Hepatocellular Carcinoma Column: Editorial

# Dissecting the pleiotropic actions of HBx mutants against hypoxia in hepatocellular carcinoma

**Ying-Ying Lee[1,2], Myth T.S. Mok[3,4], Alfred Sze-Lok Cheng[2,3,4]**

[1]Institute of Digestive Disease and Department of Medicine and Therapeutics, [2]State Key Laboratory of Digestive Disease, [3]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China; [4]Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen 518052, China

*Correspondence to:* Alfred Sze-Lok Cheng, PhD. School of Biomedical Sciences and State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Hong Kong SAR, China; Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen 518052, China. Email: alfredcheng@cuhk.edu.hk.

**Abstract:** Error-prone integration of the hepatitis B virus X protein (HBx) into the hepatocellular genome generates a multitude of mutants exerting diverse effects on the development and progression of hepatocellular carcinoma (HCC). A recent study by Lai and colleagues revealed the disparate regulatory activity of clinically-predominant HBx mutants towards hypoxia-inducible factor-1α (HIF-1α), a central regulator of tumor angiogenesis, proliferation, metastasis and differentiation. These findings have shed insight into specific viral contribution of hypoxic response during hepatocarcinogenesis.

**Keywords:** Hepatitis B virus X protein mutant (HBx mutant); hepatocellular carcinoma (HCC); hypoxia-inducible factor-1α (HIF-1α)

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer death in China and the fifth most frequent malignancy worldwide (1). Among the known risk factors hepatitis B virus (HBV) plays a pivotal role in liver carcinogenesis particularly in Asia (2). According to World Health Organization (WHO) reports, an estimated two billion people are infected with HBV and about 600 thousand people die every year due to either acute or chronic consequences of HBV infection, commonly manifested as hepatitis, cirrhosis and HCC. Clinically, only a minority of HCC patients are eligible for surgical intervention because most patients present symptoms at advanced stages. Unfortunately, the post-hepatectomy survival rate remains low as recurrence is common in surgically treated HBV-associated HCC (3).

To help improve the clinical management and treatment of HCC, extensive researches were performed to identify the specific pathogenic factors in HBV-dependent HCC. To this end, multiple transgenic mouse models that express either full HBV genome or selected HBV fragments were generated to study the HBV-induced liver carcinogenesis *in vivo* (4-6). All these transgenic mouse studies consistently demonstrated that over-expression of the HBx gene alone is sufficient to cause HCC. Following studies further showed that the HBx protein can accelerate HCC development in many aspects encompassing apoptosis, proliferation, inflammation, angiogenesis, immune responses, and multi-drug resistance (7-10). This collective evidence reveals that HBx is the major functional player responsible for the development of HBV-dependent HCC.

Solid tumors depend on the formation of new blood vessels for growth and progression. To better understand the molecular mechanisms underlying the HBx-induced HCC, researchers went on to examine the potential cross-talk between HBx and hypoxia-inducible factor-1α (HIF-1α), the central regulator of angiogenesis (11). It was found that HBx primarily relies on its C-terminal peptide to enhance the transcriptional activity of HIF-1α to promote angiogenesis through modulation of p42/p44 mitogen-activated protein kinases (MAPKs) signaling pathways (12-14). Moreover, HBx can also induce the expression of metastasis-associated protein 1 (MTA1) and histone deacetylase 1 (HDAC1)

which physically interact with and stabilize HIF-1α (15). The discovery of this HBx-HIF-1α-dependent axis not only provides novel molecular insights on hepatocarcinogenesis, but also explains how HCC development can take place in hypoxic microenvironment.

In human HCC tissues, HBx are highly prone to mutations and are known to exist in many different forms. It was previously demonstrated that artificial point mutations at certain HBx codons caused a marked loss of transactivation capability, whereas HBx mutants with carboxyl (C)-terminal truncations significantly enhanced cell proliferation and transformation (16-18), suggesting that different mutations in HBx can lead to diverse consequences at cellular levels. Nevertheless, the effects of naturally occurring HBx mutants on the target gene HIF-1α have yet been evaluated. To address this, Liu *et al*. recently performed a clinically relevant study aiming to identify *bona fide* HBx mutations and examine their functions on HIF-1α activity (19). Using nested PCR technique, Liu *et al*. identified an array of HBx mutation hotspots in over 100 HCC samples, wherein the mutations at nucleotides 1630, 1721, 1762 and 1764 were present in more than half of the tested specimens. Of note, certain HBx mutations co-existed in a subset of samples, indicating a mixture of HBx isoforms in clinical HCC. The authors then cloned the identified HBx mutants and examined their potential stimulation on HIF-1α. Interestingly, it was found that all tested HBx point mutations significantly increased HIF-1α activity, with the maximal induction generated by the dual mutations K130M/V131I. In stark contrast, C-terminal deletions of HBx concordantly attenuated the induction effect on HIF-1α when compared with wild-type (wt) HBx. By monitoring the half-life of wt and mutated HBx, it was shown that C-terminal truncations markedly reduced the protein stability of HBx, providing an explanation for the loss of stimulation on HIF-1α. To further determine the clinical significance of these identified HBx mutants, immunohistochemical analysis revealed a positive correlation between the levels of HBx isoforms and HIF-1α in HCC tissues. Moreover, the expression of HIF-1α was associated with poor survival of HCC patients. Collectively, these findings suggest that the accumulative effects of coexistent HBx mutants in human HCC are to up-regulate HIF-1α transcriptional activity thereby contributing to HCC development.

This latest study presents an important concept that random mutations arising from the error-prone integration of HBx into human genome has significant functional impact, at least in part, in the HIF-1α-mediated hepatocarcinogenesis. Given the disparate roles played by different point mutations and C-terminal truncations in HBx, it will be of future interest to determine the underlying molecular basis, particularly the influence of individual mutations on the structural integrity of HBx. Nevertheless, more detailed analyses of HBx mutants, HIF-1α and HCC characteristics in larger clinical cohorts will be necessary to validate the positive association of the collective HBx mutants, HIF-1α and poor patient survival in HCC, as the antibody-based detection by Liu *et al*. lacks HBx sequence specificity and hence the ratio of individual HBx mutants in HCC remains elusive. Moreover, whether concordant HIF-1α over-expression occurs in precancerous lesions as observed for other important cellular effector of HBx (20) remains to be explored.

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