Poster abstracts

tion by conventional PCR is labor intensive, time consuming and expensive. One hundred thousand PCR reactions require greater than 1,000 96-well plates, and 100 microliter reactions use nearly \$1 worth of enzyme per reaction. This will prohibit many labs from routinely manufacturing complete mammalian chips. We have developed a surface attachment chemistry that allows the probe DNA to be amplified directly on the slide in a single enzymatic process, making the cost and time required to prepare a slide only a few times greater than a single reaction. Using this chemistry, the probes are attached at their 5' ends to an acrylamide matrix. This approach has two theoretical advantages. First, probe molecules are attached to a volume of acrylamide rather than a two-dimensional surface, which enables a greater amount of probe to be attached per feature. In principle, this could increase the signal-to-noise ratio of hybridization reactions. Second, a 5' attachment increases the kinetics of target-probe hybridization, and may increase the specificity of hybridization compared with probes attached to the surface at many bases by UV crosslinking. We believe this will allow more stringent wash conditions, and may enhance the specificity of hybridization reactions.

We have also developed the ExpressDB database for yeast RNA expression data and loaded it with 17.5 million pieces of data reported by 11 studies, including data from Affymetrix arrays, cDNA arrays and SAGE. A web-based tool supports queries from 217 conditions (see <http://arep.med.harvard.edu/ExpressDB>). Clustering analyses of the 217 conditions by similarity of ORF expression profiles indicate that conditions cluster more closely with conditions from the same source study than they do with ostensibly similar conditions from other studies.

Stephan, Dietrich

Molecular pathophysiologic hints into Niemann-Pick Type C disease using cDNA microarray technology

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The identification of a disease gene is often only the first of many steps toward understanding the pathophysiology of a disorder, particularly when a cascade of events leads to the disease phenotype. In Niemann-Pick types A and B there is a primary genetic defect involving sphingomyelin, which results directly in neurologic dysfunction. This is not the case in Niemann-Pick Type C (NPC), where the gene *NPC1*, which regulates lysosomal cholesterol metabolism, is mutated¹. Here the link between the primary genetic defect and the severe demyelination, which is a hallmark of the disease, is not apparent. We have used cDNA microarray technology² to identify genes which could be influenced by the primary gene defect and whose altered expression could result in the phenotype. Two arrays were used, an early 1,400 human EST set, and a sequence-verified 5,000 EST set. Fluorescently labelled NPC human fibroblast cDNA (Cy5) and unaffected human fibroblast cDNA (Cy3) were hybridized to the array and ratios of the transcript level of each EST between normal and affected were determined. Transcripts that were outside of the 99.0% confidence interval were chosen on the basis of interesting biology for further consideration. Among these was the gene *CD9*, which encodes a transmembrane protein which is a major component of the myelin sheath and which is confirmed by northern analysis as significantly downregulated in NPC (20% of normal). FACS analysis confirms the abnormality at the protein level as well. The mouse model of NPC was used to verify the abnormality at the RNA level *in vivo*. This data suggests a major component of the myelin sheath is directly regulated during cholesterol metabolism and may contribute to the NPC phenotype. Another gene, encoding Galactocerebrosidase, was upregulated in NPC and may indicate a similarity in mechanism between NPC and the two other forms of the disease (A and B). Finally, the entire cholesterol biosynthetic pathway can be seen to be upregulated by looking specifically at enzymes in this path-

way in the outlier data set. We are attempting to facilitate elucidation of the link between the primary genetic defect and the molecular pathophysiology of Niemann-Pick type C disease.

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2. DeRisi, J. *et al. Nature Genet.* **14**, 457-460 (1996).

Stratowa, Christian

Correlation of clinical data with expression profiles in B-cell chronic lymphocytic leukaemia as determined by cDNA microarray analysis

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Combining data obtained by gene expression profiling with other sample-related data (for example clinical data in the case of patient samples) should increase the amount of information that can be extracted from the raw data. We performed a feasibility study by systematically comparing large-scale gene expression profiles with clinical features in human B-cell chronic lymphocytic leukaemia (B-CLL), a disease characterized by broad clinical variability but well-defined disease progression criteria. cDNA microarrays were employed to determine the expression levels of 1,024 selected genes in 54 peripheral blood lymphocyte samples obtained from patients with B-CLL. Statistical analyses were applied to correlate the expression profiles with clinical parameters including patient survival and disease staging. We were thus able to identify a set of genes whose expression levels correlated with patient survival or with disease progression. Unexpectedly, most of these genes encode either cell-adhesion molecules (L-selectin, integrin-β2) or factors inducing cell-adhesion molecules (interleukin-1β, interleukin-8, EGR1), suggesting that prognosis of this disease may be related to a defect in lymphocyte trafficking. This report demonstrates the feasibility of a systematic integration of large-scale gene expression profiles with clinical data. We anticipate that the availability of databases integrating comprehensive molecular with clinical information and subsequent computational analyses will foster our knowledge of the complex nature of human cancer.

Su, Yan A.

Identification of suppressor genes associated with the chromosome 6-mediated suppressed melanoma cell UACC903(+6) by cDNA microarray

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The development and progression of human malignant melanoma is believed to