Hepatitis B virus X protein in the proliferation of hepatocellular carcinoma cells

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1. ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common and deadly malignant neoplasms worldwide. Chronic hepatitis B virus (HBV) infection is closely associated with the occurrence of HCC. The HBV genome encodes a ubiquitous transactivator, termed the HBV X protein (HBx), that is essential for HBV replication in vivo. HBx is involved in multiple steps of carcinoma development. Even in the preneoplastic stage, HBx acts as a tumor promoter. HBx participates in several mechanisms that have been linked to cell proliferation, including gene transcription, cell cycle regulation, and several signaling pathways. Moreover, HBx mutants, especially those with mutations in the COOH-terminal end, have been implicated in hepatocarcinogenesis. Therefore, therapeutic strategies targeting HBx could be effective at multiple stages of HCC development, even as early as the preneoplastic stage.

2. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related death worldwide (1). It is highly prevalent in the Asia-Pacific region and Africa and is becoming more common in Western countries (2), with an estimated incidence of between 500,000 and 1,000,000 new cases annually. The incidence of HCC in developed countries, including Japan, Australia, European countries, Canada, and the United States, has increased over the past 20 years (3,4). In the United States alone, the annual incidence of HCC has increased by approximately 80% over the past two decades (5). The incidence and mortality rates of HCC are almost identical, reflecting the poor overall survival rates for patients with this type of tumor. Interestingly, approximately 70%–90% of HCC patients have an established history of chronic liver disease and cirrhosis,

the major risk factors for which include alcoholic liver disease and chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) (6,7).

A strong correlation between chronic HBV infection and HCC has been identified (8-11). As a result of ongoing viral replication and the immunological response to the infection, approximately 25% of chronically infected patients develop liver cirrhosis, inflammation and, ultimately, HCC. The HBV genome encodes a ubiquitous transactivator, termed the HBV X protein (HBx), that is essential for HBV replication in vivo. HBx gene is a viral non-structural gene that operates as a multifunctional regulator by modulating the activity of host cellular genes (12-14) and transactivating transcription factors such as AP-1, NF-kB, CREB, and TBP (15,16). Moreover, HBx is involved in the activation of multiple signaling pathways that are linked to cell proliferation and survival, such as RAS/RAF/MAPK, MEKK1/JNK and PI3K/Akt (17-19). HBx is often expressed from integrated fragments of the HBV genome in HCC tissues (20,21), and mice expressing HBx in their liver either develop HCC spontaneously (22) or display increased susceptibility to hepatocarcinogens (23). In this review, we will discuss the role of HBx in the proliferation of HCC cells.

3. HBX PROMOTES PROLIFERATION IN THE PRENEOPLASTIC STAGE

Cirrhosis, a consequence of progressive liver injury and fibrosis, is the single greatest risk factor for HCC (24). In most cases of HCC that are associated with cirrhosis, hepatic fibrosis is thought to represent an early step in hepatocarcinogenesis because cirrhotic changes in the liver can be caused by the accumulation and exacerbation of fibrosis (25). It has also been demonstrated that HCC patients with advanced fibrosis have shorter overall survival periods than those without fibrosis (26). The calculation of the fibrosis score is recommended for tumor-node-metastasis staging of HCC (27). Because HBV infection is a very common etiological factor in the development of HCC (28), the role of HBV, especially HBx, in liver cirrhosis has been investigated. Hepatic stellate cells (HSCs) play a central role in the process of hepatic fibrosis (29). HBx has been proposed to play a direct role in facilitating liver fibrosis through the paracrine activation of stellate cells (30) and by promoting HSC proliferation and upregulating the expression of the fibrosis-related molecules transforming growth factor-beta1 (TGF-beta1) and connective tissue growth factor (CTGF) (31).

In a mouse model, HBx was found to act as a tumor promoter during the development of diethylnitrosamine-induced preneoplastic lesions. Furthermore, HBx expression contributes to the development of hepatocarcinogen diethylnitrosamine (DEN)-mediated carcinogenesis by promoting the proliferation of altered hepatocytes rather than by directly interfering with the repair of DNA lesions (32). In addition, HBx binds to Pin1 to enhance hepatocarcinogenesis in HBV-infected hepatocytes (33). Pin1 is a protein that binds

to and inhibits the toxicity of never-in-mitosis A (NIMA), a fungal mitotic kinase (34). Pin1 specifically binds to and facilitates the isomerization of pSer/Thr-Pro motifs in a large and defined subset of phosphoproteins, which are usually substrates of proline-directed protein kinases. Proline-directed phosphorylation plays an essential role in both normal and malignant cell proliferation (35-37). Pang and colleagues (33) found that HepG2 cells expressing Pin1 and HBx exhibited a synergistic increase in cellular proliferation compared with cells expressing Pin1 or HBx alone. Furthermore, the concomitant expression of Pin1 and HBx in the nontumorigenic human hepatocyte cell line MIHA led to a synergistic increase in tumor growth. In Hep3B cells with suppressed Pin1 expression, HBx-enhanced tumor growth in nude mice was abrogated. Thus, HBx acts as a tumor promoter in the preneoplastic stage partly by interacting with functional proteins.

In an HBx-transgenic HCC mouse model, four- to fivefold enhanced expression of the c-myc transgene and increased hepatocyte proliferation was observed at the preneoplastic stage (38). Thus, c-myc could cooperate with HBx in the initiation of hepatocarcinogenesis. In hepatoma cells, HBx and c-myc were found to work cooperatively to support ribosome biogenesis and cellular transformation (39). In addition, the fibroblast growth factor (FGF)-inducible 14 (Fn14) immediate-early response gene is rapidly induced during liver regeneration in vivo and is expressed at high levels in HCC nodules in the c-myc/TGF-alpha-driven and HBx protein-driven transgenic mouse models of hepatocarcinogenesis (40). c-myc also acts as a downstream gene in HBx-SMYD3-related hepatocarcinogenesis, and HBx transfection enhanced SMYD3 and c-myc expression, decreased cell apoptosis, and increased cell proliferation in HepG2 cells (41). These findings indicate that c-myc could influence multiple aspects of cell proliferation, including gene expression, protein synthesis and cellular transformation. c-myc could act as a hub for the convergence of different mechanisms for cell proliferation, even during the preneoplastic stage, making it particularly attractive for efforts to prevent HCC at the preneoplastic stage.

4. HBX PROMOTES PROLIFERATION IN HEPATOCARCINOGENESIS

During hepatocarcinogenesis, HBx acts to promote HCC cell proliferation. Zhang and colleagues (42) found that HBx could facilitate cell proliferation in human liver tumor cells through its interaction with the Hepsin protein. In a human liver cell model, HBx was found to regulate many genes that may be involved in carcinogenesis, including COX-2, the upregulation of which leads to the enhancement of proliferation (43). HBx promotes the proliferation of HCC cells via several pathways: e.g., participating in gene transcription and affecting the cell cycle.

4.1. HBx promotes proliferation via upregulating transcription

HBx promotes transcription in HCC cells. Wu and colleagues (44) found that the deregulation of cellular

genes by oncogenic HBx may be one early event that favors hepatocyte proliferation during liver carcinogenesis. Moreover, at early steps of the transformation process, a mutation in codon 249 (AGG to AGT, arginine to serine, p.R249S) of Aflatoxin B1 was found to contribute to hepatocarcinogenesis by affecting the interaction of this protein with HBx, conferring a subtle growth advantage, although this interaction is not required for the progression to advanced HCC (45).

The eukaryotic RNA polymerase II (RNAPII) protein transcribes mRNA and microRNA genes (46,47). RNA polymerase II consists of 12 subunits, including RPB5, and functions by interacting with other transcription factors (48,49). HBx can interact directly with RPB5 (49). RPB5-mediating protein (RMP) is associated with the RNAPII subunit RPB5 and functionally counteracts the transcriptional activation promoted by HBx. RMP is a radiation-sensitive factor, and it may play essential roles in HCC growth by affecting the proliferation and apoptosis of HCC cells (50). Additionally, HBx and RMP compete with one another for association with transcription factor IIB (TFIIB) (51). During RNAPII recruitment and transcription, TFIIB plays a central role in preinitiation complex assembly, providing a bridge between the promoter-bound TFIID and RNAPII proteins (52). In vivo, TFIIB is a target of HBx, and a C-terminal domain mutation in TFIIB inhibits its transcriptional coactivation by HBx (53). TFIIB overexpression may play essential roles in the pathogenesis of hepatocellular carcinoma by affecting the proliferation of HCC cells, which is associated with HBx (54).

Among the transcription factors that are regulated by HBx, NF-kappaB has been shown to be associated with tumorigenesis by activating the expression of over 200 genes involved in immune response, inflammation, anti-apoptosis signaling, and cell proliferation (55,56). The constitutive activation of NF-kappaB has been reported in HCC cell lines expressing HBx (57) and in HCC tissues in transgenic mice (58). HBx was found to bind to VHL binding protein (VBP1), which interacts with von Hippel-Lindau protein (VHL), a protein that is associated with the regulation of NF-kappaB (59). Correspondingly, VBP1 was found to facilitate HBx-induced NF-kappaB activation and cell proliferation (60). Moreover, one of the direct transcriptional targets of NF-kappaB, iASPP, is an inhibitory member of the ankyrin-repeat-, SH3-domain- and proline-rich-region-containing protein (ASPP) family that was found to be up-regulated in HCC. Increased iASPP expression contributes to tumor progression through its proliferative and antiapoptotic activities (61).

A dominant negative mutant of I-kappaB-alpha was shown to reduce the level of colony formation by HBx-expressing liver cells, demonstrating the importance of NF-kappaB in liver cell proliferation (62). However, neither an I-kappaB-alpha (S32/36A) mutant plasmid nor NF-kappaB inhibitors (1-pyrrolidinecarbonidithioic acid and sulfasalazine) had a substantial effect on cell proliferation (63). The same study further showed that proteasome inhibitor-1 (Pro1) and MG132 enhanced the HBx-induced

endoplasmic reticulum-stress (ER-stress) response and the subsequent activation of caspase-12, -9 and -3 and reduced cell proliferation. However, camptothecin (CPT) triggered the activation of caspase-3 without inducing caspase-12 and reduced cell proliferation (63). HBx also can upregulate mammalian target of rapamycin (mTOR) signaling through I-kappaB kinase beta (IKK beta), thereby increasing cell proliferation in HCC (64). These findings, which demonstrate that HBx participates in NF-kappaB-related pathways, may inform future studies of the process of HCC development associated with inflammation.

Moreover, HBx can regulate gene expression through the regulation of microRNAs (miRNAs). miRNA microarrays were employed to evaluate the expression of cellular miRNAs in HBx-versus control-HepG2 cells, and the results showed that HBx up-regulated 7 and down-regulated 11 miRNAs, including let-7 family members. The deregulation of the expression of the let-7 family of miRNAs by HBx may represent a potential novel pathway through which HBx acts to deregulate cell proliferation leading to hepatocarcinogenesis (65). HBx also down-regulated miR-338-3p in HCC, and miR-338-3p has been shown to inhibit proliferation by regulating CyclinD1 (66). By contrast, long noncoding RNAs (lncRNAs) were found to be highly up-regulated in liver cancer; these lncRNAs have been shown to promote hepatoma cell proliferation by down-regulating p18 in vitro and in vivo. When the highly up-regulated lncRNAs were knocked down, the HBx-enhanced cell proliferation through the up-regulation of p18 was abolished (67). Thus, both miRNAs and lncRNAs can influence the effects of HBx on transcription in HCC cells, though they do not encode proteins. These studies provide a novel perspective of how HBx affects gene expression in hepatocytes to promote hepatoma cell proliferation. Some of the miRNAs that are regulated by HBx were recently found to affect the cell cycle, which will be discussed in a later section of this review.

4.2. HBx promotes proliferation by affecting cell cycle progression
The cell cycle refers to the series of events that take place in a cell leading to its division and duplication, and it consists of four distinct phases: G1 phase, S phase, G2 phase and M phase. The activation of each phase is dependent on the proper progression and completion of the previous phase. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence, called G0. HBx has been shown to promote hepatocyte proliferation by altering cell cycle progression. In primary mouse hepatocytes, HBx inhibits cell cycle progression by increasing the expression of p21(Cip-1/WAF1/MDA6) and p27(Kip-1). The reduced expression of p16, p21, and p27, which is often observed in HCC, enhances mitogen-activated protein kinase (MAPK) signaling and HBx, causing proliferation in hepatocytes (68).

In each phase of the cell cycle, HBx can act as a regulator to interfere with normal cycle events, leading to HCC development. HBx can induce G2/M arrest through the sustained activation of cyclin B1-CDK1 kinase but does
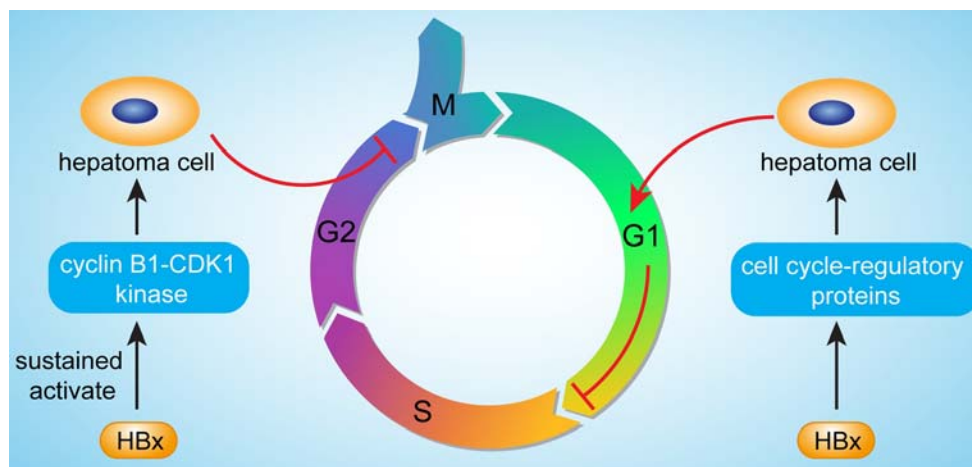
Figure 1. HBx in the cell cycle. HBx can induce G2/M arrest through sustained activation of cyclin B1-CDK1 kinase, and negatively regulated cell growth in vitro and in vivo. HBx could affecte the levels and activities of various cell cycle-regulatory proteins to induce normally quiescent hepatocytes to enter the G1 phase of the cell cycle, but it does not promote S phase entry in cultured primary rat hepatocytes.

not inhibit this kinase, as many other G2/M arrest mechanisms do, and HBx negatively regulates cell growth in vitro and in vivo (69). However, HBx also induces normally quiescent hepatocytes to enter the G1 phase of the cell cycle. HBx, both by itself and in the context of HBV replication, is able to affect the levels and activities of various cell cycle-regulatory proteins to induce normally quiescent hepatocytes to enter the G1 phase of the cell cycle, but it does not promote S phase entry in cultured primary rat hepatocytes (70) (Figure 1). In HBx transgenic mice, Wu and colleagues (71) observed a similar phenomenon, finding that HBx could block the G1/S transition in hepatocytes and caused both a failure of liver functionality and cell death in the regenerating liver.

The G1/S cell cycle progression, cell proliferation and blunted cellular senescence could be induced by decreased Notch1 intracellular domain (ICN1) in vitro through transient HBx expression. The suppression of ICN1 also reduced the senescence-like growth arrest caused by stable HBx expression in a nude mouse xenograft transplantation model and in HBV-associated HCC patient tumor samples (72). Activated Notch signaling is required for HBx to promote the proliferation and survival of human hepatic cells both in vitro and in vivo (73). One of the ligands of Notch signaling pathway, namely Jagged1, which controls cellular proliferation and differentiation, has been found to be regulated by HBx and may contribute to the development of HCC (74). On the other hand, HBx could affect G1 phase exit and promote proliferation in malignant hepatocytes by altering miRNA expression. By arresting cells in the G1 phase and inducing apoptosis, ectopically expressed miR-15a/16 suppressed the proliferation, clonogenicity, and anchorage-independent growth of HBx-expressing HepG2 cells, whereas the reduced expression of miR-16 accelerated the growth and cell-cycle progression of HepG2 cells. During this process, c-myc acts as a mediator of the HBx-induced repression of miR-15a/16 in HepG2 cells (75).

4.3. Signaling pathways in promoting proliferation

In addition to the identified functions of HBx in transcription and cell cycle progression, HBx participates in several signaling pathways, promoting the proliferation of HCC cells. HBx strongly increased the transcription of fatty acid synthase (FAS) in human hepatoma HepG2 and H7402 cells, and this up-regulation was mediated by 5-lipoxygenase (5-LOX). HBx was also shown to upregulate 5-LOX through the phosphorylated extracellular signal-regulated protein kinases 1/2 (p-ERK1/2). FAS was able to upregulate the expression of 5-LOX through a feedback mechanism in which released leukotriene B4 (LTB4) promotes the up-regulation of FAS (76). Thus, HBx promotes cell growth through a positive feedback loop involving 5-LOX and FAS. A similar positive feedback loop involving 5-LOX was also found by Shan and colleagues (77). However, this loop is distinct from the former loop in that HBx enhances and maintains liver cell proliferation via not only 5-LOX and p-ERK1/2 but also cyclooxygenase-2 (COX-2), released arachidonic acid metabolites and Gi/o proteins. Through this positive feedback loop, HBx upregulates the levels of COX-2, 5-LOX and phosphorylated extracellular signal-regulated p-ERK1/2 in liver cells and also releases arachidonic acid metabolites, including LTB4, that are able to activate p-ERK1/2. Subsequently, the activated p-ERK1/2 could upregulate the expression of COX-2 and 5-LOX, in another positive feedback mechanism (77) (Figure 2). In addition to p-ERK1/2, mitogen-activated protein kinase kinase kinase 2 (MEKK2), which is a member of the MAPK signaling pathway, was found to be involved in the HBx-induced growth of hepatoma cells. By upregulating MEKK2, HBx could promote hepatoma cell proliferation, which may be involved in hepatocarcinogenesis (78). Because MAPK regulates the activities of several translation and transcription factors, HBx is thus able to regulate translation or transcription during cell proliferation through some MAPK members.
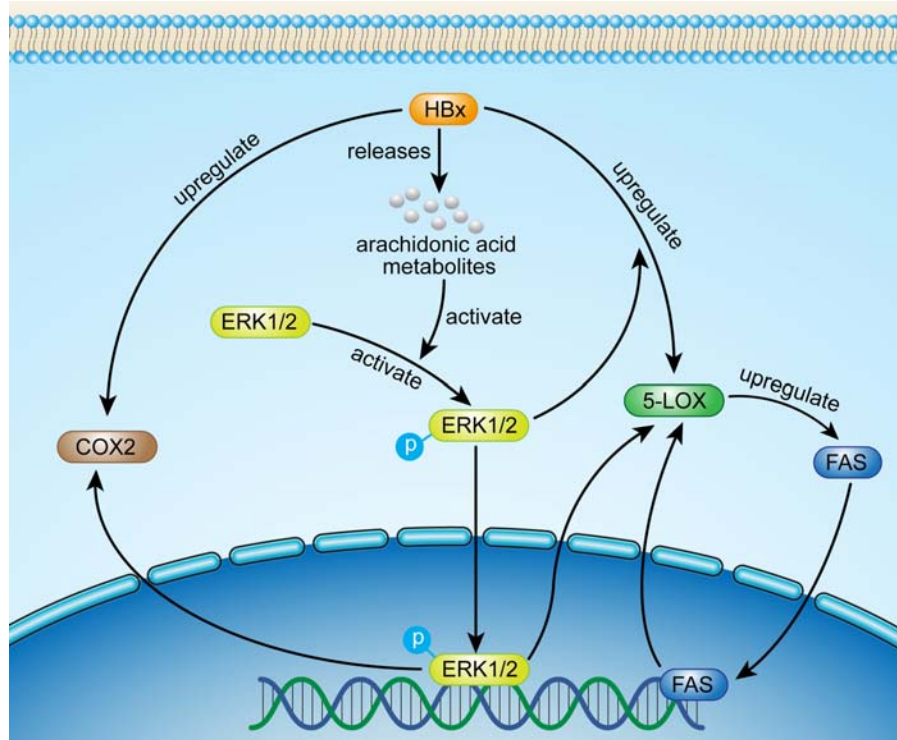
Figure 2. HBx promotes HCC cell growth through the positive feedback loop. HBx could upregulate 5-lipoxygenase (5-LOX) through phosphorylated extracellular signal-regulated protein kinases 1/2 (p-ERK1/2). And the 5-LOX was responsible for the up-regulation of FAS. FAS was able to upregulate the expression of 5-LOX through a feedback mechanism. HBx upregulates the levels of COX-2 and also releases arachidonic acid metabolites, which are able to activate ERK1/2. Subsequently, the activated p-ERK1/2 could upregulate the expression of COX-2 and 5-LOX, in another positive feedback mechanism

In addition, HBx is involved in several other signaling pathways to promote proliferation. The stimulation of cell proliferation by HBx could be linked to its function in elevating cytosolic calcium signals, the underlying molecular mechanism of which is an increase in mitochondrial calcium uptake and store-operated calcium entry that leads to higher sustained cytosolic calcium levels and stimulates HBV replication in vitro (79). By repressing wild-type beta-catenin expression and enforcing a beta-catenin-dependent signaling pathway, HBx can induce cellular changes that lead to the acquisition of metastatic and/or proliferative properties, thus contributing to the development of HCC in HepG2 cells (80). HBx can also up-regulate serine/threonine p21-activated kinase 1 (PAK1), which can regulate cytoskeletal dynamics and protect cells from anoikis, thus promoting the growth of aggressive xenograft tumors in mice (81). In the extracellular space, HBx could up-regulate LASP-1, a focal adhesion protein, through the phosphatidylinositol 3 kinase (PI3K) pathway to promote the proliferation and migration of hepatoma cells (82).

4.4. HBx mutants

In humans, HBx has been shown to be expressed in HCC tissues, and it is the sequence that is most preferentially retained in the integrated form of HBV (83-86). Several HBx mutants have been isolated from sera and/or liver tissues from patients with HCC. HBx sequences isolated from integrated HBV genomes in tumor tissues have frequently been reported to have a deletion at the 3' end (87-94). In addition, a Ser31Ala point mutation (95) and two linked point mutations, Lys130Met and Val131Ile (96-98), were also shown to be prevalent in HCC patients.

COOH-terminal amino acid deletions of HBx are common in human HCC. Tu and colleagues (99) found that the HBx COOH-terminus plays a key role in controlling cell proliferation, viability, and transformation. They further found that although full-length HBx suppressed the focus formation induced by the cooperation of the ras and myc oncogenes in primary rat embryo fibroblasts, COOH-terminally truncated HBx enhanced the transforming ability of ras and myc (99). HBx mutants with 20, 30, or 40 amino acids deleted from the COOH-terminus (HBx3'-20, -30, -40) have been described. HBx3'-20 and HBx3'-40 promoted the proliferation of HCC cells, while HBx3'-30 had the opposite effect (100-102). Moreover, HBx3'-20 and HBx3'-40 stimulated increased cell proliferation, focus formation, tumorigenicity, and invasive growth and metastasis due to the promotion of the G0/G1 to S phase transition of the cell cycle, compared with the full-length HBx. In contrast, the HBx3'-30 mutant repressed cell proliferation by triggering arrest in G1 phase. The

expression of the P53, p21(WAF1), p14(ARF), and MDM2 proteins is also altered by the expression of HBx mutants (102). These findings support the hypothesis that HBx mutants might be selected in tumor tissues and play a role in hepatocarcinogenesis by modifying the biological functions of HBx.

5. CONCLUSION

HBV is one of the leading causes of HCC. The HBx protein is the HBV protein that is most commonly implicated in the pathogenesis of HCC. HBx is involved in multiple aspects of carcinoma development, and it activates several signaling pathways that lead to the transcriptional upregulation of a number of genes, including oncogenes. Moreover, HBx mutants, especially those with COOH-terminal deletions, could be involved in hepatocarcinogenesis. Thus, therapeutic strategies targeting HBx could be effective at multiple stages of HCC development, even as early as the preneoplastic stage.

6. ACKNOWLEDGMENTS

7. REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127,2893–2917 (2010)

2. Bosch FX, Ribes J, Diaz M, Cleries R: Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 127, S5–S16 (2004)

3. Sun TT, Chu YY: Carcinogenesis and prevention strategy of liver cancer in areas of prevalence. *J Cell Physiol* Suppl3,39-44 (1984)

4. Altekruse SF, McGlynn KA, Reichman ME: Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 27, 1485-91 (2009)

5. World Health Organization. Mortality database: 2012 [updated 2012 November]; Available from: www.who.int/whosis/en.

6. Chen CJ, Yu MW, Liaw YF: Epidemiological characteristics and risk factors of hepatocellular carcinoma. *J Gastroenterol Hepatol* 12,S294–308 (1997)

7. El-Serag HB, Rudolph KL: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 132,2557–76 (2007)

8. Marotta F, Vangieri B, Cecere A, Gattoni A: The pathogenesis of hepatocellular carcinoma is multifactorial event. Novel immunological treatment in prospect. *Clin Ter* 155,187–199 (2004)

9. Cougot D, Neuveut C, Buendia MA: HBV induced carcinogenesis. J Clin Virol 34, Suppl 1,S75–78 (2005)

10. Anzola M: Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. *J Viral Hepat* 11,383–393 (2004)

11. Barazani Y, Hiatt JR, Tong MJ, Busuttil RW: Chronic viral hepatitis and hepatocellular carcinoma. *World J Surg* 31,1243–1248 (2007)

12. Koskinas J, Petraki K, Kavantzas N, Rapti I, Kountouras D: Hepatic expression of the proliferative marker Ki-67 and p53 protein in HBV or HCV cirrhosis in relation to dysplastic liver cell changes and hepatocellular carcinoma. *J Viral Hepat* 12,635–641 (2005)

13. Dewantoro O, Gani RA, Akbar N: Hepatocarcinogenesis in viral Hepatitis B infection: the role of HBx and p53. *Acta Med Indones* 38,154–159 (2006)

14. Park US, Su JJ, Ban KC, Qin L, Lee EH: Mutations in the p53 tumor suppressor gene in tree shrew hepatocellular carcinoma associated with hepatitis B virus infection and intake of aflatoxin B1. *Gene* 251,73–80 (2000)

15. Chaparro M, Sanz-Cameno P, Trapero-Marugan M, Garcia-Buey L, Moreno-Otero R: Mechanisms of angiogenesis in chronic inflammatory liver disease. *Ann Hepatol* 6,208–213 (2007)

16. Edamoto Y, Hara A, Biernat W, Terracciano L, Cathomas G: Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. *Int J Cancer* 106,334–341 (2003)

17. Tommasi S, Pinto R, Pilato B, Paradiso A: Molecular pathways and related target therapies in liver carcinoma. *Curr Pharm Des* 13,3279–3287 (2007)

18. McCubrey JA, Steelman LS, Abrams SL, Lee JT, Chang F, et al: Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. *Adv Enzyme Regul* 46,249–279 (2006)

19. Kasai Y, Takeda S, Takagi H: Pathogenesis of hepatocellular carcinoma: a review from the viewpoint of molecular analysis. *Semin Surg Oncol* 12,155–159 (1996)

20. Kew MC: Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 26,144–152 (2011)

21. Liu XH, Lin J, Zhang SH, Zhang SM, Feitelson MA: COOHterminal deletion of HBx gene is a frequent event in HBV-associated hepatocellular carcinoma. *World J Gastroenterol* 14,1346–1352 (2008)

22. Kim CM, Koike K, Saito I, Miyamura T, Jay G: HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 351,317–320 (1991)

23. Zhu H, Wang Y, Chen J, Cheng G, Xue J: Transgenic mice expressing hepatitis B virus X protein are more susceptible to carcinogen induced hepatocarcinogenesis. *Exp Mol Pathol* 76,44–50 (2004)

24. Luedde T, Schwabe RF: NF-kappaB in the liver–linking injury, fibrosis and hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 8,108–118 (2011)

25. Matsumura H, Moriyama M, Goto I, Tanaka N, Okubo H, Arakawa Y: Natural course of progression of liver fibrosis in Japanese patients with chronic liver disease type C-a study of 527 patients at one establishment. *J Viral Hepat* 7,268–275 (2000)

26. Pawlik TM, Poon RT, Abdalla EK, Zorzi D, Ikai I, Curley SAl: Critical appraisal of the clinical and pathologic predictors of survival after resection of large hepatocellular carcinoma. *Arch Surg* 140,450–457; discussion 457-458 (2005)

27. Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG: AJCC Cancer Staging Handbook: From the AJCC Cancer Staging Manual. 6th ed. New York: Springer 469 (2002)

28. Huo TI, Lin HC, Hsia CY, Wu JC, Lee PC, Chi CW, Lee SD: The model for end-stage liver disease based cancer staging systems are better prognostic models for hepatocellular carcinoma: a prospective sequential survey. *Am J Gastroenterol* 102,1920–1930 (2007)

29. Bataller R, Brenner DA: Liver fibrosis. *J Clin Invest* 115,209-218 (2005)

30. Martín-Vílchez S, Sanz-Cameno P, Rodríguez-Muñoz Yl: The hepatitis B virus X protein induces paracrine activation of human hepatic stellate cells. *Hepatology* 47,1872-83 (2008)

31. Guo GH, Tan DM, Zhu PA, Liu F: Hepatitis B virus X protein promotes proliferation and upregulates TGF-beta1 and CTGF in human hepatic stellate cell line, LX-2. *Hepatobiliary Pancreat Dis Int* 8,59-64 (2009)

32. Madden CR, Finegold MJ, Slagle BL: Hepatitis B virus X protein acts as a tumor promoter in development of diethylnitrosamine-induced preneoplastic lesions. *J Virol* 75,3851-8 (2001)

33. Pang R, Lee TK, Poon RT: Pin1 interacts with a specific serine-proline motif of hepatitis B virus X-protein to enhance hepatocarcinogenesis. *Gastroenterology* 132,1088-103 (2007)

34. Lu KP, Hanes SD, Hunter T: A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* 380,544–547 (1996)

35. Lu KP: Pinning down cell signaling, cancer and Alzheimer's disease. *Trends Biochem Sci* 29,200–209 (2004)

36. Zhou XZ, Lu PJ, Wulf G, Lu KP: Phosphorylation-dependent prolyl isomerization: a novel signaling regulatory mechanism. *Cell Mol Life Sci* 56,788–806 (1999)

37. Kim MR, Choi HS, Heo TH, Hwang SW, Kang KW: Induction of vascular endothelial growth factor by peptidyl-prolyl isomerase Pin1 in breast cancer cells. *Biochem Biophys Res Commun* 369,547–553 (2008)

38. Terradillos O, Billet O, Renard CA: The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. *Oncogene* 14,395-404 (1997)

39. Shukla SK, Kumar V: Hepatitis B virus X protein and c-Myc cooperate in the upregulation of ribosome biogenesis and in cellular transformation. *FEBS J* 279,3859-71 (2012)

40. Feng SL, Guo Y, Factor VM: The Fn14 immediate-early response gene is induced during liver regeneration and highly expressed in both human and murine hepatocellular carcinomas. *Am J Pathol* 156,1253-61 (2000)

41. Yang L, He J, Chen L, Wang G: Hepatitis B virus X protein upregulates expression of SMYD3 and C-MYC in HepG2 cells. *Med Oncol* 26,445-51 (2009)

42. Zhang JL, Zhao WG, Wu KL: Human hepatitis B virus X protein promotes cell proliferation and inhibits cell apoptosis through interacting with a serine protease Hepsin. *Arch Virol* 150,721-41 (2005)

43. Zhang WY, Xu FQ, Shan CL, Xiang R, Ye LH, Zhang XD: Gene expression profiles of human liver cells mediated by hepatitis B virus X protein. *Acta Pharmacol Sin* 30,424-34 (2009)

44. Wu CG, Salvay DM, Forgues M: Distinctive gene expression profiles associated with Hepatitis B virus x protein. *Oncogene* 20,3674-82 (2001)

45. Gouas DA, Shi H, Hautefeuille AH: Effects of the TP53 p.R249S mutant on proliferation and clonogenic properties in human hepatocellular carcinoma cell lines: interaction with hepatitis B virus X protein. *Carcinogenesis* 31,1475-82 (2010)

46. Venters BJ, Pugh BF: How eukaryotic genes are transcribed. *Crit Rev Biochem Mol Biol* 44,117–141 (2009)

47. Sims RJ 3rd, Belotserkovskaya R, Reinberg D. Elongation by RNA polymerase II: the short and long of it. *Genes Dev* 18,2437–2468 (2004)

48. Sakurai H, Ishihama A: Transcription organization and mRNA levels of the genes for all 12 subunits of the fission yeast RNA polymerase II. *Genes Cells* 6,25–36 (2001)

49. Cramer P, Bushnell DA, Fu J: Architecture of RNA polymerase II and implications for the transcription mechanism. *Science* 288,640–649 (2000)

50. Yang H, Gu J, Zheng Q, Li M: RPB5-mediating protein is required for the proliferation of hepatocellular carcinoma cells. *J Biol Chem* 286,11865-74 (2011)

51. Yang Y, Zheng L, Chen Y: Study of HBV X protein and RMP, an RPB5 mediate protein competitively interacting with general transcription factor TF2B. *Zhonghua Gan Zang Bing Za Zhi* 8,15–17 (2000)

52. Deng WS, Roberts SGE: TFIIB and the regulation of transcription by RNA polymerase II. *Chromosoma* 116,417–429 (2007)

53. Haviv I, Shamay M, Doitsh G: Hepatitis B virus pX targets TFIIB in transcription coactivation. *Mol Cell Biol* 18,1562–1569 (1998)

54. Li L, Zhang A, Cao X, Chen J, Xia Y, Zhao H, Shen A: General Transcription Factor IIB Overexpression and a Potential Link to Proliferation in Human Hepatocellular Carcinoma. *Pathol Oncol Res* (2012) [Epub ahead of print]

55. Okamoto T, Sanda T and Asamitsu K: NF-kappa B signaling and carcinogenesis. *Curr Pharm Des* 13,447-462 (2007)

56. Rosette C and Karin M: Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 274,1194-1197 (1996)

57. Um HR, Lim WC, Chae SY, Park S, Park JH and Cho H: Raf-1 and protein kinase B regulate cell survival through the activation of NF-kappaB in hepatitis B virus X-expressing cells. *Virus Res* 125,1-8 (2007)

58. Factor V, Oliver AL, Panta GR: Thorgeirsson SS, Sonenshein GE and Arsura M: Roles of Akt/PKB and IKK complex in constitutive induction of NF-kappaB in hepatocellular carcinomas of transforming growth factor alpha/c-myc transgenic mice. *Hepatology* 34,32-41 (2001)

59. An J and Rettig MB: Mechanism of von Hippel-Lindau protein-mediated suppression of nuclear factor kappa B activity. *Mol Cell Biol* 25,7546-7556 (2005)

60. Kim SY, Kim JC, Kim JK: Hepatitis B virus X protein enhances NFkappaB activity through cooperating with VBP1. *BMB Rep* 41,158-63 (2008)

61. Lu B, Guo H, Zhao J: Increased expression of iASPP, regulated by hepatitis B virus X protein-mediated NF-κB activation, in hepatocellular carcinoma. *Gastroenterology* 139,2183-2194.e5 (2010)

62. Yun C, Um HR, Jin YH: NF-kappaB activation by hepatitis B virus X (HBx) protein shifts the cellular fate toward survival. *Cancer Lett* 184,97-104 (2002)

63. Kim A, Kwon OS, Kim SO: Caspase-3 activation as a key factor for HBx-transformed cell death. *Cell Prolif* 41,755-74 (2008)

64. Yen CJ, Lin YJ, Yen CS: Hepatitis B virus X protein upregulates mTOR signaling through IKKβ to increase cell proliferation and VEGF production in hepatocellular carcinoma. *PLoS One* 7,e41931 (2012)

65. Wang Y, Lu Y, Toh ST: Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. *J Hepatol* 53,57-66 (2010)

66. Fu X, Tan D, Hou Z, Hu Z, Liu G: miR-338-3p Is Down-Regulated by Hepatitis B Virus X and Inhibits Cell Proliferation by Targeting the 3'-UTR Region of CyclinD1. *Int J Mol Sci* 13,8514-39 (2012)

67. Du Y, Kong G, You X: Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. *J Biol Chem* 287,26302-11 (2012)

68. Qiao L, Leach K, McKinstry R: Hepatitis B virus X protein increases expression of p21(Cip-1/WAF1/MDA6) and p27(Kip-1) in primary mouse hepatocytes, leading to reduced cell cycle progression. *Hepatology* 34,906-17 (2001)

69. Cheng P, Li Y, Yang L: Hepatitis B virus X protein (HBx) induces G2/M arrest and apoptosis through sustained activation of cyclin B1-CDK1 kinase. *Oncol Rep* 22,1101-7 (2009)

70. Gearhart TL, Bouchard MJ: The hepatitis B virus X protein modulates hepatocyte proliferation pathways to stimulate viral replication. *J Virol* 84,2675-86 (2010)

71. Wu BK, Li CC, Chen HJ: Blocking of G1/S transition and cell death in the regenerating liver of Hepatitis B virus X protein transgenic mice. *Biochem Biophys Res Commun* 340,916-28 (2006)

72. Xu J, Yun X, Jiang J: Hepatitis B virus X protein blunts senescence-like growth arrest of human hepatocellular carcinoma by reducing Notch1 cleavage. *Hepatology* 52,142-54 (2010)

73. Wang F, Zhou H, Xia X, Sun Q, Wang Y, Cheng B: Activated Notch signaling is required for hepatitis B virus X protein to promote proliferation and survival of human hepatic cells. *Cancer Lett* 298,64-73 (2010)

74. Gao J, Chen C, Hong L: Expression of Jagged1 and its association with hepatitis B virus X protein in hepatocellular carcinoma. *Biochem Biophys Res Commun* 356,341-7 (2007)

75. Wu G, Yu F, Xiao Z: Hepatitis B virus X protein downregulates expression of the miR-16 family in

malignant hepatocytes in vitro. *Br J Cancer* 105,146-53 (2011)

76. Wang Q, Zhang W, Liu Q: A mutant of hepatitis B virus X protein (HBxDelta127) promotes cell growth through a positive feedback loop involving 5-lipoxygenase and fatty acid synthase. *Neoplasia* 12,103-15 (2010)

77. Shan C, Xu F, Zhang S, You J: Hepatitis B virus X protein promotes liver cell proliferation via a positive cascade loop involving arachidonic acid metabolism and p-ERK1/2. *Cell Res* 20,563-75 (2010)

78. Kong GY, Zhang JP, Zhang S, Shan CL, Ye LH, Zhang XD: Hepatitis B virus X protein promotes hepatoma cell proliferation via upregulation of MEKK2. *Acta Pharmacol Sin* 32,1173-80 (2011)

79. Yang B, Bouchard MJ: The hepatitis B virus X protein elevates cytosolic calcium signals by modulating mitochondrial calcium uptake. *J Virol* 86,313-27 (2012)

80. Chen L, Hu L, Li L: Dysregulation of β-catenin by hepatitis B virus X protein in HBV-infected human hepatocellular carcinomas. *Front Med China* 4,399-411 (2010)

81. Xu J, Liu H, Chen L: Hepatitis B virus X protein confers resistance of hepatoma cells to anoikis by up-regulating and activating p21-activated kinase 1. *Gastroenterology* 143,199-212.e4 (2012)

82. Tang R, Kong F, Hu L: Role of hepatitis B virus X protein in regulating LIM and SH3 protein 1 (LASP-1) expression to mediate proliferation and migration of hepatoma cells. *Virol J* 9,163 (2012)

83. Bergametti F, Prigent S, Luber B: The proapoptotic effect of hepatitis B virus HBx protein correlates with its transactivation activity in stably transfected cell lines. *Oncogene* 18,2860–2871 (1999)

84. Paterlini P, Poussin K, Kew M, Franco D, Brechot C: Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 21,313–321 (1995)

85. Zhu M, London WT, Duan LX, and Feitelson MA: The value of hepatitis B x antigen as a prognostic marker in the development of hepatocellular carcinoma. *Int J Cancer* 55,571–576 (1993)

86. Wang WL, London WT, Feitelson MA: Hepatitis B x antigen in hepatitis B virus carrier patients with liver cancer. *Cancer Res* 51,4971–4977 (1991)

87. Wei Y, Etiemble J, Fourel G, Vitvitski-Trepo L, Buendia MA: Hepadna virus integration generates virus-cell cotranscripts carrying 3 truncated X genes in human and woodchuck liver tumors. *J Med Virol* 45,82–90 (1995)

88. Chen WN, Oon CJ, Leong AL, Koh S, Teng SW: Expression of integrated hepatitis B virus X variants in human hepatocellular carcinomas and its significance. *Biochem Biophys Res Commun* 276,885–892 (2000)

89. Hsia CC, Nakashima Y, and Tabor E: Deletion mutants of the hepatitis B virus X gene in human hepatocellular carcinoma. *Biochem Biophys Res Commun* 241,726–729 (1997)

90. Wollersheim M, Debelka U: Hofschneider PHA transactivating function encoded in the hepatitis B virus X gene is conserved in the integrated state. *Oncogene* 3,545–552 (1988)

91. Poussin K, Dienes H, Sirma H: Expression of mutated hepatitis B virus X genes in human hepatocellular carcinomas. *Int J Cancer* 80,497–505 (1999)

92. Yamamoto S, Nakatake H, Kawamoto S, Takimoto M, Koshy R, Matsubara K: Transactivation of cellular promoters by an integrated hepatitis B virus DNA. *Biochem Biophys Res Commun* 192,111–118 (1993)

93. Schluter V, Meyer M, Hofschneider PH, Koshy R, Caselmann WH: Integrated hepatitis B virus X and 3' truncated preS/S sequences derived from human hepatomas encode functionally active transactivators. *Oncogene* 9,3335–3344 (1994)

94. Tsuei DJ, Hsu TY, Chen JY, Chang MH, Hsu HC, Yang CS: Analysis of integrated hepatitis B virus DNA and flanking cellular sequences in a childhood hepatocellular carcinoma. *J Med Virol* 42,287–293 (1994)

95. Yeh CT, Shen CH, Tai DI, Chu CM, Liaw YF: Identification and characterization of a prevalent hepatitis B virus X protein mutant in Taiwanese patients with hepatocellular carcinoma. *Oncogene* 19,5213–5220 (2000)

96. Takahashi K, Akahane Y, Hino K, Ohta Y, Mishiro S: Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients, comparative analysis of 40 full-length isolates. *Arch Virol* 143,2313–2326 (1998)

97. Baptista M, Kramvis A, Kew MC: High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 29,946–953 (1999)

98. Hsia CC., Yuwen H, Tabor E: Hot-spot mutations in hepatitis B virus X gene in hepatocellular carcinoma. *Lancet* 348,625–626 (1996)

99. Tu H, Bonura C, Giannini C, Mouly H: Biological impact of natural COOH-terminal deletions of hepatitis B virus X protein in hepatocellular carcinoma tissues. *Cancer Res* 61,7803-10 (2001)

100. Liu XH, Lin J, Cao XZ, Zheng JM, Chen Y, Zhu MH: Biological impact of the COOH-terminal 40 amino acid

deletions of hepatitis B virus X protein in hepatocellular carcinoma cells. *Zhonghua Yi Xue Za Zhi* 85,825-30 (2005)

101. Liu XH, Zhu MH, Cao XZ, Zheng JM, Chen Y: Biological effects of COOH-terminal amino acid deletions of hepatitis B virus X protein on hepatocellular carcinoma cell line Huh7. *Ai Zheng* 24,1213-9 (2005)

102. Liu X, Zhang S, Lin J: Hepatitis B virus X protein mutants exhibit distinct biological activities in hepatoma Huh7 cells. *Biochem Biophys Res Commun* 373,643-7 (2008)

Abbreviations: HCC:hepatocellular carcinoma; HBV:chronic hepatitis B virus; HBx:HBV X protein; HCV: hepatitis C virus; HSCs:hepatic stellate cells; TGF-beta1:transforming growth factor-beta1; CTGF:connective tissue growth factor; DEN:diethylnitrosamine; NIMA:never-in-mitosis A; FGF:fibroblast growth factor; Fn14:FGF-inducible 14; RNAPII:RNA polymerase II; RMP:RPB5-mediating protein; TFIIB:transcription factor IIB; VBP1:VHL binding protein; VHL:von Hippel-Lindau protein; ASPP:ankyrin-repeat-, SH3-domain- and proline-rich-region-containing protein; Pro1:proteasome inhibitor-1; ER-stress:ndoplasmic reticulum-stress; CPT:camptothecin; IKK beta:I-kappaB kinase beta; mTOR:mammalian target of rapamycin; miRNAs:microRNAs; lncRNAs:long noncoding RNAs; FAS:fatty acid synthase; 5-LOX:5-lipoxygenase; p-ERK1/2:signal-regulated protein kinases 1/2; LTB4:leukotriene B4; COX-2:cyclooxygenase-2; MEKK2:mitogen-activated protein kinase kinase kinase 2; PAK1:p21-activated kinase 1

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