phases. The first phase is to classify the cDNAs and the second is to complete full-length sequencing and functional annotations. We have developed two original methods to construct full-length cDNAs efficiently: 'cap-trapper', which preferentially recognizes the Cap site of mRNA; and the 'trehalose-thermoactivated reverse transcriptase', which allows the reverse transcriptase reaction at higher (60 C) temperatures. We have constructed over 80 libraries from embryonic tissues of different developmental stages and adult tissues to ensure the greatest possible coverage of the expressed mRNA. More than 200,000 successful sequencing passes have been performed with the use of two tools developed in-house: a high-throughput plasmid preparation system and the RISA 384 capillary sequencer. Most of the sequences were performed from the 3´ end to select individual cDNAs. We have selected more than 30,000 different cDNAs. Using these sets of RIKEN full-length cDNA, we have established gene expression microarrays containing a 20 K set of RIKEN full-length cDNA unique mouse genes (http://genome.rtc.riken.go.jp). This set has been used to profile expression patterns of various adult and embryonic tissues. Target DNAs were PCR amplified and printed on Poly-L-lysine coated glass slides. Target DNAs were blocked by excess amounts of Cot1DNA. Probes were labelled by two-colour fluorescent dye using random primer and reverse transcriptase. Normalization has been achieved using a global normalization method. We have also developed a program to filter the noise. The experiment was done twice and reproducible results were extracted and clustered. We will present a large set of data that show the spatial and temporal expression patterns of mice. These mouse full-length 20 K cDNA microarrays are widely applicable to analyse the global expression profiling of normal and diseased status of mice.

Ozcelik, Hilmi

Using microarray technology to study the role of genetic polymorphisms in breast cancer risk

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Mutant alleles of dominant, highly penetrant breast cancer genes, including *BRCA1* and *BRCA2*, do not occur frequently, and hence account for only a small proportion of breast cancer cases. On the other hand, several studies have suggested an association between low-penetrant alleles and breast cancer risk. Although the contribution of low-penetrant alleles to the individual breast cancer 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. Candidate gene approach is one of the most logical and practical strategies to identify these risk-enhancing, low-penetrant variants. Until now, a major obstacle in investigating the risk associated with multiple candidate genes has been a lack of technology for large-scale genotyping of large populations. Consequently, many studies have focused efforts on only one or two genetic polymorphisms, and even in these cases the analysis was only limited to relatively small sample sizes. Microarray technology is a solution to this obstacle. We plan to exploit the high-throughput power of microarrays to simultaneously genotype 32 different genetic polymorphisms derived from 26 genes in a well-defined, representative population-based sample containing a large number of subjects. We have selected genetic polymorphisms in genes functioning in biochemical/biological pathways frequently perturbed in cancers, those genetic polymorphisms in genes encoding components of the carcinogen metabolic pathways and the immune response pathways. We have access to the Ontario Familial Breast Cancer Registry (OFBCR), which is the largest population-based breast cancer registry in Canada. We also have support from the established microarray facility of the Ontario Cancer Institute in Toronto. The objective of the proposed study is to identify low-penetrant, yet commonly occurring, genetic polymorphisms which contribute to the risk of developing breast cancer. The establishment of this approach will prepare us for large-scale genotyping involving hundreds or even thousands of candidate genes in large defined populations. This will lead to a more complex analysis of gene-gene and gene-environment interactions than is currently possible. Advances in disease aetiology will significantly expand our abilities to design strategies for the prevention of breast cancer development and progression.

Peng, Tao

Inhibition of specific mRNA translation—possible mechanism of rapamycin's inhibition of T-cell proliferation

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Rapamycin, an immunosuppresant drug, prevents interleukin 2 (IL-2) induced proliferation of T-cells. Although the signalling pathway affected by rapamycin is poorly understood, current evidence indicates that the drug acts by inhibiting the translation of specific mRNAs. To identify mRNAs translationally regulated by IL-2 and/or rapamycin, we screened high density oligonucleotide arrays with probes prepared from polysomal mRNA from the IL-2 dependent human Kit-225 and mouse CTLL-2 cells. In Mouse 11k chips containing 11,000 genes (known genes and ESTs), 5% of genes showed significant polysome profile change after rapamycin treatment. The rapamycin sensitive genes include translation control molecules, such as ribosomal proteins and elongation factors, secreted proteins, cytoplasmic signaling molecules, metabolic enzymes and transcription factors. Similar results were obtained in human Kit-225 cells. We are designing cDNA-based microarrays that contain IL-2 and rapamycin sensitive mRNAs and using these arrays to study translation control during cell growth and division.

Peterson, Todd

Resonance light-scattering particles for ultra-sensitive detection of nucleic acids on microarrays

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The emergence of microarray-based technologies has revolutionized the analysis of gene expression and DNA sequence. In advanced mircroarray applications, nucleic acids are labelled with fluorescent dyes and hybridized to cDNAs or oligonucleotides configured on a solid surface. Fluorescent signals are detected and interpreted using sophisticated instrumentation that relies on scanning confocal fluorescence microscopy and fluorescent image analysis software. In practice, the performance and accessibility of many microarray systems is encumbered by limited sensitivity of the fluorescent label(s), poor dynamic range, fluorescence quenching, photobleaching and expensive instrumentation. To address these microarray system performance issues, we have applied resonance light-scatter-