

## Molecular characteristics and stages of chronic hepatitis B virus infection

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Supported by Science and Technology Department of Qingdao Government 07-2-1-15-nsh

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Received: April 9, 2009 Revised: May 26, 2009

Accepted: June 2, 2009

Published online: July 7, 2009

**Key words:** Hepatitis B virus; Pathology; Immune tolerance; Immune clearance; Inactive hepatitis B surface antigen carriers; Reactivation; T-cell response; Cytokines

**Peer reviewer:** Thomas Bock, PhD, Professor, Department of Molecular Pathology, Institute of Pathology, University Hospital of Tuebingen, D-72076 Tuebingen, Germany

Shi YH, Shi CH. Molecular characteristics and stages of chronic hepatitis B virus infection. *World J Gastroenterol* 2009; 15(25): 3099-3105 Available from: URL: <http://www.wjgnet.com/1007-9327/15/3099.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.3099>

### Abstract

Hepatitis B virus (HBV) is a common viral pathogen that causes a substantial health burden worldwide. Remarkable progress has been made in our understanding of the natural stages of chronic HBV infection. A dynamic balance between viral replication and host immune response is pivotal to the pathogenesis of liver disease. Knowledge of the HBV genome organization and replication cycle can unravel HBV genotypes and molecular variants, which contribute to the heterogeneity in outcome of chronic HBV infection. Most HBV infections are spontaneously resolved in immunocompetent adults, whereas they become chronic in most neonates and infants at a great risk of developing complications such as cirrhosis and hepatocellular carcinoma (HCC). Those with chronic HBV infection may present in one of the four phases of infection: immune tolerance, immune clearance [hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB)], inactive carrier state, and reactivation (HBeAg-negative CHB). Understanding the dynamic nature of chronic HBV infection is crucial in the management of HBV carriers. Long-term monitoring and optimal timing of antiviral therapy for chronic HBV infection help to prevent progression of HBV-related liver disease to its later stage, particularly in patients with higher risk markers of HCC, such as serum DNA concentration, HBeAg status, serum aminotransferase, HBV genotypes, and pre-core or core mutants.

### INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem worldwide. Some individuals can develop acute HBV infection and achieve complete immune clearance of virus, yielding a life-long immunity, while others can develop chronic HBV infection depending on the host immune response. Chronic HBV infection is associated with a wide range of clinical manifestations, from an asymptomatic carrier state with a normal liver histology to severe and chronic liver diseases, including cirrhosis and hepatocellular carcinoma (HCC)<sup>[1,2]</sup>.

There is a particular concern in the Asia-Pacific region, where chronic HBV infection is prevalent, with a carrier rate of approximately 10% of chronic HBV carriers. About 25%-40% of them will eventually die of liver disease (cirrhosis with and without HCC) with a death rate of 50% for male carriers and 15% for female carriers, respectively. Chronic HBV infection is a dynamic process with a replicative or a non-replicative (or low replicative) phase based on virus-host interaction which is pivotal to the pathogenesis of liver disease. Understanding the dynamic nature of chronic HBV infection is crucial in the management of HBV carriers. Long-term monitoring and optimal timing of antiviral therapy for chronic HBV infected patients can help to prevent progression of HBV-related liver disease to its later stage<sup>[3,4]</sup>.

### PATHOLOGY OF HBV INFLAMMATORY REACTION

Viral hepatitis, characterized by diffused inflammatory

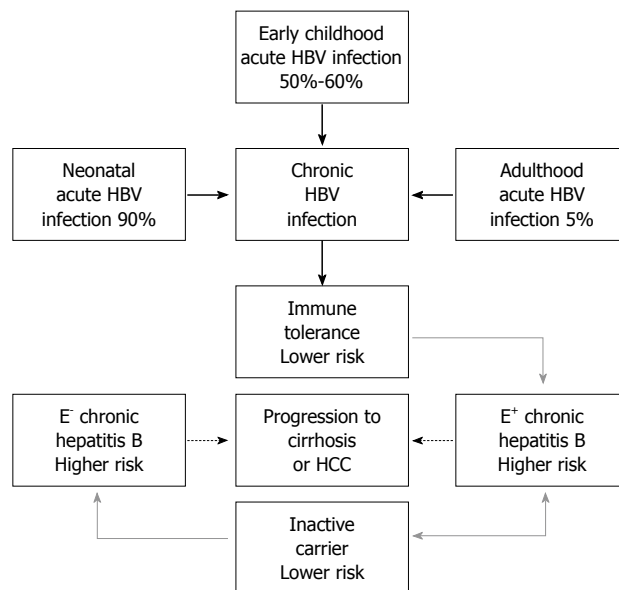
reaction, is associated with cell damage and death. It has been recently reported that HBV replication is associated with cell death, which is different from the widely accepted non-cytopathic characteristics of HBV<sup>[5]</sup>. The mechanism of cell damage is generally defined as the result of cytotoxic T-lymphocyte (CTL)-mediated immune responses to viral infection<sup>[6]</sup>. Another typical process causing cell death is apoptosis. It has been shown that HBV proteins, such as HBx and HBsP, can induce apoptosis<sup>[7]</sup>. A careful light microscopic examination of HBV genotypes A-C transfected cells can reveal rounded up and death cells which are apoptotic signs. To identify the observed cell death, FACs is used because apoptotic cells can show phosphatidylserine on cell membrane. HePG2 cells can be transfected with HBV genotypes A-C. Cells observed under a phase contrast microscope, can be stained with apoptosis markers and analyzed by flow cytometry. HBsP expression can be detected by Western blotting assay. BH3 sequences can be aligned and analyzed with the vector NT1. HBV genotypes A-C transfected cells display cell death which has been further proved as apoptosis. HBsP, a pro-apoptotic protein, is detectable during transfection of virus genomes. Different apoptotic effects are correlated with the expression of different genomes. Alignment and analysis of the HB3 domains of three virus genomes can reveal a slight variance. It has been reported that variant HBsP expression and BH3 sequence of HBV genotype may be involved in differential apoptotic effects on transfected cells<sup>[8]</sup>. However, HBV can also directly cause death of hepatocytes<sup>[6]</sup>.

## HBV TRANSMISSION AND INFECTION

In high endemic regions, such as Asia, Africa, Pacific Islands and the Arctic, early perinatal and horizontal infection in childhood is the main route of HBV transmission with a hepatitis B surface antigen (HBsAg) positive rate of 8%-15%, while in low endemic areas, such as Western countries, HBV is a predominant disease in adolescents and adults due to high risk sexual behaviors or drug injections, with a HBsAg positive rate of less than 2%<sup>[9]</sup>.

The vast majority of early perinatal or horizontal infections in childhood are the main route of HBV transmission in untreated infants whose mothers are hepatitis B e antigen (HBeAg) positive, and over 90% of them will become chronic HBV carriers. In contrast, about 90% of HBV infections may occur as acute infection and only 5%-10% may occur as chronic infection in adults. This dramatic difference in chronic rates is believed to reflect the host immunologic status and the time of infection.

Although infants whose mothers are HBeAg-positive HBV carriers are at a high risk of developing infection and subsequently become viral persistence, the age of infants at the time of HBV infection is inversely correlated with the chronic rate<sup>[10,11]</sup>. A HBc/HBe-specific Th-cell tolerance model can show the reversibility of T-cell tolerance. It has been shown that a single prenatal dose of HBeAg can result in apparent T-cell tolerance



**Figure 1** Phases of chronic hepatitis B virus (HBV) infection. Thick arrows indicate HBV infection rates in different age groups, dotted arrows indicate changes in histology, grey arrows indicate changes at risk of progressing to cirrhosis or HCC (E<sup>+</sup>: HBeAg positive; E<sup>-</sup>: HBeAg negative).

in mice at the age of 8-12 wk, but the tolerance may disappear at the age of 16 wk<sup>[12]</sup>, suggesting that T-cell tolerance can be maintained and HBcAg/HBeAg will continuously present. HBc/HBe-specific thymocytes are absent in thymus and this “repertoire renewal process” requires about 16 wk. Similarly, human fetus may be exposed to tolerogenic HBeAg in uterus not infected at birth. The longer the elapsed time before HBV infection is, the greater the probability of renewing HBc/HBe-specific T-cell repertoire is, because the neonate would no longer be exposed to HBeAg<sup>[13]</sup>.

There is an obvious difference between patients infected with HBV in adolescence or adulthood immediately entering immune clearance phase, and short duration and tendency quiescent after seroconversion from HBeAg to antibody against HBeAg (anti-HBe). Such patients are termed “healthy” carriers. In contrast, patients with early HBV infection have a prolonged immune tolerance phase and a prolonged immune clearance phase, indicating that their diseases tend to progress after HBeAg seroconversion.

## STAGES OF HBV INFECTION

Remarkable progress has been made in our understanding of the four natural stages of chronic hepatitis B (CHB): immune tolerance stage, immune clearance stage, inactive HBsAg carrier stage, and reactivation stage. However, not all CHB infection patients go through all the four stages (Figure 1).

### Immune tolerance stage

Patients with perinatal or early childhood-acquired HBV infection have an initial tolerance stage characterized by the presence of HBeAg, high serum DNA level, normal serum aminotransferase level, and minimal or

no inflammation on liver biopsy<sup>[14]</sup>. Such manifestations can rarely be seen in those who are infected with HBV in later childhood or adulthood and whose infection subsequently develop into chronic HBV infection<sup>[14]</sup>.

Although a high serum DNA level in liver disease patients with minimal or no inflammation is considered as a sequela of immune tolerance to HBeAg, it has been shown that HBeAg may promote HBV chronicity by functioning as an immunoregulatory protein<sup>[15]</sup>. For example, in transgenic mice, transplacental transfer of maternal HBeAg may preferentially behave as a tolerogen and inactive HBe/HBc-specific Th cells through at least central deletion of high-affinity HBe/HBcAg-specific CD4<sup>+</sup>T cells or clonal ignorance and anergy in periphery blood, resulting in ineffective cytotoxic T cell lysis of infected hepatocytes. Such a mechanism may be responsible for the high chronic HBV infection rate (~90%) observed in babies infected by their HBeAg positive mothers accounting for the inability of infants to clear perinatal HBV infection. After neonatal or prenatal HBV infection (absent in uterus tolerance), secretion of monomeric HBeAg in the relatively Th<sub>2</sub>-biased neonatal immune system may also have an anti-inflammatory influence on nucleoprotein-specific immune response by eliciting Th<sub>2</sub>-like cytokines. Secreted HBeAg can also enter thymus. It has been subsequently reported that HBeAg specific Th<sub>2</sub>-like cells can preferentially survive tolerance production to a greater extent than HBeAg-specific Th<sub>1</sub>-like cells<sup>[16]</sup>. Therefore, chronicity resulting from vertical transmission of HBV characterized by the predominance of HBeAg-specific Th<sub>2</sub>-like cells and secretion of anti-inflammatory cytokines, such as IL-4, IL-5, and IL-10, can enhance antibody production, and viral persistence would characterize the HBeAg-specific T-cell response. If the *status quo* of various clonal tolerance phenotypes could be maintained as long as the HBeAg concentration and/or the non-inflammatory hepatic environment remain unchanged, the immune tolerance would last 1-4 decades. During this phase, the rate of spontaneous or treatment-induced HBeAg seroconversion is less than 5%<sup>[17,18]</sup>. Patients in the immune tolerance phase are considered at a low risk of progressing to cirrhosis or HCC based on serial monitoring of virological, clinical and ultrasonographic assessments.

Although antiviral therapy is not recommended for immune tolerance patients, they should be closely monitored for progression to the immune clearance phase. Once this occurs, antiviral therapy should be considered more diligently after 6-12 mo if HBeAg seroconversion does not occur because disease progression can occur in the immune clearance phase.

### **Immune clearance stage**

As the host immune system matures, a nonspecific increase in hepatic inflammation or decrease in HBeAg serum concentration, perhaps due to the emergence of core promoter region or pre-core region mutants, may allow activation of intermediate-or low-avidity HBeAg-specific T cell clones that are not physically deleted and/or reverse the anergic state of others<sup>[17]</sup>. Therefore,

treatment modalities for chronic HBV infection should be directed at activating the relatively low-avidity HBeAg-specific T cells. Such a shift from HBeAg-specific Th<sub>2</sub> cell tolerance to Th<sub>1</sub> cell activation may recognize HBV-related epitopes on hepatocytes, and immune-mediated hepatocellular injury ensues, the so called clearance phase of CHB infection. Then, IL-2, INF and tumor necrosis factor are secreted following inflammation. The HBcAg-specific T-cell response is characterized by CTL induction, liver injury and inhibition of viral replication<sup>[6]</sup>. In patients with prenatally acquired HBV infection, transition from immune tolerance phase to immune clearance phase occurs during the second or third decade of life. Although HBV replication and viremia continue in the liver, the serum virus level becomes lower in immune clearance phase than in immune tolerance phase when viral replication is completely unopposed. The active phase of CHB is often marked by increased levels of alanine aminotransferase (ALT), necrotic inflammatory activity, and cycling HBV-DNA and HBeAg due to liver injury. Pre-core minus mutants and mutations within the core gene begin to accumulate at the time of ALT flare up because they have a better ability to evade immune clearance<sup>[19]</sup>, suggesting that nucleoprotein antigens are the major immune attacking foci during chronic HBV infection, perhaps because the nucleoprotein-specific T-cell repertoire has been eroded to a lesser extent than the envelope-specific repertoire simply due to the lower concentration of HBe/HBcAg. CD4<sup>+</sup> HBeAg-specific T cells, identified in HBeAg-single-Tg and TCR-HBeAg-Tg mice, are not deleted or anergized and remain quiescent in the presence of serum HBeAg, but can mediate seroconversion and liver injury once they are activated. These HBeAg-specific T cells escape tolerance induction due to their low avidity and/or low TCR density<sup>[18]</sup>.

This active phase is characterized by the presence of HBeAg, high serum HBV DNA and aminotransferase levels, as well as active inflammations and fibrosis in the liver. A key event in the natural history of HBeAg positive CHB patients is HBeAg seroconversion<sup>[20]</sup>. Several studies have shown that seroconversion with a marked reduction in HBV replication is associated with biochemical and histological remission of inflammatory activity in the majority of patients<sup>[2,10,20]</sup>. Most studies showed that the mean annual rate of spontaneous HBeAg seroconversion ranges 8%-15% in children or adults with an elevated ALT level<sup>[20]</sup>. Although the ALT level is normal in most Asian children, their spontaneous HBeAg seroconversion rate is less than 2% during the first 3 years of age and then increases to 4%-5%. In some cases, spontaneous flare up of hepatitis is not frequently recognized because it is usually asymptomatic. Since subsequent HBeAg seroconversion would not occur in such flare up of hepatitis, it can thus be viewed as an abortive attempt at seroconversion. However, some patients present with a symptomatic flare up of hepatitis that mimics acute hepatitis and even present with fulminant hepatic failure. Regression of fibrosis occurs several months or years after HBeAg seroconversion. These flare ups of hepatitis may precede the disappearance

of HBeAg and development of HBeAg antibody, culminating in the remission of hepatitis activity. It has been recognized that the duration of immune clearance phase and the frequency and severity of flare ups are correlated with the risk of progressing to cirrhosis and HCC<sup>[21-23]</sup>.

### **Inactive HBsAg carrier stage**

This inflammatory phase of HBV infection also leads to HBeAg seroconversion and enters into inactive HBsAg carrier status. Inactive carriers form the largest group of chronic HBV infection patients. After seroconversion, most patients remain negative for HBeAg and positive for anti-HBe antibody with an undetectable or a low HBV DNA level, while the minority have undetectable viral loads. Biopsy findings can range from mild inflammation and minimal fibrosis to inactive cirrhosis if the disease is severe during immune clearance<sup>[20]</sup>.

The progress of inactive HBsAg carrier state is usually benign. A long-term follow-up (up to 18 years) of these carriers can indicate a sustained biochemical remission and a very low risk of developing cirrhosis or HCC in them<sup>[19]</sup>. Patients even with no cirrhosis may develop liver cancer in their inactive HBsAg carrier state. In addition, approximately 20%-30% of inactive HBsAg carriers may undergo spontaneous reactivation of hepatitis B during the follow-up. Multiple episodes of reactivation or sustained reactivation can cause progressive liver damage and even decompensation. HBV reactivation is usually asymptomatic but can occasionally mimic acute viral hepatitis. Some carriers eventually become HBsAg negative and develop anti-HBs. The estimated incidence of delayed HBsAg clearance is 1%-2% per year in Western countries where HBV infection is acquired in adulthood, and 0.05%-0.8% per year in endemic areas where HBV infection is mostly acquired perinatally or in early childhood. Prognosis can be improved by loss of HBsAg as liver disease is inactive or non-progressive, but HBsAg clearance does not completely prevent occurrence of liver decompensation or HCC in patients with cirrhosis<sup>[23-25]</sup>.

### **Reactivation stage**

Chronic HBeAg-negative patients can be divided into chronic inactive HBsAg carriers and CHB patients with biochemical and intermittent virological activity<sup>[5]</sup>. HBeAg-negative chronic hepatitis may recur in one third of inactive HBV carriers without serum reversion of HBeAg<sup>[22,23]</sup>.

It is believed that seroconversion of HBeAg to HBeAb is accompanied with cessation of HBV replication and remission of liver disease. However, HBsAg-negative CHB has been recognized as an important form of chronic hepatitis, and e-antigen negativity is due to mutations in pre-core and core promoter regions. The most frequent pre-core mutation is a G-A change in nucleotide 1896 (G1896A) which creates a stop codon<sup>[23]</sup> and the most common core promoter mutation involves a substitution of nucleotides 1762 and 1764, which can result in loss of HBeAg synthesis. Loss of circulating HBeAg can decrease the induction of HBeAg-specific Th<sub>2</sub> cell activity and result in a predominance of inflammatory

Th<sub>1</sub>-like cells<sup>[16]</sup>. HBeAg-negative CHB (pre-core mutant) occurs as the predominant species during typical HBV infection with wild-type virus which is selected during the immune clearance phase (HBeAg seroconversion). Several studies have shown that HBeAg may be a target antigen on HBV-infected hepatocytes<sup>[5,15,18]</sup>. Failure to produce a target antigen may be a means of evading immune clearance. The clonal heterogeneity of HBeAg-specific T-cell tolerance may explain how a primarily tolerogenic protein can exert its pressure on the immune response to the selection of HBeAg negative mutant. For example, high-avidity HBeAg-specific T-cell clones may be tolerated and low-activity T-cell clones may be activated and involved in selecting HBeAg-negative mutant in the same patient<sup>[18,24-26]</sup>. The occurrence of HBeAg-negative mutants during chronic active HBV infection, especially in the presence of a high viral load, is correlated with an exacerbation of liver injury and a worse prognosis. Serum HBeAg can act as an efficient T-cell tolerogen which reduces the frequency of liver injury and down-regulates anti-HBc production. Anergy of HBc/HBeAg and HBeAg specific T-cells depends on HBeAg concentration and is reversible in the absence of HBeAg, which may explain the correlation between pre-core and core promoter mutations and severe liver injury<sup>[19,22,27,28]</sup>.

Progress to this phase occurs spontaneously or to inactive carriers during immune suppression. Some patients can progress directly from HBeAg positive to HBeAg negative CHB. Identification of pre-core/core promoter mutations and recognition of HBeAg negative CHB indicate that the disease occurs after HBeAg seroconversion<sup>[19]</sup>. Age is significantly higher in HBeAg-negative patients than in HBeAg-positive patients. ALT and HBV DNA levels are significantly lower in e-antigen negative patients than in e-antigen positive patients. However, spontaneous recovery is rarer, long-term prognosis is poorer, and histological lesions are more severe in HBeAg-negative patients than in HBeAg-positive patients. Necrotic inflammatory activity is almost identical in both HBeAg-negative and positive patients. However, fibrotic activity is higher in e-antigen negative patients than in e-antigen positive patients. The estimated annual incidence of cirrhosis is 2%-6% in HBeAg positive CHB patients and 8%-10% in HBeAg negative CHB patients. The higher incidence of cirrhosis in HBeAg-negative patients is related to age and fibrosis stage, suggesting that HBeAg-negative chronic hepatitis can progress to cirrhosis and HCC in the natural history of HBV infection rather than *de novo* infection with HBV variants that do not produce HBeAg<sup>[29-31]</sup>. HBeAg-specific T cell tolerance is reversible in the absence of tolerogen. Since antiviral treatment can reduce HBeAg and viral load possibly in combination with HBc/HBeAg-specific immunization, it can alleviate chronic HBV infection by shifting the cytokine profile from Th<sub>2</sub> to Th<sub>0/1</sub><sup>[26,32]</sup>.

### **Occult HBV infection**

Occult HBV infection is defined as the existence of HBV DNA in serum, although it is not considered as a phase of CHB<sup>[33,34]</sup>.

In addition to a symptomatic and serologically

Table 1 Characteristics of chronic hepatitis B at different stages

Phase	ALT	HBsAg	HBeAg	HBeAb	HBV DNA (IU/mL)	Th cell biased	Liver histology
Immune tolerance	Usually normal	Present	Present	Absent	≥ 20000	Th <sub>2</sub> cell	Normal or mild inflammation
Immune clearance	Elevated	Present	Present	Absent	≥ 20000	Th <sub>2</sub> /Th <sub>1</sub> cell	Active inflammation
Inactive HBsAg carrier	Usually normal; can have flares	Present	Absent	Present	> 20000	Th <sub>1</sub> cell	Mild inflammation or inactive cirrhosis
HBeAg <sup>+</sup> CHB	Period flares	Present	Absent	Present	> 20000 < 20000	Th <sub>1</sub> cell	Active inflammation
Occult hepatitis B	Rarely elevated	Absent	Absent	Present when HBV recovered	< 20000	Th <sub>1</sub> cell	From normal to cirrhosis HCC

evident infection, occult persistent HBV carriage has been identified since nucleic acid amplification assay enhances its sensitivity to hepadnaviral genomes and their replicative intermediates. There is evidence that occult HBV infection is a common and long-term consequence of acute hepatitis B resolution. This form of residual infection is termed as secondary occult infection (SOI). The data from the woodchuck model of HBV infection indicate that exposure to a small amount of hepadnavirus can also cause primary occult infection where virus genome but not serological makers of virus exposure are detectable without liver involvement. However, both forms of virus replicate at a low level in the lymphatic system. Serological testing for SOI can reveal the presence of antibodies to HBV core antigen (anti-HBc), which has been recognized not only as a valuable marker of prior HBV exposure but also as an indicator of progressing occult HBV infection<sup>[35]</sup>. It has been recently reported that up to 20% of individuals with occult HBV carriage are not reactive to anti-HBc or any other serological indicators of HBV exposure, and detection of naturally acquired antibodies to HBsAg (anti-HBc) does not exclude the existence of occult HBV infection<sup>[33,36]</sup>.

The severe consequences of occult HBV infection have not been fully recognized. There is evidence that occult HBV can be a source of virus contamination in blood and organ donations, as well as a reservoir from which full blown hepatitis can arise<sup>[37]</sup>. Case reports also indicate that immunosuppression caused by chemotherapy or immunomodulatory agents or immunodeficiency due to HIV infection or hematological malignancies can induce reactive occult infection<sup>[38,39]</sup>. Mild necrotic inflammation has been documented in liver samples obtained from acute hepatitis B patients many years after recovery<sup>[40]</sup>. Liver fibrosis and cirrhosis of unknown origin have been explained by occult HBV infection in many retrospective studies<sup>[35,41,42]</sup>. The oncogenic potency of occult HBV persistence becomes progressively evident and is further elevated in alcoholics and patients with other liver ailments like hepatitis C<sup>[41,42]</sup>. No reports are available on the treatment of occult HBV infection (Table 1).

## CONSEQUENCES OF CHRONIC HBV INFECTION

Individuals with chronic HBV infection are at an increased risk of developing end-stage liver diseases

including cirrhosis, hepatic failure, and HCC. It has recently been estimated that about 53% of HCC cases in the world are related to HBV infection. The lifetime risk of developing HCC is increased even in patients with cleared HBsAg or occult HBV infection. Further risk factors include chronic HCV infection, exposure to aflatoxin B<sub>1</sub>, alcohol abuse, obesity and diabetes<sup>[4,43]</sup>. Thus, it is important to identify HBV-infected patients at a higher risk of progressing to HCC.

The reason why some CHB patients progress to HCC remains unknown. Host factors, such as immune response to HBV, genetic predisposition to HCC, high HBV replication rate, mutations within the HBV genome, are related with HCC. Many observations revealed that the major factor for the development of HBV-associated HCC is the immune system<sup>[41,43,45]</sup>. Development of hepatitis, chronic hepatitis, and HCC could be exclusively observed in mice reconstituted with bone marrow and in non-transgenic animals, but not in controls, suggesting that ineffective immune response is the principle oncogenic factor during chronic HBV infection of human beings. In other words, the same T-cell response has different effects. If T cell response is strong enough, HBV can be eliminated from the liver. If not, a pro-carcinogenic effect can be induced by triggering necrotic inflammatory disease without final eradication of HBV from the liver. It can, thus, be concluded that the immune system-mediated chronic inflammation of the liver, continuous cell death and subsequent cell proliferation may increase the frequency of genetic alteration and the risk of developing cancer. However, the molecular basis of inflammatory liver carcinogenesis caused by HBV remains largely unsolved. Cytokines modulate inflammation and the presence of inflammatory cells with the production of inflammatory cytokines activates cellular oxidant-generating pathways. Reactive oxygen species that are generated in inflammatory conditions induce oxidative DNA damage and increased oxidative stress caused by chronic inflammation can produce genetic mutations and gross chromosomal alterations<sup>[4,44,45]</sup>. Extensively oxidative DNA damage has been detected in hepatocytes of HBV-transgenic mice and humans with chronic hepatitis<sup>[46]</sup>.

HBV genotype C infection is associated with a higher risk of developing HCC than HBV genotype B infection<sup>[29]</sup>. The BCP A1762T/G1764A mutant is associated with an increased risk of developing HCC



compared with the double wild type variant, whereas the pre-core G1896A mutation is associated with a decreased risk of developing HCC compared with the wild-type variant. Several mechanisms of liver carcinogen are related to the BCP A1762T/G1764A mutation which may enhance HBV virulence by increasing host immune response and viral replication, or by altering the coding region of the X antigen. Mutant BCP may augment the host immune response to HBV-infected hepatocytes by diminishing circulating HBeAg and increasing hepatocyte apoptosis and regeneration, thus leading to liver injury<sup>[47,48]</sup>. The BCP mutation appears to enhance the efficacy of viral replication either by modulating the relative levels of pre-core and core RNAs or by creating a transcription factor binding site for hepatocyte nuclear factor 1. Mutations in the BCP region overlapping the coding sequence of the X antigen of HBV may result in changes of amino acids, K130M and V131I, in the X gene. These amino acid changes may interfere with cell growth control and DNA repair, thus leading to HCC<sup>[49,50]</sup>. There is experimental evidence that HBx, a multifunctional protein with oncogenic potentials, can interact with a large number of cellular factors and modulate their normal function, thus leading to deregulation of normal cell activities and HCC<sup>[46,51]</sup>. Despite its importance in HCC development, the clinical significance of genetic variability in the *x* genetic region still remains poorly understood<sup>[52]</sup>.

Several factors, including age, male gender, repeated episodes of severe acute exacerbation, and HBV reactivation after HBeAg seroconversion, are related with the risk of developing advanced liver disease in patients with CHB. Previous studies showed that HBV genotype C infection is associated with later HBeAg seroconversion and multiple episodes of acute exacerbation without HBeAg seroconversion than genotype B HBV infection<sup>[9,21,25,30,49]</sup>. The delayed HBeAg seroconversion may prolong the inflammation process and subsequently result in more severe liver damage<sup>[30]</sup>. Several nucleotide mutations in the pre-core and core promoter regions may reduce HBeAg production and are associated with advanced liver disease<sup>[47]</sup>. In Asia, genotype C and T1762 and A1764 mutants may play a role in HBV-related liver cirrhosis, and can be used in predicting the clinical outcome of patients with chronic HBV infection.

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