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Hepatocarcinogenesis associated with hepatitis B, delta and C viruses

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Globally, over half a billion people are persistently infected with hepatitis B (HBV) and/or hepatitis C viruses. Chronic HBV and HCV infection frequently lead to fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Co-infections with hepatitis delta virus (HDV), a subviral satellite requiring HBV for its propagation, accelerates the progression of liver disease toward HCC. The mechanisms by which these viruses cause malignant transformation, culminating in HCC, remain incompletely understood, partially due to the lack of adequate experimental models for dissecting these complex disease processes *in vivo*.

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Introduction

As the second highest cause of cancer-related death worldwide, liver cancer is a substantial global health problem [1]. More than 90% of the primary liver cancers diagnosed are specifically hepatocellular carcinoma (HCC), which has an especially poor prognosis with 700,000 new cases and 600,000 deaths occurring every year [2]. A multitude of factors contribute to the development of HCC, including viral, immune, chemical and genetic etiologies. For 80% of all HCCs, infections with the viruses HCV, HBV and/or HDV — all of which have a high propensity for progressing to chronicity — are considered the root cause. Worldwide, billions of people have been exposed to HBV, HCV and HDV, resulting in approximately 170 million chronic HCV carriers and 350 million chronic HBV carriers of which 15–20 million are co-infected with HDV (Table 1). Between 15% and 40% of those chronically infected with HBV or HCV will develop serious liver disease, including cirrhosis and/or HCC. HBV/HDV co-infected patients experience rapid progression of liver disease and also have the highest mortality rate (20%) of any of the viral hepatitises [3–5] (Table 1). The chronic damage inflicted on the liver by the immune system in its (unsuccessful) attempts to clear these viruses is generally considered to be the main contributor to liver disease progression. However, it is less clear how these viruses trigger hepatocarcinogenesis. Different mechanisms by which HCV, HBV and HDV contribute to the development and progression of HCC have been proposed. These include continuous antiviral inflammatory responses, ongoing immune clearance of infected cells and hepatocyte regeneration which all occur during chronic infection and lead to genetic and epigenetic changes that put patients at increased risk for HCC establishment [6,7].

In the following sections, we briefly discuss the biology of HBV, HCV and HDV, highlight oncogenic mechanisms that lead to development of HCC in individuals infected with these viruses and provide an outlook of the tools needed to more efficiently study virally induced HCC.

Hepatitis C, B and delta viruses

HCV is an enveloped, positive-sense, single-stranded RNA virus of the *Hepacivirus* genus in the *Flaviviridae* family. Following a complex entry mechanism into hepatocytes, which involves numerous cellular host factors (reviewed in [8]), the HCV genome is released from the nucleocapsid into the cytoplasm. Here, HCV's 9.6 kb RNA genome is translated into a ca. 3000 amino acid polyprotein that is processed by viral and cellular proteases to release the viral structural proteins — core, E1, E2 — and non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. In addition, a short +1 open-reading frame (ORF) exists that produces a genome product referred to as minicore. Minicore currently has no ascribed functions, but mutations in codons 70 and 91 are associated with the development of liver cancer and lead to increased expression of this protein [9]. The structural and non-structural proteins carry out various functions in the viral life cycle, including viral genome propagation, assembly and egress of infectious virions. Although several reports have suggested the existence of extrahepatic reservoirs, including peripheral blood mononuclear cells [10,11], epithelium

Table 1

Prevalence and severity of chronic viral hepatitis

	HCV	HBV	HBV/HDV
Prevalence	170 million	240 million	20 million
Fibrosis	20–39% [105,106]	34% [107]	23.4% [108]
Cirrhosis	20–30% [109]	15–20% [110]	14–77% [111,112]
HCC	1–3% [110]	0.01–5.4% [113]	10–15% [114]
Time to HCC	≥30 years [109]	10–30 years	≥10 years [115,116]

[12] and even tissues of the central nervous system [13], it is generally thought that HCV productively replicates exclusively in hepatocytes. HCV's highly restricted tissue range is governed by a higher — albeit not exclusive — expression of essential HCV entry factors and the microRNA, miR122, which is solely expressed in hepatocytes. In contrast to HBV and HDV, the HCV life cycle does not have a nuclear stage and is completed wholly in the cytoplasm of infected hepatocytes.

HBV belongs to the *Hepadnaviridae* family and has a very compact 3.2 kb partially double-stranded DNA genome that is organized in four partially overlapping ORFs. These encode for the HBV surface polypeptides, which form three proteins, namely the small (S), medium (M), and large (L) surface antigens that are inserted into the viral envelope. Additional virally encoded components include HBV core protein which comprises the viral nucleocapsid; the X protein which has many suggested functions including epigenetic regulation of the viral genome; and the viral polymerase which is involved in viral replication and packaging [14,15]. Like HCV, HBV is a uniquely hepatotropic virus. Although transfection experiments in tissue culture suggest that HBV is able to replicate in non-hepatic cells [16,17], viral entry is dependent on the bile acid transporter sodium-taurocholate cotransporting poplypetide (NTCP) exclusively expressed in hepatocytes [18•,19••]. Interaction of the N-terminal region of preS with NTCP initiates entry during which the virus is internalized via receptor-mediated endocytosis (reviewed in [20]). Following uncoating, the nucleocapsid is transported to the nuclear membrane where the genome is then released as a relaxed circular genome (rcDNA) into the nucleus. In the nucleus, the partially double-stranded viral genome is repaired, forming covalently closed circular DNA (cccDNA), which serves as the transcriptional template for all four viral gene products as well as pre-genomic RNA (pgRNA). HBV transcripts are transported into the cytoplasm where the viral mRNAs are translated and pgRNA packaged in a nucleocapsid. Here, pgRNA is reverse transcribed into rcDNA that can either be imported into the nucleus to replenish the cccDNA pool or nucleocapsid-encased rcDNA can be enclosed by lipid bilayer containing the HBV envelope proteins and then released from the host cell [21].

HDV is a negative-sense RNA virus independent of any viral family and the sole member of the *Deltaviridae* genus. The small HDV circular genome is only ca 1.7 kb in length and is stabilized by extensive intramolecular base pairing. HDV encodes a single ORF that encodes the delta antigen (HDAg), which exists as two isoforms, the small and large HDAg [22]. HDV is considered a subviral satellite as it requires the HBV envelope proteins to form infectious virions. Consequently, the early steps of HDV entry into hepatocytes follow the same mechanism as HBV. Once within the hepatocyte, a nuclear localization signal on HDAg-L triggers the translocation of the HDV nucleocapsid to the nucleus where the viral genome is replicated. The small size of the HDV genome results in the virus' reliance on host enzymes, including cellular RNA polymerases, to successfully replicate (reviewed in [23]). The incoming RNA serves as the template for transcripts longer than the size of the virus' genome. Such multimeric, linear RNAs contain at least two copies of the antigenomic ribozyme, releasing unit-length linear RNA following self-cleavage. Antigenomic RNA is circularized and forms the template for new genomic RNAs following similar intermediate steps.

Mechanisms of HCV-associated hepatocarcinogenesis

HCV-associated hepatocarcinogenesis is a protracted process that is usually the result of decades-long persistent infection. The mechanisms driving hepatocarcinogenesis during chronic HCV infection remain incompletely understood. HCV is known to trigger strong cell-intrinsic responses mediated largely by type I and III IFN which induce a plethora of IFN stimulated genes. A variety of evasion mechanism enable HCV to blunt antiviral signaling in some cells, thereby creating a cellular environment which is more conducive for viral persistence. This antiviral innate response precedes the activation and infiltration of a variety of lymphocyte subsets, including NK cells, CD8 and CD4 lymphocytes, aiming at clearing HCV infected cells. In chronically infected patients these antiviral defenses fail as HCV rapidly acquires mutations allowing it to evade immune pressure. Clearly, the inflammatory context established in the liver during persistent HCV infection plays an important role, as HCC only develops following stages of fibrosis and cirrhosis. In efforts to clear virally infected cells, immune-mediated liver injury subsequently induces proliferation of hepatocytes, which are usually quiescent. This compensatory hepatocyte proliferation results in propagation of oncogenic mutations, leading to clonal expansion of transformed cells that eventually give rise to HCC. These oncogenic mutations may be introduced by, for example, reactive oxygen, nitrogen intermediates, and damage-associated molecular pattern molecules released by