National Academy of Clinical Biochemistry Guidelines: The Use of Microarrays in Cancer Diagnostics

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Abbreviations: DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; PCR, polymerase chain reaction; MGED, Microarray Data Standards, Annotations Ontologies and Databases; SNP, Single Nucleotide Polymorphism, TMA, Tissue MicroArray

INTRODUCTION

Microarrays were first commercially introduced in 1996 by Affymetrix (Genechip^{R} technology) (1). Depending on the biomolecule immobilized on the surface, these devices are known as DNA-chips, protein-chips or cell-chips. Cancer was one of the first areas in which microarrays have been employed for diagnostic purposes. Disease classification, prognosis, monitoring and prediction of therapeutic response are some of the areas where microarrays have the potential to become routine diagnostic tools. This technology enables substitution of linear studies of individual events to parallel and simultaneous analysis of complex systems and pathways. Regardless of the application, the resulting information comprises thousands of individual measurements and provides an intricate and complex snapshot of biological properties of the cell, tissue, organ or fluid.

PRINCIPLES OF MICROARRAYS

A microarray is a compact device that contains a large number of well-defined immobilized capture molecules (e.g. synthetic oligos, PCR products, proteins, antibodies) assembled in an addressable format. The best-known microarrays, DNA-biochips, are miniature arrays of gene fragments attached to a glass or plastic surface. These chips are used to examine gene activity (expression profiling) and identify gene mutations or single nucleotide polymorphisms (SNPs), by hybridization between the sequences on the microarray and a labeled probe (the sample of interest). There are two major methods for microarray fabrication: a) photolithography, as is used in the Affymetrix system (400,000 spots in a 1.25 x 1.25 cm area), and b) mechanical deposition or printing on glass slides, membranes etc. The major advantages of microarrays include: small volume deposition (nanoLiters, nL), minimal wasted reagents, access to many genes/proteins simultaneously, massive parallel information, automation and potentially quantification. For more detailed information on microarrays, see specialized books (2, 3) and an entire issue of *Nature Genetics* (4).

TISSUE MICROARRAYS

High-throughput analysis of tissues is facilitated by new technologies such as multi-tissue northern blots, protein arrays or real-time PCR (5-8). However, the problem with these methods is that tissues are disintegrated before analysis, preventing identification of the cell types expressing the gene of interest (9). These and other shortcomings can be overcome by tissue microarray (TMA) techniques (10). TMAs consist of up to a 1000 tiny cylindrical tissue samples (0.6 mm in diameter) assembled on a regular-sized routine histology paraffin block. Sections are cut from TMA blocks using standard microtomes. TMA sections allow simultaneous analysis of up to a 1000 tissue samples in a single experiment. The technique is therefore cost-effective. Despite the small size of arrayed samples, TMA studies generally provide reasonably representative information. TMAs are applied over a broad range of cancer research: prevalence TMAs (11-13), progression TMAs (10, 14-16), prognostic TMAs and TMAs composed of experimental tissues such as cell lines (17,18) or xenografts (19).

APPLICATIONS OF MICROARRAYS

Microarrays have been successfully applied in a variety of settings including

- Gene expression profiling (the most popular application)
- Detection of Single Nucleotide Polymorphisms (SNPs) (Pharmacogenetics)
- Sequencing by hybridization (genotyping/mutation detection)
- Protein expression profiling
- Protein-protein interaction studies
- Whole genome biology experiments

Cancer is a heterogeneous disease in many respects, including its cellularity, different genetic alterations and diverse clinical behaviors. Many analytical methods have been used to study human tumors and to classify patients into groups with similar clinical behavior. Most methods require specialized pathologist interpretation; yet none of the classifications are homogeneous enough. It has been hypothesized that the genetic heterogeneity and clinical behavior of cancer

could be better assessed by studying genome-wide gene expression profiles by microarrays (20). Although the potential of microarrays is yet to be fully realized, these tools have shown great promise in deciphering complex diseases, including cancer (20). A partial list of applications of microarrays in cancer is presented in Table 1 (21-43). As is the case with many new technologies, microarrays have many shortcomings, briefly discussed in the following section.

LIMITATIONS OF MICROARRAYS

Microarray technologies are still evolving and this presents difficulties for standardization and consensus development. There are no 'gold standards' such as reference reagents or bioinformatics algorithms. These standards are essential for comparison of data between laboratories and on different platforms (44). Recent reports suggest that microarray data are noisy and not reproducible (45,46). Furthermore, bias poses a significant threat to the validity of data generated by such technologies (47).

MICROARRAYS: NACB RECOMMENDATIONS

There is little doubt that microarrays will eventually become routine diagnostic tools, and the first commercial devices are already on the market (Table 2). However, this is still a relatively new technology and several parameters need to be further optimized and validated prior to their implementation into routine clinical practice, including: selection of optimal capture molecules, standardized hybridization protocols and standardized data collection and interpretation. For DNA and protein microarrays to be reliable tools, they must possess probe sequences that hybridize with high sensitivity and specificity, thereby allowing specific detection of their intended targets. Results must become more reproducible, robust and interchangeable between laboratories, and quality control and quality assurance systems must be established (44). Determining the appropriate level of analytical and clinical validation needed for each application raises new challenges for scientists in industry, academia and regulatory agencies (48).

Two important issues need to be considered when evaluating microarray expression data

1. Whether the results are valid or accurate for the particular biological system under study, and

2. Whether the data fundamentally describe the phenomenon being investigated (49).

Introduction of artifacts is possible at any time during an array experiment, therefore each component of the procedure must be carefully considered. The validation process can be divided into three areas: experimental quality control, independent confirmation of data and universality of results (49). Furthermore**,** before implementation of microarrays into routine practice, it will be preferable to automate the process to minimize variability and increase robustness. Array production, like any other diagnostic device, must meet minimum criteria set by the Food and Drug Administration (FDA) (50). The International Meeting on Microarray Data Standards, Annotations Ontologies and Databases (MGED) focuses on standardization of biochips and proposes appropriate guidelines (51, 52). Despite widespread applications of microarrays in research, the level of evidence of these studies for clinical application, as described by Hayes et al (53), is Level V (evidence from small pilot studies that estimate distribution of marker levels in sample population). Based on the information above, the NACB Panel has formulated the recommendations outlined in Table 3.

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Table 1. Microarray applications in cancer diagnostics

Microarray Technology	Application	Cancer	Reference
Comparative genomic hybridization	Classification	Breast	21, 22
cDNA tissue expression profiling	Classification	Breast	23
	Therapeutic response	Lymphoma	23,24
	Molecular profiling	Prostate	25
Gene expression profiling	Prognosis	Breast	26,27
	Classification	Breast	26,28
	Diagnosis	Ewing sarcoma	29
	Diagnosis	Rhabdomyosarcoma	29
	Diagnosis	Burkitt lymphoma	29
	Diagnosis	Neuroblastoma	29
	Diagnosis	GI tumor	30
	Diagnosis	Prostate	31
	Prognosis	Prostate	32,33
	Diagnosis	Bladder	31
	Treatment tailoring	Breast	31
	Classification	Colorectal	31
	Classification	Gastroesophageal	31
	Classification	Kidney	31
	Prognosis	Kidney	34
	Classification	Ovarian	31
	Classification	Pancreas	31
	Classification	Lung	31,35,36
	Molecular profiling	Prostate	37
	Development stages	B-cell lymphomas	38,39
	Mutations	BRCA 1 (breast, ovarian)	27,40,41
Prognostic signature	Prognosis	Breast	41
		Lung	42
Genome mining	Biomarker discovery	Ovarian	43

Table 2: **Some commercially available cancer diagnostic devices based on microarray technology.**

Name	Intended Use	Manufacturer
1. Amplichip CYP450	Identifies variations in genes CYP2D6 and CYP2C19 for pharmacogenomics	Roche (www.roche.com)
2. GeneChip Mapping 100K	Whole genome SNP analysis (100,000 SNPs) for establishing disease predisposition	Affymetrix (www.affymetrix.com)
3.MammaPrint CupPrint	70-gene signature for breast cancer prognosis Identifying the primary tumor	Agendia (www.agendia.com)
4.p53 GeneChip	Sequencing of p53 gene for identifying mutations	Affymetrix
5. Tumor PSA Array Tumor Monitoring Array Colorectal Cancer DNA Array cDNA Expression Array	tPSA, fPSA, CEA CEA, AFP, hCG, CA19-9, CA125, CA15-3 TP-53, APC, K-ras, BRAF Ovarian, Breast cancer	Randox (www.randox.com)

Table 3. NACB Recommendations for use of Microarrays in Cancer Diagnostics

1. Gene expression microarrays are new and promising devices used for cancer diagnosis, prognosis, prediction of therapeutic response, monitoring and selection of therapy. The level of evidence from published studies, according to Hayes et al. (53) is Level V [lowest category]. Consequently, microarrays should continue to be used as research devices, but not as tools for making clinical decisions.
2. Standardization and clinical validation of expression microarrays is warranted.
3. Quality control and quality assurance programs for expression microarrays need to be further developed.
4. Microarray automation is encouraged for improving reproducibility, throughput and robustness.
5. Tissue microarrays are devices suitable for high-throughput analysis of large numbers of samples and are recommended for use in clinical trials and retrospective studies for evaluating and validating new tumor markers by immunohistochemistry.
6. Use of microarrays for single nucleotide polymorphism (SNP) analysis is recommended for establishing haplotypes and for correlating these haplotypes to disease predisposition.
7. Microarrays are recommended to be used for high-throughput genotyping and mutation/sequence variation detection for cancer diagnostics and pharmacogenomics. More validation is necessary to ensure equivalent results between standard technologies (such as DNA sequencing) and microarray analysis.
8. Protein microarrays and other similar technologies are recommended to be used as research tools for multiparametric analysis of large numbers of proteins. The level of evidence is not as yet high enough for clinical applications.