BIO DESIGN

pISSN 2288-6982 | eISSN 2288-7105

MINI REVIEW P 20-25

Role of the Hepatitis B Virus X Protein in Viral Pathogenesis

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Hepatitis B virus (HBV) infection is a major cause of acute and chronic liver disease, with an estimated 400 million people chronically infected worldwide. Chronic HBV infection is associated with the development of liver disorders such as hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). The hepatitis B virus X protein (HBx) is a small virally encoded multifunctional regulator protein that has been implicated in HBV-associated liver pathogenesis. In this review, the various roles of HBx will be briefly discussed with respect to the pathogenesis of HBV infection. Understanding the function of HBx during HBV replication and its effect on HBV-mediated liver pathogenesis may lead to new therapeutic options for controlling HBV-associated liver disease.

INTRODUCTION

Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family of viruses and has a highly compact genome that is only 3.2 kilobases in length. This genome contains four overlapping open reading frames including the S, C, P, and X genes that encode the viral surface proteins (envelop), core protein (capsid), polymerase, and X protein (HBx), respectively. The S gene encodes a family of surface antigens (large, middle, and small) embedded in the envelope of HBV. The C gene codes for the core protein, which forms the nucleocapsid wherein viral replication occurs. The P gene encodes the viral polymerase, which has reverse transcriptase, RNase H, and DNA polymerase activities. After infection, HBV DNA enters into the host nucleus and forms covalently closed circular DNA (cccDNA) which serves as the template for viral transcription (Seeger 2007).

HBV infection is strongly associated with a variety of liver diseases such as acute or chronic inflammation, liver cirrhosis, and hepatocellular carcinoma (HCC) (Seeger 2007; Robinson 1994; Seeger 2000; Kremsdorf 2006; Brechot 2004). Among the HBV-encoded proteins, the HBV X protein (HBx) is multifunctional and serves as a key regulator of many activities such as HBV infection, genome replication, epigenetic regulation of virus and host, and liver pathogenesis (reviewed in Seeger 2007; Bouchard 2004; Feitelson 1997; Zheng 2009) (Figure 1).

Many signaling pathways are involved directly or indirectly in the multifunctional activities of HBx. Some of these representative pathways regulated by HBx are summarized in Figure 2. It is unclear whether these signaling pathways occur simultaneously or independently in infected hepatocytes; however, their activation by HBx may explain HBV-associated liver pathogenesis.

No cellular or viral proteins other than HBx have been reported to exert such a variety of functions to date. Evidence is rapidly accumulating that suggests controversial pleiotropic functions for HBx, including those involved in pro-apoptosis and tumorigenesis (anti-apoptosis). These multiple functions of HBx are probably related to differences in HBx expression levels, relative amount of host regulatory proteins, or unidentified mechanisms. It remains to be determined whether these multifunctional effects of HBx are physiologically relevant in the natural course of HBV infection. In this overview, recent progress related to the study of HBx function in viral infection, host regulation, and HBV pathogenesis will be reviewed.

HBX IN VIRUS REPLICATION

The essential role of HBx in HBV replication was first reported in woodchuck hepatitis B virus(Chen 1993; Zoulim 1994), and further studies showed the central activity of HBx during viral replication using cellular systems and a mouse model (Tang 2005; Keasler 2007).

HBx is a known transactivator that activates a variety of cellular and viral transcriptional elements including the HBV enhancers/promoters and transcription factors (Michael 2004). It has been shown to interact with several components of the basal transcriptional machinery including TFIIB, TFIIH, the RPB5 subunit of RNA polymerases, and the TATA-binding protein (TBP) (Michael 2004). HBx also stimulates the RNA pol I- and pol III-dependent promoters (Aufiero 1990). These HBx-regulated transcriptional activities are linked to stimulate a variety of

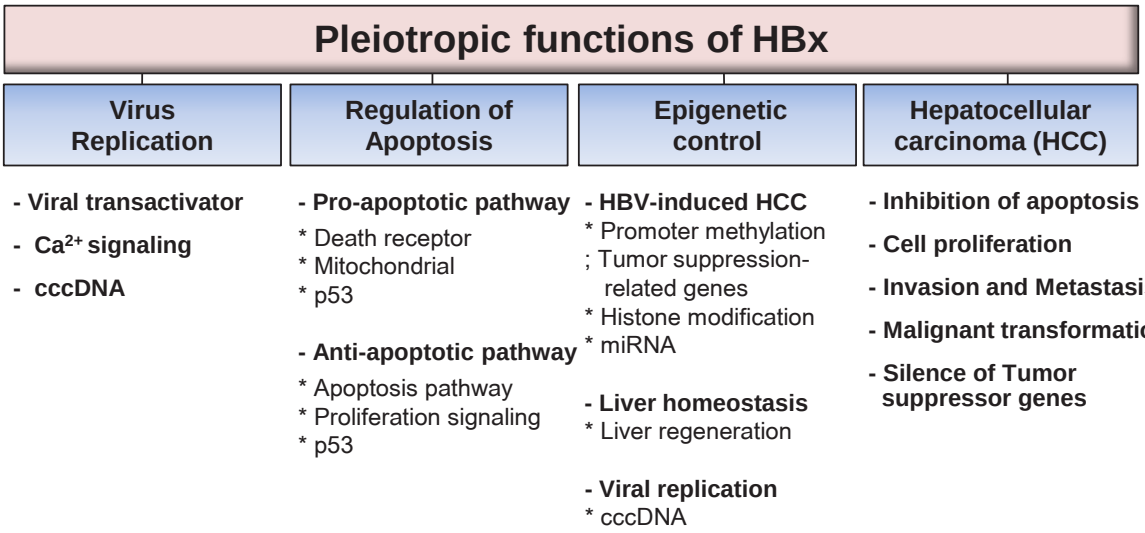

FIGURE 1 | Roles of HBx in viral pathogenesis. HBx is involved in several signaling pathways associated with (1) virus replication, (2) regulation of apoptosis, (3) epigenetic control, and (4) hepatocellular carcinoma.

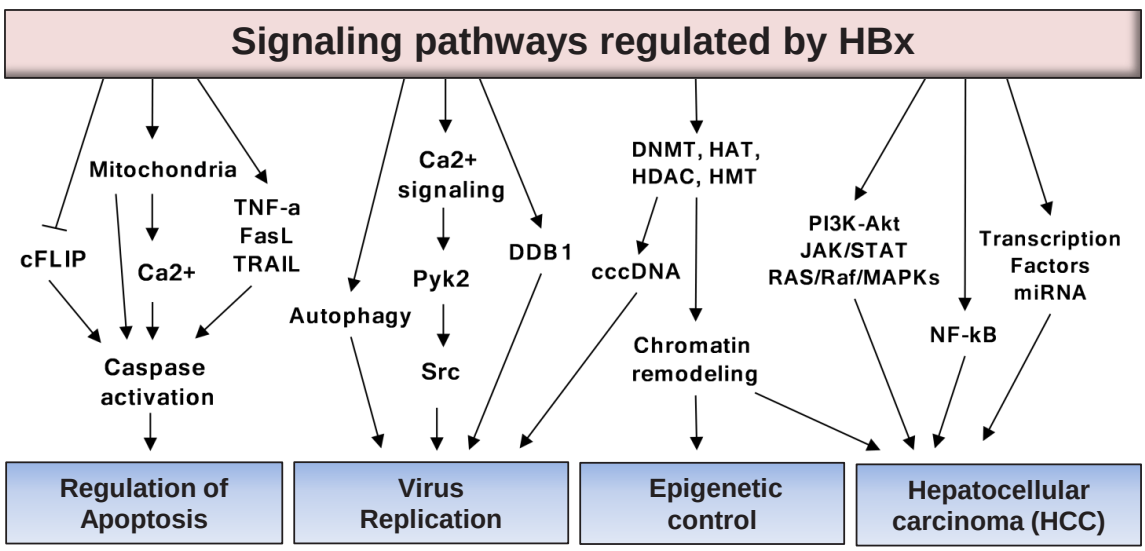

FIGURE 2 | Signaling pathways regulated by HBx. HBx regulates several signaling pathways that are involved in HBV-mediated liver disease and HBV replication.

signaling pathway such as Src, Ras, NF-κB, and NF-AT, which are directly or indirectly related to HBV pathogenesis and replication. The interaction of HBx with DNA damage binding protein 1 (DDB1) is also involved in the stimulation of viral replication by enhancing the transcription of viral RNAs (Leupin 2005).

HBx appears to stimulate virus replication through several mechanisms. For example, HBx has been shown to stimulate viral replication via the activation of the Ca^{2+} dependent signaling pathway in HepG2 cells (Bouchard 2001). HBx triggers the release of Ca^{2+} from mitochondria into the cytosol, which in turn activates the Ca^{2+}-dependent Pyk2 kinase, and phosphorylated Pyk2 subsequently activates Src kinase, which stimulates virus replication by activating the HBV polymerase (Bouchard 2001). (Figure 2).

HBx also influences epigenetic control of nuclear HBV transcription from cccDNA by modulating the recruitment of chromatin-modifying enzymes onto the viral minichromosome

(Belloni 2009). In fact, HBx expression is required for epigenetic modification of HBV cccDNA, leading to the initiation of HBV RNA transcription in HepaRG cells (Lucifora 2011). Because the cccDNA serves as a template for transcription of the HBV pregenomic RNA used for viral DNA synthesis, the functional inhibition of HBx-mediated epigenetic control of cccDNA may effectively suppress HBV replication.

HBx also stimulates virus replication by triggering autophagy through the binding-mediated activation of phosphatidylinositol 3-kinase class III (PI3KC3) (Sir 2010). Collectively, it is evident that HBx has several important functions needed to stimulate viral replication, and these essential roles highlight HBx as a potential target for the control of HBV infection.

HBX IN THE REGULATION OF APOPTOSIS

Apoptosis, or programmed cell death, is a highly regulated process that has a vital role in both organ development and clearance of dysregulated or damaged cells (Elmore 2007). A large number of studies have assessed the impact of HBx expression on the regulation of apoptosis, but it is not clearly understood whether HBx induces or inhibits the growth of human hepatocytes during the natural course of HBV infection, evidence suggests that HBx can both induce and inhibit cellular apoptosis.

The major mechanism of HBx-induced apoptosis involves the death receptor and mitochondrial pathway (Figure 2). Evidence suggests that expression of HBx induces cellular apoptosis by up-regulating death receptors (Wang 2004), sensitizing liver cells to apoptotic stimuli (Kim 2003; Su 1997), and inactivation of cellular FLIPs, the most potent inhibitor of the death receptor (Kim 2003). Alternatively, HBx induces apoptosis via disturbance of mitochondrial functions such as the loss of mitochondrial membrane potential (Terradillos 2002), mitochondrial aggregation (Takada 1999), increase of intracellular Ca^{2+} concentrations (Chami 2003), and downregulation of the expression of the mitochondrial anti-apoptotic molecule Bcl-xL (Miao 2006). Finally, HBx induces apoptosis in a p53-dependent (Chirillo 1997) as well as p53-independent manner (Su 1997; Terradillos 1998). While all these reports clearly suggest the apoptotic function of HBx, it is still controversial whether apoptosis induced by HBx expression is directly related to HBV-mediated liver disease.

Many viruses have evolved genes that effectively block or suppress the apoptosis of host cells leading to persistent infection, either by fine-tuning the environment for efficient viral replication or by allowing infected cells to escape from the host immune response. Several viral proteins have been shown to block apoptosis pathways, including E1B and E3 of adenovirus, core protein of hepatitis C virus (HCV), large T antigen of Simian Virus 40 (SV40), CrmA of cowpox virus, E6 of Human Papillomavirus (HPV), and LMP1 of Epstein-Barr virus (EBV).

HBx performs its anti-apoptotic function by interfering with several pathways. However, since HBx also activates cell cycle progression and proliferation, it is not straightforward to confirm whether the observed anti-apoptotic activities of HBx are direct or indirect. HBx inhibits apoptosis by activating cell proliferation through the SAPK/JNK (Diao 2001), NF-κB (Pan 2001) and PI3K (Lee 2001) pathways, or by directly inhibiting apoptosis mediwWators such as caspase-3 (Gottlob 1998) and p53 (Elmore 1997).

Rapidly accumulating data indicates that HBx exerts effects for both pro-apoptosis and anti-apoptosis. These seemingly contradictory functions of HBx are probably the result of varied experimental subsets, different environmental conditions or as of yet unidentified mechanisms. It also remains to be determined whether the pro- and anti-apoptotic effects of HBx are physiologically relevant in the course of natural HBV infection. Since a delicate balance between cell apoptosis and proliferation is critical for normal liver homeostasis, dysregulation of this balance by several factors including HBV infection is strongly associated with liver disease. Therefore, the similar imbalances in hepatocytes mediated by HBx may be responsible for HBV-associated liver pathology.

HBX IN EPIGENETIC CONTROL

HBx performs epigenetic modifications on both viral DNA (cccDNA minichromosome) and the host chromosome. The novel function of HBx as an epigenetic modifier has only been recently observed (Figure 2).

Many studies have demonstrated that epigenetic changes can contribute to HBV-induced HCC development. HBx induces promoter hypermethylation of some genes involved in tumor-suppressing activity including p16INK4A (Jung 2007; Park 2011), E-cadherin (CDH1) (Lee 2005; Liu 2006) and the insulin-like growth factor binding protein 3 (IGFBP-3) (Park 2007), by upregulating the expression of DNA methyltransferases (DNMTs). In parallel, several studies show that HBx does not directly influence the expression of DNMTs but instead increase recruitment of DNMT1 and/or DNMT3A to the loci of specific genes to induce changes in methylation status and expression levels. These include the ankyrin repeat-containing, SH3 domain-containing, and proline-rich region-containing protein 2 (ASPP2) (Zhao 2010), metallothionein 1F (MT1F), and interleukin-4 receptor (IL-4R) (Zheng 2009). HBx also induces hypomethylation of targeted promoters that leads to significantly elevated expression of certain genes, including several genes related to tumor promotion, such as retinal dehydrogenase 1 (ALDH1), plasma retinol-binding protein precursor (RBP), cellular retinol-binding protein I (CRBP1) (Tong 2009), and cadherin 6 (CDH6) (Zheng 2009).

Another documented effect of HBx is aberrant histone modification that could be related to HCC. For example, HBx upregulates the IL-8 and proliferating cell nuclear antigen (PCNA) genes through histone acetylation (Cougot 2007). IL-8 is upregulated in a variety of cancers and PCNA is a known component of cellular complexes involved in DNA replication and repair. HBx is also known to induce histone methyltransferase (HMT) that leads to the transactivation of c-myc oncogene (Tian 2013).

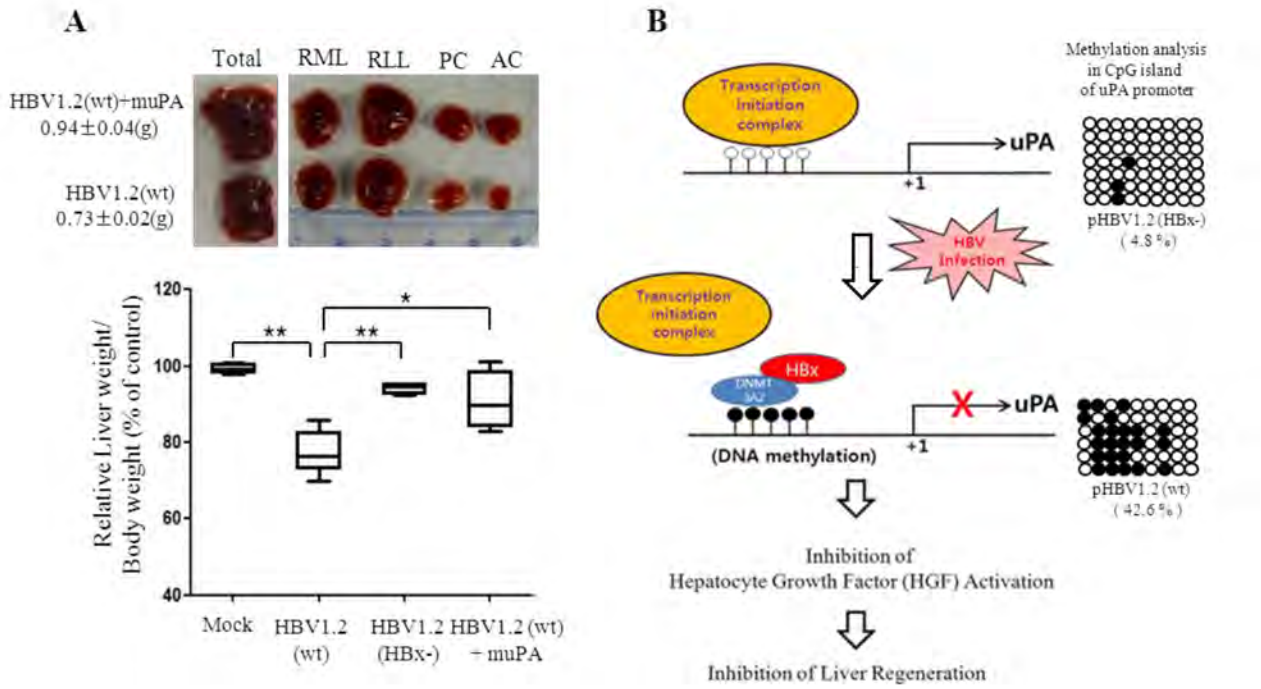

FIGURE 3 | HBx inhibits liver regeneration via epigenetic regulation of urokinase-type plasminogen activator (uPA). A. Inhibition of liver regeneration by HBx and restoration of liver regeneration by supplementation with exogenous uPA in the HBV mouse model. B. Model for the explanation of HBx-induced inhibition of liver regeneration and methylation analysis of uPA promoter by bisulfide sequencing. RML, right median lobe; RLL, right liver lobe; PC, posterior caudate lobe; AC, anterior caudate lobe. *p < 0.01, **p < 0.001. Adapted from *Park et al*. (Hepatology 2013;58:762-776).

Very interestingly, HBx was also reported to alter the host microRNA (miRNA) profile through the upregulation or downregulation of specific miRNAs that are associated with HCC development. Many host miRNAs are reported to be correlated with HBV-induced HCC. This relationship between HBx and the host miRNA profile suggests that HBx-mediated regulation of gene expression might occur through the miRNA-related mechanisms of epigenetic control (Tian 2013).

Recent studies have shown that HBx regulates HBV replication through epigenetic control of cccDNA. Nuclear HBx is recruited to the HBV minichromosome along with cellular histone acetyltransferases such as CBP/p300 and PCAF/GCN5, and the histone deacetylases HDAC1 and hSirt1 (Belloni 2009). These results suggest that HBx may control HBV replication via epigenetic regulation of viral cccDNA. HBx also increased levels of modified histone H3 (methylated, phosphorylated, and acetylated) bound to cccDNA during HBV replication in HepG2 cells, suggesting that HBx stimulates viral gene expression and replication through chromatin remodeling (Luo 2013).

Very recently, HBV pathogenesis induced by HBx-mediated epigenetic control was comprehensively studied (Park 2013). The liver has a unique ability to fully regenerate through dynamic processes (Fausto 2006; Michalopoulos 2007), and this regenerative potential of the liver is important for metabolic homeostasis. HBV infection causes acute or chronic liver damage by dysregulating hepatocyte proliferation and liver regeneration. HBx is suggested to be associated with the inhibition of liver cell proliferation triggered by partial hepatectomy (Tralhao 2002), and more recently the molecular mechanism describing HBx inhibition of liver regeneration was reported using liver cell lines and a mouse model (Park 2013) (Figure 3). This model involves HBx-regulated expression of the urokinase plasminogen activator (uPA), an enzyme essential for the activation of hepatocyte growth factor (HGF). HBx inhibits liver regeneration by suppressing uPA expression through epigenetic control on the uPA promoter through the recruitment of DNMT3A2, leading to promoter hypermethylation.

Evidence shows that the aberrant epigenetic events induced by HBx play important roles on the pathogenesis of several HBV-related diseases. Unraveling the molecular mechanisms underlying the dysregulation of liver regeneration by HBV infection will extend the understanding of pathophysiology of virus-mediated liver disorders, and ultimately provide new treatment options for patients with liver disease.

HBX AND HEPATOCELLULAR CARCINOMA(HCC)

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide, and the majority of cases are related to HBV infection. Among viral proteins, HBx is thought to play a role in the development of HCC following chronic infection. Previous studies have reported a correlation between high level of HBx expression and the development of HCC in transgenic mice (Kim 1991; Koike 1994), indicating that HBx may have an important role in liver carcinogenesis. The pathogenesis of chronic infections, particularly in relation to HBx, and the development of HCC have been extensively reviewed in the literature (Seeger 2007; Robinson 1994; Kremsdorf 2006; Brechot 2004; Feitelson 1997).

HBx interacts with many signaling pathways that directly or indirectly contribute to HCC development (Figure 2). HBx can inhibit apoptosis, enhance the invasive and metastatic potential of infected cells, promote cell proliferation, silence the function of tumor suppressor genes, and induce malignant transformation (Figure 1). HBx interacts with the p53 tumor suppressor and disrupts its function (Feitelson 1993; Wang 1994). HBx also modulates the transcriptional activation of AP-1, AP-2, and NF-κB (Lucito 1992; Natoli 1994), and upregulates the Ras/Raf/ERKs, PI3K-Akt, and JAK/STAT signaling pathways (Doria 1995; Shih 2000; Lee 1998).

Epigenetic changes induced by HBx, including aberrations in DNA methylation, histone modification, and microRNA expression, also promote the development of HCC. Epigenetic modification by HBx in host DNA probably alters the expression of both oncogenes and tumor suppressor genes, contributing to HCC pathogenesis.

CONCLUSIONS

Despite numerous studies on the HBx protein, the exact function and influence of HBx on liver pathogenesis under physiological conditions or during the natural course of HBV infection are still largely undefined. The specific roles of HBx are still under intense investigation for understanding HBV replication and the molecular pathogenesis of HCC.

HBx expression is essential for viral replication and is clearly involved in liver disease. HBx is also often integrated into the host chromosome, possibly inducing HBx-related liver damage even after the viral clearance. HBx is therefore an important therapeutic target, likely throughout the full course of infection. If compounds that functionally interfere with HBx are developed, they should be effective not only for inhibiting virus gene expression and replication, but also for promoting resolution of the chronic carrier state. Most importantly, therapeutic targeting of HBx could reverse some of the epigenetic changes that contribute to the development of HCC, so inhibiting the epigenetic function of HBx could prevent the development of HBV-induced liver cancer.

Although the HBx protein is known to interact with numerous cellular proteins disrupting their normal endogenous function, the common structural features or binding domains in HBx have not been identified because the protein structure of HBx has yet to be solved. Characterizing the structure/function relationships of HBx-host target complexes should provide greater insight into the exact nature of HBx-mediated cellular deregulation, and lead to many opportunities to circumvent the HBx-mediated events leading to HBV-associated liver pathogenesis.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No.2013R1A2A2A01068194), and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (No. 2013R1A1A3008681)

Original Submission: October 22, 2013
Revised Version Received: November 28, 2013
Accepted: December 3, 2013

REFERENCES

Aufiero, B., and Schneider, R.J. (1990). The hepatitis B virus X-gene product trans-activates both RNA polymerase II and III promoters. *EMBO J* **9**, 497-504.

Belloni, L., Pollicino, T., De Nicola, F., Guerrieri, F., Raffa, G., Fanciulli, M., Raimondo, G., and Levrero, M. (2009). Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. *Proc Natl Acad Sci USA* **106**, 19975-19979.

Brechot, C. (2004). Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. Gastroenterology 127, S56-61.

Bouchard, M.J., and Schneider, R.J. (2004). The enigmatic X gene of hepatitis B virus. *J Virol* **78**, 12725-12734.

Bouchard, M.J., Wang, L.H., and Schneider, R.J. (2001). Calcium signaling by HBx protein in hepatitis B virus DNA replication. *Science* **294**, 2376-2378.

Chen, H.S., Kaneko, S., Girones, R., Anderson, R.W., Hornbuckle, W.E., Tennant, B.C., Cote, P.J., Gerin, J.L., Purcell, R.H., and Miller, RH. (1993). The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *J Virol* **67**, 1218-1226.

Chirillo, P., Pagano, S., Natoli, G., Puri, P., Burgio, V., Balsano, C., and Levrero, M. (1997). The hepatitis B virus X gene induces p53-mediated programmed cell death. *Proc Natl Acad Sci USA* **94**, 8162-8167.

Cougot, D., Wu, Y., Cairo, S., Caramel, J., Renard, C.A., Levy, L., Buendia, M.A., Neuveut, C. (2007). The hepatitis B virus X protein functionally interacts with CREB-binding protein/p300 in the regulation of CREB-mediated transcription. *J Biol Chem* **282**, 4277-4287.

Diao, J., Khine, A.A., Sarangi, F., Hsu, E., Iorio, C., Tibbles, L.A., Woodgett, J.R., Penninger, J., and Richardson, C.D. (2001). X protein of hepatitis B virus inhibits Fas-mediated apoptosis and is associated with up-regulation of the SAPK/JNK pathway. *J Biol Chem* **276**, 8328-8340.

Doria, M., Klein, N., Lucito, R., Schneider, R.J. (1995). The hepatitis B virus HBx protein is a dual specificity cytoplasmic activator of Ras and nuclear activator of transcription factors. *EMBO J* **14**, 4747-4757.

Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicol Pathol* **35**, 495-516.

Elmore, L.W., Hancock, A.R., Chang, S.F., Wang, X.W., Chang, S., Callahan, C.P., Geller, D.A., Will, H., and Harris, C.C. (1997). Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc Natl Acad Sci USA* **94**, 14707-14712.

Fausto, N., Campbell, J.S., and Riehle, K.J. (2006). Liver regeneration. *Hepatology* **43**, S45-S53.

Feitelson, M.A., and Duan, L.X. (1997). Hepatitis B virus X antigen in the pathogenesis of chronic infections and the development of hepatocellular carcinoma. *Am J Pathol* **150**, 1141-1157.

Feitelson, M.A., Zhu, M., Duan, L.X., and London, W.T. (1993). Hepatitis B X antigen and p53 are associated in vitro and in liver tissues from patients with primary hepatocellular carcinoma. *Oncogene* **8**, 1109-1117.

Gottlob, K., Fulco, M., Levrero, M., and Graessmann, A. (1998). The hepatitis B virus HBx protein inhibits caspase 3 activity. *J Biol Chem* **273**, 33347-33353.

Jung, J.K., Arora, P., Pagano, J.S., and Jang, K.L. (2007). Expression of DNA methyltransferase 1 is activated by hepatitis B virus X protein via a regulatory circuit involving the p16INK4a-cyclin D1-CDK 4/6-pRb-E2F1 pathway. *Cancer Res* **67**, 5771-5778.

Keasler, V.V., Hodgson, A.J., Madden, C.R., and Slagle, B.L. (2007). Enhancement of hepatitis B virus replication by the regulatory X protein in vitro and in vivo. *J Virol* **81**, 2656-2662.

Kim, C.M., Koike, K., Saito, I., Miyamura, T., and Jay, G. (1991). HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* **353**, 317-320.

Kim, K.H., and Seong, B.L. (2003). Pro-apoptotic function of HBV X protein is mediated by interaction with c-FLIP and enhancement of death-inducing signal. *EMBO J* **22**, 2104-2116.

Koike, K., Moriya, K., Iino, S., Yotsuyanagi, H., Endo, Y., Miyamura, T., and Kurokawa, K. (1994). High-level expression of hepatitis B virus HBx gene and heptocellular carcinogenesis in transgenic mice. *Hepatology* **19**, 810-819.

Kremsdorf, D., Soussan, P., Paterlini-Brechot, P., and Brechot, C. (2006). Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. *Oncogene* **25**, 3823-3833.

Lee, J.O., Kwun, H.J., Jung, J.K., Choi, K.H., Min, D.S., and Jang, K.L. (2005). Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. *Oncogene* **24**, 6617-6625.

Lee, Y.H., and Yun, Y. (1998). HBx protein of hepatitis B virus activates Jak1-STAT signaling. *J Biol Chem* **273**, 25510-25515.

Lee, Y.I., Kang-Park, S., Do, S.I., and Lee, Y.I. (2001). The hepatitis B virus-X protein activates a phosphatidylinositol 3-kinase-dependent survival signaling cascade. *J Biol Chem* **276**, 16969-16977.

Leupin, O., Bontron, S., Schaeffer, C., and Strubin, M. (2005). Hepatitis B virus X protein stimulates viral genome replication via a DDB1-dependent pathway distinct from that leading to cell death. *J Virol* **79**, 4238-4245.

Liu, J., Lian, Z., Han, S., Waye, M.M., Wang, H., Wu, M.C., Wu, K., Ding, J., Arbuthnot, P., Kew, M., Fan, D., and Feitelson, M.A. (2006). Downregulation of E-cadherin by hepatitis B virus X antigen in hepatocellullar carcinoma. *Oncogene* **25**, 1008-1017.

Lucifora, J., Arzberger, S., Durantel, D., Belloni, L., Strubin, M., Levrero, M., Zoulim, F., Hantz, O., and Protzer, U. (2011). Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. *J Hepatol* **55**, 996-1003.

Lucito, R., and Schneider, R.J. (1992). Hepatitis B virus X protein activates transcription factor NF-kappaB without a requirement for protein kinase C. *J Virol* **66**, 983-991.

Luo, L., Chen, S., Gong, Q., Luo, N., Lei, Y., Guo, J., and He, S. (2013). Hepatitis B virus X protein modulates remodeling of minichromosomes related to hepatitis B virus replication in HepG2 cells. *Int J Mol Med* **31**, 197-204.

Miao, J., Chen, G.G., Chun, S.Y., and Lai, P.P. (2006). Hepatitis B virus X protein induces apoptosis in hepatoma cells through inhibiting Bcl-xL expression. *Canc Lett* **236**, 115-124.

Michalopoulos, G.K. (2007). Liver regeneration. *J Cell Physiol* **213**, 286-300.

Natoli, G., Avantaggiati, M.L., Chirillo, P., Costanzo, A., Artini, M., Balsano, C., and Levrero, M. (1994). Induction of the DNA-binding activity of c-jun/c-fos heterodimers by the hepatitis B virus transactivator pX. *Mol Cell Biol* **14**, 989-998.

Pan, J., Duan, L.X., Sun, B.S., and Feitelson, M.A. (2001). Hepatitis B virus X protein protects against anti-Fas-mediated apoptosis in human liver cells by inducing NF-kappa B. *J Gen Virol* **82**, 171-182.

Park, E.S., Park, Y.K., Shin, C.Y., Park, S.H., Ahn, S.H., Kim, D.H., Lim, K.H., Kwon, S.Y., Kim, K.P., Yang, S.I., Seong, B.L., and Kim, K.H. (2013). Hepatitis B virus inhibits liver regeneration via epigenetic regulation of urokinase-type plasminogen activator. *Hepatology* **58**, 762-776.

Park, I.Y., Sohn, B.H., Yu, E., Suh, D.J., Chung, Y.H., Lee, J.H., Surzycki, S.J., and Lee, Y.I. (2007). Aberrant epigenetic modifications in hepatocarcinogenesis induced by hepatitis B virus X protein. *Gastroenterology* **132**, 1476-1494.

Park, S.H., Jung, J.K., Lim, J.S., Tiwari, I., and Jang, K.L. (2011). Hepatitis B virus X protein overcomes all-trans retinoic acid-induced cellular senescence by downregulating levels of p16 and p21 via DNA methylation. *J Gen Virol* **92**, 1309-1317.

Seeger, C., and Mason, W.S. (2000). Hepatitis B virus biology. *Microbiol Mol Biol Rev* **64**, 51-68.

Robinson, W.S. (1994). Molecular events in the pathogenesis of hepadnavirus-associated hepatocellular carcinoma. *Annu Rev Med* **45**, 297-323.

Seeger, C., Zoulim, F., and Mason, W.S. (2007). *Hepadnaviruses. In Fields Virology*, 5th ed,; Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E., Eds.; Lippincott Williams & Wilkins, Philadelphia, PA, USA, 2, 2977-3029.

Shih, W.L., Kuo, M.L., Chuang, S.E., Cheng, A.L., and Doong, S.L. (2000). Hepatitis B virus X protein inhibits transforming growth factor-b-induced apoptosis through the activation of phosphatidylinositol 3-kinase pathway. *J Biol Chem* **275**, 25858-25864.

Sir, D., Tian, Y., Chen, W.L., Ann, D.K., Yen, T.S., and Ou, J.H. (2010). The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. *Proc Natl Acad Sci USA* **107**, 4383-4388.

Su, F., and Schneider, R. (1997). Hepatitis B virus HBx protein sensitizes cells to apoptotic killing by tumor necrosis factor α. *Proc Natl Acad Sci USA* **94**, 8744-8749.

Tang, H., Delgermaa, L., Huang, F., Oishi, N., Liu, L., He, F., Zhao, L., and Murakami, S. (2005). The transcriptional transactivation function of HBx protein is important for its augmentation role in hepatitis B virus replication. *J Virol* **79**, 5548-5556.

Takada, S., Shirakata, Y., Kaneniwa, N., and Koike, K. (1999). Association of hepatitis B virus X protein with mitochondria causes mitochondrial aggregation at the nuclear periphery, leading to cell death. *Oncogene* **18**, 6965-6973.

Terradillos, O., de La Coste, A., Pollicino, T., Neuveut, C., Sitterlin, D., Lecoeur, H., Gougeon, M.L., Kahn, A., and Buendia, M.A. (2002). The hepatitis B virus X protein abrogates Bcl-2-mediated protection against Fas apoptosis in the liver. *Oncogene* **21**, 377-386.

Terradillos, O., Pollicino, T., Lecoeur, H., Tripodi, M., Gougeon, M.L., Tiollais, P., and Buendia, M.A. (1998). p53-independent apoptotic effects of the hepatitis B virus HBx protein in vivo and in vitro. *Oncogene* **17**, 2115-2123.

Tian, Y., Yang, W., Song, J., Wu, Y., and Ni, B. (2013). Carcinoma Pathogenesis Contributing to Human Hepatocellular Aberrant Epigenetic Modifications. *Mol Cell Biol* **33**, 2810-2816.

Tong, A., Gou, L., Lau, Q.C., Chen, B., Zhao, X., Li, J., Tang, H., Chen, L., Tang, M., Huang, C., and Wei, Y.Q. (2009). Proteomic profiling identifies aberrant epigenetic modifications induced by hepatitis B virus X protein. J *Proteome Res* **8**, 1037-1046.

Tralhao, J.G., Roudier, J., Morosan, S., Giannini, C., Tu, H., Goulenok, C., Carnot, F., Zavala, F., Joulin, V., Kremsdorf, D., and Bréchot, C. (2002). Paracrine in vivo inhibitory effects of hepatitis B virus X protein (HBx) on liver proliferation: an alternative mechanism of HBx-related pathogenesis. *Proc Natl Acad Sci USA* **99**, 6991-6996.

Wang, W.H., Gregori, G., Hullinger, R.L., and Andrisani, O.M. (2004). Sustained activation of p38 mitogen-activated protein kinase and c-Jun N-terminal kinase pathways by hepatitis B virus X protein mediates apoptosis via induction of Fas/FasL and tumor necrosis factor (TNF) receptor 1/TNF-alpha expression. *Mol Cell Biol* **24**, 10352-10365.

Wang, X.W., Forrester, K., Yeh, H., Feitelson, M.A., Gu, J.R., and Harris, C.C. (1994). Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci USA* **91**, 2230-2234.

Zhao, J., Wu, G., Bu, F., Lu, B., Liang, A., Cao, L., Tong, X., Lu, X., Wu, M., and Guo, Y. (2010). Epigenetic silence of ankyrin-repeat-containing, SH3-domaincontaining, and proline-rich-region-containing protein 1 (ASPP1) and ASPP2 genes promotes tumor growth in hepatitis B virus-positive hepatocellular carcinoma. *Hepatology* **5**1, 142-153.

Zheng, D.L., Zhang, L., Cheng, N., Xu, X., Deng, Q., Teng, X.M., Wang, K.S., Zhang, X., Huang, J., and Han, Z.G. (2009). Epigenetic modification induced by hepatitis B virus X protein via interaction with de novo DNA methyltransferase DNMT3A. *J Hepatol* **50**, 377-387.

Zoulim, F., Saputelli, J., and Seeger, C. (1994). Woodchuck hepatitis virus X protein is required for viral infection in vivo. *J Virol* **68**, 2026-2030.