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REVIEW

Hepatitis B virus infection and the risk of hepatocellular carcinoma

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Abstract

Epidemiological studies have provided overwhelming evidence for a causal role of chronic hepatitis B virus (HBV) infection in the development of hepatocellular carcinoma (HCC). However, the pathogenesis of HBV infection and carcinogenesis of HBV-associated HCC are still elusive. This review will summarize the current knowledge on the mechanisms involved in HBV-related liver carcinogenesis. The role of HBV in tumor formation appears to be complex, and may involve both direct and indirect mechanisms. Integration of HBV DNA into the host genome occurs at early steps of clonal tumor expansion, and it has been shown to enhance the host chromosomal instability, leading to large inverted duplications, deletions and chromosomal translocations. It has been shown that the rate of chromosomal alterations is increased significantly in HBV-related tumors. Prolonged expression of the viral regulatory HBV x protein may contribute to regulating cellular transcription, protein degradation, proliferation, and apoptotic signaling pathways, and it plays a critical role in the development of hepatocellular carcinoma.

Key words: Hepatocellular carcinoma; Hepatitis B virus infection; Hepatitis B virus genotypic variations; Hepatitis B virus x protein

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, and the third most common cause of cancer mortality[1,2]. This tumor, which arises from hepatocytes, is often associated with liver cirrhosis resulting from chronic liver diseases. Among the environmental risk factors, the prevalence of chronic hepatitis B and C virus infections is linked directly to the incidence of HCC. There is now evidence for persistence within the tumor cells of a low level HBV multiplication potential. Hepatitis B virus (HBV) DNA replicative molecules and covalently closed circular DNA (cccDNA) are detectable by polymerase chain reaction (PCR). Moreover, the association between HCC and HBV recurrence after liver transplantation, and the detection of cccDNA in HCC cells point toward the possibility of HBV replication in tumor cells. The latter could act as potential reservoirs for HBV recurrence, especially in patients who present with a recurrence of HCC[3]. So far, chronic and persistent infection with hepatitis B virus is a major risk factor for the development of HCC.

Globally, it is estimated that 350 million people are chronically infected with the HBV[4]. Approximately 25% of

chronically HBV-infected individuals will develop HCC[5]. Chronic carriers of HBV have up to a 30-fold increased risk of HCC[6]. In areas of high HBV endemicity, persons with cirrhosis have an approximately 16-fold higher risk of HCC than the inactive carriers, and a 3-fold higher risk for HCC than those with chronic hepatitis but without cirrhosis[7]. Although the mechanisms of oncogenesis of HBV remain obscure, several factors have been identified to be associated with a high risk of developing HCC among chronic hepatitis B (CHB) patients. HBV exerts its oncogenic potential through a multi-factorial process, which includes both indirect and direct mechanisms that likely act synergistically[8].

HEPATITIS B VIRUS INFECTION AND LIVER CARCINOGENESIS

Hepatitis B virus DNA genome is able to integrate into the cellular chromosomal DNA, causing both viral and host genome rearrangements

HBV DNA is a small, circular DNA with a highly compact genetic organization and overlapping open reading frames. It shares with retroviruses the use of reverse transcription during its replication. It is well known that the HBV DNA genome is able to integrate into the cellular chromosomal DNA, causing both viral and host genome rearrangements. HBV DNA integration enhances the instability of the host chromosome, leading to large inverted duplications, deletions and chromosomal translocations. As a result of spontaneous errors in viral reverse transcription, variations in the HBV genome occur naturally. These mutations arise during the course of chronic infection with HBV. In fact, several HBV mutant strains, including those with mutations in the Pre-C/C, core promoter and deletion in the *Pre-S/S* genes, are involved in the pathogenesis of progressive liver disease and HCC development[9]. Several studies have shown that HBV DNA insertion into cellular genes was frequent, and could occur in genes encoding for proteins that were crucial for the control of cell signaling, proliferation and apoptosis[10,11].

HBV-related HCC can also arise in the absence of significant liver damage. Many of these chromosomal segments contain key players in liver carcinogenesis such as P53, PB Wnt/β-catenin, cyclins A and D1, transforming growth factor β (TGF-β) and Ras signaling[12]. In another study HBV DNA was integrated at random sites of human DNA; the *MLL4* gene was one of the targets for integration during hepatocarcinogenesis[13]. Furthermore, viral DNA integration into the cellular DNA is not necessary for viral replication, but allows for the persistence of the viral genome in the cell. Viral DNA insertion as well as cellular DNA replication occurs during liver cell proliferation, secondary to the necrosis/apoptosis of adjacent hepatocytes.

Viral genotype and the risk of hepatocellular carcinoma

The viral genotype is another factor that affects cancer risk. Genotype C has a higher risk of causing HCC than genotype B[14,15], and genotype D has a higher cancer risk than genotype A[16]. Compared to the Asian genotypes (B and C), the European genotypes (A and D) are less well established.

Hepatitis B virus genotypic variations and the risk of hepatocellular carcinoma

Specific genotypic variations in HBV have been associated with cirrhosis and HCC. These variations include, in particular, mutations in the pre-core region (Pre-C, A1896G inside the ε structure of the genome), in the basal Core promoter (A1762T/G1764A), and in ORFs encoding PreS1/PreS2/S and Pre-C/C. There is an overlap between Pre-C or basic core promoter (BCP) mutations and genotype, since these mutations appear to be more common in genotype C as compared to other genotypes[14]. The $1762^{T}/1764^{A}$ double mutations (1762 A-to-T and 1764 G-to-A) in the BCP region were commonly found to be borne by HCC patients in some high-risk populations, and were thus suggested as potential biomarkers for hepatocarcinogenesis[17,18]. Comparison of HBV isolates from different studies indicates that the mutation rate of A1762T/G1764A is 64% for genotype C, 40% for genotype B and 35% for other genotypes[19]. Kusakabe *et al*[20] investigated a population-based cohort consisting of 19 393 subjects (middle aged or older) with a follow-up of over 13 years in Japan. They found that HBV mono-infected subjects with the A1762T/G1764A double mutation could be at high risk for HCC development during the natural course of HBV infection[20]. In addition, the 1753^{V} mutations (1753-to-C/A/G) were also associated with the progression of liver disease[21]. Li *et al*[22] evaluated the roles of genetic variations of HBV in the development of HCC in Southern Guangxi China. Their study supported the hypothesis that both the 1762T/1764A double mutations and the 1753V/1752V mutations were associated with increased risk for HCC. Fan *et al*[23] found that patients with higher viral load and genotype C had a higher incidence of 1762/1764 double mutations, and that Enhancer Ⅱ and DR1 were significantly more in the HCC group than in the CHB group, which may play an important role in HCC development via nucleotide substitution. The BCP mutations could affect the core promoter that regulates the expression of both HBeAg and the core protein, and this may be related to the higher rate of replication of genotype C. Substitutions in the BCP may increase genotype virulence by deregulating the transcription of pcARN/pgARN, increasing the risk of HCC in patients infected with genotype C[24]. Thus, the BCP overlaps with the X region of the HBV genome, and mutations in the amino acid sequence at positions 130 and 131 in this region have been proposed as prognostic markers for the development of liver cancer[9].

Yang *et al*[14] found that the Pre-C mutation (A1896G) could prevent the production of HBeAg, by introducing a premature stop codon into the ORF Pre-C/C that abolished the production of HBeAg. However, HBV DNA synthesis persisted under these conditions; this may cause liver damage with progression to cirrhosis and cancer.

Mutations in Pre-S have been reported in HCC cases compared to chronic or asymptomatic cases. These mutations, including deletions in Pre-S in the integrated HBV DNA, may impair the secretion of HBsAg, leading to increased endoplasmic reticulum and oxidative stress in hepatocytes[25]. Truncated forms of Pre-S2 have also been shown to interact with cyclin A, a critical regulator of the cell division cycle, an observation that supports a role for Pre-S2 in hepatocyte hyperplasia and a likely role in the process of HBV-related tumorigenesis[26]. Thus, deletions of Pre-S may contribute to hepatocarcinogenesis by several mechanisms.

Altogether, this combination of genomic mutations, and/or deletions, together with transcriptional and post-transcriptional regulations, will therefore allow the establishment of viral persistence, and the ongoing synthesis of HBV antigens.

DNA METHYLATION AND THE RISK OF HEPATOCELLULAR CARCINOMA

DNA methylation occurs in the early stage of cancer development, including HCC. Genomic hypomethylation increases chromosome instability while localized hypermethylation decreases tumor suppressor gene expression, thus increasing the risk of HCC development[27]. Aberrant methylation of *RASSF1A* (Ras association domain family member 1) is thought to be an early event in the development of HCC[28]. The process is catalyzed by DNA methyltransferases (DNMT). DNMT inhibitors directly repress tumor angiogenesis, indicating that epigenetic modifications mediated by DNMT are involved in the regulation of gene expression during tumor angiogenesis[29]. Another significant link has been suggested between HCC development and the silencing by DNA hypermethylation of several tumor suppressor genes (*TSGs*). A number of TSGs, including $p16^{INK4A}$, *SOCS-1*, *APC*, *GSTP1* and *E-cadherin*, are silenced by DNA methylation in a large proportion of liver tumors, and this process often starts at the preneoplastic stage[30]. In some reports, a higher rate of promoter methylation for specific genes, such as $p16^{INK4A}$ and *E-cadherin*, has been observed in HBV-related tumors compared to nonviral tumors[31].

HEPATITIS B VIRUS X PROTEIN AND THE RISK OF HEPATOCELLULAR CARCINOMA

The hepatitis B virus x protein (HBx) protein is a 154 amino acid polypeptide with a molecular mass of about 17 kDa. HBx appears to play a critical role in the development of HCC. HBx is important for HBV replication and can regulate cellular transcription, protein degradation, proliferation, and apoptotic signaling pathways (reviewed by Bouchard and Schneider[32]). HBx protein does not bind directly to DNA, but rather acts on cellular promoters by protein-protein interactions and by modulating cytoplasmic signaling pathways. The cell cycle inhibition effect of HBx was validated through a liver regeneration experiment reported by Sidorkiewicz *et al*[33]. Kuo *et al*[34] reported that HBx can downregulate Wnt/β-catenin expression and suppress cell growth by not only repressing cell proliferation, but also triggering cell apoptosis. Furthermore, Hsien *et al*[35] have found that HBx protein interacts with the tumor suppressor adenomatous polyposis coli to activate Wnt/β-catenin signaling. Wnt/β-catenin has been shown to up-regulate the epithelial cell adhesion molecule in HCC cells to promote tumor initiation and stemness[36]. Thus HBx activation of Wnt/β-catenin may promote directly the transformation of liver cells into cancer initiating cells[37]. A number of ways in which HBx protein may induce antiapoptotic effects have been described. The most important of these is the ability of HBx to inhibit p53-mediated apoptosis. Recent experiments have suggested that HBx protein may increase the expression of telomerase reverse transcriptase and telomerase activity, prolonging the lifespan of hepatocytes and contributing to malignant transformation. The protein also interferes with nucleotide excision repair through both p53-dependent and p53-independent mechanisms. Carboxyl-terminal truncated HBx protein loses its inhibitory effects on cell proliferation and pro-apoptotic properties, and it may enhance the protein's ability to transform oncogenes. Dysregulation of IGF-Ⅱ enhances the proliferation and anti-apoptotic effects of oncogenes, resulting in uncontrolled cell growth. Another possible explanation for the anti-apoptotic effect of HBx protein involves the accumulation of the anti-apoptotic protein, survivin[37]. Guo *et al*[37] found that Hep3B cells expressing HBx protein increased the levels of hepatoma upregulated protein (HURP) RNA and protein, and showed resistance to cisplatin-induced apoptosis. Knockdown of HURP in these cells reversed this effect. The anti-apoptotic effect of HBx protein was shown to require activation of the p38/mitogen activated protein kinase (MAPK) pathway. In addition, the expression of survivin was upregulated by HBx protein in an HURP-dependent manner. High levels of HURP favored the expression of the anti-apoptotic survivin in HBx-expressing cells. These results indicate that HBx protein activates the expression of HURP *via* the p38/MAPK pathway, culminating in the accumulation of survivin. In recent years, evidence has accumulated that HBx protein modulates the transcription of methyl transferases, causing regional hypermethylation of DNA that results in silencing of tumor suppressor genes, or global hypomethylation. This, in turn results in chromosomal instability, thereby playing a role in hepatocarcinogenesis.

The $p16^{INK4A}$ gene is known as an abnormal tumor suppressor gene and critical cancer-related gene in human hepatocarcinogenesis. Several studies have shown that hypermethylation of the $p16^{INK4A}$ promoter is an important early event in carcinogenesis[38]. Zhu *et al*[39] found that HBx upregulates *DNMT1* and *DNMT3A* expression at both the mRNA and protein levels, and that HBx represses $p16^{INK4A}$ expression by inducing hypermethylation of

the $p16^{INK4A}$ promoter. Moreover, HBx induces the hypermethylation of the $p16^{INK4A}$ promoter through *DNMT1* and *DNMT3A*. Regulation of *DNMT1* and *DNMT3A* by HBx promotes the hypermethylation of the $p16^{INK4A}$ promoter region[39].

Among the activities of HBx, its trans-activation function may play a crucial role in hepatocarcinogenesis, because it is involved in the activation of a large number of signaling pathways and cellular genes that are involved in oncogenesis, proliferation and inflammation. For example, HBx transactivates a number of cellular promoters and enhancers containing binding sites for nuclear factor-kappa-B, activator protein 1 (AP-1), AP-2, cellular promoters of genes associated with cell proliferation such as IL-8, TNF, TGF-β, and epidermal growth factor receptor, and cytosolic signal transduction pathways including Src kinases, Cjun N-terminal kinase, JaK1/STAT and protein kinase, which have overlapping effects on cell proliferation and viability[40,41].

CONCLUSION

The studies we have reviewed here illustrate that HBV constitutes a major environmental etiological factor for primary liver cancer in humans. It will therefore be important to analyze gene expression and proteomic changes in a large series of samples from CHB at different stages, to identify suitable prognostic markers and therapeutic targets. Furthermore, detection of the viral genomes using sensitive, PCR-based, assays is mandatory to enable an accurate appraisal of their prevalence. Genomic alterations and epigenetic factors like methylation-associated gene silencing may play an important role in the deregulation of cellular functions, leading to malignant transformation. A better understanding of the complex role of HBV in liver tumorigenesis will undoubtedly contribute to the improvement of the management of liver diseases induced by CHB.

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