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Review Article

Review: DNA Microarray Technology and Drug Development

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Received 17 December 2009; accepted 15 January 2010

Abstract On the contrary to slow and non specific traditional drug discovery methods, DNA microarray technology could accelerate the identification of potential drugs for treating diseases like cancer, AIDS and provide fruitful results in the drug discovery. The technique provides efficient automation and maximum flexibility to the researchers and can test thousand compounds at a time. Scientists find DNA microarray useful in disease diagnosis, monitoring desired and adverse outcomes of therapeutic interventions, as well as, in the selection, assessment and quality control of the potential drugs. In the current scenario, where new pathogens are expected every year, DNA microarray promises as an efficient technology to detect new organisms in a short time. Classification of carcinomas at the molecular level and prediction of how various types of tumor respond to different therapeutic agents can be made possible with the use of microarray analysis. Also, microarray technique can prove instrumental in personalized medicines development by providing microarray data of a patient which could be used for identifying diseases, treatment specific to individual and trailing disease prognosis. Microarray analysis could be beneficial in the area of molecular medicines for analysis of genetic variations and functions of genes in normal individuals and diseased conditions. The technique can give satisfactory results in single nucleotide polymorphism (SNP) analysis and pharmacogenomics studies. The challenges that arise with the technology are high degree of variability with data obtained, frequent up gradation of methods and machines and lack of trained manpower. Despite this, DNA microarray promises to be the next generation sequencer which could explain how organisms evolve and adapt looking at the whole genome. In a nutshell, Microarray technology makes it possible for molecular biologists to analyze simultaneously thousands of DNA samples and monitor their behavior patterns, which brings about a tremendous improvement over the tedious "one gene per experiment" technology that prevailed previously.

Keywords: DNA Microrray, drug discovery, potential drugs, gene monitoring, pharmacogenomics, next generation sequencer

Introduction

Despite the tremendous advancement in the field of biotechnology, traditional drug discovery methods remain slow and are not effective in the synthesis and screening of newer drugs. DNA microarray technology could potentiate the identification of newer compounds which can be utilized for the treatment of incurable diseases.[1] Microarray analysis allows scientists to understand the molecular mechanisms underlying normal and dysfunctional biological processes. It has provided scientists with a tool to investigate the structure and activity of genes on a wide scale. Microarray technology could speed up the screening of thousands of DNA and protein samples simultaneously. [2]

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DNA Microarray technique

DNA Microarray consists of a small chip, about the size of a fingernail, on a support surface, having 96 or more tiny wells. Each of these wells or spots contains thousands of DNA probes or oligonucleotides which are arranged in a grid pattern on the chip.[3,4] These chips are small, solid supports onto which the sequences from thousands of different genes are immobilized at fixed locations which means a single DNA chip can provide information about thousands of genes simultaneously. DNA microarrays work on the principle of base pairing and hybridization. Microarrays are available in two different formats – oligonucleotide arrays and cDNA microarrays.[5-8] Oligonucleotide arrays are made by synthesizing specific oligonucleotides in a specific alignment on a solid surface using photolithography whereas cDNA arrays are produced by printing a double stranded cDNA on a solid support (glass or nylon) using robotic pins. The study of gene expression using microarrays is based on the competitive hybridization of differently labeled populations of cDNAs. Fluorescent dyes, usually Cy3 and Cy5, are used to distinguish cDNA pools

reverse transcribed from different mRNA samples that have been isolated from cells or tissues.[9] The labeled cDNAs are applied to the microarray and allowed to hybridize under conditions analogous to those established for Southern blotting. After the slide is washed to remove nonspecific hybridization, it is read in a laser scanner that can differentiate between Cy3- and Cy5-signals, collecting fluorescence intensities to produce a separate 16-bit TIFF image for each channel. [10-12] The relative intensities obtained for each channel are then normalized to adjust for differences in labeling and detection efficiencies so that the two data sets become comparable and ratios of intensity for each spot can be calculated. Spots that fluoresce predominately in one color or the other, indicates a gene that is differentially expressed in the sample. The result can be quantified by measuring the intensity of fluorescence, which corresponds to the amount of gene expressed in the sample. [13,14]
Different softwares can then be used to interpret this crude data. Automation and connectivity are the backbone of any microarray laboratory. The three major steps of a microarray technology are preparation of microarray, preparation of labeled probes and hybridization and finally, scanning, imaging and data analysis.[15,16]
DNA microarray technique can efficiently use bioinformatics tools to analyze large amount of data generated via an individual experiment. DNA microarray enables the comprehensive measurement of the expression levels of hundreds of genes, simultaneously. Using this technique, a comprehensive understanding of the cell can be achieved.[17-18] However, because even simple life forms, such as microorganisms, have more than a thousand kinds of genes, the data from a DNA chip cannot be analyzed without statistical and informational technology. Bioinformatics is the interdisciplinary research field integrating molecular biology with informatics.[19,20] There are many techniques in bioinformatics for the analysis of DNA microarray data; however, these are mainly divided into fold-change analysis, clustering, classification, genetic network analysis and simulation.

Applications of DNA Microarray Technology

The possibilities of microarray technology in healthcare are unlimited. The technology could prove central to genomic research, molecular disease diagnosis, clinical research, drug discovery and in development of personalized medicine. DNA microarrays have helped to define molecular features of cancer progression as well as to distinguish between the nonmetastatic and the metastatic phenotype, such as in melanoma and medulloblastoma. It has been found to be extremely useful for the identification of sets of genes whose expression define behavior of individual tumor. Due to this, classification of carcinomas at the molecular level and prediction of how various types of tumor respond to different therapeutic agents can be made possible. In addition, the technology has also been used to identify novel drug targets especially in pancreatic cancer.[21]

Also, microarray technique could be instrumental in personalized medicine development by providing microarray data of a patient which could be used for identifying diseases. Susceptibility to a given disease is unique to individual's genetic make-up. An individual's response to a drug is dependent on his or her ability to metabolize the drug. DNA microarrays could tell the whole process related to drug metabolism and subsequent specific treatment.[22]

DNA microarrays are being analyzed to understand the innate immune system, including the cellular response to various bacterial endotoxins. Despite the availability of antibiotics, bacteremia remains a serious health problem and is a leading cause of death in intensive care units. For gram-negative bacteria, these effects are mediated largely by the bacterial endotoxin lipopolysaccharide (LPS). DNA microarrays are now being used to identify other genes that may be regulated in response to endotoxins, such as LPS, with the ultimate hope of providing new drug targets for sepsis.

DNA microarray technology can also be used in the area of infectious diseases, to identify novel drug targets to pathogen and also to elucidate protective response in the host. In the current scenario, where new pathogens, for example, human mutant of the bird-flu virus, H1N1, are expected every year, DNA microarray promises as an efficient technology to detect new organisms in a short time. Microarray analysis could be beneficial in the area of molecular medicines for analysis of genetic variations and functions of genes in normal individuals and diseased conditions. This technology may also be appropriate for monitoring liver toxicity in the patients. The technique can give satisfactory results in single nucleotide polymorphism (SNP) analysis and pharmacogenomics studies i.e. correlation between therapeutic responses to drugs and genetic profiles of the patients. In other words, technique can be applied to monitor changes in gene expression in response to drug treatment.[23]

The growing needs of genomics and proteomics for the analysis of gene and protein function for global bioremediation are enhancing the need for microarray-based assays enormously. Protein microarray technology has been successfully implicated for the identification, quantification and functional analysis of protein in basic and applied proteome research. Other than the DNA chip, a large variety of protein- microarray based approaches have already been verified that this technology is capable of filling the gap between transcriptomics and proteomics. However in bioremediation, microarray based protein–protein interaction studies still need to make progress to understand the chemotaxis phenomenon of any site specific bacterium towards the environmental contaminant. The recent combined approaches of transcriptomics and proteomics with microarray technology have revealed new pathways for aerobic and anaerobic biodegradation of toxic wastes that will certainly lead to further identification of new signature proteins.[24]

Microarrays are often viewed as screens to identify markers for traditional diagnostics, such as immunohistochemistry, for routine clinical use. However, immunohistochemistry is generally non quantitative, identification of antibodies can be laborious, and multiplexing is not easy. More sophisticated and high-throughput validation methods are required. An alternative view would be to actually use microarrays in

the clinic. This would require either custom arrays for different indications or whole genome analysis of every sample coupled with an analysis of relevant genes. As commercially available, low-cost, technically simple array and easy-to-use analytic technology become accessible; its routine clinical use can be explored. In addition, the resulting data could populate large expression databases that would serve as growing, centralized, and standardized references to which new cancer samples could be compared. The feasibility of routine clinical use of microarrays, however, has yet to be established.

Advantages of DNA Microarray Technology

Microarray technique has a number of advantages over traditional technology. First, it reduces the labor-intensive process of manually transferring and handling samples, saving time and reducing errors. Second, it requires smaller amount of fluids, in microlitre quantity. Third, it is an automated process and gives high degree of flexibility. In contrast to several traditional approaches such as Northern blots which limit research to one-gene-at-a-time experiments, microarray assays allow a large scale experiment which involves monitoring the expression levels of thousands of genes simultaneously at a particular time and at a particular condition. One of the most popular uses of microarray is to measure gene expression levels in two different samples under two different conditions. It allows the study of patterns of gene expression across many experiments that survey diverse cellular responses and conditions. When used properly, gene expression profiling techniques promise a wealth of data that can be used to develop a more complete understanding of gene function regulation and interactions.[25]

Present & future DNA challenges of Microarray Technology

The major challenges that arise with the technology are high degree of variability in data obtained, frequent up gradation of methods and machines and lack of trained manpower. The procedure of micro dissecting specimens is very expensive at the moment and it is difficult to select just the diseased cells under consideration, as normal and mutated cells can be in very close proximities of each other and can even overlap.

One limitation of microarrays is the tendency to select genes that are more abundantly expressed which may be a technical hurdle that simply needs to be overcome by improved methods of labeling and signal detection. Related to this issue is the observation that despite the miniaturization of microarrays, a considerable amount of sample is still required such that protocols will recommend labeling cDNA equivalent to 10 µg of total RNA, which may be possible in some systems but difficult to achieve in others, such as for micro dissected or flow cytometer sorted cells. A whole area of research focusing on obtaining valid data from exceedingly small amounts of material has arisen, leading to the development of such methods as for in vitro amplification of RNA , reverse transcription from single cells , and the use of tyramide-based techniques that can amplify fluorescent signals up to 1,000-fold .[26]

The assumption of the close correlation between RNA expression and gene function must nevertheless be tested for genes that are required for further study. The apparent levels of RNA expression should be confirmed independently either by Northern hybridization, quantitative PCR methods, or RNAse protection assays. The complementary methods of analysis, such as in situ hybridization and immunohistochemistry, will continue to be valuable in providing spatial information likely to withstand even the most patient practice of micro dissection of cells from tissue.

From the point of view of the technology, microarrays seem to be headed toward becoming more comprehensive, not only increasing the number of elements that can be arrayed, but expanding the kinds of materials that are arrayed, such as the spotting of peptides and other small molecules at one end and the arraying of micro sections of cells and tissue at the other. At first glance, these trends appear to be driven solely by technology but these developments are driven by a realization that gene expression, however global, is only part of what defines a gene and that it will be necessary to integrate information obtained from different areas, such as from genomic structure, RNA expression profiles, protein interaction mapping, and compartmentalization of gene function in time and space, to formulate increasingly meaningful hypothesis that can be tested at several fronts and, finally, to arrive at truly define a human genome.

Table 1: Types of DNA Microarray & their applications [30]

Microarray type	Description	Application
Expression Analysis	Analysis of gene expression levels, identify diseased tissue genes	Drug development, Drug response tracking, Disease progression, Drug mechanism of action,
Mutation/Polymorphism Analysis	Detect mutations /polymorphisms in gene sequence, Single Nucleotide Polymorphism (SNP) analysis	Drug response tracking, Disease progression, Disease risk assessment, Genotyping Species identification, Population genetics
Comparative Genomic Hybridization (CGH)	Identification in the increase or decrease of the chromosomal fragments, detect chromosomal aberrations	Tumor classification, Disease diagnosis, Risk assessment

Conclusion

DNA Microarray technology enables scientists to investigate and address tissues which were once thought to be non traceable. DNA microarray promises to be the next generation sequencer which could explain how organisms evolve and adapt looking at the whole genome. DNA microarray technology could be foreseen as an automated process for screening of compound targets, diagnostic and drug development with improved efficiency, quality and reliability of data, helping to reduce overall cost of research and development.[27-29]

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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