Exploration of new HCC biomarkers

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Analysis of plasma/serum for levels of viral antigens or antibodies to viral proteins has been used extensively as an early biomarker of potential risk of HCC. In addition, detection of elevated levels of alpha-fetoprotein is commonly used for early identification of HCC. Unfortunately, both of these approaches are not highly sensitive or specific. As a result, there is continuing investigation to identify additional biomarkers that may help in the early identification of cases. The use of DNA isolated from plasma or serum for detection of gene specific methylation has been discussed previously. In addition, tumor DNA isolated from blood has been analyzed for the presence of p53 mutations and found in a subset of cases to be present years prior to diagnosis as for methylated DNA. The general level of DNA present in blood has also been suggested as a potential biomarker of cancer.

Among the newer methods being tested are the detection of specific mutations in HBV. In many cases of HCC in China and Africa a double mutation, an A to T transversion at nucleotide 1762 and a G to A transition at nucleotide 1764 (1762T/1764A) have been found. These mutations have been associated with increased severity of HBV infection and cirrhosis suggesting that they might be a useful biomarker for high risk subjects.

The field of proteomics also holds promise for the development of new biomarkers. A number of groups are developing mass spectrometry methods for the identification of serum/plasma proteomic patterns that will distinguish bloods of HCC cases from those of controls. While some interesting preliminary data have been developed for several cancers, much additional work needs to be done in this area. Another approach is the use of platforms that contain an array of large numbers of proteins that can be used to screen plasma/serum samples for the presence of antibodies to specific proteins as a diagnostic marker of disease. Early studies detected antibodies to p53 protein in the blood of about 25% of cases with bladder, lung, colon and oral cancer but in <2% of control studies. The hope is that assaying for multiple antibodies will enhance the sensitivity and specificity of this approach.

Another approach being used is gene expression studies to identify new molecular markers for HCC. Microarray techniques can determine differences in the expression profiles of HCC cell lines compared to control lines and in HCC tissues compared to control tissues. The identification of candidate genes that are overexpressed in HCC could lead to the establishment of serum assays for the corresponding proteins in blood samples.

The IARC TP53 mutation database: a resource for studying the significance of TP53 mutations in human cancers

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The tumor suppressor gene TP53 is frequently inactivated by gene mutations in many types of human sporadic cancers, and inherited TP53 mutations predispose to a wide spectrum of early-onset tumors (Li-Fraumeni et Li-Fraumeni-like Syndromes). All TP53 gene variations (somatic and germline mutations, as well as polymorphisms) that are reported in the scientific literature or in SNP databases are compiled in the IARC TP53 Database. This database provides structured data and analysis tools to study mutation patterns in human cancers and cell-lines and to investigate the clinical impact of mutations. It contains annotations related to the clinical and pathological characteristics of tumors, as well as the demographics and carcinogen exposure of patients. The IARC TP53 web site (http:/

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/www-p53.iarc.fr/) provides a search interface for the core database and includes a comprehensive user guide, a slide-show on TP53 mutations in human cancer, protocols and references for sequencing TP53 gene, and links to relevant publications and bioinformatics databases. The database interface allows download of entire data sets and propose various tools for the selection, analysis and downloads of specific sets of data according to user's query.

Recently, new annotations on the functional properties of mutant p53 proteins have been integrated in this database. Indeed, the most frequent TP53 alterations observed in cancers (75%) are missense mutations that result in the production of a mutant protein that differ from the wild-type by one single amino-acid. The characterization of the biological activities of these mutant proteins is thus very important. Over the last ten years, a great amount of systematic data has been generated from experimental assays performed in yeast and human cells to measure the impact of these mutations on various protein properties: (1) trans-activation activities (TA) of mutant proteins on reporter genes placed under the control of various p53 response-elements, (2) capacity of mutant proteins to induce cell-cycle arrest or apoptosis, (3) ability to exert dominant-negative effect (DNE) over the wild-type protein, (4) activities of mutant proteins that are independent and unrelated to the wild-type protein (gain of function, GOF). Prediction models based on interspecies protein sequence conservation have also been developed to predict the functional impact of all possible single amino-acid substitutions. These data have been used to produce systematic functional classifications of mutant proteins and these classifications have been integrated in the IARC TP53 database. New tools have been implemented to visualize these data and analyze mutation frequencies in relation to their functional impact and intrinsic nucleotide substitution rates. Thus, the database allows systematic analyses of the factors that shape the patterns and influence the phenotype of missense mutations in human cancers.

In a recent analysis of the database, we showed that that loss of TA capacity is a key factor for the selection of missense mutations, and that difference in mutation frequencies is closely related to nucleotide substitution rates along TP53 coding sequence. TA capacity of inherited missense mutations was also found to be related the age at onset of specific tumor types, mutations with total loss of TA being associated with earlier cancer onset cancers compared to mutations that retain partial trans-activation capacity. Furthermore, 80% of the most common mutants show a capacity to exert dominant-negative effect (DNE) over wild-type p53, compared to only 45% of the less frequent mutants studied, suggesting that DNE may play a role in shaping mutation patterns.

Hotspot mutations have been linked to exposure to environmental factors in several cancers: tobacco smoke in lung cancer, tobacco smoke and alcohol in head and neck cancers, aromatic-amines in bladder cancer, aflatoxine-B1 and HBV in liver cancer, and UV in skin cancer. In lung cancers, four specific mutants are observed at high frequencies, V157F, R158L, R248L and R273L. These hotspots are due to G>T transversions that have been shown to be caused by the presence of benzo(a)-pyrene diol epoxide (BPDE) adducts on guanines at these codons. BPDE is the main metabolite of benzo(a)-pyrene, one of the most potent carcinogens present in high quantity in tobacco smoke. In hepatocellular carcinomas from less developed regions, one specific mutant is observed at a high frequency, R249S. Although the precise mechanism remain unknown, its presence is attributed to an interaction between HBV infection and mutagenesis by the food contaminant carcinogen aflatoxine-B1. In these examples, all hotspot mutants are defective for TA. These observations show how mutagenesis by environmental carcinogens and selection of loss of trans-activation can shape TP53 mutation patterns.

In conclusion, the integration of standardized annotations on the functional impact of missense mutations in the IARC TP53 database provides a powerful framework for the analysis of "functional" patterns of mutations in cancers, the detection of genotype/phenotype associations and provide new insights into the factors that shape mutation patterns and influence mutation phenotype, which may have clinical interest.

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