SPECIAL ARTICLE

HEPATOCELLULAR CARCINOMA - CARCINOGENESIS UPDATED

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Hepatocellular carcinoma (HCC) is a leading cause of death in many parts of sub-Saharan Africa and Asia[1]. The majority (90%) of the primary liver cancers seen in Singapore is due to HCC[2]. Hepatocarcinogenesis is a multistep, multifactorial process. Both viral and chemical carcinogens are involved in the induction of HCC though the exact mechanism of hepatocarcinogenesis remains to be completely elucidated.

Chronic hepatitis B virus (HBV) infection has been documented as one of the most important risk factor for the development of HCC. The surface antigen of HBV (HBsAg) is frequently present in the blood and liver tissue in patients with HCC in countries where HBV carriers are common. Other evidences in supporting that HBV has an aetiologic role in HCC are: familial clustering of HCC, cirrhosis and HBV carriers; parallelism between the prevalence of HCC and the frequency of HBV carriers; frequent occurrence of HCC among HBV carriers in comparison with non-carriers within the same population and development of HCC in certain animal species infected by the indigenous hepatitis virus that is similar to human HBV. The presence of integrated HBV DNA and/or detectable viral antigens in most HCC[5-7] and cultured cell lines derived from HCC[8,9] provides further evidence for a close relationship between HBV and HCC. Recent studies have also shown that the persistent expression of HBxAg (hepatitis B X gene product) in the liver of chronic HBV infection may be important in the carcinogenesis of HCC[10,11]. This indicates that HBV probably has a direct oncogenic role in the pathogenesis of HCC other than through the pathway of chronic liver injury, regeneration and cirrhosis. An increased incidence of HCC in HBsAg-negative individuals with serological evidence of past HBV infection (positive anti-HBs and anti-HBc) has also been described. With the use of polymerase chain reaction, HBV has been shown to persist at low levels in a number of these subjects[12-14].

Human HBV does not contain known oncogenes in its genome. The mechanisms by which HBV contributes to the pathogenesis of HCC remain unclear. However, the virus may act as an initiator through integration of the HBV genome in hepatocytes resulting in insertional mutagenesis[15,16] and/or chromosomal deletions and translocation[17]. HBV may also initiate hepatocarcinogenesis after integration by expression of HBxAg and/or preS/S products, which have been shown to trans-activate a variety of virus and host promoters[18-20]. Both integration and/or HBV gene expression may alter the patterns of expression of oncogenes and/or tumour suppressor genes important to the development of HCC. Chronic HBV infection may also act in the promotion and/or progression stage of carcinogenesis by enhancing the growth of preneoplastic or neoplastic cells.

There are many epidemiological characteristics of HCC suggesting that other risk factors besides HBV infection are involved in the development of HCC. These are: 1) striking male to female difference in HCC incidence despite their similarity in HBV carrier prevalence[21], 2) wide variation in age of onset of HCC in endemic areas even though most HBV carriers are infected perinatally or during early childhood[22], 3) great discrepancy in HCC incidence among Chinese migrants in Hong Kong, Taiwan, Singapore, Shanghai and USA[22], 4) extraordinarily high HCC mortality in the Southern provinces of China (especially GuangXi)[23,24]. These observations cannot be explained by the HBsAg prevalence alone and other independent risk factors including aflatoxin exposure, hepatitis C (HCV) infection, alcohol drinking are probably involved also in the pathogenesis of HCC[3].

Aflatoxins (mycotoxins derived from *Aspergillus flavus*) have been well-documented to cause extensive hepatic necrosis when administered to animals in large doses or to result in HCC when dosed chronically. *Aspergillus flavus* and aflatoxin B_1 (AFB_1) were frequent contaminants of grains and legumes, particularly in Asia and sub-Saharan Africa, where climate and food storage techniques allow for higher quantities of AFB_1 in the food supply. High incidence of HCC is found to closely parallel the prevalence of AFB_1 - contaminated food products, thus supporting a strong epidemiological link between AFB_1 ingestion and HCC[25 28]. Several evidences support a close relationship between AFB_1 and HCC: (1) rats treated with AFB_1 have HCC developed in a dose-dependent fashion; (2) AFB_1 treatment of transgenic mice with integrated HBV DNA greatly enhanced the development of HCC as compared with mice not treated with AFB_1; (3) epidemiological studies from Swaziland showed regional differences in HCC incidence that correlate more closely with the dietary AFB_1 intake than with the incidence of HBV infection; (4) Recently, high aflatoxins and the presence of an uniform point mutation (G to T) of third base of condon 249 of the P53 tumour suppressor gene in HCC was found[29 40]. This finding is supported by *in vitro* studies indicating that the specific point mutation of P53 was preferentially targeted to form adducts with aflatoxin B_1[31,32]. However, these same regions are also noted to have high rates of hepatitis B infection. Inconsistent results are also observed in case-control studies[3, 33-35] which may be due to difficulty in assessing the aflatoxin exposure at the population or individual levels accurately. Aflatoxin exposure may also

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interact with HBV in the hepatocarcinogenesis in areas where such exposure is common[36,37]. Among individuals who were HBsAg-negative, the relative risk of HCC in conjunction with positive urinary aflatoxin was 1.9, whereas for those who were HBsAg-positive, detection of urinary aflatoxin increased the relative risk to 60.1[38]. Serum AFB_1 measurement may be insensitive in assessing AFB_1 exposure[39]. Urinary aflatoxin metabolites and DNA - adducts reflect only recent dietary exposure to aflatoxin while assays for serum albumin adducts of aflatoxin can assess dietary intake of aflatoxin over a longer period of time which may show an even stronger association between aflatoxins and HCC. Aflatoxin B_1 DNA adduct in smeared tumour tissue from patient with HCC were detected at a rate of 70% in a recent Taiwanese study[41]. However, the causal role for aflatoxins in HCC remains to be clarified.

Several case reports of HCC in patients with Non A, Non B hepatitis (NANBH) have been published[42-45]. Since the development of a diagnostic immunoassay for the detection of specific HCV antibodies (anti-HCV), a link between chronic HCV infection and the development of HCC has been established. High prevalence of anti-HCV in HBsAg - negative patients with HCC have been reported[46-51] and mainly in areas where HBV infection is not endemic. The substantially increased incidence of HCC in Japan during recent decades, especially among males, has been attributed to an increasing frequency of chronic hepatitis C infection in this population. The interval between transfusion and diagnosis of HCC is around 30 years in a large cohort of Japanese patients[51] and similar lag time between exposure to HCV and HCC development is also noted among haemophiliac patients[52]. Although there is convincing epidemiological evidence for a direct or indirect relationship between HCV and the development of HCC, their causal relationship remains to be determined.

The possible interaction between the HCV and HBV in the pathogenesis of HCC remains unclear. A synergistic effect between the viruses was described in patients in United States, Greece and Taiwan[53-55] while no significant interaction between the viruses was found in Italian or French patients with HCC[56,57]. Integration of the HCV genome does not occur, and it acts indirectly through the pathway of chronic liver injury, regeneration and cirrhosis which has been considered premalignant. So far, cirrhosis has been found to coexist in almost all anti-HCV positive HCC patients in contrast to 60%-85% of HBsAg-positive HCC patients, suggesting that cirrhosis is a pre-requisite factor for HCC development in subjects with HCV infection. Thus, the pathogenesis role of HCV is probably different from that of HBV.

Although ethanol has not been implicated as a carcinogen by itself, a number of epidemiological studies suggest an indirect role of ethanol in hepatocarcinogenesis. HCC risk is significantly increased by alcohol consumption[58,59]. It should be noted that HCC can be seen in alcoholics without cirrhosis[60]. Thus, alcohol definitely has a role in the development of HCC, but its relative importance may be less than was once thought. The association is however not observed in case-control studies in countries where HBV infection are endemic[31,61,62]. In a rodent chemical carcinogenesis model, ethanol acts as a promoter[63] through induction of various enzymes, alternation of DNA repair and the immunosurveillance system and dietary deficiencies.

With progress in the understanding of basic molecular mechanisms of hepatocarcinogenesis, the biochemical aberrations that predispose the liver to the development of HCC will in due time be more fully understood. Recognition of the conditions associated with HCC can lead to early diagnosis of patients at risk and prevention of this malignancy.

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