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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