

Hepatocellular carcinoma: susceptibility markers

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Genetic polymorphisms of the carcinogen-metabolizing enzymes cytochrome P450 (CYP), glutathione S-transferase (GST) M1 and N-acetyltransferase (NAT2) as well as p53 polymorphisms have been studied experimentally as susceptibility markers for hepatocellular carcinoma development in hepatocellular carcinoma cell lines and in mouse hepatocellular carcinomas. In addition, these susceptibility markers have been studied in hepatocellular carcinoma patients, in the context of coexisting alcohol consumption, smoking and/or HBV infection. To date, there is no clear evidence that susceptibility markers have an overall impact on hepatocarcinogenesis, but in subgroups of individuals, such as smokers, susceptibility markers are emerging indicators for hepatocellular carcinoma risk definition.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common tumours in the world and usually represents a late complication of chronic progressive liver disease (Schafer & Sorrell, 1999). Although less frequent in the United States and Europe, these tumours have an annual incidence of up to 500 cases per 100 000 population in certain regions of Asia and sub-Saharan Africa. The reasons for this high incidence are chronic infections with hepatitis B virus (HBV), hepatitis C virus (HCV) and the delta virus (HDV), as well as HBV-HCV coinfections (Di Bisceglie, 1998). The clinical course of HBV and HCV infection depends in part on molecular characteristics of the viruses, in part on the patients' HLA haplotype (Kuzushita *et al.*, 1998) and in part on other coexisting risk factors. Well recognized non-viral exogenous agents associated with HCC development are alcohol and aflatoxin B₁ (AFB₁). In the western countries, alcohol-induced liver injury is a leading cause of liver cirrhosis and the most important HCC risk factor (Donato *et al.*, 1997). In southern China and Africa, dietary ingestion of high levels of AFB₁ may present a special environmental hazard, particularly in chronically HBV-infected individuals. Other exogenous factors have also been incriminated and include dietary iron overload (Mandishona *et al.*, 1998), long-term use of oral contraceptives (Mant & Vessey, 1995; Waetjen &

Grimes, 1996) and high-dose anabolic steroids. The development of hepatic cirrhosis, particularly in association with inherited genetic diseases, such as α -1-antitrypsin deficiency or haemochromatosis, places the individual at a greatly increased risk for HCC development. The major clinical HCC risk factor is liver cirrhosis, since 70–90% of HCCs develop in a macronodular cirrhosis. In addition, HCCs are more frequent in males than in females and the incidence generally increases during the last decades of life and with age (Poynard *et al.*, 1997; Taylor-Robinson *et al.*, 1997; De Vos Irvine *et al.*, 1998; Deuffic *et al.*, 1998; El-Serag & Mason, 1999). The etiological risk factors may exist either alone or in combination, e.g., HCV infection and alcohol use (Corrao & Arico, 1998) or HBV infection and exposure to AFB₁ (Sun *et al.*, 1999), further increasing HCC risk.

As susceptibility markers of HCC development, carcinogen-metabolizing enzymes have been analysed in HCC cell lines and in mouse HCCs as well as in HCC patients. In HCC patients, susceptibility markers have been studied in the context of coexisting alcohol consumption, smoking and/or HBV infection. In the following, genetic polymorphisms of cytochrome P450 (CYP), glutathione S-transferase (GST) M1, N-acetyltransferase (NAT2) and p53 are discussed as susceptibility markers of HCC development.

Cytochrome P450

The expression and polymorphisms of cytochrome P450 (CYP) isozymes CYP1A1, CYP2A6, CYP2B6, CYP2D6, CYP2E1, CYP3A4, CYP3D4 and CYP1A or CYP3D7 as carcinogen-metabolizing enzymes have been studied *in vitro* as well as in animal models and HCC patients.

The status of CYP enzymes was studied in a transgenic mouse model (ATX mice) carrying the HBV X gene under the control of the α -1-antitrypsin regulatory elements (Chomarat *et al.*, 1998). The HBx protein is suspected to play a central role in hepatocarcinogenesis through its *trans*-activation of cellular genes, including oncogenes, as well as through *cis*-acting elements. Indeed, the HBV X gene has been shown to *trans*-activate the expression of specific CYP isozymes. In ATX mice, however, no *trans*-activation of CYP1A or CYP2A5 was observed. These data indicate that HBx expression alone is insufficient to induce *trans*-activation of CYP genes (Chomarat *et al.*, 1998). As detailed below, there is evidence, however, that *p53* polymorphism at codon 72 interacts with CYP1A1 and carotenoid levels in smoking-related hepatocarcinogenesis (Yu *et al.*, 1999b).

Epidemiological evidence indicates that dietary AFB₁ and mutations in the *p53* tumour-suppressor gene are involved in hepatocarcinogenesis. However, the correlation between AFB₁-DNA adduct formation or *p53* mutations and their activation pathways has not been elucidated to date. In this context, Mace *et al.* (1997) established SV40-immortalized human liver epithelial cell lines stably expressing CYP1A2, CYP2A6, CYP2B6 and CYP3A4 proteins. These cell lines were highly sensitive to the cytotoxic effects of AFB₁, with formation of DNA adducts and CYP-dependent mutations of the *p53* gene. These findings indicate that the differential expression of specific CYP genes in human hepatocytes can modulate AFB₁-induced cytotoxicity, DNA adduct levels and the frequency of *p53* mutations (Mace *et al.*, 1997).

Since cigarette smoking has been associated with increased HCC risk in some epidemiological studies, and *CYP1A1* is involved in biotransformation of tobacco-derived polycyclic aromatic hydrocarbons (PAHs), *CYP1A1* polymorphisms have been studied in HBV-infected patients (Yu *et al.*, 1999a). *CYP1A1* genotypes were associated with increased HCC risk in smokers but not in non-

smokers, indicating that tobacco-derived PAHs can increase HCC risk among HBV-infected patients and that *CYP1A1* polymorphism is an important modulator of the hepatocarcinogenic effect of PAHs (Yu *et al.*, 1999a).

In mouse liver tumours, the hepatic CYP2A6 enzyme is invariably overexpressed, suggesting that the oxidative metabolism of procarcinogens in the liver may be involved in hepatocarcinogenesis. In contrast, in human HCCs, CYP2A6 overexpression has been found not to be an invariable phenotype (Raunio *et al.*, 1998). In another study of HCC patients and healthy individuals, however, subjects with two active *CYP2D6* genes (rapid metabolizers) were found to be at increased risk for HCC development, especially in patients without evidence of HBV or HCV infection (Agundez *et al.*, 1996).

Susceptibility to HCC development has also been studied in relation to *CYP2E1* genotype. In cigarette smokers but not in non-smokers, homozygosity for the c1/c1 genotype was associated with significantly increased risk for HCC, indicating that tobacco-derived PAHs may play a role in HCC development (Yu *et al.*, 1995). For *CYP2E1* genotypes, no such association was found in Korean HCC patients, irrespective of HBV and HCV infection or chronic alcohol consumption (Lee *et al.*, 1997).

Glutathione S-transferase M1

Genetic polymorphisms of glutathione S-transferases (GST) are involved in carcinogen metabolism. GST status has been studied in a transgenic mouse model (ATX mice) carrying the HBV X gene under the control of the α -1-antitrypsin regulatory elements (Chomarat *et al.*, 1998). In this model, no *trans*-activation of GST was observed, indicating that HBx expression alone is insufficient to induce *trans*-activation of GST genes (Chomarat *et al.*, 1998).

Susceptibility to HCC development was further studied by *GSTM1* genotyping of HCC patients as well as healthy individuals. *GSTM1*-null genotype was not associated with increased HCC risk (Yu *et al.*, 1995). As detailed below, however, there is a significantly increased HCC risk in patients with the *p53* Pro allele at codon 72 in smokers with the *GSTM1*-null phenotype (Yu *et al.*, 1999a).

N-Acetyltransferase 2

N-Acetyltransferase 2 (NAT2) is a polymorphic enzyme which is expressed in the liver in a genotype-dependent manner and is involved in activation and inactivation of carcinogens through N-acetylation. In a study of HCC patients and healthy individuals, subjects with inactivating mutations of NAT2 (slow acetylators) were found to be at increased risk for HCC development, especially in patients without evidence of HBV or HCV infection (Agundez *et al.*, 1996).

p53

An analysis of the Pro variant allele of the p53 polymorphism at codon 72 in HBV-infected patients with or without HCC revealed no overall increased HCC risk in one study (Yu *et al.*, 1999a). However, there were synergistic effects on HCC development for the p53 Pro allele at codon 72 and chronic liver disease as well as for HCC family history in first-degree relatives. A significantly increased HCC risk in patients with the Pro allele was further observed in smokers with the GSTM1-null phenotype. p53 polymorphism at codon 72 also interacts with CYP1A1 and carotenoid levels in smoking-related hepatocarcinogenesis (Yu *et al.*, 1999a).

Conclusion

There is no clear evidence to date that susceptibility markers have an overall impact on hepatocarcinogenesis. In subgroups of individuals, however, such as smokers, susceptibility markers are emerging indicators for hepatocellular carcinoma risk definition. The establishment of new hepatocellular carcinoma animal models and larger-scale studies in hepatocellular carcinoma patients stratified according to individual characteristics, such as smoking, alcohol ingestion, HBV or HCV infection and others, should allow clearer definition of the role of susceptibility in the management of individuals at risk of hepatocellular carcinoma development.

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