Molecular Biology of Hepatitis B Virus

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Chronic infection with the hepatitis B virus (HBV) affects an estimated 257 million people worldwide and can lead to liver diseases such as cirrhosis and liver cancer. Viral replication is generally considered not to be cytopathic. Nevertheless, some HBV proteins have direct carcinogenic effects, and chronic inflammation resulting from disrupted antiviral responses and aberrant innate immune reactions lead to HBV infection-related disease and mortality in up to 25% of cases. HBV is an enveloped virus. The enveloped core particle contains the viral polymerase and the partially double stranded HBV DNA genome. The DNA is generated from an RNA template and thus the virus should be classified as a retrovirus. The virus has one accessory protein, the X protein (HBx), which is the main viral oncogene. HBx expression is essential to initiate and maintain viral RNA transcription.

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1. Introduction

Worldwide, more than 296 million people are infected with hepatitis B virus (HBV), and each year an estimated 820,000 people die as a consequence. Most of these deaths are due to chronic HBV infections (CHB), in which continuing viral replication and the resulting inflammation lead to severe liver damage and liver cancer, which occurs in about 25% of the chronically infected individuals [1]. It is poorly understood why the immune system fails to clear the infection. Antiviral responses do develop, but the cells that can recognize HBV do not function properly [2][3]. This leads to a “status quo”, in which HBV specific immune cells suppress viral replication but fail to clear the infection [4]. In resource-rich settings, HBV infection can be treated with nucleoside or nucleotide analogues (NAs), which prevent viral replication but do not affect the stable HBV DNA in already-infected hepatocytes. Due to the natural turnover of hepatocytes, the number of infected cells becomes less and less, and after years of therapy, often no markers of HBV replication can be found in the serum anymore. However, small amounts of HBV genomic DNA persist in liver parenchymal cells, and if therapy is discontinued, the viral infection is re-established from this DNA. Therefore, to reduce the chances of developing HBV infection-related pathology, NAs have to be taken lifelong. A safe and effective vaccine that can protect children right after birth is available; however, this strategy cannot prevent all perinatal transmissions, especially those occurring before or during delivery, and does not suffice as a means to contain the current epidemic [5]. Although in some countries, the introduction of perinatal HBV vaccination as standard care has greatly reduced HBV incidence, the number of HBV cases is increasing worldwide. Indeed, whereas the incidence of—and the mortality due to—other infections such as human immunodeficiency virus (HIV) and tuberculosis (TB) are declining, the incidence of—and mortality due to—viral hepatitis, mainly HBV, are rising. The WHO has urged scientists and policy makers worldwide to reckon these treat-and-device strategies to combat HBV.

If not treated, HBV infection can lead to liver fibrosis, liver cirrhosis, and finally to liver failure. Most HBV-related deaths, however, are attributable to a specific type of liver cancer: hepatocellular carcinoma (HCC), which develops in about 10–25% of HBV-infected individuals [6][7]. The odds of developing HBV infection-related HCC differ for the different HBV genoypes [8]. The median survival of untreated HCC is 8 months. The 5-year survival rate of HCC patients is about 14% in the US, and is lower in developing countries where HCC is more common [9]. Due to the high mortality rate, HCC is the fourth leading cause of cancer-related death worldwide, and the vast majority of these cancers are caused by HBV infection.

Many aspects of HBV infection contribute to the development of HBV-related HCC. The ongoing immune responses cause liver inflammation and dysregulate various processes. Importantly, in most HBV infection-related HCCs, HBV DNA can be found integrated in the host genome. Such integrations may contribute to HCC by affecting the expression of oncogenes near the integration site [10], but also the expression of viral proteins may significantly contribute to the development of HBV-related HCC [11][12]. Thus, the development of HBV-related HCC is a multifactorial process to which several different aspects of HBV replication may contribute.

2. Molecular Biology of HBV

With only about 3200 bases, HBV has the smallest known genome of a human DNA virus. In the infected hepatocyte, HBV exists as a small circular DNA molecule, the covalently closed circular DNA (CCCDNA), from which RNA is transcribed. These RNAs are translated into proteins or serve as the template to make new DNA, which is packaged in newly formed virus particles. HBV particles consist of an enveloped core particle, which can consist of 180 (T = 3) or 240 (T = 4) HBV core protein (C) monomers. Playing an important role in HBV diagnostics, the core protein is often referred to as the core antigen or HBcAg. In the cytoplasm, the core particle assembles around a complex formed by the viral polymerase (P) and the viral pregenomic RNA (pgRNA). Only after the formation of the viral core particle is the viral pgRNA reverse-transcribed by the P protein. Completion of the reverse transcription and the partial completion of the second (+) DNA strand lead to structural changes in the outside of the core particle and induce its envelopment.

The viral envelope consists of a host cell-derived lipid bilayer membrane and three different envelope proteins called surface (S) proteins; the (small) S, and the S1 and S2 proteins. S and S1 are n-terminally truncated forms of the S2 proteins that are produced by alternative initiations of transcription and/or translation. When considered as a soluble antigen, the S protein is referred to as S antigen (HBsAg) in analogy to HBcAg.

HBV entry is initiated by the binding of the preS1 domain of the HBV large surface protein to the bile acid transporter sodium taurocholate cotransporting polypeptide (NTCP) [13]. Subsequently, the HBV core particle is released into the cytoplasm and migrates to the nucleus; in this regard, the exact mode and site of release have not yet been conclusively characterized. However, it has been shown that the core particle disassembles in association with the nuclear pore and releases the partially double-stranded HBV DNA in the nucleus. Here, the viral DNA is repaired by cellular enzymes to form the fully double-stranded circular minichromosome called cccDNA. Transcription from the cccDNA is tightly regulated by the chromatin state, DNA methylation, and level and

activity of transcription factors. All these regulatory mechanisms are heavily affected by extracellular signals, and as such, interaction of the HBV-infected cell with its environment is a major factor in the regulation of HBV replication. HBV expresses two nonstructural proteins: the accessory X protein (HBx), and the e antigen (HBeAg), which is a truncated form of the core protein that is secreted. HBx is essential for the initiation and maintenance of HBV RNA transcription [14]. The best-understood function of HBx is inducing the degradation of the Smc5/6 complex, which in the absence of HBx binds to the HBV cccDNA and strongly suppresses or blocks viral RNA transcription [15][16]. Besides regulating HBV RNA transcription, it has been observed that HBx expression can cause many, poorly understood effects on cells, such as the activation of cellular signalling pathways, disruption of the cell cycle, cell-cell interaction, and more. However, it is not clear how such effects may benefit viral replication, and even though they are observed in infected patients, they often do not seem to occur in other natural models of HBV infection [17], indicating that induction of cellular signalling pathways in vivo may depend on specific conditions and interactions between the infected cell and its environment. Interestingly, although the effect of HBx on HBV RNA transcription is occurring inside the infected cell, HBx expression also affects cells in a paracrine manner. For instance, HBx expression in hepatocytes induces collagen expression in HSCs in a paracrine manner [18], and can affect hepatocyte proliferation [19].

Compared to regulatory functions by the HBx, the functions of the HBeAg are less well understood. HBeAg is translated from the HBV core gene, when RNA transcription is initiated from an alternative transcription initiation site than that of the core RNA. This leads to translation of the core protein from an alternative 5′ in-frame start codon. This protein is differentially processed; the c- and n terminal parts are cleaved off and dimers of this truncated protein are excreted. Transcription of the e antigen is regulated by the basal core promoter (BCP) [20], and intriguingly, is often lost during or after the immune active stage of the infection. The odds of losing e antigen expression differs for the different HBV genotypes [8], and specific mutations in the basal core promoter are associated with increased risk of developing HBV infection-related HCC [21]. HBeAg expression is associated with increased viral loads, and is generally believed to contribute to immune anergy by overloading the immune system. In line with such a “strategy”, HBV-infected cells also secrete massive amounts of HBsAg, which are embedded in the membrane of rod- and cone-shaped particles. These massive amounts of excreted antigens can induce tolerance by affecting the tolerization of adaptive immune cells. On top of that, HBsAg also affects local nonparenchymal cells that may otherwise contribute to antiviral responses, such as liver sinusoidal endothelial cells (LSEC) [22]. Some reports also report the excretion of empty and HBV DNA-containing, non-enveloped HBV core particles, and large protein complexes consisting of the HBV core and e antigens, the so-called HBV Core-related antigens (HBcrAg) [23][24]. Moreover, the excretion of intact HBV core particles by HBV replicating cells has been observed in vitro [25], but if this takes place in vivo remains elusive, and whether such particles play a role in HBV replication or pathology is unclear [25]. Like the secretion of exosomes, which is the best-known group of extracellular vesicles, the secretion of HBV virions occurs via multivesicular bodies (MVBs) and depending on Alix and the ESCRT III complex, which are also essential for exosome release [26][27][28][29]. Subviral particles (SVPs), on the other hand, are formed at the membranes of the endoplasmic reticulum, which is why their release occurs via the Golgi network [30][31][32]. This use of cellular secretion mechanisms for the release of its own gene products

once again demonstrates the marked adaptation of HBV to the host and is nowadays the subject of intensive research.

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