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Is Hepatitis C Virus Carcinogenic?

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Abstract

Although infection with hepatitis C virus (HCV) has become a leading cause of hepatocellular carcinoma, the mechanisms by which it results in carcinogenesis remain a subject of debate. Here, we explore the possibility that HCV replication impairs cellular DNA damage responses, thereby promoting instability of the infected host cell genome, and that HCV exerts a direct cancer-promoting effect in addition to eliciting immune-mediated inflammation and apoptosis of hepatocytes contributing to hepatocellular carcinogenesis.

No one would argue with the notion that chronic infection with hepatitis C virus (HCV) causes hepatocellular carcinoma (HCC). Early observations of the association between post-transfusion “non-A non-B” hepatitis and HCC in Japan from the 1980s[1] have, unfortunately, proved to be all too true, and in many industrialized countries (including the U.S. and Japan), HCV infection is now the leading risk factor for HCC. The age-adjusted incidence rate of HCC has tripled in the U.S. over the past 30 years[2], reflecting the spread of HCV among Americans decades earlier. Most cases occur in patients with well-established cirrhosis, by itself a very strong risk factor for liver cancer. However, this is not always the case. Eight percent of patients developing HCC in the prospective HALT-C study lacked any evidence of cirrhosis, although all had an Ishak fibrosis score of at least 3 when enrolled in the study[3]. Adjusting for other risk factors, such as alcohol intake, active HCV infection increases the risk of HCC about 18-fold[4]. Thus, the question is not whether HCV infection causes liver cancer, but rather how it does this. Is HCV directly carcinogenic? Or does infection simply set in motion a brisk inflammatory and pro-fibrotic response that causes cancer?

Human viruses fall along a continuum in terms of their potential to cause cancer, and how they go about doing this. Some, like high-risk papillomaviruses or the gamma herpesviruses Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus, could be considered directly carcinogenic, because the expression of particular viral gene products can overwhelm normal controls and directly drive unbridled cellular proliferation. At the other

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end of the spectrum, liver cancer associated with hepatitis B virus (HBV) may arise primarily as a result of the inflammatory response it evokes, despite a clear ability to integrate parts of its genome into chromosomal DNA. HCV appears to fall in the middle of this continuum - both eliciting *indirect effects* in the guise of inflammatory and pro-fibrotic host responses that contribute substantially to carcinogenesis, but also exerting *direct effects* upon the infected cell that may promote its malignant transformation.

The role played by inflammation in liver cancer is well documented. Elegant studies have shown that chronic immune-mediated liver damage is both necessary and sufficient for the development of HCC in HBV transgenic mice, and that other potential mechanisms of carcinogenesis such as insertional mutagenesis or viral transactivation are not required[5]. Inflammation may also be the root cause of the increased risk of primary liver cancer associated with obesity and type 2 diabetes[6, 7]. Fibrosis is a key feature of the wound healing response initiated by inflammation within the liver, and it is tightly associated with the development of HCC due to all causes. Exactly how inflammation, fibrosis and liver cancer are linked remains unsettled, although NF-κB, a master regulator of inflammation, may play a central role through its influence on the life and death of both parenchymal and nonparenchymal cells[8]. Given the strongly pro-fibrotic nature of chronic hepatitis C, these links are undoubtedly active in the development of HCV-associated HCC.

Multiple studies suggest that cirrhotic patients who achieve a sustained antiviral response (SVR) to therapy have approximately a 3-fold reduction in the risk of HCC[9, 10]. This demonstrates the importance of the role played by the virus, but it doesn't distinguish between direct effects of viral protein expression versus the immune response to viral antigens. The continued occurrence of HCC following elimination of the virus may reflect residual pro-carcinogenic effects of cirrhosis, or alternatively, the length of time required for newly developing HCC to become clinically apparent[3]. Other evidence comes from a longitudinal, community-based cohort study in Asia that found that the risk of HCC among seropositive individuals to be associated independently with both the serum viral RNA level and serum alanine aminotransferase (ALT)[11]. These data support a role for both direct and indirect (i.e. inflammation-related) mechanisms of carcinogenesis.

One way to look at the potential contribution of direct vs. indirect mechanisms to HCV-associated HCC is to ask whether there are significant differences in the cancers that arise in patients with HCV vs. HBV infection. If in both cases, cancer results from the effects of chronic immune-mediated liver damage and unresolved wound healing responses, one would anticipate few differences in the underlying genetics of these cancers. While we are poised to learn much more about this from whole cancer genome sequencing efforts, there are tantalizing clues that suggest significant differences. One difference appears to be in the expression of the liver-specific microRNA, miR-122, an indispensable host cell factor that is essential for HCV replication. Conserved in sequence from zebrafish to humans, miR-122 is abundantly expressed in hepatocytes where it comprises over 50% of mature miRNAs and represses numerous liver-specific genes[12]. Its role in HCV replication is independent of its regulation of hepatic genes and requires its binding near the 5’ end of the HCV RNA genome[13]. Recent work in our laboratory indicates that miR-122 recruits argonaute 2 to the viral RNA, protecting it from cellular RNA decay machinery[14]. miR-122 is essential to the

HCV life-cycle and its therapeutic silencing with an antisense locked nucleic acid (LNA) oligonucleotide has potent antiviral effects in HCV-infected chimpanzees[15].

miR-122 also has important tumor suppressor properties in the liver. Loss of miR-122 expression appears to contribute to the malignant phenotypes of tumor cells, as reconstituting its expression may reverse anchorage-independent growth, migration, invasion, and tumor formation in nude mice[16, 17]. miR-122 also regulates cyclin G1, and thus influences the stability and transcriptional activity of p53[18]. While several studies indicate that miR-122 expression is typically reduced in liver cancer (reviewed in [19]), two reports suggest that its expression is preserved in HCV-associated cancers[17, 20]. Why should this be so, if HCV-associated cancer, like HCC due to many other causes, results from chronic inflammation and unresolved liver injury? One speculative possibility is that the replication of HCV is in some way intimately involved, directly, in carcinogenesis.

A quantitative analysis of nonmalignant liver tissues collected during surgical resection of HCV-related HCC indicated that only a small fraction of hepatocytes express detectable HCV antigens[21]. Such studies are plagued by technical difficulties inherent in detecting the low abundance of viral antigens expressed in the liver, but they raise a fundamental question that has yet to be answered: does cancer arise in an HCV-infected hepatocyte, or in uninfected bystander cells that are present in much greater numbers? Although more cancers need to be studied, the apparent preservation of miR-122 expression in HCV-associated HCC, despite its loss in HCC due to other causes, may be a clue that cancer arises within HCV-infected hepatocytes. Hepatocellular carcinogenesis is a multistep process[22]. Early loss of miR-122 expression during the progression toward cancer would restrict viral replication and prevent any subsequent direct contributions from viral protein expression. On the other hand, cells maintaining miR-122 expression would be at risk for continued direct effects of HCV, and would be selected during the progression toward cancer.

This speculative hypothesis is buttressed by the fact that some lineages of transgenic mice expressing HCV proteins are at risk for HCC despite the absence of immune-mediated inflammation and fibrosis[23, 24]. These lineages include mice with high-level expression of the viral core protein, as well as mice expressing a very low abundance of the complete viral polyprotein. The development of HCC in these transgenic mice in the absence of immune-mediated inflammation suggests that the expression of viral proteins may have a direct carcinogenic effect. However, steatosis is prominent in these mice[23, 24]. Thus, alternative inflammatory mechanisms operative in the development of HCC associated with metabolic syndrome, as mentioned above, may be at work here as well.

How could the expression of HCV proteins contribute directly to cancer? There is no evidence that HCC results from continued expression of a viral oncogene, such as occurs with the oncogenic papillomaviruses and gamma herpesviruses. Nor does it result from integration of viral sequences into the host cell genome, as HCV is an RNA virus that exploits an RNA lifecycle confined exclusively to the cytoplasm. However, one aspect of HCV biology that is increasingly evident is the manner in which it has evolved to hijack the functions of numerous cellular proteins (even miRNAs, as described above) to promote its survival in the liver. Viral proteins interact with signaling pathways to disable innate

immune responses, and with a host of cellular proteins to facilitate viral entry, translation, RNA synthesis and the assembly and release of infectious virus. Some of these interactions involve cellular proteins that have significant roles in controlling cell proliferation or that have tumor suppressor properties[19]. One good example is the interaction of the viral RNA-dependent RNA polymerase, NS5B, with the retinoblastoma protein, Rb.

Rb is typically a nuclear protein, but it is synthesized in the cytoplasm where it is bound specifically by NS5B[25, 26]. E6-associated protein (E6AP, or UBE3A), a HECT-type E3 ubiquitin ligase that mediates papillomavirus degradation of p53, is recruited to this complex and directs ubiquitylation of Rb, targeting it for degradation by the proteasome and reducing Rb abundance in HCV-infected cells[26] (Fig. 1). Only Rb is affected, not other pocket-binding proteins, but the net effect is an increase in activity of E2F-responsive promoters[25]. As Rb controls the G1 to S-phase transition, this mechanism may have evolved to overcome infection-induced blocks to cell cycle progression[27, 28]. Acute loss of Rb also impairs immune responses, and thus may facilitate virus escape from immunity[29]. However, Rb also regulates DNA damage responses, the G2 to M transition, and the mitotic spindle checkpoint[30] (Fig. 1). These are critical controls in prevention of cancer, directing cells to repair DNA damage or chromosome mis-segregration or to induce apoptosis before cell division. The risk of oxidative DNA damage is high in the infected liver, not only due to inflammation as discussed above, but also possibly due to direct effects of HCV proteins[31]. NS5B-mediated loss of Rb expression, if it occurs in vivo as it does in infected cell cultures[26], likely renders the infected hepatocyte unable to mount a normal DNA damage response and can be expected to promote genomic instability and increase the risk of HCC. Loss of hepatic Rb expression enhances genomic instability and diethyl-nitrosamine (DEN)-induced tumorigenesis in mice[32]. Several studies suggest that HCV may also disrupt the function of p53, a second major tumor suppressor in the liver (reviewed in[19]). The loss of p53 function could be synergistic with loss of Rb, but is not as well documented.

While it might seem paradoxical that HCV would directly disrupt the function of one key tumor suppressor (Rb), while the expression of another (miR-122) is preserved during the progression to cancer, such a scenario can be explained by how these host cell components influence the replication of the virus. The virus gains no evolutionary advantage from causing cancer. It regulates Rb, presumably, because this makes the host cell a more hospitable environment for replication[33]. miR-122 expression is preserved, because without it there is no further virus replication[15], and no possible direct oncogenic consequences of virus infection.

Apoptosis likely plays a key role in HCV pathogenesis. A fraction of infected cells undergo apoptosis in cell culture[34] and in mice with chimeric human livers[35]. This may occur via cell autonomous pathways induced by the presence of the virus, or result from sensitization of infected cells to extrinsic signals elicited as a result of the immune response to the virus[36]. The balance of pro- vs. anti-apoptotic signals determines whether an infected cell undergoes apoptosis or survives to divide. Virus-specific pro-survival effects, mediated in part by Rb loss, represent a mechanism by which HCV could promote cancer directly by enhancing survival of infected cells in the face of the pro-apoptotic effects of oxidative stress or DNA damage. On the other hand, pro-apoptotic signals induced by virus replication represent an

alternative mechanism by which HCV infection could promote cancer by causing the death of hepatocytes, thereby resulting in compensatory proliferation in an environment of inflammation and associated oxidative stress. Such a mechanism has been suggested for DEN-mediated carcinogenesis, where apoptosis was found to drive hepatocellular carcinogenesis in mice[37]. Also supporting this idea are recent studies showing that stimulation of hepatocyte turnover can promote HCC in mice[38].

So, is HCV a “carcinogenic” virus? There are strong arguments for both direct and indirect effects of the virus contributing to hepatocellular carcinogenesis (Fig. 2), and we will likely never know which if either predominates. Is this an important question? Regardless of the mechanisms involved, direct acting antiviral drugs are changing our expectations for hepatitis C therapy and one would hope that they will lessen the future incidence of HCV-associated liver cancer. Sustained antiviral responses should reduce if not ultimately eliminate the risk of HCC in infected persons. Some would argue that this would make the question moot. However, for those patients who fail DAA therapy, or those who for whatever reason never have access to it, a better understanding of the mechanisms of carcinogenesis and how HCV-associated HCC differs from HCC due to other causes may provide new avenues for prevention, and possibly even treatment, of this devastating cancer.

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Abbreviations

CDK	cyclin-dependent kinase
DEN	diethyl-nitrosamine
E6AP	E6-associated protein
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HECT	homologous to E6AP carboxyl terminus
NF-κB	nuclear factor-κB
NS	nonstructural protein
Rb	retinoblastoma protein

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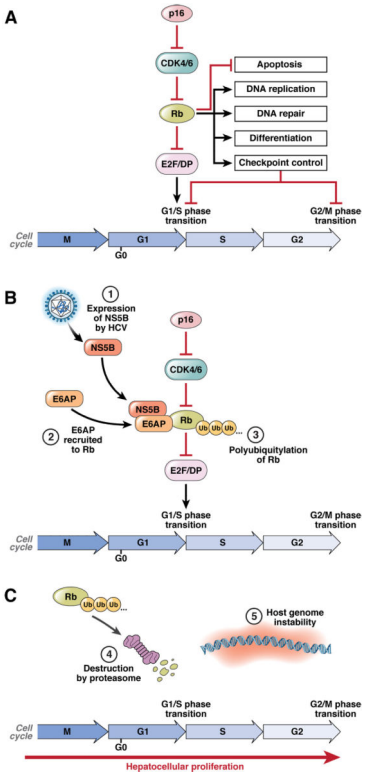

Figure 1. Disruption of the Rb pathway by HCV NS5B

(A) Rb regulates multiple cellular processes, including cell cycle progression, apoptosis, DNA replication and repair, cellular differentiation and senescence. Rb binds to and represses E2F/DP transcription factor complexes. During the G1/S phase transition, Rb phosphorylation by cyclin-dependent kinases (CDKs) results in dissociation of Rb from E2F/DP complexes. The derepressed E2F/DP complexes are free to initiate transcription of S phase-specific genes, thus allowing cell cycle progression. **(B)** NS5B interacts with Rb in the cytoplasm of HCV-infected cells, and recruits the E3-ubiquitin ligase E6AP to facilitate ubiquitylation of Rb. **(C)** Ubiquitylation targets Rb for proteasome mediated degradation. Loss of Rb results in dysregulation of the many processes that Rb controls. For example, the unscheduled activation of E2F/DP-dependent gene expression can compromise cell cycle checkpoints that guard against chromosomal instability as well as induce p53-dependent apoptosis.

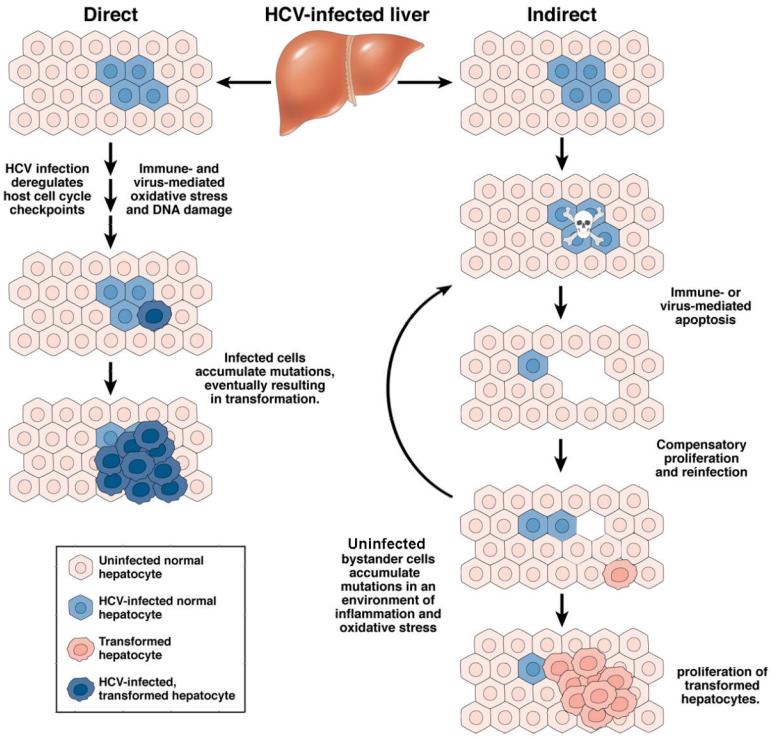

Figure 2. Direct versus indirect mechanisms of carcinogenesis in the HCV-infected liver

Competing scenarios depicting how liver cancer might arise within an infected cell as the direct result of viral protein expression (**left**), or in uninfected hepatocytes proliferating in response to the apoptotic death of infected cells (**right**). Direct vs. indirect mechanisms of carcinogenesis are not mutually exclusive and are subject to considerable overlap. The risk of cancer arising via either scenario is likely to be enhanced by the presence of cirrhosis, immune-mediated inflammation, and oxidative stress.