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Review

Biological Roles of Hepatitis B Viral X Protein in the Viral Replication and Hepatocarcinogenesis

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Hepatitis B virus is a pathogenic virus that infects 300 million people worldwide and causes chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Hepatitis B virus encodes four proteins. Among them, the HBx protein plays a central role in the HBV pathogenesis. Because the HBx protein is considered to play a central role in the induction of viral replication and hepatocarcinogenesis, the regulation of its function could be a key factor in the development of new interventions against hepatitis B. In this review, HBx protein-related viral replication and hepatocarcinogenesis mechanisms are described, with a focus on the recently reported viral replication mechanisms related to degradation of the Smc5/6 protein complex. We also discuss our recent discovery of a compound that inhibits HBx protein-induced degradation of the Smc5/6 protein complex, and that exerts inhibitory effects on both viral replication and hepatocarcinogenesis. Finally, prospects for future research on the HBx protein are described.

Key words: HBx, Smc5/6, DDB1, nitazoxianide, DNA repair

Hepatitis B virus (HBV) has four large open reading frames (ORFs) in its genome. The S gene, C gene, and P gene encode multiple types of HBs antigens, core proteins, and polymerase proteins, respectively. The fourth ORF is the X region, which encodes the HBx protein. The HBx protein has various important physiological functions [1], including the regulation of viral protein expression and viral replication, the protection of HBV from the immune system, and the development of liver cancer. The HBx protein often acts indirectly (acts in trans) at sites distant from its subcellular localization [2]. For example, the HBx protein acts to activate various intracellular signals in the cytoplasm, and as a result contributes to the induction of inflammation and hepatocarcinogenesis. On the other hand, in the nucleus, HBx binds to transcription complexes and thereby regulates the expression of various genes. It also regulates the activities and the expression of proteins involved in epigenome modifications, such as histone deacetylase and DNA methyltransferase, and regulates the expression levels of various downstream genes [3]. Collectively, these functions indicate that the HBx protein is closely involved in both the viral replication and pathogenesis of chronic hepatitis [4].

One of the key features of HBV is its integration of the viral genes into the host genome [5]. Most current therapeutic development against HBV aims at eliminating covalently closed circular DNA (cccDNA). However, cccDNA is not the only source of the viral protein expression; viral genes integrated into the host genome are another important source. In particular, the X gene is located immediately upstream of the nick of the viral minus strand, and is therefore a region that is fre-

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quently integrated into the host genome. Because functionally intact HBx protein is indeed produced from the integrated viral genomic sequences, it is assumed that the regulation of the HBx protein is necessary even after the elimination of the cccDNA is achieved. In any case, because the HBx protein undoubtedly plays a central role in viral replication, regulation of this protein is extremely important to achieve a functional cure or complete elimination of the hepatitis B virus.

HBx Protein and Viral Replication

The HBx protein functions to maintain the expression of viral products from viral genes and viral replication. For example, the HBx protein activates the promoter activity of the X and C genes [6]. The HBx protein is also considered to play an essential role in viral replication and its maintenance, based on the finding that the chronicity of woodchuck hepatitis virus, an HBV-related virus, disappears upon the mutation and destruction of WHx, the woodchuck hepatitis virus X protein corresponding to HBx [7].

HBV cccDNA exists in the nucleus of the infected cells as minichromosomes. HBx protein is also present in the nucleus and associates with the transcriptional complexes to contribute to the expression of viral RNA from HBV-DNA. Moreover, binding with several histone deacetylases, or binding with protein arginine methyltransferases that suppress the transcription of viral RNA results in efficient viral replication [8]. There is also a report describing that the HBx protein promoted the formation of cccDNA by activating the DNA helicase activity of the TFIIH subunit and utilizing the DNA repair factor TDP2.

Binding between the HBx and DDB1 Proteins

Studies using woodchucks and in vitro replication models using plasmids have shown that the binding of HBx protein to the damaged DNA binding protein 1 (DDB1) is crucial for HBV replication. The DDB1 protein was originally reported as one of the recognition factors in base excision repair, but recently the DDB1 protein was shown to act as an adapter for cullin 4A RING E3 ligase (CRL4) and to bind to the DDB1 cullin accessory factor (DCAF) receptor. CRL4 regulates various cellular functions such as damaged DNA repair, and the HBx protein itself has been suggested to act as a DCAF receptor and influence the DDB1-DCAF pathway [9].

Smc5/6 Protein Degradation and Viral Replication

It has been reported that various viruses target CRL4 to positively regulate viral replication. In 2016, two studies reported important research results related to CRL4 on HBx protein. The Smc5/6 complex, which is a structural maintenance of chromosomes complex that suppresses transcription from cccDNA, is proteolyzed via the binding of the HBx protein and DDB1 protein, and promotes transcription of viral RNAs [10, 11]. However, because more than 100 other types of host proteins that bind to the HBx protein have been reported, it is highly likely that new regulatory factors related to HBx protein about the transcription of viral RNAs from the cccDNA will be discovered in the future.

HBx Protein and Hepatocarcinogenesis

Because HBx transgenic mice develop liver cancer, the HBx protein is considered to have carcinogenic potential, but the mechanism is still unclear. From the point of view of cell proliferation, there are many reports that the HBx protein may affect various intracellular signals, resulting in faster cell turnover. On the other hand, from the viewpoint of cell survival, the HBx protein has been shown to have both pro-apoptotic and anti-apoptotic actions. That is, the HBx protein activates the NF-κB pathway and exerts anti-apoptotic, while increasing the expression of the Bax protein with its pro-apoptotic action and reducing the expression of anti-apoptotic Bcl-xl. Originally, HBV infection itself does not have cytotoxicity, but the anti-apoptotic action of the HBx protein is necessary for persistent infection. Maintaining such a balance with cell survival may explain the appearance of carcinogenesis after a long clinical course.

In addition, the expression of the HBx protein increases the expression of DNMT and controls the expression of DNMT through promoter methylation of various genes associated with carcinogenesis. In addition, there are many studies showing that the HBx protein increases or decreases the expression levels of various microRNAs and long non-coding RNAs to regulate the expression levels of their target gene products,

resulting in carcinogenesis.

Inhibitors of the Binding between HBx and DDB1 Proteins for Potential Clinical Application

As mentioned above, the host Smc5/6 protein complex suppresses the transcription of viral RNAs from HBV cccDNA. In contrast, the HBx protein induces the degradation of the Smc5/6 protein complex via binding with the DDB1 protein. That means there is a battle between the host and viruses (Fig. 1). Although the binding between the HBx and DDB1 proteins has been known for a long time, recent studies have shown that the binding between the HBx and DDB1 proteins is essential for efficient viral RNA transcription [10, 12]. Therefore, we hypothesized that compounds that inhibit the binding between the HBx and DDB1 proteins would prevent the Smc5/6 protein degradation and would suppress the transcription of viral RNA. After screening for compounds that inhibit this protein–protein binding, we found that a compound called nitazoxanide inhibits the binding and also reduces the expression of the viral RNA and viral proteins [13]. Nitazoxanide is a drug manufactured by the company Romark in the United States and has been approved as an antiprotozoal drug in both the United States and Europe. In fact, it was reported that 12 chronic hepatitis B patients in Egypt were administered nitazoxanide and in some cases HBsAg indeed disappeared [14]. Although nitazoxanide has not yet been approved in Japan, a Phase II trial of nitazoxanide as a candidate drug for a novel treatment against HBV is underway globally.

In our own recent experiments, we focused on the fact that a step called neddylation is necessary for the degradation of Smc5/6 proteins. We tested MLN4924 (Pevonedistat), a neddylation inhibitor used for the treatment of blood diseases, in an in vitro HBV replication system. As expected, MLN4924 inhibited degradation of the Smc5/6 proteins and potently suppressed the expression of HBV-RNA [15]. Theoretically, these compounds, which inhibit the production of viral RNA from cccDNA, do not affect the amount of cccDNA, but experimentally, nitazoxanide and MLN4924 decreased the amount of cccDNA. This suggests the possibility that these compounds directly affect cccDNA levels by unknown mechanisms or the possibility that viral RNA or viral proteins are involved in the maintenance and stability of cccDNA. In fact, there are reports that the core protein and X protein, which are viral proteins, are involved in the generation and maintenance of cccDNA.

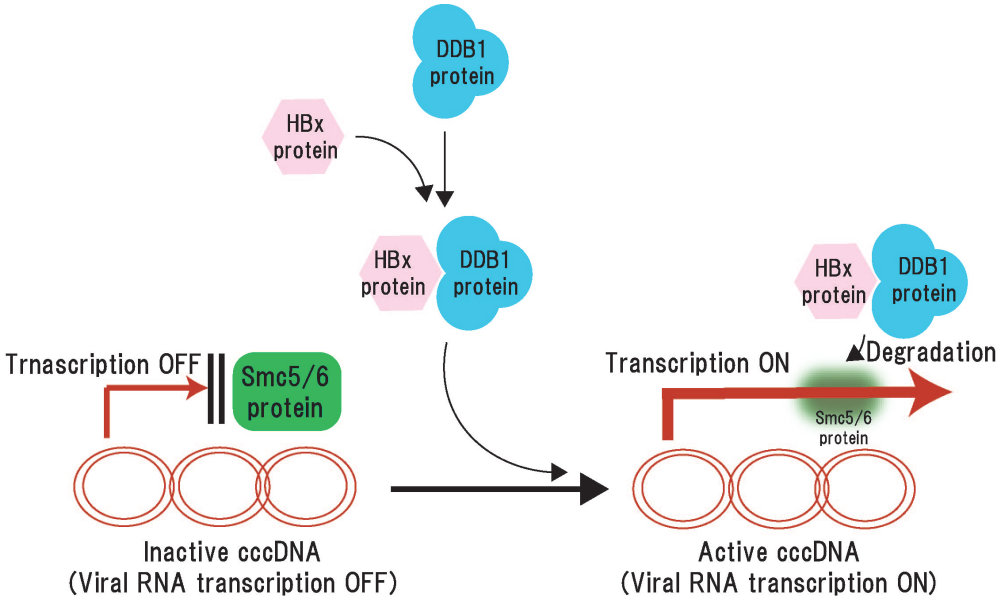

Fig. 1 Efficient viral RNA transcription after Smc5/6 degradation by the HBx protein. The HBx protein binds to the host protein DDB1 and induces degradation of the Smc5/6 protein complex that suppresses viral RNA transcription, thereby promoting viral RNA transcription.

Accumulation of DNA Damages by Degradation of the Smc5/6 Protein Complex

While it has been suggested that HBx-triggered degradation of the Smc5/6 protein complex positively affects viral replication, we wondered if there are any influences on the host cells by the degradation of Smc5/6 protein complex. It has been reported that the Smc5/6 proteins are involved in the recognition of the initiation of the DNA repair mechanism when double-stranded DNA breaks occur. Through various overexpression and knockout experiments, we confirmed that the downregulation of the Smc5/6 proteins by the HBx protein caused the accumulation of mutations in the genomic DNA, leading to the cellular transformation [16]. These results suggest that the degradation of the Smc5/6 proteins by the HBx protein has two implications: for the virus side, it promotes viral replication, and for the host side, it is linked to the malignant transformation due to the accumulation of gene mutations (Fig. 2).

Conclusion

There is no doubt that the HBx protein plays an important role in the process of HBV infection, both in terms of viral replication and carcinogenesis (Fig. 3). Therefore, it is very important to develop new therapeutic methods targeting the HBx protein. However, based on the results of research to date, the biological functions of the HBx protein are extremely diverse, and the effects of this protein may differ depending on the stage during the course of chronic infection and on the surrounding environment. If the degradation of the Smc5/6 proteins by the HBx protein contributes to both the enhancement of viral replication and initiation of hepatocarcinogenesis, this would appear to be a theoretical phenomenon, since a single event would account for both the effects on the viral side and those on the host side. However, this is just one of the many possible mechanisms of hepatocarcinogenesis involving the HBx protein. If a more natural HBV infection model could be established in the near future, elucidation of the pathophysiology and the development of novel intervention methods would be possible from the perspective of the control of both viral replication and liver carcinogenesis induced by the HBx protein.

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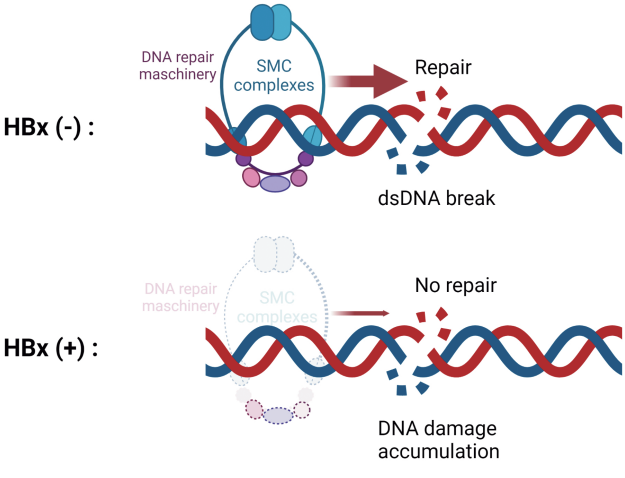

Fig. 2 HBx-mediated degradation of Smc complexes leads to the accumulation of DNA damages. Because Smc complexes are involved in the repair of dsDNA breaks, HBx-mediated degradation of SMc complexes leads to the accumulation of DNA damages, resulting in carcinogenesis.

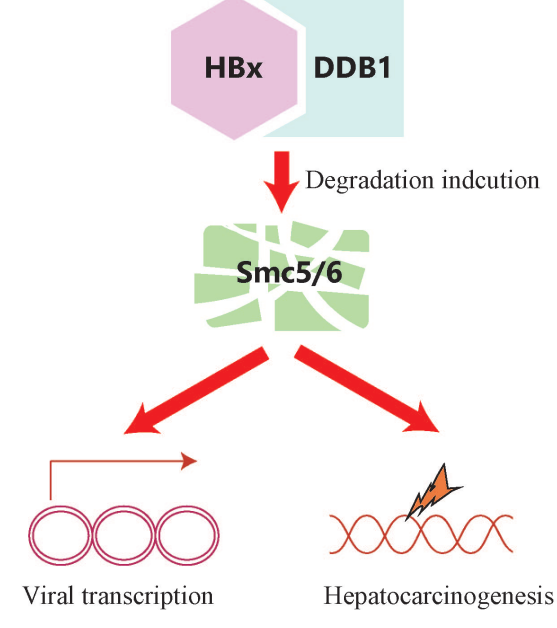

Fig. 3 Viral replication and hepatocarcinogenesis induced by degradation of the Smc5/6 protein complex. HBx-mediated degradation of Smc5/6 proteins not only promotes the transcription of viral RNA, but also causes accumulation of DNA damages in the host cells, leading to viral replication and carcinogenesis.

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