higher in Asians than in African Americans, whose rates are 2 times higher than those in whites. The reason for this ethnic variability likely includes differences in the prevalence and acquisition time of major risk factors for liver disease and HCC.

Sex

In almost all populations, males have higher liver cancer rates than females, with male:female ratios usually averaging between 2:1 and 4:1. At present, the largest discrepancies in rates (>4:1) are found in medium-risk European populations. Typical among these ratios are those reported from Geneva, Switzerland (4.1:1), and Varese, Italy (5.1:1). Among 10 French registries listed in Volume VIII of Cancer in Five Continents, 9 report male:female ratios of >5:1. In contrast, typical ratios currently seen in high-risk populations are those of Qidong, China (3.2:1), Osaka, Japan (3.7:1), Gambia (2.8:1), and Harare, Zimbabwe (2.4:1). Registries in Central and South America report some of the lowest sex ratios for liver cancer. Typical ratios in these regions are reported by Colombia (1.2:1) and Costa Rica (1.6:1).

The reasons for higher rates of liver cancer in males may relate to sex-specific differences in exposure to risk factors. Men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes, and have increased iron stores. However, experiments show a 2- to 8-fold increase in HCC development in male mice.[7] These data support the hypothesis that androgens influence HCC progression rather than sex-specific exposure to risk factors. Nonenvironmental endogenous factors that may affect male risk adversely include higher body mass index and higher levels of androgenic hormones. Several stud-

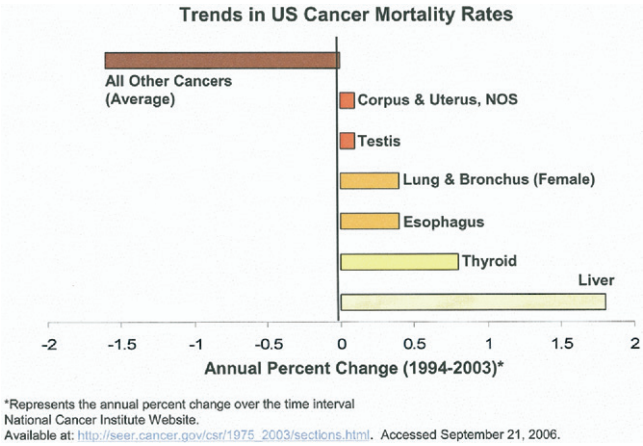

Figure 2. Liver cancer has the fastest growing death rate in the United States. The overall cancer mortality rate has declined in the United States (top horizontal bar). However, annual per change in mortality rate is increasing in few cancer sites, the fastest of which is liver cancer.

ies conducted in Taiwan[8,9] reported a positive association between increased circulating testosterone levels and HCC in HBV-infected men.

Age

The global age distribution of HCC varies by region, incidence rate, sex, and, possibly, by etiology.[2] In almost all areas, female rates peak in the age group 5 years older than the peak age group for males. In low-risk populations (eg, United States, Canada, and United Kingdom), the highest age-specific rates occur among persons aged 75 and older. A similar pattern is seen among most high-risk Asian populations (eg, Hong Kong and Shanghai). In contrast, male rates in high-risk African populations (eg, Gambia, Mali) tend to peak between ages 60 and 65 before declining, whereas female rates peak between 65 and 70 before declining. These variable age-specific patterns likely are related to differences in the dominant hepatitis virus in the population, the age at viral infection, and the existence of other risk factors. Notably, although most HCV carriers became infected as adults, most HBV carriers became infected at very young ages.

Distribution of Risk Factors

Major risk factors for HCC vary by region. In most high-risk areas, the dominant risk factor is chronic HBV infection. In Asia, HBV infection largely is acquired by maternal-child transmission, whereas sibling-to-sibling transmission at young ages is more common in Africa. Consumption of AFB_1 -contaminated foodstuffs is the other major HCC risk factor in most high-rate areas.

Unlike the rest of Asia, the dominant hepatitis virus in Japan is HCV. HCV began to circulate in Japan shortly after World War II.[10] Consequently, HCC rates began to increase sharply in the mid-1970s, with an anticipated peak in HCV-related HCC rates projected around 2015, although recent data suggest the peak already might have been reached.

In low-rate HCC areas, the increasing number of persons living with cirrhosis is the likely explanation for the increasing incidence of HCC. This has resulted from a combination of factors including an increasing incidence of cirrhosis caused by HCV and, to a lesser extent, HBV infection, as well as a general improvement in survival among cirrhosis patients. It has been estimated that HCV began to infect large numbers of young adults in North America and South and Central Europe in the 1960s and 1970s as a result of intravenous drug use.[11] The virus then moved into national blood supplies and circulated until a screening test was developed in 1990, after which time rates of new infection decreased dramatically. Currently, it is estimated that HCV-related HCC in low-rate countries will peak around 2010.[12]

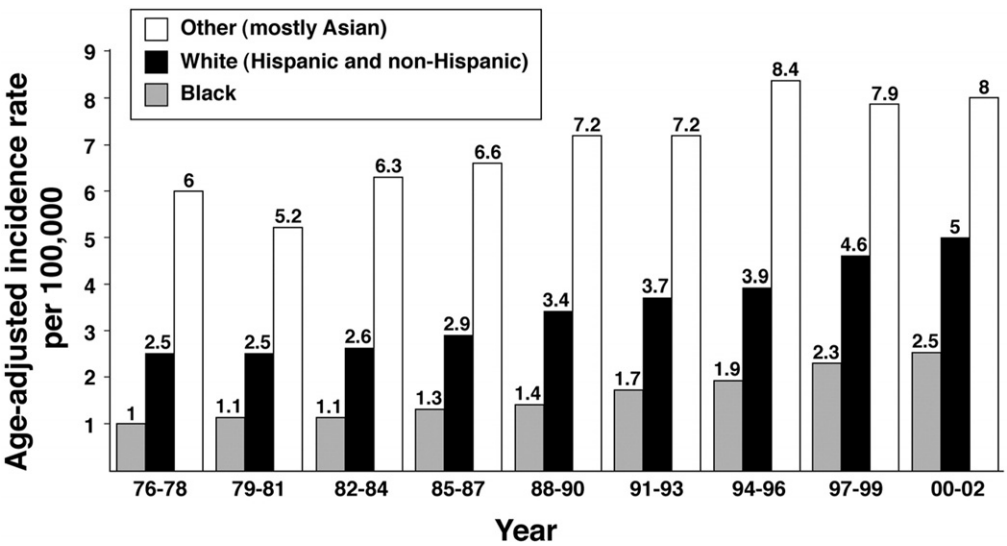

Figure 3. Average yearly, age-adjusted incidence rates for HCC men and women in the United States shown for 3-year intervals between 1975 and 2002. Whites include approximately 25% Hispanics whereas Other race is predominantly (88%) Asian. Source: Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence—SEER 13 Regs Public-Use, Nov 2004 Sub (1973–2002 varying), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2005, based on the November 2004 submission.

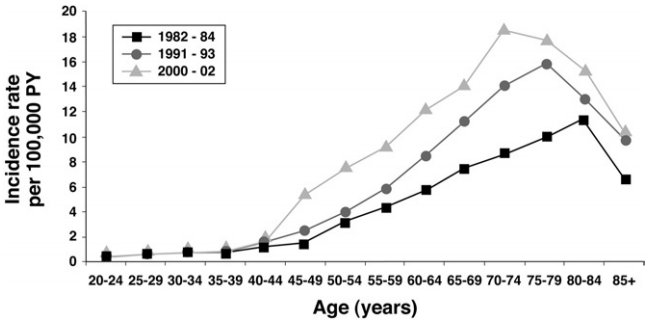

Figure 4. Temporal trends in the age distribution of HCC in the United States. A shift toward younger ages is observed concomitant with the recent increase in the overall incidence of this malignancy.

HCC in the United States

HCC is the fastest growing cause of cancer-related death in men in the United States (Figure 2). Age-adjusted HCC incidence rates increased more than 2-fold between 1985 and 2002 (Figure 3).[13,14] The average annual, age-adjusted rates of HCC verified by histology or cytology increased from 1.3 per 100,000 during 1978–1980 to 3.3 per 100,000 during 1999–2001.[15] The increase in HCC started in the mid-1980s, with the greatest proportional increases occurring during the late 1990s. The largest proportional increases occurred among whites (Hispanics and non-Hispanics), whereas the lowest proportional increases occurred among Asians. The mean age at diagnosis is approximately 65 years, 74% of cases occur in men, and the racial distribution is 48% white, 15% Hispanic, 13% African American, and 24% other race/ethnicity (predominantly Asian) (Figure 3). During recent years as incidence rates increased, the age distribution of HCC patients has shifted toward relatively younger ages, with the greatest proportional increases between ages 45 and 60 (Figure 4).

Four published studies examined secular changes in HCC risk factors in the United States.[16–19] Two studies were from large, single-referral centers where viral risk factor ascertainment was based on serology findings, and the other 2 studies were from national databases in which risk factors were ascertained from International Classification of Diseases 9th revision codes in billing or discharge records. In all 4 studies, the greatest proportional increases occurred in HCV-related HCC, whereas HBV-related HCC had the lowest and most stable rates (Figure 5). Overall, 15%–50% of HCC patients in the United States have no established risk factors.

Risk Factors of HCC

HCC is unique in that it largely occurs within an established background of chronic liver disease and cirrhosis (~70%–90% of all detected HCC cases) (Table 1). Major causes of cirrhosis in patients with HCC include hepatitis B, hepatitis C, alcoholic liver disease, and possibly nonalcoholic steatohepatitis. Less common causes include hereditary hemochromatosis, α-1 antitrypsin deficiency, autoimmune hepatitis, and some porphyrias.

HBV

Globally, HBV is the most frequent underlying cause of HCC, with an estimated 300 million persons with chronic infection worldwide. Case-control studies have shown that chronic HBV carriers have a 5- to 15-fold increased risk of HCC compared with the general population. The great majority, between 70% and 90%, of HBV-related HCCs develop in patients with cirrhosis. However, HBV is a notorious cause for HCC in the absence of cirrhosis.

The increased HCC risk associated with HBV infection particularly applies to areas where HBV is endemic. In these areas, it usually is transmitted from mother to newborn (vertical transmission) and up to 90% of infected persons follow a chronic course. This pattern is different in areas with low HCC incidence rates where HBV is acquired in adulthood through sexual and parenteral routes (horizontal transmission), with more than 90% of acute infections resolving spontaneously. The annual HCC incidence in chronic HBV carriers in Asia ranges between .4% and .6%. This figure is lower in Alaskan natives (.26%/y), and is lowest in Caucasian HBV carriers.[20]

Several other factors have been reported to increase HCC risk among HBV carriers including male sex; older age (or longer duration of infection); Asian or African race; cirrhosis; family history of HCC; exposure to aflatoxin, alcohol, or tobacco; or co-infection with HCV or hepatitis D virus (HDV). HCC risk also is

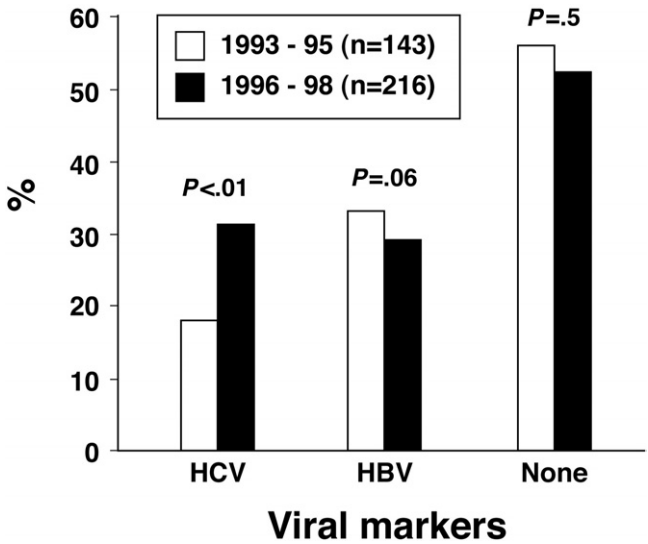

Figure 5. Progression of HCV-related liver disease. Modified from Hassan et al. (Reprinted with permission from J Clin Gastro [http://lww.com]).[18]

Table 1. Incidence of HCC in Patients With Compensated Cirrhosis

	Study A	Study B	Study C
Number of patients	384	112	103
Follow-up period, y	5.0	4.5	3.3
Decompensation, % per year	3.9	4.4	5.0
HCC, % per year	1.4	2.3	3.3

NOTE. Results are from 3 cohort studies conducted in 3 countries (study A, Italy; study B, United States; and study C, France). Study A data from Fattovich et al,[182] study B data from Hu and Tong,[183] and study C data from Serfaty et al.[33]

increased in patients with higher levels of HBV replication, as indicated by the presence of hepatitis B e antigen (HBeAg) and high HBV-DNA levels. In addition, it has been suggested in Asian studies that genotype C is associated with more severe liver disease than genotype B.[21]

In the natural history of chronic HBV infection, spontaneous or treatment-induced development of antibodies against hepatitis B surface antigen (HBsAg) and HBeAg leads to improved clinical outcomes. A meta-analysis of 12 studies with 1187 patients who received interferon and 665 untreated patients followed up for 5 years found a lower HCC incidence in treated (1.9%; 95% confidence interval [CI], .8%–3.0%) than untreated patients (3.2%; 95% CI, 1.8%–4.5%). However, this difference was not statistically significant.[22]

The risk of HCC is substantially lower in persons who are immune to HBV. Many studies have shown, by using sensitive amplification assays, that HBV DNA persists as occult HBV infection for decades among persons with serologic recovery (HBsAg negative) from acute infection. Occult HBV is associated with anti-HBc and/or anti-HBs.[23] A single multinational investigation found the prevalence of occult HBV in liver tissue to be 11% in Italy, 5%–9% in Hong Kong, and 0% in the United Kingdom. Supporting an association with occult HBV, a high proportion of individuals with HCV infection who develop HCC have demonstrable HBV DNA and proteins in their neoplastic and adjacent nonneoplastic liver tissue. Mild hepatitis and fibrosis have been reported in some subjects more than 10 years after recovery from an acute HBV infection.[24] However, although some studies have linked the development of HCC in individuals with chronic HCV infection to occult HBV, others have not found an association. For example, in the seminal study by Beasley,[25] the incidence of HCC was significantly lower in immune persons compared with carriers (5 vs 495 per 100,000 per year).[25]

Hepatitis B vaccination is widely recognized as the most effective measure to prevent HBV infection. Confirming mortality reductions from HBV-associated complications, including HCC, in adults may require several decades. However, the results in the Taiwanese children provide the first evidence that HCC is preventable via effective vaccination.[26] Ten years after the initiation of the immunization program, the incidence of HCC among 6- to 14-year-olds in Taiwan declined dramatically. The average incidence of HCC declined from .7 per 100,000 children from 1982 to 1986 to .36 from 1990 to 1994. There was a similar decline in the mortality associated with HCC. One study[27] that used mathematic modeling estimated that for the year 2000, there were 620,000 persons who died worldwide from HBV-related causes: 580,000 (94%) from chronic infection-related cirrhosis and HCC. In the surviving birth cohort for the year 2000, the model estimated that without vaccination, 64.8 million would become HBV infected and 1.4 million would die from HBV-related disease. Routine infant hepatitis B vaccination, with 90% coverage and the first dose administered at birth, would prevent 84% of global HBV-related deaths.

HCV

Chronic HCV infection is a major risk factor for the development of HCC. Markers of HCV infection are found in a variable proportion of HCC patients; for example, 44%–66% in Italy,[28,29] 27%–58% in France, 60%–75% in Spain, and in 80%–90% of HCC patients in Japan.[10] A higher but undefined proportion of HCC patients might have had HCV detected by polymerase chain reaction testing of liver tissue and/or serum, even if antibody to HCV (anti-HCV) was nondetectable. In a meta-analysis of 21 case-control studies in which second-generation enzyme immunoassay tests for anti-HCV were used, HCC risk was increased 17-fold in HCV-infected patients compared with HCV-negative controls (95% CI, 14–22).[30]

The likelihood of the development of HCC among HCV-infected persons is difficult to determine because of the paucity of adequate long-term cohort studies; however, the best estimate is from 1% to 3% after 30 years (Figure 6). HCV increases HCC risk by promoting fibrosis and eventually cirrhosis. Once HCV-related cirrhosis is established, HCC develops at an annual rate of 1% to 4%; although rates of up to 7% have been reported in Japan. Rates of cirrhosis 25–30 years after infection range between 15% and 35%.[31] The highest incidence rates for cirrhosis and HCC were observed in HCV-contaminated blood or blood-products recipients (14 and 1 per 1000 person-years for cirrhosis and HCC, respectively) and in hemophiliacs (5 and .7 per 1000 person-years, respectively). The lowest rates have been reported in women who received a one-time contaminated anti-D immune globulin treatment (1 and 0 per 1000 person-years, respectively).

In HCV-infected patients, host and environment factors appear to be more important than viral factors in determining progression to cirrhosis. These factors include older age, older age at the time of acquisition of infection, male sex, heavy alcohol intake (>50 g/day),

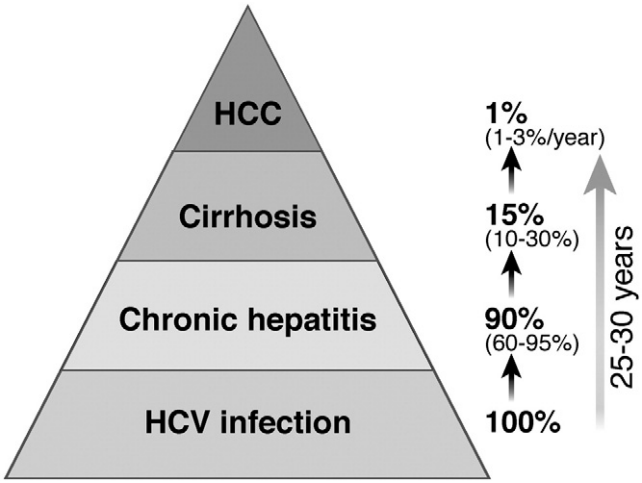

Figure 6. Proportion of patients with HCC related to viral hepatitis. In this study all US-born patients who were seen at MD Andersen Medical Center in Houston, Texas, were tested for serologic evidence of HCV and HBV. Only HCV-positive HCC increased during the study period. Reprinted with permission from Hassan et al.[20]

diabetes, obesity, and co-infection with human immunodeficiency virus or HBV.[32] There is no strong evidence that HCV viral factors such as genotype, load, or quasispecies are important in determining the risk of progression to cirrhosis or HCC.

Successful antiviral therapy in patients with HCV-related cirrhosis may decrease HCC risk moderately among patients treated with interferon,[28,29,31–38] but the evidence is weak because it comes mostly from observational and nonrandomized clinical trials. The lack of randomization in most of these studies may exaggerate treatment benefits because it is likely healthier patients who tend to get treated more frequently than those with advanced liver disease (who are known to be more likely to develop HCC).

Alcohol

Heavy alcohol intake, defined as ingestion of more than 50–70 g/day for prolonged periods, is a well-established HCC risk factor. It is unclear whether risk of HCC is altered significantly in those with low or moderate alcohol intake. Although heavy intake is associated strongly with the development of cirrhosis, there is little evidence of a direct carcinogenic effect of alcohol otherwise.

There is also evidence for a synergistic effect of heavy alcohol ingestion with HCV or HBV, with these factors presumably operating together to increase HCC risk by more actively promoting cirrhosis. For example, Donato et al[30] reported that among alcohol drinkers, HCC risk increased in a linear fashion with daily intake of more than 60 g. However, with the concomitant presence of HCV infection, there was an additional 2-fold increase in HCC risk over that observed with alcohol use alone (ie, a positive synergistic effect).

Toxic Exposures

Aflatoxin. AFB_1 is a mycotoxin produced by the *Aspergillus* fungus. This fungus grows readily on foodstuffs such as corn and peanuts stored in warm, damp conditions. Animal experiments showed that AFB_1 is a powerful hepatocarcinogen, leading the International Agency for Research on Cancer to classify it as carcinogenic.[39]

Once ingested, AFB_1 is metabolized to an active intermediate, AFB_1 -*exo*-8,9-epoxide, which can bind to DNA and cause damage, including producing a characteristic mutation in the p53 tumor-suppressor gene (p53 249^{ser}).[40] This mutation has been observed in 30%–60% of HCC tumors in aflatoxin-endemic areas.[41,42]

Strong evidence that AFB_1 is a risk factor for HCC has been supplied by person-specific epidemiologic studies performed in the past 15 years. These studies were performed after the development of assays for aflatoxin metabolites in urine, AFB_1 -albumin adducts in serum, and detection of a signature aflatoxin DNA mutation in tissues.

The interaction between AFB_1 exposure and chronic HBV infection was shown in short-term prospective studies in Shanghai, China. The urinary excretion of aflatoxin metabolites was associated with a 4-fold increase in HCC risk whereas HBV infection increased the risk 7-fold. However, individuals who both excreted AFB_1 metabolites and were HBV carriers had a dramatic 60-fold increased risk of HCC.[43]

In most areas where AFB_1 exposure is a problem, chronic HBV infection also is highly prevalent. In addition to HBV vaccination as the major preventive tactic, persons already chronically infected could benefit by eliminating AFB_1 exposure. Efforts to accomplish this goal in China[6] and Africa[42] have been launched.

Vinyl chloride. An association has been established between vinyl chloride exposure in factory workers and angiosarcoma of the liver, but not with other histologic liver tumors. A meta-analysis conducted by Boffetta et al[44] summarized the results from studies of the association between occupational exposure to vinyl chloride in relation to cancer mortality. The Standardized Mortality Ratio for liver cancers other than angiosarcoma (based on 2 large cohorts) was 1.35 (95% CI, 1.04–1.77).[45] However, these nonangiosarcoma liver tumors included HCC, bile ducts, gallbladder, and liver cancer of unknown histology. Given the small number of cancers, potential for tumor misclassification, and the borderline statistical significance, a significant association between HCC and other nonangiosarcoma tumors and vinyl chloride exposure is highly unlikely.

Nonalcoholic Fatty Liver Disease

It has been suggested that many cryptogenic cirrhosis and HCC cases represent more severe forms of nonalco-

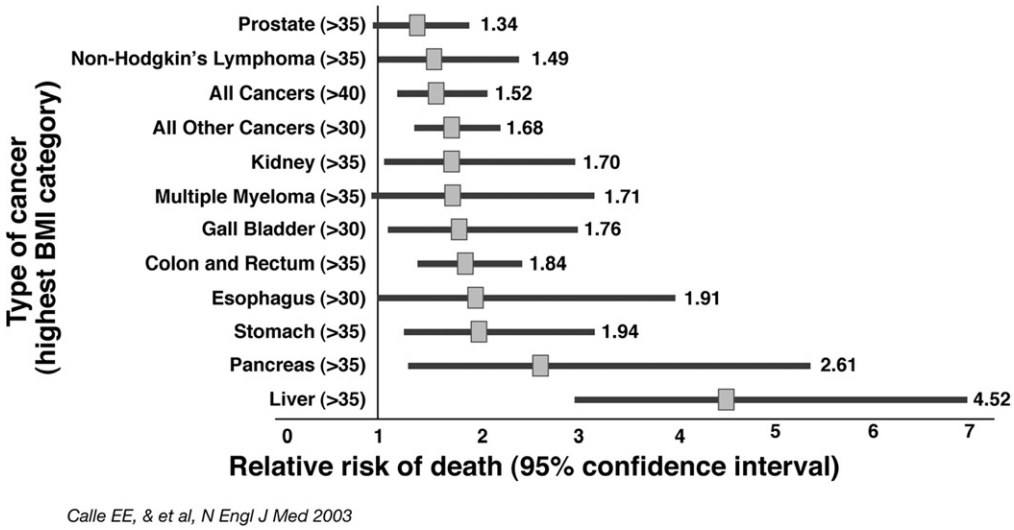

Figure 7. Mortality from cancer in obese US men. Relative risks (and 95% CIs) are shown for several types of cancer; the highest relative risk was observed for liver cancer. Modified from Calle et al.[63]

holic fatty liver disease (NAFLD), namely nonalcoholic steatohepatitis (NASH). Studies in the United States evaluating risk factors for chronic liver disease or HCC have failed to identify HCV, HBV, or heavy alcohol intake in a large proportion of patients (30%–40%). Once cirrhosis and HCC are established, it is difficult to identify pathologic features of NASH. Several clinic-based case-control studies have indicated that HCC patients with cryptogenic cirrhosis tend to have clinical and demographic features suggestive of NASH (predominance of women, diabetes, and obesity) as compared with age- and sex-matched HCC patients of well-defined viral or alcoholic etiology.[46–48] For example, Regimbeau et al[49] examined 210 patients who underwent resection for HCC, of whom 18 (8.6%) had no identifiable cause for chronic liver disease, and found a higher prevalence of obesity (50% vs 17% vs 14%) and diabetes (56% vs 17% vs 11%) compared with patients with alcoholic and viral hepatitis, respectively.[48] Evidence of progression from NAFLD to HCC from prospective studies is scant. There are case reports[50,51] and small case series describing the development of HCC several years after the NASH diagnosis.[52] In a community-based retrospective cohort study, 420 patients diagnosed with NAFLD in Olmsted County, Minnesota, were followed up for a mean duration of 7.6 years. In that study, liver disease was the third leading cause of death (as compared with the 13th leading cause of death in the general Minnesota population), occurring in 7 (1.7%) subjects. Twenty-one (5%) patients were diagnosed with cirrhosis, of whom 2 developed HCC.[53]

However, the most compelling evidence for an association between NASH and HCC is indirect and comes from studies examining HCC risk with 2 conditions strongly associated with NASH: obesity and diabetes.

Obesity

In a large prospective cohort study of more than 900,000 individuals in the United States followed up for a 16-year period, liver cancer mortality rates were 5 times greater among men with the greatest baseline body mass index (range, 35–40) compared with those with a normal body mass index[54] (Figure 7). In the same study, the risk of liver cancer was not as increased in women, with a relative risk of 1.68.[54] Two other population-based cohort studies from Sweden and Denmark found excess HCC risk (increased relative risk, 2- to 3-fold) in obese men and women compared with those with a normal body mass index.[44,55]

Strongly associated with obesity, especially abdominal obesity, insulin resistance is known to contribute significantly to hepatic steatosis.[56,57] The development of increased levels of steatosis has been associated with more severe necroinflammatory activity[49,58] and fibrosis.[58,59–64] Fibrosis progression rates also have appeared to be higher when marked steatosis was present,[65] with some studies suggesting that the increase in steatosis itself may be an indicator of fibrosis progression.[59] Finally, liver disease occurs more frequently in those with more severe metabolic disturbances,[66] with insulin resistance itself shown to increase as the disease progresses.[66]

Several developed countries, most notably the United States, are in the midst of a burgeoning obesity epidemic. Although the evidence linking obesity to HCC is relatively scant, even small increases in risk related to obesity could translate into a large number of HCC cases.

Diabetes Mellitus

Diabetes has been proposed as a risk factor for both chronic liver disease and HCC through the development of NAFLD and NASH.

Several case-control studies from the United States, Greece, Italy, Taiwan, and Japan examined the association between diabetes, mostly type II, and HCC. At least 8 studies found a significant positive association between diabetes and HCC, 2 studies found a positive association

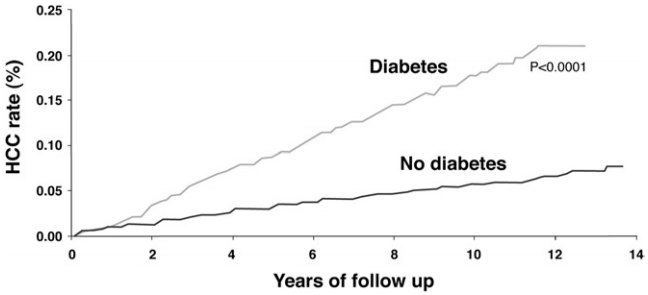

Figure 8. Diabetes and the risk of HCC. The study examined 173,463 patients with diabetes and 650,620 without diabetes. No patient had acute or chronic liver disease recorded before, during, or within 1 year of his or her index hospitalization. Reprinted with permission.[8]

that did not quite reach significance, and 1 study found a significant negative association. A potential bias in cross-sectional and case-control studies, however, is difficulty in discerning temporal relationships between exposures (diabetes) and outcomes (HCC). This problem is relevant in evaluating HCC risk factors because 10%–20% of patients with cirrhosis have overt diabetes and a larger percentage have impaired glucose tolerance. Thus, diabetes also may be the result of cirrhosis.

Cohort studies, which intrinsically are better suited to discern temporal relationships between exposure and disease, also have been conducted. All compared HCC incidence in cohorts of diabetic patients with either the expected incidence given HCC rates in the underlying population or with the observed HCC incidence among a defined cohort without diabetes.[67] Three studies conducted among younger or smaller cohorts found either no or a low number of HCC cases. At least 4 other cohort studies examined a large number of patients for relatively long time periods, with 3 studies finding a significantly increased risk of HCC with diabetes (risk ratios, 2–3).[67–69] We recently conducted a study of HCC incidence in a large cohort of VA patients (173,643 patients with and 650,620 patients without diabetes). The findings of this study indicated that HCC incidence doubled among patients with diabetes and was higher among those with a longer duration of follow-up evaluation (Figure 8).[67]

Although most studies have been conducted in low-HCC rate areas, diabetes also has been found to be a significant risk factor in areas of high HCC incidence such as Japan. Although other underlying risk factors such as HCV may confound the association between diabetes and HCC, they do not seem to fully explain observed associations between diabetes and HCC. Taken together, available data suggest that diabetes is a moderately strong risk factor for HCC.[70] Additional research is required to examine issues related to the duration and treatment of diabetes, as well as possible confounding by diet and obesity.

Tobacco

The relationship between cigarette smoking and HCC has been examined in more than 50 studies in both low- and high-rate HCC areas. In almost all countries, both positive-association and lack-of-association findings have been reported. Among studies reporting positive associations, several found effects were limited to population subgroups defined by HBV status, HCV status, genetic polymorphism, or other exposure. Given the concurrence of tobacco smoking and alcohol drinking, the confounding effect of alcohol cannot be excluded completely. Taken together, available evidence suggests that any effect of smoking on HCC is likely to be weak and limited to a subset of the general population. However, because 2 studies conducted exclusively among women reported positive associations, it has been suggested that attributable risk among women may be higher than that in men.[71,72]

Oral Contraceptives

There is experimental rationale for a possible role of oral contraceptives (OCs) in liver neoplasia. Nuclear estrogen receptors exist in hepatocytes and are increased in HCC, suggesting a hormonal responsiveness of hepatic neoplastic tissue. Estrogen and progesterone components of OCs have been shown to induce and promote liver tumors in animals.[73] Estrogens are thought to cause liver neoplasia by increasing proliferation rates, thereby increasing rates of spontaneous mutations.[74]

Several human studies have reported an increased risk of developing benign liver tumors, such as hepatic cell adenoma and focal nodular hyperplasia in women using OCs.[75] OC use also has been linked in small case series to malignant liver tumors such as HCC, mixed hepatocellular and ductal carcinoma, cholangiocarcinoma, and hepatoblastoma.[76]

The epidemiologic evidence for an association between OCs and increased risk of HCC is unclear. Several studies, generally using small sample sizes, investigated a possible link between OCs and HCC. The association between OC use and HCC risk recently was examined in a review of 12 case-control studies that included 739 cases and 5223 controls.[77] The pooled estimate of ORs (age- and sex-matched only) from all 12 studies was 1.57 (95% CI, .96–2.54, $P = .07$). Eight of these studies reported adjusted ORs (in addition to age and sex); the pooled estimate was 1.45 (95% CI, .93–2.27, $P = .11$). Six studies showed a significant 2- to 20-fold increase in HCC risk with longer durations (>5 y) of OC use. The evidence is inconclusive to establish a relation between OCs and the risk of HCC. Furthermore, there is no information on newer, low-dose OCs.

Hemochromatosis

There are very few population-based risk estimates of HCC in individuals homozygous for known *HFE* mu-

tations, and no information on the risk in carriers (heterozygous). A population-based study conducted in Sweden using multiple national data sources indicated a 1.7-fold increase in the incidence of HCC among 1800 individuals with hereditary hemochromatosis (mostly homozygous for the C282Y mutation). Much higher HCC risk estimates were reported from selected samples of patients with hemochromatosis who were seen in referral centers; however, these estimates are likely to be inflated as a result of selection bias. The proportion of patients with HCC who have HFE mutations is unclear and is likely to be small for homozygous mutations (3% in a small referral-based case series,[78] and 5% in a series of 144 cases[79]). Whether the prevalence of HFE mutations is higher than expected in patients with HCC in the absence of cirrhosis has been suggested but remains unclear.[80] Similarly, there is only preliminary evidence, which needs to be confirmed in larger studies, for a positive interaction between HFE mutations and viral hepatitis to increase the risk of HCC.[81] The proportion of HCC cases heterozygous for HFE is larger but whether significantly greater than controls is not clear. The prevalence of C282Y heterozygotes in clinical case series has ranged from 4%,[82] to as high as 11.8%,[79] and 12.5.[83] In comparisons with controls with cirrhosis (and no HCC), studies have shown conflicting findings between failure to show a statistically significant association,[82] and a 3-fold increase.[83] There is no evidence for an increased prevalence of H63D mutations in HCC cases as compared with controls.[83]

Diet

The role of diet, except for alcohol drinking and aflatoxin contamination, in the etiology of HCC is unclear. Dietary anti-oxidants, specifically selenium and retinoic acid, have been shown to inhibit liver tumors in animals. In a cohort study of men in Taiwan, lower vegetable intake was associated significantly with an increased risk of HCC and this effect was limited to hepatitis B virus chronic carriers and cigarette smokers.[84] In that study, baseline levels of serum retinol were associated inversely with future risk of HCC in chronic hepatitis B carriers. In another report on the same cohort, low baseline serum levels of selenium were also predictive of increased HCC risk.[85] A favorable effect of high intakes of milk and yogurt, white meats, eggs, and fruits has been suggested by a hospital-based case-control study.[86] Another study among Japanese atomic bomb survivors reported an approximately 50% HCC risk reduction in those with high consumption of isoflavone-rich miso soup and tofu, adjusting for HBV and HCV viral infections.[87] On the other hand, a smaller case-control conducted in Athens, Greece, did not support an association of specific food groups or particular nutrients with the risk of HCC.

Coffee drinking. Several epidemiologic studies have reported a reduced risk of increased liver enzyme levels and a reduced risk of cirrhosis. Animal studies have suggested that coffee reduces liver carcinogenesis. Further, coffee drinking also has been associated with reduced insulin levels and risk of type 2 diabetes. At least 9 epidemiologic studies conducted in Japan and Southern Europe reported on the association between coffee intake and HCC. Coffee drinking has been associated with a reduced risk of HCC in at least 5 case-control studies (25%–75% risk reduction with 2–4 cups of coffee/day as compared with none).[88–92] Three cohort studies have reported on the association between coffee intake and future risk of HCC.[93–95] Of those, 2 studies showed a significant reduction in HCC risk with coffee intake of 1 or more cups of coffee, and the second study showed a dose-response relationship (20% reduction with 1–2 cups and a 75% reduction with ≥5 cups[94]). The third publication reporting on 2 cohorts showed a reduced HCC risk with coffee drinking that was of borderline significance.[93] Overall, the data suggested a modest reduction of HCC risk with coffee drinking. Most of these studies examined general population controls, which may not be the most appropriate comparator given their low risk for HCC and chronic liver disease. However, the inverse association between coffee consumption and HCC persisted in the studies that presented results stratified by liver disease[88,93–95] or used a second control group of patients with liver disease.[90]

Genetic Epidemiology of HCC

Epidemiologic research has shown convincingly that the great majority of adult-onset HCC cases are sporadic and that many have at least 1 established nongenetic risk factor such as alcohol abuse or chronic HCV and HBV infection. However, most people with these known environmental risk factors never develop cirrhosis or HCC, whereas a sizable minority of HCC cases develop among individuals without any known risk factors.

Genetic variation has long been suspected to influence the variable risk for HCC observed both within and across populations. There is preliminary evidence indicating that host genetic factors may contribute to progression of HCV. A large study (500 patients with cirrhosis and 500 pair-matched controls with nonhepatic disease) reported a significant increase in the prevalence of cirrhosis among first-degree relatives of patients with cirrhosis, mostly HCV-related (odds ratio, 17; 95% CI, 4.2–12.9).[96] Another study reported familial clustering and potential maternal linkage for insulin resistance for NAFLD, cirrhosis, and among patients with NAFLD (as compared with healthy controls).[97] In addition, numerous familial HCC clusters have been reported; such families also tend to show clustering of hepatitis B carriers. However, 2 analyses have found evidence of genetic sus-

ceptibility to HCC, while taking into account HBV infection.[98,99]

It has, however, only recently become possible to perform large-scale epidemiologic studies to evaluate genetic risk factors given rapid advances within the field of genomics including completion of the Human Genome Project, public accessibility to information on millions of human single-nucleotide polymorphisms, development of high-throughput DNA microarrays, and a dramatic reduction in the cost of genetic testing.[100–105]

The majority of HCC studies have been case-control studies conducted in populations with a high HCC rate (Asian and African) or a medium HCC rate (European). Typically, they have examined only a few polymorphisms in a few genes selected because of the following: (1) their role in the key liver function of detoxification including phase I and phase II enzymes such as cytochrome P-450s, N-acetyl transferases, and glutathione S-transferases (GSTs), (2) their role in biological pathways potentially relevant in chronic liver disease and carcinogenesis including inflammatory response (eg, interleukin 1β, IRN) and DNA repair (eg, XRCC1), or (3) their role in mitigating or exacerbating the effects of exposure to specific etiologic risk factors for HCC such as alcohol or aflatoxin (eg, ADH3, ALDH2, EPHX1).

Results from the genetic epidemiology studies evaluating varied polymorphisms, as risk factors for HCC largely have been equivocal, with findings of a positive association, association only within a limited subset of the population, or no or negative association reported. The lack of reproducibility is attributed to inadequate sample sizes 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.[102,104] Furthermore, virtually all of these studies have lacked power to detect interactions; it is estimated that several thousand cases and controls are required to adequately assess the effects of gene–gene or gene–environment interactions. Other contributing factors include population stratification or population-based differences in the relative distribution of alleles (eg, among different racial groups), and the use of nonrepresentative control groups, and variable genetic penetrance.

Given genetic epidemiology studies often are highly underpowered, meta-analysis has been recognized as an important tool to define more precisely the effect of individual polymorphisms on relative risk of disease and to identify potentially important sources of between-study heterogeneity.[106,107] We recently completed a meta-analysis evaluating the effect of the 2 most frequently evaluated polymorphisms for HCC risk to date: the dual-deletion GST polymorphisms GST-M1 (14 studies) and GST-T1 (13 studies).[108] Individual studies for both polymorphisms reported variable findings and therefore the observed heterogeneity necessitated the use of a random-effects model. Pooled estimators suggested a possible small excess risk with either GST-M1 or GSTT1 null genotypes, although findings approached significance only for GSTT1 (OR_{GSTM1}, 1.16; 95% CI, .89–1.53; OR_{GSTT1}, 1.191; 95% CI, .99–1.44). Although overall pooled estimators for GSTM1 and GSTT1 suggest a possible small excess of HCC with the null genotype, additional studies with larger samples and conducted in other populations are needed to clarify further the role of both polymorphisms in the etiology of HCC and to investigate gene–environment interactions.

Genetic epidemiology studies in HCC, similar to several other conditions, have fallen short of early expectations that they rapidly and unequivocally would result in identification of genetic variants conveying substantial excess risk of disease and thereby establish the groundwork for effective genetic screening for primary prevention. However, recent identification of genetic risk factors for some chronic diseases such as Alzheimer's disease and breast cancer as well as the small effect exerted by individual genes and hence the very large sample size required to examine these effects have given rise to cautious optimism[103] that genetic epidemiology ultimately will provide important information on the etiopathogenesis of many chronic diseases, including HCC.

Molecular Mechanisms of Hepatocarcinogenesis

As we can see from the epidemiology, hepatocarcinogenesis is linked tightly to chronic liver damage but rarely develops in healthy liver during normal aging. One possible explanation for this tight correlation is that cancer development in the liver requires cell division, leading to the step-wise acquisition of genetic hits necessary for cellular transformation. In favor of this hypothesis, chemical-induced carcinogenesis in rodents is accelerated when liver cell division is induced by partial hepatectomy.[109] However, the frequency of replication errors in human HCC is low, thus making it unlikely to be a major mechanism of hepatocarcinogenesis.[110] Instead, human HCCs are associated with a high prevalence of chromosome copy number alterations and translocations.

The most common and unifying condition associated with hepatocarcinogenesis is cirrhosis, which develops after long latencies (20–40 y) of chronic liver disease. HCC risk remains low during chronic liver disease but exponentially increases at the cirrhosis stage. To understand the molecular basis of hepatocarcinogenesis it is of utmost importance to decipher the molecular mechanisms that increase liver tumor formation at the cirrhosis stage (Figure 9).

Molecular Mechanisms Inducing Hepatocarcinogenesis at the Cirrhosis Stage

Liver cell proliferation is increased during chronic hepatitis, but cirrhosis is characterized by decreasing hepatocyte proliferation,[111] indicating that the regenerative capacity of the liver exhausts at the cirrhosis stage. In the following section we describe 3 basic mechanisms that could accelerate carcinogenesis at the cirrhosis stage (Figure 9).

Telomere Shortening: Limitation of Regenerative Reserve and Induction of Chromosomal Instability

The telomere hypothesis of cellular aging provides a rational explanation for the limited regenerative reserve of liver cells. Telomere shortening limits the proliferative capacity of primary human cells (including hepatocytes) to a finite number of cell divisions.[112,113] Accelerated telomere shortening of liver cells occurs during chronic liver disease, resulting in an accumulation of senescent hepatocytes at the cirrhosis stage.[114] When telomeres reach a critically short length, chromosome uncapping induces DNA damage signals, cell-cycle arrest, senescence, or apoptosis.[115,116]

Telomere dysfunction also activates DNA repair pathways leading to chromosomal fusions.[117,118] When cells with fused chromosomes enter the cell cycle, breakage of fused chromosomes can lead to chromosomal gains and losses as well as chromosomal translocations in the daughter cells.[119] It has been shown that telomere shortening accelerated the initiation of liver tumors in telomerase-deficient mice in response to carcinogen treatment.[120] It is tempting to speculate that telomere dysfunction also could induce chromosomal instability in human hepatocytes at the cirrhosis stage, thereby initiating HCCs.[121] In support of this hypothesis, it has been shown that telomeres are critically short in human HCC[122,123] and on the single-cell level telomere shortening correlated with increasing aneuploidy in human HCC.[124]

Impaired Hepatocyte Proliferation: Cancer Induction by Loss of Replicative Competition

The decrease in hepatocyte proliferation itself could enhance cancer formation at the cirrhosis stage. In accordance with this assumption, chemicals inhibiting hepatocyte proliferation accelerate carcinogen-induced liver tumor formation in rats[125] as well as the expansion and transformation of transplanted hepatocytes.[126] Similarly, leukemogenesis in mice was accelerated in the context of impaired cycling of hematopoietic progenitor cells.[127] It is conceivable that abnormally proliferating hepatocytes would not expand in a healthy regenerating liver but would expand quickly and eventually transform in the growth-restrained environment of the cirrhotic liver. Telomere shortening and hepatocellular senescence contribute to the loss of replicative reserve during cirrhosis.[7,128] p21 is the primary target of p53, inducing cell-cycle arrest and senescence in response to telomere shortening.[129] In agreement with the hypothesis that impaired hepatocyte proliferation induced by telomere shortening could accelerate the cancer risk, a correlation between increased p21 expression and HCC risk has been observed in human cirrhosis.[130]

In addition to telomere shortening, aging itself impairs hepatocyte proliferation in mice by inducing CCAAT/enhancer binding protein α–mediated repression of E2 promoter binding factor.[131] A decrease in liver regenerative capacity during aging could explain the acceleration of cirrhosis progression in aged patients with chronic hepatitis[132] and could promote hepatocarcinogenesis. In agreement with this assumption, the clonogenic potential of transplanted hepatocytes is increased in liver of aged rats compared with young rats.[133] Notably, activation of the phosphoinositide-3-kinase/protein kinase β (PI3K)/Akt pathway—a frequent molecular alteration in human HCC—can neutralize the growth-inhibitory activity of CCAAT/enhancer binding protein α in liver tumors.[134]

Altered Milieu Conditions: Promoting Tumor Cell Proliferation

Alterations of the microenvironment (local factors in the liver) and the macroenvironment (systemic acting factors) could represent another factor increasing the cancer risk at the cirrhosis stage.

The restoration of liver mass is regulated tightly. In mouse models, liver regeneration after partial hepatectomy stops precisely when liver mass is restored. Similarly, in human liver transplant recipients liver size adapts to the recipient size. The molecular mechanisms controlling the regulation of liver mass have not been identified. Unlike the pancreas, liver organ size is not regulated by the size of the stem cell pool generated during development.[135] These findings indicate that liver organ mass is controlled by an interplay of growth factors. A possible scenario for liver mass regulation suggests that hepatic metabolites activate some feedback mechanisms (growth factors) that induce the growth or involution of the liver. In principle, it is possible that these mechanisms become activated in response to loss of liver cell mass and loss of liver function during cirrhosis, thus creating a growth-stimulatory macroenvironment that may accelerate cancer formation.

Cirrhosis also changes the microenvironment, which impacts on tumor formation. One of the hallmarks of cirrhosis is the activation of stellate cells, resulting in increased production of extracellular matrix proteins, cytokines, growth factors, and products of oxidative stress.[136] Many of these components have been shown to alter the proliferation of hepatocytes and could promote

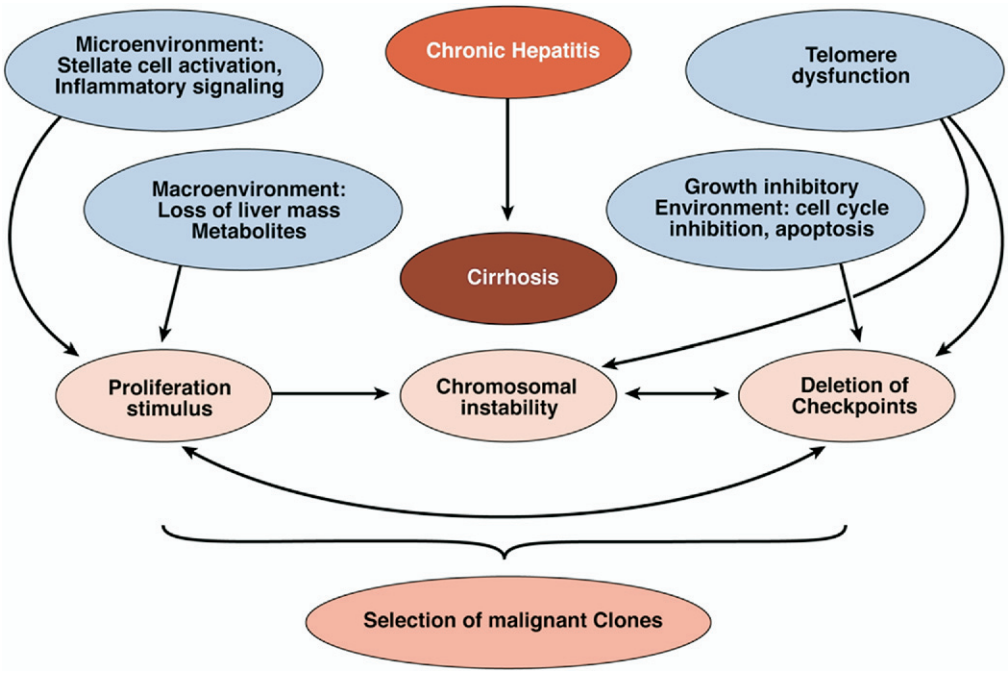

Figure 9. Molecular mechanisms inducing hepatocarcinogenesis at the cirrhosis stage. Molecular mechanisms that could explain the increased cancer risk at the cirrhosis stage are summarized. These mechanisms include cell-intrinsic and cell-extrinsic alterations. On the cell intrinsic level, cirrhosis is associated with telomere shortening. Telomere shortening leads to an activation of the cell cycle and apoptosis checkpoints that restrain the proliferative capacity of liver cells and in turn select for hepatocytes carrying deletions of checkpoint genes. In addition, telomere shortening induces chromosomal instability, which can be accelerated further by loss of checkpoint function. On the cell extrinsic level, cirrhosis induces alterations of the microenvironment including altered cytokine secretion from activated stellate cells as well as inflammatory signaling from infiltrating immune cells. Moreover, the decrease in liver function at the cirrhosis stage could increase toxic metabolites in blood serum, inducing alteration of the macroenvironment. Both alterations of the macroenvironment and the microenvironment could stimulate proliferation of hepatocytes, thus leading to a further selection of genetically altered (pre-) malignant clones.

tumor formation.[137] Moreover, inflammatory responses induced by the immune system and hepatitis could influence the progression of HCC. Along these lines, deletion of suppressor of cytokine signalling-3 (SOCS3)—a negative regulator of interleukin-6–related cytokines—promoted hepatitis-induced hepatocarcinogenesis in mice.[138] In addition, it has been shown that a unique immune response signature of the liver microenvironment correlates with venous metastases, recurrence, and prognosis of human HCC.[139] The influence of inflammatory signaling on hepatocarcinogenesis can be context dependent; deletion of nuclear factor κ B–dependent inflammatory responses enhanced HCC formation in carcinogen-treated mice.[140] Similarly, deletion of NF-kappa β essential modulator/I kappa β kinase (NEMO/IKK), an activator of nuclear factor κ B, induced steatohepatitis and HCC in mice.[141] In contrast, inhibition of nuclear factor κ B impaired HCC progression in a mouse model of cholestatic hepatitis.[142]

It has been shown that telomere shortening of human fibroblast enhances secretion of proinflammatory proteins that alter the proliferation and differentiation of cocultured epithelial cells.[143] Similar mechanisms could be active in human cirrhosis characterized by hepatocyte telomere shortening and senescence.[114,144]

Molecular Alterations in HCC and Its Possible Role in Promoting Carcinogenesis at the Cirrhosis Stage

In the following section we briefly categorize some of the most common molecular alterations in human HCC and discuss the potential role of these alterations in promoting cancer formation at the cirrhosis stage.

Loss of Cell-Cycle Checkpoints

Telomere shortening and the activation of cell-cycle checkpoints impair hepatocyte proliferation during cirrhosis.[111,114] The abrogation of DNA damage checkpoints could represent a selective advantage allowing clonal expansion of genetically altered hepatocytes at this stage. An abrogation of cell-cycle checkpoints is one of the hallmarks of hepatocarcinogenesis. Among the most commonly affected checkpoints are as follows (Figure 10).

p53, p14 alternate reading frame, mouse double minute-2. The p53 pathway is a major tumor-suppressor pathway that (1) limits cell survival and proliferation (replicative senescence) in response to telomere shortening,[115] (2) induces cell-cycle arrest in response to oncogene activation (oncogene-induced senescence),[145] and (3) protects genome integrity. The p53 pathway is af-

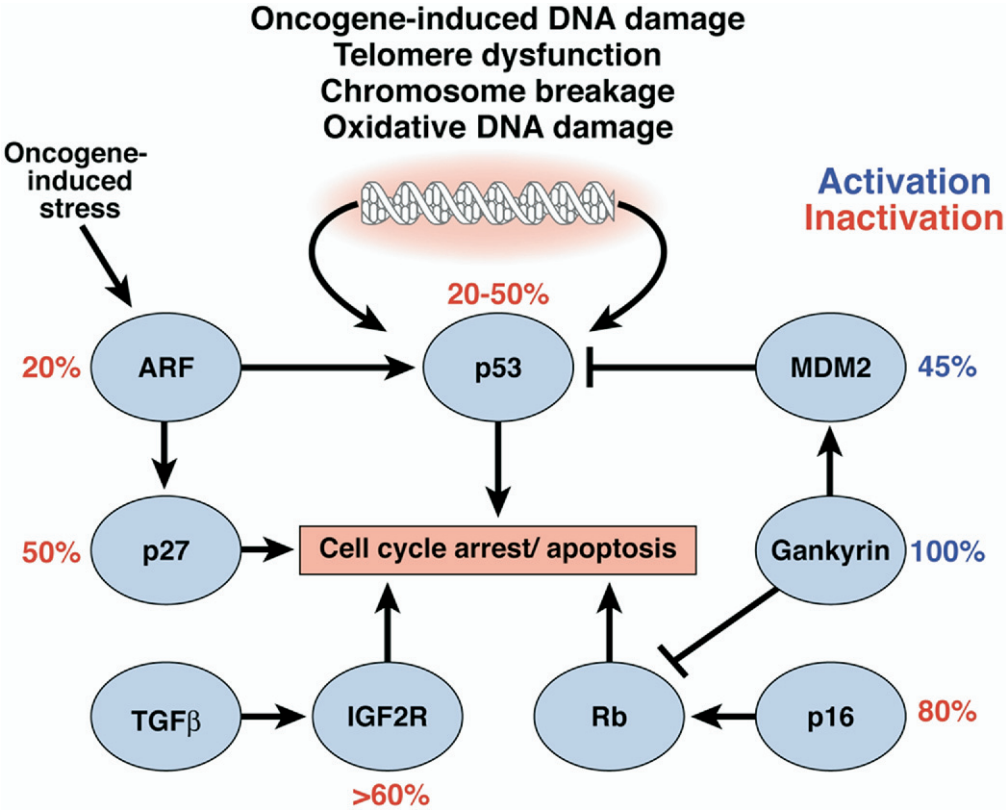

Figure 10. Alterations of cell cycle and apoptosis checkpoints associated with hepatocarcinogenesis. The most frequently occurring lesions that lead to dysfunction of the cell cycle and apoptosis checkpoints in human HCC are summarized. Four major checkpoints are affected in 60%–100% of human HCC including the p53, the Rb, the p27, and the transforming growth factor β/IGF2R pathways.

fected at multiple levels in human HCC as follows: (1) p53 mutations occur in aflatoxin[41]-induced HCC (>50%) and with lower frequency (20%–40%) in HCC not associated with aflatoxin, (2) microdeletions of p14ARF occur in 15%–20% of human HCC but rarely in p53 mutant HCC,[146] (3) increased Mdm2 expression has been observed in human HCC,[147] (4) the vast majority of human HCC overexpresses gankyrin,[148] which inhibits both Rb-checkpoint (see later) and p53-checkpoint function.[149,150] It has been shown that p53 mediates senescence in response to telomere dysfunction in mouse liver cells.[151] Thus, impairment of p53 checkpoint function at the cirrhosis stage could provide a selective advantage allowing proliferation of hepatocytes with critically short telomeres. p53 deletion increases chromosomal instability and epithelial cancer formation in telomere dysfunctional mice.[119] It seems likely that loss of p53 checkpoint function at the cirrhosis stage would lead to an expansion of hepatocytes with dysfunctional telomeres, chromosomal instability (CIN), and initiation of HCC. In agreement with this model, expression of p21 (a downstream target of p53) is increased during cirrhosis but is lost in premalignant liver tumors (small cell changes) and HCC.[123] In addition to its effect on p53 checkpoint function, deletion of p14AR7 also affects the expression of p27.[152] The down-regulation of p27 expression is a negative prognostic factor in HCC.[153]

Rb/p16. The p16/Rb checkpoint is another major pathway limiting cell proliferation in response to telomere shortening, DNA damage, and oncogene activation.[115,145] In human HCC, the Rb pathway is disrupted in more than 80% of cases, with repression of p16 by promoter methylation being the most frequent alteration.[154] Moreover, expression of gankyrin (an inhibitor of p53 and Rb checkpoint function) is increased in the vast majority of human HCCs,[149] indicating that the Rb checkpoint is dysfunctional in the vast majority of human HCCs. It is conceivable that an impairment of the Rb checkpoint would allow an expansion of hepatocytes with dysfunctional telomeres at the cirrhosis stage. In agreement with this assumption, an increase in p16 expression occurs in cirrhosis, but p16 expression is diminished in premalignant liver tumors (small cell changes) and HCC.[123] In addition, increased expression of Id1 (inhibitor of p16) correlated with the HCC risk in cirrhosis patients.[155]

Insulin-like growth factor 2 receptor. Loss of heterozygosity in the insulin-like growth factor 2 receptor (IGF2R) locus is a frequent and early event in human hepatocarcinogenesis occurring in more than 60% of dysplastic nodules and HCC.[156] IGF2R impairs cell proliferation by promoting degradation of the IGF2 mitogen and by activation of transforming growth factor β signaling.[157] Loss of IGF2R could cooperate with IGF2 overexpression, which is a common feature in human HCCs.[158] In addition, an abrogation of IGF2R could represent a selective advantage at the cirrhosis stage by impairing antiproliferative and proapoptotic signals in-

duced by transforming growth factor β, which is overexpressed in cirrhosis. Interestingly, IGF2R is located in the subtelomeric region of chromosome 6 in human beings,[159] indicating that telomere shortening could influence recombination rates and loss of heterozygosity of this locus.

Resistance to Apoptosis

The frequent inactivation of p53 in human HCC indicates that abrogation of p53-dependent apoptosis could promote hepatocarcinogenesis. In agreement with this assumption, it was shown that hepatoma subtracted-cDNA library clone one (HSCO)—an inhibitor of p53-dependent apoptosis—frequently is overexpressed in human HCC.[160] In addition, p53-independent pathways can induce apoptosis in response to telomere dysfunction.[115,161] The role of impairments of p53-independent apoptosis for hepatocarcinogenesis remains to be defined. It was shown that Hint2 (an apoptosis sensitizer acting at the mitochondria) is down-regulated in human HCC, correlating with poor prognosis.[162]

The transforming growth factor β pathway frequently is activated at the cirrhosis stage and induces apoptosis by activating Smad3-mediated BCL2 repression.[163] It remains to be determined how tumor cells escape activation of this pathway during cirrhosis and whether it involves the deletion of IGF2R. Apoptosis resistance also could involve insulin-receptor signaling and activation of the Akt pathway.[164]

Activation of Oncogenic Pathways

In general, the activation of oncogenic pathways in human HCC appears to be more heterogeneous compared with other cancer types. A possible explanation is that oncogene activation may occur late in human hepatocarcinogenesis as a consequence of chromosomal instability induced by telomere shortening and checkpoint dysfunction (see earlier). Some of the more frequently

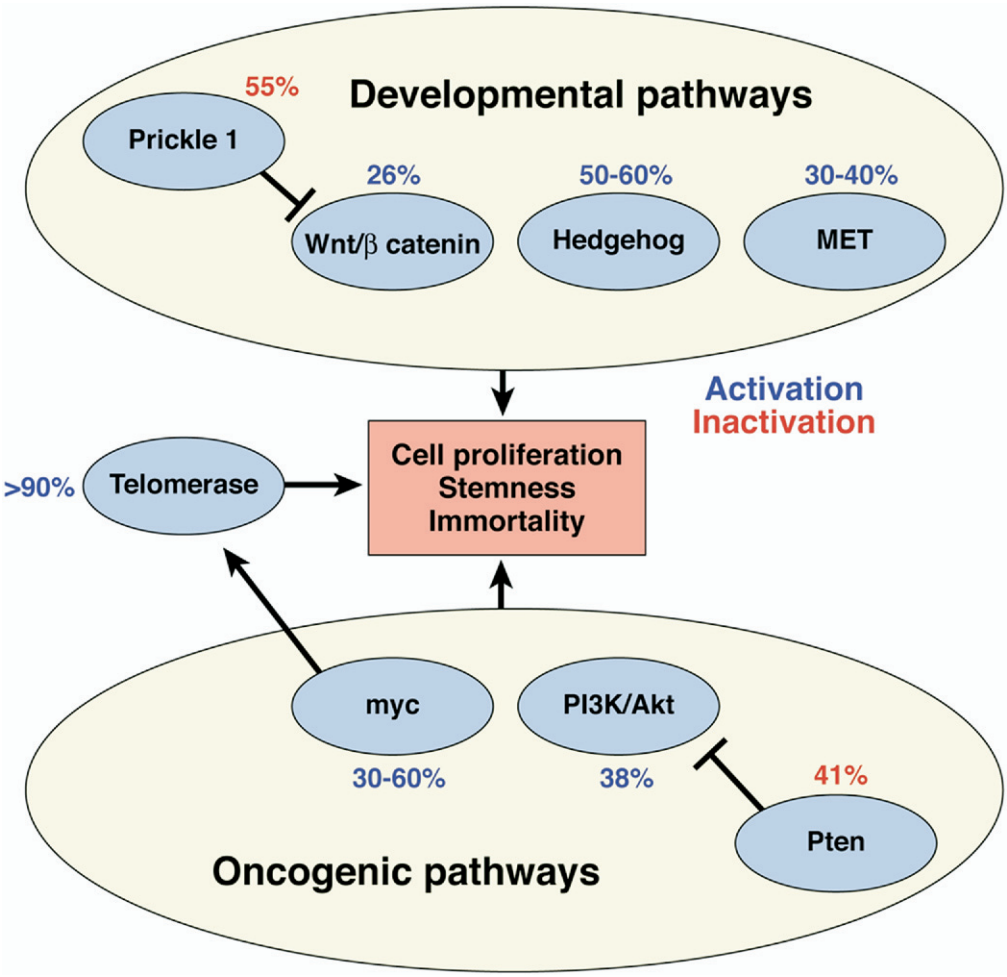

Figure 11. Alterations of developmental and oncogenic pathways associated with hepatocarcinogenesis. The most frequently occurring lesions that lead to an activation of developmental and oncogenic pathways in human HCC are summarized. Four major pathways are activated in 20%–60% of human HCC including the Akt, the myc, the β-catenin, the hedgehog, and the met pathways. In addition, telomerase activation is a very frequent event in human hepatocarcinogenesis. Although telomerase by itself is not an oncogene, it is an essential step during the immortalization of tumor cells.

found oncogenic pathways in human HCC include the following (Figure 11).

Wnt/β-catenin. The β-catenin pathway is regulated tightly during early liver development.[165] Activation of the β-catenin pathway frequently occurs in mouse and human HCC involving somatic mutations,[166] as well as transcriptional repression of negative regulators.[167] Liver-targeted deletion of adenomatosis polyposis coli (APC) and the consequent activation of the β-catenin pathway is sufficient to induce hepatocarcinogenesis in mouse models.[168] Other developmental pathways that have been implicated in human HCC are the hedgehog pathway[169] and the c-met proto-oncogene/hepatocyte growth factor receptor (MET) pathway.[170]

The activation of developmental pathways suggests that some HCC may arise from liver stem cells. Normal liver regeneration does not require stem cells because somatic hepatocytes retain the ability to re-enter the cell cycle. The growth-inhibitory environment in cirrhotic liver may lead to an activation and transformation of liver stem cells. First studies have provided evidence for the existence of stem cell populations in human HCC[171,172] and a stem cell gene expression signature in a subset of human HCC.[173]

PI3K/Akt. An activation of Akt signaling and impaired expression of phosphatase and tensin homolog (PTEN) (a negative regulator of Akt) have been reported in 40%–60% of human HCC.[174] Activation of the Akt pathway suppresses transforming growth factor β–induced apoptosis and growth-inhibitory activity of CCAAT/enhancer binding protein α (see earlier). Both effects could promote tumor formation at the cirrhosis stage. Activation of the Akt pathway has been linked to an activation of β-catenin signaling in intestinal stem cells,[175] suggesting that 2 of the most frequently activated oncogenic pathways in hepatocarcinogenesis might be interconnected.

Myc is a potent oncogene inducing hepatocarcinogenesis in mouse models and it was shown that HCCs induced by myc depend on continuous activation of the oncogene.[176] The data on human HCC are heterogeneous and further studies are required. A correlation between myc-activation and tumor size/prognosis has been reported.[177] One mechanism of myc activation in human HCC is the amplification of the chromosome locus,[178] which might be induced by telomere dysfunction and dysfunctional checkpoints. Along these lines, increased copy numbers of mouse chromosome 15 (carrying the myc-gene locus) is a frequent aberration in murine HCC induced in the context of p53 mutation and telomere shortening.[161]

Given the similarities in mouse and human HCC, an interspecies comparison will be a useful tool for further identification of oncogenes and tumor suppressors relevant to human hepatocarcinogenesis.[179] In addition, gene expression profiling represents a powerful approach to decipher the molecular pathways of hepatocarcinogenesis.[170,173,179]

Tumor Cell Immortality

More than 90% of human HCC show an activation of telomerase, and up-regulation of telomerase reverse transcriptase is a bonafide marker of human HCC.[148] Telomerase can synthesize telomeres de novo and expression of telomerase reverse transcriptase is the rate-limiting step for activation of telomerase and immortalization of human cells. Experiments on primary human cells have shown that telomerase is an essential component to generate transformed human cells with unlimited proliferative potential.[180] Telomerase is inactive in normal human liver but activation occurs during the transition from premalignant lesions to HCC.[148] Experimental data from mice have shown that telomere shortening increases the initiation of liver tumors but telomerase deficiency limits the progression of early lesions toward macroscopic tumors.[120] A current hypothesis indicates that telomerase activation is necessary to limit telomere dysfunction and to prevent the accumulation of excessive levels of chromosomal instability and DNA damage that would impair tumor growth independent of p53 checkpoint function.[161,181]

Summary

The pathophysiology of hepatocarcinogenesis is linked tightly to the evolution of cirrhosis. Several mechanisms at the cirrhosis stage appear to accelerate cancer formation including the following: (1) telomere dysfunction inducing chromosomal instability, (2) a growth-inhibitory environment selecting for proliferative cells, (3) alterations of the microenvironment and macroenvironment stimulating cellular proliferation (Figure 10). A detailed understanding of how these alterations select for distinct molecular pathways during the development of HCC could improve the screening, prevention, and treatment of HCC in patients with chronic liver disease and cirrhosis.

References

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–543.
2. Parkin DM. Cancer Incidence in five continents. IARC scientific publications volume VIII[No. 155]. 2002. Lyon: IARCPress.
3. Okuda K, Nakanuma Y, Miyazaki M. Cholangiocarcinoma: recent progress. Part 1: epidemiology and etiology. J Gastroenterol Hepatol 2002;17:1049–1055.
4. McGlynn KA, Tsao L, Hsing AW, Devesa SS, Fraumeni JF Jr. International trends and patterns of primary liver cancer. Int J Cancer 2001;94:290–296.
5. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 1997;336:1855–1859.
6. Yu SZ. Primary prevention of hepatocellular carcinoma. J Gastroenterol Hepatol 1995;10:674–682.

7. Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. Science 2000;287:1253–1258.
8. Yu MW, Chen CJ. Elevated serum testosterone levels and risk of hepatocellular carcinoma. Cancer Res 1993;53:790–794.
9. Yu MW, Yang YC, Yang SY, et al. Hormonal markers and hepatitis B virus-related hepatocellular carcinoma risk: a nested case-control study among men. J Natl Cancer Inst 2001;93: 1644–1651.
10. Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62(Suppl 1):8–17.
11. Armstrong GL, Alter MJ, McQuillan GM, Margolis HS. The past incidence of hepatitis C virus infection: implications for the future burden of chronic liver disease in the United States. Hepatology 2000;31:777–782.
12. Wong JB, McQuillan GM, McHutchison JG, Poynard T. Estimating future hepatitis C morbidity, mortality, and costs in the United States. Am J Public Health 2000;90:1562–1569.
13. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–750.
14. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127(5 Suppl 1):S27–S34.
15. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–823.
16. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004; 127:1372–1380.
17. El-Serag HB, Mason AC. Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 2000; 160:3227–3230.
18. Hassan MM, Frome A, Patt YZ, El-Serag HB. Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. J Clin Gastroenterol 2002;35:266–269.
19. Kulkarni K, Barcak E, El-Serag H, Goodgame R. The impact of immigration on the increasing incidence of hepatocellular carcinoma in the United States. Aliment Pharmacol Ther 2004;20: 445–450.
20. McMahon BJ, Alberts SR, Wainwright RB, Bulkow L, Lanier AP. Hepatitis B-related sequelae. Prospective study in 1400 hepatitis B surface antigen-positive Alaska native carriers. Arch Intern Med 1990;150:1051–1054.
21. Kao JH, Chen PJ, Lai MY, Chen DS. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. J Clin Microbiol 2002;40:1207–1209.
22. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol 2001;34:593–602.
23. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis 2002;2:479–486.
24. Yuki N, Nagaoka T, Yamashiro M, et al. Long-term histologic and virologic outcomes of acute self-limited hepatitis B. Hepatology 2003;37:1172–1179.
25. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61:1942–1956.
26. Kane MA. Global control of primary hepatocellular carcinoma with hepatitis B vaccine: the contributions of research in Taiwan. Cancer Epidemiol Biomarkers Prev 2003;12:2–3.
27. Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol 2005;34: 1329–1339.
28. Ikeda K, Saitoh S, Arase Y, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–1130.
29. Bruno S, Battezzati PM, Bellati G, et al. Long-term beneficial effects in sustained responders to interferon-alfa therapy for chronic hepatitis C. J Hepatol 2001;34:748–755.
30. Donato F, Tagger A, Gelatti U, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002;155:323–331.
31. Freeman AJ, Dore GJ, Law MG, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. Hepatology 2001; 34(4 Pt 1):809–816.
32. Cramp ME. HBV + HCV = HCC? Gut 1999;45:168–169.
33. Serfaty L, Aumaitre H, Chazouilleres O, et al. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. Hepatology 1998;27:1435–1440.
34. No authors listed. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. International Interferon-alpha Hepatocellular Carcinoma Study Group. Lancet 1998;351: 1535–1539.
35. Imai Y, Kawata S, Tamura S, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 1998;129:94–99.
36. Niederau C, Lange S, Heintges T, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–1695.
37. Valla DC, Chevallier M, Marcellin P, et al. Treatment of hepatitis C virus-related cirrhosis: a randomized, controlled trial of interferon alfa-2b versus no treatment. Hepatology 1999;29:1870–1875.
38. Okanoue T, Itoh Y, Minami M, et al. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. J Hepatol 1999;30:653–659.
39. Overall evaluations of carcinogenicity an updating of IARC monographs. IARC Monographs 1987;1-42[Suppl 7],83-87. Lyon: IARCPress.
40. Garner RC, Miller EC, Miller JA. Liver microsomal metabolism of aflatoxin B 1 to a reactive derivative toxic to Salmonella typhimurium TA 1530. Cancer Res 1972;32:2058–2066.
41. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 1991;350:429–431.
42. Turner PC, Sylla A, Diallo MS, Castegnaro JJ, Hall AJ, Wild CP. The role of aflatoxins and hepatitis viruses in the etiopathogenesis of hepatocellular carcinoma: A basis for primary prevention in Guinea-Conakry, West Africa. J Gastroenterol Hepatol 2002; 17(Suppl):S441–S448.
43. Qian GS, Ross RK, Yu MC, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 1994;3:3–10.
44. Wolk A, Gridley G, Svensson M, et al. A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control 2001; 12:13–21.
45. Boffetta P, Matisane L, Mundt KA, Dell LD. Meta-analysis of studies of occupational exposure to vinyl chloride in relation to cancer mortality. Scand J Work Environ Health 2003;29:220–222
46. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002;36:1349–1354.

47. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123: 134–140.
48. Regimbeau JM, Colombat M, Mognol P, et al. Obesity and diabetes as a risk factor for hepatocellular carcinoma. Liver Transpl 2004;10(2 Suppl 1):S69–S73.
49. Fiore G, Fera G, Napoli N, Vella F, Schiraldi O. Liver steatosis and chronic hepatitis C: a spurious association? Eur J Gastroenterol Hepatol 1996;8:125–129.
50. Cotrim HP, Parana R, Braga E, Lyra L. Nonalcoholic steatohepatitis and hepatocellular carcinoma: natural history? Am J Gastroenterol 2000;5:3018–3019.
51. Zen Y, Katayanagi K, Tsuneyama K, Harada K, Araki I, Nakanuma Y. Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. Pathol Int 2001;51:127–131.
52. Shimada M, Hashimoto E, Taniai M, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2002;37:154–160.
53. Adams LA, Lymp JF, St SJ, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–121.
54. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–1638.
55. Moller H, Mellemgaard A, Lindvig K, Olsen JH. Obesity and cancer risk: a Danish record-linkage study. Eur J Cancer 1994; 30A:344–350.
56. Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117–1123.
57. Ratziu V, Trabut JB, Poynard T. Fat, diabetes, and liver injury in chronic hepatitis C. Curr Gastroenterol Rep 2004;6:22–29.
58. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33:1358–1364.
59. Hwang SJ, Luo JC, Chu CW, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. J Gastroenterol Hepatol 2001;16:190–195.
60. Poynard T, Ratziu V, McHutchison J, et al. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003;38: 75–85.
61. Westin J, Nordlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. J Hepatol 2002;37: 837–842.
62. Wong VS, Wight DG, Palmer CR, Alexander GJ. Fibrosis and other histological features in chronic hepatitis C virus infection: a statistical model. J Clin Pathol 1996;49:465–469.
63. Ong JP, Younossi ZM, Speer C, Olano A, Gramlich T, Boparai N. Chronic hepatitis C and superimposed nonalcoholic fatty liver disease. Liver 2001;21:266–271.
64. Ohata K, Hamasaki K, Toriyama K, et al. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer 2003;97:3036–3043.
65. Rubbia-Brandt L, Quadri R, Abid K, et al. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. J Hepatol 2000;33:106–115.
66. Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology 2004;39:179–187.
67. El Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460–468.
68. Adami HO, Chow WH, Nyren O, et al. Excess risk of primary liver cancer in patients with diabetes mellitus. J Natl Cancer Inst 1996;88:1472–1477.
69. Wideroff L, Gridley G, Mellemkjaer L, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997;89:1360–1365.
70. El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol 2006;4: 369–380.
71. Evans AA, Chen G, Ross EA, Shen FM, Lin WY, London WT. Eight-year follow-up of the 90,000-person Haimen City cohort: I. Hepatocellular carcinoma mortality, risk factors, and gender differences. Cancer Epidemiol Biomarkers Prev 2002;11:369–376.
72. Tanaka K, Hirohata T, Fukuda K, Shibata A, Tsukuma H, Hiyama T. Risk factors for hepatocellular carcinoma among Japanese women. Cancer Causes Control 1995;6:91–98.
73. Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004;127(5 Suppl 1):S72–S78.
74. De BV, Welsh JA, Yu MC, Bennett WP. p53 mutations in hepatocellular carcinoma related to oral contraceptive use. Carcinogenesis 1996;17:145–149.
75. Korula J, Yellin A, Kanel G, Campofiori G, Nichols P. Hepatocellular carcinoma coexisting with hepatic adenoma. Incidental discovery after long-term oral contraceptive use. West J Med 1991;155:416–418.
76. Rosenberg L. The risk of liver neoplasia in relation to combined oral contraceptive use. Contraception 1991;43:643–652.
77. Maheshwari S, Kramer J, El-Serag HB. The association between oral contracepatives and hepatocellular carcinoma: a meta-analysis. J Hepatol (In press).
78. Cauza E, Peck-Radosavljevic M, Ulrich-Pur H, et al. Mutations of the HFE gene in patients with hepatocellular carcinoma. Am J Gastroenterol 2003;98:442–447.
79. Willis G, Bardsley V, Fellows IW, Lonsdale R, Wimperis JZ, Jennings BA. Hepatocellular carcinoma and the penetrance of HFE C282Y mutations: a cross sectional study. BMC Gastroenterol 2005;5:17.
80. Blanc JF, De Ledinghen V, Bernard PH, et al. Increased incidence of HFE C282Y mutations in patients with iron overload and hepatocellular carcinoma developed in non-cirrhotic liver. J Hepatol 2000;32:805–811.
81. Fracanzani AL, Fargion S, Stazi MA, et al. Association between heterozygosity for HFE gene mutations and hepatitis viruses in hepatocellular carcinoma. Blood Cells Mol Dis 2005;35:27–32.
82. Boige V, Castera L, de RN, et al. Lack of association between HFE gene mutations and hepatocellular carcinoma in patients with cirrhosis. Gut 2003;52:1178–1181.
83. Hellerbrand C, Poppl A, Hartmann A, Scholmerich J, Lock G. HFE C282Y heterozygosity in hepatocellular carcinoma: evidence for an increased prevalence. Clin Gastroenterol Hepatol 2003;1: 279–284.
84. Yu MW, Hsieh HH, Pan WH, Yang CS, Chen CJ. Vegetable consumption, serum retinol level, and risk of hepatocellular carcinoma. Cancer Res 1995;55:1301–1305.
85. Yu MW, Horng IS, Hsu KH, Chiang YC, Liaw YF, Chen CJ. Plasma selenium levels and risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. Am J Epidemiol 1999; 150:367–374.
86. Talamini R, Polesel J, Montella M, et al. Food groups and risk of hepatocellular carcinoma: A multicenter case-control study in Italy. Int J Cancer 2006;119:2916–2921.
87. Sauvaget C, Nagano J, Hayashi M, Spencer E, Shimizu Y, Allen N. Vegetables and fruit intake and cancer mortality in the Hiro-

shima/Nagasaki Life Span Study. Br J Cancer 2003;88:689–694.
88. Gallus S, Bertuzzi M, Tavani A, et al. Does coffee protect against hepatocellular carcinoma? Br J Cancer 2002;87:956–959.
89. Gelatti U, Covolo L, Franceschini M, et al. Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: a case-control study. J Hepatol 2005;42:528–534.
90. Tanaka K, Hara M, Sakamoto T, et al. Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case-control study in Japan. Cancer Sci 2007;98:214–218.
91. Ohfuji S, Fukushima W, Tanaka T, et al. Coffee consumption and reduced risk of hepatocellular carcinoma among patients with chronic type C liver disease: A case-control study. Hepatol Res 2006;36:201–208.
92. Montella M, Polesel J, La VC, et al. Coffee and tea consumption and risk of hepatocellular carcinoma in Italy. Int J Cancer 2007;120:1555–1559.
93. Shimazu T, Tsubono Y, Kuriyama S, et al. Coffee consumption and the risk of primary liver cancer: pooled analysis of two prospective studies in Japan. Int J Cancer 2005;116:150–154.
94. Inoue M, Yoshimi I, Sobue T, Tsugane S. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. J Natl Cancer Inst 2005;97:293–300.
95. Kurozawa Y, Ogimoto I, Shibata A, et al. Coffee and risk of death from hepatocellular carcinoma in a large cohort study in Japan. Br J Cancer 2005;93:607–610.
96. Pagliaro L, Pasta L, D'Amico G, Madonia S, Pietrosi G. Familial clustering of (mostly) HCV-related cirrhosis. A case-control study. J Hepatol 2002;37:762–766.
97. Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease 10. Clin Gastroenterol Hepatol 2006;4:1162–1169.
98. Yu MW, Chang HC, Liaw YF, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. J Natl Cancer Inst 2000;92:1159–1164.
99. Yu MW, Chang HC, Chen PJ, et al. Increased risk for hepatitis B-related liver cirrhosis in relatives of patients with hepatocellular carcinoma in northern Taiwan. Int J Epidemiol 2002;31:1008–1015.
100. Burton PR, Tobin MD, Hopper JL. Key concepts in genetic epidemiology. Lancet 2005;366:941–951.
101. Dawn TM, Barrett JH. Genetic linkage studies. Lancet 2005;366:1036–1044.
102. Cordell HJ, Clayton DG. Genetic association studies. Lancet 2005;366:1121–1131.
103. Davey SG, Ebrahim S, Lewis S, Hansell AL, Palmer LJ, Burton PR. Genetic epidemiology and public health: hope, hype, and future prospects. Lancet 2005;366:1484–1498.
104. Hattersley AT, McCarthy MI. What makes a good genetic association study? Lancet 2005;366:1315–1323.
105. Hopper JL, Bishop DT, Easton DF. Population-based family studies in genetic epidemiology. Lancet 2005;366:1397–1406.
106. Khoury MJ, Little J. Human genome epidemiologic reviews: the beginning of something HuGE. Am J Epidemiol 2000;151:2–3.
107. Little J, Khoury MJ, Bradley L, et al. The human genome project is complete. How do we develop a handle for the pump? Am J Epidemiol 2003;157:667–673.
108. White DL, Li D, Nurgelieva Z, El-Serag HB. The glutathione S-transferase variants as possible risk factors for hepatocellular carcinoma: a HuGE systematic review and meta-analysis. Gastroenterology 2007;132:A179–180.
109. Barbason H, Rassenfosse C, Betz EH. Promotion mechanism of phenobarbital and partial hepatectomy in DENA hepatocarcinogenesis cell kinetics effect. Br J Cancer 1983;47:517–525.
110. Kawai H, Suda T, Aoyagi Y, et al. Quantitative evaluation of genomic instability as a possible predictor for development of hepatocellular carcinoma: comparison of loss of heterozygosity and replication error. Hepatology 2000;31:1246–1250.
111. Delhaye M, Louis H, Degraef C, et al. Relationship between hepatocyte proliferative activity and liver functional reserve in human cirrhosis. Hepatology 1996;23:1003–1011.
112. Wege H, Chui MS, Le HT, Strom SC, Zern MA. In vitro expansion of human hepatocytes is restricted by telomere-dependent replicative aging. Cell Transplant 2003;12:897–906.
113. Levy MZ, Allsopp RC, Futcher AB, Greider CW, Harley CB. Telomere end-replication problem and cell aging. J Mol Biol 1992;225:951–960.
114. Wiemann SU, Satyanarayana A, Tsahuridu M, et al. Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. FASEB J 2002;16:935–942.
115. Wright WE, Shay JW. The two-stage mechanism controlling cellular senescence and immortalization. Exp Gerontol 1992;27:383–389.
116. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. Nature 2003;426:194–198.
117. Smogorzewska A, Karlseder J, Holtgreve-Grez H, Jauch A, de LT. DNA ligase IV-dependent NHEJ of deprotected mammalian telomeres in G1 and G2. Curr Biol 2002;12:1635–1644.
118. Maser RS, Wong KK, Sahin E, et al. DNA-dependent protein kinase catalytic subunit is not required for dysfunctional telomere fusion and checkpoint response in the telomerase-deficient mouse. Mol Cell Biol 2007;27:2253–2265.
119. Artandi SE, Chang S, Lee SL, et al. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 2000;406:641–645.
120. Farazi PA, Glickman J, Jiang S, Yu A, Rudolph KL, DePinho RA. Differential impact of telomere dysfunction on initiation and progression of hepatocellular carcinoma. Cancer Res 2003;63:5021–5027.
121. Satyanarayana A, Manns MP, Rudolph KL. Telomeres and telomerase: a dual role in hepatocarcinogenesis. Hepatology 2004;40:276–283.
122. Plentz RR, Caselitz M, Bleck JS, et al. Hepatocellular telomere shortening correlates with chromosomal instability and the development of human hepatoma. Hepatology 2004;40:80–86.
123. Plentz RR, Park YN, Lechel A, et al. Telomere shortening and inactivation of cell cycle checkpoints characterize human hepatocarcinogenesis. Hepatology 2007;45:968–976.
124. Plentz RR, Schlegelberger B, Flemming P, et al. Telomere shortening correlates with increasing aneuploidy of chromosome 8 in human hepatocellular carcinoma. Hepatology 2005;42:522–526.
125. van Gijssel HE, Maassen CB, Mulder GJ, Meerman JH. p53 protein expression by hepatocarcinogens in the rat liver and its potential role in mitoinhibition of normal hepatocytes as a mechanism of hepatic tumour promotion. Carcinogenesis 1997;18:1027–1033.
126. Laconi S, Pani P, Pillai S, Pasciu D, Sarma DS, Laconi E. A growth-constrained environment drives tumor progression in-vivo. Proc Natl Acad Sci U S A 2001;98:7806–7811.
127. Bilousova G, Marusyk A, Porter CC, Cardiff RD, DeGregori J. Impaired DNA replication within progenitor cell pools promotes leukemogenesis. PLoS Biol 2005;3:e401.
128. Satyanarayana A, Wiemann SU, Buer J, et al. Telomere shortening impairs organ regeneration by inhibiting cell cycle re-entry of a subpopulation of cells. EMBO J 2003;22:4003–4013.

129. Choudhury AR, Ju Z, Djojosubroto MW, et al. Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. Nat Genet 2007;39:99–105.
130. Wagayama H, Shiraki K, Yamanaka T, et al. p21WAF1/CTP1 expression and hepatitis virus type. Dig Dis Sci 2001;46:2074–2079.
131. Iakova P, Awad SS, Timchenko NA. Aging reduces proliferative capacities of liver by switching pathways of C/EBPalpha growth arrest. Cell 2003;113:495–506.
132. Poynard T, Mathurin P, Lai CL, et al. A comparison of fibrosis progression in chronic liver diseases. J Hepatol 2003;38:257–265.
133. Pasciu D, Montisci S, Greco M, et al. Aging is associated with increased clonogenic potential in rat liver in vivo. Aging Cell 2006;5:373–377.
134. Wang GL, Iakova P, Wilde M, Awad S, Timchenko NA. Liver tumors escape negative control of proliferation via PI3K/Akt-mediated block of C/EBP alpha growth inhibitory activity. Genes Dev 2004;18:912–925.
135. Stanger BZ, Tanaka AJ, Melton DA. Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. Nature 2007;445:886–891.
136. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209–218.
137. Giannelli G, Bergamini C, Fransvea E, Sgarra C, Antonaci S. Laminin-5 with transforming growth factor-beta1 induces epithelial to mesenchymal transition in hepatocellular carcinoma. Gastroenterology 2005;129:1375–1383.
138. Ogata H, Kobayashi T, Chinen T, et al. Deletion of the SOCS3 gene in liver parenchymal cells promotes hepatitis-induced hepatocarcinogenesis. Gastroenterology 2006;131:179–193.
139. Budhu A, Forgues M, Ye QH, et al. Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. Cancer Cell 2006;10:99–111.
140. Sakurai T, Maeda S, Chang L, Karin M. Loss of hepatic NF-kappa B activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. Proc Natl Acad Sci U S A 2006;103:10544–10551.
141. Luedde T, Beraza N, Kotsikoris V, et al. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell 2007;11:119–132.
142. Pikarsky E, Porat RM, Stein I, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–466.
143. Parrinello S, Coppe JP, Krtolica A, Campisi J. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J Cell Sci 2005;118(Pt 3):485–496.
144. Schnabl B, Purbeck CA, Choi YH, Hagedorn CH, Brenner D. Replicative senescence of activated human hepatic stellate cells is accompanied by a pronounced inflammatory but less fibrogenic phenotype. Hepatology 2003;37:653–664.
145. Di MR, Fumagalli M, Cicalese A, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 2006;444:638–642.
146. Tannapfel A, Busse C, Weinans L, et al. INK4a-ARF alterations and p53 mutations in hepatocellular carcinomas. Oncogene 2001;20:7104–7109.
147. Jablkowski M, Bocian A, Bialkowska J, Bartkowiak J. A comparative study of P53/MDM2 genes alterations and P53/MDM2 proteins immunoreactivity in liver cirrhosis and hepatocellular carcinoma. J Exp Clin Cancer Res 2005;24:117–125.
148. Llovet JM, Chen Y, Wurmbach E, et al. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. Gastroenterology 2006;131:1758–1767.
149. Higashitsuji H, Itoh K, Nagao T, et al. Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. Nat Med 2000;6:96–99.
150. Higashitsuji H, Higashitsuji H, Itoh K, et al. The oncoprotein gankyrin binds to MDM2/HDM2, enhancing ubiquitylation and degradation of p53. Cancer Cell 2005;8:75–87.
151. Lechel A, Satyanarayana A, Ju Z, et al. The cellular level of telomere dysfunction determines induction of senescence or apoptosis in vivo. EMBO Rep 2005;6:275–281.
152. Kalinichenko VV, Major ML, Wang X, Petrovic V, Kuechle J, Yoder HM, Dennewitz MB, Shin B, Datta A, Raychaudhuri P, Costa RH. Foxm1b transcription factor is essential for development of hepatocellular carcinomas and is negatively regulated by the p19ARF tumor suppressor. Genes Dev 2004;18:830–850.
153. Ito Y, Matsuura N, Sakon M, Miyoshi E, Noda K, Takeda T, Umeshita K, Nagano H, Nakamori S, Dono K, Tsujimoto M, Nakahara M, Nakao K, Taniguchi N, Monden M. Expression and prognostic roles of the G1-S modulators in hepatocellular carcinoma: p27 independently predicts the recurrence. Hepatology 1999;30:90–99.
154. Azechi H, Nishida N, Fukuda Y, et al. Disruption of the p16/cyclin D1/retinoblastoma protein pathway in the majority of human hepatocellular carcinomas. Oncology 2001;60:346–354.
155. Matsuda Y, Yamagiwa S, Takamura M, et al. Overexpressed Id-1 is associated with a high risk of hepatocellular carcinoma development in patients with cirrhosis without transcriptional repression of p16. Cancer 2005;104:1037–1044.
156. Yamada T, De Souza AT, Finkelstein S, Jirtle RL. Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. Proc Natl Acad Sci U S A 1997;94:10351–10355.
157. Dennis PA, Rifkin DB. Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci U S A 1991;88:580–584.
158. Breuhahn K, Vreden S, Haddad R, et al. Molecular profiling of human hepatocellular carcinoma defines mutually exclusive interferon regulation and insulin-like growth factor II overexpression. Cancer Res 2004;64:6058–6064.
159. Acquati F, Malgaretti N, Hauptschein R, Rao P, Gaidano G, Taramelli R. A 2-Mb YAC contig linking the plasminogen-apoprotein(a) gene family to the insulin-like growth factor 2 receptor (IGF2R) gene on the telomeric region of chromosome 6 (6q26-q27). Genomics 1994;22:664–666.
160. Higashitsuji H, Higashitsuji H, Nagao T, et al. A novel protein overexpressed in hepatoma accelerates export of NF-kappa B from the nucleus and inhibits p53-dependent apoptosis. Cancer Cell 2002;2:335–346.
161. Lechel A, Holstege H, Begus Y, Schienke A, Kamino K, Lehmann U, Kubicka S, Schirmacher P, Jonkers J, Rudolph KL. Telomerase deletion limits progression of p53-mutant hepatocellular carcinoma with short telomeres in chronic liver disease. Gastroenterology 2007;132:1465–1475.
162. Martin J, Magnino F, Schmidt K, et al. Hint2, a mitochondrial apoptotic sensitizer down-regulated in hepatocellular carcinoma. Gastroenterology 2006;130:2179–2188.
163. Yang YA, Zhang GM, Feigenbaum L, Zhang YE. Smad3 reduces susceptibility to hepatocarcinoma by sensitizing hepatocytes to apoptosis through downregulation of Bcl-2. Cancer Cell 2006;9:445–457.
164. Chen RH, Su YH, Chuang RL, Chang TY. Suppression of transforming growth factor-beta-induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway. Oncogene 1998;17:1959–1968.

165. Micsenyi A, Tan X, Sneddon T, Luo JH, Michalopoulos GK, Monga SP. Beta-catenin is temporally regulated during normal liver development. Gastroenterology 2004;126:1134–1146.
166. de La CA, Romagnolo B, Billuart P, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci U S A 1998;95:8847–8851.
167. Chan DW, Chan CY, Yam JW, Ching YP, Ng IO. Prickle-1 negatively regulates Wnt/beta-catenin pathway by promoting Dishevelled ubiquitination/degradation in liver cancer. Gastroenterology 2006; 131:1218–1227.
168. Colnot S, Decaens T, Niwa-Kawakita M, et al. Liver-targeted disruption of Apc in mice activates beta-catenin signaling and leads to hepatocellular carcinomas. Proc Natl Acad Sci U S A 2004;101:17216–17221.
169. Sicklick JK, Li YX, Jayaraman A, et al. Dysregulation of the Hedgehog pathway in human hepatocarcinogenesis. Carcinogenesis 2006;27:748–757.
170. Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. J Clin Invest 2006;116:1582–1595.
171. Yin S, Li J, Hu C, et al. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. Int J Cancer 2007;120:1444–1450.
172. Chiba T, Kita K, Zheng YW, et al. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. Hepatology 2006;44:240–251.
173. Lee JS, Heo J, Libbrecht L, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 2006;12:410–416.
174. Hu TH, Huang CC, Lin PR, et al. Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. Cancer 2003;97:1929–1940.
175. He XC, Yin T, Grindley JC, et al. PTEN-deficient intestinal stem cells initiate intestinal polyposis. Nat Genet 2007;39:189–198.
176. Shachaf CM, Kopelman AM, Arvanitis C, et al. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature 2004;431:1112–1117.
177. Wang Y, Wu MC, Sham JS, Zhang W, Wu WQ, Guan XY. Prognostic significance of c-myc and AIB1 amplification in hepatocellular carcinoma. A broad survey using high-throughput tissue microarray. Cancer 2002; 95:2346–2352.
178. Wilkens L, Flemming P, Gebel M, et al. Induction of aneuploidy by increasing chromosomal instability during dedifferentiation of hepatocellular carcinoma. Proc Natl Acad Sci U S A 2004; 101:1309–1314.
179. Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, Fan ST, Luk JM, Wigler M, Hannon GJ, Mu D, Lucito R, Powers S, Lowe SW. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell 2006;125:1253–1267.
180. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. Nature 1999;400:464–468.
181. Djojosubroto MW, Chin AC, Go N, et al. Telomerase antagonists GRN163 and GRN163L inhibit tumor growth and increase chemosensitivity of human hepatoma. Hepatology 2005;42:1127–1136.
182. Fattovich G, Giustina G, Schalm SW, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Hepatology 1995;21:77–82.
183. Hu KQ, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. Hepatology 1999;29: 1311–1316.

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