15. GENE EXPRESSION IN THYROID TUMORS

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INTRODUCTION

Endocrinologists and pathologists would welcome a simple reliable test of the nature and potential of thyroid nodules at the first encounter. Even with satisfactory fine needle aspirations a definitive cytological diagnosis may not be always be possible and prognostication is thus limited. As discussed in other chapters in this book (2 & 16), several molecular markers examined in surgical specimens have been proposed to be specific for histologic types of thyroid tumors and/or malignancy. None, however, is diagnostic.

In the event the approaches to examine a limited number of molecular markers at a time are by means not robust enough to apply to the cytological harvest of thyroid FNA. Because of the very nature of malignant transformation (see Chapter 1), it is expected that gene products in many cellular pathways would be involved, only some of which turn out to be tissue-specific or tumor-subtype specific.

The exploration of the transcriptome of tumors or normal tissue during development, or following treatment with drugs or hormones holds much promise to the better understanding of physiologic and pathologic processes (1–4).

This chapter discusses the application of this technique to thyroid tumors.

DNA MICROARRAYS

In essence, cDNA or oligonucleotides are spotted onto glass slides, silicon wafers or nylon membranes and are then exposed to florescently-labelled mix of RNA (or cDNA made thereof) from biological specimens. Each DNA latches onto the RNA or cDNA

that matches its sequence. Based on the location and intensity of the signal the source gene and its activity can be detected. Many protocol refinements and software programs have been introduced to ensure internal consistency, reproducibility, statistical analysis, gene annotation and ontological linkages of the huge amounts of raw data (3,4).

THE APPLICATIONS OF DNA MICROARRAYS

DNA microarray is a tool most commonly used to monitor levels of gene expression levels. DNA chip technology can be helpful in documenting DNA copy number, DNA/protein interactions and genetic polymorphisms (4). It holds promise in the search for gene promotor regions and screening DNA/chromosomes for gene expression (4).

One of the first and still most used tools applied to microarray data visualization is hierarchical clustering. In one dimension, sample output is grouped to similarity and in the other according to the overall similarity of expression across samples. An important objective of this approach is to identify similarly regulated genes across specimens examined (3). Sophisticated computational treatment of the data has, however, failed to establish this "guilt by association" as a valid spin-off from microarray analysis (5). An evolutionary approach identifying orthologs that have retained their function goes a long way to answering the criticism of the identification of co-expressed genes by microarrays (6).

Cluster analysis of differentially expressed genes has, nevertheless, been helpful in a number of areas of clinical medicine, particular in oncology (3,7,8). Hierarchical clustering has been helpful in:

1. Tumor classification or subclassification
2. Identification of potentially important genes characterizing a tumor, susceptibility to drugs and metastatic potential
3. Identification of new drug targets to provide new therapeutic tools.
4. Identifying biomarkers for establishing or confirming diagnosis and outcome of therapy.

Microarray technology as currently used has provided novel insight into B-cell lymphoma, breast cancer, melanoma and other human malignancies (2,8–11)

GENE EXPRESSION PROFILING IN THYROID CANCER

A limited number of studies have reported on DNA microarray studies in thyroid cancer: two on follicular tumors, one on papillary carcinoma (PTC) and only one that examined a range of benign and malignant thyroid disease. The numbers of samples analyzed in each study was small to moderate in size. Most of the studies quoted are, however, robust and pass muster for the stringent rules stipulated for reporting of gene expression studies (4).

Papillary thyroid carcinoma

Huang et al. (12) used oligonucleotide DNA chips containing more than 12,000 genes to profile 8 papillary carcinomas and matching normal thyroid tissue. They found the