



Hepatitis B Virus X Gene: A Key Player in Hepatocellular Carcinoma Progression

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Abstract

Chronic Hepatitis B virus (HBV) infection is a major global health issue, affecting around 296 million people worldwide. It increases the risk of developing liver cancer. The Hepatitis B virus X gene in the HBV genome is an important regulator and oncogene, essential for chronic HBV infection and liver cancer progression. Understanding its function will help in developing targeted therapies for HBV-related liver cancer. Determining the incidence of HCC with different genotypes and HBx mutations is necessary. In this review, the Hepatitis B virus X gene is signified and implicated as the principal role in causing HCC. This review aims to evaluate the significance of Hepatitis B virus X to that of other HBV genes. HBV gene mutations such as P, S, and C are responsible for viral replication, antigen expression, and a disrupted immune response, respectively. Compared to other genes, the X gene of the hepatitis virus has the worst clinical results that significantly lead to Hepatocellular carcinoma. Most previous research has concluded that HBx significantly leads to chronic HBV infection. Therefore, the HBV risk factors, prevalence, and HBx mutations are essential in spreading the chronic infection. Further studies on the HBx gene is still required to explore in countries like Pakistan in terms of leading to disease like hepatocellular carcinoma.

Keywords: Hepatitis B virus; Hepatocellular carcinoma; HBx protein; Pakistan

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1. INTRODUCTION

Hepatitis B Virus belongs to the Hepadnaviridae family and is a DNA virus. HBV reproduces its genome through reverse transcription. It has a completely formed minus strand and a partially developed plus strand. The HBV genome is circular and double-stranded, measuring 3.2 kb ¹. The prevalence of various HBV genotypes varies around the world. North America, Northern Europe, Africa, and India all have genotype A. Southeast Asia has a higher prevalence of genotypes B and C. In contrast, southern Europe, India, and the Middle East have a higher prevalence of genotype D ².

According to the previous study, patients with genotype A have more chronic genotypes. Patients with genotypes C, D, and F have worse clinical results because they are bound to foster cirrhosis and HCC than individuals with different genotypes ³. The only known natural host is a human. HBV replicates only in liver tissue after entering the liver through circulation. HBV hijacks lipid transport routes to reach hepatocytes,

including lipoproteins and macrophages' reverse cholesterol transport system⁴. HBV infection is linked to an increased risk of liver fibrosis in patients with metabolic-related fatty liver disease⁵. HBV integrations in liver disease like HCC exhibit different sequences at junctions. The presence of the vh-DNA, a combination of virus-host chimaera DNA, may serve as a unique identifier for different cases of HCC⁶. HBx is believed to have a direct or indirect involvement in the initiation and progression of HCC.

HBx promotes cancer by increasing genomic instability, activating telomerase, altering the epigenome, and regulating cell death and proliferation. It degrades the Smc5/6 protein complex, leading to poor DNA damage repair and increased cancer risk. The F132A variant, which doesn't degrade Smc5/6, can be used to study the role of HBx in hepatocellular carcinoma growth and HBV spread⁷. In a recent study, researchers employed techniques such as molecular docking, molecular dynamics simulation, MM/GBSA, and T-SNE to investigate potential antiviral compounds capable of inhibiting the HBx protein's function. Their complex stability and conformation clustering analysis pinpointed three promising agents: SC75741, Punicalagin, and Ledipasvir. Among these, SC75741 demonstrated the highest potency with a binding energy of -9.9 kcal/mol. These findings hold promise for developing more effective anti-HBV drugs, indicating a potential breakthrough in combating hepatitis B virus infections⁸.

Various risk factors contribute to the greater incidence of hepatitis B and C in contemporary society. It includes prior dental work, household contacts, surgery, transfusion history (blood and its components), and sexual interaction⁹. A study in southern Iran indicates that the probability of risk factors like tattooing causing HBV infection among pregnant women was surprisingly low. Furthermore, none of the pregnant women who tested positive for HBV experienced symptoms and were entirely unaware of their infection¹⁰. The systematic studies exhibit genetic diversity with various prevalent genotypes; specific genes or genotypes are associated with a higher risk of chronic diseases like liver cirrhosis (LC) and Hepatocellular Carcinoma (HCC).

2. HBV GENOME AND GENES

The HBV genome has four ORFs that overlap. Because of the circular DNA and partially positive and negative organization of strands, they do overlap (Fig.1). These ORFs encode core proteins, viral envelope proteins, viral polymerase, and HBx proteins¹¹. The S ORF encodes surface envelope proteins, including HBsAg. The P ORF encodes polymerase protein. The C gene contains pre-core and core regions and can produce different proteins due to multiple translation initiation codons. Translation of hepatitis B virus (HBV) RNA can result in the production of two essential proteins, either the viral nucleocapsid (HBcAg) or the hepatitis B e antigen (HBeAg). The core protein can form a capsid-like structure and has a group of positively charged amino acids at its C-terminus. The HBxAg, encoded by HBV X ORF functions in the viral life cycle, is not fully understood, but it is essential for HBV infection and may be involved in its cancer-causing potential¹².

The polymerase gene overlaps with the S gene or envelope gene. That is why any mutation in the RT domain of the polymerase gene can ultimately affect the amino acid sequence of HBsAg¹³. The core protein is produced from the core gene, while the pre-core/core protein is generated from the pre-core ATG. The pre-core/core protein is cleaved to form hepatitis B e antigen (HBeAg). The expression of core protein or HBeAg depends on the transcript¹⁴. The covalently closed circular (ccc) HBV DNA in the nucleus generates four HBV mRNA size variations (0.7, 2.1, 2.4, and 3.5kb). The HBV genome has an exposed central hydrophilic region, the S gene. It spans residues 110 to 155 and contains the 'a' determinant, crucial for triggering immunity. It is typical for nucleotide changes in this area to impair the binding of or prevent the detection of hepatitis B surface antigen (HBsAg) in diagnostic assays. Because the S gene and the polymerase gene overlap, mutations in the S gene can drastically change the polymerase protein's function. The HBV core protein, one of HBV's encoded proteins, can trigger interferon and cytokine production in human hepatoma cells and mouse models, potentially contributing to persistent HBV infection and liver cancer development. The inflammation caused by these host molecules is linked to the development of hepatocellular carcinoma (HCC) in HBV infection. Researchers are actively studying the connection between the HBV core protein and cytokine production to better understand HBV infection's progression and develop potential therapies¹⁵. Other than these genes, a highly conserved gene is called HBx. It produces the polypeptide having 154 amino acids known as HBx. It has a molecular weight of 17 kDa¹⁶⁻¹⁸. HBx gene is still the most crucial gene to study in causing HCC.

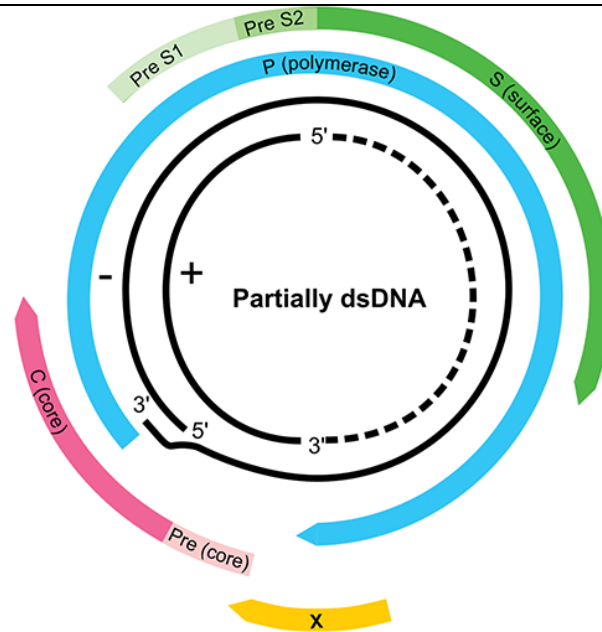


Fig. 1. HBV Genome, including its open reading frames.

2.1. Mutations in HBV Genes

All four overlapping open reading frames encoded for core protein, surface antigen, X protein, and viral polymerase have HBV mutations. A lack of standardized terminology and discrepancies in assay sensitivities undermine the clinical importance of HBV mutations. Studying mutations in HBV genes in the same species has also been challenging. It is because of mutations in more than one area of the HBV genome^{19,20}. Previous systematic reviews reported mutations in these genes could impact viral replication, antigen expression, and clinical outcomes. Understanding the relationship between HBV mutations and the development of liver disease, including chronic infection and hepatocellular carcinoma, is crucial for effective clinical therapy and management of HBV-related conditions. Table 1 shows the HBV genes and their respective functions and mutations.

Table 1 HBV Genes: Functions^{21,22} and Mutations

HBV Genes	Open Reading Frames	Encoded Viral Proteins	Functions	Mutation in HBV Genes
S Gene	ORF S	HBsA, Pre S1 and Pre S2 (a component of the envelope protein)	Expression of surface antigens	Mutation in the S gene can affect the expression of HsAg and hence cause occult hepatitis B infection or reactivation of hepatitis B ²³ .
C Gene	ORF C	(HBcAg) and HBeAg	Genome replication and progeny virion production	Mutations in the C gene of HBV can alter the core protein's structure, allowing HBV to evade immune detection. The virulent strains can consistently provoke host immunological responses and cause severe liver disease ²⁴ .
Polymerase Gene	ORF Polymerase	viral polymerase protein (Pol)	DNA synthesis/ RNA degradation, viral and RNA binding, pgRNA packaging	Mutations in the P gene can alter infectivity, viral secretion, and resistance to anti-HBs antibodies ²⁵ .

X Gene	ORF X	HBx protein	Pathogenesis: Cell cycle progression, apoptosis, and signal transduction	Liver cirrhosis and HCC ²⁶ .
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3. HBV RELATED HCC IN PAKISTAN

HBx shows high expression in HCC tissues. It significantly stimulates HCC invasion and metastasis in vivo and in vitro with oncogene activity ²⁷. HCC is more prevalent in males, older individuals, and urban areas. Diabetes, hypertension, and obesity increase the risk of HCC. The higher incidence of HCC in Pakistan is due to factors like hepatitis B and C infections, exposure to aflatoxin, and the high prevalence of diabetes and obesity ²⁸. The prevalence of HCC is rising in Pakistan; its chronicity is consistent with our community's increased exposure to HCC risk factors. According to recent findings, hepatobiliary malignancies may be the widespread malignancy in our society among adult males. According to data from a study, age-standardized rates of HCC in Pakistan are 2.8 for women and 7.6 per 100,000 people per year for men. These investigations are primarily based on hospital-derived data. As a result, they do not accurately represent the most recent population-based prevalence of HCC ²⁹.

HCC is seen in Pakistan's 3.7% to 16% of malignant tumours, with viral hepatitis being the most common cause. In Pakistan, B-related cirrhosis (22%) or viral hepatitis C (68%) causes roughly 87% of HCC. Another study in Pakistan, including Hyderabad and Sindh, found a significant prevalence of viral-associated HCC (HBV 43% and HCV 66%) ³⁰. A study from Karachi found an increase in HCC admissions and the most significant incidence of virally associated HCC. These findings show that most cases are found when the disease is already advanced or leading to chronicity, with few effective treatment options ³¹. The age-standardized rates of hepatocellular carcinoma (HCC) in Pakistan have shown an increasing trend. A study conducted in Pakistan found that the age-standardized incidence rate of HCC was 5.8 per 100,000 population. This rate is higher than the global average, estimated at around 2.7 per 100,000 population. The provided sources do not directly compare the age-standardized rates of HCC in Pakistan to global trends. However, the information provided in the sources allows us to infer that the rates in Pakistan are higher than the worldwide average. The studies conclude that men are more prone than women to acquiring HCC globally, and comparable evidence is evident in Pakistan. HCC is infrequently seen throughout the first forty years of life, except in South Asia, where liver cirrhosis is widespread. As a result, in Pakistan, 5.7 males and 3.7 females have an age-adjusted incidence of HCC ³². The studies also draw attention to determining HBx as the root cause of chronic HBV infection/HCC. Table 2 shows the following studies, which signify and determine HBx as the leading cause of HCC.

Table 2 HBx causing HCC ^{33, 34}

HBx Activity	Causing HCC	Study Design
Modification and activation of α integrin subunits and β 1 integrin subunits, respectively	Triggering invasion and metastasis	Chang cell line expressing HBx in vitro
Nuclear factor- κ B/p65-mediated upregulation of Capn4		In vitro HBx-expressing HepG2 and H7402 cell lines
Activation of the CD44 cell-surface adhesion molecule		Chang cell line that is HBx-expressing in vitro

HBx can interact with chromatin-modifying enzymes and influence regulatory non-coding RNAs, including microRNAs and long ncRNAs. It can impact chromatin structure and gene expression. HBx also interacts with signal-transduction pathways like p53, Wnt, and nuclear factor- κ B, which regulate chromatin modifications and gene expression ³⁵. These interactions between HBx and chromatin-modifying enzymes control viral and cellular gene expression, ultimately aiding HBV replication. The HBx protein can initiate epigenetic alterations that disrupt miRNA expression, subsequently influencing downstream epigenetic modifications in the development of HBV-related liver cancer (HBV-HCC). The misregulation of miRNA triggered by HBx can

impact and be impacted by epigenetic changes, resulting in the adjustment of gene expression in both the virus and the host. This intricate interplay involving HBV infection, epigenetic alterations, disease progression, and the immune response contributes to severe conditions in HBV-HCC³⁶.

4. HBV X GENE AND HCC

It is thought that HBx is engaged in several functions, including transcriptional activation, cell cycle control, signalling, and DNA repair³⁷.

4.1. Function of HBx

Comparative to other genes of HBV, as mentioned in Table 1, HBx has an epigenetic role. It interacts with chromatin-modifying enzymes to target the epigenetic regulation of cellular gene expression. The function of HBx can enhance the expression of microRNA-155. It also suppresses the PTEN/PI3K-AKT pathway in cellular and clinical terms. A recent study found that the HBx protein expression in yeast models significantly affects the shape of the mitochondria. Increasing DNA methylation and reducing GAS5 expression, HBx may improve HCC cell survival and invasion. Boosting HBx-downregulated GAS5 might successfully restrict cell viability. As a result, it invades by binding to YBX1 and activating the YBX1/p21 signalling pathway³⁸⁻⁴¹. These studies depict the essential role of HBx in leading to lethal cellular conditions.

4.2. HBx Mutation and Expression Leading to HCC

In Pakistani HBV isolates, patients with HBV-positive disease frequently have mutations in the Enh-II region of the X gene. It is linked to LC and HCC (Fig.2). The mutations identified in CH patients are concerning since many people may develop LC and HCC due to these mutations⁴². In recent literature, four new mutations were found related to HBx. The B-cell epitope of the HBx protein had two unique alterations, C1491G and C1500T. (aa 26–48). In the enhancer II area, a new mutation called G1658T was discovered (Enhancer II: 1627–1774). The K95N-causing G1658T mutation also manifests in the HBx protein's T helper cell (TH) epitope (aa 91–105). The mutation Glu80 to Asp G1613T was also identified⁴³. These specific HBx mutations may affect how HBV interacts with the host's innate immune system. It might restore IFN- production, and ultimately, the increased proinflammatory cytokine production might help revive worn-out immune responses and break through to the immunological-tolerant phase⁴⁴. According to a recent study, the C1653T mutation of HBx increases the likelihood of HCC. The C1653T (H94Y) mutation in HBx, found in the EnH II region, can worsen outcomes in liver cancer (HCC). It affects the binding of nuclear factors and causes a His94Tyr amino acid change in HBx. This mutation boosts HBx-induced cell growth, invasion, migration, and apoptosis in HCC cells. In a mouse model, it also promotes tumour growth. The mutation increases fibrosis, intracellular ROS production, alters MCP-1 and interleukin 18 expression in HepG2 cells, contributing to HCC. The success of apatinib treatment for HCC patients may be assessed using this mutation as a possible biomarker. In combination, the HBx mutations C1653T+T1674G+A1762T/G1764A induce cancer-promoting inflammation. It can increase the expression of PAI1 and CDC20, ultimately promoting carcinogenesis^{45, 46}.

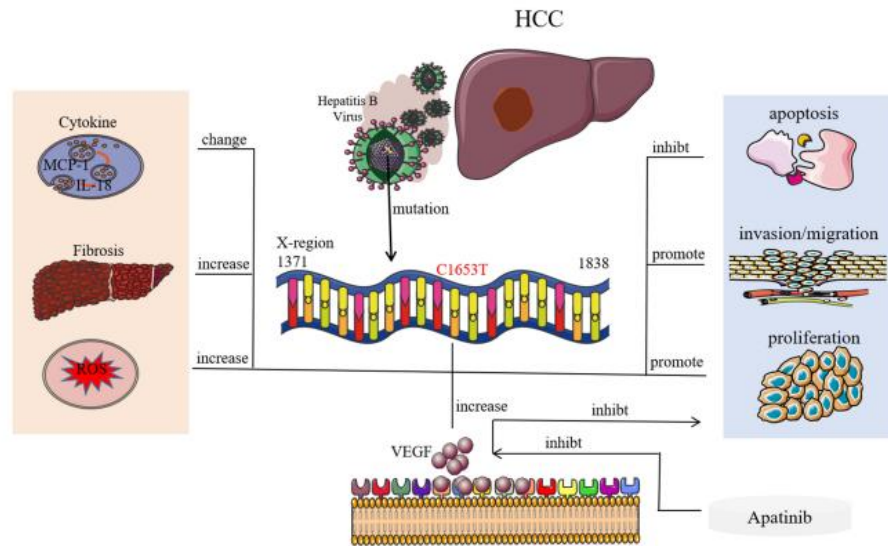


Fig. 2. Mutations in HBx region of HBV; causing proliferation of cells and counter effect of treatment.

The technique of Immunohistochemistry has confirmed HBx expression in HCC tissues. An experimental study used a recombinant adenovirus vector to introduce the HBV X gene into SMMC-7721 HCC cells. It demonstrated its invasive and metastatic potential in a mouse model²⁷. These findings highlight the essential role of HBx in the progression of HCC. It also targets the HBV X gene as the therapeutic agent. There is a high need to introduce a course of therapy or diagnosis to regulate this specific gene that will ultimately control the HBV infection or prevent it from progressing to the chronic stage.

5. CONCLUSIONS

It is crucial to comprehend the precise role of HBx in the onset, progression, and drug resistance of Liver cancer and chronic HBV infection. The genome of HBV is based on four genes producing seven proteins. As the HBV genes overlap, any mutation in a single gene can affect the function of another gene. This review aims to compare the significant role of Hepatitis B virus X with other HBV genes. The mutations in HBV genes like P, S, and C are responsible for viral replication, antigen expression, and disturbed immunological response, respectively. Comparative to other genes, the X gene of the hepatitis virus has the worst clinical outcomes. Also, it demonstrates the HBx gene's essential role in chronic infections like Hepatocellular carcinoma. The previous findings have shown the HBx expression in HCC tissues. It may significantly increase HCC invasion and metastasis via oncogene activity. This review demonstrates and calls for further research into the Hepatitis B virus X gene (especially in countries like Pakistan), which promotes the chronicity of hepatitis B virus infection. The review claims the HBV X gene is a potential therapeutic target in HCC.

CONFLICT OF INTEREST

The authors have no conflict of interest.

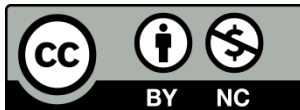
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