Gene profiling of liver deseases

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Motivation

There is an increasing interest in complementing conventional histopathological evaluation with molecular tools in an attempt to increase the sensitivity and specificity of cancer staging for diagnostic and prognostic purposes. Oligo-array methodology enables investigators to study also expression profile and activation of thousands of genes simultaneously. In particular, the identification of cancer-related specific expression patterns might allow the elucidation of molecular mechanisms underlying cancer progression. To gain insight into the molecular mechanisms of hepatocarcinogenesis and to identify potential HCC markers, we performed a microarray analysis on 80 surgical liver sample biopsies from HCV-correlated HCC, HCV-positive liver tissue and GI-metastatic liver tissues obtained from patients enrolled at the INT in Naples. RNA from individual tumors was compared with RNA isolated from adjacent non-tumor tissue and with RNA from normal liver tissue. Expression of 36.000 oligos representing 90% of human genome was analyzed using the advanced analysis tools of the BRB Array tools, at the Immunogenetics Section Departement of Trasfusion Medicine, Clinical Center , National Institutes of Health Bethesda, MD, on home-made oligo-chips.

Methods

Hybridized arrays were scanned at 10-µm resolution on a GenePix 4000 scanner (Axon Instruments) at variable PMT voltage to obtain maximal signal intensities with less than 1% probe saturation. Resulting jpeg and data files were deposited at microarray data base (mAdb) http://nciarray.nci.nih.gov, whose evaluations numbers are retrieved for further median centering, filtering of intensity (>200) and spot elimination (bad and no signal). For Unsupervised Analysis gene selection is obtained by low-stringency filtering (80% gene presence across all experiments and at least one experiment with ratio fold change >3) and the selected genes are further analyzed. Hierarchical cluster analysis was conducted on these genes according to Eisen. Differential expressed genes were visualized by Treeview. Supervised class comparison is performed utilizing the BRB ArrayTool developed at NCI, Biometric Research Branch, Division of Cancer Treatment and Diagnosis. Gene clusters, identified by the univariate t-test, were challenged with two alternative additional tests: the univariate permutation test (PT) or the global multivariate PT. The multivariate PT was calibrated to restrict the false discovery rate to 10%. The pathway analysis was done using the gene set expression comparison kit implemented in BRB-Array-Tools. The list of upregulated genes was further analyzed by “Igenuity System Database” to identify their human pathways. The t-test significance threshold was set at 0.001.

Results

Consistent differences were found between the expression patterns of HCC and those seen in non-tumor liver tissues. A number of specific cancer-related molecular biomarkers as well as modified cellular networks and signaling pathways were identified in HCC tissues. Samples from HCV-related HCC showed strong up-regulation of genes involved in specific metabolic pathways, such as Aryl Hydrocarbon receptor signaling, 14-3-3 mediated signaling and protein Ubiquitination pathway. Samples from HCV-related cirrhosis showed strong up-regulation of genes involved in Antigen Presentation, Protein Ubiquitination, Interferon signaling, IL-4 signaling, Bacteria and Viruses life cycle and chemokine signaling pathways. A time course analysis was performed to find a marker of tumoral progression between healthy donors (CTR), HCV related HCC and Cirrhosis related HCV liver samples. CTR represented the early time point, the cirrhosis the intermediate point and the HCC the final time point. Each point set represented the average of all samples (>6) belonging to the specific group. This time course analysis identified 49 genes differentially expressed during the natural history of the liver progression to cancer. In conclusion, Microarray approach allows quantitative and simultaneous analysis of gene expression of a large amount of genes and the systematic studies of expression patterns are extremely useful to identify molecular events and key pathways involved in cellular functions induced by specific etiopathogenetic events. In this study, informative data on the global

pattern of gene of HCV-related HCC have been obtained. They may be extremely helpful for the identification of exclusive activation markers to characterize gene expression programs associated with HCC progression. Thus, such genomic approach can represent a step toward a better understanding of the molecular pathophysiology as well as an improved methodology for detection, diagnosis, and classification of HCC.

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