REVIEW

Chronic hepatitis B virus and hepatitis C virus infections and cancer: synergy between viral and host factors

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Abstract

Hepatitis B virus (HBV) or hepatitis C virus (HCV) infections represent major causes of chronic liver disease and hepatocellular carcinoma. Despite inducing shared pathological events leading to oncogenic transformation, these two viruses present profound differences in their molecular features, life cycle and interplay with host factors, which significantly differentiate the prognostic and therapeutic approach to the related diseases. In the present review, we report the main mechanisms involved in the multistep process leading from HCV/HBV infection and cancer development, discussing side-by-side the analogies and differences between the two viruses. Such events can be broadly categorized into (a) direct oncogenic effects, involving integration in the host genome (in the case of HBV) and chromosomal instability, interference with oncosuppressor pathways, induction of oxidative stress, promotion of angiogenesis, epithelial–mesenchymal transition, alterations in the epigenetic asset and interaction with non-coding RNAs; and (b) indirect activities mostly mediated by host events, including chronic inflammation sustained by peculiar cytokine networks (such as interleukin-6 and lymphotoxins), metabolic dysfunctions promoted by steatohepatitis, interplay with gut microbiota and fibrotic events (mainly in HCV infection). This scenario suggests that the integrated study of viral and host factors may lead to the successful development of novel biomarkers and targets for therapy.

Keywords: Chronic inflammation, hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, tumours
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Direct oncogenic activities of hepatitis B and hepatitis C viruses

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major aetiological agents of chronic liver disease and hepatocellular carcinoma (HCC). HCC is the fifth most prevalent tumour type and the third leading cause of cancer-related deaths worldwide. In Africa and Asia, where HBV is endemic, 60% of HCC is associated with HBV, 20% is related to HCV; in the USA, Europe, Egypt and Japan, more than 60% of HCC is associated with HCV, about 20% is related to HBV [1].

Hepatitis B virus is a hepadnavirus that replicates via an RNA intermediate. The HBV genome is a partially double-stranded relaxed circular DNA molecule of 3.2 kb; when HBV enters hepatocytes the relaxed circular DNA is converted into a covalently closed circular DNA. This covalently closed circular DNA is organized as a mini-chromosome by histone and non-histone viral and cellular proteins [2] and serves as template for transcription of all viral mRNAs. It persists during therapy using nucleos(t)ide analogues that suppress HBV replication, therefore disease recurrence is possible even after successful treatment and clearance of hepatitis B surface antigen [3]. HBV can promote HCC in many ways, including through its integration in the host genome. HBV–DNA integration into human chromosomes has been detected in 80–90% of HBV-related HCCs and insertions have been associated with genetic alterations within the cell genome, including generalized genomic instability, gene and chromosomal deletions, translocations, amplifications of cellular DNA, and generation of fusion

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transcripts [4]. Genome-wide next-generation sequencing approaches have identified recurrent sites for HBV–DNA integration [5]. Genes reported to be frequently altered are *TERT*, *MLL4*, *CCNE1*, *NTRK2*, *IRAK2* and *MAPK1* [6]. The risk of HCC development may remain elevated also in individuals that are negative for circulating HBsAg even if the virus persists in the liver: this condition is known as occult HBV infection (OBI) [7–9]. Several studies indicate that OBI represents an important risk factor for HCC development because long-term persistence of occult HBV may exert both an indirect role, supporting a mild necroinflammation, and a direct oncogenic effect, mediated by its integration into the host genome, as well as by maintaining the expression of transforming proteins like X-protein [10,11]. Moreover, consistent epidemiological studies have shown that OBI is associated with a more severe liver disease in HCV-infected patients, suggesting that OBI may synergize with HCV-mediated hepatitis in co-infected subjects [12–14].

Hepatitis C virus is an enveloped virus with positive-sense RNA genome of 9.6 kb that encodes for structural (Core, E1, E2/p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins. It is a virus with an exclusively cytoplasmic life cycle, and maintains itself as an endoplasmic reticulum-associated episome. The mechanisms of HCV-induced HCC involve the interplay of host, environmental and viral factors. Unlike HBV, HCV does not integrate into the host genome but, like HBV, can induce chromosomal instability by direct effects of its proteins on genes that regulate organization of centrosomal processes and the mitotic spindle during cell cycle progression [15].

Both host and viral factors have been recognized to impact HCC development/progression in HBV and/or HCV-infected individuals. Several host and environmental components, including metabolic syndrome, tobacco or alcohol intake, and genetic factors, contribute to determine HCC risk in both HCV and HBV infections. Conversely, viral factors seem to differentially affect HCC development in the two infections: indeed, although cirrhosis plays a major role in HCV-related carcinogenesis, viral factors represent strong prognostic indicators of HCC development in HBV infection, also in the absence of cirrhosis [1]. They include high levels of viral replication [16], the viral genotype (with the B genotype found prevalent in cohort of HCC carriers [17]), and the presence of specific point mutations in the basal core protein promoter [18,19] or deletions in pre-S sequence [20]. This scenario clearly anticipates the idea that the two viruses, and respectively HBV or HCV, may preferentially rely on direct virus-related oncogenic activities rather than on indirect inflammatory and fibrotic events, when arranging liver carcinogenesis.

Both HBV and HCV contribute to HCC induction by directly promoting host gene expression pathways and cellular phenotypes that have been recognized as hallmarks of cancer, i.e. growth factor-independent proliferation, resistance to growth inhibition, tissue invasion and metastasis, angiogenesis, reprogramming of energy metabolism, resistance to apoptosis, and induction of oxidative stress [21]. The contribution of HBV to HCC involves the expression of hepatitis B x (HBx) and pre-S or S polypeptides, while the HCV core protein and non-structural proteins NS3 and NS5A contribute to oncogenic processes. Indeed, HBx, HCV core and NS5A were reported to interact with wild-type p53 and limit its functions [22], including nucleotide excision repair and transcription-coupled repair [23]. Oxidative stress is induced by both HBV and HCV by altering Ca^{2+} signalling and increasing reactive oxygen species levels that in turn trigger endoplasmic reticulum stress [24,25]. However, endoplasmic reticulum integrity is important for virus replication, therefore both viruses trigger the unfolded protein response and autophagy to restore endoplasmic reticulum functions [26,27].

Hypervascularization is a major characteristic of HCC. HBV and HCV (HBx, HCV Core, E1, NS3, NS5A) promote angiogenesis by upregulating hypoxia inducible factor 1α [28–30] that orchestrates the response to hypoxia by transcriptionally upregulating vascular endothelial growth factor and cyclo-oxygenase 2, and by activating matrix metalloproteinases [30]. On the basis of this evidence, anti-angiogenic treatments have been proposed in HCC, such as Sorafenib that is the first molecular target drug approved for the treatment of advanced HCC. However, the efficacy and safety of anti-angiogenic therapies remain controversial and substantial evidence of primary and acquired resistance to Sorafenib has been reported [31].

Hepatocellular carcinoma is a complex and heterogeneous tumour with frequent intrahepatic spread and extrahepatic metastasis. Liver fibrogenesis, tumour progression and metastasis are induced by epithelial–mesenchymal transition, which occurs in response to sustained inflammation, ultimately leading to organ destruction. Both HBV and HCV promote epithelial–mesenchymal transition by induction of transforming growth factor-β_1 and SNAIL and by activation of RAS-ERK, phosphoinositide 3-kinase (PI3K)-AKT and Wingless-type MMTV integration site family (Wnt) signalling pathways [32–34]. In human HCC, mutations in Wnt/β-catenin signalling are common (15–33% *CTNNB1*, 15% *AXIN1* and 1–2% *APC*) [35]. HBx can activate β-catenin by upregulation of Upregulated gene clone 11 (URG11), inactivation of E-cadherin complexes, upregulation of SNAIL and activation of the proto-oncogene Src [21], while NS5A can trigger β-catenin activation by upregulating Twist family BHLH transcription factor 2 (TWIST2) [32] and by activating PI3K-AKT [36]. Stabilization of β-catenin promotes the transcription of stemness-related genes (e.g.

POU5F1 and *NANOG*), while upregulating an epithelial cell adhesion molecule, which in turn contributes to the stemness of cancer cells through binding to target genes [37]. Moreover, β-catenin can enhance the transcription of hypoxia inducible factor 1α, therefore promoting cell survival and epithelial–mesenchymal transition, favouring the stemness of cancer cells and overcoming hypoxia [38,39].

Epigenetic changes are known to contribute to HBV-induced and HCV-induced HCC. Epigenetic modifications of tumour suppressor genes may permit the constitutive expression of oncogenes that is characteristic of tumour progression. The heritable epigenetic changes acquired, along with tumour expansion, result in a permanent phenotypical change. Both viruses upregulate DNA methyltransferases [40,41], and indeed DNA hypermethylation in the promoter region of specific oncosuppressor genes was found in HBV- and HCV-related HCC [42]. For example, HCV core protein and HBx suppress the cyclin-dependent kinase inhibitors INK4a and p21 through promoter methylation, an event that in turn determines the inactivation of the Retinoblastoma tumour suppressor [21]. Moreover, both HBx and HCV core induce DNA methylation in the E-cadherin promoter [42], ultimately activating β-catenin.

HBV and HCV can alter the expression of selected mitochondrial RNAs and long non-coding RNAs that affect virus persistence and oncogene/oncosuppressor pathways in HCC [43,44]. For instance, miR122 has been shown to be an essential host factor for HCV infection and an antiviral target because it stimulates virus replication by stabilizing the viral RNA in infected cells [45]. A recent work has proposed a model for HCV-induced miR-122 sequestration that may result in global de-repression of host miR-122 targets, providing a fertile environment for the long-term oncogenic potential of HCV [46]. HBV infection downregulates the expression of miR122, which interferes with viral replication, and viral load inversely correlates with miR122 expression in HBV-infected patients [47]. Given its central role in liver biology, miR122 represents an interesting therapeutic target for the treatment of liver diseases and HCC [45]. Given the role of deregulated miRNAs in host–virus interaction and HCC development, these molecules have the potential to become novel diagnostic and prognostic biomarkers and therapeutic targets in virus-related diseases.

Current treatments of hepatitis B comprise pegylated interferon-α and oral nucleoside/nucleotide analogues, which often lead to viral suppression, decrease the risk of HCC as well as post-surgery HCC recurrence but rarely lead to complete virus eradication [48]. A highly efficacious vaccine has been developed for HBV, but the implementation of vaccination programmes is not uniform, and vaccine non-responders and vaccine-escape mutants have limited the effectiveness of the vaccine [49,50]. Prevention strategies, large data sets from next-generation sequencing analysis of HBV–DNA integration sites, eradication of the covalently closed circular DNA molecule as well as identification of new biomarkers represent the main goal for the treatment of HBV-induced HCC. For HCV, with the development of new direct-acting antiviral agents, sustained virological response rates have risen to >90%. The US Food and Drug Administration approved Sofosbuvir for the treatment of patients with HCV genotypes 1, 2, 3 and 4; however, further genetic and functional studies are needed to identify novel biomarkers and prevention strategies in HCV-associated cancer.

HBV/HCV-driven inflammatory response and liver carcinogenesis

The tight link between chronic inflammation and tumour initiation/promotion has been established from a huge amount of data [51,52]. Cells of both innate and adaptive arms of the immune response are recruited and/or locally expanded in tissues in response to injuries of different origin (chemical damage, metabolic pressure, infectious trigger, or other) and arrange a series of activities, originally directed to the neutralization of the instigating factor and to efficient tissue repair, but later turning into deleterious chronic low-grade inflammation and regenerative mechanisms, fostering oncogenic transformation [51,52]. Several pieces of evidence indicate that HBV and HCV infections promote hepatocellular carcinogenesis not only directly, through the direct oncogenic activities of specific viral proteins, but also indirectly, by establishing chronic low-grade inflammation and fibrosis. Pioneer studies in mouse models have clearly demonstrated that the transgenic expression of HBV proteins was not sufficient per se to recapitulate the HBV-related liver disease, and that the HBV-specific T-cell response was required to initiate in HBV-transgenic mice a process of chronic hepatitis, sustained by non-HBV-specific T cells and other players such as neutrophils, macrophages and natural killer cells, finally leading to HCC development [53,54]. Similarly, a very low incidence of HCC has been reported in several lines of HCV-transgenic mice, spontaneously developing steatosis in the absence of overt inflammation [55]; again supporting the idea that chronic inflammation crucially determines the oncogenic power of HCV infection. Many diverse immunological mechanisms, instigated by HBV/HCV infections, may participate in HCC promotion [52]. Hepatic levels of the cytokines lymphotoxins α and β were specifically increased in human HBV-related and

HCV-related chronic liver diseases and HCC, and transgenic mice that highly express lymphotoxins specifically in the liver displayed strong inflammatory infiltration, hepatocellular damage and spontaneous HCC development [56]. Lymphotoxins mainly acted by triggering the hepatocyte-intrinsic IKKβ-dependent nuclear factor-κB pathway, which in turn promoted chemokine release and recruitment of inflammatory cells responsible for hepatic remodelling and HCC development [56]. HCV infection was directly able to induce lymphotoxin expression in a human hepatocyte cell line, even though immune cells also contributed to lymphotoxin provision [56]. In particular, NS5B, the RNA-dependent RNA polymerase of HCV, was directly able to induce lymphotoxin expression in hepatocytes [57]. Similarly, other HCV viral proteins can directly induce pro-inflammatory signalling pathways leading to nuclear factor-κB activation and to the release of pro-tumorigenic cytokines [58,59]. While lymphotoxins may exert more relevant functions at early phases of virus-related inflammation, other cytokines and chemokines may rather contribute to amplify the immune-hepatocyte cross-talk at later stages. In a mouse model of chemical hepatocarcinogenesis, interleukin-6 (IL-6) was found to be responsible for the gender disparity in HCC development, through the repressive effect of oestrogens on IL-6 secretion by Kupffer cells [60], and high levels of serum IL-6 predicted HCC induction in chronic HBV [61] and HCV [62] infections. Many other cytokines, such as tumour necrosis factor, IL-1 and IL-23, produced in conditions of chronic inflammation, may contribute to tumour initiation/promotion, through converging to the activation of pro-tumorigenic NF-κB and signal transducer and activator of transcription 3 pathways [52].

The HBV/HCV-related chronic inflammation may lead to oncogenic transformation not only through the direct instigation of transforming events but also indirectly, through metabolic alterations occurring in steatohepatitis and through the tissue remodelling processes that are associated with hepatic cirrhosis. However, such indirect events may have a higher impact in HCV than in HBV infections. Indeed, the risk of HCC development is related to the extent of viral replication and virus-directed immunity in the case of HBV infection, while being more closely associated with host factors such as metabolic dysfunctions and fibrotic processes in the case of HCV infection [63]. Both viruses contain steatogenic proteins that reshape hepatic lipid metabolism and induce hepatic steatosis, which contributes to the induction of oncogenic pathways in a hepatocyte-intrinsic fashion [64]. However, virus-driven steatosis may also contribute to carcinogenesis extrinsically, through the establishment of hepatic and/or systemic metabolic inflammation that plays pro-tumorigenic roles. Indeed, chemical liver carcinogenesis is strongly enhanced by dietary or genetic caloric overload [65] and obesity increases the HCC risk [63]. Importantly, obesity and diabetes synergize with HCV infection, more than with HBV infection, in determining HCC risk [66]. In a mouse model of combined steatohepatitis and obesity, CD8 T cells and NKT cells were found to be responsible not only for inflammation and HCC development but also for triggering lipid metabolism alterations in hepatocytes [67]. This scenario is complicated by recent observations indicating that obesity may affect cancer risk not only through metabolic or inflammatory dysfunctions but also through the involvement of a *guilty bystander*, which is the gut microbiota. Exposure to *Helicobacter hepaticus* significantly enhances HCC development in HCV-transgenic mice [68] and dietary overload reshuffles the gut microbial colonization leading to the release of bacterial metabolites that in turn promote HCC development [69].

A relevant difference between HBV-related and HCV-related HCC is the impact of cirrhosis, more strictly associated to HCC in the case of chronic HCV infection [63,70]. HCV viral proteins directly induce, in hepatic stellate cells, the release of profibrogenic mediators and also of inflammatory cytokines and chemokines that further amplify cycles of tissue damage/repair [71]. The cytokine transforming growth factor-β may be considered as a critical cytokine bridging cirrhosis and carcinogenesis [72]. Interestingly, transforming growth factor-β plays a variety of immunosuppressive activities, therefore turning cirrhosis into a tolerant microenvironment that may favour the escape of emerging tumour clones from immune control. In line with this idea, our recent data have underscored the accumulation of regulatory T cells, crucial suppressors of anti-tumour immune responses, not only in HCC but also in cirrhosis of HCV-infected patients, indicating that immunosuppression may represent an early event in the multistep process leading from HCV-related cirrhosis to cancer [73]. The study of host-related factors/networks contributing to the oncogenic evolution of HCV/HBV-induced chronic liver diseases may lead to the discovery of novel biomarkers for predicting disease progression and, importantly, of potential targets for immunomodulatory therapies.

Transparency declaration

The authors declare that they have no conflicts of interest.

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