Review Article
Regulatory mechanisms for HBV replication

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Abstract: Hepatitis B virus (HBV) is one of the smallest enveloped DNA viruses and infects more than 350 million people worldwide. HBV infection causes acute and chronic hepatitis, and is one of the major causes of cirrhosis and hepatocellular carcinoma. Currently available therapies can achieve viral suppression, but not termination of HBV infection. HBV replicates its genome via an RNA intermediate in infected hepatocytes. Complex virus-host interactions ensure that the virus is able to fulfill its replication requirements while successfully evading antiviral innate immune responses. An in-depth understanding of the molecular mechanisms regulating HBV replication is necessary for designing more effective therapeutic strategies, which would in turn improve the management of patients with chronic HBV infection and eventually help achieve viral eradication. The regulation of HBV transcription is a critical step in the viral life cycle and has been extensively investigated. The replication of HBV can also be regulated by other mechanisms, including regulation of the stability of HBV transcripts and viral protein, regulation of capsid assembly, and regulation of HBV DNA synthesis. In addition, recent studies have revealed that HBV replication is subject to epigenetic regulation by several mechanisms, including DNA methylation, histone acetylation, and microRNAs (miRNAs). This review focuses on the molecular mechanisms underlying the regulation of HBV replication, with special emphasis on the epigenetic mechanisms.

Keywords: Hepatitis B virus, replication, histone modification, DNA methylation, microRNA

Introduction

Hepatitis B virus (HBV) infection is a major global health problem; 350 million patients with chronic HBV infections are at a high risk of developing liver cirrhosis and hepatocellular carcinoma (HCC). HBV belongs to the *Hepadnaviridae* family, a small group of liver-tropic viruses that share a distinctive strategy for replication. Currently available therapies can only achieve viral suppression and not termination of HBV infection; lifelong therapy is required in the majority of infected persons [1]. One major conceptual advance in HBV therapy has been the recognition of the existence of a complex network of genetic and epigenetic events that influence HBV replication. This review summarizes the current understanding of the molecular mechanisms underlying the regulation of HBV replication, with special emphasis on the epigenetic mechanisms.

Viral life cycle

The life cycle of HBV begins with viral entry into cells. The first step of the life cycle involves low-affinity binding of HBV particles to heparan-sulfate proteoglycans, which is followed by high-affinity binding of virus particles to specific receptors. Sodium taurocholate cotransporting polypeptide (NTCP) has been identified as one of the high-affinity receptors for HBV; NTCP binds to HBV preS1 peptides via its N-terminal myristic acid (N-myr) at the glycine 2 (myr-preS12-48) moiety [2]. Following attachment, viral envelope proteins are fused with the cell membrane. The viral core particle housed within the endosomal membrane is then actively transported to the cell nucleus. This transport is mediated by a nuclear localization signal on the capsid protein, and the encapsidated HBV genome gains access to the nucleus via the importin pathway using the nuclear transport receptors Imp-β/Imp-α [3]. The capsid dissociates in the nucleus and the relaxed circular DNA (rcDNA) is converted into covalently closed circular DNA (cccDNA) by host cell DNA repair enzymes [4]. The cccDNA assumes the role of a minichromosome and functions as the template for viral transcription. The HBV genome is transcribed into four major RNAs, including the

3.5-kb precore (preC) and pregenomic RNA (pgRNA), the 2.4-kb pre-S1 mRNA, the 2.1-kb pre-S2/S mRNA, and the 0.7-kb X mRNA [5]. The pgRNA encodes both the core and polymerase proteins, whereas the precore mRNA encodes the precore precursor. The 2.4-kb and 2.1-kb mRNAs encode the three forms of HBV surface antigens, and the 0.7-kb transcript functions as the template for the translation of HBV X protein. Subsequently, the polymerase/pgRNA complex is encapsidated into newly formed capsids, where the pgRNA is subsequently reverse-transcribed into rcDNA. After concomitant degradation of the pgRNA, a single-stranded DNA with minus polarity and a complementary plus-strand DNA are synthesized to form the HBV genome (rcDNA). Finally, maturing nucleocapsids bud through the endoplasmic reticulum membrane, acquiring their outer envelope and incorporating viral envelope proteins in the process [3-5].

Transcriptional regulation of HBV replication

Regulation of HBV transcription by cis-elements

HBV transcription is initiated by the following four promoters: core, S1, S2, and X. In addition, two enhancers (enhancer [EN] I and ENII) and cis-acting negative regulatory elements play pivotal roles in the regulation of viral gene transcription [5]. All of the HBV regulatory cis-elements are embedded within protein-coding sequences [5]. Synthesis of the 3.5-kb RNA is regulated by two partially overlapping but distinct core promoters that overlap the 3' end of the X open reading frame (ORF) and the 5' end of the pre-C/C ORF; these promoters direct the transcription of the preC and pgRNAs. The core promoter consists of the basal core promoter (BCP) and an upstream regulatory sequence. The BCP lacks the canonical TATA box sequence, and each of the pg and preC transcripts is produced from distinct promoter sequences that include TATA box-like and initiator elements [5]. The preS1 promoter is the only HBV promoter that contains a classical TATA-box sequence [6], and it regulates the transcription of the 2.4-kb pre-S1 mRNA. The unusual preS2 promoter is positioned within the overlapping polymerase and preS1 ORFs, and it shares extensive homology with the simian virus 40 late promoter. The preS2 promoter contains a CCAAT motif and two transcription initiation elements that can

act independently of each other. In addition to stimulating the transcription of the 2.1-kb transcript, the CCAAT motif also represses the transcription of the 2.4-kb transcript [7, 8]. The transcription of HBx is under the control of the X promoter, which is located upstream of the transcription initiation site and overlaps with the 3'-end of ENI. ENI (~200 bp) is the main stimulating element, and it significantly upregulates the preC and HBx mRNAs, but has little effect on surface protein mRNAs. The location of ENI partially overlaps the X promoter; ENI harbors binding sites for ubiquitous and liver-specific transcription factors [9]. ENII is located upstream of the core promoter, and it preferentially increases the transcriptional activity of the preS1, preS2, X, and core promoters [5]. A negative regulatory element (NRE) that is present upstream of the core promoter suppresses core promoter activity in an orientation-independent manner. The NRE contains three different functional subregions: NRE-a, NRE-b, and NRE-c. These cis-elements individually produce a weak suppressive action, but together, they act synergistically to exert an 11-fold inhibitory effect on transcriptional regulation by the core promoter [5].

Regulation of HBV transcription by trans-acting factors

Various ubiquitous and liver-enriched transcription factors modulate HBV transcription by binding to different cis-regulatory elements. The core promoter harbors multiple response elements for various ubiquitous and liver-enriched transcription factors, including chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1), farnesoid X receptor α (FXRA), retinoid X receptor α (RXRA), peroxisome proliferator-activated receptor α (PPARα), hepatocyte nuclear factor 4α (HNF-4α), hepatocyte nuclear factor 3β (HNF-3β), CCAAT/enhancer-binding protein α (C/EBPα), CCAAT/enhancer-binding protein β (C/EBPβ), nuclear orphan receptor TR4, and Sp1 [5, 10]. The basic transcriptional activity of the preS1 promoter requires a hepatocyte nuclear factor-1 (HNF-1) binding site upstream of the TATA-binding protein (TBP) motif [5, 11]. In addition, the preS1 promoter also contains binding sites for neurofibromin 1 (NF1), Sp1, nuclear factor-Y (NF-Y), hepatocyte nuclear factor 3 (HNF-3), and nuclear factor Prospero-related homeobox protein (Prox1) [12]. The preS2 promoter con-

tains binding sites for the following transcriptional factors: NF-Y and CCAAT binding factor (CBF) [13]. X-promoter binding protein (X-PBP), NF1, C/EBP, artificial transcription factor (ATF), activator protein 1 (AP1), and p53 can bind to the X promoter and regulate the transcription of HBx [14, 15].

In addition to binding to promoters, transcription factors can also regulate HBV transcription by binding to enhancers. Transcription factors that bind to ENI include HNF-3, regulatory factor X 1 (RFX1), NF1, HNF-4, RXRa, PPAR, signal transducer and activator of transcription-3 (STAT-3), and COUP-TF [5]. In addition, the following transcription factors bind to ENII: liver receptor homologue 1 (LRH-1), FXRα, RXRα, HNF-4α, C/EBPα, C/EBPβ, hepatocyte nuclear factor 3α (HNF-3α), HNF-3β, nuclear respiratory factor-1 (NRF1), Prox1, Sp1, and FOXO1 [10].

HBx is a trans-activator that is required for HBV replication *in vivo*. In contrast to other trans-acting factors, HBx does not bind DNA directly; rather, it exerts its transcription effects by binding to and modifying the function of transcription factors. In addition, HBx may affect HBV transcription by modulating different signaling pathways [16, 17].

Regulation of the stability of HBV transcripts

In addition to transcriptional regulation, host factors can regulate the stability of HBV transcripts. For example, myeloid differentiation primary response protein 88 (MyD88), which is induced by alpha interferon (IFN-α), exhibits antiviral activity against HBV by accelerating the decay of viral pgRNA in the cytoplasm [18]. IFN-α treatment or interferon promoter stimulator 1 (IPS-1) activation induces the expression of zinc finger antiviral protein (ZAP), which binds to the ZAP-responsive elements of HBV pgRNA to mediate RNA decay [19].

Regulation of viral protein stability and capsid assembly

Nucleophosmin binds to HBV core protein dimers and promotes HBV capsid assembly [20]. Cellular inhibitor of apoptosis protein 2 (cIAP2) inhibits HBV replication by accelerating the ubiquitin-proteasome-mediated decay of HBV polymerase and attenuating the encapsidation of HBV pgRNA [21]. SR-domain protein kinase (SRPK) 1 and 2 are two important kinases involved in cellular RNA splicing. SRPK 1 and 2 bind to HBV core protein and suppress HBV replication by reducing the packaging efficiency of pgRNA without affecting the formation of viral core particles [22]. Hdj1 and hTid1, which belong to the Hsp40/DnaJ chaperone-family, specifically bind to the viral core and HBx proteins and promote their degradation [23]. Hepatocystin/80K-H interacts with HBx protein and promotes its degradation; overexpression of hepatocystin/80K-H inhibits HBV replication [24]. MxA interacts with hepatitis B core antigen, leading to the accumulation of HBcAg in the perinuclear compartments and suppressing core particle formation [25].

Regulation of HBV DNA synthesis

Hsc70 and Hsp90 can enhance the *de novo* synthesis of HBV at the reverse transcription stage (conversion of pgRNA to DNA) [26]. Hsp60 promotes HBV replication through the activation of HBV polymerase before its encapsidation into the HBV core particle [27]. In addition to introducing G-to-A hypermutations into the nascent minus strand DNA of HBV, some human apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) proteins, including APOBEC3B, APOBEC3C, APOBEC3F, APOBEC3G, and APOBEC3H, can inhibit HBV DNA production; this function is independent of their deaminase activities [28].

Epigenetic regulation of HBV replication

Regulation of hepatitis B virus replication by histone modification

The HBV genome forms a cccDNA minichromosome that serves as the template for viral mRNA synthesis. Numerous studies have revealed that the HBV minichromosome consists of both histone and nonhistone proteins. In addition to the HBV core and HBx proteins, the histone proteins (including H1, H2A, H2B, H3, and H4), several cellular transcription factors (CREB, ATF, YY1, STAT1, and STAT2), and chromatin modifying enzymes (PCAF, p300/CBP, HDAC1, SIRT1, and EZH2) have been shown to bind to cccDNA in human hepatoma cells containing replicating HBV [29-33]. Because histone modifications play pivotal roles in transcriptional control, it is conceivable that histone modifiers can regulate HBV tran-

Table 1. microRNAs involved in regulation of HBV replication

MicroRNAs that repress HBV replication by targeting HBV transcripts			MicroRNAs that repress HBV replication by targeting cellular genes			MicroRNAs that promote HBV replication by targeting cellular genes		
MicroRNA	Target	Ref	MicroRNA	Target	Ref	MicroRNA	Target	Ref
MiR-199a-3p	SHBs	43	MiR-122	HO-1	58	MiR-1	FXRA	53
MiR-210	LHBs	43		Cyclin G(1)	50	MiR-372/373	NFIB	51
MiR-125a-5p	Pol & HBs	44		SOCS3	66	MiR-501	HBXIP	52
MiR-122	Pol & Core	45	MiR-141	PPARA	55	MiR-15b	HNF1α	49
MiR-15a	Pol & HBx	46	MiR-125b	SCNN1A	59	MiR-581	EDEM1, Dicer	40
MiR-20a	HBx & Pol	47	MiR-26b	CHORDC1	56			
MiR-92a-1	HBx & Pol	47	MiR-130a	PGC1α & PPARγ	54			
MiR-1231	Core	48	MiR-155	C/EBP-beta	60			

scription. Pollicino and colleagues demonstrated that HBV replication is regulated by the acetylation status of H3/H4 histones bound to the viral cccDNA, both in cell-based replication systems and in the liver of chronically HBV-infected patients [29]. Cellular histone acetyltransferases (CBP, p300, and PCAF/GCN5) are recruited onto the cccDNA minichromosome in an HBx-dependent manner, and the kinetics of p300 recruitment on cccDNA parallels those of HBV replication. p300 recruitment is severely impaired, whereas the recruitment of histone deacetylases hSirt1 and HDAC1 is enhanced, and cccDNA-bound histones are rapidly hypoacetylated in cells replicating the HBx mutant [30]. Two recent studies have demonstrated that IFN-α can epigenetically regulate HBV replication [31, 34]. IFN-α treatment resulted in active recruitment of the transcriptional corepressors, i.e., HDAC1, SIRT1, and polycomb repressor complexes 2 (EZH2 and YY1), to HBV cccDNA, in addition to hypoacetylation/hypermethylation of cccDNA-bound histones [31, 34].

Regulation of HBV replication by DNA methylation

The 3.2-kb HBV genome contains three major CpG islands, which overlap the start site of the small surface (S) gene (island I), span a region that overlaps EN I/II and is proximal to the core promoter (island II), and cover the start codon of the polymerase (P) gene and upstream region of SP1 promoter (island III) [35]. Methylation of CpG islands 1 and 2 has been detected in HBV DNA extracted from liver biopsy samples from chronic hepatitis B patients, suggesting that increased methylation of HBV DNA may decrease the production of viral proteins [35].

Another cohort study in advanced hepatitis B patients confirmed that low serum HBV DNA levels and low virion productivity are significantly associated with elevated cccDNA methylation [36].

Regulation of HBV replication by DNA methylation has also been validated by *in v*itro experiments. Compared with non-methylated DNA, transfection of methylated HBV DNA led to reduced HBV mRNA levels and decreased surface and core protein expression in HepG2 cells, and decreased secretion of HBV viral proteins into the cell supernatant [37]. Targeting of the C-terminus of DNA methyltransferase 3a (Dnmt3a) to the HBV X promoter via a six-zinc-finger peptide resulted in epigenetic suppression of HBV expression. These data provide direct evidence that DNA methylation regulates HBV replication. HBV infection can also affect the expression of host DNA methyltransferases (DNMTs). Using a model system that mimics natural HBV infection, Vivekanandan et al. demonstrated that DNMTs, including DNMT1, DNMT2, and DNMT3, were significantly upregulated in response to HBV infection. Cotransfection experiments with full-length HBV and DNMT3 demonstrated downregulation of viral protein and pgRNA production [38].

Regulation of HBV replication by microRNAs (miRNAs)

MiRNAs are a group of small noncoding regulatory RNAs that have critical functions in various physiological and pathological processes. MiRNAs are transcribed as primary miRNAs that are processed in the nucleus by Drosha, resulting in the liberation of ~70-nucleotide (nt) stem loop structures called precursor miRNAs.

The precursor miRNAs are then transported into the cytoplasm, where they are processed by Dicer, resulting in a ~21-nt RNA duplex. One strand of the duplex, the mature miRNA, preferentially enters the RNA-induced silencing complex to mediate the cleavage or translational repression of mRNAs by binding to their 3'-untranslated regions (UTRs) [39].

Although our small RNA deep sequencing data indicated that HBV does not encode miRNAs [40], HBV products altered miRNA expression profiles. With regard to chronic HBV infection or HBV-related HCC, the miRNA profiles in liver tissue or serum from numerous studies are controversial and inconsistent [41]. Numerous studies have revealed that the HBx protein can alter the expression of several host miRNAs. We also demonstrated that hepatitis B surface antigen (HBsAg) regulated the expression of 142 miRNAs in HepG2 cells [42]. Emerging evidence has revealed that miRNAs play pivotal roles in regulating HBV replication, as discussed in detail below and summarized in **Table 1**.

MiRNAs repress HBV expression by binding to HBV transcripts

Several studies have revealed that cellular miRNAs can inhibit HBV replication by directly targeting HBV transcripts. MiR-199a-3p and miR-210 were shown to effectively reduce HBsAg expression and suppress viral replication by targeting the HBsAg coding region and pre-S1 region, respectively [43]. MiR-125a binds to the transcript encoding the surface antigen and interferes with its expression, thereby inhibiting viral replication [44]. MiR-122 negatively regulates viral gene expression and replication by targeting the sequence located at the coding region of the mRNA encoding viral polymerase and the 3' untranslated region of the mRNA encoding the core protein [45]. Other miRNAs, including miR-15a, miR-16-1, miR-1231, and the miR-17-92 cluster, can also target HBV mRNAs and inhibit HBV replication [46-48].

Indirect regulation of HBV replication by cellular miRNAs

In addition to targeting HBV transcripts directly, cellular miRNAs can regulate HBV replication via indirect mechanisms by targeting transcription factors and nuclear receptors. MiR-15b promotes HBV replication by augmenting HBV ENI activity via direct targeting of HNF1α [49]. MiR-122 may exert its effect on HBV indirectly via downregulation of its target, cyclin G1, thereby interrupting the interaction between cyclin G1 and p53 and abrogating p53-mediated inhibition of HBV replication [50]. MiR-122 was shown to miR-372 and -373 promote HBV gene expression by targeting nuclear factor I/B [51]. MiR-501 may promote HBV replication by targeting HBXIP [52]. MiR-1 was shown to enhance HBV core promoter transcription activity by augmenting farnesoid X receptor alpha (FXRA) expression [53]. MiR-130a reduced HBV replication by targeting two major metabolic regulators, PGC1α and PPARγ, both of which can potently stimulate HBV replication [54]. MiR-141 was shown to suppress HBV replication by reducing HBV promoter activity through downregulation of PPARA [55]. Cysteine- and histidine-rich domain containing 1 (CHORDC1) was shown to increase viral antigen expression, transcription, and replication by elevating HBV enhancer/promoter activity. MiR-26a and miR-26b inhibit HBV transcription and replication by targeting CHORDC1 [56]. In addition to targeting FXRA, miR-1 enhances HBV transcription by inhibiting HDAC1 expression [53].

In addition to regulating HBV transcription, miRNAs can also regulate HBV replication via other mechanisms. In a previous study, we demonstrated that miR-581 targets Dicer and endoplasmic reticulum degradation-enhancing alpha-mannosidase-like protein 1 (EDEM1), and represses their expression [40]. Dicer can process 7SL RNA into small fragments, which in turn interfere with the formation of signal recognition particles and inhibit protein secretion. Overexpression of miR-581 enhances HBsAg secretion by reducing the levels of Dicer-processed 7SL RNA fragments. EDEM1 promotes HBsAg degradation; therefore, overexpression of miR-581 stabilizes HBsAg by downregulating EDEM1 [40]. Heme oxygenase-1 (HO-1) was shown to repress HBV replication directly in hepatocytes at a posttranscriptional step by reducing the stability of the HBV core protein and thus blocking the replenishing of nuclear HBV cccDNA [57]. MiR-122 suppresses HBV replication by targeting HO-1 [58]. MiR-125b inhibits HBV expression by targeting the sodium channel non-voltage-gated 1 alpha

(SCNN1A) [59]. In addition to regulating HBV transcription by targeting C/EBP-β, miR-155 enhances innate antiviral immunity by promoting the JAK/STAT signaling pathway and by targeting SOCS1. Furthermore, miR-155 mildly inhibits HBV infection in human hepatoma cells [60, 61].

The same stimulus can regulate HBV replication via different mechanisms

We have summarized above the molecular mechanisms underlying the regulation of HBV replication. It is worth mentioning that the same stimulus can regulate HBV replication via different mechanisms. For example, tumor necrosis factor alpha (TNF-α) suppresses HBV DNA replication without inducing cell lysis by disrupting the formation or stability of cytoplasmic viral capsids through activation of nuclear factor-κB [62]. It also induces the expression of cIAP2, which in turn promotes the ubiquitin-proteasome-mediated decay of HBV polymerase, inhibits the encapsidation of HBV pgRNA, and suppresses HBV protein and RNA synthesis [21, 63]. Moreover, TNF-α can block HBV replication by promoting the destabilization of pre-existing cytoplasmic viral nucleocapsids containing viral RNA and DNA, as well as the empty nucleocapsids [64]. Another example is alpha interferon (IFN-α), which suppresses HBV transcription by inhibiting the activity of HBV enhancers and by epigenetic modification of cccDNA minichromosomes [65]. It also inhibits HBV nucleocapsid formation and destabilizes HBV transcripts [65]. Interestingly, it has been reported that IFN-α treatment induced a marked decrease of miR-122, which may negatively affect the anti-HBV function of IFN-α [66].

Conclusions and perspective

Although numerous studies have revealed that cellular miRNAs directly or indirectly influence the HBV life cycle by targeting HBV transcripts or host factors, the issue of whether viruses are subject to restriction by endogenous miRNAs in infected human cells is controversial. Cullen et al. recently reported that the replication of many human viruses is refractory to inhibition by endogenous cellular miRNAs [67]. Our previous studies revealed that knockdown of Dicer, a key enzyme involved in miRNA biogenesis, only slightly increased the secretion of HBsAg without affecting the levels of HBV transcripts

[40]. Therefore, further studies are needed to elucidate the role of miRNAs in HBV replication.

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Disclosure of conflict of interest

None.

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References

[1] Wang FS, Fan JG, Zhang Z, Gao B and Wang HY. The global burden of liver disease: the major impact of China. Hepatology 2014; 60: 2099-2108.

[2] Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J and Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife 2012; 1: e00049.

[3] Ezzikouri S, Ozawa M, Kohara M, Elmdaghri N, Benjelloun S and Tsukiyama-Kohara K. Recent insights into hepatitis B virus-host interactions. J Med Virol 2014; 86: 925-932.

[4] Locarnini S and Zoulim F. Molecular genetics of HBV infection. Antivir Ther 2010; 15 Suppl 3: 3-14.

[5] Moolla N, Kew M and Arbuthnot P. Regulatory elements of hepatitis B virus transcription. J Viral Hepat 2002; 9: 323-331.

[6] Siddiqui A, Jameel S and Mapoles J. Transcriptional control elements of hepatitis B surface antigen gene. Proc Natl Acad Sci U S A 1986; 83: 566-570.

[7] Lu CC, Chen M, Ou JH and Yen TS. Key role of a CCAAT element in regulating hepatitis B virus surface protein expression. Virology 1995; 206: 1155-1158.

[8] Zhou DX and Yen TS. The hepatitis B virus S promoter comprises A CCAAT motif and two initiation regions. J Biol Chem 1991; 266: 23416-23421.

[9] Cai YN, Zhou Q, Kong YY, Li M, Viollet B, Xie YH and Wang Y. LRH-1/hB1F and HNF1 synergistically up-regulate hepatitis B virus gene transcription and DNA replication. Cell Res 2003; 13: 451-458.

[10] Curtil C, Enache LS, Radreau P, Dron AG, Scholtes C, Deloire A, Roche D, Lotteau V, Andre P and Ramiere C. The metabolic sensors FXRalpha, PGC-1alpha, and SIRT1 cooperatively regulate hepatitis B virus transcription. FASEB J 2014; 28: 1454-1463.

[11] Raney AK, Easton AJ and McLachlan A. Characterization of the minimal elements of the hepatitis B virus large surface antigen promoter. J Gen Virol 1994; 75: 2671-2679.

[12] Raney AK and McLachlan A. Characterization of the hepatitis B virus major surface antigen promoter hepatocyte nuclear factor 3 binding site. J Gen Virol 1997; 78: 3029-3038.

[13] Bock CT, Kubicka S, Manns MP and Trautwein C. Two control elements in the hepatitis B virus S-promoter are important for full promoter activity mediated by CCAAT-binding factor. Hepatology 1999; 29: 1236-1247.

[14] Fukai K, Takada S, Yokosuka O, Saisho H, Omata M and Koike K. Characterization of a specific region in the hepatitis B virus enhancer I for the efficient expression of X gene in the hepatic cell. Virology 1997; 236: 279-287.

[15] Takada S, Kaneniwa N, Tsuchida N and Koike K. Hepatitis B virus X gene expression is activated by X protein but repressed by p53 tumor suppressor gene product in the transient expression system. Virology 1996; 216: 80-89.

[16] Tang H, Oishi N, Kaneko S and Murakami S. Molecular functions and biological roles of hepatitis B virus x protein. Cancer Sci 2006; 97: 977-983.

[17] Rawat S and Bouchard MJ. The hepatitis B virus (HBV) HBx protein activates AKT to simultaneously regulate HBV replication and hepatocyte survival. J Virol 2015; 89: 999-1012.

[18] Li J, Lin S, Chen Q, Peng L, Zhai J, Liu Y and Yuan Z. Inhibition of hepatitis B virus replication by MyD88 involves accelerated degradation of pregenomic RNA and nuclear retention of pre-S/S RNAs. J Virol 2010; 84: 6387-6399.

[19] Mao R, Nie H, Cai D, Zhang J, Liu H, Yan R, Cuconati A, Block TM, Guo JT and Guo H. Inhibition of hepatitis B virus replication by the host zinc finger antiviral protein. PLoS Pathog 2013; 9: e1003494.

[20] Jeong H, Cho MH, Park SG and Jung G. Interaction between nucleophosmin and HBV core protein increases HBV capsid assembly. FEBS Lett 2014; 588: 851-858.

[21] Wang Z, Ni J, Li J, Shi B, Xu Y and Yuan Z. Inhibition of hepatitis B virus replication by cIAP2 involves accelerating the ubiquitin-proteasome-mediated destruction of polymerase. J Virol 2011; 85: 11457-11467.

[22] Zheng Y, Fu XD and Ou JH. Suppression of hepatitis B virus replication by SRPK1 and SRPK2 via a pathway independent of the phosphorylation of the viral core protein. Virology 2005; 342: 150-158.

[23] Sohn SY, Kim SB, Kim J and Ahn BY. Negative regulation of hepatitis B virus replication by cellular Hsp40/DnaJ proteins through destabilization of viral core and X proteins. J Gen Virol 2006; 87: 1883-1891.

[24] Shin GC, Ahn SH, Choi HS, Lim KH, Choi do Y, Kim KP and Kim KH. Hepatocystin/80K-H inhibits replication of hepatitis B virus through interaction with HBx protein in hepatoma cell. Biochim Biophys Acta 2013; 1832: 1569-1581.

[25] Li N, Zhang L, Chen L, Feng W, Xu Y, Chen F, Liu X, Chen Z and Liu W. MxA inhibits hepatitis B virus replication by interaction with hepatitis B core antigen. Hepatology 2012; 56: 803-811.

[26] Wang YP, Liu F, He HW, Han YX, Peng ZG, Li BW, You XF, Song DQ, Li ZR, Yu LY, Cen S, Hong B, Sun CH, Zhao LX, Kreiswirth B, Perlin D, Shao RG and Jiang JD. Heat stress cognate 70 host protein as a potential drug target against drug resistance in hepatitis B virus. Antimicrob Agents Chemother 2010; 54: 2070-2077.

[27] Park SG, Lee SM and Jung G. Antisense oligodeoxynucleotides targeted against molecular chaperonin Hsp60 block human hepatitis B virus replication. J Biol Chem 2003; 278: 39851-39857.

[28] Janahi EM and McGarvey MJ. The inhibition of hepatitis B virus by APOBEC cytidine deaminases. J Viral Hepat 2013; 20: 821-828.

[29] Pollicino T, Belloni L, Raffa G, Pediconi N, Squadrito G, Raimondo G and Levrero M. Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. Gastroenterology 2006; 130: 823-837.

[30] Belloni L, Pollicino T, De Nicola F, Guerrieri F, Raffa G, Fanciulli M, Raimondo G and Levrero M. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. Proc Natl Acad Sci U S A 2009; 106: 19975-19979.

[31] Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, Petersen J, Raimondo G, Dandri M and Levrero M. IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest 2012; 122: 529-537.

[32] Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G and Dandri M. Control of cccDNA

function in hepatitis B virus infection. J Hepatol 2009; 51: 581-592.

[33] Bock CT, Schwinn S, Locarnini S, Fyfe J, Manns MP, Trautwein C and Zentgraf H. Structural organization of the hepatitis B virus minichromosome. J Mol Biol 2001; 307: 183-196.

[34] Liu F, Campagna M, Qi Y, Zhao X, Guo F, Xu C, Li S, Li W, Block TM, Chang J and Guo JT. Alpha-interferon suppresses hepadnavirus transcription by altering epigenetic modification of ccc-DNA minichromosomes. PLoS Pathog 2013; 9: e1003613.

[35] Vivekanandan P, Thomas D and Torbenson M. Hepatitis B viral DNA is methylated in liver tissues. J Viral Hepat 2008; 15: 103-107.

[36] Kim JW, Lee SH, Park YS, Hwang JH, Jeong SH, Kim N and Lee DH. Replicative activity of hepatitis B virus is negatively associated with methylation of covalently closed circular DNA in advanced hepatitis B virus infection. Intervirology 2011; 54: 316-325.

[37] Vivekanandan P, Thomas D and Torbenson M. Methylation regulates hepatitis B viral protein expression. J Infect Dis 2009; 199: 1286-1291.

[38] Vivekanandan P, Daniel HD, Kannangai R, Martinez-Murillo F and Torbenson M. Hepatitis B virus replication induces methylation of both host and viral DNA. J Virol 2010; 84: 4321-4329.

[39] Ha M and Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.

[40] Wang YQ, Ren YF, Song YJ, Xue YF, Zhang XJ, Cao ST, Deng ZJ, Wu J, Chen L, Li G, Shi KQ, Chen YP, Ren H, Huang AL and Tang KF. MicroRNA-581 promotes hepatitis B virus surface antigen expression by targeting Dicer and EDEM1. Carcinogenesis 2014; 35: 2127-2133.

[41] Zhang X, Hou J and Lu M. Regulation of hepatitis B virus replication by epigenetic mechanisms and microRNAs. Front Genet 2013; 4: 202.

[42] Wu J, Zhang XJ, Shi KQ, Chen YP, Ren YF, Song YJ, Li G, Xue YF, Fang YX, Deng ZJ, Xu X, Gao J and Tang KF. Hepatitis B surface antigen inhibits MICA and MICB expression via induction of cellular miRNAs in hepatocellular carcinoma cells. Carcinogenesis 2014; 35: 155-163.

[43] Zhang GL, Li YX, Zheng SQ, Liu M, Li X and Tang H. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. Antiviral Res 2010; 88: 169-175.

[44] Potenza N, Papa U, Mosca N, Zerbini F, Nobile V and Russo A. Human microRNA hsa-miR-125a-5p interferes with expression of hepatitis B virus surface antigen. Nucleic Acids Res 2011; 39: 5157-5163.

[45] Chen Y, Shen A, Rider PJ, Yu Y, Wu K, Mu Y, Hao Q, Liu Y, Gong H, Zhu Y, Liu F and Wu J. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. FASEB J 2011; 25: 4511-4521.

[46] Wang Y, Jiang L, Ji X, Yang B, Zhang Y and Fu XD. Hepatitis B viral RNA directly mediates down-regulation of the tumor suppressor microRNA miR-15a/miR-16-1 in hepatocytes. J Biol Chem 2013; 288: 18484-18493.

[47] Jung YJ, Kim JW, Park SJ, Min BY, Jang ES, Kim NY, Jeong SH, Shin CM, Lee SH, Park YS, Hwang JH, Kim N and Lee DH. c-Myc-mediated overexpression of miR-17-92 suppresses replication of hepatitis B virus in human hepatoma cells. J Med Virol 2013; 85: 969-978.

[48] Kohno T, Tsuge M, Murakami E, Hiraga N, Abe H, Miki D, Imamura M, Ochi H, Hayes CN and Chayama K. Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA. J Viral Hepat 2014; 21: e89-97.

[49] Dai X, Zhang W, Zhang H, Sun S, Yu H, Guo Y, Kou Z, Zhao G, Du L, Jiang S, Zhang J, Li J and Zhou Y. Modulation of HBV replication by microRNA-15b through targeting hepatocyte nuclear factor 1alpha. Nucleic Acids Res 2014; 42: 6578-6590.

[50] Wang S, Qiu L, Yan X, Jin W, Wang Y, Chen L, Wu E, Ye X, Gao GF, Wang F, Chen Y, Duan Z and Meng S. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1) -modulated P53 activity. Hepatology 2012; 55: 730-741.

[51] Guo H, Liu H, Mitchelson K, Rao H, Luo M, Xie L, Sun Y, Zhang L, Lu Y, Liu R, Ren A, Liu S, Zhou S, Zhu J, Zhou Y, Huang A, Wei L, Guo Y and Cheng J. MicroRNAs-372/373 promote the expression of hepatitis B virus through the targeting of nuclear factor I/B. Hepatology 2011; 54: 808-819.

[52] Jin J, Tang S, Xia L, Du R, Xie H, Song J, Fan R, Bi Q, Chen Z, Yang G, Liu J, Shi Y and Fan D. MicroRNA-501 promotes HBV replication by targeting HBXIP. Biochem Biophys Res Commun 2013; 430: 1228-1233.

[53] Zhang X, Zhang E, Ma Z, Pei R, Jiang M, Schlaak JF, Roggendorf M and Lu M. Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. Hepatology 2011; 53: 1476-1485.

[54] Huang JY, Chou SF, Lee JW, Chen HL, Chen CM, Tao MH and Shih C. MicroRNA-130a can inhibit hepatitis B virus replication via targeting PGC1alpha and PPARgamma. RNA 2015; 21: 385-400.

[55] Hu W, Wang X, Ding X, Li Y, Zhang X, Xie P, Yang J and Wang S. MicroRNA-141 represses HBV replication by targeting PPARA. PLoS One 2012; 7: e34165.

[56] Zhao F, Xu G, Zhou Y, Wang L, Xie J, Ren S, Liu S and Zhu Y. MicroRNA-26b inhibits hepatitis B virus transcription and replication by targeting the host factor CHORDC1 protein. J Biol Chem 2014; 289: 35029-35041.

[57] Protzer U, Seyfried S, Quasdorff M, Sass G, Svorcova M, Webb D, Bohne F, Hosel M, Schirmacher P and Tiegs G. Antiviral activity and hepatoprotection by heme oxygenase-1 in hepatitis B virus infection. Gastroenterology 2007; 133: 1156-1165.

[58] Qiu L, Fan H, Jin W, Zhao B, Wang Y, Ju Y, Chen L, Chen Y, Duan Z and Meng S. miR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. Biochem Biophys Res Commun 2010; 398: 771-777.

[59] Zhang Z, Chen J, He Y, Zhan X, Zhao R, Huang Y, Xu H, Zhu Z and Liu Q. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. Arch Virol 2014; 159: 3335-3343.

[60] Sarkar N, Panigrahi R, Pal A, Biswas A, Singh SP, Kar SK, Bandopadhyay M, Das D, Saha D, Kanda T, Sugiyama M, Chakrabarti S, Banerjee A and Chakravarty R. Expression of microRNA-155 correlates positively with the expression of Toll-like receptor 7 and modulates hepatitis B virus via C/EBP-beta in hepatocytes. J Viral Hepat 2015; 22: 817-27.

[61] Su C, Hou Z, Zhang C, Tian Z and Zhang J. Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. Virol J 2011; 8: 354.

[62] Biermer M, Puro R and Schneider RJ. Tumor necrosis factor alpha inhibition of hepatitis B virus replication involves disruption of capsid Integrity through activation of NF-kappaB. J Virol 2003; 77: 4033-4042.

[63] Liu X, Shao J, Xiong W, Yu S, Hu Y, Liu J, Wang X, Xiang L and Yuan Z. Cellular cIAP2 gene expression associated with anti-HBV activity of TNF-alpha in hepatoblastoma cells. J Interferon Cytokine Res 2005; 25: 617-626.

[64] Puro R and Schneider RJ. Tumor necrosis factor activates a conserved innate antiviral response to hepatitis B virus that destabilizes nucleocapsids and reduces nuclear viral DNA. J Virol 2007; 81: 7351-7362.

[65] Pei RJ, Chen XW and Lu MJ. Control of hepatitis B virus replication by interferons and Toll-like receptor signaling pathways. World J Gastroenterol 2014; 20: 11618-11629.

[66] Hao J, Jin W, Li X, Wang S, Zhang X, Fan H, Li C, Chen L, Gao B, Liu G and Meng S. Inhibition of alpha interferon (IFN-alpha)-induced microRNA-122 negatively affects the anti-hepatitis B virus efficiency of IFN-alpha. J Virol 2013; 87: 137-147.

[67] Bogerd HP, Skalsky RL, Kennedy EM, Furuse Y, Whisnant AW, Flores O, Schultz KL, Putnam N, Barrows NJ, Sherry B, Scholle F, Garcia-Blanco MA, Griffin DE and Cullen BR. Replication of many human viruses is refractory to inhibition by endogenous cellular microRNAs. J Virol 2014; 88: 8065-8076.