



six candidate genes by real-time polymerase chain reaction quantitation analysis of four GBM samples. Investigation of these genes should help provide important insights into the biological mechanisms at work, facilitate identification of tumors that are susceptible or resistant to radiation therapy and aid in the design of approaches to enhance specifically the radiosensitivity of these deadly neoplasms.

Grandori, Carla

[1]

## Gene expression profiles at various stages of lymphomagenesis in *Em-myc* transgenic mice

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To define molecular signatures of *c-myc*-induced B-cell neoplasias we have explored, using DNA microarrays, gene expression profiles at various stages of tumorigenesis utilizing the transgenic *Em-myc* model<sup>1</sup>. In this system *c-myc* is expressed, under the control of the immunoglobulin heavy-chain enhancer, selectively in B-cell precursors, and it causes the rapid appearance of B-cell lymphomas. Overt tumors are preceded by a marked polyclonal expansion of pre-B cells both in the bone marrow and in the spleen. However, these cells are not yet tumorigenic. Within a few months the *Em-myc* mice develop a monoclonal or oligoclonal lymphoma. We monitored gene expression changes using an 11,000-gene chip (Affymetrix) of pre-B cells derived from the bone marrow of *Em-myc* mice before tumor development and cells from lymphomas, consisting primarily of pre-B cells. Both samples were then compared with normal pre-B lymphocytes obtained from bone marrow of wild-type mice. The data analysis allowed a display of gene expression changes that accompany these various stages of tumor development. Preliminary results indicate that specific signaling pathways are selectively altered in the tumor samples or in the hyperplastic pre-B cells. In addition, comparison of the pre-B cells expressing *Em-myc* with wild-type pre-B cells highlighted gene expression changes that might be direct consequences of deregulated *c-myc* expression and therefore provided new candidate *Myc* target genes. We will present a discussion of the relevance of our study for the understanding of *c-myc*-induced tumorigenesis.

1. Adams, J.M. *et al. Nature* **318**, 533–538 (1985).

Graveel, Carrie

[2]

## Analysis of gene expression alterations in mouse and human hepatocellular carcinomas

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Liver cancer is the fifth most common cancer worldwide, with 437,000 cases reported in 1990. Yet a mutational model has not yet been developed for liver cancer, as it has for certain other cancers, such as colon cancer. A thorough understanding of the molecular events leading to neoplastic transformation of the liver requires a detailed comparison of the gene expression pattern in normal liver cells with that in cancer cells. We have performed gene expression profiling of normal and neoplastic livers. Using oligonucleotide microarrays, we compared liver tumors (from diethylnitrosamine-treated C3H/He mice) with three different states of the normal liver: quiescent adult, regenerating adult and newborn.

Although each comparison revealed hundreds of differentially expressed genes, only 22 genes were found to be deregulated in the tumors in all three comparisons. We also employed representational difference analysis to clone fragments of messenger RNAs differentially expressed in liver tumors versus regenerating livers. Although many of the same mRNAs were identified as in the oligonucleotide microarray experiments, we also cloned several new mRNAs that are differentially regulated in liver tumors. We have cloned the mouse complementary DNA of *novel 4* and are currently isolating the human homologue of this unknown gene. We are using representational difference analysis and oligonucleotide microarrays to identify genes whose expression is deregulated in the development of human hepatocellular carcinomas. Using these models and techniques, we hope to identify common genetic alterations in the progression of liver cancer in both humans and mice.

Gregg, Jeff

[3]

## Molecular profiling of a mouse model for metastatic breast carcinoma

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Breast cancer is among the most common human cancers. One of the significant predictors of prognosis is distant metastasis. As cancer is the consequence of a broad dysregulation of cell signaling pathways, the ability of cells to metastasize may be due to changes in a limited number of pathways related to invasiveness and metastasis. Our study focuses on gaining a better understanding of these pathways using a mouse model for mammary tumor metastasis. We are working with two transplantable mouse mammary tumor lines with significant differences in metastatic potential. Met-1 tumors develop 100% pulmonary metastases while Db-7 tumors exhibit significantly fewer pulmonary metastases (9%). In order to extract genes differentially expressed in both Met-1 and Db-7, we performed two suppression subtractive hybridization (SSH) experiments. From each of the subtracted libraries, 2600 clones were PCR-amplified and arrayed. By fluorescently labeling the unsorted Met-1 and Db-7 libraries and hybridizing them onto the microarray, we were able to demarcate the truly differentially expressed genes. Clones found to be greater than two-fold differentially expressed were then sequenced for identification. Differential expression of each gene was verified by RT-PCR, Northern blot, and Western blot. In order to determine the function of a subset of these genes, anti-sense and sense constructs were introduced into cell lines derived from these tumors. Migration assays were performed and the metastatic potential of each gene was assessed. We have identified several interesting genes that are both differentially and functionally important for the metastatic phenotype in this model.

Hager, Jeff

[4]

## Genomics of islet cell carcinogenesis

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All cancers are thought to develop through a series of distinct stages, which are the result of an accumulation of both genetic and epigenetic changes. This is evident in a mouse model of pancreatic islet cell tumorigenesis, RIPTag, in which the focal nature of the endocrine pancreas has allowed accurate identification, quantifica-