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TOPIC HIGHLIGHT

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Molecular mechanism of hepatitis B virus X protein function in hepatocarcinogenesis

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Abstract

Many factors are considered to contribute to hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC), including products of HBV, HBV integration and mutation, and host susceptibility. HBV X protein (HBx) can interfere with several signaling pathways associated with cell proliferation and invasion, and HBx C-terminal truncation has been suggested to impact the development of HCC. This review focuses on the pathological functions of HBx in HBV-induced hepatocarcinogenesis. As a transactivator, HBx can affect regulatory non-coding RNAs (ncRNAs), including microRNAs and long ncRNAs. HBx is also involved in epigenetic modification and DNA repair. HBx interacts with various signal-transduction pathways, such as the p53, Wnt, and nuclear factor-κB pathways. We conclude that HBx hastens the development of hepatoma.

Key words: Hepatocellular carcinoma; Hepatitis B virus; Hepatitis B virus X protein; Hepatocarcinogenesis

Core tip: The mechanisms underlying hepatitis B virus (HBV)-induced malignant transformation remain ambiguous, but research has suggested that HBV X (HBx) protein has a crucial function in the pathogenesis of hepatocellular carcinoma. This review focuses on the pathological functions of HBx in HBV-induced hepatocarcinogenesis.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most

common cause of cancer mortality[1]. Chronic hepatitis B virus (HBV) infection has been demonstrated to be a risk factor for liver carcinogenesis, accounting for 55% of cases worldwide. Notably, 80% or more of such cases are found in the eastern Pacific region and sub-Saharan Africa, areas with the highest tumor incidence[2,3]. The mechanisms underlying HBV-induced malignant transformation remain ambiguous, but previous research has suggested that HBV X (HBx) protein has a crucial role in the pathogenesis of HCC[4]. Here, we review the molecular mechanisms of HBx in the pathogenesis of HCC.

HBx GENE AND HBx PROTEIN

HBV is considered to be the smallest DNA virus and contains a 3.2 kb circular double-stranded viral DNA genome, including a long minus-strand that is complementary to viral mRNA sequences and a short plus-strand. The open reading frame (ORF) of HBx is 465 bp long, from nucleotides 1376 to 1837, and is translated into a 154 amino acid (aa) protein. The *HBx* gene is located upstream of gene *C* and close to the sticky end of the viral genome, where it also overlaps with other genes, including viral polymerase, Pre C, ORF5, and ORF6. Although HBx cannot directly bind to the DNA helix, it can activate other protein factors to further bind to their or other promoters and enhancers. Thus, HBx can trans-regulate gene transcription[5].

The plus-strand HBx viral genome contains several transcriptional regulation element sequences, including gene expression basic core promoter, core upstream regulatory sequence, negative regulatory element, enhancer Ⅱ, direct repeat 1 (DR1), and DR2. Also, the 5′ end of the *HBx* gene overlaps with the ORF of DNA polymerase P[6,7]. Thus, the *X* gene of HBV contains the longest overlapping region between structural and functional sequences in the viral genome. More importantly, because of the overlap between the coding region and regulation elements in the *X* gene of HBV, any DNA mutation and/or deletion can affect functionally both gene and transcriptional regulation.

HBx AND DNA REPAIR

Current studies indicate that DNA repair is one of the driving mechanisms of carcinogenesis. Accumulation of DNA damage causes genomic instability and eventually leads to mutations. Recent studies showed that the expression level of HBx positively correlated with that of 8-hydroxy-2 deoxyguanosine (8-OHdG), a key oxidative stress indicator that causes DNA mis-pairing. Meanwhile, a high level of HBx inhibited human DNA glycosylase α activity, which caused suppression of DNA repair machinery, long-term DNA damage, and tumorigenesis[8].

Jung *et al*[9] reported that HBx with C terminal truncation does not induce reactive oxygen species (ROS) production and has no effect on level of 8-OHdG. This indicated an important role for the HBx C terminal region in oxidative stress-induced ROS production, consequential mitochondrial DNA damage, and HCC pathogenesis. Another study also reported that HBx can regulate p53 expression and further depress the DNA repair capability[10].

HBx AND METHYLATION

Epigenetic studies allow us to understand how DNA methyltransferases (DNMTs) involved in DNA methylation can control gene expression through chromatin structural modification, changes in regional DNA accessibility, changes in DNA stability, and shifts in DNA-protein interactions. HBx can affect the cell cycle, proliferation, invasion, apoptosis, *etc*. of HCC cells by regulating DNMTs involved in DNA methylation of specific genes. A recent publication demonstrated that HBx can upregulate DNMT1 and DNMT3A through transactivation[11]. Wei *et al*[12] demonstrated that downregulation of miR-101 by HBx can lead to abnormal DNA methylation by miR-101-targeting of DNMT3A and promotion of HCC malignancy. A similar study showed that HBx upregulated DNMT1 and DNMT3A at both the transcriptional and translational levels, leading to induction of p16 (INK4A) promoter methylation and subsequent inhibition of p16 expression[13].

HBx AND NON-CODING RNAs

Non-coding RNAs (ncRNAs) compose a large group of RNAs transcribed from non-coding regions of the human genome. ncRNAs account for about 90% of the genome and can be categorized in two types: 18-200 nucleotide small ncRNAs, including microRNAs (miRNAs), small interfering (siRNAs), Piwi-interacting RNAs, small nuclear RNAs, small nucleolar RNAs, *etc.*; 200 nucleotide to 100 kb long ncRNAs (lncRNAs), including mRNA-like ncRNAs, long no-poly A tail ncRNAs, *etc.*[14,15]. Most of these RNAs have been rarely studied, and although their functions remain entirely unclear, they have a variety of important biological functions.

MiRNAs play a critical role in the control of gene expression and signal transduction in HCC carcinogenesis. Several *in vitro* studies demonstrated that HBx can promote early stage HCC progression by inducing high levels of miR-21 expression, which inhibiteds programmed cell death 4 in cancer cells[16,17]. Upregulated miR-21 and miR-222 also can directly target tumor suppressor p27 and Kipl, a key regulator of the cell cycle, to contribute to cancer progression[18]. In a previous study, Bandopadhyay *et al*[19] found that miR-21 and miR-222 were downregulated when HepG2 cells were transfected with HBx and HBV plasmid DNA or HepG2.2.15 cells were infected with HBV. This result was confirmed in clinical plasma samples from HCC patients. Interestingly, similar downregulated effects

also were observed in transfected HepG2 cells and patients' plasma for miR-145, whereas miR-145 was upregulated in an infected HepG2.2.15 cell line. These results suggested that HBx can control multiple miRNAs in different manners to promote HCC progression[19]. Additionally, an animal model showed that HBx inhibited the tumor suppressor p53 to control the expression of miR-148a and to increase the expression of hematopoietic pre-B cell leukemia transcription factor-interacting protein. This resulted in activation of Akt, extracellular-related kinase, and mammalian target of rapamycin signaling pathways to enhance tumor cell growth, invasion, and metastasis[20]. A recent study also showed that HBx can downregulate miR-192, suggesting that HBx may be anti-apoptotic in HCC[21].

lncRNAs play crucial roles in human cancers. It has been reported that the lncRNA highly up-regulated in liver cancer (HULC) was dramatically upregulated in HCC[22]. Du *et al*[23] reported that HBx can increase expression of HULC *via* the cAMP-response element binding protein activated promoter of lncRNA HULC. Furthermore, downregulation of P18, a gene downstream of HULC, can promote liver cell proliferation. Another lncRNA (termed lncRNA-Dreh) can be downregulated by HBx, which enhanced HCC cell invasion and migration *in vitro*[24]. It is known that deregulation of lncRNA is one of the key factors in HCC tumor initiation and progression.

HBx MUTANTS AND TUMOR IMITATION

HBV infection-induced HCC usually occurs within 10-30 years after the initial HBV infection. During this period, mutations of the HBV genome accumulate. Two dominant types of HBx mutations can be detected in chronic hepatitis: type Ⅰ are single nucleotide mutations at multiple sites, and type Ⅱ are C-terminal truncations that cause relatively higher levels of protein accumulation in the tumor region. Liver cells with these two types of mutations may have proliferative advantage in colony formation.

Previous studies have shown that HBV genome integration is random, and there are no specific integration sites or rules. HBx and HBV core gene (HBc) mutations and deletions commonly occur in viral genome integration[25-28]. A polymerase chain reaction DNA amplification study of 45 tumor samples and sequencing results of 19 samples showed a high frequency of HBx mutation in HCC. Those mutations were mostly located close to the carboxyl terminus. It is believed that a strong correlation exists between HBx mutation and liver cell cancer transformation[29]. Similarly, we determined that the hot spot of HBx mutation is highly regional. Blood tests of HBx mutations from patients in Europe and Africa showed a higher incidence of mutation at 130 and 131 aa of HBx for mild hepatitis patients and accumulation of HBx C-terminal truncation in HCC peri-tumor tissues[30-34]. In contrast, a study of 153 HCC patients from Vietnam showed more 130 and 131 aa mutations in tumor tissue, with only four out of 48 samples having HBx C-terminal truncation accumulation[35]. A report from Hong Kong claimed that more than 54 mutations were detected in 95.2% of tissue samples and 95.3% of blood samples from 113 patients, where there was at least one mutation in most of the samples[36]. There were 12 mutation sites in tissue samples and nine mutation sites in blood samples, which suggested a mutation-driven pathogenesis for HCC. Another study demonstrated that mutations were complicated and changeable in both HCC and peri-carcinoma liver tissue (PCLT). C-terminal truncation is more frequently found in HCC than in benign liver tissues. However, there is no single site mutation of a nucleic acid or amino acid that results in a distribution discrepancy between HCC and PCLT[37]. The reports described above indicated a regional distribution of HBx mutants, which reflects the high degree of complexity of HBV caused HCC.

The results of a comparative study between HBx C-terminal truncation and full-length HBx transfection indicated that each mutation plays a different role in cancer cell biology[38,39]. Specifically, overexpression of HBx 20 aa and 40 aa C-terminal deletion mutants can enhance cell growth, colony formation, tumor volume, and G1 to S phase cell cycle transition. In contrast, an HBx 30 aa C-terminal deletion mutant can inhibit cell proliferation. These results suggested that 125-134 aa of HBx is important for cell proliferation. More recent studies showed that HBx spontaneous deletion mutations were typically located in the same region. Liu *et al*[40] and Wang *et al*[41] reported that the HBx 127 mutant contributed to tumor cell proliferation metastasis more than wild-type HBx by promoting cell growth through a positive feedback loop involving 5-lipoxygenase, fatty acid synthase, and miR-215. This finding is consistent with a report from Fu *et al*[42] that concluded that the HBx-d382 deletion mutant (128-145 aa) enhanced cell proliferation. The dual mutations K130M/V131I strengthened the capability of HBx, as they upregulated the expression and transcriptional activity of hypoxia-inducible factor 1-alpha (HIF-1α). The C-terminal truncation and deletion mutations, however, weakened the ability of HBx to upregulate HIF-1α. Furthermore, the C-terminus was found to be essential for HBx stability and transactivation. A positive correlation was found between the HBx mutants and HIF-1α expression in clinical HCC samples[43]. In brief, it is believed that C-terminal truncation and deletion promoted tumor malignancy. However, the detailed mechanism needs to be investigated further.

HBx AND THE P53 SIGNALING PATHWAY

Mutations in the tumor suppressor gene *p53* are the most common in all types of cancers. p53 disorder plays an important role in the tumorigenesis of HCC.

Many studies have indicated a complex transactivation between HBx and p53, where HBx directly inhibits p53 activity by binding to its C-terminus[44]. In addition, overexpression of the p53 target gene murine double minute 2 can induce degradation of HBx in HCC[45]. Kew *et al*[46] investigated the effect of wild-type and mutant HBx on p53 and found that HBx mutants, but not wild-type HBx, can inhibit p53 expression and its downstream signaling.

Recent studies suggested that overexpression of a HBx C-terminal mutant in HHT4 cells, a normal liver cell line, significantly increased the colony forming efficiency (CFE), whereas its corresponding wild-type allele CNT significantly decreased the CFE in HHT4 cells. Meanwhile, the p53-249Ser mutant interacted with HBx mutants to regulate cell proliferation and mitochondrial stability[47]. A report from another group showed that the HBx gene overlapped with the HBV core promoter region. Thus, core promoter mutations can also lead to HBx mutants that further upregulate S-phase kinase-associated protein 2 (SKP2). SKP2 can downregulate p53 though ubiquitination and consequentially promote tumorigenesis[48].

HBx AND THE NUCLEAR FACTOR-κB SIGNALING PATHWAY

Nuclear factor (NF)-κB is one of the driving transcriptional factors in cancer biology and participates in cross talk with multiple pathways to control tumor initiation, development, invasion, and metastasis. Previous studies showed that HBx interacts with NF-κB to increase the expression of metastasis-associated protein 1 (MTA1). MTA1 is a major chromatin modulator that plays important roles in inflammation and tumor initiation. NF-κB cross talk with Notch signaling has also been demonstrated, and Notch 1 signaling can be blocked by HBx transfection in the normal liver cell line L02[49]. Lim *et al*[50] demonstrated that endogenous P22-FLIP, a cleavage product of c-FLIP$_L$, can interact with HBx to activate NF-κB signaling. Further investigation showed that P22-FLIP, HBx, and NEMO, a regulatory subunit of IκB kinase (IKK), also known as IKKγ, can form a trimer complex to activate NF-κB signaling and promote tumor formation.

Lee *et al*[51] showed that NF-κB is highly associated with HBx131, HBx130, HBx5, HBx94, and HBx38 mutants as well as the HBx130-HBx131 double mutation and the HBx5-HBx130-HBx131 triple mutation. These double and triple mutations increased HCC incidence to 3.75 and 5.34 times the normal risk level, respectively. HBx5 mutants and double mutants showed much higher NF-κB activity than wild-type and triple-mutation HBx. Notably, triple-mutation HBx cannot enhance NF-κB activity.

Many studies have demonstrated that HBx can promote HCC cell invasion and metastasis through NF-κB signaling. Zhang *et al*[52] reported that HBx activated NF-κB binding to the calpain small subunit 1 (Capn4) promoter and, thus, upregulated expression of Capn4 in HCC cell. This HBx-induced Capn4 upregulation can be significantly blocked by specific siRNA knockdown of NF-κB or pyrrolidinedithiocarbamic acid (PDTC). Studies from other groups also showed that HBx increased the expression of NF-κB target genes, including vascular endothelial growth factor (VEGF), matrix metalloproteinase 2 (MMP2), MMP9, and MMP14. In addition, PDTC inhibited HBx stimulation of NF-κB signaling, which led to a decrease in the expression of VEGF, MMP9, and MMP14 but not MMP2. PDTC also showed an anti-angiogenic effect in HepG2 tumor xenograft nude mice. These results demonstrated that HBx promoted tumor cell invasion, angiogenesis, and metastasis by activating NF-κB signaling and upregulating downstream target genes VEGF and MMPs[53]. HBx also can associate with peroxidase to enhance the level of ROS. This led to greater activation of NF-κB and the formation of a positive feedback loop in cancer cells. Peroxidase-associated HBx upregulated MMPs and downregulated E-cadherin to enhance tumor cell invasion[54].

HBx AND THE Wnt SIGNALING PATHWAY

Highly preserved Wnt signaling has important functions in embryo development, and abnormal Wnt signaling can stimulate tumorigenesis. Wnt signaling molecules can be divided in two categories: (1) canonical Wnt/β-catenin signaling molecules, including Wnt-1, Wnt-3a, Wnt-8a Wnt-8b, *etc.*[55]; and (2) non-canonical Wnt signaling molecules, including Wnt-4, Wnt5a, Wnt-11[56], as well as Wnt/Ca^{2+}, Wnt/planar cell polarity, and others[57,58].

Many studies have shown that HBx competitively binds to adenomatous polyposis coli to disassociate β-catenin from its degradation complex, resulting in nuclear β-catenin accumulation and activation of Wnt signaling to induce tumor transformation[59]. In addition, overexpression of HBx with Wnt-1 can activate Wnt/β-catenin signaling in Huh7 cells by stabilizing cytoplasmic β-catenin. Furthermore, stabilization of β-catenin by HBx can be achieved by inhibiting glycogen synthase kinase 3 activity *via* the activation of Src kinase[60].

Liu *et al*[61], Geng *et al*[62] and Lin *et al*[63] found that the Wnt5a gene is regulated by HBx mutants through gene expression library screening. Further research showed that Wnt-5a may suppress tumor progression in HBV-induced HCC[61-63]. An immunohistochemical study of 114 HCC samples demonstrated that Wnt-5a as well as its receptor, receptor tyrosine kinase-like orphan receptor 2 (ROR2), were downregulated in 80.7% (92/114) of samples. The expression of Wnt-5a was negatively correlated with β-catenin expression and positively correlated with E-cadherin

expression. Thus, the expression of Wnt-5a and ROR2 is associated with patient prognosis. Huh7 HCC cells transfected with Wnt-5a have a decreased proliferation rate, and Wnt-5a siRNA knockdown can increase cell proliferation[64]. These findings suggested that HBx mutants can control tumor growth *via* signaling through the Wnt pathway.

CONCLUSION

HBx is the only expressed HBV viral protein in malignant HCC and has been shown to be a key molecule in HCC carcinogenesis. However, the molecular mechanism of HBx-induced HCC progression remains unclear. HBx is maintained as an important player in HCC tumorigenesis. HBx functions in HCC through its nuclear translocation, protein-protein interactions, regulation of transcription factors, induction of chromosome instability, and nuclear localized HBx-involved signal transduction, thereby controlling cancer cell proliferation, transformation, invasion, and metastasis. After studying HBx mutants and their associated molecular pathways, it is clear that these mutants have different biological functions and activities compared to wild-type HBx and that they may play important regulatory roles in the pathogenesis of HCC.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]

2 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218]

3 **Kew MC**. Epidemiology of chronic hepatitis B virus infection, hepatocellular carcinoma, and hepatitis B virus-induced hepatocellular carcinoma. *Pathol Biol* (Paris) 2010; **58**: 273-277 [PMID: 20378277 DOI: 10.1016/j.patbio.2010.01.005]

4 **Zhang XD**, Wang Y, Ye LH. Hepatitis B virus X protein accelerates the development of hepatoma. *Cancer Biol Med* 2014; **11**: 182-190 [PMID: 25364579 DOI: 10.7497/j.issn.2095-3941.2014.03.004]

5 **Seeger C**, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51-68 [PMID: 10704474]

6 **Venard V**, Corsaro D, Kajzer C, Bronowicki JP, Le Faou A. Hepatitis B virus X gene variability in French-born patients with chronic hepatitis and hepatocellular carcinoma. *J Med Virol* 2000; **62**: 177-184 [PMID: 11002246]

7 **Kramvis A**, Kew MC. The core promoter of hepatitis B virus. *J Viral Hepat* 1999; **6**: 415-427 [PMID: 10607259]

8 **Cheng B**, Zheng Y, Guo X, Wang Y, Liu C. Hepatitis B viral X protein alters the biological features and expressions of DNA repair enzymes in LO2 cells. *Liver Int* 2010; **30**: 319-326 [PMID: 19968784 DOI: 10.1111/j.1478-3231.2009.02167.x]

9 **Jung SY**, Kim YJ. C-terminal region of HBx is crucial for mitochondrial DNA damage. *Cancer Lett* 2013; **331**: 76-83 [PMID: 23246371 DOI: 10.1016/j.canlet.2012.12.004]

10 **Capovilla A**, Carmona S, Arbuthnot P. Hepatitis B virus X-protein binds damaged DNA and sensitizes liver cells to ultraviolet irradiation. *Biochem Biophys Res Commun* 1997; **232**: 255-260 [PMID: 9125143]

11 **Tian Y**, Yang W, Song J, Wu Y, Ni B. Hepatitis B virus X protein-induced aberrant epigenetic modifications contributing to human hepatocellular carcinoma pathogenesis. *Mol Cell Biol* 2013; **33**: 2810-2816 [PMID: 23716588 DOI: 10.1128/MCB.00205-13]

12 **Wei X**, Xiang T, Ren G, Tan C, Liu R, Xu X, Wu Z. miR-101 is down-regulated by the hepatitis B virus x protein and induces aberrant DNA methylation by targeting DNA methyltransferase 3A. *Cell Signal* 2013; **25**: 439-446 [PMID: 23124077 DOI: 10.1016/j.cellsig.2012.10.013]

13 **Zhu YZ**, Zhu R, Shi LG, Mao Y, Zheng GJ, Chen Q, Zhu HG. Hepatitis B virus X protein promotes hypermethylation of p16(INK4A) promoter through upregulation of DNA methyltransferases in hepatocarcinogenesis. *Exp Mol Pathol* 2010; **89**: 268-275 [PMID: 20620135 DOI: 10.1016/j.yexmp.2010.06.013]

14 **Nana-Sinkam SP**, Croce CM. Non-coding RNAs in cancer initiation and progression and as novel biomarkers. *Mol Oncol* 2011; **5**: 483-491 [PMID: 22079056 DOI: 10.1016/j.molonc.2011.10.003]

15 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438]

16 **Li CH**, Xu F, Chow S, Feng L, Yin D, Ng TB, Chen Y. Hepatitis B virus X protein promotes hepatocellular carcinoma transformation through interleukin-6 activation of microRNA-21 expression. *Eur J Cancer* 2014; **50**: 2560-2569 [PMID: 25087183 DOI: 10.1016/j.ejca.2014.07.008]

17 **Qiu X**, Dong S, Qiao F, Lu S, Song Y, Lao Y, Li Y, Zeng T, Hu J, Zhang L, Zhang L, Fan H. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* 2013; **32**: 3296-3305 [PMID: 23604124 DOI: 10.1038/onc.2013.150]

18 **Galardi S**, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafrè SA, Farace MG. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. *J Biol Chem* 2007; **282**: 23716-23724 [PMID: 17569667]

19 **Bandopadhyay M**, Banerjee A, Sarkar N, Panigrahi R, Datta S, Pal A, Singh SP, Biswas A, Chakrabarti S, Chakravarty R. Tumor suppressor micro RNA miR-145 and onco micro RNAs miR-21 and miR-222 expressions are differentially modulated by hepatitis B virus X protein in malignant hepatocytes. *BMC Cancer* 2014; **14**: 721 [PMID: 25260533 DOI: 10.1186/1471-2407-14-721]

20 **Xu X**, Fan Z, Kang L, Han J, Jiang C, Zheng X, Zhu Z, Jiao H, Lin J, Jiang K, Ding L, Zhang H, Cheng L, Fu H, Song Y, Jiang Y, Liu J, Wang R, Du N, Ye Q. Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis. *J Clin Invest* 2013; **123**: 630-645 [PMID: 23321675 DOI: 10.1172/JCI64265]

21 **Xie QH**, He XX, Chang Y, Jiang X, Lin JS. [HBx gene down-regulates miR-192 expression and inhibits apoptosis of human hepatoma cell line HepG2]. *Zhonghua Gan Zang Bing Zazhi* 2011; **19**: 857-860 [PMID: 22433310 DOI: 10.3760/cma.j.issn.1007-3418.2011.11.015]

22 **He Y**, Meng XM, Huang C, Wu BM, Zhang L, Lv XW, Li J. Long noncoding RNAs: Novel insights into hepatocelluar carcinoma. *Cancer Lett* 2014; **344**: 20-27 [PMID: 24183851 DOI: 10.1016/j.canlet.2013.10.021]

23 **Du Y**, Kong G, You X, Zhang S, Zhang T, Gao Y, Ye L, Zhang 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* 2012; **287**: 26302-26311 [PMID: 22685290 DOI: 10.1074/jbc.M112.342113]

24 **Huang JF**, Guo YJ, Zhao CX, Yuan SX, Wang Y, Tang GN, Zhou WP, Sun SH. Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. *Hepatology* 2013; **57**: 1882-1892 [PMID: 23239537 DOI: 10.1002/hep.26195]

25 **Murakami S**. Hepatitis B virus X protein: a multifunctional viral regulator. *J Gastroenterol* 2001; **36**: 651-660 [PMID: 11686474]

26 **Tu H**, Bonura C, Giannini C, Mouly H, Soussan P, Kew M, Paterlini-Bréchot P, Bréchot C, Kremsdorf D. Biological impact of natural COOH-terminal deletions of hepatitis B virus X protein in hepatocellular carcinoma tissues. *Cancer Res* 2001; **61**: 7803-7810

[PMID: 11691796]
27 **Wang Q**, Zhang T, Ye L, Wang W, Zhang X. Analysis of hepatitis B virus X gene (HBx) mutants in tissues of patients suffered from hepatocellular carcinoma in China. *Cancer Epidemiol* 2012; **36**: 369-374 [PMID: 22178505 DOI: 10.1016/j.canep]
28 **Xie Y**, Liu S, Zhao Y, Guo Z, Xu J. X protein mutations in hepatitis B virus DNA predict postoperative survival in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 10325-10331 [PMID: 25034530 DOI: 10.1007/s13277-014-2331-0]
29 **Liu XH**, Lin J, Zhang SH, Zhang SM, Feitelson MA, Gao HJ, Zhu MH. COOH-terminal deletion of HBx gene is a frequent event in HBV-associated hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1346-1352 [PMID: 18322946 DOI: 10.3748/wjg.14.1346]
30 **Hsia CC**, Yuwen H, Tabor E. Hot-spot mutations in hepatitis B virus X gene in hepatocellular carcinoma. *Lancet* 1996; **348**: 625-626 [PMID: 8774611]
31 **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* 1998; **143**: 2313-2326 [PMID: 9930189]
32 **Kim DC**, Chung WJ, Lee JH, Jang BK, Hwang JS, Kang KJ, Kwon SY. Clinicopathological characteristics of PIK3CA and HBx mutations in Korean patients with hepatocellular carcinomas. *APMIS* 2014; **122**: 1001-1006 [PMID: 24673525 DOI: 10.1111/apm.12245]
33 **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* 1999; **29**: 946-953 [PMID: 10051502]
34 **Iavarone M**, Trabut JB, Delpuech O, Carnot F, Colombo M, Kremsdorf D, Bréchot C, Thiers V. Characterisation of hepatitis B virus X protein mutants in tumour and non-tumour liver cells using laser capture microdissection. *J Hepatol* 2003; **39**: 253-261 [PMID: 12873823]
35 **Song LH**, Duy DN, Binh VQ, Luty AJ, Kremsner PG, Bock CT. Low frequency of mutations in the X gene, core promoter and precore region of hepatitis B virus infected Vietnamese. *J Viral Hepat* 2005; **12**: 160-167 [PMID: 15720531]
36 **Chen GG**, Li MY, Ho RL, Chak EC, Lau WY, Lai PB. Identification of hepatitis B virus X gene mutation in Hong Kong patients with hepatocellular carcinoma. *J Clin Virol* 2005; **34**: 7-12 [PMID: 16087118]
37 **Wang D**, Cai H, Yu WB, Yu L. Identification of hepatitis B virus X gene variants between hepatocellular carcinoma tissues and pericarcinoma liver tissues in Eastern China. *Int J Clin Exp Pathol* 2014; **7**: 5988-5996 [PMID: 25337243]
38 **Liu X**, Wang L, Zhang S, Lin J, Zhang S, Feitelson MA, Gao H, Zhu M. Mutations in the C-terminus of the X protein of hepatitis B virus regulate Wnt-5a expression in hepatoma Huh7 cells: cDNA microarray and proteomic analyses. *Carcinogenesis* 2008; **29**: 1207-1214 [PMID: 18477650 DOI: 10.1093/carcin/bgn111]
39 **Liu X**, Zhang S, Lin J, Zhang S, Feitelson MA, Gao H, Zhu M. Hepatitis B virus X protein mutants exhibit distinct biological activities in hepatoma Huh7 cells. *Biochem Biophys Res Commun* 2008; **373**: 643-647 [PMID: 18602370 DOI: 10.1016/j.bbrc.2008.06.087]
40 **Liu F**, You X, Chi X, Wang T, Ye L, Niu J, Zhang X. Hepatitis B virus X protein mutant HBxΔ127 promotes proliferation of hepatoma cells through up-regulating miR-215 targeting PTPRT. *Biochem Biophys Res Commun* 2014; **444**: 128-134 [PMID: 24434140 DOI: 10.1016/j.bbrc.2014.01.004]
41 **Wang Q**, Zhang W, Liu Q, Zhang X, Lv N, Ye L, Zhang X. 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* 2010; **12**: 103-115 [PMID: 20126469]
42 **Fu XY**, Tan DM, Hou ZH, Hu ZL, Liu GZ, Ouyang Y, Liu F. [Effect of microRNA on proliferation caused by mutant HBx in human hepatocytes]. *Zhonghua Gan Zang Bing Zazhi* 2012; **20**: 598-604 [PMID: 23207154 DOI: 10.3760/cma.j.issn.1007-3418.2012.08.012]
43 **Liu LP**, Hu BG, Ye C, Ho RL, Chen GG, Lai PB. HBx mutants differentially affect the activation of hypoxia-inducible factor-1α in hepatocellular carcinoma. *Br J Cancer* 2014; **110**: 1066-1073 [PMID: 24346287 DOI: 10.1038/bjc.2013.787]
44 **Elmore LW**, Hancock AR, Chang SF, Wang XW, Chang S, Callahan CP, Geller DA, Will H, Harris CC. Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc Natl Acad Sci USA* 1997; **94**: 14707-14712 [PMID: 9405677]
45 **Xian L**, Zhao J, Wang J, Fang Z, Peng B, Wang W, Ji X, Yu L. p53 Promotes proteasome-dependent degradation of oncogenic protein HBx by transcription of MDM2. *Mol Biol Rep* 2010; **37**: 2935-2940 [PMID: 19842060 DOI: 10.1007/s11033-009-9855-1]
46 **Kew MC**. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 144-152 [PMID: 21199526 DOI: 10.1111/j.1440-1746.2010.06546.x]
47 **Jiang W**, Wang XW, Unger T, Forgues M, Kim JW, Hussain SP, Bowman E, Spillare EA, Lipsky MM, Meck JM, Cavalli LR, Haddad BR, Harris CC. Cooperation of tumor-derived HBx mutants and p53-249(ser) mutant in regulating cell proliferation, anchorage-independent growth and aneuploidy in a telomerase-immortalized normal human hepatocyte-derived cell line. *Int J Cancer* 2010; **127**: 1011-1020 [PMID: 20017137 DOI: 10.1002/ijc.25118]
48 **Yan J**, Yao Z, Hu K, Zhong Y, Li M, Xiong Z, Deng M. Hepatitis B Virus Core Promoter A1762T/G1764A (TA)/T1753A/T1768A Mutations Contribute to Hepatocarcinogenesis by Deregulating Skp2 and P53. *Dig Dis Sci* 2015; **60**: 1315-1324 [PMID: 25567052]
49 **Luo J**, Zhou H, Wang F, Xia X, Sun Q, Wang R, Cheng B. The hepatitis B virus X protein downregulates NF-κB signaling pathways through decreasing the Notch signaling pathway in HBx-transformed L02 cells. *Int J Oncol* 2013; **42**: 1636-1643 [PMID: 23450368 DOI: 10.3892/ijo.2013.1842]
50 **Lim KH**, Choi HS, Park YK, Park ES, Shin GC, Kim DH, Ahn SH, Kim KH. HBx-induced NF-κB signaling in liver cells is potentially mediated by the ternary complex of HBx with p22-FLIP and NEMO. *PLoS One* 2013; **8**: e57331 [PMID: 23483900 DOI: 10.1371/journal.pone.0057331]
51 **Lee JH**, Han KH, Lee JM, Park JH, Kim HS. Impact of hepatitis B virus (HBV) x gene mutations on hepatocellular carcinoma development in chronic HBV infection. *Clin Vaccine Immunol* 2011; **18**: 914-921 [PMID: 21490166 DOI: 10.1128/CVI.00474-10]
52 **Zhang F**, Wang Q, Ye L, Feng Y, Zhang X. Hepatitis B virus X protein upregulates expression of calpain small subunit 1 via nuclear factor-kappaB/p65 in hepatoma cells. *J Med Virol* 2010; **82**: 920-928 [PMID: 20419804 DOI: 10.1002/jmv.21753]
53 **Liu LP**, Liang HF, Chen XP, Zhang WG, Yang SL, Xu T, Ren L. The role of NF-kappaB in Hepatitis b virus X protein-mediated upregulation of VEGF and MMPs. *Cancer Invest* 2010; **28**: 443-451 [PMID: 20073580 DOI: 10.3109/07357900903405959]
54 **Han JM**, Kang JA, Han MH, Chung KH, Lee CR, Song WK, Jun Y, Park SG. Peroxisome-localized hepatitis Bx protein increases the invasion property of hepatocellular carcinoma cells. *Arch Virol* 2014; **159**: 2549-2557 [PMID: 24810099 DOI: 10.1007/s00705-014-2105-4]
55 **Peifer M**, Polakis P. Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. *Science* 2000; **287**: 1606-1609 [PMID: 10733430]
56 **Kühl M**, Sheldahl LC, Park M, Miller JR, Moon RT. The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 2000; **16**: 279-283 [PMID: 10858654]
57 **Kühl M**, Sheldahl LC, Malbon CC, Moon RT. Ca(2+)/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus. *J Biol Chem* 2000; **275**: 12701-12711 [PMID: 10777564]
58 **Katoh M**. WNT/PCP signaling pathway and human cancer (review). *Oncol Rep* 2005; **14**: 1583-1588 [PMID: 16273260]
59 **Grigoryan T**, Wend P, Klaus A, Birchmeier W. Deciphering the

function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. *Genes Dev* 2008; **22**: 2308-2341 [PMID: 18765787 DOI: 10.1101/gad.1686208]

60 **Hsieh A**, Kim HS, Lim SO, Yu DY, Jung G. Hepatitis B viral X protein interacts with tumor suppressor adenomatous polyposis coli to activate Wnt/β-catenin signaling. *Cancer Lett* 2011; **300**: 162-172 [PMID: 20971552 DOI: 10.1016/j.canlet.2010.09.018]

61 **Liu XH**, Pan MH, Lu ZF, Wu B, Rao Q, Zhou ZY, Zhou XJ. Expression of Wnt-5a and its clinicopathological significance in hepatocellular carcinoma. *Dig Liver Dis* 2008; **40**: 560-567 [PMID: 18294932 DOI: 10.1016/j.dld.2007.12.011]

62 **Geng M**, Cao YC, Chen YJ, Jiang H, Bi LQ, Liu XH. Loss of Wnt5a and Ror2 protein in hepatocellular carcinoma associated with poor prognosis. *World J Gastroenterol* 2012; **18**: 1328-1338 [PMID: 22493546 DOI: 10.3748/wjg.v18.i12.1328]

63 **Lin X**, Wang Q, Cao Z, Geng M, Cao Y, Liu X. Differential Expression of Wnt Pathway Genes in Sporadic Hepatocellular Carcinomas Infected With Hepatitis B Virus Identified With OligoGE Arrays. *Hepat Mon* 2013; **13**: e6192 [PMID: 23483081 DOI: 10.5812/hepatmon.6192]

64 **Bi L**, Liu X, Wang C, Cao Y, Mao R, Li P, Geng M. Wnt5a involved in regulation of the biological behavior of hepatocellular carcinoma. *Int J Clin Exp Pathol* 2014; **7**: 987-995 [PMID: 24696716]

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