Chronic infection with the hepatitis B virus has been linked epidemiologically to the development of hepatocellular carcinoma for more than 30 years. Although the mechanisms by which chronic hepatitis B viral infection results in hepatocellular carcinoma are unclear, there is good evidence that the virus itself exerts a direct hepatocarcinogenic effect, and this has implications for prevention. First, programs of universal infant vaccination have been shown to be effective in reducing the rate of hepatocellular carcinoma among children. This benefit should be translated into adulthood among vaccine recipients. Second, it has been suggested that antiviral therapy against hepatitis B may reduce the risk of hepatocellular carcinoma. Antiviral therapy against hepatitis B is effective in causing prolonged lowering of serum levels of hepatitis B virus DNA. There are emerging data showing that prolonged antiviral therapy may reduce the risk of hepatocellular carcinoma among certain patients with chronic hepatitis B. (HEPATOLOGY 2009;49:S56-S60.)

Introduction

As early as 1970, chronic infection with the hepatitis B virus (HBV) was noted to be associated with the development of hepatocellular carcinoma (HCC).1 Subsequent studies during the 1970s and 1980s found that more than 80% of patients with HCC in high-incidence areas, such as East Asia and sub-Saharan Africa, were seropositive for hepatitis B surface antigen (HBsAg), whereas population controls typically had rates of HBsAg of 10% to 15%. Furthermore, more than 90% of HCC cases had antibody to hepatitis B core antigen detected in serum, serological evidence of active or previous HBV infection, substantially more frequently than in controls. A powerful substantiation of the association between HBV infection and HCC was the result of a prospective cohort study reported by Beasley and colleagues in 1981.2 These investigators followed more than 22,000 male municipal workers in Taiwan and found that those who were seropositive for HBsAg had rates of HCC that were highly significantly greater than the rates in uninfected controls. They calculated the relative risk for HCC among those who were HBV-infected to be 63 in comparison with uninfected controls.

Epidemiology

HCC is one of the most frequent solid tumors occurring worldwide. In 2002, the most recent year for which comprehensive data are available, the estimated number of cases of HCC that developed worldwide that year was 625,000.3 Because of the exceedingly high mortality rate of HCC, the incidence rate of this cancer is almost equivalent to the mortality rate. More than 80% of these cases occurred among individuals in developing countries, and the male-to-female ratio was approximately 2.4 to 1. In the United States, the American Cancer Society has estimated that about 18,000 deaths occurred because of cancer of the liver and intrahepatic bile ducts (mostly HCC) in the United States in 2008, again with a strong male preponderance.4 Of some concern is the fact that the rate of HCC deaths appears to have increased by about 40% over the period of 1990 to 2004, whereas the overall rate of cancer deaths has declined by about 18% during this same period. Although much of this increase in the United States has been attributed to hepatitis C, there is speculation that it may be due in part to an increase in HBV-related HCC, particularly among immigrants from endemic countries.
More recent cohort studies have confirmed the high risk of HCC in HBsAg-positive individuals as originally identified in the Beasley study. An example is the Haimen City cohort, which included about 11,000 HBsAg-positive subjects followed over a mean period of 8 years.5 The relative risk of HCC in HBsAg-positive persons compared to HBsAg-negative controls was 18.8 for men and 33.2 for women. Interestingly, a long-term follow-up study of apparently healthy blood donors in Italy found that only 0.6% developed HCC over an average period of follow-up of 29 years.6 This rate was no different than the 0.6% rate of HCC in a group of HBsAg-negative blood donor controls followed for a similar period of time. The reason for this apparently lower risk of HCC is not known, but it may be due to the Western cohort having milder, inactive hepatitis B in comparison with the typical Asian cohorts. Furthermore, in Western populations, hepatitis B is usually acquired during adolescence or adulthood, commonly through sexual contact, rather than in infancy from maternal-infant spread. These differences in epidemiology may modulate the risk of HCC.

Risk Factors for HCC

Known risk factors for HCC include chronic viral hepatitis, cirrhosis, heavy alcoholism, nonalcoholic fatty liver disease, and certain inherited metabolic conditions such as hemochromatosis and alpha-1-antitrypsin deficiency. The proportion of cases of HCC associated with these risk factors has been estimated. In Africa and East Asia, the largest attributable fraction is due to hepatitis B (60%), whereas in the developed Western world, approximately 20% of cases can be attributed to HBV infection.7

In a retrospective survey of HCC referred to 13 liver disease centers in the United States, 700 cases were identified, 20.1% of which were seropositive for HBsAg (including 4.7% who were also seropositive for antibody to hepatitis C virus)8 (Fig. 1). Interestingly, among Asians, 55.1% had HBsAg versus only 9.2% of whites, while African Americans had intermediate rates of HBsAg positivity. Many of the Asians with HBV-related HCC were foreign-born. These findings highlight the heterogeneity of patients with HCC in the United States.

Pathogenesis

The mechanism by which HBV infection causes HCC is not completely known (reviewed by Blum et al.9; see Table 1). Evolution to HCC may be the direct effect of the virus itself, or it may be an indirect effect through the process of the inflammation, regeneration, and fibrosis associated with cirrhosis due to the HBV infection. HBV DNA has been shown to become integrated within the chromosomes of infected hepatocytes, the integration of viral genetic material occurring in a critical location within the cellular genome. For example, integration of HBV DNA has been observed within the retinoic acid receptor alpha gene and within the human cyclin A gene, both playing crucial roles in cellular growth. However, in many if not most cases, the HBV DNA integration site does not appear to be in a critical location, and the process appears to be random. Furthermore, the length and the components in the HBV DNA integrant vary considerably, and the viral DNA may be rearranged, deleted, or present in repeats.10 These findings suggest that it is not the process of integration itself that leads to HCC.

The hepatitis B x (HBx) gene product has been implicated in causing HCC because it is a transcriptional activator of various cellular genes associated with growth control.11 The HBx gene expression is also associated with activation of the Ras–Raf–mitogen-activated protein kinase pathway, an important cellular pathway that has been implicated in hepatocarcinogenesis. In addition, HBx has been found to interact with p53, interfering with its function as a tumor suppressor. Another viral gene

Table 1. Possible Mechanisms of HBV-Induced HCC9

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<thead>
<tr>
<th>Direct</th>
<th>Integration of HBV DNA into chromosomes of hepatocytes</th>
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<tbody>
<tr>
<td></td>
<td>● Integration within or near functional cellular genes</td>
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<tr>
<td>HBx protein</td>
<td>● HBx is a transcriptional activator.</td>
</tr>
<tr>
<td></td>
<td>● It activates the Ras-Raf-MAPK pathway.</td>
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<td></td>
<td>● It interacts with p53, a tumor suppressor.</td>
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<td></td>
<td>The truncated HBsAg gene product is a transactivator.</td>
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<tr>
<th>Indirect</th>
<th>Inflammation and regeneration associated with chronic HBV infection</th>
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<td>Via cirrhosis associated with chronic HBV infection</td>
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hepatitis C is a common cause of cirrhosis. In keeping with the suggestion that HCC may be directly related to HBV infection is the observation from several studies that elevated serum levels of HBV DNA (a marker of higher levels of HBV replication) are associated with a higher risk of HCC. A recent longitudinal study of 3653 HBsAg-positive subjects in Taiwan found that an elevated serum level of HBV DNA (>10,000 copies/mL; ~2000 IU/mL) at baseline was a strong predictor of subsequent development of HCC and the association was independent of serum hepatitis B e antigen status, serum aminotransferases levels, or the presence of cirrhosis. Although these data were compelling, serial measurements of serum HBV DNA levels were not available from the cohort. Thus, data are lacking on the impact of persistently high serum HBV DNA levels versus changing or fluctuating serum HBV DNA levels.

Another line of evidence suggesting a direct hepatocarcinogenic role of HBV is the association of certain genotypes with higher rates of HCC. Thus, in Asian cohorts, HBV genotype C is generally thought to increase the risk of HCC above that of genotype B. It has been speculated that this may be because patients infected with genotype C are likely to remain seropositive for hepatitis B e antigen for longer periods and thus have higher serum levels of HBV DNA for a greater period of time. However, studies in other populations have found genotype B or even genotype F to be more strongly associated with HCC. Thus, the exact role of HBV genotype in hepatocarcinogenesis remains to be clarified.

Consistent with the hypothesis that HBV-related HCC may occur indirectly via cirrhosis is the observation that approximately 70% of cases of HBV-related HCC occur in association with cirrhosis, although the rate of cirrhosis appears to be lower in younger patients with HCC. Cirrhosis, independently of its cause (alcohol, hepatitis C, or metabolic errors), is associated with a high rate of HCC. Thus, the high rate of HCC in persons with chronic HBV infection may merely reflect the fact that hepatitis C is a common cause of cirrhosis.

Animal Models

HBV is a member of the hepadnavirus family, which includes several rodent and avian viruses such as woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus, and duck hepatitis B virus (DHBV). Each of these agents may result in chronic infection, but only WHV is consistently associated with the development of HCC. Thus, data derived from woodchucks experimentally infected with WHV indicate that almost all animals with chronic infection will develop HCC after approximately 3 to 4 years of life, whereas the cancer is not found in animals that have never been infected. Interestingly, ducks chronically infected with DHBV do not appear to be at high risk for HCC. The difference in HCC risk between woodchucks and ducks with chronic hepadnavirus infection may be due to the fact that DHBV lacks the HBx gene or a similar analog, and this provides further supporting evidence for a role of the HBx gene product in hepatocarcinogenesis.

Screening and Surveillance for HCC

Because individuals with chronic HBV infection can be readily identified and because they are known to be at risk for HCC, surveillance programs have become institutionalized as a means of identifying HCC at an early stage when more treatment options are available. An example is a surveillance program for HBsAg-positive persons in Alaska that has now been in existence for several decades. Experience with this program has shown that HCC can be routinely detected at an early stage, and this has led to improved patient survival in comparison with the era before surveillance.

The American Association for the Study of Liver Diseases has promulgated recommendations for the screening of HBsAg-positive individuals in a practice guideline published in 2005. Essentially, HBsAg-positive patients who have active liver disease or who are older or have a family history of HCC are recommended for regular surveillance with ultrasound examination every 6 to 12 months (Table 2).

Prevention

Considerable progress has been made in the prevention of HBV-related HCC, specifically through the implementation of large-scale programs of vaccination against hepatitis B. Thus, many countries have introduced universal infant vaccination, particularly in regions in which there is a high incidence of HCC. One of the best examples is in Taiwan, where universal infant vaccination was

<table>
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<th>Table 2. AASLD Recommendations for HCC Surveillance Among HBsAg Carriers</th>
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<tr>
<td>Asian males over the age of 40 years</td>
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<tr>
<td>Asian females over the age of 50 years</td>
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<tr>
<td>All patients with cirrhosis who are seropositive for HBsAg</td>
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<tr>
<td>Those with a family history of HCC</td>
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<tr>
<td>Those who were born in Africa and are over the age of 20 years</td>
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<tr>
<td>Patients with high serum levels of HBV DNA and ongoing hepatic injury</td>
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Abbreviations: AASLD, American Association for the Study of Liver Diseases; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma. 

In keeping with the suggestion that HCC may be directly related to HBV infection is the observation from several studies that elevated serum levels of HBV DNA (a marker of higher levels of HBV replication) are associated with a higher risk of HCC. A recent longitudinal study of 3653 HBsAg-positive subjects in Taiwan found that an elevated serum level of HBV DNA (>10,000 copies/mL; ~2000 IU/mL) at baseline was a strong predictor of subsequent development of HCC and the association was independent of serum hepatitis B e antigen status, serum aminotransferases levels, or the presence of cirrhosis. Although these data were compelling, serial measurements of serum HBV DNA levels were not available from the cohort. Thus, data are lacking on the impact of persistently high serum HBV DNA levels versus changing or fluctuating serum HBV DNA levels.

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introduced in the mid-1980s. This national program has been shown to be associated with a decrease in the rate of HBsAg positivity among children and infants from nearly 10% in 1984 to only 1.3% in 1994.\textsuperscript{24,25} In conjunction with this decrease in hepatitis B, there has been a significant reduction in the incidence of childhood HCC, much of which was HBV-related. Thus, the rate of childhood HCC (between the ages of 6 and 14 years) was 0.7 per 100,000 population in the early 1980s prior to the introduction of the vaccine, whereas between 1996 and 1999, it fell to 0.19 per 100,000 population.\textsuperscript{24,25} It is reasonable to expect that as these vaccinated children grow into adulthood, they will remain protected against hepatitis B and the risk of HCC. Similar success is also likely in the other countries that have introduced universal infant vaccination. Some barriers to full implementation of a policy of universal infant vaccination against hepatitis B in underdeveloped countries include the cost of the vaccine, the availability of health programs to deliver the vaccine, and technical issues such as the lack of reliable refrigeration in areas in which the vaccine is administered.

The other main approach to preventing HBV-related HCC is antiviral therapy of hepatitis B. The risk of developing HCC appears to be greatest among individuals with the highest serum levels of HBV DNA. From these findings, it follows that the risk of HCC might be reduced by therapy that successfully lowers HBV DNA levels. The introduction of the nucleoside analogs as therapy for chronic hepatitis B has provided a safe and effective means of accomplishing this outcome. The largest and most compelling study suggesting that antiviral treatment might decrease the risk of HCC was a randomized, controlled trial of lamivudine versus placebo in patients with advanced chronic hepatitis B and high serum levels of HBV DNA.\textsuperscript{23} The primary outcome of the study was progression of liver disease, including an increase in the Child-Pugh score, bleeding from esophageal varices, and the development of HCC. The study was halted early because of an excess of serious outcomes among the placebo recipients. Indeed, the rate of HCC by that time was 3.9% among lamivudine recipients versus 7.4% among placebo recipients, and this difference reached statistical significance ($P = 0.047$).

Summary and Needs for Future Research

HBV is the single most common cause of HCC worldwide. HBV-related HCC is most common in developing countries, particularly in the Far East and sub-Saharan Africa. Although the pathogenesis of HBV-related HCC remains uncertain, there is strong evidence of HBV itself being a direct cause of HCC. Population-based vaccination programs against HBV have been associated with reductions in the incidence of HCC, and it is thought that widespread programs of universal infant vaccination will have the potential to dramatically reduce the incidence of HCC in the future. Although the impact of antiviral therapy is also uncertain, there is good evidence that prolonged suppression of HBV replication with nucleoside or nucleotide analogs may reduce the risk of HBV in patients with chronic hepatitis B.

The most important challenges remaining in the area of hepatitis B and HCC are the development of improved means of early detection and treatment. Currently, HBV-related HCC is often detected late at a time when surgical interventions and liver transplantation are no longer feasible. Inexpensive, easily applied, and noninvasive biomarkers for HCC in high-risk patients would be helpful in identifying patients at a stage at which the tumor can be resected or more successfully treated. Better imaging methods that reliably separate small HCCs from regenerative nodules (which are common in patients with cirrhosis) would complement screening using biomarkers. Therapies for HCC are also critically needed. Standard cancer chemotherapy is largely ineffective against HCC, and newer, noncytolytic approaches are now becoming practical and have great promise. Finally, better approaches to universal HBV vaccination and identification and treatment of chronic hepatitis B are needed to allow for the application of the many advances that have been made in the understanding, prevention, and treatment of this important form of cancer.

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