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The role of HBV cccDNA in occult hepatitis B virus infection

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Abstract
Occult hepatitis B virus (HBV) infection (OBI) refers to the presence of replication-competent HBV DNA in the liver, with or without HBV DNA in the blood, in individuals who tested negative for HBV surface antigen (HBsAg). In this peculiar phase of HBV infection, the covalently closed circular DNA (cccDNA) is in a low state of replication. Several advances have been made toward clarifying the mechanisms involved in such a suppression of viral activity, which seems to be mainly related to the host's immune control and epigenetic factors. Although the underlying mechanisms describing the genesis of OBI are not completely known, the presence of viral cccDNA, which remains in a low state of replication due to the host's strong immune suppression of HBV replication and gene expression, appears to be the causative factor. Through this review, we have provided an updated account on the role of HBV cccDNA in regulating OBI. We have comprehensively described the HBV cell cycle, cccDNA kinetics, current regulatory mechanisms, and the therapeutic methods of cccDNA in OBI-related diseases.

Keywords Occult hepatitis B · HBV cccDNA kinetics · Cell cycle · Regulation mechanism · Therapy

Introduction

OBI is a global public health issue with a higher incidence rate [1–3]. The rate of infections due to OBI has increased gradually since Hoofnagle first proposed, in 1978 [4], that HBV can transmit from anti-HBc-positive blood donors. From clinical reports to mechanistic research, the pathogenesis of occult hepatitis is gradually being explored. Some scholars have reported that patients with occult hepatitis B are one of the main sources of HBV infection and can spread the virus through multiple routes, such as from mother-to-child, blood transfusion, organ transplantation, and hemodialysis [5, 6]. Various factors are known to contribute to the outbreak of OBI [7–10], although only a few are mentioned below: (1) diverse types of OBI outbreaks; (2) low levels of HBV DNA content and viral protein; (3) HBV DNA sequence variation; (4) HBV virus gene integration with the host-cell genes; (5) PBMCs and lymphocytes and other tissue cells are infected by HBV; (6) abnormal host immune response; (7) interference by other viruses; (8) the quality of the detection reagents and the lowest detection limit; (9) HBV immune complex; (10) the inhibition of HBsAg expression and secretion; and (11) the regulatory effect of HBV cccDNA. The present review extensively describes the HBV cell cycle, cccDNA kinetics, current regulatory mechanisms, and therapeutic methods of cccDNA in OBI-related diseases.

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HBV cccDNA cell cycle

The early steps of HBV entry

Upon HBV entry into the host, the Dane particles, which are one of the particulate forms of HBsAg in the blood of patients infected with hepatitis B virus (HBV), are

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concentrated on the low-affinity glycosaminoglycans (GAGS) on the cell surface. The PreS1 domain of the HBV large envelope protein (LHBs) and its receptor sodium taurocholate cotransport polypeptide (NTCP) share a high affinity, and its auxiliary receptor epidermal growth factor receptor (EGFR) is bound [11–15]. This internalization complex is linked to N-glycosylated NTCP with E-cadherin [16]. This combination allows NTCP to relocate to the plasma membrane. Moreover, a new study revealed that NTCP is needed for de novo infection [17]. Whether the coreceptors and host cytokines are required for hepatitis B to enter liver cells has not been fully elucidated [18].

Steps in the cell where the HBV enters

The HBV-NTCP-EGFR complex enters the cell mainly through reticulin-mediated endocytosis [19–21]. After virus entry into the host hepatocytes, a loose ring DNA (RC-DNA) is delivered to the nucleus. Within the nuclei, the formation and assembly of nucleocapsids and the synthesis of HBV cccDNA are conducted through the following steps (Fig. 1): the virus uses the host DNA-Taq polymerase along other enzymes to form cccDNA. Once formed, cccDNA acts as a template for pregenomic RNA

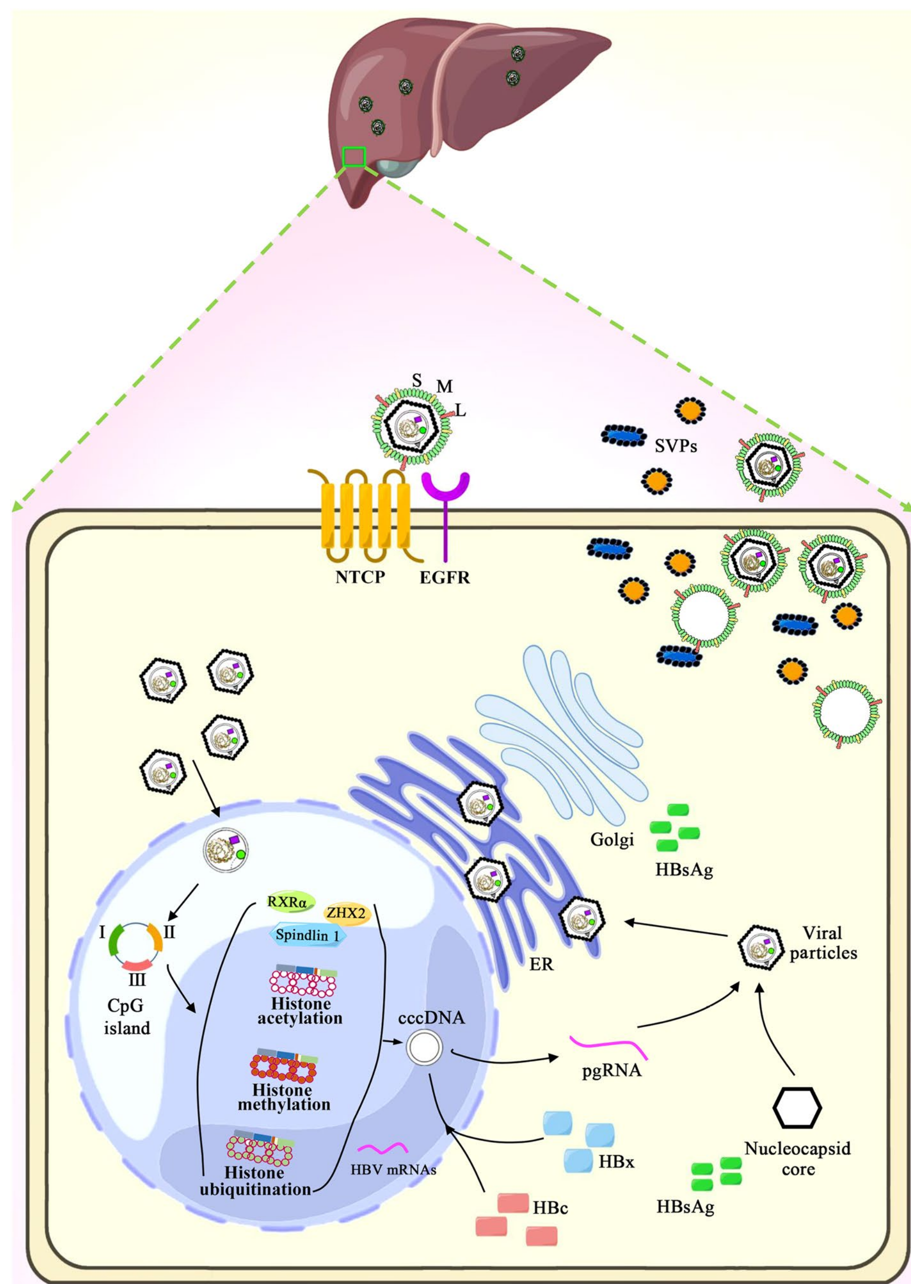

Fig. 1 Schematic representation of the regulation of cccDNA in occult HBV infection. *NTCP* sodium taurocholate cotransporting polypeptide, *EGFR* epidermal growth factor receptor, *ZHX2* zinc fingers and homeoboxes 2, *RxRα* retinoic acid X receptor α, *ER* endoplasmic reticulum, *SVPs* subviral-particles

(pgRNA) and four different lengths of viral messenger RNA (mRNA) synthesis, by the action of host-cell RNA polymerase, thereby forming a reverse transcription template and a translation template.

① Reverse transcription template: Reverse transcription of HBV pregenomic RNA (pgRNA) forms negative single-stranded HBV DNA and then completes the synthesis of positive-stranded DNA. This step provides a template for HBV replication.

②Translation template: Four main messenger RNAs (MRNAs) encode viral proteins, including hepatitis B surface antigens (HBsAg), e antigen (HBeAg), core antigens (HBcAg), HBV polymerase (Pol), and regulatory protein X (HBx) [26]. It is important that HBcAg and HBx proteins regulate HBV DNA replication and HBsAg expression through HBV cccDNA [22–34].

The kinetics of cccDNA loss

HBV cccDNA is surrounded by several nucleosomes, which consists of histones H3, H4, H2A, H2B, H1, and HBcAg, forming a strong minichromosome [35, 36]. In addition, HBV cccDNA is related to HBx, hepatitis B core protein (HBc), and to some host transcription and epigenetic regulation factors. The viral cccDNA plays the key role in the de novo infection and maintenance of HBV infection. The turnover time of cccDNA pools is necessary to achieve the goal of a "complete" or "virological" cure strategy. Since the kinetics of cccDNA in patients is not completely known, the goal of completely curing HBV is difficult. Therefore, there is a need for cell-based models or animal models as well as clinical trials that illustrate the pathway of HBV cccDNA into cells and the intracellular regulation for developing novel antiviral therapies for "virological" cures of HBV infection.

In a unique hepatocellular culture system, a HepG2-NTCP-K7-cell model was used to research the entire HBV life cycle, which included the replication and decay dynamics. After infection, HBV maintained a steady cccDNA level, with a 40-day half-life [37]. Similarly, in animal models, researchers have attempted to determine an accurate half-life model. However, owing to the differences between individuals and species, the half-life varies. In animal models, surprisingly, ducks, woodchucks, or infected chimpanzees have half-lives of no more than 2 months [38–40]. These data suggest that the half-life of hepatocytes can be determined by the size of PCNA-positive nuclei (proliferating cell nuclear antigen) [39]. Notably, a mouse model with AAV-rcccDNA has been developed, and rcccDNA has been reported to persistently exists in mouse hepatic tissues. Thus, it is a favorable platform for studying cccDNA persistence and for developing new drugs and treatments to completely clear HBV [41].

NAs can inhibit rcDNA synthesis and are the most commonly used anti-HBV treatment in clinical trials. Interestingly, during LAM or LdT antiviral therapies, the serum HBV RNA mutant rtM204I/V kinetics in patients were well related to HBV replication and HBsAg expression. It has been demonstrated that the cccDNA half-life time was 6.9–21.7 weeks, and the patients were studied for 5.6–11.1 weeks for their OBI-related illnesses [42] (Table 1). These evidence suggests that the prestored cccDNA pool may decline faster than that forecasted previously. The current data indicated that, with the research and development of powerful antiviral drugs, eliminating cccDNA is a promising future approach.

HBV cccDNA dynamics of different treatment stages in OBI-related patients

HBV cccDNA plays an important role in HBsAg expression and DNA replication. The correlations were evident among HBV cccDNA and the HBsAg levels and DNA loads in different treatment stages. In CHB patients who never received antiviral therapy, HBV cccDNA was found to be positively correlated with HBsAg in HBeAg-positive and HBeAg-negative patients. However, HBV cccDNA was not correlated with DNA replication in HBeAg-negative patients [43]. When the antiviral therapy was applied to CHB patients, HBV cccDNA was found to be positively correlated with the serum HBsAg levels in HBeAg-negative and lower serum levels of HBeAg CHB patients [44]. In addition, in some nucleos(t)ide analog patients, the range of HBsAg decline was not relevant to cccDNA reduction. However, among the hospitalized CHB patients, intrahepatic different cccDNA levels were not significantly correlated with the quantity of intrahepatic HBsAg expression. In contrast, there was a positive correlation between intrahepatic cccDNA and serum HBV DNA levels [45]. For CHB patients, after 48 weeks of treatment or in treatment-naïve patients, the relationship remains controversial among HBV cccDNA, HBsAg, and HBV DNA [46–52]. These phenomena may be relevant

Table 1 Kinetics models of cccDNA loss with a half-life time

Source	Models	Half-life time
Cell	HepG2-NTCP-K7	40-day
Animal	Ducks	No more than 2 months
	Woodchucks	
	Infected chimpanzees	
Patient	RNA mutant-rtM204v	6.9–21.7 weeks
	OBI-related illnesses	5.6–11.1 weeks

to the cccDNA half-life time and regulation mechanisms among cccDNA, DNA loads, and HBsAg expression (Fig. 2; Table 2).

Regulation mechanisms of HBV cccDNA in OBI-related diseases

The research on the regulation of HBV cccDNA epigenetic factors mostly employ transfection, immunohistochemistry, chromatin immunoprecipitation (ChIP), and chromatin immunoprecipitation sequencing (ChIP-Seq)techniques to analyze in vitro hepatocyte model transfection, chimeric mice with human liver models, CHB models, and other models to simulate the interaction in vivo so as to draw conclusions. However, due to the lack of sufficient animal-based studies or extracorporeal models for OBI research, some of the current HBV cccDNA regulation data are based on the comparison of patients as the research objects with CHB to obtain some macroscopic conclusions. However, most of them were based on the CHB research model for OBI. Presently, there are only a few direct reports that describe the role of HBV cccDNA epigenetic factors in the pathogenesis of OBI.

It is well-known that cccDNA is in a low replicative state in OBI-related diseases. HBV cccDNA is a foundation for

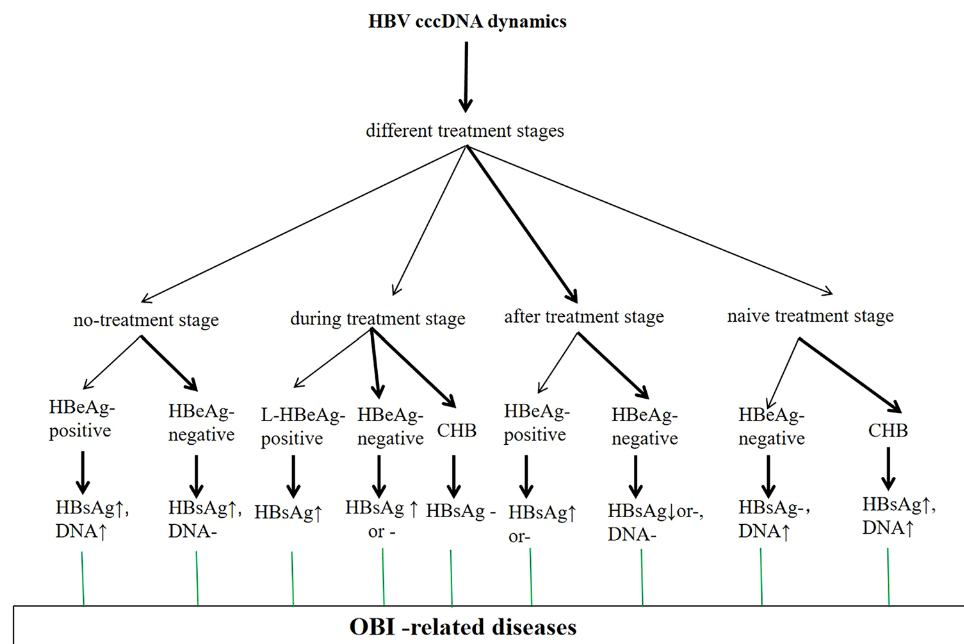

Fig. 2 The cccDNA dynamics of different treatment stages in OBI-related diseases

Table 2 The correlation among HBV cccDNA, HBsAg, and HBV DNA in different treatment stages of OBI-related diseases

Stages	HBeAg	HBV cccDNA	HBVDNA	HBsAg	References
No-treatment	Positive	↑	↑	↑	[43]
	Negative	↑	–	↑	[43]
During antiviral therapy	L-positive	↑		↑	[44]
	Negative	↑		↑	[44]
		↑		–	[45]
After treatment	Positive	↑		↑	[47]
	Positive	↑		↑	[50]
	Negative	↑	–	–	[47, 48]
	Positive	↑		↑	[49]
Naïve-treatment	Negative	↑	↑	–	[51]
		↑	↑	↑	[52]
		↑	↑	↑	[46]

"↑", positive correlation; "↓",negative correlation; "–", no relation among HBV cccDNA, HBsAg, and HBV DNA in OBI-related diseases

the transcription and replication of HBV. It accumulates in the infected intrahepatic nucleus in the form of a stable episome, which constitutes minichromosomes through histones, nonhistone proteins, and cellular proteins. A recent study revealed that the content of replicated viral templates (cccDNA) in OBI patients is extremely low, which, in turn, lowers the viral transcription activity [53]. When the body's immunity is low, the risk of HBV reactivation in OBI patients increases due to of the presence of latent cccDNA [54]. This increases the risk of acute hepatitis and liver failure. The expression of viral genes is under HBV transcriptional regulation. Several studies have demonstrated that other related antigens and post-translational modifications with histones, non-histones, methylation, host transcription factors, ubiquitination, and others affect the transcription and replication of cccDNA. Together, they regulate the progression of occult hepatitis B (Fig. 1; Table 3).

HBV cccDNA regulates the OBI viral load

Histone acetylation, ubiquitination regulation, and protein methylation

The acetylation state of histones bound by cccDNA regulates HBV replication and transcription activities. SIRT3 is a host factor that restricts HBV replication, involving a reduction in the binding of YY1, which is a host transcription factor and RNA polymerase II, to cccDNA [55]. However, SIRT1, the TIP60 complex, HDAC11, and HAT1 promote OBI activation. Among them, sirtuin 1 is a class III histone deacetylase that may promote the replication of HBV in liver cells [56]. In addition, the TIP60 complex is bound to the HBV promoter and inhibits HBV transcription driven by the precore/core promoter [57]. In addition, histone deacetylase 11 (HDAC11) affects the specificity of DAC11 and reduces the acetylation level of histone H3 bound by cccDNA [58]. Moreover, protein acetyltransferase 1 (HAT1) plays key roles in host chromatin assembly, reducing HBV replication and cccDNA accumulation and modulating the acetylation of histones H3K27/H4K5/H4K12 of cccDNA minichromosomes [59–61].

Np95/ICBP90-like RING Finger Protein (NIRF) is a new E3 ubiquitin ligase that can negatively regulate HBV transcription and/or replication. Recent studies have suggested that it affects polyubiquitinated protein degradation, carcinogenesis, cell proliferation, cell cycle, and epigenetic modification [62]. The research demonstrated that NIRF not only inhibits HBV replication through interaction with HBcAg but also reduces acetylation of H3 histones combined with HBV cccDNA. This observation helps prepare a new guide for studying the epigenetic

Table 3 HBV DNA response and HBsAg expression with cccDNA epigenetic regulation in OBI-related diseases

Classification	Examples	Models	HBV cccDNA	HBV DNA	HBsAg	References
Histone acetylation, regulation	①SIRT3	Cell	↑	↓	↓	[55]
	②SIRT1	Cell/human liver-chimeric mouse	↓	↑	↓	[56]
	③TIP60 complex			↓	↓	[57]
	④HDAC11	Cell		↓		[58]
	⑤HAT1	Cell		↓		[59]
		Cell				
Histone Ubiquitination Regulation	①NIRF	Cell		↓	↓	[62]
CpG Islands/Histone-methylation status	①LSD1	OBI	↓	↓	↓	[63]
	②PRMT5	Cell	↓	↓	↓	[64]
	③Sirt2.5	Cell	↓		↓	[65]
	③CpGII	CHB			↓	[70]
	④CpGIII	CHB				[70]
Regulatory protein and core protein regulation	①HBx	Cell	↑	↑	↓	[23–28]
	②HBx mutations	Cell	↑	↓	↓	[72]
	(HBxStop, MT16-19,21–23,25–30)	Cell	↓		↓	[72]
	HBx MT3,20,24					[31]
	③HBc					
Host transcription factor regulation	①ZHX2, Spindlin1	Cell/Mouse	↑	↓		[66, 67]
	②RXRα	Cell	↑	↑		[68]

"↑", positive correlation; "↓", negative correlation; "–", no relation among HBV cccDNA, HBsAg, and HBV DNA in OBI-related diseases;

Sirtuin 1 SIRT1, *Sirt2 isoform 5* Sirt2.5, *Sirtuin 3* SIRT3, *Histone Deacetylase 11* HDAC11, *Histone Acetyltransferase 1* HAT1, *Np95/ICBP90-like RING Finger Protein* NIRF, *Protein arginine methyltransferase 5* PRMT5, *Zinc Fingers And Homeoboxes 2* ZHX2, *retinoic acid X receptor α* RxRα, *OBI* Occult hepatitis B virus infection, *CHB* Chronic Hepatitis B

modification of HBV cccDNA and lays foundation for the establishment of new anti-HBV therapeutic strategies.

Some enzymes regulate the histone methylation status and affect the replication and transcription of HBV cccDNA, including LSD1, PRMT5, and Sirt2.5. Past studies have demonstrated that inhibiting or reducing the level of LSD1 restricts the viral gene expression. This observation is related to the transcriptional repression marker H3K9 methylation and the reduction of the activation markers H3 and H3K4 modifications on the viral promoter [63]. Protein arginine methyltransferase 5 (PRMT5) plays a negative role in HBV replication, which inhibits cccDNA transcription and disrupts pregenomic RNA evolution [64]. Sirt2 isoform 5 (Sirt2.5) is a non-nuclear protein export signal that has a catalytic-splicing effect that reduces the output of HBV mRNA and cccDNA. This phenomenon is caused by storing the transcription repression markers directly and/or indirectly [65].

HBx and HBc regulation

The regulatory protein HBx can enhance the replication or transcription of HBV cccDNA in recurring OBI patients with a low viral load, resulting in abnormal liver functions. HBx is composed of 154 amino acid proteins with an N-terminus and a C-terminus, which can be tested in infected hepatocytes [23]. Past research has demonstrated that HBx activates host gene transcription and the cccDNA expression. HBX can also increase DLEU2 in infected hepatocytes. However, after HBV infection, HBx can increase HBV cccDNA transcription by inhibiting SETDB1 and allowing the establishment of active chromatin. In addition, HBX can activate the HBV cccDNA core promoter by closing the C-1619 methylation in cccDNA so as to stimulate viral replication [22, 24–28].

HBc has a positive effect on HBV replication. It has been shown to interact with cccDNA and regulate the transcription of HBV [31]. HBc forms an important part of HBV cccDNA minichromosomes, which combine early with HBV double-stranded DNA [32, 33]. Their binding occurs preferentially in the CpG island 2. In addition, its function is related to the proportion of HBc, the combination of CREB-binding protein (CBP), and the hypomethylated state of CpG island 2 in HBV cccDNA small chromosome [34].

Host transcription factors

The role of host transcription factors in the epigenetic modification of cccDNA is unclear. cccDNA and host-cell histones assemble into chromatin. However, only little is known about the relationship between histone post-translational modification and the regulation of HBV chromatin. Relevant studies have demonstrated that ZHX2 is expressed in large amounts in adult hepatocytes. It can be combined with cccDNA to decrease the HBV promoter activity. In addition, ZHX2 inhibits the output of histone regulatory genes containing p300/CBP that bind to cccDNA and leads to epigenetic suppression of cccDNA [66]. In addition, Spindlin1 was identified as an HBx interaction partner and binds to cccDNA. Interestingly, it plays a negative role in HBV transcription during infection. In addition, Spindlin1 decreases the level of histone H4K4 trimethylation, which indicates that Spindlin1 affects the epigenetic regulation [67]. The transcription factors ZHX2 and Spindlin1 inhibit HBV cccDNA replication and transcription.

Some host transcription factors regulate and promote HBV cccDNA replication and transcription, such as retinoic acid X receptor α (RxRα). On the other hand, RxRα is a nuclear receptor rich in the liver, and it regulates the replication and transcription of HBV through the activity of HBV enhancer 1, the core promoter, and the acetyltransferase on viral minichromosomes. The recruitment to trigger epigenetic changes in cccDNA [68].

Furthermore, The CpG island methylation pattern of OBI is different from that of other diseases. Past studies have demonstrated that there are different CpG methylation patterns between the OBI and CHB. The OBI and non-OBI patients exhibited methylation of the HBV CpG islands 1 and 2. Among them, the occult HBV sequence contains island 2 with a higher degree of methylation. In contrast, the non-occult HBV sequence contains a higher degree of methylation in island 1. This observation suggests that OBI and CHB may have diverse methylation-modification styles. It also suggests that, in several cases, although the immune system is suppressed, the virus retains significant genetic changes, which limits its replicating abilities [69]. Furthermore, high levels of CpG island II methylation were present in low HBV DNA patients. In addition, past in vitro studies verified that CpG island II methylation drastically inhibited cccDNA transcription such that DNA replication was lower in OBI-related diseases [70]. The relationship between a variety of models of nonexclusive genetic and epigenetic changes in the HBV DNA sequence of occult hepatitis B remains unclear.

HBV cccDNA regulates the expression level of HBsAg

CpG island methylation patterns

HBV infection is preserved by the existence of HBV cccDNA. There exists a relationship between CpG islands and viral HBsAg expression. Furthermore, host DNA methyltransferase causes viral methylation, resulting in less viral expression [70, 71]. A past study revealed that the methylation density of HBV cccDNA CpG islands may induce negative correlation between the HBsAg expression and CpG island III in

OBI-related diseases. However, the CpG islands I and II had no effects on the HBsAg levels in the same patients. CpG III is situated upstream of the HBV large-surface protein promoter (LHBs-SP1); therefore, the methylation of CpG III decreased the transcription of surface mRNA. Therefore, it is suggested that the viral gene expression is regulated by the level of CpG island III methylation [70].

HBX mutation and histone-methylation status

HBx plays the key role in HBV replication through the H-box motif and CUL4-DDB1 ubiquitin ligase [29]. For example, HBV regulatory protein X targets the protein structure of chromosome 5/6 (Smc5/6), thus leading to the degradation and ubiquitination by DDB1-CUL4-ROC1 E3 ligase to reinforce viral transcription of cccDNA [20]. Furthermore, HBx mutations made a strong correlation between cccDNA and HDAC1 to regulate the HBV viral load [72].

Therapeutic methods regulating cccDNA in OBI-related diseases

HBV is the main cause of liver diseases. HBV cccDNA elimination is the key part of the complete cure for HBV-infected patients. The clinical application of HBV cccDNA mainly includes monitoring HBV infection in organ transplant recipients and other immunosuppressive patients [73]. In addition, evaluating the effect of antiviral treatment and relapsing after stopping the drug and adjusting the treatment plan in time are some other alternatives to improve treatment efficacy [74]. To evaluate the risk of OBI associated with other liver diseases [75, 76], we have compared the similarities and differences between OBI and other liver diseases and conducted mechanistic researches [34]. Presently, most of the current treatment methods are "functional cure". Although some antiviral drugs have been used clinically, there is still a long way to go before "complete cure" could be achieved. The reason why OBI cannot be completely eliminated is that the pre-existing antiviral drugs are relatively insensitive for targeting HBV cccDNA, which results in persistent HBV infection [70]. At present, it is necessary and desirable to develop a new method that directly targets HBV cccDNA. Through recent studies on the apparent regulation of HBV cccDNA, silencing cccDNA may be a feasible treatment method. The current approach to treatment research in this field is summarized below:

Histones and immunomodulators maintain a low level of HBV DNA replication in OBI patients

Class I histone deacetylase inhibitors can induce increased acetylated H4 and HBV replication bound by cccDNA. Finally, in OBI-related diseases, histone hypoacetylation in the liver tissues and histone deacetylase 1 recruitment bound to cccDNA have been associated with the occurrence of diseases [77].

The application of the immunomodulator IFN-α not only reduces the apparent modification of cccDNA but also reduces the cccDNA activity by reducing the binding of STAT1 and STAT2 [78]. In addition, interferon-α can eliminate HBV cccDNA by regulating histone H3K79 succinylation, thereby allowing the epigenetic regulation of HBV cccDNA [79]. Furthermore, alcohol intake can strengthen intracellular HBV transcription, while recombinant human interferon IFN-α2b can inhibit ethanol-enriched HBV cccDNA by blocking the HBx/MSL2/cccDNA/HBV/HBx-positive feedback loop [80]. Furthermore, IFN-α2b can cause histone H4K8 de2-hydroxyisobutyrylation in cccDNA minichromosomes to inhibit HBV transcription and replication by increasing histone H4K8 de2-hydroxyisobutyrylation [81].

Although α-IFN is an extremely useful treatment approach for HBV infection, it has major side effects. Therefore, the regulation of cccDNA and the study of host cofactors are expected to have significant clinical value for researches on anti-hepatitis B therapeutic drugs [55].

Treatment with interleukin-6 (IL6), sharply reduces the level of cccDNA-bound histone acetylation and the 3.5-kb/pgRNA. In addition, IL6 has a certain inhibitory effect on HBV replication [82]. Moreover, curcumin has certain anti-inflammatory properties. Past studies have demonstrated that it can inhibit *HBV* replication by downregulating cccDNA-bound histone acetylation and may be developed as a viral drug for targeting cccDNA [83].

Drugs regulated by HBV cccDNA were related to HBV replication and HBsAg production

The NTCP inhibitor myrcludex B, which competitively blocks virus invasion of the hepatocyte-specific receptor NTCP, is in phase-III clinical trials, which imply a complete cure for de novo infection [84]. Flavocoxid is composed of catechin, baicalein, and two flavonoids, and past evidence has demonstrated that flavocoxid alone or in combination with entecavir may reduce cccDNA, such that it inhibits HBV DNA and the HBsAg expression in OBI-related illnesses [85]. In addition, 6-aminonicotinamide, a new inhibitor, reduces in vitro HBV DNA replication and the HBsAg levels by decreasing the transcription factor PPARα [86]. Finally, Junceellolide B significantly decreases HBV cccDNA-transcribed products [87]. On the other hand, the present evidence suggests that DIC, an NQO1 inhibitor, can silence cccDNA transcription by accelerating the degradation of HBx [88]. This evidence may provide a new therapeutic schedule for "virological" cure strategies.

Furthermore, some reports have demonstrated that lymphotoxin-β-receptor activation promotes HBV cccDNA decay. This observation may provide new headways in preventing the re-occurrence of HBV infection [89, 90].

CRISPR/Cas9 editing and the employment of transcription inhibitors provide new directions for OBI treatment

Past recent studies have demonstrated that clustered and regularly spaced short palindrome repeats (CRISPR)/CRISPR-related Cas9 nuclease (CRISPR/Cas9) may be the most promising choice for target and subsequently deplete the cccDNA storage [91]. The Cas9II system is one of the most studied systems in mammals and humans. CRISPR/Cas9 nuclease can effectively target HBV cccDNA to reduce its expression. Deep sequencing has demonstrated that the St CRISPR/Cas9 system is the safest anti-HBV active system that can provide a new possibility in the targeted therapy of HBV cccDNA.

The use of transcription inhibitors targeting viral DNA inhibits the replication of HBV [92]. These repressors (TALEs) target the open reading frame on the surface of the virus and are placed under the transcriptional control of a constitutively active promoter or a liver-specific promoter. This is a new and effective method for the epigenetic modification of HBV DNA to inactivate the virus in vivo. This method has therapeutic value and can prevent the development of potential problems associated with accidental mutagenesis of gene editing.

Conclusions and perspectives

Although it is known that HBV enters hepatocytes by endocytosis, the relationship between HBV entry into hepatocytes and the host cofactors remains to be fully understood. HBV forms HBV cccDNA under the action of host enzymes in the nucleus. Due to the peculiar nature of its gene structure, the regulatory effects of histones, non-histones, and host factors on HBV cccDNA are not yet clear, which makes the complete elimination of HBV cccDNA a problem in OBI patients. Currently, the focus of the occult HBV infection research remains mainly on HBV cccDNA gene mutation, immune regulation, the increasing apparent regulation of HBV cccDNA, HBV immune complex and detection limitation. Since, increased focus of the research remains on histone acetylation, methylation, transcription factors, and transcription inhibitors, it is possible to reduce HBV cccDNA replication or even transcription such that these situations make HBV DNA replication and HBsAg lower. In addition, in recent years, with the rapid development of CRISPR/Cas9 gene editing technology, new insight has been provided for the complete HBV cccDNA clearance. Presently, the drugs that have an effect on HBV cccDNA mainly include immunomodulators, histone modulators, and targeted transcription inhibitors. In addition, some known treatment methods are not in practice. Currently, most research models of occult hepatitis are based on chronic hepatitis and liver cancer, and there are only a few direct reports on the molecular levels of HBV cccDNA on occult hepatitis. The differences and association studies of HBV cccDNA in the pathogenesis of occult hepatitis, hepatic hepatitis, and liver cancer are also not yet clear. Therefore, researchers need to deeply consider the establishment of occult hepatitis models. On one hand, we may have established a new indicator or detection method to diagnose OBI using a specific model. On the other hand, this model can be used to analyze the epigenetic regulation pathogenesis of HBV cccDNA to search for a new therapy.

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Author contributions PH analyzed the data and wrote the manuscript; PZ and YF contributed to drawing; NH, WY, ZX and YZ were responsible for collecting, collating and checking the data; ZZ and JS conceptualized and designed the study and critically revised the manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request and could be provided by Zhenhua Zhang (zzh1974cn@163.com) or Jilu Shen (shenjilu@ahmu.edu.cn). Source data for the graphs and charts are provided in Fig. 1-2 and Tables 1–3.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Consent to participate Not applicable.

Consent for publication All authors have agreed to the consent of the manuscript.

Ethical approval Not applicable.

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