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

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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

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confirming the impact of environmental exposures on gene methylation.

DNA isolated from serum or plasma of cancer patients frequently contains the same genetic and epigenetic aberrations as DNA isolated from an individual's tumor. The process by which tumor DNA is released into circulating blood is unclear but may result from accelerated necrosis, apoptosis or other processes. p53 mutation and p16 promoter hypermethylation have been detected in paired tumor and plasma of HCC cases. More recently, we investigated promoter hypermethylation in DNA isolated from the serum of HCC patients who provided repeated blood samples prior to diagnosis and controls enrolled in a cancer screen program in Taiwan. Among cases, aberrant methylation was found in serum DNA one to nine years before clinical HCC diagnosis. RASSF1A had the highest frequency of hypermethylation with 70% of cases having at least one positive sample compared to 44% for p16 and 22% for p15. For the controls, promoter hypermethylation was found in 6 and 4% of subjects for RASSF1A and p16, respectively; none had methylation of p15. An ROC curve that included clinical risk factors (age, HBsAg status, anti-HCV status, smoking, alcohol status) and hypermethylation biomarkers gave an overall predictive accuracy of 89% with sensitivity and specificity 84% and 94%, respectively. The analysis of epigenetic changes on RASSF1A, p16 and p15 tumor suppressor genes in serum DNA may be a valuable biomarkers for early detection in populations at high risk of HCC. In addition, the area of global hypomethylation remains largely unexplored in HCC.

TP53 and Beta-catenin mutations in liver tumours

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HBV and HCV play key roles in the etiopathogenesis of Hepatocellular carcinoma (HCC) . Studies mostly based on cases from Western countries suggest distinct genetic pathways of carcinogenesis involving either TP53 or CTTNB1 mutations. Inappropriate reactivation of Wnt pathway due to mutations in CTNNB1 (Beta-Catenin) gene itself is also frequently reported. Mutant Beta-catenin escapes to ubiquitination and down regulation by GSK3-B, it accumulates and trans-activates variety of oncogenes involved in neoplasmic transformation mimicking Wnt pathway activation. Taking into consideration viral infection, chromosome instability and TP53 /Beta-catenin alterations, Laurent-Puig et al. described two distinct HCC profiles in a serie of 137 HCC cases , the first one associates HBV infection with frequent chromosomal alteration and distributes with TP53 mutations, the second would be observed in HBV negative large sized tumors and distributes with Beta-catenin mutations.

We have investigated the status of HBV and HCV infections and of genetic alterations in TP53 and CTTNB1 in 26 patients with HCC from Thailand. In tumours, HBV DNA was found in 19 cases (73%) and HCV RNA in 4 cases (15.4% cases), 3 of whom were co-infected. Among the 19 HBV positive cases, sequencing of S gene showed genotype C in 82% and genotype B in 18%. Furthermore, 5/19 cases were negative for HBsAg and were categorized as occult HBV infections. TP53 mutations were detected in 9 cases (34,6%) including 7 mutations at codon 249 (AGG to AGT, arginine to serine), considered as "fingerprint" of mutagenesis by aflatoxin metabolites. All cases with 249ser mutation had overt HBV infection. CTNNB1 mutations were found in 6/26 cases (23%), 4 of whom also had TP53 mutation. There was no significant association between CTTNB1 mutations and viral infection status. These results suggest that mutagenesis by aflatoxin may have an impact greater than recognized sofar in the etiopathogenesis of HCC in Thailand. Furthermore, TP53 and CTNNB1 mutations do not appear as mutually exclusive, and TP53 249ser mutation is associated with overt HBV infection. Thus, HCC in this context may develop according to a sequence of genetic events that includes both TP53 and CTNNB1 mutations.

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