Li *et al. Infectious Agents and Cancer* (2024) 19:56
https://doi.org/10.1186/s13027-024-00618-y

Infectious Agents and Cancer

REVIEW Open Access

Insight into the mechanisms regulating liver cancer stem cells by hepatitis B virus X protein

Xiaocui Li[1†], Delong Kong[1,4†], Wei Hu[2], Kuiyang Zheng[1,3], Hongjuan You[1], Renxian Tang[1,3*] and Fanyun Kong[1*]

Abstract

Hepatocellular carcinoma (HCC) is a heterogeneous disease with high recurrence and mortality. It is well known that a large proportion of HCCs are caused by hepatitis B virus (HBV) infection. In particular, the HBV X protein (HBX), a multifunctional molecule produced by the virus, plays a leading role in hepatocarcinogenesis. However, the molecular mechanisms underlying HBX-mediated HCC remain not fully elucidated. Recently, liver cancer stem cells (LCSCs), a unique heterogeneous subpopulation of the malignancy, have received particular attention owing to their close association with tumorigenesis. Especially, the modulation of LCSCs by HBX by upregulating CD133, CD44, EpCAM, and CD90 plays a significant role in HBV-related HCC development. More importantly, not only multiple signaling pathways, including Wnt/β-catenin signaling, transforming growth factor-β (TGF-β) signaling, phosphatidylinositol-3-kinase (PI-3 K)/AKT signaling, and STAT3 signaling pathways, but also epigenetic regulation, such as DNA and histone methylation, and noncoding RNAs, including lncRNA and microRNA, are discovered to participate in regulating LCSCs mediated by HBX. Here, we summarized the mechanisms underlying different signaling pathways and epigenetic alterations that contribute to the modulation of HBX-induced LCSCs to facilitate hepatocarcinogenesis. Because LCSCs are important in hepatic carcinogenesis, understanding the regulatory factors controlled by HBX might open new avenues for HBV-associated liver cancer treatment.

Keywords Hepatocellular carcinoma, HBX, Signaling pathways, Epigenetic regulation, Liver cancer stem cells

Introduction

Hepatocellular carcinoma (HCC), which accounts for no less than 75% of primary liver cancers globally, is a leading cause of tumor-associated deaths [1]. Surgical resection is a suitable option when the disease is discovered at an early stage. Unfortunately, many patients with this malignancy are often diagnosed at an advanced stage [2]. Although patients with advanced HCC can receive targeted therapy or immunotherapy, their therapeutic effects are disappointing. HCC is a heterogeneous disease. Currently, liver cancer stem cells (LCSCs), also called liver cancer stem-like cells or tumor-initiating cells [1], are identified as a small and unique subpopulation of HCC cells with the capacity for high self-renewal, unlimited differentiation, and hepatocarcinogenesis. Understanding the traits and regulatory mechanisms of

[†]Xiaocui Li and Delong Kong contributed equally to this work.

*Correspondence:
Renxian Tang
tangrenxian-t@163.com
Fanyun Kong
kongfanyun@xzhmu.edu.cn
[1]Jiangsu Key Laboratory of Immunity and Metabolism, Department of Pathogenic Biology and Immunology, Xuzhou Medical University, Xuzhou, Jiangsu, China
[2]NanJing Drum Tower Hospital Group Suqian Hospital, The Affiliated Suqian Hospital of Xuzhou Medical University, Suqian, China
[3]National Demonstration Center for Experimental Basic Medical Sciences Education, Xuzhou Medical University, Xuzhou, Jiangsu, China
[4]Experimental Animal Center, Xuzhou Medical University, Xuzhou, Jiangsu, China

LCSCs can help us develop novel strategies for treating the malignancy [3].

LCSC identification and characterization have been conducted for nearly two decades. Accumulated evidence shows that one reason for the occurrence of LCSCs is the transformation of hepatic stem/progenitor cells (HPCs)/oval cells. Mature hepatocytes with genetic or epigenetic changes are considered another possible origin of LCSCs during the hepatic injury or regeneration process [4, 5]. The distinct origin of LCSCs indicates its heterogeneity, which results in the lack of universal markers for LCSC identification. Until now, various LCSC surface markers, including but not limited to EpCAM, CD133, DLK1, CD90, CD44, OV6, CD24, ABCG2, and CK19, have been identified [6]. Because different LCSC surface markers have distinct functional characteristics, the identified LCSCs using distinct markers are highly heterogeneous and might exhibit different phenotypes [7]. Despite these limitations, the identified LCSC markers have laid the groundwork for current studies on LCSCs [3].

Hepatitis B virus (HBV), hepatitis C virus (HCV) infection, and nonalcoholic fatty liver disease are the main risk factors for HCC [8]. Increasing evidence indicates that LCSCs are closely associated with HCC development induced by these risk factors [3]. In addition, various cellular factors, including signaling pathways, epigenetic regulators, transcription factors, metabolic regulators, and secretory molecules, can drive the plasticity and reversibility of LCSC phenotypes in HCC [3, 9]. Given the importance of LCSCs in tumor metastasis, recurrence, and resistance to conventional treatment [4], a better understanding of the cellular factors that contribute to the regulation of LCSCs mediated by different risk factors facilitates the development of suitable approaches for targeting HCC.

Relationship of HBX with LCSCs in HBV infection

The occurrence and progression of HCC are closely related to HBV infection in Asia and Africa [2, 10, 11]. Although a variety of factors, including viral gene integration, genomic mutation, chronic inflammation, epigenetic alteration, and tumor-related signaling pathway activation, have been discovered in the pathogenesis of HBV-induced HCC [12–14], the exact relevant mechanisms are still not well clarified. Previous studies indicate that LCSCs are crucial for HBV-mediated HCC [8, 15]. Moreover, some LCSC markers can be used to predict HBV-associated HCC outcomes. For example, in HBV-positive malignancies, EpCAM is closely associated with AFP, vascular invasion, advanced tumor node metastasis stage, and poor tumor differentiation [16]. Compared to the peritumoral EpCAM-negative group, the EpCAM-positive group in paracancerous tissues with HBV infection has a worse overall survival (OS) and poor recurrence-free survival (RFS) [17]. In addition to EpCAM, clinical evidence shows that CD133 is related to poor disease-free survival (DFS) and OS [18], and CK19 is related to shorter DFS [19] in HBV-related HCC.

Four overlapping open reading frames (ORFs), P, C, S, and X, exist in the HBV genome. Functionally, these ORFs contribute to the production of viral polymerase (HBp), viral core protein (HBc), e-antigen (HBeAg), envelope antigens (HBsAg, pre-S1, and pre-S2), and HBX protein [20–23]. As a multifunctional viral protein, HBX has a vital role in viral infection, replication, and associated tumorigenesis [20–22, 24]. Furthermore, accumulating evidence shows that it can cause genetic dysregulation, result in epigenetic aberrations, and interfere with several signaling pathways involved in cellular growth, apoptosis, autophagy, and invasion to facilitate carcinogenesis [25, 26].

In particular, based on cell models [27], clinical samples [28], and mouse models [29], it has been shown that the appearance of LCSCs induced by HBX has an important contribution to hepatocarcinogenesis. In HBV-associated cancer tissues, clinically significant associations between HBX and different LCSC markers, including EpCAM and OV6, were also identified [30, 31]. Therefore, illustrating the mechanisms responsible for the modulation of LCSCs caused by HBX may not only deepen the understanding of the pathogenesis of HBV-associated malignancies but also benefit the development of therapeutic approaches to facilitate HCC management.

As mentioned, no universal marker can distinguish LCSCs from HCC cells because of the heterogeneity of LCSCs. However, until now, CD133, EpCAM, CD44, and CD90, have been used mostly for the identification and isolation of LCSCs [32]. Here, we mainly presented an up-to-date overview of the molecular mechanisms (Fig. 1), including signaling pathways and epigenetic alterations, in modulating LCSCs by HBX by controlling the expression of CD133, EpCAM, CD44, and CD90 in HCC cells.

The signaling pathways in regulating LCSCs mediated by HBX

To date, the hyperactivation of multiple signaling pathways, including Wnt/β-catenin signaling, transforming growth factor-β (TGF-β) signaling, phosphatidylinositol-3-kinase (PI3-K)/AKT signaling, and STAT3 signaling pathways influenced by HBX have been demonstrated to have significant effects in the process of HBV-associated hepatocarcinogenesis [16, 33]. In particular, increasing evidence indicates that these signaling pathways are responsible for maintaining the stemness of HBX-induced LCSCs via upregulation of different LCSC surface markers, including CD133, CD90, CD44, and

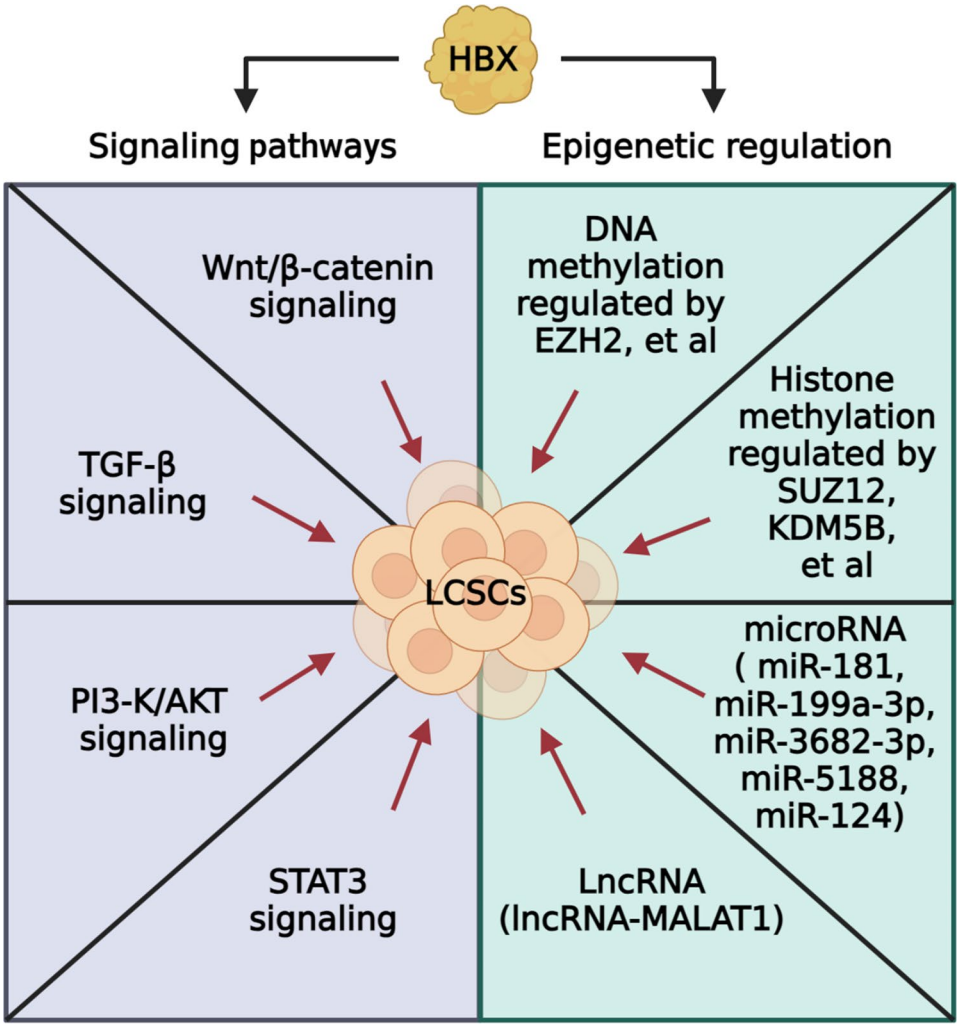

Fig. 1 The factors contribute to the regulation of LCSCs mediated by HBX. The signaling pathways, including Wnt/β-catenin signaling, TGF-β signaling, PI3-K/AKT signaling, and STAT3 signaling pathways participate in regulating LCSCs mediated by HBX. The epigenetic regulation, such as DNA and histone methylation, as well as ncRNA, including microRNA and lncRNA, also contributes to the modulation of HBX-induced LCSCs

EpCAM. Here, we summarized the contribution of these pathways to the regulation of HBX-induced LCSCs.

Wnt/β-catenin signaling

Investigations have demonstrated that Wnt/β-catenin signaling has a large effect on the initiation and progression of several cancers. β-catenin is a very significant core molecule of this signaling. Without extracellular Wnt signals, β-catenin in the cytoplasm is restricted by a "destruction protein complex", which consists of GSK3β and APC molecules, and then degraded in a ubiquitination-dependent manner. After Wnt molecules bind to the LRP5/6 and FZD receptor complex, the activated signals disrupt the "destruction protein complex". Then, β-catenin translocates into the cell nucleus, in which it forms a protein complex with TCF/LEF molecules to activate the transcription of different downstream genes [34, 35]. Until now, HBx has been discovered to upregulate the levels of β-catenin in various ways. For example, HBX is capable of binding to APC to displace β-catenin from its "destruction protein complex" [36]. The viral protein activates the β-catenin promoter to promote its expression by increasing URG11 levels [37]. By sensitizing Notch1 signaling, HBX enhances the activity of the Wnt/β-catenin pathway [38]. In HCC cells, ETV4, which is upregulated by HBX [39], and PTEN [40], as well as GSK3β, which are inhibited by the viral protein [41], also contribute to the regulation of this signaling activation. Besides these, the study from our group suggests that HBX can elevate the levels of β-catenin via LASP1 [42] (Table 1).

A previous study showed that HBV can downregulate DDX5 expression to activate this signaling and strengthen the expression of the LCSC marker EpCAM [43]. Particularly, in HBX-transfected cell models, the viral protein was observed to sensitize the Wnt/β-catenin

Table 1 The factors involved in the control of LCSC-associated signaling pathways mediated by HBX

Target signaling	Target molecules	The effect of target molecules on identified signaling	The role of HBX on target molecule	References
Wnt/β-catenin	APC, PTEN, GSK3β	Inhibition	Inhibition	[36, 40, 41]
Wnt/β-catenin	URG11, Notch1, ETV4, LASP1	Activation	Upregulation/ Activation	[37–39, 42]
Wnt/β-catenin	MDM2, MYH9	Activation	Upregulation/ Interaction	[31, 45]
PI3-K/AKT	PTEN, miR-132	Inhibition	Inhibition	[48, 49]
PI3-K/AKT	AFP, AFPR, LASP1	Activation	Upregulation	[42, 50, 51]
TGF-β	PPM1a	Inhibition	Inhibition	[58]
TGF-β	SMAD4	Activation	Upregulation/ Interaction	[59]
TGF-β	Egr	Activation	Activation	[60]
STAT3	IL-6, ROS, LASP1, SH2D5	Activation	Upregulation	[30, 42, 65, 66]

signaling pathway to benefit the anti-apoptosis of stem cells [44]. Lin et al. found that HBX could interact with MYH9 and promote its levels to activate β-catenin and facilitate the increase of multiple LCSC markers, including CD44, EpCAM, and CD133 [45]. Wang et al. showed that HBX enhances the levels of MDM2 by binding with the protein to inhibit its ubiquitination. MDM2 further activates β-catenin and elevates the levels of LCSC markers, including CD90, EpCAM, and CD133 [31]. Until now, although Wnt/β-catenin signaling has been demonstrated to benefit the modulation of HBX-induced LCSC markers, the detailed mechanisms are still not fully elaborated. Therefore, in-depth studies on the control of LCSCs via this signaling pathway in HBX-associated HCC are needed.

PI3-K/AKT signaling

The PI3-K/AKT pathway is an important signaling pathway that controls numerous cellular processes, including cell survival, autophagy, growth, and differentiation. PI3-K is a cytosolic protein complex comprising P85 and p110 protein subunits. Upon activation, the p85-p110 complex induces the generation of phosphatidylinositol 3,4,5-triphosphate (PIP3). Sequentially, PIP3 recruits and sensitizes AKT. After activation, AKT further sensitizes its substrates to modulate the expression of downstream genes [46]. Studies have shown that HBX could upregulate miR-155 to suppress PTEN and then activate PI3-K/AKT signaling (Table 1) [47]. The viral protein induces the upregulation of reactive oxygen species (ROS) to inactive PTEN and further sensitizes this signaling [48]. In addition, the inhibition of miR-132 induced by HBX contributes to PI3-K/AKT signaling activation [49]. The upregulation of AFP [50], AFPR, and LASP1 mediated by HBX can also sensitize PI3-K/AKT signaling [42, 51].

More importantly, HBX is crucial for sensitizing this signaling to facilitate the upregulation of LCSC markers. For example, HBX contributes to the upregulation of IL-23 by activating ERK1/2. The suppression of PI3-K/AKT signaling by its inhibitors significantly decreases the sensitization of ERK1/2 and the levels of IL-23 [52]. However, the upregulation of IL-23 promotes the malignant properties of HBV-positive HCC cells via attenuating HNF4α to increase the proportion of LCSCs by increasing the expression of CD133 [53]. In addition, HBX elevates the malignant proliferation capability of stem cells by increasing Cyclin D1 via activating the PI3-K/Akt pathways [54]. Dependent on this signaling [27], AFP, which is elevated by the viral protein, promotes the initiation of LCSCs by upregulating CD44, CD133, and EpCAM. Because of the importance of PI3-K/Akt signaling in the modulation of LCSCs stimulated by HBX, the components involved in this signaling are viable candidates for therapeutic intervention.

TGF-β signaling

TGF-β signaling is a highly conserved signaling pathway, and abnormalities of this signaling pathway are closely related to the initiation and progression of liver malignancies [55]. In general, the signaling is triggered by the cytokine TGF-β, which binds to two receptors, TGFβRI and TGFβRII, leading to the transcription of target genes in a SMAD-dependent or SMAD-independent manner. The signaling exhibits anti-tumor effects at the early stages of hepatocarcinogenesis but has a pro-oncogenic activity during the late stages of HCC development [56]. HBX is shown to switch TGF-β signaling from the anti-tumor p-Smad3C-p21 pathway to the pro-oncogenic p-Smad3L-c-Myc pathway in the early stage of tumorigenesis [57]. Furthermore, multiple potential mechanisms facilitate the sensitization of this signaling modulated by HBX. On the one hand, HBX inhibits PPM1a levels by elevating its ubiquitination to induce the over-sensitization of the TGF-β signaling [58]. On the other hand, HBX can enhance SMAD4 expression through TFII-I and directly binds to SMAD4, stabilizing the protein and then

increasing the activity of this signaling [59]. Additionally, with the activation of the Egr transcription factor binding site, HBX is capable of upregulating TGF-β expression to facilitate sensitization of this signaling (Table 1) [60].

Studies have reported that TGF-β signaling is a key signaling pathway that modulates and maintains HBX-induced LCSCs. For example, TGF-β, cooperating with HBX, can induce the malignant transformation of HPCs into LCSCs by increasing EpCAM and CD90 levels to facilitate epithelial-mesenchymal transition (EMT). In addition, the sensitization of the JNK/c-Jun pathway participates in HPC malignant transformation mediated by HBX and TGF-β [16]. Moreover, TGF-β could stimulate HBX-positive HCC cells to enhance the expression of the LCSC marker CD133 [61]. Owing to the significant relevance of TGF-β signaling in HBX-induced LCSCs, efforts to target this signaling using pharmacological inhibitors for therapeutic interventions are needed to investigate.

STAT3 signaling

STAT3 is a ubiquitously expressed signaling molecule that can be activated by multiple cytokines, nonreceptor-like tyrosine kinases, and growth factor receptors in almost all body cells. After activation, the signaling molecule translocates into the nucleus and then binds to DNA to drive the transcription of downstream genes that contribute to not only cellular differentiation, cell cycle, proliferation, and angiogenesis but also metastasis [62, 63]. During infection, HBV can sensitize STAT3 signaling. The activated signaling further causes the transcription of different target genes to regulate viral replication, antiviral immunity, and tumorigenesis [62, 64].

In particular, HBX could increase the production of reactive oxygen species (ROS) in HCC cells to induce the sensitization of STAT3 signaling [65]. HBX also enhances STAT3 signaling activation by upregulating SH2D5 and LASP1 expression (Table 1) [42, 66]. In HBX-transgenic mice, the viral protein upregulates IL-6 and activates STAT3 signaling to enhance the malignant transformation of stem cells by upregulating EpCAM to facilitate hepatocarcinogenesis [30]. Mutations in the HBX gene also correlate with the development of HCC [67]. Especially, STAT3 signaling, along with the activation of Nanog, had a central effect in elevating stemness properties with the increase of CD133 mediated by HBX with C-terminal mutations [68, 69]. It should be noted that although the effect of STAT3 signaling on the regulation of LCSCs in HBX-induced tumorigenesis has been identified, a full understanding of how this unique pathway affects HBX-induced LCSCs is still required.

In addition to the above signaling pathways, accumulating investigations indicate that the mitogen-activated protein kinase (MAPK) signaling pathway, including ERK, JNK, and p38 signaling, as well as the nuclear factor kappa B (NF-κB) signaling pathway, also participates in the regulation of LCSCs [70–74]. Moreover, these two signaling pathways could be activated by HBX to control the expression of a variety of factors implicated in different biological processes that benefit hepatocarcinogenesis [42, 75–82]. However, to date, whether the activations of the MAPK and NF-kB signaling pathways are involved in the modulation of LCSCs mediated by HBX during the development of HCC, is still unclear, and it is worth exploring in the future.

The epigenetic alterations in modulating LCSC induced by HBX

Epigenetic regulation is a dynamic physiological process that regulates the accessibility of host DNA but does not alter DNA sequences to induce gene expression. Epigenetic mechanisms include (1) DNA methylation; (2) Modifications of histones mediated by methylation, phosphorylation, ubiquitination, and acetylation at different sites; (3) Alterations of noncoding RNAs (ncRNA), which participate in the regulation of RNA transcription and processing [83, 84]. Studies indicate that HBX-induced epigenetic reprogramming is essential for HBV-associated hepatic carcinogenesis [26]. The emerging data support the involvement of epigenetic alterations caused by DNA and histone modification, as well as ncRNA, in HBX-mediated modulation of LCSC markers, including EpCAM, CD133, and CD44. Here, we summarized the updated findings in HBX-mediated epigenetic regulation to control LCSCs.

DNA and histone methylation

DNA Methylation is a heritable epigenetic regulation that occurs when methyl groups are added to cytosine residues in host genomes by DNA methyltransferases [83]. To date, despite ample evidence supporting the effect of DNA methylation on HCC development [85], the relevance of DNA methylation with HBX-induced LCSCs is still not fully elucidated. A recent study from Fan et al. indicated that HBX upregulated EpCAM expression by enhancing DNA demethylation with the help of the methyltransferase EZH2. Furthermore, DNA demethylation was regulated by RelA, which formed a protein complex with EZH2 and TET2 [86] (Table 2). Despite this study sheds light on the mechanisms by which HBX regulates the LCSC marker EpCAM via DNA methylation, further studies are necessary to identify additional DNA methylation modifiers and characterize their roles in modulating LCSCs induced by HBX in HBV-associated malignancy.

DNA is packaged in the nucleus around histones, which controls how DNA can be read by transcription machinery. Histone modifications controlled by methylation, phosphorylation, acetylation, ubiquitination,

Table 2 The factors involved in the regulation of LCSC-associated epigenetic alterations induced by HBX

Epigenetic factors	Target molecules involved in epigenetic regulation	The role of HBX on target molecule	References
DNA methylation	EZH2	Inhibition	[86]
Histone methylation	SUZ12	Inhibition	[88]
Histone methylation	KDM5B, WDR5	Activation/Upregulation	[89, 90]
microRNA	miR-181, miR-5188, miR-199a-3p, miR-3682-3p	Upregulation	[16, 28, 93, 94]
microRNA	miR-124	Inhibition	[95]
LncRNA	lncRNA-MALAT1	Upregulation	[95]

and sumoylation can modulate the tightness or looseness of the histone package to determine whether DNA can be transcribed freely. A specific arginine or lysine residue at the N-terminus of histones is often methylated, and gene transcription activation or repression is correlated with differences in residue methylation. For instance, transcriptional activation is related to lysine methylation at H3 lysine 4 (H3K4) and H3K36, and there is a link between H3K9 and H3K27 methylation and transcriptional repression [87]. In recent years, with technological advancements, the study of histone methylation has become an area of interest in HCC [85]. In particular, current evidence indicates that polycomb repressive complex 2 (PRC2), consisting of EZH2, SUZ12, and EED, mediates the modification of H3K27me3 to repress EpCAM expression in HCC cells. However, HBX can downregulate SUZ12 and ZNF198 to upregulate EpCAM [88]. Another study showed that, in HCC cells, HBX induces the elevation of EpCAM by activating the histone demethylase KDM5B [89], which is associated with H3K4me2/3 modification. HBX can also promote H3K4me3 modification on the promoter of EpCAM by stabilizing WDR5, a significant subunit of H3K4 methyltransferase complexes (Table 2) [90]. Histone methylation contributes to the regulation of LCSCs induced by HBX. Thus, the pharmacological control of histone methylation is a novel treatment strategy for HBX-associated hepatocarcinogenesis. In addition to methylation, histones can also be regulated by acetylation, phosphorylation, sumoylation, and ubiquitination, as mentioned above [85, 87]. However, whether these modifications also participate in modulating LCSCs mediated by HBX remains unclear.

ncRNA

Based on high-throughput RNA sequencing approaches, a variety of ncRNAs, including microRNAs with an average length of 22 base pairs [91] and long noncoding RNA (lncRNA) about 200 base pairs, have been identified [92]. Although these ncRNAs do not encode proteins, they play posttranscriptional regulatory roles in gene expression. Furthermore, they have crucial effects on hepatocarcinogenesis, and many ncRNAs have abnormal expression patterns in HBV-associated malignancies [92].

Importantly, recent investigations have indicated that ncRNAs are essential for HBX-mediated regulation of LCSCs. For instance, Arzumanyan et al. showed that HBX up-regulates miR-181 to elevate the stemness of LCSCs by increasing the expression levels of EpCAM [28]. miR-199a-3p contributes to HBX-induced malignant transformation of HPC into LCSCs with the help of TGF-β [16]. Lin et al. showed that HBX is capable of increasing miR-5188 expression to impair the levels of FOXO1 and then promoting the cellular stemness of LCSCs by increasing CD133 and CD44 expression [93]. Chen et al. found that HBX enhances the levels of miR-3682-3p to stimulate the stemness of HCC cells by elevating CD133 and CD44 [94]. In addition, it was observed that HBX can induce LCSC properties with the increase of CD133, EpCAM, and CD44 by upregulating lncRNA-MALAT1 while declining miR-124 (Table 2) [95]. To date, although these studies of ncRNAs have provided valuable information on LCSC modulation mediated by HBX, the mechanisms involved in LCSC modulation mediated by these ncRNAs are still incompletely understood, and further explorations are needed.

Conclusions and future perspectives

Experimental studies have demonstrated that HBX, a multifunctional molecule encoded by HBV, contributes significantly to hepatocarcinogenesis [96, 97]. Targeting HBX and its associated factors is a promising clinical treatment strategy for HBV-induced malignancy [98]. Despite great advances in understanding the effect of HBX on carcinogenesis have been made, the underlying mechanisms remain not fully illuminated. The pathophysiology of HCC is complex. In recent years, LCSCs have received particular attention because of its significant association with HCC recurrence, metastasis, and prognosis [4]. Especially, the appearance of LCSCs in HBV-related HCC is closely relevant to HBX. Therefore, understanding how HBX affects LCSCs may provide strategies to inhibit LCSC-mediated self-renewal, tumorigenesis, and metastasis in HBV-related HCC.

Here, we reviewed studies indicate that HBX can initiate and maintain LCSCs by controlling the expression of various LCSC markers. It should be noted that, until now, studies on the effect of HBX on the expression of LCSC

surface markers are mainly focused on CD133, EpCAM, CD44, and CD90. Whether other LCSC markers can be modulated by HBX is also worth exploring. Because of the uncertainty of LCSC origin, a single LCSC marker identified in the current research might only represent a unique subpopulation of LCSCs that could be controlled by HBX. Therefore, to better identify and isolate HBX-associated LCSCs and investigate their characteristics in detail, optimized combinations of LCSC surface markers are needed in the future. Furthermore, these identified surface markers of LCSCs have different biological characteristics [9]. To eliminate LCSCs induced by HBX, a strategy targeting more than one LCSC marker is a more effective way in HCC. Moreover, accumulated evidence indicates that to facilitate the upregulation of the LCSC markers CD90, CD133, EpCAM, and CD44, Wnt/β-catenin signaling, TGF-β signaling, PI3-K/AKT signaling, and STAT3 signaling pathways can be activated by HBX. In addition, epigenetic regulation, including DNA and histone methylation, as well as ncRNA, also contributes to the modulation of CD133, CD44, and EpCAM in HBX-induced LCSCs.

Moreover, the current investigations indicated that stem cell-derived hepatocytes effectively support HBV replication and can be used as a novel tool to examine the life cycle of HBV [99, 100]. These studies implied that the virus may induce the appearance of stem cells to facilitate its replication or infection. In addition to HBX, HBsAg, and preS1 can also induce the expression of LCSC markers, including CD90, EpCAM, and CD133, in HCC [101, 102]. However, the associated mechanisms by which HBsAg and preS1 regulate LCSCs are not fully understood. Therefore, to better understand how HBV modulates LCSCs, comprehensive work assessing the function of different viral proteins during HBV infection to regulate LCSCs is worthwhile. To date, several clinical trials targeting cancer stem cells are currently ongoing [103]. A better understanding of the properties of HBX-induced LCSCs may help develop effective therapeutic approaches against HBV-associated malignancies.

Acknowledgements
The Figure was created by BioRender (https://biorender.com/).

Author contributions
KZ, RT, and FK: Conceived and designed the review; XL, DK, and WH: Searched the literatures and collected the data; XL, HY, and FK: Wrote the paper and made the illustrations.

Funding
The work was supported by the Natural Science Foundation of the Jiangsu Higher Education Institutions (21KJA310004), National Natural Science Foundation of China (82372245), the Natural Science Foundation of Jiangsu Province (BK20211347), a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the Qing Lan Project of Jiangsu Province.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 28 June 2024 / Accepted: 21 October 2024
Published online: 11 November 2024

References

1. Liu YC, Yeh CT, Lin KH. Cancer stem cell functions in hepatocellular carcinoma and comprehensive therapeutic strategies. Cells. 2020;9:1331.
2. Dai X, Guo Y, Hu Y, Bao X, Zhu X, Fu Q, et al. Immunotherapy for targeting cancer stem cells in hepatocellular carcinoma. Theranostics. 2021;11:3489–501.
3. Lee TK, Guan XY, Ma S. Cancer stem cells in hepatocellular carcinoma - from origin to clinical implications. Nat Rev Gastroenterol Hepatol. 2022;19:26–44.
4. Cheng Z, Li X, Ding J. Characteristics of liver cancer stem cells and clinical correlations. Cancer Lett. 2016;379:230–8.
5. Nio K, Yamashita T, Kaneko S. The evolving concept of liver cancer stem cells. Mol Cancer. 2017;16:4.
6. Xiang Y, Yang T, Pang BY, Zhu Y, Liu YN. The Progress and Prospects of Putative Biomarkers for Liver Cancer Stem Cells in Hepatocellular Carcinoma. Stem Cells Int. 2016; 2016: 7614971.
7. Lv D, Chen L, Du L, Zhou L, Tang H. Emerging Regulatory mechanisms involved in Liver Cancer Stem Cell properties in Hepatocellular Carcinoma. Front Cell Dev Biol. 2021;9:691410.
8. Sukowati CH, El-Khobar K, Jasirwan COM, Kurniawan J, Gani RA. Stemness markers in hepatocellular carcinoma of Eastern vs. western population: etiology matters? Ann Hepatol. 2024;29:101153.
9. Fang X, Yan Q, Liu S, Guan XY. Cancer Stem cells in Hepatocellular Carcinoma: intrinsic and extrinsic molecular mechanisms in Stemness Regulation. Int J Mol Sci. 2022; 23.
10. Harputluoglu M, Carr BI, Hepatitis B. Before and after Hepatocellular Carcinoma. J Gastrointest Cancer. 2021;52:1206–10.
11. Gnyawali B, Pusateri A, Nickerson A, Jalil S, Mumtaz K. Epidemiologic and socioeconomic factors impacting hepatitis B virus and related hepatocellular carcinoma. World J Gastroenterol. 2022;28:3793–802.
12. Jiang Y, Han Q, Zhao H, Zhang J. The mechanisms of HBV-Induced Hepatocellular Carcinoma. J Hepatocell Carcinoma. 2021;8:435–50.
13. Jia L, Gao Y, He Y, Hooper JD, Yang P. HBV induced hepatocellular carcinoma and related potential immunotherapy. Pharmacol Res. 2020;159:104992.
14. Wang M, Xi D, Ning Q. Virus-induced hepatocellular carcinoma with special emphasis on HBV. Hepatol Int. 2017;11:171–80.
15. Yamashita T, Nault JC. Stemness of liver cancer: from hepatitis B virus to wnt activation. J Hepatol. 2016;65:873–5.
16. Dong KS, Chen Y, Yang G, Liao ZB, Zhang HW, Liang HF, et al. TGF-beta1 accelerates the hepatitis B virus X-induced malignant transformation of hepatic progenitor cells by upregulating miR-199a-3p. Oncogene. 2020;39:1807–20.
17. Dai XM, Huang T, Yang SL, Zheng XM, Chen GG, Zhang T. Peritumoral EpCAM Is an Independent Prognostic Marker after Curative Resection of HBV-Related Hepatocellular Carcinoma. Dis Markers. 2017; 2017: 8495326.
18. Yeh CT, Kuo CJ, Lai MW, Chen TC, Lin CY, Yeh TS, et al. CD133-positive hepatocellular carcinoma in an area endemic for hepatitis B virus infection. BMC Cancer. 2009;9:324.
19. Su H, Han C, He Y, Liang T, Mo S, Yang C, et al. Molecular mechanism of CK19 involved in the regulation of postoperative recurrence of HBV-associated primary hepatocellular carcinoma in Guangxi. Ann Transl Med. 2021;9:1780.
20. Kong F, Li Q, Zhang F, Li X, You H, Pan X, et al. Sirtuins as potential therapeutic targets for Hepatitis B Virus infection. Front Med (Lausanne). 2021;8:751516.

21. Kong F, Zhang F, Liu X, Qin S, Yang X, Kong D, et al. Calcium signaling in hepatitis B virus infection and its potential as a therapeutic target. Cell Commun Signal. 2021;19:82.
22. You H, Qin S, Zhang F, Hu W, Li X, Liu D, et al. Regulation of pattern-recognition receptor signaling by HBX during Hepatitis B Virus infection. Front Immunol. 2022;13:829923.
23. You H, Wang X, Ma L, Zhang F, Zhang H, Wang Y, et al. Insights into the impact of hepatitis B virus on hepatic stellate cell activation. Cell Commun Signal. 2023;21:70.
24. Kong F, You H, Kong D, Zheng K, Tang R. The interaction of hepatitis B virus with the ubiquitin proteasome system in viral replication and associated pathogenesis. Virol J. 2019;16:73.
25. Sivasudhan E, Blake N, Lu Z, Meng J, Rong R. Hepatitis B viral protein HBx and the molecular mechanisms modulating the hallmarks of hepatocellular carcinoma: a comprehensive review. Cells. 2022;11:741.
26. Yang L, Zou T, Chen Y, Zhao Y, Wu X, Li M, et al. Hepatitis B virus X protein mediated epigenetic alterations in the pathogenesis of hepatocellular carcinoma. Hepatol Int. 2022;16:741–54.
27. Zhu M, Li W, Lu Y, Dong X, Lin B, Chen Y, et al. HBx drives alpha fetoprotein expression to promote initiation of liver cancer stem cells through activating PI3K/AKT signal pathway. Int J Cancer. 2017;140:1346–55.
28. Arzumanyan A, Friedman T, Ng IO, Clayton MM, Lian Z, Feitelson MA. Does the hepatitis B antigen HBx promote the appearance of liver cancer stem cells? Cancer Res. 2011;71:3701–8.
29. Ahodantin J, Lekbaby B, Bou Nader M, Soussan P, Kremsdorf D. Hepatitis B virus X protein enhances the development of liver fibrosis and the expression of genes associated with epithelial-mesenchymal transitions and tumor progenitor cells. Carcinogenesis. 2020;41:358–67.
30. Wang C, Yang W, Yan HX, Luo T, Zhang J, Tang L, et al. Hepatitis B virus X (HBx) induces tumorigenicity of hepatic progenitor cells in 3,5-diethoxycarbonyl-1,4-dihydrocollidine-treated HBx transgenic mice. Hepatology. 2012;55:108–20.
31. Wang C, Wang MD, Cheng P, Huang H, Dong W, Zhang WW, et al. Hepatitis B virus X protein promotes the stem-like properties of OV6(+) cancer cells in hepatocellular carcinoma. Cell Death Dis. 2017;8:e2560.
32. Jeng KS, Chang CF, Sheen IS, Jeng CJ, Wang CH. Cellular and molecular biology of cancer stem cells of hepatocellular carcinoma. Int J Mol Sci. 2023;24:1417.
33. Zhang XD, Wang Y, Ye LH. Hepatitis B virus X protein accelerates the development of hepatoma. Cancer Biol Med. 2014;11:182–90.
34. You H, Li Q, Kong D, Liu X, Kong F, Zheng K, et al. The interaction of canonical Wnt/beta-catenin signaling with protein lysine acetylation. Cell Mol Biol Lett. 2022;27:7.
35. Duspara K, Bojanic K, Pejic JI, Kuna L, Kolaric TO, Nincevic V, et al. Targeting the wnt signaling pathway in Liver Fibrosis for Drug options: an update. J Clin Transl Hepatol. 2021;9:960–71.
36. Hsieh A, Kim HS, Lim SO, Yu DY, Jung G. Hepatitis B viral X protein interacts with tumor suppressor adenomatous polyposis coli to activate Wnt/beta-catenin signaling. Cancer Lett. 2011;300:162–72.
37. Du R, Huang C, Bi Q, Zhai Y, Xia L, Liu J, et al. URG11 mediates hypoxia-induced epithelial-to-mesenchymal transition by modulation of E-cadherin and beta-catenin. Biochem Biophys Res Commun. 2010;391:135–41.
38. Sun Q, Wang R, Luo J, Wang P, Xiong S, Liu M, et al. Notch1 promotes hepatitis B virus X protein-induced hepatocarcinogenesis via Wnt/beta-catenin pathway. Int J Oncol. 2014;45:1638–48.
39. Zheng C, Liu M, Ge Y, Qian Y, Fan H. HBx increases chromatin accessibility and ETV4 expression to regulate dishevelled-2 and promote HCC progression. Cell Death Dis. 2022;13:116.
40. Sun Y, Yu M, Qu M, Ma Y, Zheng D, Yue Y, et al. Hepatitis B virus-triggered PTEN/beta-catenin/c-Myc signaling enhances PD-L1 expression to promote immune evasion. Am J Physiol Gastrointest Liver Physiol. 2020;318:G162–73.
41. Jung JK, Kwun HJ, Lee JO, Arora P, Jang KL. Hepatitis B virus X protein differentially affects the ubiquitin-mediated proteasomal degradation of beta-catenin depending on the status of cellular p53. J Gen Virol. 2007;88:2144–54.
42. You H, Yuan D, Bi Y, Zhang N, Li Q, Tu T, et al. Hepatitis B virus X protein promotes vimentin expression via LIM and SH3 domain protein 1 to facilitate epithelial-mesenchymal transition and hepatocarcinogenesis. Cell Commun Signal. 2021;19:33.
43. Mani SKK, Yan B, Cui Z, Sun J, Utturkar S, Foca A, et al. Restoration of RNA helicase DDX5 suppresses hepatitis B virus (HBV) biosynthesis and wnt signaling in HBV-related hepatocellular carcinoma. Theranostics. 2020;10:10957–72.
44. Shen L, Zhang X, Hu D, Feng T, Li H, Lu Y, et al. Hepatitis B virus X (HBx) play an anti-apoptosis role in hepatic progenitor cells by activating Wnt/beta-catenin pathway. Mol Cell Biochem. 2013;383:213–22.
45. Lin X, Li AM, Li YH, Luo RC, Zou YJ, Liu YY, et al. Silencing MYH9 blocks HBx-induced GSK3beta ubiquitination and degradation to inhibit tumor stemness in hepatocellular carcinoma. Signal Transduct Target Ther. 2020;5:13.
46. Schuurbiers OC, Kaanders JH, van der Heijden HF, Dekhuijzen RP, Oyen WJ, Bussink J. The PI3-K/AKT-pathway and radiation resistance mechanisms in non-small cell lung cancer. J Thorac Oncol. 2009;4:761–7.
47. Niu LJ, Huang T, Wang L, Sun XF, Zhang YM. HBX suppresses PTEN to promote the malignant progression of hepatocellular carcinoma through mi-R155 activation. Ann Hepatol. 2022;27:100688.
48. Ha HL, Yu DY. HBx-induced reactive oxygen species activates hepatocellular carcinogenesis via dysregulation of PTEN/Akt pathway. World J Gastroenterol. 2010;16:4932–7.
49. Wei X, Tan C, Tang C, Ren G, Xiang T, Qiu Z, et al. Epigenetic repression of miR-132 expression by the hepatitis B virus x protein in hepatitis B virus-related hepatocellular carcinoma. Cell Signal. 2013;25:1037–43.
50. Zhu M, Guo J, Li W, Lu Y, Fu S, Xie X, et al. Hepatitis B virus X protein induces expression of alpha-fetoprotein and activates PI3K/mTOR signaling pathway in liver cells. Oncotarget. 2015;6:12196–208.
51. Zhu M, Guo J, Li W, Xia H, Lu Y, Dong X, et al. HBx induced AFP receptor expressed to activate PI3K/AKT signal to promote expression of src in liver cells and hepatoma cells. BMC Cancer. 2015;15:362.
52. Xia L, Tian D, Huang W, Zhu H, Wang J, Zhang Y, et al. Upregulation of IL-23 expression in patients with chronic hepatitis B is mediated by the HBx/ERK/NF-kappaB pathway. J Immunol. 2012;188:753–64.
53. Jiang Q, Sun Y, Guo Z, Chen R, Ma S, Fu M, et al. IL-23 enhances the malignant properties of hepatoma cells by attenuation of HNF4alpha. Oncotarget. 2018;9:28309–21.
54. Wang HY, Yang SL, Liang HF, Li CH. HBx protein promotes oval cell proliferation by up-regulation of cyclin D1 via activation of the MEK/ERK and PI3K/Akt pathways. Int J Mol Sci. 2014;15:3507–18.
55. Gonzalez-Sanchez E, Vaquero J, Fernandez-Barrena MG, Lasarte JJ, Avila MA, Sarobe P et al. The TGF-beta pathway: a pharmacological target in hepatocellular carcinoma? Cancers (Basel). 2021;13:3248.
56. Zhang K, Zhang M, Luo Z, Wen Z, Yan X. The dichotomous role of TGF-beta in controlling liver cancer cell survival and proliferation. J Genet Genomics. 2020;47:497–512.
57. Murata M, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, Mori S, et al. Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. Hepatology. 2009;49:1203–17.
58. Liu Y, Xu Y, Ma H, Wang B, Xu L, Zhang H, et al. Hepatitis B virus X protein amplifies TGF-beta promotion on HCC motility through down-regulating PPM1a. Oncotarget. 2016;7:33125–35.
59. Chaomin W, Wenhao N, Jialei H, Ting Z, Honglei F, Zhuang H et al. Spatiotemporal modulation of SMAD4 by HBx is required for cellular proliferation in hepatitis B-related liver cancer. Cell Oncol (Dordr). 2022;45:573–89.
60. Yoo YD, Ueda H, Park K, Flanders KC, Lee YI, Jay G, et al. Regulation of transforming growth factor-beta 1 expression by the hepatitis B virus (HBV) X transactivator. Role in HBV pathogenesis. J Clin Invest. 1996;97:388–95.
61. Rawal P, Siddiqui H, Hassan M, Choudhary MC, Tripathi DM, Nain V, et al. Endothelial cell-derived TGF-beta promotes epithelial-mesenchymal transition via CD133 in HBx-Infected Hepatoma cells. Front Oncol. 2019;9:308.
62. Chang Z, Wang Y, Zhou X, Long JE. STAT3 roles in viral infection: antiviral or proviral? Future Virol. 2018;13:557–74.
63. Wang HQ, Man QW, Huo FY, Gao X, Lin H, Li SR et al. STAT3 pathway in cancers: Past, present, and future. MedComm (2020). 2022; 3: e124.
64. Feng D, Kong X, Weng H, Park O, Wang H, Dooley S et al. Interleukin-22 promotes proliferation of liver stem/progenitor cells in mice and patients with chronic hepatitis B virus infection. Gastroenterology. 2012; 143: 188 – 98 e7.
65. Waris G, Huh KW, Siddiqui A. Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-kappa B via oxidative stress. Mol Cell Biol. 2001;21:7721–30.
66. Zheng Y, Ming P, Zhu C, Si Y, Xu S, Chen A, et al. Hepatitis B virus X protein-induced SH2 domain-containing 5 (SH2D5) expression promotes hepatoma cell growth via an SH2D5-transketolase interaction. J Biol Chem. 2019;294:4815–27.
67. Ali A, Abdel-Hafiz H, Suhail M, Al-Mars A, Zakaria MK, Fatima K, et al. Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma. World J Gastroenterol. 2014;20:10238–48.

68. Ng KY, Chai S, Tong M, Guan XY, Lin CH, Ching YP, et al. C-terminal truncated hepatitis B virus X protein promotes hepatocellular carcinogenesis through induction of cancer and stem cell-like properties. Oncotarget. 2016;7:24005–17.
69. Ching RHH, Sze KMF, Lau EYT, Chiu YT, Lee JMF, Ng IOL, et al. C-terminal truncated hepatitis B virus X protein regulates tumorigenicity, self-renewal and drug resistance via STAT3/Nanog signaling pathway. Oncotarget. 2017;8:23507–16.
70. Guo J, Guo M, Zheng J. Inhibition of bone morphogenetic protein 2 suppresses the stemness maintenance of Cancer Stem cells in Hepatocellular Carcinoma via the MAPK/ERK Pathway. Cancer Manag Res. 2021;13:773–85.
71. Zhang L, Li H, Ge C, Li M, Zhao FY, Hou HL, et al. Inhibitory effects of transcription factor Ikaros on the expression of liver cancer stem cell marker CD133 in hepatocellular carcinoma. Oncotarget. 2014;5:10621–35.
72. Lai FB, Liu WT, Jing YY, Yu GF, Han ZP, Yang X, et al. Lipopolysaccharide supports maintaining the stemness of CD133(+) hepatoma cells through activation of the NF-kappaB/HIF-1alpha pathway. Cancer Lett. 2016;378:131–41.
73. Zhang X, Hu B, Sun YF, Huang XW, Cheng JW, Huang A, et al. Arsenic trioxide induces differentiation of cancer stem cells in hepatocellular carcinoma through inhibition of LIF/JAK1/STAT3 and NF-kB signaling pathways synergistically. Clin Transl Med. 2021;11:e335.
74. Marquardt JU, Gomez-Quiroz L, Arreguin Camacho LO, Pinna F, Lee YH, Kitade M, et al. Curcumin effectively inhibits oncogenic NF-kappaB signaling and restrains stemness features in liver cancer. J Hepatol. 2015;63:661–9.
75. Tu W, Gong J, Tian D, Wang Z, Hepatitis B, Virus X. Protein induces SATB1 expression through activation of ERK and p38MAPK pathways to suppress Anoikis. Dig Dis Sci. 2019;64:3203–14.
76. Kuo TC, Chao CC. Hepatitis B virus X protein prevents apoptosis of hepatocellular carcinoma cells by upregulating SATB1 and HURP expression. Biochem Pharmacol. 2010;80:1093–102.
77. Chung TW, Lee YC, Kim CH. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. FASEB J. 2004;18:1123–5.
78. You H, Kong F, Zhou K, Wei X, Hu L, Hu W, et al. HBX protein promotes LASP-1 expression through activation of c-Jun in human hepatoma cells. J Cell Physiol. 2018;233:7279–91.
79. Liang DY, Sha S, Yi Q, Shi J, Chen Y, Hou Y, et al. Hepatitis B X protein upregulates decoy receptor 3 expression via the PI3K/NF-kappaB pathway. Cell Signal. 2019;62:109346.
80. Duan L, Wu R, Zhang X, Wang D, You Y, Zhang Y, et al. HBx-induced S100A9 in NF-kappaB dependent manner promotes growth and metastasis of hepatocellular carcinoma cells. Cell Death Dis. 2018;9:629.
81. Kong F, You H, Zhao J, Liu W, Hu L, Luo W, et al. The enhanced expression of death receptor 5 (DR5) mediated by HBV X protein through NF-kappaB pathway is associated with cell apoptosis induced by (TNF-alpha related apoptosis inducing ligand) TRAIL in hepatoma cells. Virol J. 2015;12:192.
82. Gao J, Xiong Y, Wang Y, Wang Y, Zheng G, Xu H. Hepatitis B virus X protein activates notch signaling by its effects on Notch1 and Notch4 in human hepatocellular carcinoma. Int J Oncol. 2016;48:329–37.
83. Agborbesong E, Li LX, Li L, Li X. Molecular mechanisms of epigenetic regulation, inflammation, and cell death in ADPKD. Front Mol Biosci. 2022;9:922428.
84. Shi Y, Zhang H, Huang S, Yin L, Wang F, Luo P, et al. Epigenetic regulation in cardiovascular disease: mechanisms and advances in clinical trials. Signal Transduct Target Ther. 2022;7:200.
85. Wolinska E, Skrzypczak M. Epigenetic changes affecting the development of hepatocellular carcinoma. Cancers (Basel). 2021;13:4237.
86. Fan H, Zhang H, Pascuzzi PE, Andrisani O. Hepatitis B virus X protein induces EpCAM expression via active DNA demethylation directed by RelA in complex with EZH2 and TET2. Oncogene. 2016;35:715–26.
87. Park J, Lee K, Kim K, Yi SJ. The role of histone modifications: from neurodevelopment to neurodiseases. Signal Transduct Target Ther. 2022;7:217.
88. Zhang H, Diab A, Fan H, Mani SK, Hullinger R, Merle P, et al. PLK1 and HOTAIR accelerate proteasomal degradation of SUZ12 and ZNF198 during Hepatitis B Virus-Induced Liver Carcinogenesis. Cancer Res. 2015;75:2363–74.
89. Wang X, Oishi N, Shimakami T, Yamashita T, Honda M, Murakami S, et al. Hepatitis B virus X protein induces hepatic stem cell-like features in hepatocellular carcinoma by activating KDM5B. World J Gastroenterol. 2017;23:3252–61.
90. Gao W, Jia Z, Tian Y, Yang P, Sun H, Wang C, et al. HBx protein contributes to liver carcinogenesis by H3K4me3 modification through stabilizing WD repeat domain 5 protein. Hepatology. 2020;71:1678–95.
91. Liu F, Chen J, Luo C, Meng X. Pathogenic role of MicroRNA Dysregulation in Podocytopathies. Front Physiol. 2022;13:948094.
92. Wong CM, Tsang FH, Ng IO. Non-coding RNAs in hepatocellular carcinoma: molecular functions and pathological implications. Nat Rev Gastroenterol Hepatol. 2018;15:137–51.
93. Lin X, Zuo S, Luo R, Li Y, Yu G, Zou Y, et al. HBX-induced miR-5188 impairs FOXO1 to stimulate beta-catenin nuclear translocation and promotes tumor stemness in hepatocellular carcinoma. Theranostics. 2019;9:7583–98.
94. Chen Q, Yang SB, Zhang YW, Han SY, Jia L, Li B, et al. Mir-3682-3p directly targets FOXO3 and stimulates tumor stemness in hepatocellular carcinoma via a positive feedback loop involving FOXO3/PI3K/AKT/c-Myc. World J Stem Cells. 2022;14:539–55.
95. He B, Peng F, Li W, Jiang Y. Interaction of lncRNA-MALAT1 and miR-124 regulates HBx-induced cancer stem cell properties in HepG2 through PI3K/Akt signaling. J Cell Biochem. 2019;120:2908–18.
96. Chaturvedi VK, Singh A, Dubey SK, Hetta HF, John J, Singh MP. Molecular mechanistic insight of hepatitis B virus mediated hepatocellular carcinoma. Microb Pathog. 2019;128:184–94.
97. Geng M, Xin X, Bi LQ, Zhou LT, Liu XH. Molecular mechanism of hepatitis B virus X protein function in hepatocarcinogenesis. World J Gastroenterol. 2015;21:10732–8.
98. Medhat A, Arzumanyan A, Feitelson MA. Hepatitis B x antigen (HBx) is an important therapeutic target in the pathogenesis of hepatocellular carcinoma. Oncotarget. 2021;12:2421–33.
99. Wang J, Qu B, Zhang F, Zhang C, Deng W, Dao Thi VL et al. Stem Cell-Derived Hepatocyte-Like Cells as Model for Viral Hepatitis Research. Stem Cells Int. 2019; 2019: 9605252.
100. Ni Y, Urban S. Stem cell-derived hepatocytes: a promising novel tool to study Hepatitis B virus infection. J Hepatol. 2017;66:473–5.
101. Liu Z, Dai X, Wang T, Zhang C, Zhang W, Zhang Q, et al. Hepatitis B virus PreS1 facilitates hepatocellular carcinoma development by promoting appearance and self-renewal of liver cancer stem cells. Cancer Lett. 2017;400:149–60.
102. Anfuso B, El-Khobar KE, Ie SI, Avellini C, Radillo O, Raseni A, et al. Activation of hepatic stem cells compartment during hepatocarcinogenesis in a HBsAg HBV-transgenic mouse model. Sci Rep. 2018;8:13168.
103. Tsui YM, Chan LK, Ng IO. Cancer stemness in hepatocellular carcinoma: mechanisms and translational potential. Br J Cancer. 2020;122:1428–40.

Publisher's note