Review Article

Hepatitis B virus preS/S gene mutations and their clinical implications

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Abstract: Hepatitis B virus (HBV) infection remains a major health problem worldwide. HBV is one of the smallest enveloped DNA viruses, and also one of the principal pathogens causing acute and chronic hepatitis. Hepatitis B surface antigen (HBsAg) is a hallmark for the diagnosis of HBV infection, and the quantification of serum HBsAg is regarded as a reliable marker of disease progression and predictor of the outcome. However, the mutations in the preS/S genomic region can occur in HBV DNA sequences from the patients with chronic HBV infection, including genetic recombination, base pair deletion, and point mutations. These variants carrying the modified surface antigen may induce immune escape or occult HBV infection (OBI). Furthermore, the preS/S variants may be the cause of fulminant hepatitis (FH) and fibrosing cholestatic hepatitis (FCH). The mutations in the preS/S region would increase the difficulty of preventing and treatment of HBV infection. In this review, we summarized the prevalence of the mutations in the preS/S region and their effect for the diagnosis or progression of HBV infection.

Keywords: Hepatitis B virus (HBV); mutations in preS/S region; immune escape; occult HBV infection (OBI)

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Introduction

The human hepatitis B virus (HBV) is one of the smallest enveloped DNA viruses, and one of the principal pathogens causing acute and chronic hepatitis. Although prophylactic vaccine can prevent the HBV infection and reduce the incidence effectively, about 2% of the vaccinated people in some areas can develop chronic HBV infection. There are about 240 million people suffering from chronic HBV infection worldwide, and nearly 25% of whom have chronic liver disease and/or cirrhosis, which could progress to hepatocellular carcinoma. So HBV infection remains a major health problem worldwide (1,2). Hepatitis B surface antigen (HBsAg) is a hallmark for the diagnosis of HBV infection, and the quantification of serum HBsAg is regarded as a reliable marker of disease progression and predictor of the outcome. In addition, the clearance of HBsAg and the HBsAg-seroconversion are believed to be the ultimate goals of antiviral therapy, as they represent that host immune system can control the active HBV replication successfully (3). A large amount of evidence indicated that the emergence of mutations in the PreS/S genomic region, including amid acids substitution, insertion and deletion, is a frequent event that may be occur as the consequence of antiviral treatment or immunoprophylaxis, or occur spontaneously (4). In this review, we will describe the prevalence of the preS/S gene mutations and their clinic implications.

HBV virology and the feature of preS/S

HBV is a member of the Hepadnaviridae family, and characterized by a high liver tropism and species-specificity. Only the human been and chimpanzees are fully susceptible to infection with HBV, and the liver is thought to be the exclusive target organ for HBV virus, although a small amount of HBV has been found in other tissues such as mononuclear cells, pancreas, and kidney. HBV virion is an approximately 42 nM particle in diameter and its genome is a partially double-stranded relaxed, circular DNA (rcDNA) which comprised by approximately 3.2 kb base pairs (5). HBV genome is located in the nucleocapsid (core) which is surrounded by the envelop proteins. There are four partially overlapping open-reading frames (ORF) in the HBV genome: the preS/S, preCore/Core, Pol and X, and they encode the viral envelope proteins, the preCore and Core protein, viral polymerase, and the regulatory X protein respectively. Particularly, the preS/S ORF can be translated from three different starting codons and encode three different envelope proteins respectively (Larger, Middle, and Small protein). So the C-terminal domains of the three proteins are the same, but the N-terminal extensions are different. The Larger protein (L) consists of 389 to 400 aa depending on the different serotypes, and Middle (M) protein consists of 281 aa and Small (S) protein consists of 226 aa. The 55 hydrophilic aa at the N-terminal domain of the M protein is named preS2, and the extension of hydrophilic residues containing 108 or 119 aa (depending on the genotype) at the N-terminal domain L protein is called preS1 (6,7).

The HBV envelope proteins are typical membrane proteins and have a relatively complex topology. The envelope protein synthesis is performed at the endoplasmic reticulum (ER) via a distinct mechanism that is different from viral replication. Three distinct particles containing envelop proteins can be found in the serum of HBV infected patients, spherical particles, filamentous particles, and Dane particles. The first two particles are formed by the HBV envelope proteins but don't contain the HBV genome, so they lost the capacity of infection. The Dane particles are infectious virions containing complete construction and HBV genome. The spherical and filamentous particles are termed as subviral particles (SVP) and their amount is 1,000 to 10,000 times as the infectious virions. The SVPs can form the immune complexes circulating the host body and induce small vessel vasculitis; also, the SVPs can promote the host immune tolerance (5,7). Meanwhile, most of the envelope proteins present both in SVP and virions are S protein, only 20% of the envelope proteins are constituted by M and L protein. Notably, nearly all of the commercial HBsAg quantification assays use the antibodies which target the epitopes in the S protein, so these quantitative immunoassays can detect all of circulating envelope proteins including the immune complexes, but not distinguish the HBsAg in the virions or in the SVPs (8-10).

Three highly conserved areas in HBsAg are important for the HBV genotyping and sub-genotyping which located on the residues 25–43, 69–109 and 144–157. Genotype-specific substitutions reside in between these locations in a bare major hydrophilic region (MHR) which extend from 110–155 residues and encompasses the "a" determinant, and this determinant is the crucial site for the attachment of anti-HBs antibody (5,11). The mutations in "a" determinant are common and can reduce its ability of binding to anti-HBs polyclonal and/or monoclonal antibodies which used in commercial diagnostic assays, and finally result in failure to detect HBsAg. In addition to the point mutations have been found in the HBsAg, nucleotides insertion or deletion can also occur and induce frame shift mutations. The cysteines are conserved in the HBsAg aa sequence and are very important for the formation of the loops in the "a" determinant. The mutations can lead the replacement of cysteine residues with serine, and consequently result in the changes of the molecule conformation and antigenicity lost. The lysine/arginine substitutions at residues 122 or 160 can determine the variations of the d/y or w/r subtype. The hypervariability of the HBV genome can help HBV to escape from the selection pressures caused by the host immune system, vaccination, and antiviral treatments. The genome of HBV strains is diversity worldwide and we can separate these strains into different genotypes, subgenotypes and subtypes according to the sequence of HBsAg. Mutations within HBsAg may decrease its ability recognizing and binding by neutralizing antibody. Detection of HBsAg is affected by several factors, including the HBV genotypes or subtypes, the sensitivity and specificity of the immunoassay, the capture and detection antibodies, and the conditions of immunoassay (4,12,13).

The preS/S mutation prevalence and immune escape

The preS/S sequences exhibit the highest heterogeneity of the HBV genome. Many studies have confirmed that genetic recombination, base pair deletion, and point mutations in the preS/S regions can occur in HBV DNA

sequences from the patients with chronic HBV infection. In some areas, the mutations in the preS/S are up to 10.8–11.2% among the patients who did not receive vaccine or HBIg (14,15). The plasma-derived vaccines can induce significantly higher prevalence of the "a" determinant mutations in children than the recombinant vaccines. The variants and the wild type virus can co-exist in the host. The vaccine-induced anti-HBs antibodies will be waning gradually, and it targets the variants initially. So the variants will be taken over, and the wild type virus will eventually become the predominant quasispecies (9,16). Several studies from the countries that implemented the Expanded Program on Immunization have confirmed that, people can be infected by variants with the G145R mutation or some other mutations in HBsAg "a" determinant, even those people have vaccinated and developed the anti-HBs antibodies successfully. But the prevalence of the preS/S mutations is different among these studies. Many factors may contribute to the discrepancies between these results, including the regional differences, the vaccination strategies and lasting time of the immunization program. The study in Singapore shows that the prevalence of "a" determinant variants was 39%, but in Taiwan it was 21.22%, in UK it was 22.12%, and in South Africa it was 0%. Interestingly, "a" determinant variants are rarely found in the area with high HBV prevalence, even from the individuals who have accepted the vaccination and were borne to HBV infected mothers. Moreover, the truth of prevalence of the "a" determinant variants can decrease the efficacy of vaccination has not caught people's attention, because vaccine-induced variants are hardly to induce HBV infection breakthrough. The key factors contribute to the vaccination failure include the host gene background, babies infected with HBV before delivery, babies born by the mothers with high viral load, improper vaccination strategy and so on. Taken together, the results from these different studies illuminated that the prevalence of vaccine escape variants were negligible during the practice of the vaccination program worldwide (9,17-20).

For the antiviral therapy, nucleos(t)ide analogous (NA) can suppress HBV replication and reduce the alanine aminotransferase activity quickly. But NAs need to be administrated for long-term as they can't eliminate the cccDNA in the nucleus of hepatocytes. Long-term antiviral treatment with NAs induces the polymerase gene mutate and leads to the emergence of the drug-resistance. Drug-resistance mutation is a common problem among the patients receiving NAs treatment. Because of the existence of the overlapping of the polymerase gene and the surface gene, the compensatory mutations can occur in the surface gene during the NAs treatment. Some of these mutations are located in the area of 'a' determinant and can help the HBV to escape from the host immunity (21).

The distal part of the A-B inter-domain had the overlapping areas accompanying with the B domain of RT (residues 80–236); and residues 72–228 of the RT and surface proteins, respectively. The aa residues of 208, 51, 52, 56, and 120 are notorious for various surface therapy-escape mutations of which the rtA181T/sW172 mutant has a dominant negative secretion effect which leads to the clearance of HBV antigen from the serum, where vaccine-escape-like mutants might be selected (21,22). Many studies have compared the replication capabilities of the wild type and surface mutants. The results showed that these mutants, including E164D, I195M, W196S, M198I, E164D, and I195M, which were selected during lamivudine treatment, had the minimal capabilities to bind to anti-HBs antibody, just as the mutant G145 which can escape from the vaccine immunity. So the variants selected by lamivudine may not be neutralized by the anti-HBs antibody which was induced by vaccine. For the other NAs, including adefovir, tenofovir, entecavir, and telbivudine, it's not clear whether these antivirals have the potential to select HBV variants which reduce the capabilities of binding to the anti-HBs antibody (9,23) (*Table 1*).

The preS/S mutation and occult HBV infection (OBI)

As the development of the high sensitive techniques, HBV DNA can be detected from the patients with HBsAg negative in the serum. We defined the individuals with HBV DNA positive persistently in the liver and HBsAg negative as OBI, whatever the HBV DNA in the serum is positive or negative (13). The mechanisms of OBI are not clear yet. Patients infected with HBV variants which had defective replication activity or cannot express S antigen may develop OBI. But most of the HBV quasispecies isolated from the OBI patients were replication-competent viruses. Patients infected with HBV variants producing surface antigen which cannot be recognized by the conventional detection assays may explain the development of OBI. But these explanations need to be confirmed further (8,13,54).

In recent years, OBI has attracted people's attention worldwide because it may be a threat of the HBV infection in transfusion medicine. The HBV strains from OBI patients cannot be detected by the commercial assays, so

Table 1 PreS/S mutations and their clinical implications

Mutations	Clinical implications
E164D (24), I195M (24), M198I (24), W196S (24), T116N (25), P120S/E (26), I/T126A/N/I/S (27-29), Q129H/R (29-31), M133L/T (31,32), K141E (33), P142S (33), D144A/E (32,34), G145R/A (29,31,35,36), M103I (34), L109I (34), T118K (34), P120A (34), Y134H/N (34,37), S143L (34), S171F (34), C48G (34), V96A (34), L175S (34), G185E (34), V190A (34), L108V/I (31), T87I (31), I84T (31), A91T (31), T68V (31), S78N (31), V60A (31), T31I/T (31), P34N/T (31), G31E (31), Q2K/B (31), T140I (31,37), G130N (29), F134I (29)	Immune escape
Y100S (38,39), Q101R (38), P105R (39,40), T115N/A (31,41), T116N (38), P120L (42), R122P (38), T123N/A (31,43), I126S/T (44), P127H/L (45), Q129P/R (29,31,46,47), M133T (48), S143L (38), S167L (38), R169H (48), G145R/A (31,48), C124Y (49), D144A (50), G119R (49), C124Y (49), I126S/A (31,49), C139R (51), S78N (31), L108I (31), A90V (31), Q118L (31), T87S (31), A90T (31), KL45F (31), P47T (31), N56T (31), G73E (31), P94S (31), I110L (31), S117N (31), S114T (52), P127S/T (52), M133T (52)	OBI
A1762T (53), G1764A (53), G1862T (53), G1896A (53)	Fulminant hepatitis

these OBI patients may be selected as blood donors and their blood containing HBV may transfuse to the other patients. In the blood donors, the prevalence of OBI ranges from 0.0002% to 0.084% in Europe. China is one of the highest HBV endemic regions, and the prevalence of OBI in the blood donors is as high as 0.18% (38,55-58). Most of the studies about HBV mutation just focused on limited parts of the HBV genome, so we can't get enough information to analyze relationship between mutation and OBI. A recent survey showed that the prevalence of specific mutation in OBI patients was 8.3–20.8%, but in the overt infected patients it ranged from 0% to 3.7%. It's worth to mention that there is no unified standard to determine which mutations are associated with OBI. Some scholars considered the mutations located in "a" determinant as OBI-associated mutations, but others thought they may include the mutations in the whole MHR (13,38).

A study including more the 400,000 blood-donors found that twenty mutations in genotype D strains were associated with OBI strongly. Most of these mutations were located in "a" determinant, but can also be found in other area of HBsAg. Interestingly, these mutations reported in this study were quite district from those found in OBI patients who were infected with genotypes B or C. In the author's opinion, the mutations associated with OBI may be unique among every genotype. Nevertheless, the mutation G145R/A was thought to be one of the major mutations associated with OBI, and it could be found both in genotypes B and C (49,59,60). On the other hand, the mutations in the MHR were found to be responsible for decreasing the surface antigen levels in serum. Huang et al and colleges found some mutations in the MHR can reduce the detection sensitivity of commercial assays, and others can influence the secretion of surface antigen and/or virion itself (49).

Cassini et al isolated HBV strain from liver samples and found C695T mutation which can lead to the appearance of stop codon in HBs gene at aa181. This additional stop codon can result in truncated S protein production, and decrease the levels of HBsAg both in the serum and in the liver. In this study, HBV DNA can be found in the peripheral blood mononuclear cells (PBMCs) and liver tissue gotten from the OBI patients. So the authors strongly suggested that testing the PBMCs and liver tissue can help for diagnosis of OBI (61). In addition, preS antigen also played a crucial role for the expression, synthesis, and secretion of the S protein. And the mutation in preS may have the potential association with the OBI development. The effect of preS region for the OBI development needs to be investigated further (60,62) (*Table 1*).

The preS/S mutation in acute and chronic hepatitis B

More and more evidence showed that the mutations in preS and S region can be found in severe forms of HBV related acute and chronic liver diseases, and these mutations can affect the development or even the outcome of diseases. Indeed, mutations within preS can be found in a majority of patients chronic infected with HBV. Several genetic defects may be responsible for the mutation emergence within preS, including deletions of aa in the preS1 and/or preS2 area, and point mutations. The point mutations at the start codon of preS2 also bad been found and it prevented M protein expression completely (63,64).

Mutations in preS, especially that abolish the expression of M protein, have been found in the patients with

fulminant hepatitis (FH). Pollicino *et al.* and colleges analyzed the whole genome of HBV isolated from a surgeon and his mother, both of them suffered from FH and died of it. They found the viruses from these two patients had a double mutation in the preS2 start codon (ATG → ACA) which prevent the production of the M protein (65). Several other studies also found the preS2 defective viruses from the patients of FH, and their outcomes were similar with reports by Pollicino *et al.* (66,67). These studies showed that infection by preS2 defective virus is frequently associated with FH, indicating that this variant might play a pathogenetic role in cases of acute liver failure. Until now, people can't confirm that preS2 defective viruses can induce acute liver failure in the animal models. In the HBV transgenic mouse model, HBV surface proteins accumulated in the hepatocytes, and lead the hepatocytes to enhance the sensitivity to IFN-γ produced by the cytotoxic T lymphocytes. The livers from these mice showed pan lobular necrosis and hepatic failure. According to it, we can get a hypothesis that preS2 defective viruses which can't express M protein may overproduce L protein, which promote the L protein accumulated in the hepatocytes and induce hepatic failure occurred. In addition, the specific immune response for M protein, including the T cell response and B cell response, are very important during the early stage of HBV infection. The viruses without M protein may be targeted ineffectively by the specific T cells, which may result in more severe course of the infection (4). These hypotheses can make an explanation for the association between preS2 defective mutations and the FH, but they need to be confirmed by experimental studies.

Fibrosing cholestatic hepatitis (FCH) is a rapidly progressive and usually fatal form of viral hepatitis, and characterized by specific histologic manifestation of HBV infection consisting of periportal fibrosis, hepatocyte ballooning, cholestasis, a relatively scant inflammatory infiltrate, and marked overexpression of HBV antigens in hepatocytes. FCH had been reported in immunosuppressed patients, including liver, kidney, and bone-marrow transplanted patients. The accumulation of massive HBV antigens in the hepatocytes caused by the preS mutation can also lead to a direct cytopathic effect and result in the hepatocytes impairment, just as the FCH, and this hypothesis has been confirmed by Bock CT et al in the human hepatoma cell line. It's worth to mention that treated the patients undergoing liver transplantation with NAs can not only inhibit HBV replication, but also prevent FCH development effectively (68,69).

The HBV virions with preS mutations infected the human liver and induce viral proteins and replicative intermediates retention in the hepatocytes. In this condition, the massive intracellular viral proteins may be cytotoxic and result in liver injury, which is considered to be the mechanism for preS mutant infection can induce worsen outcome of the CHB. Actually, the association between preS mutant infection and liver cirrhosis has been found in a series of studies, which has also been confirmed by some prospective researches. In addition, more and more evidence showed that the "complex HBV variants", which contain mutations both in preS and BCP area, are more danger than the variants with mutations only in one area. The "complex HBV variants" have more potential to improve CHB progression toward worse outcomes, including liver failure and cirrhosis (4,70,71) (*Table 1*).

Conclusions and perspective

HBV sequences from the OBI patients demonstrate numerous mutations that lead to immune escape, down-regulating the expression of HBsAg, or impaired HBV packaging. In addition, these mutations may be associated with changes in the core or polymerase gene, and may play an important role for the diseases progression and outcomes. The variants with mutations in the "a" determinant can produce modified surface antigen, whose antigenicity has decreased and can't be recognized and targeted by the anti-HBs neutralizing antibody induced by the vaccine immunity. Its consequence is the occurring of immune-escape. It's difficult to detect these variants by the commercially available immunoassays which target the HBsAg. And this is a stronger risk factor for the prevalence of OBI. It's necessary to screen the preS/S mutation in the CHB patients. Identifying the patients infected with these specific HBV variants can help doctors to evaluate their diseases and give them appropriate treatment, and also it may be useful for excluding the OBI patients from the blood donors and preventing these specific HBV variants transmissions.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aob.2017.10.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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