Abstracts: Session II

tion and physical isolation of such stages. All islets express the oncoprotein T antigen, but with decreasing frequency in hyperplastic islets (50–70%), angiogenic islets (10%) and end-stage tumors (2–4%). A long-term goal of our studies is to identify the genetic and epigenetic alterations that are required at each of these steps en route to tumor formation. Two genetic alterations have been identified in this model in a genome-wide screen for regions of loss of heterozygosity (LOH). One resides on chromosome 16 (*LOH16*) and is lost in the transition between hyperplastic islet and angiogenic islet. The other is on chromosome 9 (*LOH9*) and is lost as angiogenic islets develop into tumors. To identify the tumor suppressor genes contained within *LOH9* and *LOH16*, we are mapping the fine structure of the two regions by analysis of simple sequence length polymorphisms and comparative genomic hybridization with BAC-based arrays. We have developed and validated the use of murine BACs spotted on glass slides as a sensitive platform for comparative genome hybridization, with which we can readily detect loss of *LOH9* and *LOH16* in islet tumors. To identify genes whose altered expression may be critical to tumor development we have also profiled expression of approximately 22,000 genes and expressed sequence tags on microarrays using messenger RNAs derived from distinct RIPTag tumor stages. We will present patterns of gene expression and potential candidates for functional studies.

He, Huiling

[5]

Gene expression analysis of glucocorticoid-induced apoptosis by a gene microarray technique

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Glucocorticosteroids induce apoptosis in immature lymphocytes and are therefore commonly used to treat patients who have lymphoid leukemia or lymphoma. To understand the genetic pathways and molecular mechanisms of glucocorticoid-induced apoptosis, we used the gene microarray technique to analyze gene expression in a well-established mouse lymphoma cell line, S49.1. In initial experiments S49.1 cells were treated with the glucocorticoid hormone, dexamethasone or vehicle for 12 h, and total RNA was extracted. Biotinylated antisense RNAs were hybridized to Affymetrix mouse U74 sub A chips, which contain about 12,000 known genes and expressed sequence tags. Following dexamethasone treatment, 69 genes had variances greater than 2-fold, and 40 genes had variances greater than 2.5-fold. Among these 40 genes, 30 were induced and 10 were repressed in the dexamethasone-treated cells. Included among the known genes with altered expression were members of the immunoglobulin superfamily, transcription factors and receptors coupled to G protein. Among the expressed sequence tags with altered expression were tags with a high degree of homology to potassium channel proteins and β -catenins or other new genes. Earlier time points of the gene expression patterns will be analyzed by the same approach in anticipation of finding other genes with potential involvement in the apoptosis pathway induced by glucocorticoids.

Hedenfalk, Ingrid

[6]

Classification of hereditary breast cancers based on gene expression

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A large number of hereditary breast cancers are due to mutations in the two breast cancer susceptibility genes, *BRCA1* and *BRCA2*. However, many hereditary breast cancer cases cannot be accounted for by mutations in these genes, and these are believed to be due to as yet unidentified breast cancer predisposition genes (*BRCAx*). Using complementary DNA microarrays to classify hereditary breast cancers, we show that *BRCA1* and *BRCA2* tumors display distinct gene expression profiles and can be classified on the basis of gene expression patterns. We are analyzing several hereditary breast cancers that have tested negative for mutations in *BRCA1* and *BRCA2*. We tested primary tumors against a microarray of 6,512 genes and employed mathematical analyses to determine a set of genes capable of discriminating mutation-positive breast cancers. Using permutation analysis of multivariate classification functions, we established that the expression profiles of *BRCA1* and *BRCA2* tumors were significantly different statistically. With analysis of variance between gene expression levels and genotype we ascertained those genes capable of separating *BRCA1* from *BRCA2* breast tumors. Examination of this gene list suggested functional differences between *BRCA1* and *BRCA2* tumors, including differences in p53 regulation and homeostasis, and (for *BRCA1*) the possible establishment of a constitutive stress-type state. We extended our gene expression results to encompass pathological findings by generating a tissue microarray of approximately 200 breast tumor specimens. We are currently determining if *BRCAx* breast tumors can be classified and subjected to linkage analysis to recognize new breast cancer predisposition loci. Gene expression analysis has for the first time revealed a consistent and significant gene expression difference between *BRCA1* and *BRCA2* cancers, a finding with therapeutic implications.

Hegi, Monika

[7]

Molecular pathways in human glioblastoma assessed by gene expression profiling

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Glioblastoma is the most malignant form of astrocytic brain tumor; patients have a mean survival time of one year after diagnosis. Extensive genetic analyses have recognized few genetic alterations and have not yielded any predictive factors. A more comprehensive approach includes analysis of differential gene expression, reflecting regulatory differences. We seek to identify differentially expressed genes relevant for tumor development or possessing predictive value, in order to gain insights into the molecular mechanisms of tumor progression and uncover new therapeutic targets. Using complementary DNA macroarrays (Clontech) we have constructed gene expression profiles in glioblastoma biopsies from a homogenous group of patients enrolled in a pilot clinical trial (concomitant and adjuvant temozolomide and irradiation for newly diagnosed glioblastoma). We subjected the expression profiles to cluster analysis to shed light on the mechanistic aspects of