Role of Hepatitis B virus X Protein in DNA Repair During Hepatocellular Carcinoma Development

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Abstract

Hepatocellular carcinoma (HCC) is the most lethal cancer in the world. Hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol intake, and aflatoxin-B exposure have been identified as distinct causative factors for HCC. HBV infections play an important role in the development of HCC. HBV X protein (HBx) is a multifunctional protein that can modulate various cellular processes and plays a crucial role in the pathogenesis of HCC. HBx protein promotes cell cycle progression, inactivates negative growth regulators, and inhibits tumor suppressor genes such as p53. It has been shown recently that HBx modulates transcription of methyltransferases, causing regional hypermethylation of DNA that result in silencing of tumor suppressor genes. HBx is known to interact with DNA helicase components of transcription factor IIH (TFIIH), a basal transcriptional factor and an integral component of DNA excision repair.

Introduction

Hepatitis B virus (HBV) infection in humans is a major health problem and is one of the principal causative agents of liver disease. It is estimated that over 500 million individuals are infected with HBV worldwide and 1 million deaths are annually attributed to the effects of HBV infection [1-3]. The virus is associated with both acute and chronic liver diseases. Although the sequence of events in the development of hepatocellular carcinoma (HCC) remains poorly defined, a significant correlation has been made between long-term carriage of the virus and the development of HCC [1]. Modes of HBV infection are generally from mother to infant, by sexual routes and body piercing, or acupuncture. Several mechanisms by which HBV infection could lead to the development of HCC have been proposed. These mechanisms include insertional mutagenesis upon integration, trans-activation of the cellular genes, activation of signaling pathways, inactivation of tumor suppressor proteins, synergy with environmental carcinogenesis and host immune response.

One of the open reading frames of the HBV genome encodes a protein termed HBx. HBx is required for viral infection and has been implicated in virus-mediated liver oncogenesis. The HBx protein has been detected in liver tissue from patients with chronic HBV infection, cirrhosis and hepatoma [4-9]. It is now generally acknowledged that HBx supplied in trans can increase gene expression of a wide variety of viral and cellular promoters and enhancer elements [10,11]. HBx has been shown to possess pleiotropic functions including impairment of cell cycle progression [12], interaction with transcription machinery [8-11,13], and cell signal transduction and apoptosis mechanisms [14-17]. Furthermore, HBx associated physically with p53 resulting in the sequestration of p53 in the cytoplasm [18], inhibition of p53 function including its DNA binding and transactivation activities [19] as well as p53 interaction with XBP protein [19]. Several studies suggested a potential role of HBx cellular DNA repair process. HBx has shown to directly bind the TFIIH components xeroderma pigmentosum complementation group B (XPB) and group D (XPD), which are required for basal transcription as well as for the strand-unwinding step of the core nucleotide excision repair (NER) [20,21]. It also binds to a probable DNA repair factor ultraviolet-DNA-damaged binding protein (UV-DDB), Tumor Necrosis Factor-α (TNF-α).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a common malignancy and a leading cause of death worldwide. Recent epidemiological data have demonstrated that liver cancer incidence is continuously rising and will continue to do so for more than a decade, not only in Asia and Africa but also in North America and Europe [1]. HCC generally presents with poor prognosis, and no effective treatment is available for most HCC patients because this tumor remains refractory to current chemotherapeutic regimens [29]. However, HCC is frequently diagnosed when an advanced stage of the disease precludes local therapy and is unresponsive to surgery or chemotherapeutic agents. Current chemotherapeutic regimens [29]. However, HCC is frequently diagnosed when an advanced stage of the disease precludes local therapy and is unresponsive to surgery or chemotherapeutic agents.

Keywords: Hepatitis B Virus; Hepatocellular Carcinoma; HBx protein; TFIIH; Transcriptional transactivation; DNA repair.

Abbreviations: Hepatitis B virus (HBV); Hepatocellular Carcinoma (HCC); Hepatitis C Virus (HCV); Transcription Factor IIH (TFIIH); Excision Repair Cross Complementing (ERCC); Nucleotide Excision Repair (NER); Xeroderma Pigmentosum (XP); Activator Protein-1 (AP-1); Fatty Acid Synthase (FAS); Mitogen Activated Protein Kinase (MAPK); Replication Protein A (RPA); Ultraviolet-DNA-Damaged Binding protein (UV-DDB); Tumor Necrosis Factor-α (TNF-α).

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Ablative surgical interventions that could improve patient outcome. In this context, advances in our understanding of the molecular basis of HCC are urgently needed to develop early tumor markers and novel targeted agents with improved therapeutic efficiency [30,31].

Evidence has been provided for deregulation of various signaling pathways in HCC. Such as Wnt/β-catenin signaling [32], the p53 pathway [33], transforming growth factor beta (TGF-β) signaling, Ras/MAPK signaling and PTEN/AKT and mTOR pathways [34]. Additionally, altered expression of growth factors such as HGF, IGFs and amphiregulin, as well as genes involved in angiogenesis may participate in the development and progression of HCC [35] (reviewed in reference [1]).

The role of DNA damage response during the early stages of carcinogenesis has been reported in many types of cancer [36]. Genomic instability is a characteristic feature of HCC and has been proposed to contribute to the malignant transformation [37-39]. DNA double-strand break (DSB) constitutes a serious threat to genomic integrity. Inactivation of DSB repair is related to uncontrolled cell growth and increased cancer risk [40,41]. There has been compelling evidence that defective DSB repair accelerates liver carcinogenesis [42-45]. It has been demonstrated that dysregulation of Ku, the major component of DSB repair, renders hepatocytes sensitive to DNA damages induced by liver carcinogenesis [37]. A growing body of evidence further support a relationship between different Ku expression and HCC [46,47]. It has been shown recently that polymorphisms of XRCC5, Ku subunit, modulate the risk of development of HCC. In particular, the association were found more significant in HBV infected subjects than non-infected subjects [37].

Hepatitis B virus (HBV)

HBV is the prototype member of the Hepadnaviridae, a family of hepatotropic viruses. The genome of hepadnaviruses is a relaxed circular, partially doubled-stranded DNA genome that replicates via an RNA intermediate [48]. The HBV genome is around 3.2 kb in length and presents a highly compact genetic organization with 4 overlapping open reading frames (ORFs) that cover the entire genome. The pre-S/S ORF encodes the three viral surface proteins, the pre-C/C ORF encodes the e-antigen (HBsAg) and the core antigen (HBcAg), the P ORF encodes the terminal protein (TP) and the viral polymerase that possesses DNA polymerase, reverse transcriptase and RNaseH activities. The X gene encodes a small protein that is essential for virus replication but whose function remains partially understood. Finally, a viral protein termed HBSP has been shown to be encoded by a spliced viral transcript [49]. HBx is 154 amino acids in size, with a molecular mass of approximately 17.5 kDa. Comparative analyses of HBV X gene sequences from mammalian hepadnaviruses of different species revealed areas of high conservation, including presumptive helical domains located in the amino and carboxy-terminal regions, and a potential coiled-coil motif [50,51]. Several groups have reported that HBx undergoes phosphorylation and acetylation when it was expressed in insect cells [52]. HBx is found to be located in the cytoplasm of cells and in the nucleolus (reviewed in [53]). HBx protein has been found to play an important role in HBV transcription and replication. Early studies have shown that HBx stimulates the activity of viral promoters and enhancers, and that the closely related virus WHV deficient for the expression of WHx cannot replicate in the animal host [54]. More recently, it was found that the replication of X-deficient HBV genomes was strongly compromised in established cell lines and in the mouse liver and that HBx provides in "trans" was able to restore HBV replication to wild-type levels [55-57]. In most studies, this effect has been attributed to the trans-activator activity of HBx protein (reviewed in [1]).

HBV DNA integration into human host chromosomes occurs in the infected liver since early stages of natural acute infections [38,59]. Multiple integrations have been detected in chronic hepatitis tissue [60,61], and integrated HBV sequences have been seen in most (about 80%) HBV-related HCC [62,63].

HBx and transcription factors

HBx protein is multifunctional protein that can modulate various types of transcriptional factors (Figure 1). HBx does not bind directly to the DNA instead; HBx activates the transcription of host genes by its interactions with many transcription factors. The transactivation function of HBx may play an important role in HCC because it is involved in the activation of a large number of signaling pathways and cellular genes that are involved in oncogenesis, proliferation, inflammation and immune responses [64-66]. For example, it has been reported previously that HBx interacts with several basal transcriptional factors, such as transcription factor IIIB (TFIIIB), transcription factor IIH (TFIIH) [20,67], and RNA polymerase subunit RBP5 [68,69], tumor suppressor protein p53 [70,71], TATA binding protein (TBP) [72] and a putative DNA repair protein UV-DDB [23,73]. HBx interacts also with cAMP response element-binding protein (CREB), activating transcription factor-2 (ATF-2) [74-76], C/EBP and AP-2 to modify their activities [77]. It has been shown that HBx interacts with CREB-binding protein (CBP/p300) to synergistically enhance CREB activity [66]. It has been shown that HBx increases CREB/ATF DNA-binding activity as well as to enhance the recruitment of CBP/p300 to CREB/ATF bound to cellular DNA [66,78]. The modulation of CREB/ATF activity by HBx represents an important aspect of HBx activities since the CREB/ATF family members play an essential role in liver metabolism and proliferation, and CREB has been implicated in HCC [79]. Furthermore, evidence has been presented to indicate that

Figure 1: Signaling pathways regulated by HBx. HBx can stimulate signal transduction pathways such as MAPK and NF-κB. HBx also binds to various protein targets such as transcription factors, coactivators and components of the basal transcription machinery. HBx may play an important role in DNA repair by interacting with TFIIH, UVDBB1 and p53.
HBx and p53 Interaction

p53 is a well-known tumor suppressor gene, and mutational inactivation of p53 function or deletion of the gene increases susceptibility to cancer. p53 protein levels are upregulated by a post-translational mechanism in response to a range of stressors including DNA damage-inducing chemical compounds, UV irradiation, hypoxia and oncogenic viruses. p53 is a transcription factor that upregulates transcription of genes whose products block the cell cycle at G1/S phase boundary, thereby allowing repair of DNA lesions before DNA replication. Cells with irreparable damage are usually eliminated by p53-dependnet apoptosis [85]. HBx has been shown to inhibit [86-88] as well as induce [16,89,90] apoptosis. The mechanism of regulation of apoptosis by HBx was reported to be via both p53-dependnet [15,91] and p53-independent [17] pathways. In the case of HCC: the frequency of p53 mutations is not as high as those observed in other types of cancer, suggesting that there may be a unique mechanism for p53 inactivation in HCC.

It has been shown previously that HBx binds to the C-terminus of p53 and induces its sequestration from the nucleus to the cytoplasm, thereby leading to inhibition of its effects on cell cycle arrest and DNA repair [92]. HBx also represses p53-mediated transcriptional activity, inactivates the sequence-specific DNA binding activity of p53 and the associations between p53 and the nucleotide excision repair gene products XBP and XPD [93]. HBx was shown to repress two components of the transcription-repair factor TFIIH, XBP, and XPD, both in p53-proficient and p53-deficient liver cells. This inhibition is observed while HBx maintains its transactivation function. Expression of HBx in liver cells results in down-regulation of endogenous XBP and XPD mRNAs and proteins. In liver tissue from HBx transgenic mice do not exhibit tumor development unless coupled with other factors such as exposure to the hepatocarcinogen diethylaminoethyl nitrosamine [102] or when combined with c-myc induction [103]. It has been suggested previously that HBx does not directly cause cancer but plays a role in liver oncogenesis as a cofactor or tumor promoter [104]. Chronic HBV infection may present a long-term opportunity for an initiating event to occur, and HBx may act by modifying cellular regulatory/control mechanisms facilitating the culmination of the transformation process in the cell. In this regard, a highly probable tumor-initiating event is DNA damage.

DNA repair system is the primary defence against accumulation of mutations in genomic DNA and activation of cellular carcinogenesis. Deficiencies in DNA repair pathways have been linked to common cancer predisposition syndromes. Notable among these are the hereditary nonpolyposis colorectal cancer (HNPCC) and skin cancer or xeroderma pigmentosum (XP) [105,106]. DNA repair occurs by two different pathways: global repair removes DNA adducts from anywhere in the genome including from non-transcribed strand of active genes, where transcription coupled-repair removes DNA adducts from the transcribed strand of active genes [106-108]. Studies have demonstrated that some of the essential DNA repair proteins in yeast and mammalian cells are a part of basal transcription factor TFIIH [67,108,109].

The nucleotide excision repair (NER) pathway is responsible for the repair of a number of DNA lesions [110]. NER is an enzymatic pathway involving more than 30 proteins, including the single-stranded DNA-binding protein, RPA. DNA repair in HCC is likely to be closely linked to DNA repair pathways, which may underlie the development of the disease. Studies have shown that HBx can antagonize the pro-apoptotic function of HBx [95]. Moreover, inhibition of the PI3K and SAPK/JNK pathways by HBx exerts anti-apoptotic functions against transforming growth factor-β (TGF-β) [88] and Fas/FasL signaling [96,97]. It has been shown that HBs can stimulate cellular calcium signaling pathways to release calcium ions into the cytosol, leading to activation of local adhesion kinase and proline-tyrosine kinase 2 and the subsequent activation of Src tyrosine kinases with their downstream Ras-Raf-MAPK signaling pathways [98,99].

HBx and DNA repair

It has been shown that mice carrying HBx as a transgene show a direct correlation between the level of HBx expression and the likelihood to develop HCC [100,101]. However certain lineages of HBx transgenic mice do not exhibit tumor development unless coupled with other factors such as exposure to the hepatocarcinogen diethylaminoethyl nitrosamine [102] or when combined with c-myc induction [103]. It has been suggested previously that HBx does not directly cause cancer but plays a role in liver oncogenesis as a cofactor or tumor promoter [104]. Chronic HBV infection may present a long-term opportunity for an initiating event to occur, and HBx may act by modifying cellular regulatory/control mechanisms facilitating the culmination of the transformation process in the cell. In this regard, a highly probable tumor-initiating event is DNA damage.

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using normal DNA replication factors and undamaged complementary strand as template (Figure 2) [80].

In humans, the defects in XPD/ERCC2 and XPD/ERCC3 genes lead to xeroderma pigmentosum (XP) [113] and Cockayne’s Syndrome (CS) [105,107]. Both conditions are manifested by the inability of the cells to efficiently repair damaged DNA.

HBx has been shown to enhance cell susceptibility to the cytotoxic effect of genotoxic agents, e.g. UV or aflatoxins, that induce bulky adducts. This effect has been linked to impaired regulation of DNA repair and associated cell cycle checkpoint mechanisms [20,67,73,77], and/or the proapoptotic effect of HBx [114]. DNA damage induced by bulky adducts are preferred substrates for NER mechanism, where the TFIIH repair complex plays an essential role [82]. Inhibition of TFIIH activity by HBx may inhibit DNA repair and hence promote cells to undergo apoptosis. While several studies have focused on the transactivation capacity of the HBx protein in carcinogenesis, recent studies showed that HBx is capable of transcriptional repression while maintaining its transactivation functions on NF-kB and API responsive elements [115]. The implication of transactivation in carcinogenesis is demonstrated primarily in transient systems and there is evidence that HBx-induced transactivation is not sufficient for cell transformation [116].

We have shown recently that HBx inhibits DNA repair pathway [117]. In the absence of UV damage, cells expressing HBx were found to be similar to control cells in cell growth measured by colony formation assay. Similar observations were reported by Lee and co-workers [104]. They demonstrated that HBx expression did not affect the morphology, viability, and cell cycle/apoptosis profiles or DNA repair machinery of UV-ununtreated HepG2 cells. However, HBx-expressing cells exhibited increased sensitivity to UV damage and reduced DNA repair capacity. The observation that HBx suppresses XPB and XPD in liver tissue from HBx-transgenic mice supports the biological relevance of our recent findings [117]. XPB and XPD helicase and ATPase activities, but not the TFIIH kinase, are required for NER function [82-84,118]. The XP and XPD proteins of the TFIIH complex are also involved in transcription-coupled repair [117]. Several transcription factors responsive elements are present in both XPB and XPD promoters, including Sp1. Sp1 transcription factor has been shown to be a specific target for HBx protein resulting in impairment of its DNA binding properties [12].

It has been shown previously that HBx protein interferes with NER through both p53-independent and p53-dependent mechanisms. HBx protein inhibits cell cycle checkpoint mechanisms required for DNA repair and binds to damaged DNA, interfering with NER and facilitating the accumulation of host DNA mutations [119,120]. HBx also binds to several cellular proteins known to be involved in DNA repair pathways including human homology of the UV-DDB [121].

It has been shown that pre-S1 and pre-S2 mutant HbsAG caused oxidative stress and DNA damage in Ground glass hepatocytes (GGHs), the pathological hallmarks for late phases of chronic HBV infection [122]. The DNA repair gene oggi was greatly induced by over-expression of pre-S mutant HbsAg. Other studies have reported that a defect in the oggi DNA repair gene is involved in various types of human carcinogenesis [123].

Previous studies showed that the HBx induced oxidative stress and high levels of ROS production, leading to oxidative DNA damage [124]. The DNA repair enzymes, human 8-oxooguanine DNA glycosylase 1 (hOGG1) and DNA glycosylase a (hMYHa) are crucial for repairing oxidative DNA damages. It has been shown recently that expression of HBx in non-tumor hepatic LO2 cells induced malignant transformation by promoting the accumulation of 8-hydroxydeoxyguanosine (8-OhdG) in LO2/HBx cells, which was accompanied by significant lower levels of hMYHa, indicating the presence of HBx-related oxidative DNA damage in human hepatic cells [125]. Therefore, efficient DNA repair for damaged DNA should play an important role in cancer prevention. These studies and our study [117] suggest that HBx may act as the promoting factor by inhibiting DNA repair causing DNA damage and accumulation of errors, thereby contributing to HCC development.

Mapping of the functional domains of HBx

Many studies showed that HBx plays an important role in HCC pathogenesis by interacting with cellular oncogenes [23,71,72] and that its functional domain involved in oncogenesis is at the middle of HBx protein [20,73].

Tang and co-worker has mapped the coactivation domain within the C-terminal, two thirds of which (aa51-138) is identified to that of the transactivation. In contrast, the N-terminal of HBx has the ability to down regulate transactivation and was defined as the negative regulatory domain [126].

It has been shown recently that the COOH-terminal truncated HBx plays a critical role in the HCC carcinogenesis via the activation of cell proliferation [127]. Alteration of HBV X gene has been detected more frequently in tissue samples of cirrhosis and/or HCC than in those of mild liver disease [128]. However, the mechanism of HBx in HCC carcinogenesis is still unclear, although many studies have associated it to ability of HBx trans-activating cellular oncogenes and signaling cascades that stimulate cell proliferation and lead to HCC

Figure 2: Model for HBx inhibition of NER. Three protein complexes are involved in DNA-damage recognition in NER pathway, XPA, XPC and RPA (I). These proteins recruit the transcription factor IIH (TFIIH) that incorporate two helicases XPD and XPF that unwind a 30 bp DNA fragment around the DNA damage (II). After DNA unwinding, damaged-DNA strand is excised by XPG and XPF-ERCC1 complex (III). After excision, damaged-DNA strand is removed and replaced by re-synthesizing the template complementary DNA strand by polymerase complex (IV). HBx inhibits NER by inactivating the DNA damage-binding (DDB) protein. HBx also downregulates the expression of HBx and XPD and interfering with TFIIH function leading to accumulation of errors, thereby contributing to HCC development.
carcinogenesis [1,76,129-131]. It has been demonstrated that the full-length HBx contains two function domains: oncogenic domain (the NH2 terminal through middle peptide) and proapoptotic domain (the COOH-terminal peptide). There is a balance between these two functions in HBV-infected hepatocytes. When the proapoptotic domain is deleted by an unknown mechanism during the viral integration, the balance is broken and the oncogenic function becomes dominant, leading to the subsequent development of HCC.

**Summary and Conclusions**

To date, a few mechanisms of HBV-induced HCC have been proposed. Early studies proposed that insertional mutagenesis of the HBV genome into human chromosomes might cause inactivation of tumor suppressor/proto-oncogenes [132-134]. However, later studies have shown that integration of HBV genome is genome-wide and unlikely attacks a specific tumor suppressor or proto-oncogene [134,135]. HBx initiates transactivation as well as induction of signal transduction pathways such as Ras/Raf-1 [136,137].

It has been suggested that inhibition of DNA repair mechanisms by HBV products may contribute to observed synergetic interaction between chronic infection with HBV and exposure to liver carcinogenesis [37]. We have recently defined the inhibitory role of HBxs in DNA excision repair process, thus hampering the cellular ability to repair the damaged DNA more effectively during HBx expression. Therefore, efficient DNA repair for damaged DNA should play an important role in cancer prevention. Our findings suggest that HBxs may act as the promoting factor by inhibiting DNA repair causing DNA damage and accumulation of errors, thereby contributing to HCC development. As I described in this review, HBx has multifunctional role in the development of HCC. Cancer cells are often defective in both signaling and repair pathways. Understanding the effect of HBxs on these two pathways could open ways to make tumours more vulnerable to combinational therapy.

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