Hepatitis B virus genetic mutations and evolution in liver diseases

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Hepatitis B virus (HBV) belongs to the genus Orthohepadnavirus of the Hepadnaviridae family and is approximately 3.2 kb in length. Owing to a lack of proofreading capacity during reverse transcription and a high replication rate, HBV exhibits as quasispecies. To detect the genetic mutations of HBV, many methods with different sensitivities and throughputs were developed. According to documentary records, HBV mutation and evolution were important via parameters in predicting disease progression and therapeutic outcome. In this review, we separately discussed the correlation between HBV genomic mutations in four open reading frames and liver disease progression. Since some of the results were controversial from different laboratories, it remains to be seen whether functional analyses will confirm their role in modifying the course of infection.

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Key words: Hepatitis B virus; Mutation; Genotype; Liver disease; Risk markers

Core tip: Understanding the characteristics of hepatitis B virus (HBV) is crucial for early diagnosis and optimized treatment. In this review, we reviewed the technologies being used in the evolutionary and mutational analysis of HBV and introduced a high throughput method of deep sequencing for HBV (ultra-highthroughput next generation sequencing technology). And then, we separately discussed the correlation between HBV genomic mutations in four open reading frames and liver disease progression. Since some of the results were controversial from different laboratories, it remains to be seen whether functional analyses will confirm their role in modifying the course of infection.

INTRODUCTION

Hepatitis B virus (HBV) belongs to the genus Orthohepadnavirus of the Hepadnaviridae family and is approximately 3.2 kb in length with four overlapping open reading frames.
frames (ORFs) encoding the polymerase (P), core (C), surface antigen (S), and X protein. Based on ≥ 8% intergenotype divergences in the entire genome, HBV has been classified into at least ten different genotypes, named A-J[7]. In addition, subgroups have been reported in different genotypes of HBV[8,9]. As documented in many studies, HBV exhibits a mutation rate more than 10-fold higher than that of other DNA viruses and exists as quasispecies, owing to a lack of proofreading capacity during reverse transcription and a high replication rate.

METHODS FOR DETECTION HBV GENETIC MUTATIONS

HBV DNA was almost simultaneously cloned and sequenced in 1978 by three pioneers[7-10]. From then on, several methods have been developed to determine the HBV genome and its genetic mutations[11-13], including polymerase chain reaction (PCR) amplification and direct Sanger sequencing[14,15], restriction fragment length polymorphism[16], line probe assay[17,18], enzyme-linked immunoassay[19], clone-based sequencing (CBS)[20,21], real-time PCR (RT-PCR) assay, fluorescence resonance energy transfer (FRET)-based RT-PCR assay[22], and hybridization-fluorescence polarization assay[23]. Among these methods, direct PCR sequencing detects mutations present in ≥ 20% of the circulating virus population (on average). Clone-based sequencing has a higher sensitivity for detecting low-prevalence HBV mutations and has been commonly used for detecting HBV heterogeneity. However, its throughput limitation and time-consuming nature can not be satisfied with the growing need for HBV complexity and diversity analysis. In recent years, ultra-high-throughput next generation sequencing (NGS) technology is used in the HBV heterogeneity analysis[24,25]. It is more sensitive and efficient in terms of low abundant variation detection (< 20% minority variants) than that by CBS method[26], and can simultaneously detect mutations in different HBV gene regions[27], thus sheds light on the future clinical application of NGS in HBV quasispecies studies.

HBV GENOTYPING IN LIVER DISEASES

HBV genotype is an important viral parameter in predicting disease progression and therapeutic outcome[28-33]. Many population-based or community-based long-term cohort studies showed that genotype is one of the high risk factors for liver disease progression. More than a decade followed-up studies revealed that persons in the inactive phase of hepatitis B with genotype B were at a high risk of reactivation[34], and HIV-infected patients with HBV genotype B were more likely to experience acute exacerbations of hepatitis and liver disease-related death than those with genotype C coinfection[35]. Other cohort studies revealed that compared with genotypes A and B cases, HBV genotypes C and D infection is associated with higher prevalence of basal core promoter mutation and a higher risk of hepatocellular carcinoma (HCC)[36-38]. These observations suggest pathogenic differences between HBV genotypes[39].

Several studies of standard interferon therapy showed that genotypes A and B were associated with better response to Peg-IFN-α-2a therapy and higher rates of HBeAg seroconversion compared to genotypes C and D[39,40,41], and HBV genotype B was an independent factor for HBeAg clearance[42]. Interestingly, other studies of pegIFN-α reported that genotypes A and D but not genotype B were associated with a higher rate of HBeAg seroconversion[43,44]. These discrepant results may be due to several intrinsic features and weaknesses in the majority of clinical trials conducted, such as different ethnicities and different patient enrollment criteria. Thus, guidelines from three regional bodies-AASLD, APASL and EASL-all stop short of recommending genotyping as part of the management of chronic hepatitis B[45,46]. Still, additional multicenter data on the relation between HBV genotypes and treatment response are needed before testing for HBV genotypes in clinical practice is recommended.

HBV GENETIC MUTATIONS AND EVOLUTION IN LIVER DISEASES

Many investigations demonstrated that during the progression of liver diseases, genetic mutations and evolution were observed in the HBV gene-coding regions, and some of them could be risk markers for liver injury (Table 1).

PreS1/S2/S ORF

The HBV S-ORF is composed of three forms of HBV surface genes: pre-S1, preS2, and S domain. The pre-S domain is the essential binding site for hepatocyte receptors and contains several epitopes for T or B cells. Mutations at this region may directly influence HBV infection and liver disease progression. Pre-S deletion was observed in chronic hepatitis B infection, fulminant hepatitis B, acute hepatitis B and HCC[47-49]. Several cross-sectional studies have shown an association between pre-S mutation and HCC[50,51]. Longitudinal observations demonstrated a gradual combination of pre-S deletion during the development of HCC, and patients with pre-S mutations had significantly higher 5-year cumulative incidences of HCC than those without (26.5% vs 5.7%, P < 0.001)[52]. Variation and deletion in the 3’ terminus of pre-S are also associated with occult HBV infection[53]. Besides deletion variation, a novel preS1 mutation, W4P/R was observed with the progression of liver diseases and male predominance from a Korean chronic cohort through a molecular epidemiologic study. These W4P/R mutants were significantly related to severe liver diseases [HCC and liver cirrhosis (12.4%, 19/153 patients) vs chronic hepatitis and carrier (1.1%, 1/94 patients), P < 0.001]. Interestingly, all of the W4P/R mutants were found only in the male gender, not in the female gender, which may in part provide the likely explanation for the relatively high ratio of male to female incidence in HCC.
Table 1 Possible risk markers for liver injury

| ORF       | Major Mutations | Clinical status | Ref. |
|-----------|-----------------|-----------------|------|
| PreS1/S2/S|                 |                 |      |
| preS deletion | CHB [49-51] |                 |      |
| FHB       |                 |                 | [52,53] |
| AHB       |                 |                 | [54,55] |
| HCC       |                 |                 | [56-61,78] |
| W4P/R     |                 |                 | [62] |
| male predominance | HCC and LC |                 | [23] |
|           |                 |                 |      |
| S         |                 |                 |      |
| T207A     | LC              | [63]            |      |
| T770C     | HCC             | GenBank no.AY206393 |      |
| C695      | Occult infection | HCC and LC | [64] |
| T207A     | HBsAg(-)        | [65-67]         |      |
| X         |                 |                 |      |
| A1762T/G1764A | LG, HCC | mild liver histology | [68,69,71,75-79] |
| C1653T    | LG, HCC         | [75,77-79]      | [72] |
| T1735V    | LG, HCC         | [75,77-80]      |      |
| G1386M    | LG, HCC         | [75,77-80]      |      |
| B1499     | LG, HCC         | [75]            |      |
| G1613A    | LG, HCC         | [77,79]         |      |
| A1727G    | LG, HCC         | [76,77]         |      |
| G1757A    | LG, HCC         | [76,77]         |      |
| C1766T    | LG, HCC         | [76-78]         |      |
| T1758A    | LG, HCC         | [75-76]         |      |
| A1727G    | HCC             | [76]            |      |
| C1773T    | HCC             | [76]            |      |
| preC/C    |                 |                 |      |
| A1986T    | FHB, HCC        | [37,80]         |      |
| G1389A    | HCC             | [37]            |      |
| C193A or C1914T | HCC | [37] |      |
| A2149C/T  | HCC             | [37]            |      |
| A2188T/C  | HCC             | [37]            |      |
| C2198A    | HCC             | [37]            |      |
| C2444A/T  | HCC             | [37]            |      |
| core antigen | CHB          | [82,83]         |      |
| internal deletions | HCC | [84] |      |
| immune-suppressed patients | [85] | |      |
| P         |                 |                 |      |
| G741H     | HCC             | [89,93]         |      |
| CHB       | [90-93]         | | | |

ORF: Opening reading frame; HCC: Hepatocellular carcinoma; CHB: Chronic hepatitis B; FHB: Fulminant hepatitis B; AHB: Acute hepatitis B; LC: Liver cirrhosis.

X ORF

HBV-X protein is associated with the pathogenesis of HBV related diseases, especially hepatocellular carcinoma in chronic patients. Genetic variability of the X gene includes genotypic specific variations and mutations emerging during chronic infection. The double mutation of nucleotide A1762T/G1764A in basal core promoter (BCP) is frequently observed in HBV sequences isolated from patients with chronic HBV infection, fulminant hepatitis, HCC, and in reactivation of HBV with a fulminant course\(^{66,69}\), which results in mutations at two codons in the carboxyl functional region of X protein (K130M and V131I). At present, there are conflicting opinions regarding 1762T/1764A hotspot mutations. Some studies suggest that these mutations decrease HBeAg expression and slightly increase viral DNA replication, and are mostly found in patients who seroconvert to anti-HBe and develop HCC\(^{70-72}\). By contrast, other studies indicate that these mutations are not associated with HBeAg/anti-HBe status or HBV DNA or HCC\(^{73,74}\). Other mutations including M1386, T1485, B1499, A1613, T1653, G1727, A1757/T1764/G1766, T1773, G or C1753, and T1766/A1768 mutations have been reported to be associated with the development of HCC\(^{75-79}\), which are alone and/or in combination to be the predictive markers for hepatocarcinogenesis.

PreCore/core ORF

HBV precore/core ORF encoding proteins, hepatitis B e antigen (HBeAg) and core antigen (HBcAg), are two indicators of active viral replication. Mutations in the C region were mainly distributed in MHC restricted region. In particular, mutations in the MHC class II restricted region (in M2RR) were found to be significantly related to HCC. Six (preC-W28*, C-P5H/L/T, C-E83D, C-197F/L, C-L100I and C-Q182K/*) and seven types (preC-W28*, preC-G29D, C-D32N/H, C-E43K, C-I97F/L, C-L100I and C-Q182K/*) of mutations in the preC/C region were found to be related to HCC and to affect the HBeAg serostatus, respectively\(^{17,80}\). However, children with HBV infection in the immune-tolerance phase had not have pre-w28* mutation, suggests that this mutation may be the result of life-long chronic HBV infection\(^{80}\). Also, a heterogeneous population of core antigen internal deletions (CID) has been found to be highly prevalent in chronic HBV carriers\(^{81,82,83}\), HCC patients\(^{84}\) and immunosuppressed patients\(^{8,83}\), suggesting that the host immune pressure against T cells is the major driving force of preC/C mutation\(^{86-88}\).

Polymerase ORF

Mutations in the reverse transcriptase domain and different HBV genotypes may result in changes in amino acid sequence and protein configuration of HBV polymerase. Prior research has suggested that lamivudine is the major cause of YMDD mutations in HBV P-ORF.
and lamivudine-related YMDD mutation is an independent risk factor for HCC.[98] However, the mechanism remains unclear. Further research has revealed that strains with YMDD mutations also exist in patients with chronic HBV infection not previously treated with lamivudine.[99-101] A recent research showed that spontaneous YMDD mutations were detected in LC and HCC patients, and genotype C strains in HCC patients had a significantly higher spontaneous YMDD mutation rate than in LC patients, and was associated with a 7.775-fold higher risk for the development of HBV-related HCC than patients infected by other type HBV strains (P = 0.013, 95% CI: 1.540-39.264). This may have been caused by different genotype strains having different biological properties, pathogenicity and carcinogenicity.[102]

In all, understanding the characteristics of hepatitis B virus is crucial for early diagnosis and optimized treatment. There is great need to develop methodologies that take into account both factors from the host and the pathogen. Many of candidate mutations seem unexpected given our current knowledge of the molecular genetics of HBV. Thus, it remains to be seen whether functional analyses will confirm their role in modifying the course of HBV. Thus, it remains to be seen whether functional analyses will confirm their role in modifying the course of HBV. It remains uncertain whether functional analyses will confirm their role in modifying the course of HBV.

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