Elkahloun, Abdel G. [55]

Gene expression profile analysis of 13 human breast cancer specimens using laser capture microdissection and cDNA arrays

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The advent of high-density complementary DNA microarray technology, with its capacity for simultaneous monitoring of thousands of genes, provides a unique opportunity for high-throughput expression analysis of cancer. Although most current microarray studies have been performed with genetic material obtained *in vitro*, a major advance in functional genomic investigations would be the ability to perform array-based expression analysis with genetic material obtained *in vivo* and originating from morphologically distinct cellular populations, including various stages of cancer progression. Until recently, *in vivo* analysis of tumor-specific genomic alterations, array-based or otherwise, has been hampered by the inability to obtain specific cell types accurately from cancerous tissue. The recent development of laser capture microdissection allows for accurate and rapid procurement of specific cell populations within complex tissue and provides the opportunity to perform array-based expression profiling of genetic material obtained *in vivo*. We describe the combined use of laser capture microdissection and high-throughput complementary DNA microarrays to monitor gene expression levels in 13 breast cancer specimens. By using different data visualization tools (clustering and multidimensional scaling) we demonstrate that expression profiles of more than 8,000 genes can be successfully generated using non-amplified RNA derived from distinct cell populations within several different morphological stages of human breast cancer.

English, Jessie [56]

Expression profiling of lung cancer cell lines

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We determined the molecular profiles of seven human lung cancer cell lines using commercially available large-scale DNA arrays (Incyte Genomics) composed of 60,000 elements (= 45,000 genes). Relative gene expression was determined by comparing the expression of normal primary epithelial lung cell lines to that of three SCLC cell lines and four NSCLC cell lines. Few genes were differentially expressed when independently derived primary lung cell lines (NHBE and SAEC) were compared with each other. However, we detected substantial differences in gene expression when comparing tumor cell lines with normal cell lines. The largest gene expression changes occurred in cell-surface markers and cytoskeletal elements. Of genes with greater than eightfold differential expression in at least one cell line, over 15% were members of five gene families: cytokeratins, laminin 5, fibronectin, integrins and annexins. A hierarchical clustering algorithm was used to analyze gene expression changes (threefold or greater) across eight probe pairs. We grouped probe pairs into clusters to categorize relationships among cell lines. The three SCLC cell lines formed one cluster and the four NSCLC cell lines clustered together. The normal cell lines seemed to be distinct from both SCLC and NSCLC. Using cluster analysis of individual genes we identified a cluster containing genes involved in mitotic pathways and up-regulated in most tumor cell lines. Four genes were represented two or more times within this cluster and placed in adjacent rows. The capacity of this method of statistical analysis to group these genes within the same cluster and as adjacent records supports the validity and reproducibility of our experimental approach.

Farnham, Peggy [57]

Use of chromatin immunoprecipitation to study transcriptional deregulation in cancer cells

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Many human cancers are caused by deregulation of specific transcription factors. To study the mechanisms by which transcriptional deregulation causes neoplasia, we are using a chromatin immunoprecipitation protocol to complement three different types of gene expression profiling. We first generated a weight matrix based on a set of known E2F binding sites and then used this weight matrix to search the genome for potential E2F binding sites. Using the chromatin immunoprecipitation assay, we confirmed the identification of a set of new E2F target genes. As a second approach, we have used the chromatin immunoprecipitation protocol to characterize the transcription complexes bound to β-catenin target genes that were identified by others using gene expression profiling of tissue culture cells. Using primary tumor samples, we show a direct recruitment of both β- and γ-catenin to certain cellular promoters. Finally we have used oligonucleotide microarrays and representational difference analysis to identify a set of genes that are highly upregulated in liver tumors. Our current goals are to identify common regulatory regions in the promoters that regulate these messenger RNAs specific to liver tumors and to characterize the components of the transcription complexes bound to these promoters using our chromatin immunoprecipitation protocol.

Fox, Jay [58]

Melanoma gene expression during matrix degradation and contraction

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We have previously demonstrated that the expression of MMPs, MT-MMPs and integrins in human melanoma cell lines is differentially regulated depending on the type and assembly state of the extracellular matrix on which the cells are seeded. We