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Name of Journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 91754

Manuscript Type: EDITORIAL

Hepatitis B virus genotypes in precision medicine of hepatitis B-related hepatocellular carcinoma: Where we are now

HBV genetic diversity and HCC

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Abstract

Hepatitis B virus (HBV) infection is a major player in chronic hepatitis B that may lead to the development of hepatocellular carcinoma (HCC). HBV genetics are diverse and it is classified into at least 9 genotypes (A to I) and 1 putative genotype (J), each with specific geographical distribution and possible different clinical outcomes in the patient. This diversity may be associated with the precision medicine for HBV-related HCC and the success of therapeutical approaches against HCC, related to different pathogenicity of the virus and host response. This Editorial discusses recent updates on whether the classification of HBV genetic diversity is still valid in terms of viral oncogenicity to the HCC and its precision medicine, in addition to the recent advances in cellular and molecular biology technologies.

Key Words: hepatitis B virus; hepatocellular carcinoma; genotypes; pathogenesis; precision medicine

Sukowati CHC, Jayanti S, Turyadi T, Muljono DH, Tiribelli C. Hepatitis B virus genotypes in precision medicine of hepatitis B-related hepatocellular carcinoma: Where we are now. *World J Gastrointest Oncol* 2024; In press

Core Tip: This article discusses recent updates on the classification of HBV based on its genetic diversity (genotype) and its relationship with current data in HBV pathogenicity, HCC, and HCC treatment. This article discusses recent updates on the classification of HBV based on its genetic diversity (genotype) and its relationship with current data in HBV pathogenicity, HCC, and HCC treatment.

INTRODUCTION

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Hepatitis B virus (HBV) infection is a major player in chronic hepatitis B, liver fibrosis and cirrhosis, and finally the development of hepatocellular carcinoma (HCC). Data from World Health Organization (WHO) fact sheet estimated that in 2019 around 300

million people were living with chronic hepatitis B infection with 1.5 million new infections each year. HBV infection led to an estimated 820,000 deaths, mostly from liver cirrhosis and HCC ^[1], accounting for around 50% of HCC cases globally ^[2]. Even though highly effective vaccination against hepatitis B is available and even mandatory in many countries, chronic HBV infection remains the major cause of mortality ^[3].

The pathogenesis of HBV in triggering sequent liver damage occurs in an indirect mechanism related to necro-inflammation and immunological response of the host, leading to liver fibrosis and cirrhosis. However, being a DNA virus, HBV can directly cause oncogenic transformation where HBV DNA fragments might integrate into human DNA at the early steps of clonal tumor expansion. This integration may induce host genomic instability and insertional mutagenesis of diverse oncogenes ^[4], as we previously reviewed ^[5].

On the other hand, HCC itself, within the same patient and among patients, is very heterogeneous in terms of natural history, molecular classification, histology, and cellular profile. This influences disease progression, classification of the disease, prognosis, and natural cellular susceptibility to drugs ^[6]. Naturally, this is associated with the therapy. In this era of precision medicine, therapeutical approaches for HBV-related HCC can also be influenced by the natural heterogeneity of the HBV. This Editorial discusses recent updates on whether the classification of HBV genetic diversity is still valid in terms of viral pathogenicity to the HCC and its treatment.

HBV GENOTYPES

HBV belongs to the *Hepadnaviridae* family with around 3.2 kilobases of compact and partially double-stranded genome. HBV genome consists of four overlapping Open Reading Frames (ORFs) in the HBV genome: P, S, C, and X each encodes for different protein(s) important in viral replication and pathogenesis ^[7]. In various aspects, HBV is unique including its genetic material, genome organization, genome replication, and genetic control ^[8].

Based on genome-wide sequence divergence of at least 7.5%, HBV is classified into nine genotypes (A–I) and one putative genotype (J). Except for genotypes E, G, and putative J, all HBV genotypes are also further subdivided into subgenotypes, with DNA divergence between 4 and 7.5% [9,10]. Among these, genotypes A to E account for about 96.2% of all global chronic hepatitis B [11], thus the main genotypes found in HCC.

HBV genotypes are related to geographic distributions, ethno-geographic ranges, population migration, and HBV evolution [12]. Genotype A, divided into subgenotypes A1 to A8, is distributed in northern and northwestern Europe, South Africa, and Brazil, and includes. HBV/B (genotype B), divided into subgenotypes B1 to B10, is found primarily in Asia and the Arctic. HBV/C, composed of subgenotypes C1 to C17 is primarily distributed in the Pacific islands, and HBV/D, consisting of D1 to D12, is widely distributed in the Mediterranean, India, and Russia. HBV/E and HBV/F are distributed in the African continent, where E is restricted to West and Central Africa while F is mainly distributed all over Africa and includes subgenotypes F1 to F6. HBV/G is widespread in Europe, America, Africa, and Asia. HBV/H is mainly distributed in America. HBV/I is mainly distributed in Asia, Vietnam, Laos, southwestern China, and eastern India, and includes subgenotypes I1 to I3. HBV/J was recently found in Japan [10,13].

HBV GENOTYPES AND PATHOGENESIS TO HCC

Following the classification of HBV genotypes, studies to understand the association between HBV genotypes and the progression of liver diseases, especially for HCC, have also been attempted. Since HCC development is complex involving long-term progression, the results, as expected, are varied. In addition, HBV genotypes are also linked with core and basal core promoter (BCP) mutations. It was shown that the core promoter and BCP A1762T/G1764A mutation usually occurs nearly 10 years before the identification of HCC and may be an early episode of hepatocarcinogenesis [14].

For the last 2 decades, various studies have been performed in several regions, especially in China, India, and Alaska, showing in particular the association between

HBV genotypes and HCC occurrence. One of the earliest studies showed that from 154 HCC case patients, followed in 14 years from around 4800 HBV carriers in Taiwan, HBV/C ¹³ was associated with an increased risk of HCC compared with other HBV/A and HBV/B (adjusted OR = 5.11), strongly associated with higher plasma HBV DNA levels [15]. This study on the risk of HBV/C was then confirmed by several other reports in Asian populations, including in mainland China, Vietnam, and Japan, also in the last five years [16–21]. However, a study in China showed that a heterozygous mutation status of reverse transcriptase rt169/rt180 was associated with the increased risk of HCC, regardless of HBV genotypes [22].

In the Alaskan population, several studies showed that the HCC risk was higher for HBV/F, followed by HBV/C and HBV/A, compared to HBV/B and HBV/D [23,24]. In the Indian population, however, HBV/D was the predominant genotype associated with HCC cases [25], even though another study showed that patients with HBV genotypes A and D showed similarities in clinical, histological severity, and therapeutic responses [26]. A study in Africa showed that carriers of HBV/A1 and HBV/E display unique clinical features. Even though HBV/E-infected individuals had HBV high viral loads, and transmit HBV perinatally, HBV/A1-infected individuals with low viral loads and horizontal transmission, exhibited a higher extent of liver damage and a higher risk of developing HCC [27].

Here, we need to emphasize, that the above studies were majorly country-based, analyzing the predominant genotypes that exist within countries, thus limiting a direct comparison comprising a larger number of samples with all (or almost all genotypes) from a wider geographical area. However, as previously demonstrated by Wong *et al*, a meta-analysis study analyzing 43 studies with a total of 14,545 patients with HBV genotypes of A to H, still showed that HBV/C was ⁷ associated with a higher risk of HCC as compared with other major HBV genotypes A, B, and D. This partially explains the higher risk of HCC in patients from Southeast Asia, where HBV/C is predominant. The patient was infected with HBV/C, often at higher risk for more active liver disease and cirrhosis [28].

PRECISION MEDICINE FOR HBV-RELATED HCC

⁹ Patients with distinct HBV genotypes experience varying prognoses, immune responses, and clinical symptoms, consequently influencing HBeAg seroconversion, HBsAg seroclearance, and resistance to antiviral drugs [13,29]. Connections have been noted between the risk of HCC in youth and HBV/B, as well as, in later stages of life, with genotype C. Even Specifically, associations have been observed among subgenotypes and HCC risk with subgenotype Ba in younger individuals and subgenotype Ce in those at a more advanced age [30].

To date, the sole recourse to prevent or delay the onset of HCC in individuals with chronic HBV infection is antiviral therapy. Interferon-alpha (IFN- α) and nucleosides/nucleotide analogues (NUCs, *e.g.* entecavir, tenofovir) are the treatment modalities currently available for chronic HBV infection [31,32]. Nevertheless, it is important to note that treatment responses can vary among different HBV genotypes.

HBV/B and HBV/E are categorized as strong responders for IFN- α and NUCs meanwhile HBV/C and HBV/D are weak responders for both therapeutic approaches [33]. HBV/A patients show better virological responses after IFN- α treatment with 62.5% of HBV DNA reduction compared to HBV/B, HBV/C, and HBV/E. Furthermore, the rates of HBsAg clearance following IFN- α treatment exhibit variability across genotypes. Notably, HBV/A and HBV/B demonstrate clearance rates at 22% and 33%, respectively, surpassing HBV/C and HBV/E at 0% [34]. Meanwhile, the pegylated IFN (peg-IFN) treatment still shows good treatment response among CHB children with HBV/C by giving 67% of HBeAg seroconversion [35], which is twice higher than adults [36]. Indicating the use of peg-IFN is still effective for children with chronic HBV/C infection. Meanwhile, treatment responses also manifest in the utilization of different NUCs among West African patients with CHB. In a comprehensive 5-year cohort study involving CHB patients with genotype E, it was observed that tenofovir (TDF) demonstrates superior suppression of HBsAg and HBV DNA compared to entecavir (ETV) [34].

As the efficacy of currently available treatment varies among the genotypes, several novel therapies have emerged to tackle the issue. Genotype-specific effects are evident in the current utilization of a therapeutic vaccine called NASVAC containing HBsAg and HBcAg from HBV/A. In clinical trials, NASVAC demonstrated superior efficacy compared to Peg-IFN in reducing HBV DNA levels. This effect was particularly pronounced among CHB patients with HBV/D, as opposed to those with HBV/A and HBV/C [37]. In the HBV genotype humanized model, the use of core protein allosteric modulators (CpAMs) efficiently inhibited HBeAg secretion for HBV/A, HBV/B, HBV/D, and HBV/E compared to ETV. However, it did not demonstrate significant advantages over ETV in suppressing HBV DNA [38].

PERSPECTIVE IN MOLECULAR AND CELLULAR BIOTECHNOLOGY

Advances in biotechnology and molecular biology continue to elucidate further characterization of both the pathogen (HBV) and the host (human liver cells). A robust approach to studying the pathogenicity and searching for treatment related to HBV genotypes is by taking advantage of *in vitro* and *in vivo* manipulation of the virus, as well as in animal models.

To understand how each genotype may act differently, several recent studies by the group of Liang *et al.* at the NIH had successfully produced ⁴ stable cell lines producing high titers of HBV genotype A2, B2, C1, E, F1b, and H by transfecting plasmids in permissive HepG2 cells. They showed differences in ³ genotype-associated variations in viral antigen production, infection kinetics, and responses to human IFN- α treatment [47]. This study was then followed by a deeper analysis of the replicative capacity of infectious patient-derived HBV clones covering genotypes A to E. All clones produced ¹² viral particles, and they were infectious in HepG2-NTCP cells, primary human hepatocytes, and human chimeric mice. However, among genotypes, HBV/D exhibited higher infectivity than other genotypes *in vitro*, although it was comparable to HBV/A and HBV/B *in vivo*. Together with HBV/A, HBV/D also induced more pronounced intrahepatic and proinflammatory cytokine responses leading to a rapid clearance [38].

Previously, it was shown that HBV/C could generate more ROS and DNA double-strand breaks than HBV/D in Huh7 and HepG2 [48].

Liu *et al.* [38] have demonstrated how HBV genotypes affect host immune responses by using human-derived HBV genotype clones transfected in hepatic cell lines as well as *in vivo* (chimeric mice). Mice exposed to HBV/A and HBV/D exhibit a more noticeable inflammatory reaction, by increasing proinflammatory cytokines (IFN- γ , TNF- α , IL-6) and chemokines (CCL2 and CXCL10), in comparison to HBV/B, HBV/C, and HBV/E [38].

The pan-D-subgenotypes reverse transcriptase inhibitor has also been identified as a potent anti-HBV agent, demonstrating consistent inhibitory effects across various HBV D-subgenotypes *in vitro*. This suggests the possibility of further optimization, making it a promising drug candidate for treating HBV/D-infected patients, regardless of the specific D-subgenotypes [39].

Recently, an RNA interference therapy also emerged for CHB. VIR-2218 is an experimental N-acetylgalactosamine-conjugated RNA interference therapeutic to treat chronic HBV infection. The treatment targets a region in the HBV genome common to all viral transcripts, making it effective against HBV genotypes A to J [40]. In participants with cHBV infection, VIR-2218 demonstrated dose-dependent decreases in HBsAg levels. Nonetheless, none of the participants exhibited serum HBsAg loss or hepatitis B surface antibody seroconversion[41].

CONCLUSION

To conclude, as previously documented, HBV is an ancient virus with deep ancestry where its diversity is generated by features of the unique viral replication cycle as well as by cellular host factors [42]. Thus, besides the variation of the HBV genome itself, the clinical outcome of developing HCC can be influenced by the genetic background of the host. Human genetic polymorphisms that are associated with HBV clearance and persistent infection were reviewed previously [43]. Since the discovery of sodium taurocholate co-transporting polypeptide (NTCP), as the cellular receptor for HBV entry

into host hepatocytes ^[44], several recent studies also showed that the NTCP S267F variant exhibited protective effects against HBV was associated with a reduced risk of LC and HCC ^[45,46].

Until now, even though until now the association between HBV genotypes, pathogenicity, and precision medicine for HCC is still debatable, the variability of treatment responses tends to emphasize the significance of HBV genotypes in clinical outcomes. We consider that HBV genotype can be put into account as a factor to predict the outcome of the disease. This can also be an additional factor in determining the choice of treatment. However, the implication of this approach for widely used is still challenged by the limitation of facilities, the cost of HBV genotyping, and the high cost of treatment, especially in low-middle-income countries.

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