Ringner, Markus

[16]

A layered perceptron approach to categorizing cDNA microarray measurements

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We present a supervised regression model for classifying complementary DNA microarray measurements and a method to rank genes according to their importance for the classification. To allow for a supervised regression model with no overfitting, we reduce the dimensionality of the measured samples (as given by number of genes) by principal component analysis and use the ten dominant components as inputs to multilayered perceptron models. We classify samples using a threefold validation procedure, and the procedure is repeated such that numerous multilayered perceptron models are calibrated. Thus each sample is in many validation sets and all these predictions are used as a committee to classify a sample. The sensitivity of the classification on the different genes is determined by the absolute value of the partial derivative of the output with respect to the gene expressions, averaged over samples and multilayered perceptron models. In this way the genes can be ranked. The method is successfully applied to classify solid tumors in the category of small blue round-cell tumors as well as sporadic breast tumors. In addition, the method correctly classifies additional blind tests and also identifies whether a blind test is different from the disease categories used for calibration.

Romagnolo, Donato

[17]

Microarray expression profiles in breast cancer MCF-7 cells following exposure to benzo[a]pyrene and its diol epoxide

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Maintenance of genome integrity in mammalian cells may be compromised by the concomitant accumulation of DNA damage and loss of repair functions. Polycyclic aromatic hydrocarbons are classic DNA-damaging and tumor-promoting agents. Benzo[a]pyrene (B[a]P), a prototype PAH, induces several genotoxic responses, including cell cycle arrest, oxidative stress and DNA adduct formation. Exposure of MCF-7 cells to B[a]P (1–5 μ M) and the B[a]P diol epoxide (BPDE) (50–100 nM) induced transient S-phase arrest, which was followed by accumulation in G2 and M and 70% loss of cell viability. In cells treated with B[a]P and BPDE, we observed segregation of nucleolar material and a drastic reduction in the number of ribosomal fibrillar centers. To identify the expression profile in cells treated with B[a]P or BPDE, we analyzed the levels of expression of 1,152 selected genes using a GeneMAP CancerArray in control and treated cells. Changes in gene expression were evaluated twice in duplicate ($n=4$). Compared with the expression levels in untreated cells, transcripts that were upregulated by both B[a]P and BPDE included members of the cytochrome P450, AP-1 (Fra-1, Fra-2) and glutathione S-transferase families; PARP; and G1/S-specific cyclins. In contrast, tran-

script levels for BRCA-1, G2/M cyclins, bcl-2, junB and fosB were downregulated in cells treated with B[a]P or BPDE. Treatment with the metabolite BPDE tended to elicit a stronger response than treatment with B[a]P. Analysis of expression profiles by microarray may be useful to understand the interactions among overlapping pathways and assess exposure to environmental carcinogens.

Sakamoto, Kathleen

[18]

Protechials: A novel approach to pharmacological inhibition of protein function

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A central goal of the pharmaceutical and biotechnology industries is to identify small, stable, cell-permeable molecules capable of inhibiting the activity of cellular proteins that contribute to cancer. We propose a general method for developing inhibitors that exploits the unique characteristics of the ubiquitin-dependent proteolytic system of eukaryotic cells. The ultimate purpose of our research is to identify a cell-permeable molecule that binds to the substrate-docking site of a ubiquitin ligase. By covalently linking this molecule to other compounds that bind specific cellular proteins, we intend to develop a new class of drugs that can trigger the destruction of any protein in eukaryotic cells for which there exists a small, cell-permeable ligand. Our goal is to develop an experimental system to prove that this new concept for drug design is experimentally feasible. To this end, we have used an IkBa phosphopeptide that binds with high affinity to the substrate-docking domain of the ubiquitin ligase bTrCP. We have chemically linked this peptide to the fungal metabolite fumagillin, which binds specifically to the cellular enzyme methionine aminopeptidase-2 (MetAP-2). Our results indicate that the peptide-fumagillin chimera tethered the enzyme to bTrCP and targeted it for ubiquitination and degradation. We hope to expand this approach to develop new strategies to treat cancer.

Salceda, Susana

[19]

Identification of differentially expressed genes in breast cancer

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Breast cancer is the most prevalent cancer, and it ranks second among the causes of cancer deaths in women in the United States. Analysis of differentially expressed genes in breast cancer could help in identifying potential diagnostic markers. We used complementary DNA database mining and the polymerase chain reaction-based suppression subtractive hybridization method (Clontech) to identify differentially expressed genes. Mining the cDNA database, we identified genes or expressed sequence tags (ESTs) that showed breast tissue specificity and upregulation in cancer. Simultaneously we sequenced 10,000 clones for each of seven subtracted libraries prepared using different approaches. A set of new ESTs, with no

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match with sequences in the Genbank/EMBL database, was selected. Further analysis of the genes and expressed sequence tags identified with either approach was performed using a real-time polymerase chain reaction. We used a minimum of 120 samples in the expression profile for each gene or EST, which included a panel of normal tissues and matching samples (cancer and normal adjacent from the same individual) for breast, endometrium, cervix, uterus, colon and stomach, among other tissues. Eleven genes or ESTs showed upregulation in 45–100% of the breast cancer samples when compared with the normal adjacent tissue. Three of them showed high specificity for breast tissue. Full-length cloning and antibody production for these genes are in progress. Further evaluation will allow us to determine their potential use in early detection, differential diagnosis, disease monitoring and surveillance of breast cancer.

Samatar, Ahmed

[20]

Identification of TGF- β II as a transcriptional target of Akt

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Akt/PKB is a serine–threonine kinase that plays a critical role in cell survival signaling by phosphorylating and inactivating components of the cell death machinery. Akt has been shown to phosphorylate a subfamily of forkhead transcription factors and prevent their nuclear localization, leading to repression of genes involved in apoptosis, such as *Fas* ligand. Other genes involved in forkhead-mediated apoptosis have yet to be identified. Using bioinformatics approaches, we have identified genes containing forkhead factor binding sites in their promoter sequences. One gene identified by this search is that coding transforming growth factor β II (TGF- β II). We show that activated Akt can downregulate TGF- β II promoter activity and that this repression is mediated by the three forkhead factor binding sites present in its promoter region. TGF- β II (but not TGF- β I) messenger RNA levels are significantly downregulated in pancreatic and breast cancer cell lines expressing activated Akt (through either gene amplification or inactivation of the *PTEN/MMAC1* tumor suppressor gene). Tumor cell lines expressing activated Akt and an intact TGF- β II signaling pathway are responsive to TGF- β -mediated growth inhibition. Our studies demonstrate that repression of TGF- β II promoter in tumors expressing activated Akt is a new mechanism to abrogate the growth inhibition or apoptotic effects (or both) of TGF- β .

Samimi, Goli

[21]

Gene expression profiling of oxaliplatin-resistant cell lines

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We compared the gene expression patterns of oxaliplatin-resistant cell lines with the patterns of their sensitive counterparts in an attempt to discover universal mechanisms that characterize resistant cells. Isogenic oxaliplatin-resistant variants of four ovarian carcinoma cell lines and one squamous cell carcinoma line were selected by repeated exposures of increasing concentrations of oxaliplatin (resistance levels ranged from 4-fold to 20-fold). We harvested RNA from all five pairs and assayed gene expression through hybridization to Affymetrix HuGeneFL

arrays. Using global, unsupervised, two-way hierarchical clustering we observed that resistant cell lines clustered with their corresponding parental cell lines, indicating that the number of changes in gene expression leading to resistance are probably few and that these changes are diluted in the global gene expression profile of the entire cell. In addition, Venn diagram analysis revealed that very few genes were commonly up- or downregulated across all cell lines. However, employing a recently developed supervised clustering method that uses principal component analysis to extract groups of genes with maximal variance across cell lines, we found a small cluster of genes that were consistently differentially expressed in most but not all resistant cell lines. Although this group of genes contains some interesting members, it is unlikely that these genes are the sole determinants of oxaliplatin resistance. Finally, comparison of the numbers of differentially expressed genes from each pair that were associated with specific MeSH terms supported our conclusion that no common mechanism of resistance could be detected within the five resistant pairs.

Sanchez-Carbayo, Marta

[22]

Expression profiling of osteosarcoma-transfected cells with *MDR1* and *NEO*

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Using complementary DNA microarrays we compared the expression patterns of the osteosarcoma cell line U2OS and three derived subclones obtained by transfection of *MDR1*, *NEO* and *MDR1/NEO* and selected with doxorubicin and neomycin. The expression arrays included 8,976 sequence-verified clones comprising both known genes and expressed sequence tags. We performed Northern blots to confirm the expression changes obtained for certain genes. *MDR1* expression was associated with changes in the expression of genes related to different processes, including regulation of certain cell adhesion genes such as *zyxin*; drug transport proteins such as *MRP1*; metabolic enzymes such as monoamine oxidase A; apoptotic signaling genes such as *I-TRAF*; and tumor suppressor genes such as *KISS-1*. The presence of *MDR1* and downregulation of *MRP1* were also confirmed by immunohistochemistry. Cells transfected with *MDR1* alone or co-transfected displayed more related expression profiles than cells transfected only with the *NEO* gene, as confirmed by hierarchical clustering. This study provides further characterization of genetic events associated with *MDR1* expression, in addition to identifying new targets and potential pathways involved in the molecular mechanism of multidrug resistance.