

## MINI-REVIEW

# Hepatitis B Virus Gene Mutations and Hepatocarcinogenesis

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### Abstract

Chronic hepatitis B virus (HBV) infection has long been the most common cause of hepatocellular carcinoma (HCC). However, some aspects of the pathogenesis of HBV infection and genesis of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) are still inconclusive. An increasing number of published studies indicate that hepatitis B virus mutations are associated with risk of HCC. These variations include, in particular, mutations in ORF S,C,X gene regions. This mini-review summarizes results of clinical studies and molecular mechanisms on the possible relations of HBV mutations with the development of hepatocellular carcinoma.

**Keywords:** Hepatocellular carcinoma - hepatitis B virus - gene mutations

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### Introduction

Hepatocellular carcinoma (HCC). is the fifth most common solid tumor worldwide, and represents the third leading cause of cancer mortality (Forner et al., 2012). The most prominent factors associated with HCC include chronic hepatitis B and C virus infection, chronic alcohol consumption, aflatoxin-B1-contaminated food and virtually all cirrhosis-inducing conditions. In addition, Hepatitis B virus (HBV). is a major etiologic agent that is endemic in China, Southeast Asia and in parts of Africa. Individuals with chronic HBV infection are at increased risk of developing HCC, especially those with chronic liver disease and cirrhosis (Tsukuma et al., 2005; Arzumanyan et al., 2013).

Hepatocarcinogenesis as a process in individuals with chronic HBV infection is complex, and involves both host and viral factors. However, the exact role of HBV in the development of HCC remains enigmatic. Several hypotheses have been proposed to explain the potential mechanism, including expression of viral protein, such as, HBx protein from open reading frame (ORF). X gene, to modulate cell proliferation and viability, integration of HBV DNA into the host genome to alter the function of endogenous genes or induce chromosomal instability and accumulation of genetic damage due to hepatic inflammation mediated by virus specific T cells (Ishikawa, 2010; Zhu et al., 2012; Arzumanyan et al., 2013). However, it is unclear whether genetic characteristics of HBV, including HBV genotype and specific genetic mutations, contribute to the risk of HCC. In this article, the role of HBV mutations in the development of HCC will be reviewed.

### General Features of Hepatitis B Virus

Hepatitis B virus is a small, relaxed circular, partially double stranded 3.2 kb DNA virus that contains a highly compact genetic organization. There are four partially overlapping open reading frames (ORFs), consisting of the surface gene (S), precore and core gene (C), polymerase gene (P), and X gene (Tang et al., 2006; Tong et al., 2007). Among them, ORF P that encodes the RT (reverse transcriptase). domains of the polymerase overlaps completely with the ORF S that encodes HBV surface proteins (Michel and Tiollais, 1987). Moreover, viral sequences HBV X gene overlaps the ORF C region, and mutations in both ORF X and ORF C often occur together (Hussain et al., 2009). The four protein-coding regions are shown between the inner and outer circles (Seeger and Mason, 2000). HBV replicates via the reverse transcriptase enzyme system which lacks proofreading ability; therefore, new virions possess diverse genetic variability (Hannoun et al., 2000). This variability is in the form of deletions, insertions, or synonymous or non-synonymous nucleotide substitutions.

### Characteristics of Pre-S/S Gene

The envelope proteins are produced from a single open reading frame, S gene region, with three different translation sites, pre-S1, pre-S2, and S. It produces three forms of HBV surface proteins (HBs), which are the large (L), middle (M). and small (S). HBs (Schmitt et al., 1999). The major component of the envelope protein is S protein which consists of 226 amino acids and drives particle budding. The M protein is composed of the S

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protein with an additional of 55 amino acids termed pre-S2 attached to the N-terminus. The L protein is the M protein with an additional 108 or 119 genotype-dependent amino acids attached to the N-terminus (Ni et al., 2010). It has been shown that amino acid substitutions within the “a determinant” domain in the HBV S region may lead to conformational changes in the S protein (Tian et al., 2007). Mutants of pre-S deletion, rtA181T/sW172stop, rtM204I is in ORF S gene region.

## **Pre-S Mutants in HBV-Related Hepatocarcinogenesis**

The pre-S1 and pre-S2 region is the region with the most variability in the HBV genome (Lauder et al., 1993). Many studies have been reported on the association of pre-S mutations with severity of liver disease. Several meta analysis studies found that mutations at the promoter sites of pre-S1 and pre-S2 are significantly associated with an increased risk of HCC (Lin et al., 2007; Liu et al., 2009). The Pre-S mutation generally presents in form of deletions (Chen et al., 2006; Yeung et al., 2011). Specifically, the pre-S2 deletion mutation was also associated with increased risk of HCC in Asian children. (Abe et al., 2009). Additionally, amino acid substitution from Phenylalanine to Lysine at codon 141 in pre-S2 region (F141L). is also associated with HCC in patients infected with HBV genotype C (Mun et al., 2011). On the other hand, some studies have detected that the pre-S internal deletion mutants are unlikely to play an important role in hepatocarcinogenesis in clinical study (Tatsukawa et al., 2011). Numerous studies for mechanisms shown that deletions mutations in Pre-S in the integrated HBV DNA, may impair the secretion of HBsAg, leading to increased endoplasmic reticulum and oxidative stress in hepatocytes (Fang et al., 2008). Truncated forms of Pre-S2 have also been shown to interact with cyclin A, a critical regulator of the cell division cycle, an observation that supports a role for Pre-S2 in hepatocyte hyperplasia and a likely role in the process of HBV-related tumorigenesis (Wang et al., 2005). Moreover, Hung et al demonstrates that HBV pre-S2 deletion increased Bcl-2 expression which plays an important role in resistance to 5-fluorouracil-caused cell death. Their studies provide an important chemotherapeutic strategy in HBV pre-S2 deletion associated tumor (Hung et al., 2011). Thus, deletions of Pre-S may contribute to hepatocarcinogenesis by several mechanisms. Furthermore, pre-S mutations were not significantly associated postoperative recurrence of HCC (Heo et al., 2013).

## **Secondary Mutations in Pre-S/S Gene and Risk of HCC**

The mutation at rtA181T/surface truncation mutation (rtA181T/sW172stop). can result in a stop mutation in the envelope region of the S gene (SW172stop), and has also been reported in a substantial proportion of adefovir dipivoxil - and lamivudine-resistant patients (Yeh et al., 2000; Villet et al., 2008). which was independently

associated with severe liver consequences, especially occurrence of HCC, in drug-resistant patient (Yeh et al., 2011). LAM and ADV remains to be a popular anti-HBV agent in many parts of the world because of its low cost, excellent safety profile in long term use, and absence of oncogenic potential in animal studies. However, because of widespread use, an increasing number of LAM-resistant patients have emerged. Previously, Lai et al demonstrates that tumorigenicity of NIH3T3 cells stably transfected with plasmid, which carried the rtA181T/ sW172stop, in nude mice (Lai and Yeh, 2008). Additionally, Hosaka et al. (2010) demonstrate that YIDD (rtM204I). mutants, cirrhosis and age > 50 years increased the risk of HCC in cirrhotic patients receiving adefovir add-on lamivudine.

## **General Features of Precore/Core and Basal Core Promoter Genes Region of Hepatitis B Virus**

The basic core promoter (BCP) and its adjacent precore (pre-C) region are crucial for replication of HBV. BCP binds various liver factors and pre-C forms e structure in pregenomic RNA (pgRNA) as the encapsidation signal (Seeger and Mason, 2000). Changes in viral replication may influence the progression of liver diseases (Jammeh et al., 2008; Tsai et al., 2008). Mounting evidence has emerged to demonstrate that BCP and pre C mutants are predisposed to severe and progressive liver diseases after HBV infection, causing an increased risk for hepatocellular carcinoma (HCC) (Tong et al., 2007; Fang et al., 2008; Yuen et al., 2008). T1762/A1764 double mutation, T1753V, G1896A, G1899A, G1613A and C1653T occur in ORF C gene region.

## **A1762T/G1764A Double Mutations in Pre-C/C Region and Risk of HCC**

The A1762T/G1764A double mutations (1762 A-to-T and 1764 G-to-A)., which are the most commonly studied molecular variants in the basal core promoter (BCP). region and overlaps with the open reading frame encoding HBV X gene, were commonly found to be borne by HCC patients, and were thus suggested as potential biomarkers for hepatocarcinogenesis (Fang et al., 2008; Yuan et al., 2009). Clinical studies have indicated that strong association between mutation in the A1762T/G1764A mutations and risk of HCC (Dong et al., 2008; Liu et al., 2011; Yin et al., 2011). Kusakabe et al investigated a population-based cohort consisting of 19 393 subjects (middle aged or older). with a follow-up of over 13 years in Japan. They found that HBV mono-infected subjects with the A1762T/G1764A double mutation could be at high risk for HCC development during the natural course of HBV infection (Kusakabe et al., 2011). Contradictory results were obtained from studies which found that lack of an association between A1762T/G1764A mutations and HCC development (Chan et al., 2004). For example, Fan et al found that patients with higher viral load and genotype C had a higher incidence of A1762T/G1764A double mutations, which may not be related with development of HCC (Fan et al., 2011). In addition, The postoperative

recurrence or survival period may not be affected by the double mutation A1762T/G1764A in patients with HBV-associated HCC treated with curative surgical resection (Mathews et al., 2012).

## Secondary Mutations in Pre-C/C Genes and Risk of HCC

Clinical studies have indicated that the 1753V mutations (1753-to-C/A/G). were also associated with the progression of liver disease, such as HCC (Tanaka et al., 2006; Malik et al., 2012). Additionally, Li et al (Xu et al., 2010). evaluated the roles of genetic variations of HBV in the development of HCC supported the hypothesis that both the A1762T/G1764A double mutations and the 1753V/1752V mutations were associated with increased risk for HCC. Mounting studies have shown that precore G1896A is involved in HBeAg negativity by introducing a stop codon in the pre-C region, and significantly increases the risk for HCC in chronic hepatitis B patients (Du et al., 2007; Tong et al., 2007). Someone found that the Pre-C mutation (A1896G). could prevent the production of HBeAg, by introducing a premature stop codon into the ORF Pre-C/C that abolished the production of HBeAg, this may cause liver damage with progression to cirrhosis and cancer (Yang et al., 2008). Besides, G1899A also locates at precore region of HBV genome and associated with increased risk of HCC compared with HBV patients without G1899A (Liao et al., 2012). For the mechanisms that underlie the interaction between G1899A and the onset of HCC still remain elusive. Additionally, C1914G mutations in the basal core promoter and precore regions were usually associated with advanced forms of liver disease and had an increased risk of HCC (Malik et al., 2012). G1613A and C1653T double mutations, which were recently identified, were frequently found in patients with HCC. Moreover, A single G1613A mutation was associated with future emergence of HCC (Tatsukawa et al., 2011), and a single C1653T mutation in the ORF C gene region are independent factor for development of HCC. However, no significant association was found between the C1653T mutant and HCC risk in HBeAg positive patients (Tanaka et al., 2006; Shi et al., 2013).

## Characterize Mutations in the HBV X Gene of Hepatitis B Virus

Hepatitis B virus X gene is the smallest of four kinds of HBV functional genes, with a length of 465 bp encoding a 154 amino acid protein with an N-terminal negative regulatory domain and a C-terminal transactivation domain (Bouchard and Schneider, 2004). The HBV X protein, HBV X gene express, is a multifunctional regulator that is essential for viral replication and plays an important role in regulating gene transcription, participating in cell signaling, and controlling cell proliferation and apoptosis (Murakami, 2001). A large number of evidences reveal that HBV X protein was reported as a strong risk factor of hepatocellular carcinoma (HCC) (Ng and Lee, 2011). What's more, the gene mutants in HBV X gene that were

highly associated with hepatocarcinogenesis, have been reported in many studies (Yeh et al., 2000; Kim et al., 2008; Choi et al., 2009).

## Mutations in HBV X Gene and Risk of HCC

Many groups have reported that mutants in HBx gene have been associated with HCC. In addition to point mutations, deletions, especially C-terminal truncations or insertions, have been frequently detected in tissues and sera samples in HCC patients. This suggests that deletions or insertions in the HBx gene may play a pivotal role in hepatocarcinogenesis (Tu et al., 2001; Ma et al., 2008). However, some study shown that deletions were located in X gene regions were found that was unlikely associated the risk of hepatocellular carcinoma (Tatsukawa et al., 2011).

## Secondary Mutations in HBV X Gene and Risk of HCC

The codon-38 change of HBV X protein, the amino acid change was attributable to a change cytosine to thymine at nucleotide 1485 in HBx gene is an independent risk factor for the development of HCC (Muroyama et al., 2006). The C-terminal domain of the HBV X gene overlaps the BCP region, and mutations in both HBx and BCP often occur together (Park and Chung, 2007; Hussain et al., 2009). Thus, the BCP overlaps with the X region of the HBV genome, and mutations in the amino acid sequence at positions 130 and 131 (at codon 130 [AAG → ATG] and 131 [GTC → ATC]). in this region have been proposed as prognostic markers for the development of liver cancer (Kuang et al., 2004; Minemura et al., 2005; Lee et al., 2011). In addition, study found that HBx-A31 (containing a mutation at codon 31). was detected more frequently in patients with HCC (Yeh et al., 2000).

## Summary

Evidences confirm that hepatitis B virus can cause hepatocellular carcinoma (HCC). Multiple factors, including HBV mutants, suggest that hepatitis B virus induce hepatocarcinogenesis in chronic hepatitis B patients. Mounting clinical studies shown that strong association between HBV mutations and hepatocellular carcinoma, particularly, mutants were pre-S deletion mutation and A1762T/G1764A double mutations. However, for the pathogenic mechanisms that underlie the interaction between HBV mutants and the onset of HCC still remain elusive. Thus, further prospective studies are needed to confirm the role of these mutations in the development of HCC. Hopefully, hepatitis B virus gene mutations might serve as a useful molecular marker for predicting the clinical outcomes of patients with chronic HBV infection.

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