

Molecular mechanism of HBx protein function in HBV related hepatocellular carcinoma carcinogenesis

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality.¹ Based on significant differences in the geographic distribution of HCC incidence, chronic hepatitis B virus (HBV) infection has been identified as a major risk factor for HCC.^{2,3} The mechanisms underlying HBV-induced malignant transformation remain ambiguous; however, previous research has suggested that HBV X (HBx) protein has a crucial function in the pathogenesis of HCC.⁴ This minireview will focus on studies on the molecular mechanisms of HBx in HCC pathogenesis.

HBx gene and HBx protein

HBV contains a 3.2-kb circular double-stranded viral DNA genome and is considered to be the smallest DNA virus. The X gene of HBV contains the longest overlapping region between structural and functional sequences in the viral genome.⁵ More importantly, due to overlapping of the coding region and regulation elements in the X gene of HBV, any DNA mutation and/or deletion can affect its functions at both the gene and transcriptional regulation levels.^{6,7}

Effects of HBx

Previous research on the pathogenesis of HBx-induced HCC and on other aetiological forms of the tumour had suggested the role of several factors in HCC pathogenesis, including DNA repair, HBx methylation, non-coding RNA and HBx mutation.

Current studies indicate that DNA repair is one of the driving mechanisms of carcinogenesis. Accumulation of DNA damages results in genomic instability and eventually leads to mutations. Na et al.⁸ suggested that the physical interaction of HBx and poly ADP-ribose polymerase 1 (PARP1) accelerates DNA damage by inhibiting recruitment of the DNA repair complex to the damaged DNA sites, which may lead to the onset of hepatocarcinogenesis. Epigenetic changes suggest that HBx can upregulate DNA methyltransferase 1 (DNMT1) and DNMT3A through transactivation.⁹ Therefore, HBx affects DNMTs to inactivate tumour suppressor genes or cause chromosomal instability, thus serving an increasingly important function in hepatocarcinogenesis.¹⁰ With the development of new techniques, the interaction between HBx and ncRNAs, including micro RNAs and long ncRNAs (lncRNA), is becoming evident in the development of HCC. Micro RNAs and lncRNAs play a critical role in the control of gene expression and signal transduction in HCC carcinogenesis. Several *in vitro* studies have demonstrated that miR-19a, miR-122 and miR-223 are differentially regulated by HBx protein and are involved in cell proliferation in hepatoma cells.¹¹ HBx-upregulated lncRNA UCA1 promotes cell growth and tumorigenesis by recruiting EZH2 and repressing p27Kip1/CDK2 signalling.¹² During the period of initial HBV infection, mutations of the HBV genome accumulate. There are two dominant types of HBx mutations in chronic hepatitis: type I are single nucleotide mutations at multiple sites and type II are C-terminal truncations causing the accumulation of higher levels of protein in the tumour region. In

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short, HBx mutations promote tumour malignancy.^{13,14} However, the detailed mechanism needs further investigation.

HBx and signalling pathway

HBx is one of the driving key factors in cancer biology and participates in cross-talk with multiple signalling pathways to control tumour initiation, development, invasion and metastasis. Regarding the mechanism of action of various signalling pathways in HBx studies, p53, nuclear factor (NF)- κ B and Wnt signalling pathways have been the primary focus areas.

Several studies have indicated a complex transactivation between HBx and p53; HBx directly inhibits p53 activity by binding to its C-terminus.¹⁵ In addition, over expression of the p53 target gene murine double minute 2 can induce the degradation of HBx in HCC.¹⁶ Zhang et al.¹⁷ reported that HBx activates the binding of NF- κ B to the calpain small subunit 1 (Capn4) promoter to promote HCC cell invasion and metastasis. In addition, the combined effects of HBV and TNF may play important roles in the activation of metabolic pathways through the activation of NF- κ B.¹⁸

Highly preserved Wnt signalling has important functions in embryonic development, whereas abnormal Wnt signalling can stimulate tumorigenesis. Our study found that the Wnt-5a gene is regulated by HBx mutations through gene expression library screening. Further research showed that Wnt-5a may suppress tumour progression in HBV-induced HCC.¹⁹⁻²¹ An immunohistochemical study of 114 HCC samples demonstrated that Wnt-5a and its receptor, receptor tyrosine kinase-like orphan receptor 2 (ROR2), were down regulated in 80.7% (92/114) of samples. The expression of Wnt-5a is 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 exhibit a decreased proliferation rate, while Wnt-5a siRNA knockdown can increase cell proliferation.²² These findings suggest that HBx mutations can control tumour growth via signalling through the Wnt pathway.

Conclusion

HBx is the only expressed HBV viral protein in malignant HCC and has been demonstrated to be a key molecule in HCC carcinogenesis. However, the molecular mechanism of HBx-induced HCC progression remains unclear. HBx plays an important role in HCC tumorigenesis through its nuclear translocation, protein-protein interactions, regulation of transcription factors, induction of chromosome instability and nuclear-localised HBx-mediated signal transduction. These processes ultimately control cancer cell proliferation, transformation, invasion and metastasis. Based on the several studies regarding HBx mutations and the involved molecular mechanisms, it has been found that these mutations have different biological functions and activities compared to wild-type HBx and they may play important regulatory roles in the pathogenesis of HCC.

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