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The Application of Microarray Analysis to Pediatric Diseases

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Historically, studies to decipher genetic alterations related to human disease have been limited to single genes or proteins. Completion of the Human Genome Project, which began in 1990, has resulted in sequencing of the human genome and the generation of numerous technological, genetic, and bioinformatics resources. These advances have made global transcriptional analysis a reality with the emergence of several approaches that allow investigators to analyze hundreds to thousands of genes in parallel. These techniques include serial analysis of gene expression (SAGE) [1], differential display [2], and DNA microarrays [3–5]. Over the past decade, DNA microarrays have become an extensively applied, mainstream component of biomedical research. The knowledge gained through use of this technology has improved understanding of human biology and disease. DNA microarray technology likely will play an important role in the development of new and effective diagnostic, preventive, and therapeutic approaches [6,7]. This article briefly reviews the state of the technology and its successful translations in pediatric research.

A microarray is essentially a miniaturized high-density dot blot, consisting of thousands or tens of thousands of probes that are immobilized on a two-dimensional solid matrix. Each probe is specific for, and capable of detecting the presence of an RNA transcript in the sample(s) being analyzed. The samples may compare experimental versus control tissue culture cells or diseased versus healthy cells or tissue. The DNA microarray has evolved

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into two commonly used formats: spotted cDNA or oligonucleotide arrays [5] and light-directed in situ synthesized oligonucleotide arrays [8,9].

Spotted arrays use high-speed robotics to mechanically or piezoelectrically [10] deposit small volumes of probe solutions onto the array surface. Numerous manufacturers have developed robotic arrayers that enable researchers to construct arrays in their own laboratories. Array construction is technically challenging, and the quality of the array has a direct impact on the reliability of the gene expression data generated [11–18]. Microscope slides, because of their low inherent fluorescence, typically are used to provide the solid support. These are coated with poly-L-lysine, amino silanes or amino-reactive silanes, which enhance surface hydrophobicity to limit spot spreading and to improve adherence of the DNA by providing a positive charge [19,20]. The printed DNA probe can be amplified products from cDNA libraries (typically greater than 200 base pairs [bp]in length) or oligonucleotides (typically 30 to 70 bp in length). As their costs have decreased, oligonucleotides have become the more popular choice over the past few years. Oligonucleotides exhibit numerous advantages over cDNAs in that cumbersome clone library management and amplification can be avoided. Oligonucleotides can be designed to exclude homologous sequences between genes, thereby enhancing specificity. In addition, a given gene can be represented by a set of different oligonucleotides targeting different regions or exons, allowing for the detection of splice variants, or discrimination of closely related genes. After printing, the DNA is cross-linked to the support using ultraviolet irradiation, followed by a blocking step using succinic anhydride to reduce the positive charge at unoccupied sites on the slide surface so that labeled sample targets do not bind nonspecifically to the array.

After the array is prepared, RNA samples are extracted from the two tissues that are to be compared. For spotted arrays, these typically are labeled differentially during reverse transcription with cyanine dye-tagged nucleotides, yielding cDNAs that are labeled with either Cy3 or Cy5. The dye-labeled cDNA targets then are cohybridized to the same array. The advantage of cohybridization is that the comparison is direct and avoids experimental variation potentially introduced by hybridizing a single sample to a single array. After hybridization, the array is washed, then analyzed with a fluorescence scanner (Fig. 1). Specially designed software is used to determine the relative amounts of an mRNA species in the original two samples for every gene on the array, this typically is defined as a normalized intensity ratio between the two fluorophores for each array element [5,16,19,21,22].

Many laboratories do not have the equipment, expertise, or desire to create their own custom arrays. This has created a large demand for commercially prepared microarrays. The Affymetrix GeneChip (Affymetrix, Santa Clara, California), the most widely used commercial system, uses an in situ synthesized oligonucleotide array and highly optimized protocols that allow investigators to rapidly generate reliable expression data. In situ synthesized oligonucleotide arrays use ultraviolet light passed through a series