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

REGINA M. SANTELLA[1]

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-

1. Professor, Department Environmental Health Sciences, Mailman School of Public Health, Columbia University. USA. rps1@columbia.edu

ate. Species differences in susceptibility to the effects of AFB1 are partially related to differences in expression of specific GSTs that are able to conjugate the epoxide to glutathione. The exo epoxide is able to intercalate into DNA and this is followed by reaction of the C8 position of the epoxide with the N7 position of guanine. NMR studies of oligonucleotide duplexes containing the adduct have demonstrated that the adduct exists with the aromatic portion intercalated on the 5' face of the guanine residue with Watson-Crick base pairing maintained. However, this covalent adduct is chemically unstable due to the charge on the ribose ring. As a result, the guanine can be released from the DNA leaving an apurinic site. This released guanine adduct can be detected in the urine and serves as a biomarker of exposure to AFB1. Alternatively, the ribose ring opens forming a stable formamidopyrimidine (FAPY) adduct. This adduct somewhat stabilizes the DNA duplex. Time course studies in animals have demonstrated that the N7 adduct is rapidly removed, probably because it causes more distortion in the helix, while the FAPY adduct is more persistent. The FAPY adduct has been detected in rat liver weeks after exposure. In addition, oxidative metabolism of AFB1 results in oxidative stress and increased levels of oxidative DNA damage including 8-oxodeoxyguanosine are found in cells treated with AFB1.

All these types of DNA damage are mutagenic primarily resulting in G to T transversions but G to A or G to C mutations are also observed at a lower frequency. Mutations adjacent to the site of adduct formation can also occur probably due to the bulkiness of AFB1 adducts. Studies in animals have demonstrated a linear dose-response between AFB1-DNA adducts and liver tumors. Studies in bacteria have demonstrated that the FAPY adduct is more mutagenic than AFB1-Gua. In bacteria, the FAPY adducts is the strongest block to replication even in the presence of bypass polymerases and thus a prime candidate for the extreme toxicity of AFB. The nucleotide excision repair pathway is the major pathway responsible for removal of bulky AFB1 adducts while base excision repair removes oxidative DNA damage and apurinic sites. Thus, in vivo differences in activities in these repair pathway will impact on HCC risk. AFB1 exposure is also associated with a specific codon 249 AGG (arginine) to AGT (serine) mutation in the p53 tumor suppressor gene that inactivates the protein. This mutation is specifically found in HCC from regions with high AFB1 exposure and not from regions with low AFB1 exposure.

Methylation in hepatocellular carcinoma

REGINA M. SANTELLA[1]

The development of HCC is a complex, multistep, multistage process. The molecular pathogenesis of HCC appears to involve multiple genetic aberrations in the molecular control of hepatocyte proliferation, differentiation and death and the maintenance of genomic integrity. This process is influenced by the cumulative activation and inactivation of oncogenes, tumor suppressor genes and other genes. p53, a tumor suppressor gene, is the most frequently mutated gene in human cancers. There is also a striking sequence specific binding and induction of mutations by AFB1 at codon 249 of p53 in HCC.

Epigenetic alterations are also involved in cancer development and progression. Methylation of promoter CpG islands is associated with inhibition of transcriptional initiation and permanent silencing of downstream genes. It is now known that most important tumor suppressor genes are inactivated, not only by mutations and deletions but also by promoter methylation. Several studies indicated that p16, p15, RASSF1A, MGMT, and GSTP1 promoter hypermethylation are prevalent in HCC. In addition, geographic variation in the methylation status of tumor DNA indicates that environmental factors may influence the frequent and concordant degree of hypermethylation in multiple genes in HCC and that epigenetic-environmental interactions may be involved in hepatocarcinogenesis. We have found significant relationships between promoter methylation and AFB1-DNA adducts

* Professor, Department Environmental Health Sciences, Mailman School of Public Health, Columbia University. USA. rps1@columbia.edu