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

Huiling He, Michael Malone, Steven Ostrowski & Clark Distelhorst

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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 microarrays (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

tumor development in association with data on gene alterations, such as p53 mutations and deletion of the *p16/p14ARF* locus, which are hallmarks of previously defined genetic pathways. Our preliminary results with eight tumors confirm upregulation of some genes already known to be important in the development of glioblastoma and the exclusivity of some genetic pathways (p53 mutations versus *EGFR* overexpression). We have created expression profiles of 120 differentially expressed genes using the Cluster program from Stanford University, and we have grouped tumors according to genetic pathways. We will link patterns of differential gene expression to clinical outcomes to identify subtypes of tumors with distinct clinical behavior.

Helou, Khalil

[8]

The *Hgfr/Met* oncogene is the target for gene amplification in DMBA-induced rat sarcomas

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Analysis of chromosome rearrangements in tumors is an important means for revealing genetic pathways underlying tumorigenesis and tumor progression. In a set of 17 rat sarcomas induced by exposure to 7,12-dimethylbenz[*a*]anthracene (DMBA), we had previously found homogeneously staining regions (which are generally accepted as cytogenetic signs of gene amplification) in 5 tumors, using cytogenetic analysis. By employing comparative genomic hybridization, we detected regional increases in DNA copy number of the proximal part of rat chromosome 4 (RNO4) in the tumors harboring homogeneously staining regions. We detected amplification of the *Hgfr/Met* oncogene, located at RNO4q21.2, by fluorescence *in situ* hybridization (FISH) in all five tumors. In four of them, several flanking genes located in the near vicinity of *Hgfr/Met*, including *Cav1* (q21.1), *Wnt2* (q21.2;q21.3) and *Cftr* (q21.3), were also amplified, although amplification was seen in a smaller fraction of the cells than *Hgfr/Met* amplification. In the fifth tumor (BL150T), *Hgfr/Met* was amplified in all cells and was the sole amplified gene of those tested. In addition the *Hgfr/Met* FISH signals in BL150T were tightly clustered and formed compact and intense spots compared with the signals seen in the other four tumors. Application of the free chromatin FISH technique to BL150T showed that the genomic *Hgfr/Met* probe stained the extended chromatin fibers of up to 1.5 megabases with an almost uninterrupted signal, indicating that the BL150T amplicon was build up solely from *Hgfr/Met* gene sequences. Our results indicate that the *Hgfr/Met* oncogene may be the primary target for amplification in a subset of rat DMBA sarcomas.

Hermann, Thomas

[9]

Effects of the RXR-specific ligand Targretin on gene expression in carcinogen-induced mammary tumors in the rat

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Targretin (LGD1069), a high-affinity ligand for the retinoid X receptors (RXRa, RXRb and RXRg), is highly effective in the prevention and treatment of carcinogen-induced breast cancer in various rat models. To understand the molecular basis of

the tumor response to Targretin, we used microarray analysis to compare the gene expression profiles of tumors regressing in response to Targretin with those of either vehicle-treated tumors or tumors resisting Targretin treatment. The vehicle-treated and Targretin-resistant tumors had very similar profiles, but the tumors responding to Targretin exhibited broad changes in gene expression. These included genes involved in proliferation, markers of differentiation, tumor markers and expressed sequence tags of unknown function. We selected a subset of these genes for further evaluation. Quantitative analysis by real-time polymerase chain reaction confirmed the differential expression for the majority of the selected genes. However, a small fraction was identified as false positives. We evaluated a subset of the confirmed genes, as well as additional genes with related biological activities, by immunohistochemistry and laser capture microdissection of tumor sections, and their regulation in better-defined *in vitro* systems is now under investigation.

Herrmann, John

[10]

A genomics approach to therapeutic antibody target discovery

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Functional genomics is expected to bring about significant advances in the discovery and development of new anticancer agents. Genomics-based drug target discovery will revolutionize how cancer is detected and classified, resulting in more finely tailored therapies. The explosion of information generated by large-scale functional genomics technologies has led to an exponential increase in the number of potential genes and proteins available for pharmaceutical and diagnostic research development. In tapping this potential, the primary challenge is to develop a strategy to integrate and extract meaning from the human genomic sequence information generated since the start of the Human Genome Project, relying on pragmatic strategies to sort and triage this information. Using a suite of integrated, high-throughput genomics technologies developed at CuraGen, we have devised streamlined approaches to identify new gene sequences rapidly, link them to specific diseases using reverse biology and finally validate them as effectors of disease progression. Application of these technologies has enabled us to identify 24 antibody drug targets for further evaluation and possible development as anticancer agents. We will discuss the role of some of the targets in cancer progression.

Hester, Susan

[11]

Studies of normal gene expression in the rat nasal epithelium using cDNA array technology

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The nasal epithelium is an important target site for chemically induced toxicity and carcinogenicity. Gene expression data are being used increasingly in studies of such conditions. In order to provide normal baseline data for this target organ, we investigated gene expression profiles in nasal transitional and respiratory epitheli-