Glynne, Richard

Monitoring gene expression and amplification through mid-density oligonucleotide array platforms

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Array technologies create an opportunity to identify diagnostic and prognostic markers, as well as drug targets, from clinical samples. We have developed a mid-density spotted oligonucleotide array platform for monitoring gene expression and genomic amplification and loss events in human tumours. The spotted oligo arrays are sensitive, robust and allow rapid incorporation of new sequence information. We have also designed a single custom Affymetrix GeneChip™ array to monitor 35,000 genes and ESTs by empirically choosing probes on the basis of consistent performance over a wide range of samples. Integration of gene expression data from Affymetrix and Eos oligo arrays, in conjunction with genome amplification data, has identified a number of transcripts with increased mRNA expression and genomic representation in breast cancer. We have further generated a normal tissue atlas database that allows us to screen potential drug leads for possible toxic side effects. Candidate disease genes identified by DNA microarrays are then used to interrogate arrayed archival tumour tissues in a highly parallel fashion by *in situ* hybridisation. These tumour tissue microarrays allow for the rapid characterisation of expression at single cell resolution. In addition, gene expression can be associated with tumour grade and stage on hundreds of archival tumours with known clinical outcomes. Using these multiple array technologies we have identified a number of transcripts expected to encode tumour-specific proteins which are potential targets for therapeutic intervention, diagnosis and prognosis of breast cancer.

Gohil, Kishorchandra

mRNA expression profile of a human cancer cell line in response to a phytochemical extract with antioxidant properties

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Herbal extracts with antioxidant properties are enjoying increasing popularity as remedies for acute and chronic illnesses such as depression, memory loss, cardiovascular disorders and immunological deficiencies. The molecular targets of these complex mixtures of botanical extracts are poorly understood. We have evaluated a well-characterised herbal extract for its ability to affect the mRNA expression profile of a human cancer cell line. We used the Affymetrix hu6800 set of DNA microarrays chip to define changes in the mRNA expression profile of cells exposed to the herbal extract for 6 hours, 48 hours and 72 hours. The target RNA extracts hybridised to 32.3% of the probes on the chip. Of the 2,200 transcripts, 400 transcripts were altered by the extract over time. Two transcripts for antioxidant enzymes were up-regulated (> threefold at 6 hours). Eighteen transcripts with a high basal expression were down-regulated and this group is associated with invasive growth. Sixteen transcripts expressed at low levels behaved as in the previous group and included transcripts for members of the MAP kinase family and signal transducing proteins. Eighteen transcripts were up-regulated and peaked at 48 hours followed by return to basal levels; this group consisted of transcription factors. The largest group of 274 transcripts was up-regulated approximately twofold through the course of the exposure to the extract and included a broad group of transcripts. Our data demonstrate that the Affymetrix hu6800 microarrays can be used to define the molecular targets of a complex mixture of a botanical extract on human cells in culture. This strategy can also be used for a more rational evaluation of the biological activities of sub-fractions and the individual components of such a complex herbal extract.

Gokgoz, Nalan

Characterisation of differential gene expression of soft tissue sarcomas by microarray technology

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Soft tissue sarcomas (STS) are a group of malignant mesenchymal tumours that present a dilemma for clinical management. Current staging systems, based largely on morphological tumour characteristics, cannot accurately classify tumours nor predict an individual patient's risk for eventual metastases. Consequently, it is a challenge to identify patients with STS who should be considered for intensive adjuvant treatment protocols. Characterisation of specific gene expression patterns in STS will improve classification of these tumours and be of value in identifying patients at high risk for developing metastases.

Our aim is to generate comprehensive gene expression profiles of the molecular alterations that occur during the development and progression of human soft tissue sarcoma. We plan to accomplish this by taking advantage of two major resources — a national STS tumour bank with corresponding clinical information and a recently developed microarray facility.

STS specimens for this study will be obtained from the Canadian Sarcoma Group (CSG) Soft Tissue Sarcoma Tumour Bank that is a resource of tumour tissue and clinical correlative data situated at Mount Sinai Hospital, Toronto, Canada. The CSG tumour bank has been actively collecting tissue and pertinent patient information prospectively since 1993. As of December 1998, this bank had collected 701 tumour samples from 609 patients with STS, and is continuing to collect 110 STS cases per year. The CSG bank includes separate computer databases for clinical/pathologic and laboratory information, and stores frozen tumour and normal tissues, blood, as well as histological slides, paraffin embedded tissue and pathology reports from the collaborating centres. Most sarcomas in the tumour bank are large which generally allows for abundant frozen tumour tissue.

We plan to take advantage of the developing technology of cDNA microarrays that allows differential gene expression to be quantitated for thousands of genes simultaneously between multiple samples. A gene array facility, which is situated at the Ontario Cancer Institute, Toronto, is capable of producing high quality chips with up to 20,000 ESTs with an XYZ robot and generates data with a very fast and sensitive reader at low cost to collaborating members of the facility. Even without knowing the identity of the majority of the ESTs examined, patterns of expression can be identified that are characteristic of the behavioural aspects of a tumour and may be prognostically important. As well, studying these gene cohorts will help identify molecular pathways as well as novel genes important in tumourgenesis.