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Molecular profiling

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Keywords

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Introduction

Molecular profiling uses measurement of global expression patterns towards identification of the individual genes that mediate particular aspects of cellular physiology. The vast data sets generated by integrating the expanding genetic database and the new expression technologies represents a significant challenge for investigators. The use of clinical specimens, critical for evaluating the molecular anatomy of human disease, ideally includes multiple samples such that molecular findings can be assessed for their frequency among patients and/or correlated with particular features of a disease. Thus, integration of clinical information, histopathology, developing technologies and laboratory methods, and bioinformatics algorithms is essential for profiling efforts. There are also significant technical challenges associated with expression profiling of clinical samples, and few experimental data supporting the possibility of this approach. This paper is intended to provide such evidence, and discuss the possible applications of these new technologies.

Aims

To asses the feasibility of molecular profiling of microdissected cell populations using cDNA library sequencing as an initial gene expression method and prostate cancer as a disease for study.

Comments

This paper provides an important proof of principle for the application of molecular profiling to examine the relationship between gene expression profiles and cellular behaviour in human tissue samples. The data presented uses prostate cancer as an example, but with obvious correlations with ongoing work on breast samples. Generation of cDNA libraries from pure, microdissected normal, pre-malignant and malignant epithelial cells has been shown to provide identification of tissue- and stage-

specific differentially expressed genes. Integration with genomic data and microarray-based profiling will facilitate both gene hunting and understanding disease-specific physiological responses. Clearly, the application of this approach to breast cancer will be critical for understanding the pathogenesis and progression of the disease.

Methods

Samples from five different prostate cancer patients were randomly selected from the NCI (National Cancer Institute) frozen tissue bank, and cells captured by manual or laser capture microdissection. Twelve microdissection-based libraries were produced from epithelial components of radical prostatectomy or biopsy specimens, including normal epithelium, premalignant foci, locally invasive cancer, and metastatic cancer. cDNA libraries were produced using protocol no.1 from the Cancer Genome Anatomy Project (CGAP) website. cDNA library quality was determined by measuring gene diversity, and was measured by sequencing a minimum of 500 randomly selected clones per library.

Results

A total of 29,183 successful sequences were performed. Analysis of the number and frequency of genes expressed showed that all of the libraries exhibited a high level of complexity. The majority of genes were observed only once or twice in each library, and the overall gene diversity average was 39.1%, which compares favorably with standard libraries derived from whole tissue specimens or cultured cells.

Discussion

The data from this initial feasibility study show that genes with a wide range of expression can be profiled by this approach, from genes observed at high levels that are known to be abundant in prostate epithelium, to a large number of low-abundance genes that were observed infrequently. The ability to recover complex transcriptosomes from microdissected cell populations provides "encouraging news for investigators interested in molecular profiling of clinical samples". Determination of a prostate epithelial unigene set (a catalogue of genes expressed in normal and malignant prostate epithelium) served as a foundation for multiple analyses of gene expression. These included analyses of prostate-unique gene expression; integration of genome data, expression profiles and disease; cDNA microarray-based profiling; single nucleotide polymorphisms; and differential gene expression. All of the present prostate data is provided on the CGAP website.

References

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