HCV-infection and hepatocellular carcinoma

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Epidemiology

Following the discovery of the hepatitis C virus (HCV) in 1989, several studies have shown an important seroepidemiological association between HCV infection and hepatocellular carcinoma (HCC). Worldwide, HCV infection ranks second only to HBV infection in its association with HCC. In southern Europe and Japan between 50% to 75% of cases are associated with HCV infection (Colombo M. *et al.*, 1989 ; Bruix J. *et al.*, 1989 ; Saito I. *et al.*, 1990 ; Nishioka K. *et al.*, 1991). Moreover, infection with HCV has been found as a major risk factor for the development of HCC. The current available information about the risk of HCC has come from prospective studies of patients with transfusion associated hepatitis C and of patients suffering from chronic liver disease. In general, HCV infection precedes the development of HCC by two or three decades after transfusion (Tong M.J. *et al.*, 1995). The EUROHEP study of patients with compensated HCV-related cirrhosis showed a 5-year cumulative incidence of HCC of 7% (Fattovich G. *et al.*, 1997). The incidence of HCC in untreated patients with compensated cirrhosis is about 2.5 per 100 person-years in Western countries, but is as high as 6.9 per 100 person-years in Japan (Nishiguchi S. *et al.*, 1995). We will undoubtedly encounter an accumulating prevalence of HCV-related HCC during the next three decades, unless the development of efficient antiviral drugs, vaccines and antitumoral agents.

Before discussing the possible oncogenetic mechanisms, it is important to underscore additional risk factors for the development of HCV-related HCC, such as age, male gender, current or previous infection with HBV and alcohol consumption (Kew M.C. *et al.*, 1997 ; Chiaramonte M. *et al.*, 1999).

Molecular mechanisms of HCV-related HCC

HCV replicates in both hepatoma cells and in non-cancerous hepatocytes from anti-HCV positive patients with HCC (Ohishi M *et al.*, 1999). The oncogenesis may be related to the HCV induced chronic inflammation, liver cell necrosis and regeneration. It is tempting to consider, but as yet unproven, that continuous or recurring cycles of necrosis of hepatocytes may render the DNA of the liver susceptible to spontaneous or mutagen induced mutations. Due to the enhanced cell turnover the time to repair the damaged DNA may be compromised.

In this way, the stage is set for an accumulation of mutations in highly proliferating cells (Idilman R. *et al.*, 1998).

The emergence of HCC in the absence of cirrhosis and in nearly normal livers of HCV carriers (De Mitri M. *et al.*, 1995 ; Elrefaie A. *et al.*, 1996) indicate that HCV may be directly involved in the hepatocarcinogenesis. The absence of integration of the HCV genome in the host cell DNA and the lack of reverse transcriptase activities of the encoded proteins suggest a major role for viral proteins interfering with cell cycle regulation (Chou W.-H. *et al.*, 1991). For some putative HCV gene products — such as NS3, core and NS5A — there is evidence of a possible involvement in the hepatocarcinogenesis.

NS3 encodes a serine-protease which is important for the processing of the HCV polyprotein. Stable expression of the N-terminal part of NS3 in murine NIH 3T3 cells can induce a transformed phenotype (Sakamuro D. *et al.*, 1995).

The *HCV core protein* is a 191 amino acid long, highly basic and non-glycosylated nucleocapsid protein. It has been demonstrated that transfection of the HCV core gene may transform NIH 3T3 cells leading to tumor formation in nude mice (Ray R.B. *et al.*, 1996). In addition, HCV core gene, in cooperation with the H-ras oncogene may also transform primary rat fibroblasts, although some controversy exists (Chang J. *et al.*, 1998). Recently, Moriya K. *et al.* (1998) reported the development of HCC in two independent lines of mice transgenic for HCV core gene. In addition, this mice had developed hepatic steatosis early in life as a histological characteristic feature of chronic hepatitis C. The mechanism by which the HCV core protein could interfere with the cell cycle regulation is only partially understood. Previously, the deduced amino acid sequence of the HCV core protein has revealed that it may function as a gene regulatory protein since there is a putative DNA binding motif, and three potential nuclear localization signals (Chang SC *et al.*, 1994). This hypothesis is supported by *in vitro* studies in which transfection of HCV core gene resulted in suppression of the expression

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and replication of HBV genome in HuH-7 cells (Shih C.M. *et al.*, 1993). Furthermore, Ray R.B. *et al.* (1995) have demonstrated transcriptional regulation of cellular and viral promoters by the HCV core protein in HepG2 cells. Recently, HCV core protein was found to repress WAF1/Cip1/Sid1 (p21), a known inhibitor of cyclin-dependent kinases and may as such promote cell growth (Ray R.B. *et al.*, 1998a). Both inhibition and enhancement of tumor necrosis factor-mediated apoptosis by the HCV core protein have been described (Ray R.B. *et al.*, 1998b ; Zhu N.L. *et al.*, 1998). We have demonstrated that non- tumorigenic HepG2-derived cells, which were stable transformed by the HCV core gene, exhibited a markedly increased carcinogenic potential in SCID mice as compared to cells transfected with the empty vector. The alpha-fetoprotein (AFP) mRNA was found upregulated in the HCV core gene transfected cell lines, while the albumin gene expression remained unaffected. In addition, the subcellular distribution of the HCV core protein was not the determining factor for the oncogenic property (Verslype C. *et al.*, 1 999).

Recently, studies investigating interferon (IFN)-resistance have suggested that the *NS5A protein* from IFN-resistant strains of genotype la and lb, may be involved in HCV-related HCC. NIH 3T3 cells expressing the NS5A protein exhibited a transformed phenotype and formed solid tumors *in vivo* (Gale M. *et al.*, 1999). The mechanisms of this oncogenic property, together with the concept of IFN-resistance, appear to be partially linked to the repression of the IFN-induced protein kinase PKR (protein kinase induced by double stranded RNA). PKR has previously been found to mediate the antiviral actions of interferon through phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF-2), which results in an inhibition of mRNA translation and a concomitant block in HCV replication. The NS5A protein from IFN-resistant HCV genotypes has been found to bind and inhibit PKR. In addition to its antiviral actions, PKR has now been defined as a tumor suppressor and an apoptotic checkpoint on cell proliferation (Gale M. *et al.*, 1999).

Prevention

Despite some preliminary evidence that interferon may offer protection against the development of HCC (International Interferon-alpha HCC study group, 1998), the best advice one can give to patients with chronic hepatitis C is to limit alcohol consumption, as this appears to be an important additional risk factor for the development of HCV-related HCC. In addition, HCV patients should be vaccinated against hepatitis B.

The above mentioned viral proteins (NS3, core and NS5A) which may play a direct role in the development of HCV- related HCC, are possible targets for anti-viral therapeutical agents.

However, while awaiting the development of an effective vaccine and new drugs to control the HCV pandemic, the battle actually evolves in favor of HCV and a substantial rise in the incidence of HCC is expected in the coming decade.

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