

Hepatitis B Virus-related Hepatocellular Carcinoma: Epidemiology and Pathogenic Role of Viral Factors

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Chronic hepatitis B virus (HBV) infection is the primary risk factor for the development of hepatocellular carcinoma worldwide. After decades of chronic hepatitis, about 30–40% of patients progress into liver cirrhosis, and of them, around 1–5% subsequently develop hepatocellular carcinoma (HCC) annually. Since the carcinogenic process involves the interplay between the hepatitis virus and the host hepatocytes, both genomes contribute to the final pathogenic outcome, either individually or synergistically. Studying the genetic factors predisposing hepatocarcinogenesis in both host and viral genomes will help illuminate the critical carcinogenic mechanisms, and create molecular targets for future therapy. In this article, we thus review the epidemiology of HBV-related HCC and viral factors involved in hepatocarcinogenesis. [*J Chin Med Assoc* 2007;70(4):141–145]

Key Words: epidemiology, genomics, hepatitis B virus, hepatocellular carcinoma, viral factor

Global Trends in the Etiology and Incidence of HCC

Hepatocellular carcinoma (HCC) is the 5th most common cancer in men and 8th in women in the world, with an estimated 0.5–1 million new cases per year.^{1,2} Overall, 75–80% of global HCC cases are attributable to persistent viral infections with either hepatitis B virus (HBV) (50–55%) or hepatitis C virus (HCV) (25–30%).³ However, strong geographic correlations have been found between the incidence of HCC and the prevalence of hepatitis B surface antigen (HBsAg) or antibody to hepatitis C virus (anti-HCV).^{3–11} For example, in HBV-endemic areas such as Taiwan, chronic HBV infection has the strongest association with the development of HCC and accounts for 75–80% of all HCC cases.^{3–6} But in certain parts of Southern Taiwan where chronic HCV infection is prevalent, evidence

of chronic HCV infection is found in 30–50% of HCC cases.^{3–6}

Geographical variability in the incidence of HCC has been noted in recent decades, which is largely explained by the changing distribution and the natural history of HBV and HCV infection. HCC is the first human cancer amenable to prevention by using HBV vaccines. From a global perspective, the burden of chronic HBV infection should begin to decline because of the increasing utilization of HBV immunization since the early 1980s.^{12,13} Recent studies demonstrated that the Taiwanese mass vaccination program against HBV has significantly reduced the carrier rate of HBsAg in children and adolescents and, as anticipated, the incidence of childhood HCC.^{14,15} After universal hepatitis B immunization in Taiwan, the average annual incidence of HCC in children 6–14 years of age declined gradually (0.70 per 100,000 children in

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1981–1986, 0.57 in 1986–1990, and 0.36 in 1990–1994). Nevertheless, a substantial decrease in HCC incidence in adults may be observed 3–4 decades later.^{16,17} Similar effects will be seen in other HBV-endemic areas.

In contrast, the incidence of HCC has risen in the past 10–20 years in several developed countries such as the United States, Japan, England, and France where HBV is not endemic.^{17–26} Although the reasons for the trend are not clear, a recent review suggested that a cohort effect related to HCV infection may likely contribute to the increasing disease burden in these countries.¹⁷

Viral Factors in HBV-related HCC

Chronic infection with HBV is a major risk factor for the development of HCC. The hepatocarcinogenesis in HBV infection has been extensively analyzed, and multiple factors appear to play a role. A major factor is chronic necroinflammation and subsequent fibrosis/liver cell proliferation. Nevertheless, HCC only occurs in a small proportion of HBsAg carriers. Since the hepatocarcinogenic process involves the interplay between HBV and host hepatocytes, both genomes may contribute to the final pathogenic outcomes, either individually or synergistically. It is therefore reasonable to speculate that apart from host factors, viral factors are very likely involved in HBV-related hepatocarcinogenesis. In the following section, we will summarize the current understanding on the role of viral proteins and viral genomic variants in hepatocarcinogenesis.

HBV proteins associated with hepatocarcinogenesis

In addition to carcinogenic events indirectly produced by decades of chronic inflammation accompanied by persistent necrosis and regeneration, HBV may encode oncogenic viral proteins that possibly contribute to hepatocarcinogenesis. For example, HBx is a well-known viral non-structural gene that operates as a multifunctional regulator modulating gene transcription, cell responses to genotoxic stress, protein degradation, apoptosis, and several signaling pathways.^{27–30} While the specific mechanisms are still unknown, its critical role in liver malignant transformation has been clearly demonstrated by transgenic mice with HBx overexpression.^{31,32} Recently, the deletion at the pre-S region of HBsAg has been identified to be more prevalent in patients with progressive liver diseases than in inactive carriers.³³ The resulting truncated HBsAg is found to accumulate in endoplasmic reticulum (ER)

and induce ER stress;³⁴ however, its significance in hepatocarcinogenesis warrants further clarification.

Role of HBV genotype, basal core promoter (BCP) mutation and viral load in hepatocarcinogenesis

In addition to viral oncogenic proteins, several viral factors, including genotype, BCP mutation, and viral load have been confirmed to be associated with hepatocarcinogenesis. Based on genomic sequence divergence,^{35,36} there are 8 HBV genotypes (A–H) with distinct geographical and ethnic distributions: genotypes A and D prevail in Africa, Europe, and India; genotypes B and C in Asia; genotype E only in West Africa; and genotype F in Central and South America.³⁷ HBV genotype has been shown to affect clinical outcome and treatment responses. In Asia, genotype C is found to be more commonly associated with severe liver diseases, liver cirrhosis, and HCC compared with genotype B,^{28,31–34,38} in Western countries, genotype D is more associated with severe liver disease and a higher incidence of HCC than genotype A.³⁹ Intriguingly, in a large Taiwanese community cohort, the risk of HCC starts to increase when viral load is greater than 10,000 copies or 2,000 IU/mL.³⁸ In addition to viral genotype, specific viral genomic mutations also correlate with HCC risk, particularly BCP T1762/A1764 mutation.^{40,41} Nevertheless, these correlations need to be confirmed by prospective cohort studies and await further *in vitro* functional investigations.

Integrated analysis of HBV factors in HCC

In previous studies, genotype C, BCP T1762/A1764 mutation, and high viral loads have been shown to carry an increased risk of HCC.^{38,40,42,43} However, confounders may exist when analyzing only one or few viral factors each time. In our cross-sectional retrospective hospital-based study, we comprehensively investigated the independent and interactive effects of each known viral factor on the development of HCC.⁴⁴ We found that advanced age, male gender, precore A1896 mutation, BCP T1762/A1764 mutation, and viral load $> 10^5$ copies/mL were independently associated with the development of HCC. Compared with a viral load $< 10^5$ copies/mL and BCP A1762/G1764 wild-type strain, the adjusted odds ratio of HCC development was > 30 in patients with a viral load $> 10^5$ copies/mL and BCP T1762/A1764 mutant.

Viral factors in young-onset HCC

A previous study revealed that age-related differences in the clinicopathologic characteristics of HCC patients existed.⁴⁵ However, little is known about the role of

viral factors in the carcinogenesis of HCC in young people. To clarify this issue, 183 HBV-related HCC patients and 202 HBV carriers were enrolled.⁴⁶ We compared the serum viral loads in young (≤ 40 years of age) and old (> 40 years of age) age groups and showed that high serum HBV DNA levels were associated with the development of HCC in old patients rather than young patients. In addition, our previous study demonstrated that genotype B was significantly more common in patients with HCC aged < 50 years compared with age-matched inactive carriers in Taiwan (80% vs. 52%; $p=0.03$).⁴⁰ This predominance was even more remarkable in younger patients with HCC, being 90% in those aged < 35 years, and most were non-cirrhotic. These data suggested that certain genotype B HBVs may be associated with the development of HCC in young non-cirrhotic carriers.⁴⁰ Similar findings were reported in Taiwanese pediatric patients.⁴⁷ Among 460 HBV carrier children being followed up to 15 years, 26 children with HBV-related HCC were documented, and genotype B was the major genotype (74%). Hence, viral factors in association with the development of HBV-related HCC in young patients are different from their old-aged counterparts.

Viral factors in non-cirrhotic HCC

Studying HBV-related non-cirrhotic HCC may help clarify the effect of viral factors in HCC development. In a hospital-based age- and genotype-matched setting, we examined the role of BCP T1762/A1764 mutation, precore A1896 mutation, and serum viral load in non-cirrhotic hepatocarcinogenesis by comparing 44 patients with HBV-related non-cirrhotic HCC, 45 with chronic hepatitis B, and 42 with HBV-related cirrhotic HCC.⁴⁸ By multiple logistic regression analysis, male gender, BCP T1762/A1764 mutation, and viral load $> 10^5$ copies/mL were independently associated with the risk of non-cirrhotic HCC. We thus suggested that virologic characteristics might be similar between cirrhotic and non-cirrhotic HCC patients.

Role of pre-S deletion in HCC

In addition to the above common viral factors, pre-S deletion of HBV has recently been shown to be associated with the progress of liver disease and the development of HCC in HBV carriers. The interactions among pre-S deletion, PC mutation, and BCP mutation in various stages of chronic HBV infection were thus investigated in 46 HBV chronic carriers and 106 age-matched carriers with different stages of liver diseases including 38 with chronic hepatitis, 18 with liver cirrhosis, and 50 with HCC.³³ By logistic regression

analysis, patients with pre-S deletion and BCP mutation were significantly associated with the development of progressive liver diseases than those without. Combination of mutations rather than single mutation was associated with the development of progressive liver diseases, especially in combination with pre-S deletion. Sequencing analysis showed that the deleted regions were more often in the 3' terminus of pre-S1 and the 5' terminus of pre-S2. Our data indicate that patients with progressive liver diseases including HCC have a higher frequency of pre-S deletion.

Summary and Perspectives

Our serial studies strongly indicate that several viral factors are critically involved in the development of HCC.^{37,42-44,46,48,49} However, why these viral factors contribute to higher risks of HCC development remains largely unknown. Previous studies suggested the following mechanisms. First, integration of HBV DNA into host cellular DNA, in some situations, acts to disrupt or promote expression of cellular genes. Second, truncated pre-S2/S, X, and spliced proteins expressed from these integrated genes may have a direct effect on cellular functions and account for their association with HCC.⁵⁰ Taken together, more investigations are needed to clarify the role of these viral factors in each stage of liver disease progression and in hepatocarcinogenesis. Furthermore, since host genomic background also contributes to final pathogenic outcome, integrating the genetic factors in both host and viral genomes leading to a predisposition to hepatocarcinogenesis will help illuminate the critical carcinogenic mechanisms.

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