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Review

The Biological Function of Hepatitis B Virus X Protein in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is one of the major malignant tumors that lead to death. Chronic hepatitis B virus infection is an important risk factor for HCC initiation. HBx protein, encoded by the HBV *X* gene, is a significant factor that promotes HBV-related HCC, although the exact molecular mechanism remains unclear. This article summarizes the pathological roles and related mechanisms of HBx in HCC. HBx plays a carcinogenic role by promoting cell proliferation, metastasis, and angiogenesis and inhibiting apoptosis in HCC. A detailed study of the biological functions of HBx will help to elucidate the mechanism of hepatocarcinogenesis and lead to the development of novel therapeutic targets for the treatment of HBV-related HCC.

Key words: Hepatocellular carcinoma (HCC); Hepatitis B virus (HBV); Carcinogenic mechanism; Hepatitis B virus X (HBx) protein

INTRODUCTION

Liver cancer is one of the most common fatal tumors worldwide. It ranks second in terms of cancer-related mortality and morbidity due to liver cancer has been ranked sixth among the major types of cancers[1]. GLOBOCAN 2012, published by the International Agency for Research on Cancer (IARC), reported approximately 782,500 new cases of liver cancer and 745,500 deaths in 2012 worldwide[2]. Hepatocellular carcinoma (HCC) accounted for 70%–90% of primary liver cancers[3]. Chronic hepatitis B virus (HBV) infection is a risk factor for HCC induction[4]. It is estimated that currently there are over 350 million HBV carriers globally[5]. However, the underlying mechanism of HBV-associated HCC remains unclear. Hepatitis B virus X (HBx) protein, encoded by the HBV *X* gene, is a multifunctional protein responsible for HBV-related hepatocarcinogenesis[6]. Numerous studies have thoroughly explored the roles that HBx played in the initiation and development of HCC and its molecular mechanisms. In this article, we comprehensively summarize the biological function of HBx in HCC by regulating a variety of biological processes, such as cell proliferation, cell invasion or metastasis, angiogenesis, and cell apoptosis.

THE HBV GENOME AND MOLECULAR MECHANISMS OF HBV INFECTION-RELATED HCC (HBV-HCC)

HBV, belonging to the Hepadnaviridae family, is a partially double-stranded and looped DNA virus. Its genome contains four overlapping open reading frames (ORFs) encoding four proteins. These are pre-S/S (virus surface protein), C (virus core protein), P (virus polymerase), and X (HBx protein).

HBV infection is involved in the carcinogenesis of HCC by chronic inflammation, integration of DNA into the host genome and viral proteins (see Fig. 1), through the following concrete mechanisms. (1) Cytotoxic T lymphocytes, induced by immune system activation after HBV infection, attack HBV-infected hepatocytes. Necrosis of liver cells then causes inflammation, resulting in further liver cirrhosis by activating fibroblasts leading to liver fibrosis. This is consistent with the fact that over

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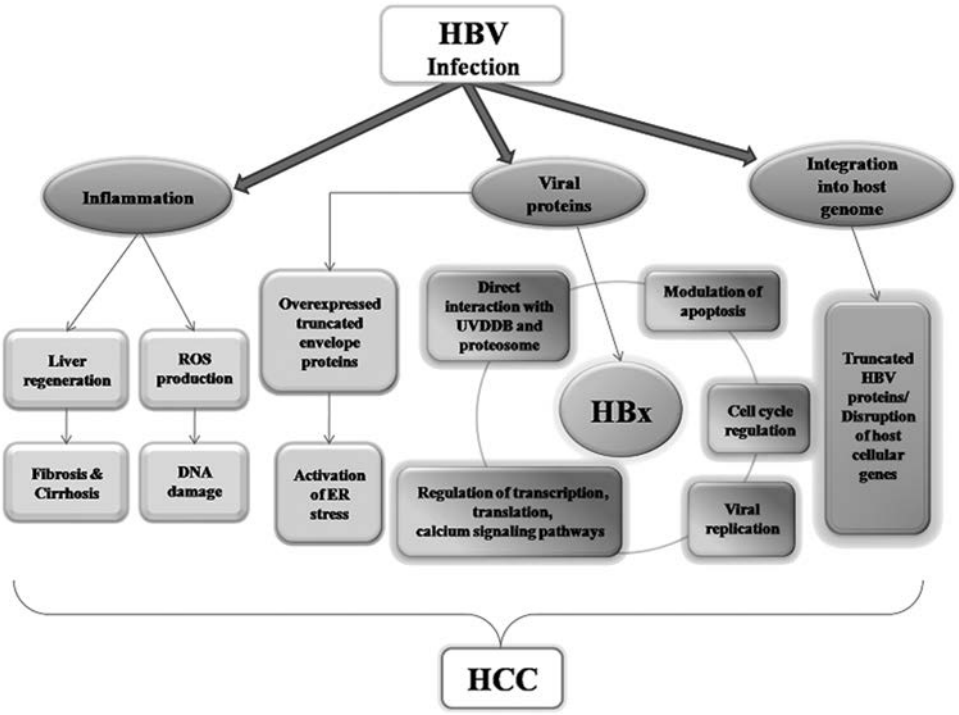

Figure 1. Schematic representation of related carcinogenetic mechanisms of hepatitis B virus infection in hepatocellular carcinoma.

80% of HCC patients may have liver fibrosis cirrhosis[7]. Additionally, inflammation and immune responses caused by HBV infection can generate oxidative stress and produce reactive oxygen species (ROS). It is well known that ROS causes oxidative damages to DNA by interrupting repair mechanisms[8]. The accumulation of DNA mutations also results in the development of HCC. (2) The HBV genome integrates into the host genome in 85% to 90% of HBV-HCC cases, whereas host genes in the HBV integration region can undergo further mutation[9–11]. This causes host cell genome instability and disorders of various intracellular signaling pathways, which are beneficial for promoting HCC development[12]. (3) Viral proteins encoded by the HBV genome also play carcinogenic roles. The surface protein is often localized on the endoplasmic reticulum with its truncated mutant, causing endoplasmic reticulum stress followed by the oxidative damage of DNA and host cell genome instability[13,14]. HBx has long been considered a key protein in malignant transformation and is an important factor in the onset of HBV-HCC[15]. Next, we summarize the biological function and carcinogenic mechanisms of HBx in HBV-HCC.

HBx PROTEIN

The X ORF of HBV genome encodes the HBx protein. HBx is a small, approximately 17-kDa multifunctional protein. A number of studies have shown HBx stimulated HBV replication and transcription[16,17]. Recent studies have concluded that HBx plays an important role in the carcinogenesis of HBV-HCC. Among the Hepadnaviridae family, only woodchuck hepatitis virus and HBV encode the HBx protein. Intriguingly, virus-associated liver cancer occurs only in hosts infected by these two viruses[18]. Transgenic mouse experiments have confirmed that HBx overexpression can induce HCC. Additionally, HBx can increase the rate of HCC formation in c-myc transfection mice and reduce the incubation period of HCC occurrence by diethylnitrosamine[19,20]. Although HBx is closely related to the onset and progression of HBV-HCC, the exact molecular mechanisms remain unknown. Here we will combine the current knowledge to elucidate the pathological function and related mechanisms of HBx in HCC from different perspectives.

HBx AND CELL PROLIFERATION

Recent studies have demonstrated that HBx plays critical roles in carcinogenesis through the promotion of HCC cell proliferation. Du et al. reported that lncRNA highly upregulated in liver cancer (HULC) promoted proliferation of hepatoma cells through suppression of p18 and HULC was upregulated by HBx[21]. Hu and colleagues reported that HBx upregulated lncRNA UCA1. UCA1 was physically associated with enhancer of zeste homolog 2 (EZH2), which suppresses p27Kip1 and results in the enhancement of cell proliferation[22]. Huang et al. found that HBx could upregulate the expression of

DBH-AS1 and consequently promoted cell proliferation through activation of MAPK signaling in HCC[23].

MicroRNAs are a new class of small noncoding RNAs that are 18 to 25 nucleotides in length. Recent work has revealed that expression of several miRNAs was altered in HBV-HCC; simultaneously aberrant expression of miRNAs played an important biological role by acting on downstream target genes. HBx plays critical roles in promoting cell proliferation through regulating miRNAs. HBx promotes aberrant HCC cell proliferation via the downregulation of miR-132, miR-429, miR-205, miR-15b, and miR-145 and the upregulation of miR-221[24–29].

HBx AND CELL INVASION OR METASTASIS

HBx promotes HCC invasion and metastasis by multiple mechanisms. Liu et al. reported that HBx increases the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) by activating the nuclear factor B (NF- B) pathway. The upregulation of VEGF and MMPs promotes HCC cell invasion and metastasis[30]. Han and colleagues found that the HBx protein is also localized to peroxisomes to increases the level of cellular ROS. The peroxisome-localized HBx increased the expressions of MMPs and decreased the expression of E-cadherin, which facilitated HCC cell invasion[31]. Zhang et al. reported that HBx promoted HCC cell migration through the upregulation of calpain small subunit 1 (Capn4)[32].

HBx also plays a critical role in enhancing HCC cell invasion through regulating miRNAs and lncRNAs. Xu and colleagues found that HBx suppressed p53-mediated activation of miR-148a, which resulted in the upregulation of hematopoietic pre-B-cell leukemia transcription factor-interacting protein (HPIP). HPIP increased the expression of mTOR through the AKT/ERK/FOXO4/ATF5 pathway, which resulted in HCC cell invasion and metastasis[33]. Arzumanyan et al. reported that HBx suppressed miR-373, a positive regulator of E-cadherin expression. The downregulation of E-cadherin is associated with the enhancement of the cell invasion ability of HCC[34]. Kong et al. found that the upregulation of miR-29a by HBx promotes HCC cell by targeting PTEN[35]. A study by Zhao and colleagues reported that HBx elevates oncoprotein AEG-1 expression to promote HCC cell migration by downregulating miR-375 and miR-136[36]. Huang and colleagues discovered that the lncRNA Dreh was downregulated by HBx using chip sequencing technology and HBx transgenic mice. They also found that lncRNA Dreh inhibited metastasis via the downregulation of vimentin[37].

HBx AND ANGIOGENESIS

HCC is a kind of highly vascularized solid tumor. Angiogenesis is important for the growth and metastasis of HCC. Previous studies showed that HBx stimulated angiogenesis during hepatocarcinogenesis. It was reported that HBx stimulated the transcription of VEGF, a known angiogenic factor[38]. Moon and colleagues found that HBx induced angiogenesis by increasing the transcriptional activity and protein level of hypoxia-inducible factor-1 (HIF-1)[39]. A study by Lu et al. found that HBx upregulated endothelin 1 (END1) indirectly by suppressing miR-1. END1 plays a crucial role in promoting angiogenesis in HCC by activating the PI3K/AKT pathway[40]. Study from another group reported that HBx contributed to angiogenesis by downregulation of Lethal-7 through activating STAT3[41]. Moreover, HBx increases the expression of MTA1 (metastasis-associated protein 1) coregulator via NF- B signaling by the suppression of miR-661 to induce angiogenesis[42]. HBx induces the expression of miR-7, miR-107, and miR-21 and results in the downregulation of mammary serine protease inhibitor (Maspin). The repressed Maspin makes a contribution to angiogenesis in HCC[43].

HBx AND CELL APOPTOSIS

HBx-induced apoptosis contributes to HCC carcinogenesis mainly because of the interaction between the HBx and p53 pathways. The p53 pathway, composed of the well-known tumor suppressor protein p53 and its downstream target genes including $p21^{CIP/WAF1}$ [44], Bax[45], TGF [46], and EGFR[47], is activated when the cell receives exogenous and endogenous stimulation. The main functions of p53 are to monitor DNA replication and cell division, block cell cycle progress, and induce apoptosis to maintain genomic integrity[48]. p53 drives progression of HCC. Previous studies have found that the p53 pathway is involved in the development of HCC[49]; additionally, interaction between p53 and HBx has been widely confirmed. At the transcriptional level, HBx can inhibit p53 promoter activity and significantly downregulate the mRNA level of p53 in HBx-transfected cells[50]. At the protein level, HBx can directly interact with p53 and inhibit p53 function[51]. p53 can no longer induce cell apoptosis after being inhibited by HBx—this may be crucial for the occurrence of early HCC[52–54].

Upon DNA damage by carcinogens or radiation, p53 is activated and causes transcription activation of the downstream target genes including $p21^{CIP/WAF1}$. $p21^{CIP/WAF1}$ inhibits the cyclin–CDK complex that induces phosphorylation of pRb. Subsequently release of E2F1 is blocked, resulting in cell cycle arrest at the G_1 phase, which can ultimately induce apoptosis[44]. A study on HBV-infected HCC tissues found that mRNA expression of $p21^{CIP/WAF1}$ was reduced compared to that in the normal liver tissue[55]. The interaction between HBx and the p53 carboxy terminus may explain why the activation of$p21^{CIP/WAF1}$ transcription by p53 is suppressed, thus inhibiting the

expression of p21[CIP/WAF1 53]. Similarly, p53 modulates apoptosis by interaction with ASPP1 and ASPP2. Nude mice experiments have confirmed that sensitivity of malignant cells to apoptotic stimuli decreased and tumor growth was promoted after knockdown of the ASPP1 and ASPP2 genes[56]. Zhao and coworkers found that, by analyzing 51 pairs of carcinoma samples and adjacent normal tissues, the expression of ASPP1 and ASPP2 was reduced due to promoter hypermethylation. Interestingly, HBx overexpression in hepatoma cells induced methylation of the ASPP2 promoter region[57]. Hence, HBx may inhibit p53-mediated apoptosis by downregulating ASPP2. In addition, HBx could inhibit Fas-mediated apoptosis by the upregulation of the SAPK/JNK pathway[58] and prevent cell apoptosis by upregulating SATB1 and HURP (hepatoma upregulated protein) expression in HCC[59].

HBx-induced apoptosis also contributes to chemotherapy drug resistance in HCC. As mentioned above, HBx can directly bind to p53 and cause inactivation of p53[51], or it can inhibit p53 through regulating Cox-2, thus inhibiting p53-induced apoptosis[60]. Furthermore, Cheng and colleagues discovered that knockdown of HBx increased apoptosis induced by cisplatin[61]. MDR1 (multidrug resistance 1) is upregulated when tumor cells switch from chemotherapeutic drug sensitivity to resistance. HBx was found to activate HIF-1 that promotes expression of MDR1 in the H411E cell line[62]. Another study showed that in the Hep3B cell line, HBx increased antiapoptosis upon cisplatin treatment by upregulating HURP via the p38/MAPK pathway. In addition, HBx also promoted the expression of survivin, an antiapoptosis protein[59]. Huang and colleagues discovered that HBx caused resistance to bortezomib in HBV-HCC, and the resistance could be antagonized by an inhibitor of MEK signaling[63].

CONCLUSION AND PROSPECTIVE

Multiple molecules and signaling pathways are involved in the formation and progression of HBV-HCC, and HBx is a key factor in hepatocarcinogenesis. We have summarized the various malignant biological functions of HBx in HCC, including regulation of cell proliferation, invasion and metastasis, apoptosis, and angiogenesis in the related molecular mechanisms.

However, the exact functions and molecular mechanisms of HBx in HCC have not yet been elucidated. Further study of the biological functions of HBx will help to elucidate the mechanism of hepatocarcinogenesis and promote the development of novel therapeutic targets for the treatment of HBV-HCC. In addition to present studies concentrating on trials of HBx in vitro, the functions of HBx must also be thoroughly explored in vivo.

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