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Epigenetic Regulation of Hepatitis B Virus Replication

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Abstract

Hepatitis B virus (HBV) is the most important cause of chronic viral hepatitis worldwide. The genome of HBV is 3.2 kb partially double-stranded DNA, which is translocated to the nuclei of infected hepatocytes and converted to complete double-stranded DNA, aka covalently closed circular DNA (cccDNA). Typical course of chronic HBV infection results in inactive carrier state with clearance of viral particles in the bloodstream. However, the cccDNA can be detected in the hepatocytes from inactive carriers by sensitive methods. It has been increasingly known that epigenetic mechanisms contribute to the control of HBV replication in the inactive stage of HBV infection. Histone modification and DNA methylation have been identified in the HBV cccDNA, leading to modification of transcriptional activity. The understanding of epigenetic control of transcription will shed light on the development of new therapeutic strategy against HBV cccDNA.

Keywords: hepatitis B virus, covalently closed circular DNA, histone modification, inactive carrier

1. Introduction

Hepatitis B virus (HBV) is the most important cause of chronic viral hepatitis worldwide. About 240–350 million people are infected with HBV globally [1]. Development of potent nucleos(t)ide analogs (Nas) has revolutionized the treatment of HBV, but current treatment cannot eradicate the DNA genome of HBV, i.e., covalently closed circular DNA (cccDNA) from the nuclei of infected hepatocytes. Since there have been no innate clearing mechanisms identified for foreign double-stranded DNA in mammalian cells, theoretically HBV cccDNA in the liver stem cells will dilute out but persist indefinitely in some portion of the hepatocytes [2]. Therefore, prolonged use of current NA therapy is recommended without interruption, which is very costly.

It has been known that transcriptional activity of HBV cccDNA varies according to the stage of natural history of chronic hepatitis B (CHB) [3, 4]. Interestingly, many patients with chronic hepatitis B are free from circulating HBV during the natural course despite the presence of HBV cccDNA in the infected nuclei [5]. These findings raise the possibility that replication of HBV is regulated at the transcriptional level. Genetic changes, i.e., DNA mutation, are an attractive explanation for the variable transcriptional activity since the reverse transcriptase activity of HBV is error-prone. However, no universal mutations have been identified associated with transcriptional suppression [6]. Consequently, epigenetic control has been proposed as the mechanism of these variable transcriptional activities in CHB patients [7], and this article covers the current knowledge of epigenetic mechanisms contributing to the transcriptional control of HBV replication.

2. Organization of HBV cccDNA and its transcriptional control

Hepatitis B virus (HBV) is a partially double-stranded circular DNA virus [8]. The viral DNA goes into nuclei of infected hepatocytes where it is converted to cccDNA [8]. The cccDNA is a viral minichromosome, which takes the form of “beads-on-a-string” conformation of nucleosomal packaging [9], analogous to DNA packaging by mammalian nucleosome. HBV core protein and X protein along with histone H3 and H4 are components of HBV minichromosome [9–11]. A variety of cellular transcription factors bind HBV cccDNA, which in turn control transcriptional activity of HBV promoters: the preC/pregenomic, S1, S2, and X promoters [12]. The core promoter initiates transcription of preC and pregenomic RNA, the template for the viral genome by reverse transcription. Ubiquitous transcription factors such as specificity protein 1 (SP1), nuclear factor kappa B (NF- κ B), activator protein 1 (AP-1), and liver-enriched transcription factors such as hepatocyte nuclear factor 3 (HNF3), CAAT enhancer-binding protein (C/EBP), and several nuclear receptors such as hepatocyte nuclear factor 4 (HNF4), peroxisome proliferator-activated receptors (PPAR) and retinoid X receptors (RXR α), farnesoid acid receptor (FXR), small heterodimer partner (SHP), and testicular orphan receptor 4 (TR4) can bind core promoter [13, 14].

3. Epigenetic control of HBV transcription: histone modification

As described above, ultrastructure of HBV cccDNA simulates that of mammalian nucleosome, suggesting the possibility of histone molecules as a main factor for transcriptional control [9]. Indeed, Pollicino et al. demonstrated the feasibility of HBV-associated histone modification as a major transcriptional regulator of HBV [11]. Genome-wise search for posttranslational modification (PTM) of HBV-infected liver cell lines has revealed that active marks of transcription such as H3K4me3, H3K27ac, and H3K122ac are abundant in active chromatin, especially in the core promoter region [19]. Interestingly, however, the repressive marks of transcription, i.e., H3K27me3 and H3K9me2, are depleted in the HBV cccDNA, suggesting that modified

Histone change	Modifier	HBV response and mechanisms	References
H3K4me3	MLL3	Activation (H3K4me modification of the NTCP promoter by MLL3 may facilitate HBV infection in vivo.)	[19, 30]
	Zinc finger and homeoboxes (ZHX2)	Repression (ZHX2 inhibited trimethylation of H3K4. Overexpression of ZHX2 also decreases the acetylation levels of H3K27 and H3K122.)	[26]
H3K27ac	EZH2	Activation (Knockdown of EZH2 resulted in upregulation of HBeAg and ABsAg, indicating a repressive function of EZH2 on HBV gene expression.)	[28, 31]
H3K122ac	P300/CBP	Activation	[19, 32, 33]
AcH3/AcH4 H3K4me3	HBx	Activation	[20–22, 24]
H4R3me2s	PRMT5	Repression (PRMT5-mediated histone H4 dimethyl Arg3 symmetric (H4R3me2s) represses cccDNA transcription.)	[28]
H3K9me3	SETDB1	Repression (upon HBV infection, cellular mechanisms involving SETDB1-mediated H3K9me3 and HP1 induce silencing of HBV cccDNA transcription through modulation of chromatin structure.)	[24]
	SIRT3	Repression (SIRT3 is a novel host factor epigenetically restricting HBV cccDNA transcription by acting cooperatively with histone methyltransferase.)	[25]
H3K27me3	Suz12	Repression (downregulation of Suz12 and Znf198 enhances HBV replication.)	[34]
H3K79me	KDM2B	Repression (KDM2B as an H3K79 demethylase and link its function to transcriptional repression via SIRT1-mediated chromatin silencing.)	[27]
etc	BCP mutation	Repression (BCP mutations decrease viral replication capacity possibly by modulating the acetylation and deacetylation of cccDNA-bound histones.)	[29]
	IFN α	Repression	[19, 35, 36]

Table 1. Histone modification affecting HBV transcription.

histones regulate HBV transcription mainly in favor of active replication. Pol2 enrichment is co-localized at the H3K27me-enriched transcription start site of precore/pregenomic area, and treatment with interferon alpha reduces the active PTMs, also suggesting that active marks of histone modification contribute to the transcriptional activity of HBV.

Much is unknown regarding the effects of histone modification on the binding of these transcription factors. Hepatitis X protein (HBx) is the most studied modulator of HBV-bound histone [20, 21]. HBx is bound to the cccDNA and enhance transcription by increasing histone acetylation and recruiting cellular coactivators p300, CBP, and PCAF [22], by inhibiting protein arginine methyltransferase 1 and reducing H4 methylation [23]. HBx also increases histone acetylation and H3K4me3 and decreases HP1 binding and

H3K9me3 on the cccDNA [24]. Other host transcription factors, mainly suppressors, that act via epigenetic control of HBV include SIRT3 [25], zinc finger and homeobox 2 (ZHX2) [26], KDM2B [27], protein arginine methyltransferase 5 (PRMT5) [28], and SETDB1 [24]. Interestingly, mutations in the basal core promoter are also reported to be associated with histone modification [29]. The effect of histone modification on HBV replication is summarized in **Table 1**.

4. Epigenetic control of HBV transcription: cccDNA methylation

Isolation and testing of HBV cccDNA shows methylation not only of HBV DNA integrated in host chromosome [39, 40] but also of HBV cccDNA. Methylation is speculated to affect replicative activity of HBV [38], and this hypothesis was confirmed in the hepatoma cell lines [41] and human liver tissue [15, 16]. HBV cccDNA has three CpG islands which harbor methylation in human liver and hepatoma cell lines [37, 38]. Methylation of HBV in the CpG islands of cccDNA is associated with suppressed transcriptional activity of HBV [15, 16] (for recent reviews, see [17, 18]). Especially, methylation of CpG island II is associated with reduced HBV replicability [42].

Although DNA methyltransferases, i.e., DNMT1, DNMT3a, and DNMT3b, are expressed in normal tissues [45], the level of expression is higher in HCC [46], which may explain the increased levels of methylation in hepatocellular carcinoma (HCC) compared to noncancerous tissues [43, 44]. In addition, since the hepatic expression of DNMT3a and DNMT3b, the de novo methylators, increases with age [47], methylation of HBV may also increase with age [42], which might explain suppressed replicative activity of HBV in the later stage of natural history. Degree of methylation also depends on HBeAg positivity [15, 42] and degree of hepatic fibrosis [42].

The mechanism of HBV DNA methylation is still unknown (**Table 2**). From the specific patterns of methylation in the HBV genome [42], it can be speculated that some kind of molecular chaperon (s) may guide the de novo methylation enzymes to the specific target sequence. HBx may play a role, because it recruits DNMT3A to the regulatory promoters [48]. Small RNAs may be a plausible candidate, as suggested by our in vitro study in which short hairpin RNA induced methylation of the target site in HBV [49].

HBV area	Modifier	HBV replication	References
CpG2	HBc	Increased (The relative abundances of HBc binding to CpG island 2 were associated with the binding of CREB binding protein (CBP) and with hypomethylation in CpG island 2 of HBV cccDNA minichromosomes.)	[50]
CpG3	PEG-IFN	Decreased (PEG-IFN treatment significantly increased methylation of HBV cccDNA in CpG island III.)	[51]

Table 2. Mechanisms of methylation in HBV cccDNA.

5. Therapeutic implications and future perspectives of epigenetics in chronic hepatitis B

NAs, the most commonly used modality of CHB therapy, is costly without definite duration. Interferon induces sustained virologic response with finite duration, but the response rate is suboptimal. The realistic goal of CHB therapy is to render the patients to the clinical situation similar to inactive carrier stage, i.e., normal alanine aminotransferase levels with low or negative serum HBV DNA levels. Since epigenetic silencing may contribute to the suppressive HBV replication status of inactive carrier stage, it would be theoretically feasible and clinically useful to induce epigenetic suppression of HBV replication simulating natural inactive stage of disease. Further studies will be needed to elucidate the mechanisms and long-term consequences of epigenetic suppression of HBV replication.

6. Conclusions

Epigenetic modification is an important mechanism of host-viral interaction in the transcriptional control of HBV. Current treatment strategy focuses on the inactivation/elimination of HBV cccDNA [17, 52], and knowledge on the epigenetic control is prerequisite for the novel development of HBV cure in the foreseeable future.

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