

Hidden secret in hepatitis B viral X protein mutation and hypoxia-inducible factor-1 α in hepatocarcinoma cancer

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Abstract: Hepatitis B type virus (HBV) is an old hepato oncogenic and hepatitis agent. Hepatitis B viral X protein (HBx)-induced malignant transformation requires the excess amounts of ATP level, inducing the extremely oxygen-deprived condition in the cancer tissues and vessels. To adapt, cells go to shift the hypoxic responsive state by altered hypoxia-responsive molecules such as HIF-1. In addition, tumors avoid or suppress immune recognition in the energy-deprived condition. The hypoxia-inducible factor-1 α (HIF-1 α) regulates MAP1, histone deacetylase and MAPK pathway. In the hypoxia, the HIF-1 α interacts with HIF-1 β , allowing DNA binding at the hypoxia response elements (HREs), while HBx binds with the nHLH/PAS domain of HIF-1 α , preventing pVHL and HIF-1 α binding capacity and degradation of HIF-1 α protein. Recent work of Liu *et al.* [2013] demonstrated that HBx in hepatocellular carcinoma (HCC) tissues contained mutations, affecting the HBx transactivation capacity and C-terminal HBx mutation. In the HCC tissues, the HBx C-terminal mutation and HIF-1 α expression were related and the different C-terminal mutations of HBx exhibit the different functionality of HIF-1 α . The C-terminal region of amino acids 119-140 was important for the stability and transactivation, and the point mutations K130M/V131I enhance the functionality of HIF-1 α , while C-terminal truncation diminish the HIF-1 α function.

Keywords: Hepatitis B type virus (HBV); hepatitis B viral X protein (HBx); hypoxia-inducible factor-1 α (HIF-1 α); C-terminal mutation; HIF-1 α function

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Hepatitis B type virus (HBV) is an old hepato oncogenic and hepatitis agent. It still induces acute and chronic hepatitis, and is associated with the hepatocellular carcinoma (HCC) in the human liver cancer. Among the four proteins of the HBV genome, Hepatitis B viral X protein (HBx) is associated with hepatocellular carcinogenesis (1), inducing liver cancer in transgenic mice (2). HBx causes the progression of liver cancer through the reduced expression of tumor suppressor genes such as p53 or PTEN (3) and the enhanced expression of matrix proteinases for the invasive potential of liver cells (4,5). However, the diverse and precise function of HBx in the metastasis of liver cancer remains still unclear, especially in its unexpected and hidden secret viewpoint. Although HBV induces hypoxia-coupled hepatocellular carcinogenesis and progression,

some questions remain as to how the HBV functions on the aberrant hypoxia-responded microenvironmental tissues and organs, and selectively occupies its specific hypoxia-related targets for carcinogenesis and malignant metastasis are the subjects to uncover.

Metastasis consists of the sequential steps, such as shedding of cells from a primary tumor into circulation, survival of the cells, arrest in a new organ, extravasation into the surrounding tissue, initiation and maintenance of growth, and vascularization of the metastatic tumor (6). Malignant transformation is associated with abnormal proliferation, requiring in the excess amounts of oxygen-consumed ATP level and energy source from proximal cells. This process makes the extremely oxygen-deprived condition in the cancer tissues and vessels. In the course

of the accelerated condition, the malignantly transformed cells go to shift the hypoxic responsive state by synthesis and expression of altered hypoxia-responsive proteins or lipids. The ability of tumor cells to evade and suppress the host immune system is decided by the differential levels of energy acquisition from the microenvironmental condition between tumor cells and normal cells including proximal host cells and immune cells. In addition, tumors often avoid or suppress immune recognition in the energy-deprived condition. One such mechanism is the survival protein expression from the locally oxygen-defected tumor microenvironment. The aggressiveness of cancer in oxygen-deprived condition involves the proliferation and vascular formation, as evidenced with the accumulated reports from various tumor cells. As a representative factor in the hypoxic condition, the hypoxia-inducible factor-1 (HIF-1) expression is markedly enhanced in cancer cells, as known for human bladder, breast, liver, ovarian, pancreatic, prostate and renal cancers (7). HIF-1 α is the most hypoxic protein and involved in regulation of many cellular pathways including migration, proliferation, senescence, transformation, survival and invasion in the extremely abnormal cancer cells. Thus, this extreme environmental-resistant factor plays a pivotal role in the metastasis of cancer cells in specific and particularly oxygen-overused or worst microenvironmental vessel system.

Since HIF-1 α levels change over time in tissue cells, particularly during differentiation, transformation, invasion, and progression, an alteration of the capacity of HBx to regulate HIF-1 α stability and expression could alter progressively the ability of cells to adapt to their hypoxic microenvironment in tissues and organs. For example, cancer metastasis event requires the abnormal expression of HIF-1 α . Besides localization in cytosol site of tumor cells, HIF-1 α interacts with its counterparts of the nuclear site. The HIF-1 α regulates metastasis-associated protein 1, histone deacetylase and mitogen-activated protein kinase pathway (8). In the hypoxia, the HIF-1 α , increased by HBx in liver cells, interacts with HIF-1 α , allowing DNA binding at the hypoxia response elements (HREs) and dramatic gene expression for proliferation, differentiation, cell-cell adhesion, angiogenesis for metastatic potential (7). Interestingly, it was suggested that HBx specifically induces expression of *HIF-1 α* gene associated with the initial shift down to the invasion mode. HBx binds with the nHLH/PAS domain of HIF-1 α , preventing pVHL and HIF-1 α binding capacity and degradation of HIF-1 α protein (9). HBx expression in cancer region of HBV-derived HCC is

associated with HIF-1 α expression compared to non-HBV HCC. Clinically mutations in *HBx* gene are frequently found in HBV-associated HCC and HIF-1 α contributes to HCC development and progression. Wild-type HBx activates HIF-1 α , however, the relationship between HBx mutations and HIF-1 α activation has not been explained in *in vitro* or *in vivo* experimental models or clinical cases.

In the recent study of Liu *et al.* (Leu LP, Hu BG, Ye C, Ho RLK, Chen GG ad Lai PBS. British Journal of Cancer, 2013, doi:10.1038/bjc.2013.787, 1-8) (10), HBx expression in terms of mutation in liver cancer region of HCC was associated with specific activation of HIF-1 α . Furthermore, the HIF-1 α was specifically induced both liver tissues from HBx-mutagenic models and in *in vitro* HBx-mutation or deletion-transfected cells and also in transcriptional activation using the luciferase reporter assay. Among many angiogenic and metastatic genes, HBx induces predominantly the transcriptional expression and activities of *HIF-1 α* gene. The HIF-1 α shRNA results if obtained may suppress the HRE-related gene expression induced by HBx, blocking the adhesion or progression by HBx-transfected cells in *in vivo* xenograft metastasis system. From the results, it is postulated that HBx in HCC tissues contained mutations, affecting the HBx transactivation capacity and C-terminal HBx mutation activates cell proliferation and transformation, targeting the process of cancer cell adhesion and cancer metastasis. From the present results, the relationship between HBx mutation and HIF-1 α expression and functions has been elucidated. The cloned HBx mutants were sequenced and experimentally transfected into HCC cells. Using the system, the behavioral pattern of HIF-1 α was analyzed for the expression and activation and for the relationship between HBx C-terminal mutations and HIF-1 α expression, indicating that the C-terminal region is crucial for the stability and transactivation potentials of HBx. Even in the HCC tissues, the HBx mutation and HIF-1 α expression were the same cases as above. It is confident that different C-terminal mutations of HBx exhibit the different functionality of HIF-1 α . Essentially, the carboxyl-terminal region of amino acids 119-140 seems to be important for the stability and transactivation of HBx. This is evident from the results showing that the point mutations K130M/V131I enhance the functionality of HBx-HIF-1 α , while C-terminal truncation or deletion downregulates the function of HBx-HIF-1 α in *in vitro* HepG2 cells and tissues from the HCC patients carrying different HBx mutants. At present, although the hypoxic conditions may protect tumor cells and enhance tumor progression using the above HBx-

HIF-1 α mechanism, the host immune inactivation should be further explained in this hypoxic and extreme condition. Seemingly, the precise molecular mechanism for tumor angiogenesis regulated by hypoxic condition is poorly understood, even though angiogenesis is associated with growth, invasion, and metastasis of solid tumors (11).

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