The role of hepatitis B virus X protein is related to its differential intracellular localization

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Chronic hepatitis B virus (HBV) infection has been strongly associated with hepatocellular carcinoma. HBV encodes an oncogenic hepatitis B virus X protein (HBx), which is a multifunctional regulator that modulates signal transduction, transcription, cell cycle progress, protein degradation, apoptosis, and genetic stability through direct and indirect interaction with host factors. The subcellular localization of HBx is primarily cytoplasmic, with a small fraction in the nucleus. In addition, high levels of HBx expression lead to an abnormal mitochondrial distribution. The dynamic distribution of HBx could be important to the multiple functions of HBx at different stages of the HBV life cycle. This short review presents an overview of the differential roles of HBx as a function of its intracellular localization.

Keywords: hepatitis B virus X protein; subcellular localization

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Introduction

Hepatitis B virus (HBV) belongs to the Hepadnavirus family and is a significant cause of liver disease worldwide. Complications of HBV range from acute and chronic hepatitis to liver cirrhosis and hepatocellular carcinoma (HCC). HBV genome is a 3.2-kb circular, partially double-stranded DNA molecule with four overlapping open reading frames (ORFs) named C, S, P, and X coding for the viral core protein, e-antigen, surface antigen, reverse transcriptase, and X protein respectively. The X-ORF is located downstream of enhancer 1 (Enh1) and is partially overlapped by the P-ORF at its N terminus, and by the preC-ORF at its C terminus [1]. X-ORF is transcribed independently under the control of Enh1 and X promoter and encodes for a 154 amino-acid-long peptide called hepatitis B virus X protein (HBx), with a molecular mass of ≈17.5 kDa. The Enh1 and X promoter are enhanced by HBx as well, suggesting that HBx is under autoregulation [2]. HBx is a multifunctional regulator that modulates host processes and transactivates various cellular transcriptional elements such as AP-1, AP-2, NF-κB, and cAMP response element site [3–5]. HBx contains four regions important for transactivation, dimerization, p53 binding, and 14-3-3 protein binding motif [6]. Tu et al. [7] analyzed the effects of HBx mutants, which were selected from the tissues of both healthy and HCC patients. They demonstrated that the HBx C terminus played a key role in the regulation of its transcriptional activity and in controlling cell viability and proliferation.

Although it is not well defined, the subcellular localization of HBx seems to be mainly cytoplasmic, with a partial nuclear distribution. Moreover, the capacity of HBx for nuclear compartmentalization might be limited [8]. HBx is primarily localized in the nucleus at low expression levels but accumulates in the cytoplasm at elevated HBx levels, indicating that the subcellular localization of HBx is influenced by its abundance [9,10]. Transactivational functions of HBx may be exerted both in the cytoplasm, via signaling pathways, and in the nucleus, via DNA-binding proteins. Recent data have shown that HBx protein is also targeted to mitochondria and co-localizes with the voltage-dependent anion channel 3 (HVDAC3). In this paper, we review the differential intracellular localizations of HBx and describe the various roles of HBx in HBV infection and related diseases.

Cytoplasmic Localization of HBx

Based on its cytoplasmic localization, HBx has been shown to mediate the activation of different signaling
pathways, including Ras-Raf mitogen-activated protein kinase (MAPK), protein kinase C (PKC), NF-κB, stress-activated protein kinase/NH2-terminal-Jun kinase (SAPK/JNK), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/Akt), and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways. Activation of these signaling pathways may contribute to different cellular fates such as transformation, differentiation, survival, and apoptosis.

Ras-Raf MAPK signaling pathway
It has been shown that cytoplasmic HBx activates the Ras-Raf MAPK signaling pathway, which is essential for HBV gene expression and transformation of differentiated hepatocytes [11,12]. Activation of the pathway begins when an external protein binds to a protein tyrosine kinase receptor that activates MAPKs and a variety of other proteins. Although activated in the cytosol, the MAPKs translocate to the nucleus on activation, and phosphorylate a large number of nuclear proteins [13]. HBx activates Src kinase, and eventually Ras as well [14]. Activated Ras is also able to override the pro-apoptotic effects of HBx, leading to hepatocarcinogenesis in HBV-infected cells [15]. Oishi et al. [16] found that an N-terminal truncated mutant of HBx, HBx-D1 (amino acids 51–154), which harbors the co-activation domain, can overcome active Ras-induced cellular senescence in human immortalized cells. HBx mutants with a C-terminal deletion enhance the transforming ability of Ras and Myc [7]. Furthermore, HBx-mediated activation of the Ras-Raf MAPK signaling pathways is also associated with mediating deregulation of cell cycle checkpoint controls and stimulating cell cycling [17].

JAK-STAT signaling pathway
HBx is shown to activate the JAK1-STAT and Src kinase signaling pathways. Lee et al. found that the tyrosine phosphorylation of various STATs, including STAT3 and 5, was constitutively enhanced by HBx and observed a concomitant increase in STAT-dependent DNA binding and transcriptional activation. Furthermore, HBx specifically elevated tyrosine phosphorylation and in vitro kinase activity of JAK1. These results clearly establish HBx as an inducer of the JAK-STAT signaling pathway, which has been shown to be strongly related to HCC [18,19].

PKC signaling pathway
PKC is a large family of phospholipid-dependent kinases involved in cell growth, differentiation, and carcinogenesis. Overexpression of wild-type or mutant PKC may lead to disordered cell growth and transformation. Cong et al. [20] have found that HBx interacts with a PKC-binding protein, XAP3, transactivating the PKC signaling pathway. In addition, HBx protein is found to increase the level of endogenous PKC and subsequently activate AP-1 and NF-κB transcription factors [21].

SAPK/JNK signaling pathway
The SAPK/JNK signaling pathway has previously been suggested to be a survival pathway for cells undergoing Fas-mediated apoptosis. Kinase assays have shown that SAPK activity is highly up-regulated in cells expressing the HBx protein. Co-precipitation and confocal immunofluorescence microscopy experiments have demonstrated that HBx localizes with a cytoplasmic complex containing MEKK1, SEK1, SAPK, and 14-3-3β proteins. Mutational analysis of HBx has demonstrated that a potential binding region for 14-3-3 proteins is essential for induction of SAPK/JNK activity and protection from Fas-mediated apoptosis [22]. Oh et al. [23] showed that HBx transactivated both JNK and MAPK signal transduction pathways in association with the mobilization of cytosolic Ca2+.

PI3K-Akt-Bad signaling pathway
Lee et al. suggested that PI3K-Akt-Bad signal transduction pathway and inactivation of caspase-3 via HBx is important for survival and protection from apoptosis [24]. HBx can effectively suppress TGF-β-induced apoptosis in the Hep3B hepatoma cell line via activation of the PI3K/Akt signaling pathway [25]. Chung et al. [26] also showed that the HBx contributes to the transcriptional regulation of matrix metalloproteinase 9 through the ERK and PI3K-Akt/PKB pathway, and increases the invasive potential of cells. In addition, the proliferation of HepG2 cells promoted by hepatitis B X-interacting protein (HBXIP) is associated with activation of the PI3K/Akt signaling pathway [27].

Apoptosis signaling pathway
Apoptosis plays an important role for the living to clear excess, corrupted, and virus-infected cells. Apoptosis can be mediated through various extrinsic or intrinsic signal pathways, with activation of caspases and the possible involvement of mitochondria. HBx has been shown to alter the coordinated balance between proliferation and programmed cell death, and can either induce or block apoptosis.

Several groups reported that HBx was capable of inhibiting cell apoptosis. Gottlob et al. [28] demonstrated for the first time that HBx was a potent caspases-3 (CPP32) inhibitor. Survivin is an anti-apoptotic protein that is overexpressed in most human cancers. Zhang et al. [29] observed that HBx could up-regulate survivin expression in hepatoma tissues. Marusawa et al. showed that survivin formed complexes with a cellular protein, HBXIP, which was originally recognized for its association with HBx. Survivin-HBXIP complexes bind pro-caspase-9 and
HBx also interacts with such complexes and suppresses caspase activation in a survivin-dependent manner. Thus, HBXIP functions as a cofactor for survivin, and serves as a link between the cellular apoptosis machinery, and a viral pathogen involved in hepatocellular carcinogenesis [30]. p53 is a tumor suppressor antigen and the p53 binding region is located in the C-terminal portion of HBx. p53 and HBx interfere with each other’s transactivation directly. HBx expression is often considered as an inactivator of the p53 tumor suppressor protein. Chung et al. [31] concluded that HBx in liver cells down-regulate the expression of PTEN and activated Akt, and HBx has an effect on the p53-mediated transcription of PTEN, which, in turn, is associated with tumor suppression. HBx activates MAT2A expression through NF-κB and cAMP–response–element–binding protein (CREB) signaling pathways, resulting in the decrease of S-adenosyl-methionine production and the inhibition of hepatoma cell apoptosis [32]. Fas-mediated apoptosis is a major cause of hepatocyte damage during liver disease. The X protein from a chronic strain of HBV was determined to inhibit Fas-mediated apoptosis and promote cell survival [22].

In contrast, it has been reported that HBx interacts with various cellular signaling factors to enhance apoptosis by interacting with cellular signaling proteins such as c-FLIP [33] and Hsp60 [34]. HBx transactivates Fas ligand gene expression to sensitize UV-induced apoptosis [35]. In addition, HBx is associated with caspase activation and mitochondrial dysfunction [36]. Kim et al. [37] suggested that HBx induced apoptosis through interaction with Bax and enhancing its translocation inside the mitochondria, thereby reducing mitochondrial membrane potential. Han et al. [38] found that HBx caused apoptosis by induction of four genes: apoptotic cysteine protease (MCH4), Fas-activated serine/threonine kinase, Bak, and glutathione S-transferase. In addition, Kim et al. suggested that HBx sensitized primary mouse hepatocytes to ethanol- and TNF-α-induced apoptosis by a caspase-3-dependent mechanism, implying that these synergistic effects could enhance cell apoptosis and liver injury [37]. Chami et al. [39] indicated that HBx perturbed intracellular Ca²⁺ homeostasis, and that this effect played an important role in the control of HBx-related apoptosis. It has been reported that the pro-apoptotic activity of HBx overcomes or bypasses the inhibitory effect of Bel-2 against Fas cytotoxicity [40].

Taken together, these seemingly contradictory results suggest that HBx may modulate apoptotic pathways differentially depending on the situation (Table 1). Whether the overall function of HBx is to induce or inhibit apoptosis is still unclear. More detailed research is required to elucidate the specific role of HBx in apoptosis. The ability of HBx to modulate cell survival and apoptosis is potentially relevant for acute and chronic HBV infection as well as the development of hepatocellular carcinoma. In HCC, it is the anti-apoptotic function of HBx that is likely to be the major determinant for manifestation of the transformed phenotype.

### Table 1 Different findings on the effect of HBx on apoptosis signaling pathways

<table>
<thead>
<tr>
<th>Inhibit cell apoptosis</th>
<th>Enhance cell apoptosis</th>
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<tr>
<td>Caspases-3 (CPP32)</td>
<td>Interactive with c-FLIP and Hsp60 inhibitor</td>
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<tr>
<td>Interactive with survivin-HBXIP</td>
<td>Transactivates Fas ligand gene expression</td>
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<td>p53 inactivation</td>
<td>Interaction with Bax, reduce mitochondrial membrane potential</td>
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<tr>
<td>PTEN down-regulation</td>
<td>Induction of four genes: MCH4, FAST kinase, Bak, and glutathione S-transferase</td>
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<td>Akt activation</td>
<td>MAT2A activation</td>
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<tr>
<td>Inhibit Fas-mediated apoptosis</td>
<td>Perturbs intracellular Ca²⁺ homeostasis</td>
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<td>Overcome the inhibitory effect of Bel-2</td>
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plays a significant role in targeting to mitochondria [43]. Rahmani et al. further showed that HBx interacted with human HVDAC3, a member of human HVDAC family led to alteration of mitochondrial transmembrane potential. This observation was consistent with further data showing that HBx expression activated STAT-3 and NF-κB transcription factors via mitochondrial oxidative stress. Such functional roles of HBx have implications for transcription factors via mitochondrial oxidative stress.

This observation was consistent with further data showing that HBx expression activated STAT-3 and NF-κB increased migration of leukocytes through activation of COX2 expression via activation of transcription factor-4 [45]. HBx in mitochondria exerts a dominant pro-apoptotic effect over Bcl-2 and may play an important role in the pathogenesis of chronic hepatitis B [46]. Lee et al. [47] showed that HBx amplified TGF-β signaling through direct interaction with Smad proteins, suggesting a role of the viral protein in HBV-associated liver fibrosis. Henkler et al. [9] proposed that apoptotic or cytotoxic effects of HBx could be related to its mitochondrial localization, since this organelle played a major role in regulation of programmed cell death. The cellular chaperones, Hsp40 protein, through destabilization of viral proteins, exert inhibitory functions on viral replication and hence might play suppressive roles in HCC [48]. HBx induces c-Myc expression by activation of Ras/Raf/ERK1/2 cascades, which in turn resulted in activation of the c-Myc-mediated Hsp90 alpha promoter and up-regulation of Hsp90 alpha expression. Overexpression of Hsp90 alpha in HBx-transfected cells enhances tumor cell aggression [49].

**Nuclear Localization of HBx**

Forgues et al. [50] showed that HBx was shuttled between the cytoplasm and the nucleus through a Crm-1-dependent nuclear export pathway. HBx targeted to the nucleus by a nuclear localization signal was able to restore HBx-deficient HBV replication, while HBx containing a nuclear export signal was not [51]. HBx not only up-regulates the expression of HBV genes by transactivating its own promoters, but also modifies cellular genes to facilitate viral replication in infected hepatocytes. HBx has been shown to activate transcription of host genes indirectly by interaction with nuclear transcription factors including NF-κB, AP-1, CREB, and TATA-box-binding protein (TBP) or through activation of different signal transduction pathways. Thus, it is believed that HBx in the nucleus may function at the promoter level.

NF-κB is a ubiquitous, crucial transcription factor including RelA (p65) and p50 and takes part in almost all aspects of cell regulation, including immune cell activation, stress response, proliferation, apoptosis, differentiation, and oncogenic transformation. NF-κB normally localizes to the cytoplasm and binds to its inhibitory proteins IκBα and IκBβ, which block the migration of NF-κB to the nucleus. Our lab has recently demonstrated that HBx protein induced the expression of chemokine MIG and IP-10, and increased migration of leukocytes through activation of NF-κB [52,53]. Wang et al. [54] showed induction of NF-κB by HBx could not only facilitate infected cell survival and HBV escape from immune clearance, but also promote liver cell malignant transformation and tumor cell advantageous growth. HBx protein inhibits p53 sequence-specific transcriptional activation. In addition, inhibitory effects of HBx involving damage-specific DNA binding protein (DDB1) on DNA repair have been reported. Moreover, HBx is more sensitive to UV-induced cell death. DDB1 is a 127-kDa protein that involves in nucleotide-excision repair and HBV replication and associated with DDB2, a UV-inducible 48-kDa nuclear protein that transports DDB1 from the cytoplasm to the nucleus. HBx associates with the DDB1 subunit of DDB, indicating that interaction with DDB1 is important for establishment of infection by the virus [55]. DDB2 enhances the nuclear accumulation of HBx independent of DDB1 binding. Collectively, these results suggest that DDB1 and DDB2 participate in the nuclear functions of HBx effectively only during the late-G1 phase of the cell cycle. Tang et al. [56] demonstrated that knockdown of DDB1, significantly enhanced the HBx-siRNA-mediated inhibition of HBV replication. HBx might alter the phosphorylation status of some of the mitotic checkpoint proteins, thereby modulating their activities [57]. Several groups have reported that the role of HBx in DNA repair is independent of p53.

**HBx and Proteasome**

HBx can also interact with the proteasome complex. Various groups have shown that HBx expression deregulates cell growth. In fact, HBx has been demonstrated to directly interact with the proteasome and with cellular proteins controlling cell growth, apoptosis (p53, DNA repair), and senescence [24]. Zhang et al. [58] demonstrated specific interaction between HBx and proteasome subunit PSMA7 and PSMC1, further showing that HBx could bind to the proteasome complex in vivo, and they also showed that this interaction could lead to inhibition of the chymotryptic peptidase and protease activities of the proteasome.

**Conclusion**

HBx is a multifunctional regulatory molecule that modulates various cellular activities. Previous research suggests that HBx affects a variety of cellular processes, including...
gene transcription, cell cycle progression, DNA damage repair, cell proliferation, and apoptosis. HBx predominantly localizes in the cytoplasm and in the nucleus at low expression levels. Approximately 5% of HBx localizes to mitochondria. The dynamic distribution of HBx could be important to the multiple functions of HBx at different stages of the HBV infection and possibly carcinoma. In this report, we extensively review the subcellular localization of HBx. However, the subcellular localization of HBx is influenced primarily by its expression level and it is still difficult to relate the expression patterns of HBx with proposed functions. We anticipate that the issues discussed above will be clarified by further research on the intracellular location of HBx and function in the near future.

References

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