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REVIEW ARTICLE

Hepatitis B virus X protein-targeted therapeutic strategies toward a functional cure for chronic hepatitis B infection: A review

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Abstract

Background: Chronic hepatitis B (CHB) remains a major global health challenge, with persistent covalently closed circular DNA (cccDNA) and viral integration leading to lifelong infection, cirrhosis, and hepatocellular carcinoma (HCC). Current antiviral therapies suppress replication but rarely achieve a functional cure. The multifunctional hepatitis B virus (HBV) X (HBx) protein is well known to sustain viral replication, impair host immune responses, and promote hepatocarcinogenesis, which makes it an attractive therapeutic target. **Aim:** This review synthesizes current literature supporting HBx as a promising therapeutic target to achieve a functional cure of CHB. **Conclusion:** HBx is a high-value therapeutic target with potential to accelerate progress toward a functional cure. Destabilization or downregulation of HBx would not only attenuate its oncogenic signaling but also limit relapse after treatment discontinuation and diminish the cccDNA reservoir and viral antigen load. **Relevance for patients:** Multitargeted treatment regimens incorporating HBx-directed therapies hold the potential to achieve durable viral suppression and a functional cure, and to reduce the risk of HCC. The combined strategies could transform the long-term management and outcomes for patients with CHB.

Keywords: Hepatitis B virus; Hepatitis B virus X protein; Chronic hepatitis B; Covalently closed circular DNA; Viral persistence; Furanocoumarins; Functional cure

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1. Introduction

Hepatitis B virus (HBV) is a DNA virus that causes acute and chronic liver disease and remains a major global health burden.[1] Transmission occurs through contact with infected blood or bodily fluids, with perinatal, early childhood, sexual, and parenteral exposures being the primary routes.[2] In highly endemic regions, vertical and early childhood transmission perpetuates infection across generations, especially where immunization coverage is limited.[2] The World Health Organization estimated that in 2022, approximately 254 million people were living with chronic hepatitis B (CHB), with approximately 1.1 million annual deaths primarily due to cirrhosis and hepatocellular carcinoma (HCC).[3] The risk of developing CHB is strongly age-dependent, with more than 90% of infants infected at birth, approximately 50% in children aged 1–5 years, and <10% in adults.[3,4]

Structurally, HBV consists of an envelope surrounding a nucleocapsid that encloses a relaxed circular DNA genome.[5] The virus exhibits a strong affinity for hepatocytes and enters cells through interactions between the pre-S1 domain of its envelope glycoprotein and the sodium taurocholate co-transporting polypeptide receptor.[5,6] Once internalized, relaxed circular DNA is transported to the nucleus and converted into covalently closed circular DNA (cccDNA), a stable episomal minichromosome that acts as a template for the transcription of the 3.5 kb pre-genomic RNA (pgRNA), the 2.4/2.1 kb transcripts encoding three surface antigens (small, middle, and large), and the 0.7 kb RNA encoding the regulatory HBV X (HBx) protein.[6,7] pgRNA is reverse transcribed into new relaxed circular DNA and also serves as the mRNA for the capsid proteins and viral polymerase, essential for virion assembly. Mature nucleocapsids either acquire an envelope for secretion as infectious virions or are recycled to the nucleus to replenish cccDNA, sustaining persistent infection. In addition, HBV DNA can integrate into the host genome, contributing to chronic disease progression and HCC. Although integrated HBV DNA is replication-defective, it can significantly contribute to the circulating pool of hepatitis B surface antigen (HBsAg), particularly in hepatitis B e antigen (HBeAg)-negative individuals.[8] The persistent cccDNA pool enables the reactivation of HBV, particularly during periods of immunosuppression, by continuously producing viral RNA and progeny viruses even after antiviral treatments, which suppress viral replication but do not eliminate the cccDNA. The stability of cccDNA, integration events, and immune evasion mechanisms make HBV eradication challenging.[7] Current antiviral therapies suppress viral replication but rarely achieve complete clearance, underscoring the need for novel therapeutic approaches toward reducing HBV transmission and the associated global disease burden.[9,10]

Among potential therapeutic targets in the HBV life cycle, HBx is of particular interest due to its central role in regulating cccDNA transcription, viral replication, and in oncogenic processes underlying HCC development.[11-13] HBx can stimulate the expression of other viral genes by acting on the viral cccDNA template. Therefore, targeting HBx destabilization or downregulation would not only attenuate HBx-driven oncogenic signaling but also limit relapse after treatment discontinuation and diminish the cccDNA reservoir and viral antigen load. The present review comprehensively synthesizes current knowledge supporting HBx as a promising therapeutic target to achieve a functional cure for CHB.

2. Methodology

Original research and review articles were included if they provided experimental, mechanistic, or therapeutic insights into (i) *HBx* gene expression, (ii) HBx-mediated regulation of cccDNA, (iii) mechanisms governing intracellular HBx stability and degradation, or (iv) natural and synthetic compounds that modulate HBx function. Reports addressing RNA interference (RNAi), genome editing, epigenetic regulation, and immunotherapeutic modalities targeting HBx were also included. Information on bioactive compounds and small molecules promoting HBx degradation or repressing cccDNA transcription was collected from the original reports and curated databases. The academic research databases, including PubMed, ScienceDirect, Scopus, Web of Science, and Google Scholar, were queried using a combination of keywords, such as "HBx," "hepatitis B virus," "HBV," "HBx degradation," "cccDNA transcription," "HBx–host interactions," "HBx inhibitors," and "therapeutic targeting of HBx." We considered all relevant scholarly literature from database inception through August 2025, without restricting publication year. Exclusion criteria included non-English publications and articles lacking sufficient methodological clarity or direct relevance to HBx biology.

3. *HBV X* gene and its regulation

The regulatory HBx protein is encoded by the "X" open reading frame in the HBV genome. The expression of the *HBx* gene involves the transcription of the viral cccDNA in the nucleus of the infected cell, which serves as the template for producing viral RNAs, including the 0.7-kb HBx transcript. The *HBx* RNA transcript is then exported to the cytoplasm and translated into HBx protein in infected host cells (Figure 1). HBx regulates its own gene expression through transcriptional and post-transcriptional mechanisms, including interactions with host factors and epigenetic mechanisms, such as DNA methylation and chromatin accessibility. The *HBx* gene is regulated by its promoter and two enhancers (Enhancer I and Enhancer II), which are responsive to host factors, such as CCAAT/enhancer binding protein, hepatocyte nuclear factor 1, hepatocyte nuclear factor 3, and cyclic adenosine monophosphate response element binding protein (CREB)/activating transcription factor 2.[11,12,14]

4. Molecular characteristics of the HBV X protein

The HBx protein is a small (17 kDa) multifunctional regulatory protein that is well conserved across all mammalian hepadnaviruses but lacks sequence homology to known proteins.[11] Structurally, HBx comprises two major functional domains: An N-terminal negative regulatory domain and a C-terminal transactivation domain. Deletion of the amino-terminal 1–50 amino acids upregulates HBx transcriptional functions, suggesting that it is a negative

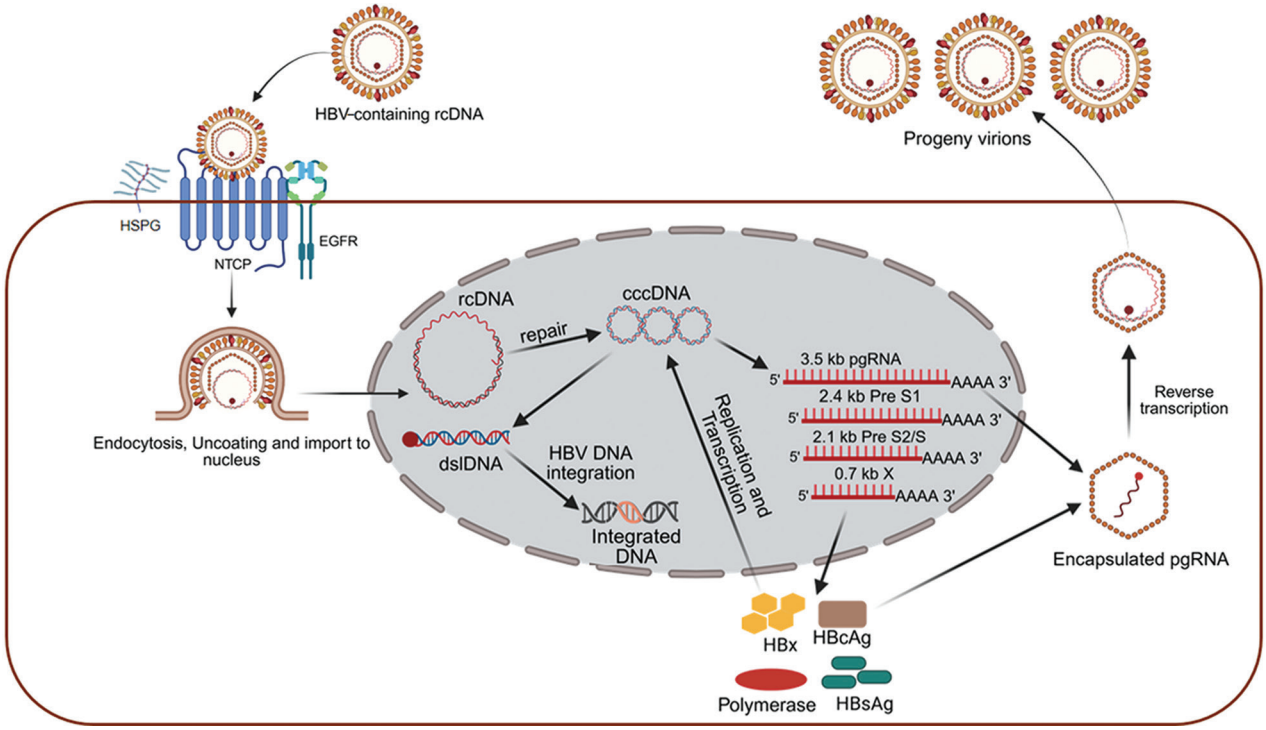

Figure 1. The hepatitis B virus (HBV) replication cycle. HBV enters hepatocytes with the help of the heparan sulfate proteoglycan (HSPG) attachment factor and sodium taurocholate co-transporting polypeptide (NTCP)/epidermal growth factor receptor (EGFR). The relaxed circular DNA (rcDNA) in virions is transported into the nucleus. rcDNA is repaired to form covalently closed circular DNA (cccDNA), which serves as a transcriptional template for pre-genomic RNA (pgRNA) and other viral mRNAs, including 0.7-kb HBV X (HBx) RNA. pgRNA is encapsidated with viral polymerase and reverse-transcribed into rcDNA within nucleocapsids. Mature nucleocapsids are either recycled to the nucleus to replenish cccDNA or enveloped and secreted as progeny virions. In parallel, HBV double-stranded linear DNA (dslDNA) can integrate into the host genome, contributing to viral persistence and pathogenesis. Created in BioRender. Giri, S. (2025) https://BioRender.com/yn90jqq. Abbreviations: HBcAg: Hepatitis B core antigen; HBsAg: Hepatitis B surface antigen.

regulatory element, whereas the C-terminal domain of HBx (amino acids 52–148) is essential for its various activities. The C-terminal region has several structural motifs, such as the H-box, Bcl-2 homology domain 3-like domain, zinc-binding motif, and alpha-helical elements.[11,12] HBx exhibits a dynamic intracellular distribution regulated by the phosphorylation of conserved residues.[15] It is predominantly cytoplasmic when highly expressed and more nuclear at lower (near-endogenous) levels.[16] HBx can directly bind to several transcription factors in the nucleus, such as activating transcription factor 2, CREB, Oct-1, p53, basic leucine zipper, and other basal transcription factors, and stimulate several cytoplasmic signal transduction pathways, such as nuclear factor kappa B (NF-κB), Janus kinase/signal transducer and activator of transcription, phosphoinositide 3-kinase/protein kinase B, Ras/Raf/mitogen-activated protein kinase, and Wnt signaling, that have a profound influence on cell proliferation, apoptosis, and viral replication.[11,14,17]

5. Regulation of intracellular HBV X protein levels

Despite its critical role in viral persistence, HBx is a short-lived protein. The intracellular abundance of HBx is tightly regulated by both the host cell's protein degradation machinery and by other viral proteins involving ubiquitination, deubiquitination, neddylation, and proteolytic degradation through proteasomal or non-proteasomal mechanisms.[7,18] These biochemical processes allow HBx to modulate various cellular processes essential for the viral life cycle and pathogenesis.

The intracellular stability of HBx protein may range from 30 min to 3 h, depending on the experimental system used. The reported half-lives of HBx range from about 30–40 min in hepatoma cells (HepG2 and Huh 7 cells) to around 1–3 h in primary human hepatocytes.[19-21] Interestingly, the woodchuck HBx protein in naturally infected hepatocytes shows a bimodal half-life—one with

a short half-life of 15–30 min and the other with a long half-life of 3 h.[22] Therefore, interference with intracellular stabilization processes or induction of destabilization mechanisms directed at HBx will open up new possibilities of developing novel therapeutic strategies for hepatitis B patients who are non-responsive to existing treatments.

5.1. Destabilization processes and promotive strategies

The destabilization of HBx protein utilizes the cellular machinery involving both viral and host factors (Figure 2). For example, the tumor suppressor p53 is known to induce degradation of HBx with the help of E3 ligase mouse double min 2 through a ubiquitin-independent proteasomal mechanism.[23] Alternatively, p53 can induce E6-associated protein-mediated ubiquitination and proteasomal degradation of HBx.[24] The intrahepatic homeobox protein muscle segment homeobox 1 acts as a restriction factor against HBV by reducing HBx protein levels through an ubiquitin-independent proteasomal degradation pathway by upregulating DnaJ heat shock protein family (Hsp40) member A4 and crystallin alpha B expression.[25] Similarly, the tumor suppressor PreS1-binding protein facilitates HBx degradation through the ubiquitin–proteasome system, resulting in the suppression of HBV DNA replication and viral gene expression.[26] Interestingly, as a part of a negative feedback mechanism to regulate viral replication, the core protein of HBV is also known to stimulate the proteasome-mediated degradation of HBx.[20]

The E3 ubiquitin ligases, such as seven in absentia homolog 1 and 2 and neuronal precursor cell-expressed developmentally downregulated gene 4, have been reported to prevent the proliferation, migration, and invasion in HBV-related HCC cell lines by promoting K48-linked polyubiquitination and proteasomal degradation of HBx.[27-29] In addition, the core protein of hepatitis C virus has been found to inhibit HBV replication by downregulating HBx levels through seven in absentia homolog 1.[30] Recent findings suggest that E3 ligase tripartite motif-containing protein (TRIM) 21 interacts with HBx and HBV DNA polymerase and triggers their ubiquitination and degradation, resulting in the downregulation of other viral antigens (HBsAg, hepatitis B core antigen, and HBeAg).[31,32] Interestingly, type I interferon (IFN)-stimulated gene TRIM5γ acts as an adaptor protein for TRIM31 and helps restrict HBV infection by targeting HBx for degradation in a multi-step process.[33] In addition, the interaction between HBx and TRIM proteins provides a new target for developing antiviral therapies for HBV. Furthermore, activating HBx-destabilizing ligases (e.g., seven in absentia homolog 2, neural precursor cell expressed developmentally downregulated protein 4, and

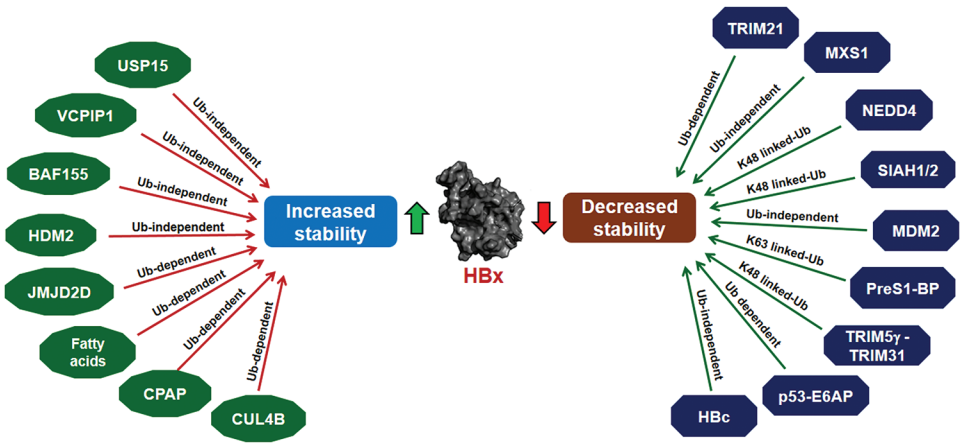

Figure 2. Regulation of the intracellular stability of hepatitis B virus X protein (HBx). Schematic representation of host and viral factors that influence the intracellular stability of HBx. Green arrows indicate HBx degradation via ubiquitin (Ub)-dependent or Ub-independent proteasomal pathways, while red arrows indicate stabilization or protection from proteolytic degradation. E3 ubiquitin ligases that mediate HBx degradation include seven in absentia homolog (SIAH) 1, SIAH2, neural precursor cell expressed developmentally downregulated protein 4 (NEDD4), tripartite motif-containing protein 5 gamma-tripartite motif-containing protein 31 (TRIM5γ–TRIM31, p53–E6-associated protein (E6AP), tripartite motif-containing protein 21 (TRIM21), PreS1-binding protein (PreS1-BP), and mouse double min 2 (MDM2), as well as non-E3 factors, such as hepatitis B virus capsid (HBc) and muscle segment homeobox 1 (MSX-1) (via DnaJ heat shock protein family member A4 [DNAJA4)] and crystallin alpha B [CRYAB]). Mechanisms include K48-linked polyubiquitination, K63-linked polyubiquitination, E6AP-mediated ubiquitination, and ubiquitin-independent proteasomal degradation. Stabilizing factors include USP15, VCPIP1, BAF155, HDM2, JMJD2D, fatty acids, CPAP, and CUL4B. Edge labels denote the specific ubiquitination type or pathway involved. These networks highlight potential therapeutic targets for regulating HBx abundance in the HBV-infected cells and viral replication. Abbreviations: BAF155: BRG1-associated factor 155; CPAP: Centrosomal P4.1-associated protein; CUL4B: Cullin 4B; Fatty acids: Fatty acids; HDM2: Human double min 2; JMJD2D: Jumonji domain–containing protein 2D; USP15: Ubiquitin-specific peptidase 15; VCPIP1: Valosin-containing protein interacting protein 1.

TRIM5γ) or mimicking their activity is likely to enhance HBx clearance, suppress HBV replication, and limit HBx-driven tumorigenesis (Figure 2).

5.2. Stabilization mechanisms and targeting strategies

Unlike the ubiquitination process, the ubiquitin moieties from the HBx protein can be removed by deubiquitinating enzymes, which prevent degradation caused by the proteasome and thereby influence its stability, function, and localization. Several deubiquitinating enzymes have been characterized that confer intracellular stability to HBx (Figure 2). For example, the ubiquitin-specific peptidase 15 is reported to prevent the proteasomal degradation of HBx, increasing its levels and augmenting its ability to activate several cellular signaling pathways, contributing to CHB.[34] Therefore, developing ubiquitin-specific peptidase 15-targeted strategies may expand the therapeutic repertoire for CHB. Similarly, the valosin-containing protein-interacting protein 1 (VCPIP1) is a novel deubiquitinating enzyme that stabilizes HBx in a ubiquitin-independent manner. VCPIP1 serves as a scaffold that promotes interactions between HBx and proteasome components (including proteasome 26S subunit adenosine triphosphate 3), thereby enhancing HBV cccDNA transcription.[35] Therefore, disrupting HBx interactions with stabilizing factors, such as VCPIP1 or proteasome 26S subunit adenosine triphosphate 3, may reduce viral persistence and provide new insights for developing ubiquitin–proteasome system-based therapeutics for HBV-mediated pathogenesis (Figure 2). In addition, understanding the ability of HBx to degrade host restriction factors also opens avenues for restoring intrinsic antiviral defense in CHB infections.

The stability and activity of HBx are also enhanced after neddylation by the human homolog of mouse double min 2 that, in turn, prevents degradation following ubiquitination by E3 ligase seven in absentia homolog 1[36] (Figure 2). Therefore, targeting the neddylation pathway holds promise for the therapy of CHB. Likewise, Jumonji C domain-containing histone demethylase protein 2D (JMJD2D) is shown to stabilize HBx protein through direct interaction, preventing TRIM14-mediated polyubiquitination and HBx degradation. JMJD2D cooperates with HBx to promote HBV cccDNA transcription by demethylating histone H3 lysine 9 (H3K9) trimethylation on the cccDNA minichromosome.[37] Therefore, targeting JMJD2D through RNAi or pharmacological inhibitors can repress HBV replication and treat CHB infection. Similarly, the scaffold protein Cullin 4B, which is a component of the Cullin-RING E3 ligase complex, interacts with HBx protein to enhance HBV replication by inhibiting HBx ubiquitination and subsequent proteasomal degradation.[38] Thus, Cullin 4B could be a potential target for inhibiting HBV replication (Figure 2).

The HBx protein is also known to promote fatty acid build-up within hepatocytes, leading to hepatic inflammation, steatosis, or fatty liver disease. Elevated levels of fatty acids subvert the ubiquitination and degradation of HBx.[39] These observations suggest new therapeutic approaches for curing CHB patients with metabolic syndrome through low-fat diet therapy. Furthermore, the interaction between centrosomal P4.1-associated protein and HBx is reported to significantly increase NF-κB activity, promoting the development and progression of HCC.[40] Altogether, these results offer opportunities to develop mechanism-based therapies. HBx protein has been shown to directly interact with the E2-EPF ubiquitin carrier protein, resulting in the stabilization of its downstream target, hypoxia-inducible factor 1 subunit alpha proteins, which promote angiogenesis and HBx-mediated tumor growth and metastasis.[41] Moreover, the chromatin remodeling factor Brg1/Brm-associated factor 155 has been found to protect HBx protein from proteolytic degradation by competing with the proteasome 20S subunit alpha 7 (Figure 2).[42] The preceding observations may help develop new strategies for treating HBV infection and chronic liver diseases.

6. Regulation of key host and viral pathways

The HBx protein modulates the host cell's signal transduction pathways, calcium and reactive oxygen species levels, and influences cell proliferation and apoptosis. It also interacts with several host transcription factors (e.g., CREB, NF-κB) to activate or repress specific host and viral genes.[11-13] HBx promotes viral replication and host cell survival, interfering with the host immune system, and epigenetically altering host gene expression (Figure 3). HBx plays a central role in viral pathogenesis by disrupting cell cycle regulation, inducing oxidative stress and DNA damage, leading to chronic infection and HCC development.[11-13] Therefore, through specific targeting of one or more regulatory pathways, it should be possible to disrupt viral replication, chronic inflammation, or disease progression as discussed in subsequent sections.

6.1. Epigenetic regulation of cccDNA

The HBx protein plays a central role in regulating the transcriptional activity of cccDNA, the persistent nuclear template of HBV.[7,43] In the absence of HBx, cccDNA exists in a repressed chromatin state, marked by hypoacetylation and H3K9 methylation, which correlates

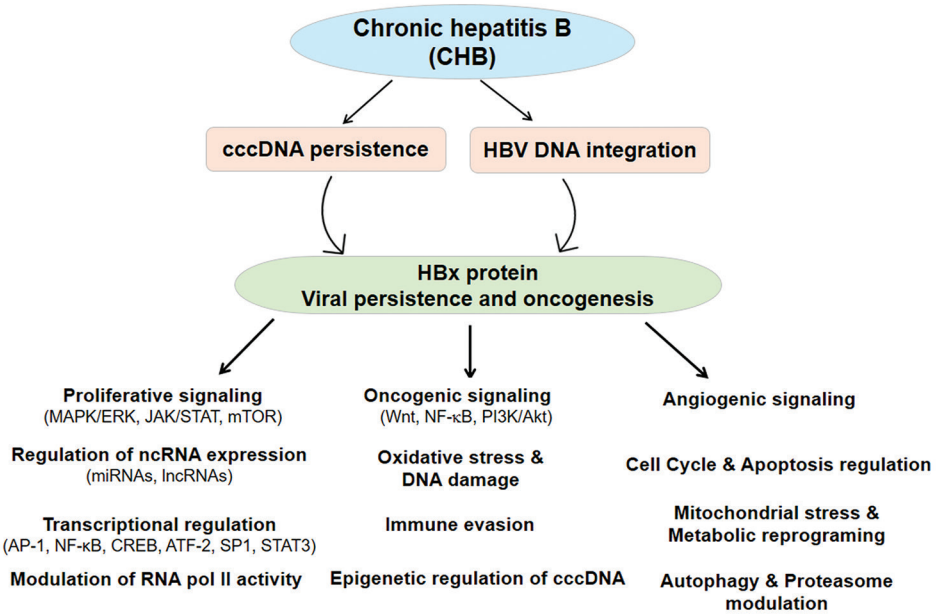

Figure 3. Role of the hepatitis B virus (HBV) X (HBx) protein in pathogenesis of chronic hepatitis B (CHB). CHB is sustained through covalently closed circular DNA (cccDNA) persistence and HBV DNA integration, both of which drive the expression of the HBx protein. HBx promotes viral persistence and hepatocarcinogenesis through multiple mechanisms, including deregulation of proliferative signaling and cell cycle, non-coding RNA expression, transcriptional regulation, epigenetic regulation of cccDNA, mitochondrial stress and metabolic reprogramming, Oxidative stress and DNA damage, immune evasion, angiogenic signaling, and many other functions. Abbreviations: Akt: Protein kinase B; AP-1: Activator protein 1; ATF-2: Activating transcription factor 2; cccDNA: Covalently closed circular DNA; CREB: Cyclic adenosine monophosphate response element binding protein; ERK: Extracellular signal-regulated kinase; JAK: Janus kinase; lncRNAs: Long non-coding RNAs; MAPK: Mitogen-activated protein kinase; miRNAs: Micro RNAs; mTOR: Mechanistic target of rapamycin; ncRNA: Non-coding RNA; NF-κB: Nuclear factor kappa B; PI3K: Phosphoinositide 3-kinase; RNA pol II: RNA polymerase II; SP1: Specificity protein 1; STAT: Signal transducer and activator of transcription.

with the recruitment of histone deacetylases, SET domain bifurcated histone lysine methyltransferase 1, and heterochromatin protein 1.[44,45] HBx binding facilitates a permissive chromatin state for viral replication and gene expression by recruiting chromatin-modifying enzymes, including p300 histone acetyltransferase and lysine-specific histone demethylase 1 to remove H3K9 methylation, and H3K4 methyltransferase Set1A to catalyze the mono-, di-, and trimethylation of lysine 4 on histone H3 (H3K4).[46-48] In addition, HBx can epigenetically alter gene expression by influencing the activity of DNA methyltransferases, which causes hypermethylation and repression of tumor suppressor genes, promoting tumorigenesis.[13]

The HBx protein also alters the epigenetic landscape of cccDNA by antagonizing host restriction factors, such as the Smc5/6 complex, and prevents transcriptional silencing.[49] One of the best-characterized HBx-host interactions involves the damage-specific DNA-binding protein 1 (DDB1), an adaptor of the cullin 4 (CUL4) A-regulator of cullins 1 E3 ubiquitin ligase complex.[50] Degradation of Smc5/6 by the HBx-DDB1-CUL4 complex removes this transcriptional block, thereby enabling productive HBV replication.[51] Consequently, these interactions are critical for persistent viral gene expression mediated by HBx.

6.2. Regulation of the host immune system

The HBx protein extensively modulates the host immune response, generally by suppressing innate immunity and evading immune destruction to promote viral persistence and disease progression. HBx can dysregulate the function of immune cells, such as dendritic cells and natural killer cells, contributing to chronic inflammation and the progression of liver disease. It achieves this through various mechanisms, including interfering with IFN signaling, altering epigenetic modifications, impairing immune cell functions, and downregulating key immune molecules such as type-1 IFN receptor and TRIM22 by suppressing tyrosine kinase 2 activity.[52] HBx can inhibit the retinoic acid-inducible gene i/melanoma differentiation-associated protein 5 pathway by interacting with essential proteins such as Sp110, thereby reducing IFN production. It can also interfere with the tumor necrosis factor receptor-associated factor 3/TANK-binding kinase 1/IFN regulatory factor 3 pathway, which is critical for initiating antiviral responses.[53,54]

The HBx protein plays a crucial role in HBV-related progression of chronic liver disease and HCC by promoting a pro-tumorigenic microenvironment, inhibiting apoptosis, and disrupting immune surveillance (Figure 3). It contributes to malignant transformation by modulating several signaling pathways, including Wnt/β-catenin, Ras/Raf/mitogen-activated protein kinase, NF-κB, Janus kinase/signal transducer and activator of transcription, phosphoinositide 3-kinase/protein kinase B, and focal adhesion kinase cascades, leading to increased proliferation, survival, invasion, and metastasis of hepatocytes.[11,14,17] In the nucleus, HBx dysregulates transcription, cell cycle checkpoints, apoptosis, and DNA repair, whereas in the mitochondria, it promotes the generation of reactive oxygen species and metabolic reprogramming.[55] HBx also induces oxidative stress, endoplasmic reticulum stress, and chronic hepatic inflammation, all of which contribute to tumor-promoting microenvironments.[56]

Furthermore, HBx also modulates immune responses by activating the NF-κB signaling pathways, protecting infected hepatocytes from apoptosis and immune-mediated clearance.[57] Its interactions with mitochondrial antiviral signaling protein can alter innate immune signaling, whereas transcriptional upregulation of host neoantigens may provoke adaptive immune responses during chronic liver disease.[58] These properties allow HBx to maintain a reservoir of infected cells, facilitating HBV persistence and increasing the risk of liver cancer. These observations suggest HBx to be a valuable therapeutic target for preventing CHB.

7. Strategies to regulate intracellular levels of HBV X protein

There are multiple strategies to regulate the intracellular HBx level and its diverse functions, which may be critical in mitigating the diverse roles of HBx in viral pathogenesis. The three main strategies are inhibiting *HBx* gene expression (RNAi, gene editing, or gene silencing); inducing proteolytic degradation of HBx protein (using small molecules); and developing therapeutic vaccines (Figure 4). The positive outcomes from new investigations could be useful in developing curative interventions for CHB.

7.1. Inhibition of the *HBV X* gene expression

As a transcriptional activator, HBx is central to HBV replication, viral gene expression, and the development of liver diseases, including hepatitis, cirrhosis, and HCC. Therefore, inhibiting HBx expression could downregulate viral load and counter HBV-associated pathogenesis. The inhibition strategies include RNAi, genome editing, and transcriptional gene silencing (Figure 4).

7.1.1. RNAi

RNAi could emerge as a preferred strategy against CHB therapy, as this could downregulate all activities associated with HBx. Small interfering RNAs (siRNAs) designed against HBx have effectively reduced HBV replication in different experimental models, confirming HBx as a critical therapeutic target for HBV.[7] Modified 5'-triphosphate siRNAs (3p-siRNAs) exhibited dual functionality, such as specific silencing of HBx and activation of the retinoic acid-inducible gene I pathway, thereby inducing type I IFN production and restoring innate immunity.[59] More broadly, siRNAs targeting all HBV transcripts suppressed HBx expression and indirectly stabilized Smc5/6, leading to the transcriptional repression of cccDNA.[51] Clinical candidates such as ALN-HBV, VIR-2218, ARC-520, and JNJ-3989 have demonstrated safe, dose-dependent HBsAg reduction, and, in some cases, prolonged suppression of viral proteins.[7,60] A phase 2 study showed that combining VIR-2218 with pegylated IFN-α2a improved HBsAg seroclearance rates.[61] GSK3389404, an antisense oligonucleotide conjugated to N-acetylgalactosamine, can also reduce HBx RNA and HBsAg levels with favorable pharmacokinetics.[62] While RNAi therapies offer strong potential, challenges remain regarding their delivery efficiency, durability, and effects on integrated HBV sequences that frequently encode HBx.[62] Early studies using hammerhead and hairpin ribozymes against HBx RNA successfully reduced HBx expression and activity, supporting the feasibility of RNA-based catalytic therapy. However, their clinical application is limited by the lack of effective delivery systems.[7]

7.1.2. Genome editing

The clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system has been used as an alternative method for directly targeting HBx and other *HBV* genes (Figure 4). HBx-specific RNAs coupled with Cas9 nucleases have been shown to excise HBx, leading to significant reductions in HBx levels, HBsAg production, and cccDNA replication in hepatoma cells.[63] However, the off-target cleavage remains a technical limitation, although multiplexed targeting of conserved viral regions may improve the efficacy of this approach. While RNAi-based drugs are already progressing through clinical trials, ribozyme and CRISPR technologies are still in the early stages but hold long-term promise.

7.1.3. Transcriptional gene silencing

In HBV-infected hepatocytes, HBx localizes to the nucleus and hijacks the host CUL4-DDB1 E3 ubiquitin ligase complex. DDB1 typically functions as an adaptor for DDB1-CUL4-associated factors, enabling precise substrate recognition.[7] HBx mimics a DDB1-CUL4-associated

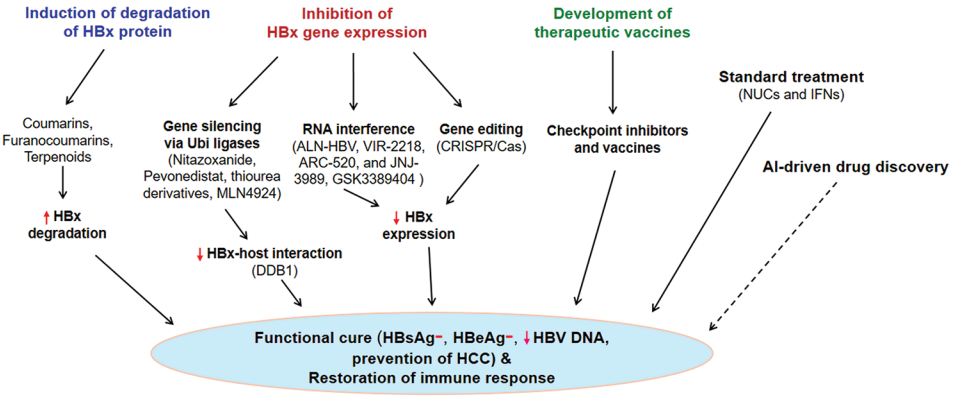

Figure 4. Therapeutic strategies targeting the hepatitis B virus X protein (HBx). Three main strategies for therapeutic targeting of HBx are the inhibition of HBx gene expression (by gene silencing, RNA interference, or gene editing), the induction of proteolytic degradation of HBx (using small molecules), and the development of therapeutic vaccines. Standard treatment for CHB infection involves antiviral medications, which are typically oral nucleos(t)ide analogs (NUCs), such as tenofovir or entecavir. Interferons (IFNs) are an alternative treatment option given to boost the host's immune system. Artificial intelligence (AI) could be considered for designing new therapeutic candidates. Abbreviations: ALN: Antisense oligonucleotide; ARC-520: RNA interference therapeutic targeting hepatitis B virus; CRISPR: Clustered regularly interspaced short palindromic repeats; DDB1: Damage-specific DNA binding protein 1; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma; JNJ-3989: RNA interference therapeutic targeting hepatitis B virus; MLN4924: NEDD8-activating enzyme inhibitor; VIR-2218: RNA interference therapeutic targeting hepatitis B virus.

factor to bind to DDB1, thereby recruiting the ubiquitin ligase machinery to target the structural maintenance of Smc5/6 for proteasomal degradation. As Smc5/6 acts as a transcriptional repressor of cccDNA, its removal by HBx relieves this repression, thereby enhancing HBV transcription and replication (Figure 4).[7] Therefore, disrupting the HBx–DDB1 interaction and maintaining cccDNA in a transcriptionally repressed state could be a promising therapeutic approach for CHB.

Recently, a number of molecules that could disrupt the HBx-DDB1 interaction have been identified. For example, nitazoxanide, a thiazolide with antiparasitic activity, also exhibited an antiviral activity against HBV by disrupting this interaction and stabilizing the Smc5/6 complex. Nitazoxanide showed a significant reduction in virion production as well as potency against multiple drug-resistant HBV variants ($EC_{50:}$ 0.15–0.31 μM).[64] A pilot clinical trial in CHB showed a rapid decline in serum HBV DNA, which was consistent with its preclinical mechanism (Table 1).[65] These findings establish nitazoxanide as a proof-of-concept inhibitor for HBx–DDB1-targeted therapy. Pevonedistat, an inhibitor of the NEDD8-activating enzyme, has been found to stabilize the Smc5/6 complex by targeting the DDB1-CUL4-ROC1 E3 ligase pathway and downregulate HBV replication.[66]

More recently, thiourea derivatives (DSA-00, DSA-02, DSA-03) have been shown to enforce episomal silencing of cccDNA by stabilizing the Smc5/6 complex, resulting in the suppression of pgRNA synthesis, HBV DNA replication, and antigen secretion (HBsAg, HBeAg).[67] These agents showed antiviral potency comparable to that of entecavir without cytotoxicity, supporting their potential in functional cure strategies (Table 1). Likewise, inhibition of neddylation mediated by the E3 ligases has been found to stabilize HBx. Inhibition of neddylation by MLN4924 is able to suppress cccDNA transcription, pgRNA synthesis, HBV DNA replication, and HBsAg production.[68] Together, these findings underscore that pharmacologic stabilization of Smc5/6 achieved through interference with HBx-DDB1 binding or modulation of HBx post-translational modifications represents a targeted strategy for durable HBV suppression.

In addition, several natural bioactive compounds have been studied for their anti-HBV activities. For example, gambogic acid exhibited dose-dependent inhibition of HBx expression by upregulating the expression of the *DTX1* gene and boosting the notch signaling pathway.[69] Likewise, ursolic acid, a pentacyclic triterpenoid derived from medicinal herbs, blocked HBx-mediated autophagy and reversed HBx-driven drug resistance, cell migration, and apoptosis regulation through p38 and extracellular signal-regulated kinase signaling.[70] With its proven hepatoprotective and antitumor effects and minimal toxicity, ursolic acid should be considered as a candidate drug for further investigation and targeting *HBx-mediated* gene regulation.

7.2. Interference with HBV X protein stability and its functions

Targeting HBx for degradation has emerged as a promising curative strategy for CHB infection because HBx plays a

Table 1. Natural products and synthetic compounds known to promote hepatitis B virus X protein degradation and suppress hepatitis B virus replication

Compound/molecule	Class/source	Mechanism of HBx degradation	Effect on HBV markers	Development stage	References
Nitazoxanide	Synthetic thiazolide anti-infective agents	Disrupts DDB1–HBx interaction, restores Smc5/6	↓ HBV transcription and proteins	Clinical (Phase I)	65
Pevonedistat	Synthetic NEDD8-activating enzyme inhibitor	Blocks DDB1–CUL4–ROC1 E3 ligase activity, restores Smc5/6	↓ HBV replication	Preclinical	66
Thiourea derivatives (DSA-00, DSA-02, DSA-03)	Synthetic small molecules	Stabilizes Smc5/6 by blocking HBx–DDB1 interaction	↓ pgRNA synthesis, ↓ HBV DNA replication, ↓ HBsAg, ↓ HBeAg	Preclinical	67
Dicoumarol	Synthetic coumarin derivative; vitamin K analog; NQO1 inhibitor	Disrupts NQO1–HBx binding, promotes 20S proteasomal degradation	↓ HBx recruitment to cccDNA, ↓ HBV RNA/DNA, ↓ HBsAg, ↓ HBcAg	Preclinical (humanized liver mouse model)	71
Esculetin	Coumarin (*Microsorium fortunei*)	Reduces HBx protein levels (likely proteasome-dependent)	↓ HBx, ↓ HBsAg, ↓ HBV DNA	Preclinical (*in vitro*)	72
Sphondin	Angular furanocoumarin (*Toddalia asiatica*)	Binds HBx at Arg72, causes, 26S proteasomal degradation	↓ HBx–cccDNA binding, ↓ HBV RNA, ↓ HBsAg	Preclinical (*in vitro* and *in vivo*)	73
Furanocoumarins (Fc-20, Fc-31)	Synthetic furanocoumarin analogs	Directly binds HBx, induces allosteric change, and causes proteasomal degradation	↓ cccDNA transcription, ↓ pgRNA, ↓ HBsAg/HBeAg, ↓ virion secretion; active vs drug-resistant strains	Preclinical (*in vitro*)	74
Asiatic acid	Triterpenoid (*Centella asiatica*)	Autophagy–lysosomal degradation of HBx; alters histone markers (↓ H3K4me3, ↑ H3K9me3, H3K27me3)	↓ HBx–cccDNA binding, ↓ cccDNA transcription	Preclinical	75
All-trans retinoic acid	Natural vitamin A derivative	Antagonizes HBx-mediated suppression of p14/p16/p21 (indirect functional inhibition)	↓ HBx-driven cell proliferation	Preclinical	76
Tranilast	Antiviral (repurposed), HBx-targeting	High-affinity binding to HBx→promotes HBx degradation	↓ HBV DNA/HBsAg in cell culture	Preclinical	84
Broad-spectrum antivirals (e.g., azithromycin, domiphen, ammonium glycyrrhizinate, valsartan)	Antiviral (repurposing/ HBx-binding)	HBx binders with a low dissociation constant; antiviral activity demonstrated	Require functional validation	Experimental	84
Rapamycin	Antiviral, immunotropic	mTOR inhibitor, enhances proteasomal degradation of HBx	↓ HBx protein, ↓ HBV replication	Preclinical	85
SC75741, punicalagin, ledipasvir	Antiviral/ HBx-binders (*in silico*/ repurposed)	Strong HBx binding (by molecular docking); predicted to block HBx-interaction regions	Potential HBx inhibitors (predicted)	Experimental validation is limited or pending	86
TLR agonists (TLR7: GS-9620/vesatolimod; TLR8: GS-9688/ Selgantolimod)	Antiviral, immunotropic	Activate innate immunity; IFNα, ISGs, augment NK/ CD8 responses to counter HBx-mediated immune suppression	↓ HBV DNA/HBsAg in animal models	Clinical-stage immune modulators	87
PD-1/PD-L1 checkpoint inhibitors (e.g., nivolumab, anti-PD-L1 antibodies)	Immunotropic	Reverse HBx-exacerbated T-cell exhaustion→restore HBV-specific $CD8^+$T cells	↑ T-cell responses, ↑ viremia	Preclinical model	87

(Cont'd...)

Table 1. ***(Continued)***

Compound/molecule	Class/source	Mechanism of HBx degradation	Effect on HBV markers	Development stage	References
IL-12 (cytokine therapy)	Antiviral, immunotropic	Boosts multifunctional T cells (↑ IFN-γ, TNF-α), lowers PD-1 expression	↓ viremia, ↑ HBV-specific T cells	Preclinical model	87
Engineered HBV-specific T cells/TCR/CAR approaches	Antiviral, immunotropic,	Redirected cytotoxic T cells target HBx/HBV-expressing hepatocytes	Killing of HBV/HBx expressing cells; ↓ HBsAg in clinical cases	Preclinical, clinical	87
siRNAs/ASOs (e.g., JNJ-3989, VIR-2218, IONIS-HBVRx, ARC-520, bepirovirsen)	Antiviral, HBx-silencing (nucleic acid)	Post-transcriptional silencing of HBV transcripts, including HBx mRNA	↓ HBx expression, ↓ HBV RNAs/ HBsAg in clinical trials or preclinical studies	Preclinical/ clinical	87-89
Therapeutic vaccines (e.g., GS-4774)	Antiviral, immunotropic	Deliver HBx/core/surface antigens to stimulate HBV-specific T cells	Immune activation observed; limited HBsAg clearance as monotherapy in trials - candidate for combination therapy	Clinical	90
Curcumin (Herbal remedy)	Antioxidant, hepatoprotective	Anti-inflammatory; downregulates HBV transcriptional co-activators	↓ HBV transcription, protects hepatocytes from oxidative damage	Preclinical/ clinical	91
EGCG (epigallocatechin-3-gallate) (Herbal remedy)	Antiviral, antioxidant	Blocks HBV receptor and clathrin-mediated endocytosis; restores autophagy/lysosomal function	↓ HBV entry↓HBV markers Assists in autophagy	Preclinical	91
Punicalagin/punicalin/ geraniin (Herbal remedy)	Antiviral	Inhibit cccDNA production promote degradation	↓ cccDNA levels↓HBV markers	Preclinical *(in vitro)*	92
Rubiadin (Herbal remedy)	Natural anthraquinone	Inhibits HBx-associated functions in hepatocytes	↓ HBsAg, ↓ HBeAg, ↓ HBV DNA	Preclinical (*in vitro*)	93
Oxymatrine (Herbal remedy)	Matrine-type alkaloid (from *Sophora flavescens*)	Inhibits HBV DNA, HBsAg, HBeAg; immunomodulatory	↓ viral replication; ↑ IFN-α; activates NK cells and macrophages;	Preclinical (*in vitro*)	94

Note: ↓ refers to reduces; ↑ refers to increases.
Abbreviations: ATRA: All-trans retinoic acid; ASOs: Antisense oligonucleotides; CAR: Chimeric antigen receptor; cccDNA: Covalently closed circular DNA; CUL4: Cullin 4; DDB1: Damage-specific DNA binding protein 1; EGCG: Epigallocatechin-3-gallate; HBcAg: Hepatitis B core antigen; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HBx: Hepatitis B virus X protein; IFNs: Interferon; IL-12: Interleukin 12; ISGs: Interferon-stimulated genes; mTOR: Mammalian target of rapamycin; NK: Natural killer; NQO1: NAD (P) H quinone dehydrogenase 1; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; pgRNA: Pregenomic RNA; ROC1: Ring-box protein 1; siRNAs: Small interfering RNAs; TCR: T-cell receptor; TLR: Toll-like receptor.

pivotal role in the HBV life cycle. Several natural and synthetic compounds have been reported to destabilize HBx through proteasomal or lysosomal pathways, thereby repressing HBV replication and viral gene expression. Coumarin-based compounds exhibit antiviral activity through diverse molecular targets. Dicoumarol, a vitamin K analog and inhibitor of nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1 (NQO1), was shown to disrupt the protective interaction between NQO1 and HBx, leading to HBx degradation through 20S proteasome.[71] Esculetin, another coumarin isolated from *Microsorium fortunei*, can also inhibit the expression of HBx protein and production of viral antigens in a dose-dependent manner (Figure 5 and Table 1).[72]

Natural furanocoumarins have a long history in traditional medicine with a wide array of bioactivities, including antiviral, anti-inflammatory, anti-proliferative, and photochemical properties, mediated by their structural variability (substituents, ring position, and isomerism). Natural furanocoumarins, such as sphondin from *Toddalia asiatica*, have been shown to bind to HBx and induce proteasomal degradation, resulting in suppression of viral transcription and HBsAg expression (Figure 5).[73] Synthetic furanocoumarin analogs include the systematic modification of substituents to optimize potency, selectivity, and pharmacological properties while minimizing off-target and toxic effects.[7] Recently, our team has identified two synthetic furanocoumarins, Fc-20 and

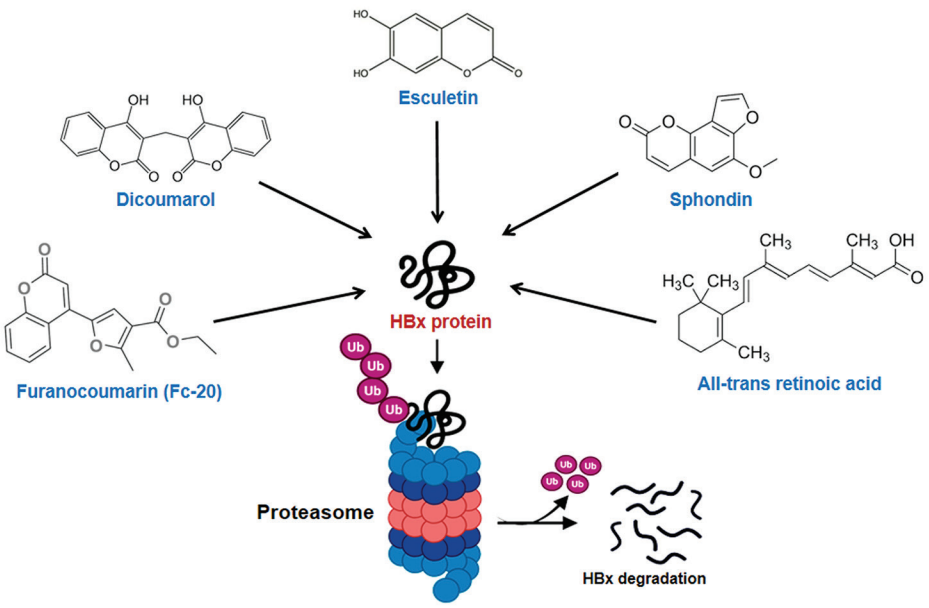

Figure 5. Inducers of hepatitis B virus X protein (HBx) destabilization. Molecular structures of some well-characterized bioactive compounds that promote proteasomal degradation of the HBx protein, including furanocoumarin (Fc-20), all-trans retinoic acid, asiatic acid, esculetin, and dicoumarol. Created in BioRender. Giri, S. (2025) https://BioRender.com/f1vc9g2.

Fc-31, that exhibited strong anti-HBV activity in cell culture by substantially reducing HBsAg and HBeAg secretion (>90%), thus outperforming the maximal reductions reported for entecavir.[74] Both compounds significantly decreased cccDNA transcriptional output, as inferred from reductions in 3.5 kb pgRNA levels, reflecting impaired replication template synthesis, and virion production, consistent with lowered intracellular viral replication intermediates (Table 1). Furthermore, these candidates retained potency against drug-resistant strains, including the tenofovir-resistant CYEI HBV mutant, positioning them as promising leads for both mono- and combination therapies.[74] Given the corroborative findings with natural analogs, such as sphondin, furanocoumarin scaffolds represent a compelling chemical series for advancing HBx-directed therapies aimed at the functional cure of CHB.

Asiatic acid, a pentacyclic triterpenoid isolated from *Centella asiatica* (a Chinese medicinal herb), can also promote HBx degradation through the autophagy-lysosomal pathway, resulting in reduced binding to cccDNA, reduced activating histone marks (H3K4 trimethylation), and increased repressive modifications (H3K9 trimethylation and H3K27 trimethylation), thereby impairing cccDNA transcription (Table 1).[75] Other natural compounds, such as all-trans retinoic acid, exhibited antiviral activity and antagonized HBx-mediated suppression of cell cycle inhibitors (p14, p16, and p21) by upregulating these proteins, suggesting indirect interference with HBx function (Table 1).[76] Collectively, these findings underscore the potential of both natural products and synthetic agents to promote HBx degradation, disrupt its interactions with host cofactors, and ultimately silence cccDNA transcription. Such strategies may complement existing antiviral efforts to achieve a functional cure for HBV.

7.3. HBV X protein-based therapeutic vaccine

One desirable goal of HBV immunotherapy has been to restore sufficient anti-HBV immunity in the CHB patients, as these patients show T cell exhaustion and a tolerogenic liver environment. HBx is considered to be a suitable immunogen for rejuvenating anti-HBV adaptive immunity in these patients. Experimental animals vaccinated with HBx demonstrated a good HBx-specific $CD4^+$ and $CD8^+$ T cell response with a significant reduction in serological biomarkers of HBV.[77] Interestingly, suppression of viral transcription, HBV replication, and viral antigen production could also be accomplished through intracellular delivery of monoclonal antibodies against HBx protein. These antibodies could also inhibit HBx–DDB1 interaction *in vitro*, suggesting their therapeutic potential.[78,79] These observations have provided proof-of-principle that targeting HBx merits further investigation.

8. Conclusion

Due to its role in viral persistence and hepatocarcinogenesis, HBx is a highly valuable therapeutic target for CHB infection.

Targeting HBx would not only mitigate both viral persistence and oncogenic transformation but also potentially improve the long-term outcomes of patients with CHB. Therefore, inclusion of HBx-directed agents within current therapeutic regimens may accelerate progress toward a functional cure. Furthermore, this could achieve durable viral suppression, limit relapse, and help restore HBV-specific immune responses.

Despite the availability of effective vaccines and nucleos(t)ide analogs as antivirals, CHB infection continues to pose a global health challenge, affecting nearly 254 million individuals and causing over 1.1 million deaths annually.[3] The persistence of cccDNA and the integration of viral genomes into host hepatocytes remain the primary barriers to cure.[80-82] Current therapeutic regimens, primarily nucleos(t)ides and IFN-α, can achieve viral suppression but rarely result in sustained HBsAg clearance.[82] Consequently, the risk of progressive liver disease and HCC persists, even with long-term therapy.[82] Recently, HBx protein has emerged as a key viral factor because of its essential role in viral replication and gene expression, as well as reprogramming of host signaling pathways to promote immune tolerance, inflammation, fibrosis, and oncogenic transformation.[11,12,17] Therefore, destabilization or downregulation of HBx would not only attenuate HBx-driven oncogenic signaling but also limit relapse after treatment discontinuation, and diminish cccDNA reservoir and viral antigen load.

Emerging approaches, such as RNAi, antisense oligonucleotides, CRISPR/Cas-based genome editing, and small molecules directed at HBx-host interactions, are being investigated as means to neutralize the oncogenic potential of HBx. Furthermore, the direct-acting antivirals that block viral entry, nucleocapsid assembly, reverse transcription, and secretion of viral antigens are at advanced stages of development.[82] While such antivirals can effectively reduce antigenemia and/or suppress viremia, none of these strategies are effective at eliminating cccDNA or integrated HBV DNA.[81,82] Therefore, combining HBx-targeted strategies into existing treatment regimens could bridge this gap. Moreover, artificial intelligence should be applied in designing therapeutic candidates with desired characteristics, such as solubility and target affinity.[83] In summary, HBx-targeting represents a critical frontier in HBV therapy with the potential to couple viral suppression with cancer prevention.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

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