Hepatitis Viruses and Human Hepatocellular Carcinoma

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Two hepatotropic viruses, hepatitis B and C viruses, are known to cause hepatocellular carcinoma in humans. Hepatocarcinogenesis is a complex, stepwise process that evolves over several to many years and precisely how hepatitis viruses contribute to malignant transformation of hepatocytes is uncertain. Hepatitis B virus is integrated into cellular DNA in the great majority of hepatitis B virus-related hepatocellular carcinomas, whereas replicative intermediates of hepatitis C virus do not insert into chromosomal DNA, making it likely that different pathogenetic mechanisms operate with the two viruses. Indeed, evidence is mounting that both direct and indirect carcinogenic mechanisms, and often the two together, are involved in virus-induced hepatocellular carcinoma. In addition, evidence is now available that hepatitis B and C viruses interact synergistically in the pathogenesis of hepatocellular carcinoma. Animal models, — other members of the Hepadnaviridae family that cause tumors in their respective animal hosts, and transgenic mice into which the sequences of hepatitis B virus DNA have been inserted — are proving useful in elucidating putative mechanisms of hepatitis B virus-related hepatocarcinogenesis. Whatever the genesis of hepatitis virus-induced hepatocellular carcinoma, it is clear that hepatitis viruses do not act alone but in conjunction with other environmental carcinogens and a number of host factors.

INTRODUCTION

Of the six human hepatitis viruses now recognised, hepatitis A and E viruses do not exist in a chronic carrier state and cause acute infections only: they are not risk factors for hepatocellular carcinoma (HCC). In contrast, hepatitis B and C viruses do exist in a chronic carrier state and are frequent causes of chronic necroinflammatory hepatic disease: they are major risk factors for human HCC. Although hepatitis D virus always occurs in tandem with hepatitis B virus and co-infection causes severe acute and chronic hepatic parenchymal disease, there is no convincing evidence that this virus contributes to hepatocarcinogenesis. Little is yet known about the newly identified hepatitis G (GB-C) virus. From the way in which it was detected, however, it is evident that chronic carriage does occur, and there is preliminary evidence that it causes chronic liver disease. A pilot study has shown this virus to be present in the serum of 4 of 14 patients with HCC.

The evidence for hepatitis B and C viruses as hepatocarcinogens will be reviewed.

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Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; WHV, woodchuck hepatitis virus; GSHV, ground squirrel hepatitis virus; anti-HCV, antibody to hepatitis C virus.
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Evidence for hepatocarcinogenicity

Chronic infection with HBV has been estimated to be responsible for up to 80 percent of global HCC. The first evidence, albeit circumstantial, of a possible link between HBV and HCC was the close parallel, at both global and local levels, between the geographical distributions of viral carriage and that of the tumor [1-3]. A causal association between HBV and HCC was soon confirmed by the cohort study of Beasley and co-workers [3] in which male Taiwanese carriers of the virus were shown to have a lifetime relative risk for developing the tumor of more than 100. Between 40 and 50 percent of these carriers, and 25 percent of those in other populations, die from HCC, cirrhosis, or both diseases. At the same time numerous studies from all parts of the world, irrespective of whether the incidence of HCC was high, intermediate, or low, demonstrated that patients with this tumor invariably showed serum and liver tissue markers of persistent HBV infection far more often than did control populations [1-3]. The closest correlations were present in ethnic Chinese and black Africans, in whom as many as 85 percent of the patients were currently infected with HBV at the time they presented with HCC and almost all of the remainder showed evidence of previous infection. In these populations HBV carriage is largely acquired early in life as a result of either perinatal infection, in ethnic Chinese [3], or horizontal infection, in black Africans [4]. It is these early-onset carriers that are at greatest risk of HCC formation. The risk for those infected later in life has been less clearly defined, but it is substantially less than for those with early infection. HBV carriage almost always precedes the development of HCC by many years, an interval consistent with a cause-and-effect relationship, and the likelihood of malignant transformation rises progressively with increasing duration of infection.

The great majority of HBV-related HCCs have sequences of viral DNA integrated into chromosomal DNA [5].

Indirect evidence for a causal relation between HBV and HCC is provided by the observation that other members of the Hepadnaviridae (to which HBV belongs) also induce HCC in their respective hosts: in eastern woodchucks (Marmota monax) infected with WHV with a frequency of virtually 100 percent within about 2 years of infection, and in Beechey ground squirrels infected with GSHV of approximately 30 percent within 5 to 6 years [6, 7]. Integrants of hepadnaviral DNA have been detected in both of these animal models. More recently, transgenic mice that developed HCC after sequences of the HBV genome were introduced into their germ-lines have provided further indirect evidence of the hepatocarcinogenicity of HBV [8, 9].

Possible mechanisms of HBV-induced hepatocarcinogenicity

Although the exact mechanisms involved in HBV-induced HCC remain uncertain, there is mounting evidence that both direct and indirect carcinogenic effects are operative.

Direct carcinogenicity: The observations that HBV-induced HCC can occur in an otherwise normal liver and that markers of HBV infection are present in serum and liver tissue as often in HCC patients without co-existing cirrhosis as in those with cirrhosis first suggested that HBV may be directly carcinogenic. This possibility was later supported by the findings both that 85 to 95 percent of HBV-related (and WHV-related) tumors contain viral DNA integrants in host DNA [5], and that transgenic mice into which the HBx gene and its regulatory sequences had been inserted develop HCC in the absence of cirrhosis or chronic hepatitis [9].

Early on, the long interval between infection with HBV and the onset of HCC implied that the virus did not contain an an acutely transforming oncogene, and this was subsequently confirmed when the viral genome was completely sequenced. The finding of viral
integrants in host DNA in HCC tissues made it probable that HBV exerts its carcinogenic effect through insertional mutagenesis. HBV DNA appears to integrate into chromosomal DNA at random sites [5], and it is therefore unlikely that insertion is exerting an effect in cis. Indeed, in three human tumors only has HBV DNA been shown to integrate in or near the regulatory sequences of a known proto-oncogene [10, 11] and in none has integration disrupted a recognised tumor suppressor gene. Moreover, fusion proteins composed of HBV- and cellular gene- products have not been detected. Nevertheless, indirect support for this possible mechanism is provided by the finding that in about 50 percent of woodchucks infected with WHV insertion of WHV DNA takes place within regulatory sequences of c-myc or N-myc 2, activating expression of these genes. Activation of c-jun and c-fos has also been described in a number of these tumors. Furthermore, transgenic mice develop HCC after the woodchuck c-myc gene in tandem with upstream WHV DNA has been incorporated into their germ-line. When ground squirrels infected with GSHV were studied, however, none of the integrants proved to be near c-myc or N-myc 2, making the overall interpretation of this possible mechanism difficult to evaluate. Moreover, in the transgenic model of Chisari and co-workers [8] expression analysis of all proto-oncogenes and tumor suppressor genes thus far implicated in hepatocarcinogenesis failed to reveal any quantitative or qualitative changes [12].

An effect in trans is, however, possible. The HBx gene can transactivate transcription of a variety of viral and cellular promoters [13], thereby perturbing the expression of cellular growth regulatory genes and hence the function of the infected cell in such a way as ultimately to induce a transformed phenotype. Support for this mechanism was provided by introducing HBx gene and its regulatory sequences into the germ-line of mice. The progeny developed, in turn, foci of altered hepatocytes, benign tumors, and finally florid HCCs, all in the absence of chronic hepatic parenchymal disease [9]. The HBV preS/S gene has also been shown, when 3'-truncated, to possess transactivating properties [14]. Both of these genes, but particularly the x gene, are often included in HBV DNA integrants, and could express HBV polypeptides or possibly HBV-cellular gene fusion proteins.

Loss or disruption of chromosomal DNA in the sequences flanking integrated HBV DNA may result in deletion of tumor suppressor genes or, by altering the physical relation between proto-oncogenes or tumor suppressor genes and their regulatory sequences, either remote from or adjacent to the site of insertion, may affect expression of these genes.

These observations suggest that abnormal expression of cellular genes may initiate aberrant growth. The HBx gene has been shown to interact in vitro with the p53 protein causing inhibition of p53-mediated transcriptional regulation [15]. An effect on apoptosis could possibly be mediated in the same way. Activation or inactivation of growth regulatory genes, whether in cis or in trans, remains an attractive hypothesis because either could confer on initiated hepatocytes a growth advantage that, coupled with an opportunity for selection, would generate cells that are at risk for further genetic or epigenetic events in the stepwise process required for tumor formation.

Although integration of viral DNA into host DNA is likely to play a pivotal role in HBV-induced HCC, it has not yet been proved to be an essential step in the process. Nor is it known when and precisely how integration takes place. Whether HBV can cause HCC in the absence of other environmental carcinogens is also uncertain. The most convincing evidence that it can are the observations that woodchucks infected with WHV as neonates and reared in an environment free of known carcinogens develop HCC [16], and that HBsAg-positive mentally disabled children living in California, USA in the apparent absence of carcinogens are at are at very high risk of developing this tumor [17]. Of
additional significance is the fact that in both of these circumstances the tumors develop in the absence of cirrhosis.

**Indirect carcinogenicity:** Most patients with HCC, whether in regions with high, low, or intermediate incidence regions of the tumor, have co-existing cirrhosis (a few have co-existing chronic hepatitis) [18]. Moreover, cirrhosis, whatever the cause, may be complicated by malignant transformation [18]. These observations lend support to the belief that HBV can induce HCC indirectly by causing chronic necroinflammatory hepatic disease. This mechanism would also explain the high incidence of HCC in patients with membranous obstruction of the inferior vena cava, in whom unremitting hepatic venous hypertension causes continuous cell necrosis and regeneration, as well as those patients with HBV-related HCC in whom viral integrants are not detected. A likely way in which chronic necroinflammatory hepatic disease could contribute to carcinogenesis would be by functioning as a promoter. Continuous or recurring cycles of necrosis of hepatocytes followed by regeneration, as typically occurs in cirrhosis or chronic hepatitis, renders the hepatocyte DNA far more susceptible both to spontaneous mutation and to exogenous damage. Furthermore, an increased hepatocyte turnover rate may, by increasing the intracellular concentration and activity of topoisomerase I, result in cleavage of viral DNA at specific motifs, thereby promoting its integration into chromosomal DNA [19]. More rapid cell division may also leave insufficient time for damaged DNA to be repaired before the cell divides again, thereby "fixing" the altered DNA in the daughter cells. In addition, increased hepatocyte division provides the opportunity for the selective growth advantage of initiated cells to be exercised.

Indirect evidence in favor of a role for necroinflammatory hepatic disease in the pathogenesis of HCC is provided by transgenic mice into which the HBV S and preS genes have been introduced [8]. The progeny overproduce large surface protein that accumulates in the smooth endoplasmic reticulum of the hepatocytes, producing severe and prolonged injury to the cells, which initiates a response characterised by inflammation, regenerative hyperplasia, and transcriptional deregulation, and which progresses ultimately to neoplasia. These findings imply that severe and prolonged injury per se may lead to unrestrained cell growth. This conclusion was supported by the subsequent expression analysis, in this model, of all proto-oncogenes and tumor suppressor genes implicated in hepatocarcinogenesis, which failed to reveal any quantitative or qualitative changes [12].

Like all forms of carcinogenesis, hepatocellular carcinogenesis is a complex multi-step process that evolves over many years. Different pathways of tumor induction, direct or indirect, probably operate in different HBV-related HCCs. Moreover, direct and indirect mechanisms are not mutually exclusive, and the two acting in concert may be responsible for most HBV-induced HCCs.

**HEPATITIS C VIRUS AND HEPTOCELLULAR CARCINOMA**

Evidence for an etiological role for hepatitis C virus (HCV) in HCC is more recent but is nonetheless persuasive. The first hint of a link between this virus and the tumor came from isolated reports in the literature of patients (and one chimpanzee) with acute non-A, non-B hepatitis following blood transfusion who, over a period of many years, developed in turn chronic hepatitis, cirrhosis, and finally HCC [20]. Stored sera from some of these patients (but not the chimpanzee) were subsequently shown to test positive for anti-HCV. More substantive evidence was provided later by case series, case/control analyses, and cohort studies that used progressively more reliable enzyme immunoassays, confirmatory recombinant immunoblot assays, and reverse transcription-polymerase chain reaction to detect HCV infection. The International Agency for Research on Cancer
of the World Health Organisation has recently concluded that HCV, like HBV, is carcinogenic in humans [21].

As is true of HBV, the proportion of patients with HCC in whom HCV can be implicated shows a pronounced geographical variation [20, 21]. In Japan, with a high incidence of HCC, and Italy and Spain, with intermediate incidences, as many as 80 percent of patients with HCC are infected with HCV, and persistent HCV is a greater risk of HCC than is chronic HBV infection. In these countries 2 to 3 percent of patients with HCV-related cirrhosis develop HCC during each year of follow-up. In contrast, in those regions in which HBV infection is hyperendemic and HCC very common, fewer than 25 percent of patients have HCV-related tumors. In the remaining countries, with low or intermediate incidences of HCC, HCV and HBV play an equal but relatively minor role, with markers of each virus being found in about one-quarter of the patients.

Patients with HCV-induced HCC are, in almost all geographical regions, older than patients with HBV-induced tumors; the difference in black Africans is about 20 years and in other populations around 10 years [20].

**Possible mechanisms of HCV-induced HCC**

Replicative intermediates of HCV, a single-stranded RNA virus of positive polarity, do not integrate into chromosomal DNA, although the virus does replicate in both normal and malignant hepatocytes [22]. If HCV is directly carcinogenic, therefore, the mechanism is likely to differ from that of HBV. Until very recently there was no well documented evidence that HCV-induced HCC occurred in livers that were not either cirrhotic or showed chronic hepatitis. However, DiMitri and co-workers have now reported examples of HCV-related tumors developing in virtually normal livers, providing the first circumstantial evidence that this virus might be capable of directly inducing neoplastic change [23].

On the other hand, the very marked propensity for acute HCV infections to progress to chronic liver disease (with a frequency now approaching 80 percent), as well as the very close link between HCV-related HCC and cirrhosis (or less often chronic hepatitis) strongly supports an indirect role for the virus in the pathogenesis of HCC. The importance of an increased hepatocyte turnover rate in HCV-induced HCC was recently emphasised in reports in which the risk of the tumor developing in patients with HCV-related chronic necroinflammatory hepatic disease correlated directly with high DNA synthetic activity as a marker of proliferative activity [24].

**Interaction between HBV and HCV in hepatocarcinogenesis**

Knowing whether HBV and HCV interact in the origin of HCC might help in elucidating the pathogenetic mechanisms involved in virally-induced hepatocarcinogenesis. Although HBV and HCV are separately major risk factors for HCC, the risk when co-infection with the two viruses occurs is uncertain. Four analyses have been published in which possible confounding influences of other variables have been taken into account in establishing the odds ratio for the development of HCC in individuals chronically infected with HBV alone, HCV alone, and the two viruses together. Two studies, one in Taiwan (which has a high incidence of HCC) [25], the other in Italy (which has an intermediate incidence) [26] showed no interaction between HBV and HCV as hepatocarcinogens. The other two, in populations with a low incidence of HCC, revealed an interactive effect, one additive (in North American patients) [27] and one multiplicative (in Greek patients) [28]. In a recent case/control study of 231 southern African blacks with HCC (a high incidence region for HCC), we demonstrated a synergistic interactive effect between the two viruses [29].
Indirect support for an interactive carcinogenic role between the two viruses comes from our observation that patients infected with both HBV and HCV are significantly younger than those infected with HCV alone. How HBV and HCV interact in the genesis of HCC is not known. Patients with dual infection react to HCV antigens alone (or predominantly), suggesting that HCV infection plays the dominant role in the immune response in patients infected with both viruses [30]. If this observation is confirmed, the promoter effect of combined HBV and HCV infection in hepatocarcinogenesis should be no greater than that with either virus alone. The evidence that HBV and HCV interact in the origin of HCC would then imply that HCV, like HBV, is capable of being directly oncogenic.

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