Along with invaluable infection studies in chimpanzees, avian and mammalian HBV-related viruses continue to offer ample opportunities for studies in naturally occurring hosts. In general, most of our progresses in hepatitis B virus research are based on infection studies with two HBV-related animal viruses: the woodchuck HBV (WHV), which infects the Eastern American woodchuck (Marmota monax), and the duck HBV (DHBV), which infects Peking ducks. Both animal models have been essential for understanding various steps of viral life-cycle and factors involved in establishment of virus infection, persistence and hepatocarcinogenesis.

Studies performed over the last ten years with HBV-replicating transgenic mice demonstrated that this small animal model is suitable to evaluate the impact of antiviral treatment strategies on HBV replication and for immunological studies upon induction of cytokines or adoptive transfer of HBV-specific cytotoxic T lymphocytes (CTLs). More recently, mouse models, based on transfection of recombinant adenoviral vector or hydrodynamic injection of naked DNA, have been developed to investigate mechanisms of viral clearance. Compared with transgenic mice, in vivo transfection systems should enable fast comparison of viral mutants for their replication competence. Nevertheless, for various reasons none of the above mentioned models are ideal, since all natural hosts of HBV-related viruses are of out-bred origin and their immune systems have not been characterized.

Genetic alterations and epigenetic changes in hepatocarcinogenesis

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Hepatocarcinogenesis as hepatocellular carcinoma (HCC) is associated with background of chronic liver disease usually in association with cirrhosis, marked hepatic fibrosis, hepatitis B virus (HBV) and/or hepatitis virus (HCV) infection, chronic inflammation, Aflatoxin B1(AFB1) exposure, chronic alcoholism, metabolic disorder of the liver and necroinflamatory liver disease. Hepatocarcinogenesis involve two mechanisms, genetic alterations (with changes in the cell's DNA sequence) and epigenetic changes (without changes in the cell's DNA sequence), but changes in the pattern of gene expression that can persist through one or more generations (somatic sense). Hepatocarcinogenesis is associated with activation of oncogenes and decreased expression of tumor suppressor genes (TSG); include those involved in cell cycle control, apoptosis, DNA repair, immortalization and angiogenesis. AFB1 is metabolized in the liver into a potent carcinogen, aflatoxin 8, 9-epoxide, which is detoxified by epoxide hydrolase (EPHX) and glutathione S-transferase M1 (GSTM1). A failure of detoxification processes can allow to mutagenic metabolite to bind to DNA and inducing P53 mutation. Genetic polymorphism of EPHX and GSTM1 can make individuals more susceptible to AFB1. Epigenetic inactivation of GSTP1 by promoter hypermethylation plays a role in the development of HCC because, it leads that electrophilic metabolite increase DNA damage and mutations. HBV DNA integration into the host chromosomal DNA of hepatocytes has been detected in HBV-related HCC. DNA tumor viruses cause cancer mainly by interfering with cell cycle controls, and activating the cell's replication machinery by blocking the action of key TSG. HBx protein is a potent co-transactivator of viral and cellular promoters such as c-yuck and c-fos. Binding HBx protein to the p53 protein may interrupt p53 induced apoptosis and may inhibit DNA repair during hepatocarcinogenesis. Liver infection may lead to enhanced cell proliferation, in presence of DNA damage from AFB1, result in increased mutations. Genetic alterations and rearrangements are present in the early steps in hepatocarcinogenesis. Genetic alterations, including two different mechanisms relate to chromosomal instability (CIN) and CpG island methylation. Genetic alterations and epigenetic changes in oncogenes and tumor suppressor genes may cause gain of functions or loss of functions respectively. HCC accu-

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mulate chromosome alterations such as chromosomal deletions, DNA rearrangements associated with HBV, DNA integration, aneuploidy, gene amplifications, mutations and microsatellite instability (MSI) as well as epigenetic changes including modulation of DNA methylation. Mutation in p53 at the third base of codon 249 in exon 7, G to T transversion (arginine to serine) linked with AFB1 exposure inactivates p53. The p53 gene may be the most important gene in human hepatocarcinogenesis. Then loss or inactivation of p53, which occurs in most of human cancer, may contribute to the genetic instability and allows genetically damaged and senescent cells to continue to replicate their DNA increasing the damage and it allow them to escape apoptosis. Studies of HCC have been identified in affecting chromosomal regions, (1p, 4q, 5q, 6q, 8p, 10q 11p, 16p, 16q, 17p and 22q). Later in hepatocarcinogenesis (HCC) tumor cells undergo increasing levels of chromosomal aberrations including loss of gene heterozygosity (LOH) of the TSG. Deletions have been reported in 8p, 17p, 4q, 1p, 13q, 16q, 6q, 16p, 1q, and 9p. For chromosome arms17p, 13q, 9p, 6q and 16p, LOH has been related to p53, RB1, p16, IGF2R and Axin1 inactivation. The b-catenin involved in intercellular interactions and signal transduction, this gene is mutated in 20-25% of HCCs at 3p. Cyclin gene has been shown to be amplified in 10-20% of HCC. LOH at the RB1 gene locus and RB1 mutations have been observed in about 15% of HCCs. Epigenetic changes in the expression of cancer- critical genes also play an important role in susceptibility to hepatocarcinogenesis induction. Changes in DNA methylation seems to be the most important mechanism for epigenetic change that could be involved in both the initiation and promotion stages of hepatocarcinogenesis. Methylation is inherited even after DNA replication by maintenance methylation. DNA methylation is often coupled with histone deacetylation and chromatin structure, and regulatory enzymes of DNA methylation (DNMT1). Exposure to environmental carcinogens may induce changes in methylation of the genes involved in hepatocarcinogenesis. Hypomethylation of promoter region leading to over expression of oncogens (c-myc). There is potentially an association between hypomethylation and CIN. Hypermethylation at CpG Island of promoter regions leads to inhibition of the binding of transcription factors directly and/or employment of the binding of protein that act to inhibit the binding of the transcription factors to cis elements. Promoter hypermethylation and loss of protein expression of TSG has been demonstrated in HCC at p16, E-cadherin (essential for adhesion functions) and 14-3-. Hypermethylation in HCC has been reported in p14, p15, SOCS1, RIZ1. However, protein expression was not assessed. Epigenetic inactivation of TSG has been recognized as contributing to tumor progression. Hypermethylation leading to an increased incidence of deamination of 5-methylcytosine to thymine, leading to C to T point mutation in TSG and/or proto-oncogenes. Dietary factors have a role in the modification of epigenetic changes. Altered expression of DNMT mRNA and DNA hypermethylation of TSGs, has been observed in HCC. The mechanism of the interaction between chemical carcinogens and changes of methylation is still unclear and need much more research. Risk factors for hepatocarcinogenesis and their genetic and epigenetic reactions remains poorly understood.

Aflatoxin B1: Mechanism of mutagenesis

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Aflatoxins are a group of toxic and carcinogenic fungal metabolites that frequently contaminate corn, peanuts and other products. Aflatoxin B1 (AFB1), the most potent of these, is metabolized by the cytochrome P450 system into a number of hydroxylated metabolites and glutathione conjugates in the process of conversion to more hydrophilic forms for urinary excretion. Unfortunately, one of these metabolites is the aflatoxin-8,9-epoxide that is produced in two forms, endo and exo. Glutathione S-transferases (GST) are able to conjugate and detoxify this reactive intermedi-

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