Microarray and its applications

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Received : 01-12-11
Review completed : 02-01-12
Accepted : 26-01-12

ABSTRACT

Microarray is one of the most recent advances being used for cancer research; it provides assistance in pharmacological approach to treat various diseases including oral lesions. Microarray helps in analyzing large amount of samples which have either been recorded previously or new samples; it even helps to test the incidence of a particular marker in tumors. Till recently, microarray's usage in dentistry has been very limited, but in future, as the technology becomes affordable, there may be increase in its usage. Here, we discuss the various techniques and applications of microarray or DNA chip.

KEY WORDS: Cancer, human genome, microarray, tissue microarray

Once the human genome sequence was completed in 2001, it paved the way for many experiments and researches; one such area was identifying the regions of DNA which control normal and disease states. Functional genomics is the study of gene function through parallel expression measurements of a genome. The most common tools used to carry out these measurements include complementary DNA microarrays, oligonucleotide microarrays, or serial analysis of gene expression (SAGE). Microarray analysis can be divided into two main steps: probe production and target (cDNA) production. Specific sequences are immobilized to a surface and reacted with labeled cDNA targets. A signal resulting from hybridization of the labeled target with the specific immobilized probe identifies which RNAs are present in the unknown target sample.

Prevention, diagnosis, and treatment in dental practice are based on an understanding of the biology of underlying oral health and disease. Few aspects of patient care will remain untouched by today's rapid advances in biological research. In the future, dentists may use inexpensive but remarkably sophisticated diagnostic tests to diagnose infection, oral lesions, and symptoms of temporomandibular dysfunction (TMD). The small variations in the DNA sequence that lead to different characteristics (such as skin color, facial features, or height) are known as polymorphisms, which also can cause or contribute to the development of many syndromes and diseases.[1] These genetic variations can be easily identified by the microarray technique.

Microarray provides a basis to genotype thousands of different loci at a time, which is useful for association and linkage studies to isolate chromosomal regions related to a particular disease. This array also can be used to locate chromosomal aberrations related to cancer, such as segments of allelic imbalance, which can be identified by loss of heterozygosity.[2] By comparative genomic hybridization techniques on genomic DNA, amplified or deleted regions in the chromosomes can be identified, such as in the case of oral cancer.[3]

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Quick Response Code:

Website: www.jpbsonline.org

DOI: 10.4103/0975-7406.100283

Technique

Gene microarray technology rests on the ability to deposit many (tens of thousands) different DNA sequences on a small surface, usually a glass slide (often referred to as a "chip"). The different DNA fragments are arranged in rows and columns such that the

How to cite this article: Govindarajan R, Duraiyan J, Kaliyappan K, Palanisamy M. Microarray and its applications. J Pharm Bioall Sci 2012;4:310-2.

identity of each fragment is known through its location on the array. Two types of microarrays are gene expression microarray and tissue microarray (TMA). Techniques like Northern blot and reverse transcriptase-polymerase chain reaction (RT-PCR) allow testing for only a few genes per experiment. But microarray or "global expression profiling" not only looks at orders of magnitude more genes than was possible previously, but also has the advantage that the genes examined are not influenced by preselection of genes.

Microarray Principle

mRNA is an intermediary molecule which carries the genetic information from the cell nucleus to the cytoplasm for protein synthesis. Whenever some genes are expressed or are in their active state, many copies of mRNA corresponding to the particular genes are produced by a process called transcription. These mRNAs synthesize the corresponding protein by translation. So, indirectly by assessing the various mRNAs, we can assess the genetic information or the gene expression. This helps in the understanding of various processes behind every altered genetic expression. Thus, mRNA acts as a surrogate marker. Since mRNA is degraded easily, it is necessary to convert it into a more stable cDNA form. Labeling of cDNA is done by fluorochrome dyes Cy3 (green) and Cy5 (red). The principle behind microarrays is that complementary sequences will bind to each other.

The unknown DNA molecules are cut into fragments by restriction endonucleases; fluorescent markers are attached to these DNA fragments. These are then allowed to react with probes of the DNA chip. Then the target DNA fragments along with complementary sequences bind to the DNA probes. The remaining DNA fragments are washed away. The target DNA pieces can be identified by their fluorescence emission by passing a laser beam. A computer is used to record the pattern of fluorescence emission and DNA identification. This technique of employing DNA chips is very rapid, besides being sensitive and specific for the identification of several DNA fragments simultaneously.

The study of the expression of most, if not all, genes in a specimen is not hypothesis-driven as most of the studies used to be,[4] but is instead referred to as "discovery-type research" or in a less flattering description as "fishing expeditions." Whereas cDNA derived from a tumor is hybridized to a chip to study gene expression levels, alterations in DNA copy number (gene amplification or deletion) can be measured by hybridizing fluorescently labeled DNA from a tumor specimen to these chips.[5,6] TMAs are constructed by transferring cores of paraffin-embedded tissue to pre-cored holes in a recipient paraffin block.[7] Over 500 cores can be placed in a single block by this technique. Sections cut from TMA blocks can then be used for immunohistochemistry (IHC) or *in situ* hybridization studies. TMAs are similar to gene expression microarrays in having samples arrayed in rows and columns on a glass slide; they differ in that each element on the TMA slide corresponds to a single patient sample, allowing multiple patient samples to be assessed for a single molecular marker in one experiment, while gene expression arrays allow assessment of thousands of molecular markers on a single patient sample per experiment.

Applications

In cancer

Tumor formation involves simultaneous changes in hundreds of cells and variations in genes. Microarray can be a boon to researchers as it provides a platform for simultaneous testing of a large set of genetic samples. It helps especially in the identification of single-nucleotide polymorphisms (SNPs) and mutations, classification of tumors, identification of target genes of tumor suppressors, identification of cancer biomarkers, identification of genes associated with chemoresistance, and drug discovery. For example, we can compare the different patterns of gene expression levels between a group of cancer patients and a group of normal patients and identify the gene associated with that particular cancer.

Gene microarrays have been used for comparative genomic hybridization. In this technique, genomic DNA is fluorescently labeled and used to determine the presence of gene loss or amplification.[6,8,9] Array-based comparative genomic hybridization (aCGH) has been used to map genetic abnormalities in a wide range of tumors, including breast carcinoma,[9] bladder carcinoma,[10] fallopian tube carcinoma,[11] gastric carcinoma,[12] melanoma,[13] and lymphoma.[14] Gene expression data can identify groups of cases with significantly different outcomes where routine histopathologic examination does not permit subclassification.

The conversion of a non-invasive tumor to an invasive tumor also warrants research. Clark *et al.* studied the gene profile of melanoma cells which became metastatic, and found a gene, *RhoC*, to be expressed more in metastatic cells than in non-metastatic melanoma cells. Microarray-based expression profiling allows us to identity families of genes as well as the important molecular and cellular events that might be important in complex processes like metastasis. Practical applications in future include diagnostic and prognostic management of patients. Clinicians will be able to use microarrays during early clinical trials to confirm the mechanisms of action of drugs and to assess drug sensitivity and toxicity. They can be used to develop a new molecular taxonomy of cancer, including clustering of cancers according to prognostic groups on the basis of gene expression profiles.[15] Areas that can be coupled with microarray technologies include classification of diseases, or molecular phenotyping; the study of gene function in relation to gene regulatory networks, or functional genomics; pharmacogenomics and developmental biology.

Antibiotic treatment

Increase in the number of resistant bacteria and superadded infections has led to failure of antibiotics. Virulence of the bacterial strains too affects the outcome of the disease process. In oral cavity where anaerobic bacteria might be the infective agent, they often are not easily culturable, especially organisms such

as actinomyces. DNA microarray analysis helps as the bacterial genomic DNA often outlasts the viability of the bacteria and a diagnosis can be made using a small amount of DNA, as opposed to the large numbers of bacteria needed for culture. In future, an abscess specimen might be sent not for culture and sensitivity testing, but rather for DNA microarray analysis.

Early detection of oral precancerous lesions

Leukoplakia or white lesions of the oral cavity may result from a myriad of reversible conditions. Currently, microscopic examination fails to identify the small subset of these lesions that progress to oral cancer. Identification of gene expression profiles or "genomic fingerprints" will allow clinicians to differentiate harmless white lesions from precancerous lesions or from very early cancer. Recent studies have illustrated the effectiveness of microarrays in oral cancer.[16,17] In future, samples taken from an incisional biopsy or brush biopsy may be sent to a laboratory for gene expression analysis. Early diagnosis and management of oral cancer is correlated with increased survival. Identification and treatment of premalignant and early cancerous oral lesions may become one of the most valuable services in future performs.

Conclusion

This review has given a small outline of the technique behind microarray and the various steps involved. The technique, though limited at present in its applications due to the cost factor, may widen its prospects once there is increase in the availability of commercial products. The ability to record large number of old samples and analyzing them for various genetic alterations helps in understanding the concept of molecular biology. Microarrays hold much promise for the analysis of diseases in the oral cavity. Classifications of oral disease by DNA, RNA, or protein profiles will greatly enhance our ability to diagnose, prevent, monitor and treat our patients. Currently, microarrays are primarily a research tool. Microarrays promise a more biologically based, individualized, and vastly improved standard for oral care, which will have great clinical impact on the way dentistry will be practiced in the future.

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Source of Support: Nil, **Conflict of Interest:** None declared.