Hepatitis B and C virus-related carcinogenesis

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Abstract

Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are the most important causes of hepatocellular carcinoma (HCC), accounting for the majority of the cases worldwide. The geographical distribution of HCC therefore coincides with the distribution of HBV and HCV infections in those areas. Similar to nonviral liver diseases, HBV and HCV infection can cause chronic injury to the liver, with subsequent progression to severe fibrosis and cirrhosis. The presence of cirrhosis is a major risk factor for the development of HCC. However, HCC can occur in the absence of cirrhosis, suggesting that both HBV and HCV may be directly involved in hepatocarcinogenesis. Several HBV factors have been implicated in hepatocarcinogenesis, including the HBx gene, the pre-S2/S gene and the HBV spliced protein. Furthermore, HBV can be integrated into the host genome, leading to changes in genomic function or chromosomal instability. By contrast to HBV, HCV cannot integrate into the host genome. Various HCV proteins, including the core, envelope and nonstructural proteins, have been shown to have oncogenic properties. For HBV infection, antiviral therapy and vaccination have been shown to decrease the risk of HCC. Antiviral therapy for HCV can also reduce the risk of HCC.

Keywords: Carcinogenesis, hepatitis B, hepatitis C, hepatocellular carcinoma, review, viral hepatitis

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, with an estimated 500 000–1 000 000 new cases annually [1,2]. The majority of HCC cases (up to 80%) occur in developing countries, with the highest incidence found in sub-Saharan Africa and Southeast Asia [3]. Although the incidence of HCC in developed western countries is low, there has been an increasing trend over the past two decades. The geographical variability observed in the incidence of HCC parallels the incidence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in those areas. These two viruses constitute the two most important risk factors for the development of HCC, accounting for over 80% of all HCC globally. Less common risk factors for HCC include nonviral factors, such as alcohol consumption, aflatoxin B1, and other chronic liver diseases.

Regardless of the aetiological agent, the development of HCC can occur through the process of increased hepatocyte turnover incited by chronic liver injury and regeneration. The continuous inflammation and oxidative stress facilitates the accumulation of genetic alterations within the hepatocyte. As a result, the major risk factor for the development of HCC is pre-existing cirrhosis [4]. Indeed, chromosomal abnormalities and a loss of alleles exist in approximately half of cirrhotic nodules, and direct outgrowth of HCC has been observed in cirrhotic nodules [5].

Although liver cirrhosis per se can cause HCC, the fact that HBV, and less commonly, HCV, can cause HCC in the absence of cirrhosis confirms the inherent oncogenic properties of these two viruses. Given that HBV is a DNA virus and HCV is an RNA virus, these two viruses are sufficiently different to cause cancer via different mechanisms at the molecular level. The mechanisms by which these viruses cause cancer are likely to involve complex interactions between the virus and the human host.

In most cancers, carcinogenesis is a multi-step process with an accumulation of genetic changes that ultimately lead to malignant transformation. The proposed mechanisms of HBV- and HCV-related hepatocarcinogenesis are summarized in Fig. 1.
Role of HBV

An estimated 350 million people worldwide are chronically infected with HBV. Of these, up to 40% will develop complications of cirrhosis and HCC [6]. The annual risk for chronic HBV carriers is less than 1%, but is higher in those patients with concurrent cirrhosis (2–3%) [7]. Approximately 70–80% of HBV-related HCC occurs in cirrhotic livers, whereas the remainder of the HCC occurs in the absence of underlying cirrhosis. The increased risk of HCC associated with HBV infection is especially prominent in areas such as Asia where HBV is endemic. In this region, HBV is transmitted either at birth or during the early years of life, thus leading to a high rate of chronic infection with the persistence of HBV in the host [8].

HBV in hepatocarcinogenesis

HBV likely causes HCC via both indirect and direct pathways. In the former, HBV incites chronic injury to the hepatocytes, with continuous necro-inflammation and regeneration activity and a resultant increase in hepatocyte turnover. The net effect of this is the accumulation of potential critical mutations in the hepatocyte genome, with subsequent malignant transformation and clonal expansion, leading to HCC.

In addition, HBV has been shown to be an oncogenic virus. The earliest evidence that linked HBV to the development of HCC was obtained in the woodchuck hepatitis virus model in which 100% of the rodents infected with woodchuck hepatitis virus developed HCC [9]. Because HBV contains partially double stranded-DNA, it can directly cause HCC by integrating its DNA into the host genome. Although the integration of HBV DNA into the host genome is not required for its replication, it does allow for the persistence of the viral genome within the host. This is supported by the fact that integrated HBV sequences can be found in approximately 80% of HBV-related HCC, and the integrated viral DNA is monoclonal in nature in individual tumours [10]. However, HBV integration can also be found in non-HCC tissue. Integration is usually multiple and occurs at multiple random sites, and can occur also during the early stages of infection. HBV integration can have several mutagenic consequences, including large inverted duplications, deletions, amplifications and translocation, resulting in chromosomal instability [11]. Malignant transformation occurs when these genetic alterations confer a selective growth advantage to the affected cell. Surprisingly, HBV integration near known oncogenes appears to be a rare event. HBV can be integrated into genes that are responsible for the control of cell proliferation and differentiation, such as the human telomerase gene hTERT (regulating cellular immortalization), MAPK1 and the cycline A gene (regulating cellular proliferation), and the gene for tumour necrosis factor receptor-associated protein 1 (regulating cellular viability) [10].

Of all the HBV genes, the HBx gene has generated the most interest in HBV-related hepatocarcinogenesis, and is the most commonly integrated gene. Over 95% of patients with HBV-related cirrhosis and dysplasia are positive for HBx, and HBx is expressed in 70% of patients with HBV-related HCC. The exact mechanism by which HBx can induce HCC development remains to be fully elucidated.
HBx is known to be a transcription activator through its interaction with a wide range of both viral and host regulatory elements [12]. These include the major histocompatibility complex, epidermal growth factor receptor, c-myc, c-jun, c-fos, TP53, AP-1, NF-KB and SPI, among others [13–15]. Through its effect on these regulator elements, HBx can interfere with the hepatocyte DNA repair system and the controlling elements of cellular proliferation [16]. In addition, HBx can bind with p53, with subsequent inhibition of p53-mediated apoptosis [17].

In addition to the HBx gene, other HBV gene elements integrating into the host genome have been incriminated in hepatocarcinogenesis. These include the truncated pre-S2/S gene, which encodes a group of regulatory proteins called transcription activators (PreS2) and also encodes the envelope proteins (LHBs, MHBs and SHBs) [18]. The PreS2 transcription activators undergo phosphorylation by protein kinase C, with subsequent activation of the signalling pathways responsible for activation of transcription factors AP-1 and NF-κB. Activation of these pathways may cause an increase in hepatocyte proliferation. In addition, overproduction of the envelope proteins, particularly LHBs and MHBs, may lead to over-accumulation in the cytoplasm of the hepatocytes, causing the histological appearance of ground-glass hepatocytes [19]. This in turn may cause cellular stress, with predisposition of the cells to undergo malignant transformation.

Another HBV protein, known as the HBV spliced protein, is expressed in chronic hepatitis B (CHB) infection. It has been suggested that this spliced protein can induce apoptosis and modulate signalling via the transforming growth factor pathway, and thus represents a novel way of promoting liver fibrosis and hepatocarcinogenesis [20].

Clinical viral factors
Apart from the complex mechanisms of hepatocarcinogenesis at the molecular level, several viral factors are associated with the increased risk of HCC development in the clinical setting. The most important and perhaps most clinically relevant is the level of serum HBV DNA. Large cohort studies have shown that higher levels of HBV DNA were associated with an increased risk of HCC [21,22]. A cut-off level of >2000 IU/mL was shown to be a strong predictor of HCC, although even lower HBV DNA levels still indicated a risk of HCC development [21,23]. Given that HBV is likely to have an oncogenic property, it is not surprising that, the higher the HBV DNA (signifying higher viral replication), the higher the risk of developing HCC. Although HBeAg positivity had previously been considered as a risk factor, it has not been shown to be an independent predictor of HCC in subsequent studies, and its effect is likely dependent on both the underlying HBV DNA levels and the HBV genotypes [21,24]. There are eight HBV genotypes (A–H), which are based on the genomic sequence divergence. Both genotype C and a high viral load have been shown to be independent predictors of HCC development [21,25]. The core promoter variants of HBV (A1762T and G1764A) have also been shown to be associated with the development of HCC [26,27].

Prevention of HBV-related HCC
The most effective measure of prevention of HBV-related HCC is the prevention of HBV infection by vaccination. A universal vaccination programme in Taiwan was demonstrated to be effective in reducing the rate of childhood HCC subsequent to its introduction [28]. Through a reduction of the seroprevalence of HBV in the general population, the incidence of HCC is likely to decline significantly, especially in areas where HBV is endemic.

In patients already infected with HBV, antiviral therapy remains the best strategy. Treatment with lamivudine has been shown to reduce the incidence of HCC in both cirrhotic and noncirrhotic CHB patients, although the effect is blunted with the development of resistance to lamivudine [29,30]. Given the importance of elevated HBV DNA as a highly significant and independent risk factor for HCC development, it is likely that the reduction of HCC risk is largely a result of the reduction of HBV DNA. With more potent antiviral drugs available (such as entecavir and tenofovir), long-term HBV DNA suppression is now possible, with a very low risk of drug resistance.

Role of HCV
There are an estimated 170 million people worldwide infected with HCV, of whom approximately 20% will progress to cirrhosis [31]. By contrast to CHB, patients with chronic hepatitis C (CHC) almost always develop HCC in the presence of established cirrhosis. The annual risk of HCC development in CHC patients with cirrhosis is in the range of 1–4%, and an estimated 1–3% of patients chronically infected with HCV will develop HCC after 30 years [32]. There is an increasing incidence of cases of HCC observed in the Western population, the majority of which are HCV-related, and the incidence is contributed to by an ageing population. Similar to HBV, the association between HCV and HCC is likely a combination of the independent effect of HCV on hepatocarcinogenesis and the indirect effect of cirrhosis.
HCV in carcinogenesis
The HCV is a positive-stranded RNA virus containing approximately 9500 nucleotides. The genome is organized into three structural proteins at the N-terminal end and four functional proteins at the C-terminal end. The structural proteins are the core (C) and envelope 1 and 2 proteins (E1 and E2, respectively), whereas the nonstructural (NS) proteins include NS2, NS3, NS4 and NS5. Unlike HBV, HCV lacks reverse transcriptase activity, and therefore HCV does not integrate into the host genome. Because the HCV is a completely cytoplasmic-replicating virus, the main hypothesis for HCV carcinogenesis is that it occurs via indirect pathways through the effects of chronic inflammation and hepatocellular injury. This is likely the major mechanism of hepatocarcinogenesis in HCV-related HCC; this assumption is supported by the fact that the presence of cirrhosis is almost a pre-requisite for HCC development.

However, it is unlikely that necro-inflammation alone is sufficient to cause HCC. In conditions such as autoimmune hepatitis, despite chronic inflammation and liver injury, HCC development is rare. Moreover, HCC can still develop in a very small proportion of non-cirrhotic patients with CHC, implicating a more direct effect from the HCV itself.

Various HCV proteins have been reported to have a role in HCC development in experimental studies involving cell culture systems and animal models. The HCV core protein is involved in viral particle assembly and generation of complete virions. However, the core protein is also involved in cell signalling, transcription activation, apoptosis, lipid metabolism and transformation [33]. In transgenic mice models, the HCV core protein has been shown to induce HCC, although the exact mechanism by which it does so remains unclear [34]. One of the potential mechanisms includes induction of oxidative stress. The HCV core protein has been shown to induce reactive oxygen species in the absence of inflammation. The oxidative stress may decrease metabolic processes within the mitochondria, with a decline in microsomal triglyceride transfer protein activity, resulting in the development of steatosis [35]. The HCV core protein has also been shown to affect the modulation of cellular gene products and several cellular regulatory pathways involved in cellular proliferation, cell cycle control and tumour formation [33]. The HCV core protein can bind to p53 and pRb tumour suppressor proteins, modulate the expression of p21/Waf, which is involved in cell cycle control, and interact with cytoplasmic signal transduction molecules to regulate transcription [36].

Apart from the core proteins, other HCV proteins have also been shown to contribute to hepatocarcinogenesis. The E2 protein can interact with CD81 and inhibit T and NK cells, thereby promoting cell survival and proliferation. The HCV NS3 protein is a multifunctional protein with protease, RNA helicase and NTPase activity. NS3 can promote hepatocarcinogenesis by its interaction with certain cellular proteins, such as p21 and p53 [37]. HCV NS5A, a membrane-associated protein, is involved in the replication of the HCV genome. The intact NS5A is cytoplasmic and bound to membranes. However, the truncated form of HCV NS5A can become localized to the nucleus to act as a transcriptional activator. NS5A can also interact with cellular signalling components and regulatory protein kinases, leading to the suppression of the host immune response and inhibition of apoptosis [38]. Therefore, an association between HCV and HCC is likely to be a result of a combination of the independent effect of HCV on hepatocarcinogenesis and the indirect effect of cirrhosis.

Risk factors in HCV-related HCC
The intake of alcohol in patients with CHC synergistically increases the risk of HCC development, and has a dose-related effect [39–41]. Co-infection with HBV also synergistically increases the risk of HCC. A meta-analysis showed that HBV/HCV co-infection was associated with a 135-fold increased risk of HCC compared to HBV and HCV mono-infection (20- and 24-fold increase, respectively), with the highest risk being in those patients with established cirrhosis [42]. In cases of co-infection with HIV/HCV, HCC is more likely to occur at a younger age and with a shorter duration of HCV infection compared to those with either HCV or HIV mono-infection [43]. The role of the HCV genotype is less clear, although some studies have shown an increased risk of HCC in patients infected with genotype 1b [39,44,45]. There is some evidence indicating that schistosomiasis may increase the risk of more severe disease in patients infected with HCV genotype 4 compared to those without parasitic infection [46]. The role of obesity and diabetes mellitus in the risk of HCC in patients with CHC remains to be fully determined, although there is some evidence that both may increase the risk [47–49].

Prevention of HCV-related HCC
Unlike HBV, there are currently no effective vaccination strategies to prevent HCV infection. The HCV polymerase enzyme lacks proofreading capabilities, which results in the profound genomic diversity of HCV. Accordingly, it is extremely difficult to produce a single effective vaccine. However, with the screening of HCV in blood transfusion services, transfusion-related HCV infection has been lowered to almost zero [50]. This has an obvious bearing on the future incidence of HCV infection in developed countries.
Interferon-based therapy is currently the standard of care for patients with CHC, and has been proven to be effective in eliminating HCV. Both conventional and pegylated interferon (IFN) therapy have been used widely, with the aim of achieving a sustained virological response (SVR). In a meta-analysis of 11 studies with 2178 patients, investigating the incidence of HCC in patients with HCV-related cirrhosis, there was a higher rate of HCC in untreated patients compared to IFN-treated patients (21.5% vs. 8.2%, respectively; OR 3.0, 95% CI 2.3–3.9). This effect was observed irrespective of whether the patients achieved SVR or not, although the effect was greater when SVR was achieved [51]. In another cohort study, treatment with conventional IFN in noncirrhotic patients infected with HCV reduced the risk of HCC by 50%, and further down to 20% in those patients who achieved SVR [52]. In another large cohort study of 2166 patients, there was a significantly lower rate of HCC after 15 years of follow-up in those who received IFN therapy compared to those not treated (13.9% vs. 23.9%, respectively; p <0.001). In those patients who achieved SVR, the rate was even lower (1.9% at 15 years) [53]. Therefore, the current treatment model for CHC appears to be effective in lowering the risk of HCC development. Given the high mortality rate associated with HCC, prevention is of paramount importance. In addition, screening programmes for at-risk individuals increase the opportunities for earlier curative intervention, with an improvement in long-term survival. Therefore, it is essential that available effective management strategies are employed, including a universal HBV vaccination programme and the identification of patients infected with HBV and HCV who may benefit from antiviral therapy.

However, it is encouraging that the currently available antiviral therapy and vaccination for HBV appear to be effective in lowering the risk of HCC development. Although many potential pathways and mechanisms have been identified in this complex sequence of events, these have only been largely studied in experimental models and in small series involving human subjects. Whether these findings obtained in vitro can be extrapolated to the in vivo environment is debatable, and the true clinical and biological relevance of these findings remains to be determined. Therefore, despite the progress made in recent decades, a coherent model of the molecular pathogenesis of HCC relating to HBV and HCV is still lacking. Future research should continue to identify aberrant genes and their protein products, as well as other risk factors, so that novel therapeutic agents may be developed.

Occult HBV Infection

Occult HBV infection is defined by the persistence of HBV DNA in the liver and/or serum without detectable serum hepatitis B surface antigen. Occult HBV has been implicated in HCC development because the highest rates of occult HBV infection have been found in patients with HCC, especially in those infected with HCV and those with alcoholic liver disease [54–56]. In these cases of HCC, HBV DNA has been detected in both tumourous and nontumourous tissue [57]. In addition, HBx protein expression and cis-activation can be found in the tumour cells of patients with HCC and evidence of occult HBV infection [58–60]. This suggests that the mechanism of hepatocarcinogenesis of occult HBV infection is similar to that of patients who are positive for hepatitis B surface antigen, and that the persistence of HBV DNA is a risk factor for the subsequent development of HCC.

Conclusions

Hepatocarcinogenesis is a multi-step process involving the accumulation of different genetic changes, which lead to malignant transformation and uncontrolled proliferation. Although many potential pathways and mechanisms have been identified in this complex sequence of events, these have only been largely studied in experimental models and in small series involving human subjects. Whether these findings obtained in vitro can be extrapolated to the in vivo environment is debatable, and the true clinical and biological relevance of these findings remains to be determined. Therefore, despite the progress made in recent decades, a coherent model of the molecular pathogenesis of HCC relating to HBV and HCV is still lacking. Future research should continue to identify aberrant genes and their protein products, as well as other risk factors, so that novel therapeutic agents may be developed.

The authors declare that they have no conflicts of interest.

References

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Takada S, Kanenwa N, Tsuchida N, Koike K. Cytoplasmic retention of the p53 tumor suppressor gene product is observed in the hepatiti-


Tai PC, Suk FM, Gerlich WH, Neurath AR, Shih C. Hypermodifica-

tion and immune escape of an internally deleted middle-envelope (M) protein of frequent and predominant hepatitis B virus variants. Viral 2002; 292: 44–58.


Kwun HJ, Jung EY, Ahn JY, Lee MN, Jang KL. p53-dependent transcrip-

Reyes GR. The nonstructural NS5A protein of hepatitis C virus: an expanding, multifunctional role in enhancing hepatitis C virus pathogen-


Sillini E, Bottelli R, Asti M et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a case–control study. Gastroen-

Zein NN, Poterucha JJ, Gross JB Jr et al. Increased risk of hepatocellu-


Pekow JR, Bhan AK, Zheng H, Chung RT. Hepatic steatosis is associ-


Pomper GJ, Wu Y, Snyder EL. Risks of transfusion-transmitted infec-


