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interrogate weak expression levels. In addition, a new weighting factor for each ratio will be introduced which provides a quality measure for the expression ratio measurement. The weighting factor enables us to process gene expression data without introducing a fixed intensity filter in analysis methods such as clustering, fingerprinting analysis and predictive analysis.

Chenchik, Alex

Novel hybridization chamber for expression profiling of glass slide microarrays

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cDNA expression microarrays are becoming popular tools for high throughput expression profiling. To simplify use of the microarrays in research lab, we developed a disposable plastic chamber, which can be used for a hybridization assay with a single slide. We compare performance of the hybridization assay using conventional glass slide-cover slide with 50 µl of hybridization mix and hybridization chamber with 1 ml of hybridization solution. We have developed a procedure for generating highly sensitive fluorescently labeled cDNA probes. Low complexity hybridization probes were generated from poly(A)+RNA using mixture of gene-specific primers. Using allylamino-modified dUTP allows one to generate the cDNA containing primary amino groups, which can be efficiently coupled with Cy3 or Cy5 succinimid ester. Under the optimal hybridization buffer composition, both approaches give similar sensitivity. The hybridization chamber approach does not require any specialized equipment and can be used in any research lab.

Childs, Geoffrey

An analysis of F9 embryonal carcinoma cell differentiation into parietal endoderm using DNA microarrays

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The F9 teratocarcinoma cell line is a well-documented model system in primary cell lineage differentiation. After exposure to *trans*-retinoic acid, these cells can develop into two distinct forms of extra-embryonic endoderm. To date, differential library screening has been the best tool available to study the numerous events occurring during the transformation from an undifferentiated tumor cell line into a fully differentiated endoderm derivative. The advent of DNA microarray technology now allows the ability to examine the changes in RNA levels of a very large number of genes simultaneously.

The differential expression profile of F9 embryonal carcinoma cells was studied during their differentiation into parietal endoderm after exposure to 10^{-7} M retinoic acid (RA) and 10^{-3} M Dibutyryl cyclic AMP. We examined the expression profile of the cells after induction for: 4, 8 and 16 hours as well as 1, 3, 4, 5, 7, 9 and 14 days, on microarrays containing 5700 mouse cDNA clones. Our results indicate two major stages in the course of differentiation into parietal endoderm. In the first 24 hours, the cells turn off many markers that are specific for several types of tissues, which are derivatives of all three primary cell types. They also show induction of several transcription factors, nucleases, proteinases and activators/inhibitors of several signal transduction pathways. Around the third day, they begin to express gene products that are associated with the final endodermal cell types.

Either undifferentiated F9 cells seem to be a plural collection of different cell types or an indeterminate type of cell expressing gene products associated with neural, muscle and gastrointestinal tissue types. After exposure to RA the expression of most of these genes stops, with the exception of some neural markers, which are further, induced during the time course. We observed an increase in expression of several genes which have been previously reported to be induced by retinoic acid in F9 cells (RAE-28, RAE-30, SPARC, laminin) as well as genes induced in other teratoma cell lines such as P19 (STRA-13, SHYC). In addition to genes that have been previously reported, we observed the induction of many novel genes such as posttranscriptional modifying enzymes, Ca+ binding proteins, components of tight junctions and proteins involved in intracellular trafficking. While 60% of the genes, that were either induced or repressed, are not currently identified, the remaining genes will provide interesting insight into the events occurring during the development of endoderm and confirms the value of microarray technology in the study of development.

Chin, Khew-Voon

Analysis of the mechanisms of drug resistance in cancer by cDNA microarray

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Drug resistance in cancer is a major obstacle to successful chemotherapy. Cancer cells exposed to antitumour drugs may be directly induced to express a subset of genes that could confer resistance, thus allowing a population of cells to survive and form the relapsed resistant tumour. Alternatively, some cancer cells may be expressing an array of genes that could confer intrinsic resistance, and exposure to cytotoxic drugs select for the survival of these cells that form the relapsed tumour. To assess whether there are differences in the mechanism of drug resistance by induction or selection, we used complementary DNA (cDNA) microarray to monitor mRNA expression in breast cancer cells that were either transiently treated with or selected for resistance to doxorubicin. Expression profiles of the human breast carcinoma MCF-7 cells transiently treated with doxorubicin were compared with those obtained from a MCF-7 cell line selected for resistance to doxorubicin. Transient treatment with doxorubicin altered the expression of a diverse group of genes in a time-dependent manner. We found a subset of transiently induced genes constitutively overexpressed in cells selected for resistance to doxorubicin, suggesting that cancer cells activate a distinct set of genes in acquiring drug resistance by either induction or selection. Our studies demonstrate the feasibility of obtaining molecular profiles of drug resistance in cancer cells by cDNA microarray, which might yield insights into the mechanisms of resistance during chemotherapy, and suggest alternative methods of treatment.

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