Abstracts: Session II

Norman, Sylvia

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Analysis of *TP53* mutations in human malignant gliomasSylvia Norman¹, Adrienne Scheck² & Jeff Braman¹¹Stratagene, La Jolla, California, USA²Barrow Neurological Institute, Phoenix, Arizona, USA

TP53 is involved in the regulation of the cell cycle, response to DNA damage and induction of apoptosis. Mutations in this gene are thought to contribute to the development, progression or both of a variety of tumors, including human malignant glioma. Molecular analyses of primary malignant gliomas have demonstrated cellular heterogeneity in gene loss, rearrangement, amplification, transcription and translation. Furthermore, the rapid recurrence of this tumor demonstrates the presence of cells that are intrinsically resistant to therapy. Whether mutations in *TP53* contribute to therapy resistance and tumor recurrence in malignant gliomas is not known. To determine if *TP53* mutations are indicative of cells with a selective advantage that contributes to the survival and/or recurrence of glial tumors, we undertook a study to compare matched cells from primary and recurrent tumors from the same patients. We will present results from our analysis of *TP53* mutations present in cells derived from both primary and recurrent glial tumors using polymerase chain reaction and sequence analysis to screen for mutations in exons 5–8 of *TP53*.

Novoradovskaya, Natalia

[65]

Using universal human reference RNA in microarray gene expression studiesNatalia Novoradovskaya¹, Nicki Chin², Terry Payette¹, Alexander Pergamenschikov², Michael Fero², David Botstein² & Jeff Braman¹¹Stratagene, La Jolla, California, USA²Stanford University, Stanford, California, USA

The expression profiles of thousands of genes can be analyzed simultaneously by hybridizing labeled complementary DNA targets to microarrays. Two-color hybridization allows comparison of two samples labeled with different fluorescent dyes on the same microarray. Normalized signals are expressed as the ratio of signals from Cy5 experimental and Cy3 reference probes. Use of a common reference sample across multiple experiments provides reliable data comparison. A reference sample should provide a hybridization signal at as many probe elements as possible. We chose 21 human cell lines, representing different tissues, to approximate the expression profile of all human genes. The expression profile of each individual cell line and that of a pool of RNAs from 21 cell lines were compared. Hybridization was performed using 24K probe element human microarrays (Stanford University) and GeneConnection Discovery microarrays (Stratagene). Signal distribution on these microarrays demonstrates that reference cDNA hybridizes to a larger number of tethered probes than cDNA from any single cell line. Ten different cell lines were chosen for Universal Human Reference RNA, providing optimal coverage on human microarrays; these can be used as a reference sample for any microarray experiment.

Nuchtern, Jed

[66]

cDNA microarray analysis of differential gene expression in *MYCN*-amplified neuroblastoma tumor cellsJed Nuchtern¹, Jason Shohet¹, Susan Stuart², M. John Hicks¹ & Susan Burlingame¹¹Baylor College of Medicine, Houston, Texas, USA²Incyte Genomics, Palo Alto, California, USA

Neuroblastoma is a common pediatric solid tumor that is frequently incurable. Biological risk stratification implicates amplification of the *MYCN* oncogene as a robust marker for poor prognosis. We have undertaken an analysis of gene expression in *MYCN*-amplified and non-amplified neuroblastoma tumors based on complementary DNA microarrays to advance our knowledge of the pathogenesis of this aggressive disease. We isolated messenger RNA from tumor cell primary cultures of two INSS stage 4 neuroblastoma patients, one with *MYCN* amplification and the other with normal *MYCN* copy number. Relative gene expression was determined after hybridization of the fluorophore-labeled cDNAs to a human cDNA microarray representing over 6,800 unique expressed genes. Using a differential gene expression ratio of 3.5 as a screening criterion, we identified 370 unique genes for further analysis. Of these, 78 were upregulated in the *MYCN*-amplified neuroblastoma and 292 had decreased expression. Amplification correlated with increased transcript levels of genes associated with cell proliferation, including cell cycle regulatory genes such as *CDC2* and *CDC28* protein kinase, and the Ki-67 antigen, as well as many other annotated molecules not previously linked to *MYCN* amplification and several uncharacterized expressed sequence tag sequences. A coherent group of genes was downregulated in the *MYCN*-amplified cells, including the genes for the class I and class II major histocompatibility complex antigens, the gene for peptide antigen transporter TAP1, and several interferon responsive transcripts. *MYCN* amplification correlates with a distinct gene expression pattern in neuroblastoma that is consistent with the clinical phenotype of these tumors. Further characterization of this motif should enable the development of new therapies for these difficult tumors.

O'Connor, Maureen

[67]

Identification of genes that are differentially expressed in response to EGF receptor signaling in A549 lung cancer cells using a combination of suppression subtractive hybridization and microarrays

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Since current treatments for almost all patients with non-small-cell lung cancer are not satisfactory, and since prognosis is adversely affected by the overexpression of EGF receptors, we are attempting to identify new genes whose expression is modulated by the EGF signaling pathway in A549 lung carcinoma cells. The cells were treated with either EGF or C225 (a neutralizing antibody that blocks the EGF receptor). We have shown that these treatments affect the epithelial-to-mesenchymal transdifferentiation of these cells. We prepared complementary DNA libraries from EGF-treated, C225Ab-treated or control cells and subjected them to suppression subtractive hybridization to identify both up- and downregulated genes. These enriched cDNAs were fluorescently labeled and used to screen 9,600 human

genes on a microarray. Thirty genes were upregulated and eighty downregulated by fourfold or more over background. To confirm subtraction efficiency, we picked approximately 700 clones from the C225-upregulated subtraction library; spotted their inserts, amplified by the polymerase chain reaction, on glass slides and hybridized them with the subtracted cDNAs. The majority of the clones exhibit high intensity when hybridized with the upregulated cDNAs and low intensity when hybridized with the downregulated cDNAs. The differential expression of these clones is now being confirmed by quantitative polymerase chain reaction with reverse transcription or northern blot analysis, and their function will be evaluated using cell differentiation and motility assays.

Onay, U. Venus

[68]

Identifying the role of single-nucleotide polymorphisms in breast cancer risk using microarray and mass spectrometry technologies

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A considerable proportion of the familial aggregation of breast cancer is probably due to alleles of low penetrance. Although their contribution to risk is relatively small, they can contribute to a large proportion of breast cancer cases in the population, because the risk-conferring alleles of these genes are common. The candidate gene approach is one of the most logical and practical strategies to identify these risk-enhancing, low-penetrant variants. A major obstacle in investigating the risk associated with multiple candidate genes has been the lack of large-scale genotyping technologies. Recently developed microarray and matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry technologies are ideally suited to the problem of high-throughput genotyping. We are relying on the power of these techniques to carry out a population-based breast cancer case-control study for 32 single-nucleotide polymorphisms (SNPs). We selected these SNPs from genes of crucial pathways that are frequently perturbed in cancers, such as those governing the cell cycle, carcinogen metabolism and the immune response pathways. We are currently developing both microarray and MALDI-TOF mass spectrometry techniques so that both can use the primer extension method to detect the allelic variants of SNPs. Both techniques are used in combination on a panel of cases involving several SNPs, to assess specificity and reproducibility. The combination of both methods will be a powerful tool for genotyping a large number of SNPs in a large number of cases. Advances in our knowledge of disease etiology will significantly expand our abilities to design strategies for the prevention of breast cancer development and the inhibition of its progression.

Orr, Michael

[69]

Discovery of 830 candidate therapeutic targets and diagnostic markers for breast cancer using oligonucleotide microarray technology

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To identify differentially expressed genes in infiltrating ductal carcinoma (IDC) of the breast, we measured the gene expression profiles of 15 IDC and 13 normal human breast tissues, using the Affymetrix GeneChip array platform for simultaneous analysis of over 60,000 genes. Fold-change comparison between normal and IDC breast tissue samples revealed 830 genes that were statistically over- or under-expressed by threefold or greater in the IDC samples. We identified 286 overexpressed genes and 544 underexpressed genes. Furthermore, the 830 genes were evaluated for tissue-specific expression by E-northern analysis of 28 different normal tissues, revealing tissue-specific candidate targets. We performed further analysis utilizing principal component analysis or hierarchical clustering with 5,467 prefiltered genes to determine if gene expression profiles could be used to distinguish between IDC and normal breast tissue samples. Both PCA and HCA indicated two distinct populations for the normal and tumor-derived samples, except for two samples, based on gene expression patterns. One aberrant tumor sample was explained by its histology, which showed only partial (<20%) involvement by malignant cells. We also employed cluster analysis to evaluate differences in samples at the single-gene level. Examination of a particular cluster of overexpressed genes displayed a multitude of differentially expressed sequence tags along with genes for topoisomerase II, cyclin B, CDC2, KI-67, and thymidine kinase, suggesting some functional similarities. Our studies provide a rational basis for additional analysis of differentially expressed genes in breast cancer, which may lead to the identification of therapeutic targets and diagnostic markers.

Parodi, Silvio

[70]

Innovative leads for antineoplastic drugs suggested by a more intimate familiarity with structural motifs of oncoproteins

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Proteins, although structurally unsuitable for their own replication, are the structures that translate an abstract DNA sequence into real action, making possible catalysis, signaling and supramolecular morphologies. Protein targets have most often been recognized by starting from antineoplastic effects initially observed at the level of an organism or a cell and working in a reductionist manner. However, no oncoproteins have been identified as cancer chemotherapy targets through this