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Molecular pathogenesis of human hepatocellular carcinoma

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Abstract

Primary hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. However, the viral-chemical etiology as well as molecular mechanisms of HCC pathogenesis remains largely unknown. Recent studies in our laboratory have identified several potential factors that may contribute to the pathogenesis of HCC. Oxidative stress and chronic inflammation have been linked to an increased risk of liver cancer. For example, oxyradical overload diseases such as Wilson disease and hemochromatosis result in the generation of oxygen/nitrogen species that can cause mutations in the p53 tumor suppressor gene. The Hepatitis B virus X gene (HBx), a viral transactivator with oncogenic potentials, has been shown to bind to and inactivate p53-mediated apoptosis. HBx mutants derived from HCC have a diminished ability to act as a transactivator. However, they still retain the ability to bind to and abrogate p53-mediated apoptosis. The comparison of gene expression profiles between HBx-expressing primary human hepatocytes and HBV-infected liver samples by cDNA microarrays indicate a unique alteration of a subset of oncogenes and tumor suppressor genes including p53. Our studies implicate both viral and endogenous chemical processes in the etiology of HCC, and p53 may be a common target for the inactivation during liver carcinogenesis.

Keywords: Hepatocellular carcinoma; Hepatitis B virus; HBx; Oxyradical overload diseases; p53; cDNA microarray

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignant diseases worldwide. It is the fifth most common cancer with an estimated 437 000 new cases diagnosed annually (5.4% of all new cancer cases). It ranks fourth in mortality rate, behind lung, stomach and colon cancers (Parkin et al., 1999; Pisani et al., 1999). HCC is a leading cause for cancer-related deaths in adults from Asia and sub-Saharan Africa (Di Bisceglie et al., 1988). The major environmental risk factors identified to be closely associated with HCC are hepatitis B (HBV) and C (HCV) viruses, which account for more than 80% of HCC cases worldwide. Other environmental agents that play a key

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role in HCC development either alone or in synergy with viral infection include aflatoxin B_1 (AFB_1) exposure (particularly in high HCC incidence geographic areas such as China), cigarette smoking and heavy alcohol consumption (Chen et al., 1997). The latter two risk factors may be more important than AFB_1 in Western countries because of the relatively low exposure to AFB_1 in these areas. HCC also is more frequent in certain genetic diseases including hemochromatosis (an iron overload disease) (Powell et al., 1996), Wilson disease (a copper overload disease) (Berman, 1988), porphyria (a decrease in activity of one of the enzymes of heme biosynthesis) (Huang et al., 1999) and α-antitrypsin deficiency (Elzouki and Eriksson, 1996). A schematic model illustrating our current understanding of genetic changes and etiology of HCC is shown in Fig. 1.

2. p53 mutation load in oxyradical overload diseases

Recent studies have linked oxidative stress and chronic inflammation with an increased risk of cancer (Christen et al., 1999). A number of oxyradical overload diseases, such as ulcerative colitis, viral hepatitis, pancreatitis, hemochromatosis and Wilson disease (WD), are associated with an increased risk of cancer. Hemochromatosis and WD are genetic disorders characterized by excess hepatic deposition of iron and copper, respectively. In addition to cirrhosis and liver failure, the risk of HCC is increased by 200-fold in hemochromatosis patients (Niederau et al., 1985). A lower incidence of HCC has been reported in WD (Cheng et al., 1992), possibly due to a reduced lifespan caused by hepatic failure and cardiac complications. Germ-line mutations in the genes responsible for both hemochromatosis and WD have been reported (Bull et al., 1993; Feder et al., 1996). Evidence of oxidative stress and the subsequent generation of reactive aldehydes have been reported in hemochromatosis and WD patients from the iron or copper overload (Britton, 1996). It is hypothesized that oxidative stress and the generation of reactive species can cause mutations in cancer-related genes or alter the function of important proteins regulating DNA repair, cell cycle and apoptosis. Because the frequency of p53

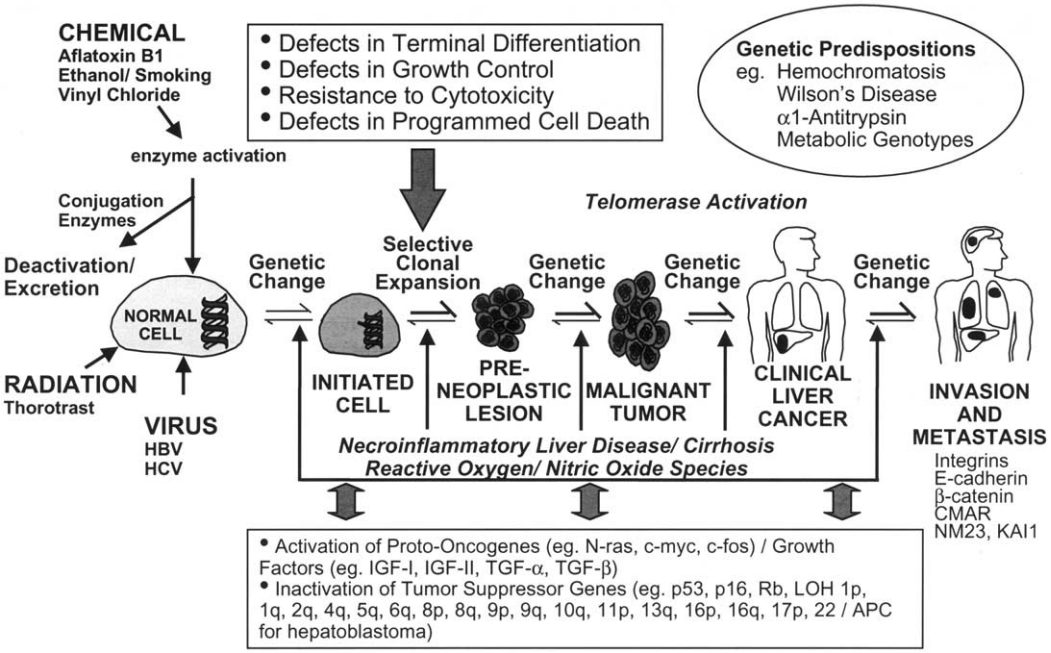

Fig. 1. Multistage hepatocarcinogenesis.

mutated alleles in nontumorous human tissue may be a biomarker of oxyradical damage and identifies individuals at increased cancer risk, we have determined the frequency of p53-mutated alleles in nontumorous liver tissue from hemochromatosis and WD patients by a highly sensitive mutation assay (Hussain et al., 2000). When compared with the liver samples from normal controls, higher frequencies of G:C to T:A transversions at codon 249 ($P < 0.001$), and C:G to A:T transversions and C:G to T:A transitions at codon 250 ($P < 0.001$ and $P < 0.005$) were found in liver tissue from 12 WD cases, and a higher frequency of G:C to T:A transversions at codon 249 ($P < 0.05$) also was found in liver tissue from eight hemochromatosis cases. Sixty percent of the WD and 28% of hemochromatosis cases also showed a higher expression of inducible nitric oxide synthase in the liver, which suggests nitric oxide as a source of increased oxidative stress. A high level of etheno-DNA adducts, formed from oxyradical-induced lipid peroxidation, in liver from WD and hemochromatosis patients has been reported previously. Furthermore, we exposed a normal lymphoblastoid cell line to 4-hydroxynonenal, an unsaturated aldehyde involved in lipid peroxidation, and observed an increase in G to T transversions at p53 codon 249 (AGG to AGT). These results are consistent with the hypothesis that the generation of oxygen/nitrogen species and unsaturated aldehydes from iron and copper overload in hemochromatosis and WD causes mutations in the p53 tumor suppressor gene.

An outcome of viral infection is hepatocellular necrosis, inflammation and liver regeneration, which over many years lead to cirrhosis. The impact of 'viral hepatitis', characterized by liver cell injury or liver edema induced by infiltration of inflammatory cells (Murakami et al., 1998), contributes to the genesis of HCC. An important mediator of HBV is the HBx protein. HBx has been shown to interact directly with and modulate the functions of mediators of the inflammatory process, including interleukin-8 (Mahe et al., 1991), intercellular adhesion molecule 1 (Hu et al., 1992) and the major histocompatibility factor (Zhou et al., 1990).

3. Molecular pathogenesis of viral hepatitis-associated liver cancer

p53 is a tumor suppressor gene whose product is involved in multiple cellular functions. The inactivation of p53, either through mutations or by binding to other viral and cellular oncoproteins, is the most common event in human cancers. We are interested in the physical and functional interactions between p53 and oncogenic HBx. We and others have shown recently that HBx binds to p53 in vivo and in vitro (Feitelson et al., 1993; Wang et al., 1994; Ueda et al., 1995; Wang et al., 1995; Elmore et al., 1997). Functional interactions of p53 and HBx also have been described. For example, HBx can inactivate p53-mediated apoptosis and inhibit nucleotide excision repair in p53-dependent and in-dependent manners (Wang et al., 1994, 1995; Elmore et al., 1997; Jia et al., 1999; Huo et al., 2001).

The integration of the HBx gene is the most frequent event in HCC. However, the integrated HBx often is truncated or contains point mutations. Previous studies indicated that these HBx mutants have a diminished co-transactivational activity. We have compared the effects of wild-type (wt) HBx and its naturally occurring mutants derived from human HCCs on transcriptional co-transactivation, apoptosis and interactive effects with p53 (Huo et al., 2001). We found that overexpression of mutant, but not wt HBx, is defective in transcriptional co-transactivation of the NF-κB-driven luciferase reporter. HBx mutants also have an attenuated pro-apoptotic activity. This deficiency may be attributed to multiple mutations in the co-transactivation domain of HBx, which leads to decreased stability of the translated product. However, wt or mutant HBx bind to p53 in vitro and retain their ability to block p53-mediated apoptosis in vivo. The abrogation of p53-mediated apoptosis by integrated HBx mutants may provide a selective clonal advantage for preneoplastic or neoplastic hepatocytes and contribute to hepatocellular carcinogenesis.

To determine the role(s) of HBx in the early genesis of HCC, we have recently utilized the NCI Oncochip microarrays that contain 2208 human cDNA clones to examine the gene expression

profiles in freshly isolated normal primary adult human hepatocytes ecotopically expressing HBx via an adenoviral system (Wu et al., 2001). The gene expression profiles also were determined in liver samples from HBV-infected chronic active hepatitis patients. Clustering algorithm analysis of the expression profiles indicates that there is a consistent alteration of a subset of oncogenes (such as c-myc and c-myb) and tumor suppressor genes (such as APC, p53, WAF1 and WT1). Our findings are consistent with the hypothesis that the deregulation of cellular genes by oncogenic HBx may be an early event that favors hepatocyte proliferation during liver carcinogenesis.

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