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Mini-review

Worldwide genetic diversity of HBV genotypes and risk of hepatocellular carcinoma

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ABSTRACT

Hepatitis B viruses (HBV) are responsible for over 50% of the worldwide attributable risk of hepatocellular carcinoma (HCC) and this figure increases even further in regions of high endemicity. Systematic sequencing of HBV genomes has identified that this common virus existed as eight distinct genotypes (denoted A–H), each regrouping variants with less than 8% divergence in their DNA sequence. These genotypes differ by their geographic distribution in populations around the globe. There is evidence that HBV genotypes also differ by their pathogenic properties, including their risk of persistence as chronic infection and their capacity to induce precursor disease or cancer. On the other hand, HBV genes may undergo mutations that become selected during the course of chronic infection and progressive liver disease. The most significant of these mutations in the context of HCC are those occurring in the pre-core (Pre-C) and basal core promoter (BCP) regions. These mutations may upregulate HBV expression and increase its virulence. These mutations may occur in all HBV genotypes but are more common in genotypes associated with more severe disease and cancer, in particular genotype C. Understanding the molecular basis of pathological variations between HBV variants is critical for prediction of disease severity. It will also be important to determine whether differences among genotypes may have an impact on the long-term protective efficacy of universal HBV vaccination.

1. Introduction

Hepatocellular carcinoma (HCC) often occurs as a sequel of chronic infection with hepatitis B virus (HBV); it is estimated that over 20% of the 400 million people with chronic hepatitis B infection will develop HCC [1,2]. In most HCC high-risk areas, the principal risk factors are HBV infection and consumption of AFB1-contaminated food [3].

The clinical course of HBV infection is variable, including acute self-limiting infection, fulminant hepatic failure, inactive carrier state and chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma (HCC) [4,5].

HBV chronic infection is characterized by persistence of Hepatitis B surface antigen (HBsAg) in the plasma over a time interval of 6 months. Although the HBsAg is detectable for several decades, the viral load decreases over the years, which coincide with the disappearance of Hepatitis B antigen e (HBeAg), a temporary increase in serum amino transferases levels and the outcome of anti-HBe. The evolution of the serological markers has been characterized

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by four phases depending on the activity of virus replication and specific immune response: immune tolerance, immune clearance, low or non replicative phase and reactivation phase [4,6] (Table 1). Chronic infection is a consequence of the disability of the immune system to control HBV infection and its frequency depends on age at which infection occurs and the route of transmission. In neonates infected perinatally, the frequency of chronic infection is as high as 90%; however, the frequency in children is 20–30% and in adults it is less than 10% [7]. Additionally, it has become evident that HBV may also persist in the form of serologically silent infections, leading to the concept of occult infection. Such infection may represent a non-negligible contribution to the population burden of HBV-related diseases and is a major concern for transfusional medicine and transplantation. In a recent statement, occult HBV infection has been defined as the presence of viral DNA in the liver, with absence of HBsAg and low titer or even absence of HBV DNA in serum [8]. Occult HBV infection occurs worldwide, and is not necessarily associated to mutations in the "a" determinant of the HBsAg [9]. Rather, it may represent an ultimate form of natural persistent infection. There is evidence supporting that occult HBV infection may accelerate the development of cirrhosis, particularly in HCV co-infected patients, but more studies are warranted to confirm this suggestion. This clinical presentation of the infection has also been associated to HCC, particularly in patients co-infected with HCV [10–13].

HBV exists as many distinct variants that differ by their capacity to become persistent and induce chronicity, as well as by the clinical manifestations of chronic infection including cancer. Prior to the definition of the genotypes, HBV strains were distinguished by serological analysis into nine hepatitis B HBsAg subtypes designated *ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4q−*, *adrq+*, and *adrq−*, determined by mutually exclusive amino acids substitution in positions 122 and 160 of the S region of HBV DNA. In 2004, Norder et al. used complete genome sequences of 234 HBV isolates as well 631 sequences of genes encoding HBsAg to assess the worldwide diversity of HBV [14]. This analysis confirmed the long history of co-evolution of HBV with humans and non-human primates. It also underlined the possible contribution of HBV variants defined on the basis of their genotypes to geographic and etiologic difference in the rate of progression of infection from acquisition of cancer to chronic liver disease and cancer. In this review, we summarize how recent knowledge of the genetic diversity of HBV has contributed to improve our understanding of the molecular epidemiology of infections and we discuss the emerging evidence of an association between some viral strains and increased frequency or rate of development of HCC.

2. HBV genome

The hepatitis B virus (HBV) belongs to the genus *Orthohepadnavirus* of the *Hepadnaviridae* family and shares with these viruses a circular genome of approximately 3.2 kb in length. It contains four overlapping open reading frames (ORF) encoding: ORF preS1/Pre-S2/S the surface antigens (HBsAg), ORF P the viral polymerase (Pol), ORF X the transactivator X protein (HBx) and ORF pre-core/core (Pre-C/C) the e Antigen (HBeAg) and Core protein (HBcAg) (Fig. 1A). Additionally, two viral enhancers (EnhI and EnhII) positively regulate transcription of the HBV promoters, including basal core promoter (BCP) that controls the transcription of both, the pre-core and core regions.

The partially double stranded DNA is generated from an intermediate RNA through reverse transcription (RT) activity of the Pol (Fig. 1B) [15]. The absence of proof reading capacity of the HBV Pol leads to a high mutation rate. On the other hand, the extreme overlapping of the ORFs of this viral genome represents a constraint for natural selection that limits the possibility of fixation of many of these mutations [16]. As a consequence of these opposite aspects, the substitution rate in HBV genome is intermediate between the ones of RNA and DNA viruses [17]. This genomic plasticity allows the generation of a quasispecies-like viral population [18], harbouring viral mutations that can occur and develop under particular selection pressures.

Several studies have pointed that recombination may play an important role in shaping the evolution of HBV [19,20]. In Human Immunodeficiency Virus (HIV), where recombination has been studied in greater detail and is a very frequent event; template switch occurring during reverse transcription seems to be the mechanism responsible for recombination. However, reverse transcription does not appear to be the event associated with recombination in hepadnaviruses, since, in contrast to HIV, it occurs after encapsidation of a single pregenomic RNA [15]. The exact mechanism of recombination of HBV genomes is not clear, but is likely to occur in the nucleus, by illegitimate replication [21] or by recombination with integrated HBV DNA [22].

Table 1
Clinical phases of HBV infection.

Phase	ALT	Viral load	HBeAg	Anti-HBe	Histological activity
Immune tolerance	Normal/low elevation	High	Positive	Negative	Normal/minimal histological activity
Immune clearance	Elevated	Fluctuating decreasing levels	Positive	Negative	Hepatic necroinflammation and variable fibrosis level
Low or non replicative	Normal	Undetectable or low levels	Negative	Positive	Inactive and minimal amount of fibrosis
Reactivation	Elevated	High	Negative	Positive	Moderate or severe necroinflammation with variable fibrosis