Chapter 2
Hepatitis B Virus-Associated Hepatocellular Carcinoma

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Abstract Liver cancer is the fifth most common cancer worldwide in men and the ninth in women. It is also the second most common cause of cancer mortality. Hepatocellular carcinoma (HCC) is the most common type of liver cancer. About 350 million people globally are chronically infected with HBV. Chronic hepatitis B virus (HBV) infection accounts for at least 50% cases of HCC worldwide. Other non-HBV factors may increase HCC risk among persons with chronic HBV infection. Both indirect and direct mechanisms are involved in HCC oncogenesis by HBV. HCC-promoting HBV factors include long-lasting infection, high levels of HBV replication, HBV genotype, HBV integration, specific HBV mutants, and HBV-encoded oncoproteins (e.g., HBx and truncated preS2/S proteins). Recurrent liver inflammation caused by host immune responses during chronic HBV infection can lead to liver fibrosis and cirrhosis and accelerate hepatocyte turnover rate and promote accumulation of mutations. Major breakthroughs have been achieved in the prevention of HBV-associated HCC with HBV vaccines and antiviral therapies.

Keywords Chronic infection • Cirrhosis • Genotype • Hepatitis B virus • Hepatocellular carcinoma • HBeAg • HBsAg • HBx • Integration • Mutation • PreS/S

2.1 Introduction

According to a survey conducted in 2012, liver cancer is the fifth most common cancer worldwide in men (7.5% of the total new cancer cases in 2012) and the ninth in women (3.4%) [1]. It is also the second most common cause of cancer

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mortality [1]. Hepatocellular carcinoma (HCC) is the most common type of liver cancer. The majority of HCC is associated with chronic infection of hepatitis B virus (HBV) or hepatitis C virus (HCV). This concise review focuses on HBV-associated HCC.

About 350 million people globally are chronically infected with HBV [2]. Chronic HBV infection accounts for at least 50% cases of HCC worldwide [3] and is the dominant risk factor for HCC in areas with endemic HBV infection such as Eastern and Southeastern Asia and sub-Saharan Africa [4].

Other non-HBV factors may increase HCC risk among persons with chronic HBV infection, including older age [5], male sex [6], cirrhosis [7], diabetes mellitus [8], exposure to environmental carcinogens (aflatoxin B1 (AFB1), heavy alcohol and tobacco consumption) [9, 10], HIV coinfection [11], and possibly HDV superinfection [12].

HBV infection is transmitted mainly vertically in endemic HBV areas, in contrast to horizontally in HBV low prevalent areas. More than 90% of vertical HBV transmission cases lead to chronic infection, whereas only 5–10% of horizontal HBV transmission cases do so. Accordingly, the average age of HBV chronic carriers who develop HCC is younger in endemic HBV areas. Men are more susceptible to HBV-associated HCC than women, probably as a result of stimulation of HBV replication by androgens and a protective role of estrogens against HBV replication [13–15]. In most cases, HBV-associated HCC develops progressively from chronic liver disease, with cirrhosis in the majority of patients (70–90%) [5]. However, cirrhosis is not a prerequisite for the development of HBV-associated HCC [7]. HBV carriers without cirrhosis, especially those who have long-lasting infection, may also develop HCC. AFB1 is the foremost environmental risk factor of HCC in some Eastern Asian areas with endemic HBV infection. AFB1 causes a specific p53 mutation and predisposes mutant hepatocytes to DNA damage [9]. AFB1 was reported to exert a synergistic carcinogenic effect with chronic HBV infection, resulting in a 60-fold increased HCC risk [16].

2.2 HBV Oncogenic Factors for HCC Development

Both indirect and direct mechanisms are involved in HCC oncogenesis by HBV. HCC-promoting HBV factors include long-lasting infection, high levels of HBV replication, HBV genotype, HBV integration, specific HBV mutants, and HBV-encoded oncoproteins. In addition, recurrent liver inflammation caused by host immune responses during chronic HBV infection can lead to liver fibrosis and cirrhosis and accelerate hepatocyte turnover rate and promote accumulation of mutations.

2.2.1 Long-Lasting Infection and High Levels of Viral Replication

Long-lasting chronic HBV infection is associated with HCC development. As aforementioned, there is a much higher rate of chronic HBV infection in endemic HBV areas due to vertical viral transmission. The lengthened HBV infection period is thought to provide more opportunities for various viral and nonviral risk factors to promote HCC oncogenesis.

Hepatitis B e antigen (HBeAg) seropositivity and higher levels of serum HBV load are associated with high risk of HCC. A long-term follow-up study among 11,893 male HBV carriers in Taiwan who were without HCC at study entry showed that the relative risk of HCC was 9.6 among men who were positive for hepatitis B surface antigen (HBsAg) alone and 60.2 among those who were positive for both HBsAg and HBeAg, as compared with men who were negative for both [17]. HBeAg seropositivity was also found associated with higher risk of early recurrence and poorer survival in patients after curative tumor resection [18]. With the routine application of HBV DNA quantification, HBeAg as a surrogate of HBV replication indicator is less utilized. The REVEAL-HBV study reported that the incidence of cirrhosis and HCC is positively and quantitatively correlated to the serum HBV DNA load in a cohort of 3653 participants with chronic HBV infection [19, 20]. Similar results were observed in a follow-up study among a prospective cohort of 1006 patients with chronic HBV infection from Hong Kong [21].

2.2.2 HBV Genotype

There are at least eight HBV genotypes (A–H), which display distinct geographical distributions [22]. Both genotypes B and C are prevalent in Eastern Asian areas. Infection with genotype C was reported to more likely result in severe liver disease, cirrhosis, and HCC than infection with genotype B [21, 23, 24]. However, a study from Taiwan reported that genotype B was associated with HCC in children with chronic HBV infection [25]. In Europe where genotypes A and D are dominant, infection with genotype D is associated with more severe liver disease or HCC than infection with genotype A [26].

2.2.3 HBV Integration

HBV replicates through reverse transcription using its pregenomic RNA as template. Progeny viral DNA in nascent capsids can be trafficked to nucleus to supplement nuclear cccDNA pool, which constitutes a reservoir of templates for HBV gene expression and replication. Unlike retroviruses, chromosomal DNA integration is

not required for HBV replication. Nevertheless, DNA integration into the genomes of host hepatocytes likely contributes to oncogenesis by HBV.

HBV DNA integration in host chromosomes has been found in the majority (85–90%) of HBV-associated HCC and probably occurs early during HBV infection [27, 28]. The genomic sites of HBV DNA integration appear random [27]. However, it is thought that HBV DNA integration into some specific genomic sites may allow the integrant-containing cells to obtain a growth advantage so that they may expand clonally. The integrated HBV DNA may induce chromosomal instability or alter the expression of host genes through *cis*-acting mechanisms. In addition, the integrated viral DNA may allow the continuous expression of viral oncoproteins such as HBx and truncated preS2/S proteins.

Recurrent HBV DNA integration occurs near actively transcribed gene-coding chromosomal regions, as well as within or near fragile genomic sites or repetitive regions, such as the Alu sequences and long interspersed nuclear elements (LINEs) [29–31]. Sequence analysis has revealed integration sites that are in the proximity of many genes involved in cell survival, proliferation, metabolism, and cell cycle regulation [29–31]. Among these genes, insertion of HBV DNA near the *hTERT* gene, encoding the catalytic subunit of telomerase, has been frequently found in HCC [29, 32]. The integration of HBV DNA into fragile genomic sites or repetitive regions may induce genomic instability or alter the expression of noncoding RNAs [33]. A HBV-human fusion transcript (HBx-LINE1) was reported to function as a long noncoding RNA (lncRNA) to influence the epithelial-mesenchymal transition and correlate with reduced patient survival and tumor formation in mice [34].

2.2.4 HBV Mutations

The reverse transcriptase of HBV lacks of proofreading activity. As a result, mutations are accumulated during chronic HBV infection and selected under the pressure of host immunity and antiviral drugs during treatment. Due to the compact and overlapping properties of HBV genome, many mutations generate defective viruses. HBV mutations that have been identified to be associated with HCC are enriched in the basal core promoter (BCP)/preC region and the preS region.

Among the many mutations in the BCP/preC region, the most common one that is significantly associated with HCC development in genotypes B and C is the T1762 and A1764 double mutation (BCP double mutation) [35, 36]. The G1896A mutation in the preC region is a common HBV mutation that creates a premature stop codon that abolishes HBeAg translation. No association exists between the G1896A mutation and HCC development [37, 38]. Several other mutations in the BCP/preC region (C1653T, T1753V) may also be associated with HCC development [38]. It is unclear how these mutations contribute to HCC development. Since the BCP/preC region contains essential HBV regulatory elements, these mutations may alter HBV gene expression and replication. In addition, because the HBx open

reading frame overlaps the BCP/preC region, some mutations may affect HBx expression or activity.

HBV mutants with point mutations, deletions, or insertions in the preS region have been frequently found in HCC [39, 40] and are associated with an increased risk of HCC [38, 39]. The preS mutations may alter the expression and secretion of HBV envelope proteins, resulting in intracellular accumulation of HBV envelope proteins, which can cause endoplasmic reticulum (ER) stress, leading to cell transformation [41, 42].

2.2.5 HBx Protein

The viral regulatory protein HBx contributes critically to HBV replication [43] and is thought to be closely related to HBV oncogenicity. It probably does not bind directly to DNA but rather acts on many cellular and viral promoters through protein-protein interactions. In the cytoplasm, HBx modulates multiple signaling pathways. These nuclear and cytoplasmic interactions result in the activation or repression of a large number of signaling pathways that play important roles in chromatin dynamics, DNA damage response, cell proliferation, viability, metabolism and migration, angiogenesis, and immune response. However, precautions should be taken concerning HBx's multiple activities. Due to the low-level expression of HBx during HBV infection and a lack of sensitive detection tools, many findings have been derived from in vitro HBx overexpression experiments and need to be verified in models that more closely mimic HBV infection and HBV-associated HCC.

HBx causes chromosomal instability by binding with different cellular proteins (Crm1, HBXIP, DDB1, p53, hBubR1) to dysregulate centriole replication, mitotic checkpoint, mitotic spindle formation, and chromosome segregation [44–47]. HBx promotes cell proliferation, viability, and migration through modulating multiple signaling pathways. HBx binds with p53 to impair p53-mediated apoptosis and checkpoint functions [48, 49]. HBx may upregulate *TERT* expression [50], but conflict results have been shown in HBx transgenic mice [51]. HBx induces CREB-dependent transcriptional activation through interacting with the CBP/p300 acetyltransferases and preventing CREB inactivation by PP1 phosphatase, resulting in expression of CREB-responsive genes involved in hepatocyte metabolism and proliferation [52, 53]. HBx can recruit DNMT3a DNA methyltransferase to suppress *all-trans* retinoic acid (ATRA)-mediated induction of p16 and p21 in HepG2 and Hep3B cells via promoter hypermethylation, resulting in inactivation of retinoblastoma protein [54]. HBx may promote cell migration and HCC cell invasive and metastatic capacity by increasing the expression of matrix metalloproteinase 3 and 9 [55, 56] and epigenetically suppressing E-cadherin expression [57]. HBx can also block tumor necrosis factor-α-mediated apoptosis [58]. On the other hand, HBx can increase cellular reactive oxygen species (ROS) levels that lead to apoptosis by

promoting mitochondria membrane depolarization or Ca2+ accumulation in mitochondria [59, 60]. HBx may also promote stemness of HCC cells [61].

HBx has been shown to promote HCC angiogenesis. HBx was reported to upregulate the stability and transcriptional activity of hypoxia-inducible factor-1α (HIF1α) and the expression of vascular endothelial growth factor (VEGF) and angiopoietin 2 (ANG2), which leads to enhanced angiogenesis [62, 63].

2.2.6 PreS/S Proteins

The PreS/S open reading frame of HBV uses alternative start codons for translation and encodes three envelope proteins (large, middle, and small) that share the 226-amino-acid sequence of the small envelope polypeptide. The contribution of wild-type or mutant PreS/S proteins to HCC development is not fully understood. Wild-type large envelope protein accumulated in the ER of hepatocytes of transgenic mice could induce ER stress and consequently cause inflammation, hyperplasia, and aneuploidy [64]. PreS2/S mutant proteins frequently found in HBV-associated HCC also accumulate in ER and may trigger a similar process [42], resulting in the upregulation of cyclin A that in turn promotes cell proliferation and chromosome instability [65, 66]. In addition, PreS2/S mutant proteins have been shown to transcriptionally activate the TERT expression [67].

2.3 Prevention

HBV-associated HCC can be prevented by vaccination against HBV infection. Vaccination of newborns against HBV has been incorporated into universal hepatitis B immunization programs of many countries and regions, which has greatly reduced the incidence of HCC in children [68]. Hepatitis B immune globulin (HBIG), in addition to hepatitis B vaccine, administered within 12–24 h after birth, has been shown to achieve 90–100% protective efficacy against perinatal transmission from mothers who are positive for HBsAg and HBeAg [69]. Recent studies showed that tenofovir treatment of HBeAg-positive mothers can successfully prevent vertical HBV transmission [70, 71].

Antiviral therapy can significantly suppress HBV replication in chronic HBV patients. Studies with patients treated with lamivudine or adefovir have shown to help prevent HCC in patients with chronic hepatitis [72, 73]. Nevertheless, nucleos(t)ide analogue therapy does not completely eliminate the risk of HCC [73]. The current first-line anti-HBV drugs, namely, entecavir and tenofovir, have been shown to improve the prevention of HCC in responders with cirrhosis [74].

2.4 Conclusions

HCC will continue to be one of the major cancers worldwide as chronic HBV infection remains a public health threat. A great deal of knowledge has been gained on the epidemiologic features and pathogenesis of HBV-associated HCC in the past three decades. However, the oncogenic mechanisms of HBV and HBV-related risk factors are not fully understood, in large part owing to a lack of animal models that recuperate clinical HBV-associated HCC. Nevertheless, major breakthroughs have been achieved in the prevention of HBV-associated HCC with HBV vaccines and antiviral therapies. With the advances in HBV virology and pathology, there will be novel prophylactic and therapeutic means for HBV-associated HCC.

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