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Review

Virus associated malignancies: The role of viral hepatitis in hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the third leading fatal cancer worldwide and its incidence continues to increase. Chronic viral hepatitis involving either hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is the leading etiology for HCC, making HCC prevention a major goal of antiviral therapy. While recent clinical observations and translational research have enhanced our understanding of the molecular mechanisms driving the initiation and progression of HCC, much remains unknown. Current data indicates that HCC tumors are highly complex and heterogeneous resulting from the aberrant function of multiple molecular pathways. This complex biology is responsible, at least in part, for the absence of highly efficient target-directed therapies for this deadly cancer. Additionally, the direct or indirect effect of HBV and HCV infection on the development of HCC is still a contentious issue. Thus, the question remains whether viral hepatitis-associated HCC stems from virus-specific factors, and/or from a general mechanism involving inflammation and tissue regeneration. In this review we summarize general mechanisms implicated in HCC, emphasizing data generated by new technologies available today. We also highlight specific pathways by which HBV and HCV could be involved in HCC pathogenesis. However, improvements to current *in vitro* and *in vivo* systems for both viruses will be needed to rigorously define the temporal sequence and specific pathway dysregulations that drive the strong clinical link between chronic hepatitis virus infection and HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common human cancers, being the fifth most prevalent tumor type and the third leading cause of cancer-related deaths worldwide [1,2]. Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), especially in the setting of established cirrhosis or advanced fibrosis, are the leading causes of HCC worldwide, and the incidence of HCC correlates with the geographic distribution of these infections [3]. Conversely, suppression of HBV replication [4,5] and a sustained viral response (SVR) in the treatment of HCV [6], are associated with a reduction in HCC among treated populations. Men are more susceptible to HCC, most probably because the major risk factors for HCC, including chronic viral hepatitis as well as alcohol consumption and non-alcoholic fatty liver disease (NAFLD) [7,8] are more common in males. However, there is substantial evidence for gender-related differences in cellular pathways associated with HCC that might make men more susceptible to HCC than women [9,10]. Early diagnosis is crucial for potentially successful curative treatment. Therefore, continuous HCC surveillance is recommended for high-risk patients, including those with cirrhosis of any etiology, chronic HCV patients with advanced liver disease and chronic HBV patients even without cirrhosis [11]. Unfortunately, despite efforts aimed at early detection of HCC, a substantial number of patients are diagnosed only when their disease is at an advanced stage. These patients are usually not amenable for potentially curative treatment modalities, such as partial liver resection, liver transplantation or radiofrequency ablation (RFA) [11], but are rather offered non-curative alternatives such as transarterial chemoembolization (TACE) and/or drug therapy with sorafenib [12]. Although associated with only a modest increase in patient survival [12], the multi-kinase inhibitor sorafenib is the first target-directed drug therapy for liver cancer, and was developed based on understanding major intracellular and extracellular signaling pathways involved in HCC pathogenesis [13]. However, despite the progress in elucidating the general mechanism(s) of HCC as well as advances in the study of HBV and HCV virology, there is still a substantial gap in our ability to mechanistically link chronic viral hepatitis with liver carcinogenesis.

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2. Pathways in HCC implicated by genomic studies

Recent advances in genetic technologies, particularly decreasing costs of sequencing have led to many studies interrogating genetic alterations in HCCs. The main pathways involved in HCC initiation or progression can be divided into several categories including cell proliferation and differentiation, inflammation, and angiogenesis, the latter being a central theme associated with this highly vascularized tumor (reviewed in [14]).

Perturbations in the epidermal growth factor (EGF) signaling pathway exemplify how cell proliferation pathways can contribute to HCC. Several studies have shown that certain polymorphisms in the EGF gene can either promote or protect from HCC [15–17], and blocking the EGF receptor has been shown to inhibit the growth of HCC cells both *in vitro* and *in vivo* [18,19]. The ubiquitous activation of Ras and Jak/STAT signaling in HCC provide additional examples of pathways involved in cell proliferation and differentiation, that when altered, affect HCC pathogenesis. Activation of these pathways is associated with DNA hyper methylation and hence silencing of their cellular inhibitors, especially in the setting of cirrhosis [20]. The PI3K-Akt pathway, intimately involved in both cell proliferation and cellular metabolism, is also activated in a significant portion of HCC samples [21]. This pathway might link metabolic alterations in the liver, such as fat accumulation to the development of liver cancer [22]. Recently, genomic analyses of HCC samples from patients with aggressive tumors and poor prognosis revealed over-expression of the fetal oncoprotein SALL4. SALL4 normally co-represses the tumor suppressor PTEN, resulting in activation of the PI3K-Akt pathway. Importantly, blocking SALL4 function with a synthetic peptide has been shown to release PTEN from co-repression, resulting in de-phosphorylation and reduced activation of Akt, accompanied by a significant shrinkage of tumors *in vivo* [23]. The wnt/β-catenin signaling pathway, important in cell differentiation and proliferation, is also frequently mutated in the tumorous tissue of HCC patients [21]. Unlike in colon cancer where mutations are frequently found in the tumor suppressor gene adenomatous polyposis coli (APC) resulting in β-catenin activation, they are rarely found in HCC tumor tissue. In contrast, other mechanisms for β-catenin activation, such as promoter over-activation [24] or mutations in AXIN1 [25] are found.

Inflammation is the hallmark of chronic hepatitis of various etiologies and is thought to be a major trigger for liver carcinogenesis. NF-κB, a major player in the cellular inflammatory cascade, promotes liver cancer in the Mdr2 knockout mouse model, suggesting a link between inflammation and cancer [26].

Angiogenesis also plays a key role in HCC development and invasive potential. A major pro-angiogenesis factor, VEGF, is elevated in the sera of HCC patients, and its serum level, as well as certain VEGF polymorphisms, appear to correlate with prognosis [27,28]. In addition, metastatic tumor antigen 1 (MTA1), a stabilizer of the angiogenesis mediator hypoxia-inducible factor-1 (HIF-1), has been found to closely correlate with post-operative recurrence of HCC and poor survival rates, especially among HBV positive HCC patients [29]. Therefore, the current evidence strongly implicates angiogenesis in HCC pathogenesis, providing clear a rationale for targeting VEGF pathways in anti-HCC therapy.

3. Hepatitis B virus is a risk factor for HCC

3.1. Epidemiology and molecular biology of HBV infection

HBV is a small DNA virus, a member of the *hepadnaviridae* family (reviewed in [30–32]). Transmission can occur by exposure to contaminated blood products, or alternatively by sexual or other modes of intimate contact [32]. In adults, acute infection usually resolves spontaneously, however, in newborns or small children, chronic infection is common and often leads to chronic hepatitis, cirrhosis and HCC [31].

The virus 3.2 kb genome contains four major open reading frames organized in a compact over-lapping gene structure (Fig. 1A). HBV gene products include the polymerase (pol), which has a reverse transcriptase activity and that drives viral replication, core that forms the viral nucleocapsid and that is also cleaved to form the secreted e antigen (HBeAg), Surface (small, middle and large) proteins that are embedded in the virus envelope and are also secreted in the form of "empty" sub-viral particles and X, encoding a protein essential for viral replication. Recently, the bile acid pump sodium taurocholate co transporting polypeptide 1 (NTCP1) has been shown to be a receptor for HBV infection [33]. Following binding to its receptor and entry into the cell, the viral nucleocapsid releases the viral DNA, that is transferred to the nucleus where it is converted into a covalently closed circular DNA (cccDNA) minichromosome (reviewed in [34] and Fig. 1B). The cccDNA serves as a template for viral transcription. This step in the virus life cycle is highly regulated and dependent on the presence of transcription factors, some of them are liver-enriched. Transcription is driven by distinct promoters controlled by two enhancers (Enhancer I and Enhancer II), resulting in a repertoire of viral transcripts that are capped with a common poly adenylation site. The 3.5 kb pre-genomic RNA (pgRNA) serves as a template for reverse-transcription, mediated

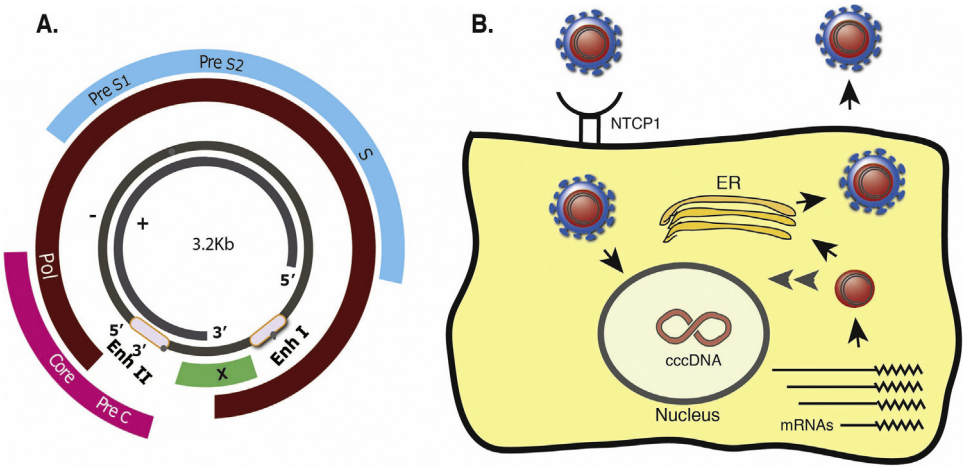

Fig. 1. Gene structure and life cycle of HBV. (A) An illustration of the small HBV genome with its unique overlapping gene structure. (B) Life cycle of HBV. See text for details (Enh – enhancer, Pol– polymerase, S – surface).

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