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Microarray: A diagnostic tool in periodontal diseases

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Abstract
Revolutionary advances are underway that will dramatically change our understanding of periodontal diseases. The phenomenal progress being made in biomedical research is in large part fueled by advances in our overall knowledge of the human genome, development of microarray technology that allows comprehensive and unbiased evaluation of global biologic pathways and networks and expanded computational abilities. Expectations are all the periodontists will be affected by advances in molecular medicines, which in turn promises to lead to more accurate diagnosis, effective disease monitoring and development of targeted and specific therapies. Present review provides a brief overview to discuss the basic concept for understanding the potential of microarray technology in periodontal diseases.

Keywords: periodontal diseases, human genome, microarray, etc.

Introduction

As periodontal disease is a bacterial infection, it is very important to know which bacteria are present before treatment begins. Using microarray, we are able to identify exactly which bacteria are causing the infection. In the majority of cases, microarray will reveal that the type of bacteria that are present in the gums can be eliminated without the use of antibiotics. In these cases, it is preferable to avoid antibiotics in order to prevent a drop in the immune system. However, some species of bacteria cannot be permanently eliminated using the treatment alone. Actinobacillus actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg) are aggressive species of bacteria that fall under this. If the test shows that these bacteria are present, then appropriate antibiotics are added to the treatment in order to produce complete healing. However, it is unknown, whether the clinical signs following periodontal therapy are associated with an expression profile of inflammatory and immunological genes that is compatible with periodontal health.

Despite the large amount of clinical data, changes in the inflammatory status following non-surgical therapy have only been sparsely studied in humans. Histological studies have indicated that the gingival infiltrate associated with dental plaque is mainly characterized by a B cells and plasma cells [1]. Following therapy, the density of CD19, CD20, CD30 and CD45RO positive cells were found to be reduced, while the overall relative number of CD3 positive cells remained unchanged [2-5]. Few studies that have attempted to analyze the expression of inflammation related genes following non-surgical therapy; they have been limited to selected genes, e.g. IL-$^{1\beta\text{IFN}}$-γ [6-7], IL-2, IL-4, IL-5, IL-6, and TNF-α [7]. A more comprehensive analysis of gene expression related to immune and inflammatory processes is, however, needed to fully appraise the genes involved in tissues homeostasis following therapy.

Therefore, the aim of this technology is to analyze the expression profiles of a broad spectrum of genes associated to immune or inflammatory processes in the gingiva of periodontitis sites following periodontal therapy and to identify candidate genes that may serve as targets for new treatment or diagnostic strategies.

Principle of microarray

A DNA microarray (synonyms DNA chip or DNA array) can be thought of as a miniaturized form of dot-blot, but in a high throughput format [8] or miniaturized gene-hybridization or gene-detection assay [9]. DNA microarrays represent DNA fragments attached to the surface in a predefined ordered fashion at high density. The principle of microarray experiments is that the mRNA or total RNA from given cells or tissue is used to produce a labelled sample, which is hybridized in parallel with a large number of DNA sequences immobilized on a solid surface in an ordered array. Global analysis of thousands of gene expressions may be performed with the accessibility of genome information and development of microarray method in a single assay [10, 11]. Each microarray experiment typically follows several steps in defined order: array fabrication, target preparation, hybridization, washing, image capture, and data analysis [12].

Array fabrication or construction of DNA microarrays (fig.1)

It is a several-step procedure which involves obtaining the DNA sequences, design of oligonucleotides or primers for generating probe DNA, selection and preparation of suitable glass surface and depositing the probe DNA on its surface. Production of an array begins with the selection of the probes

to be printed on it. In most cases, these are chosen directly from databases such as Gen Bank, [13] dbEST, [14] and UniGene [15] Which are the resource backbones of the array technologies. [16] Additionally, full-length cDNAs (complementary DNA), collections of expressed sequence tags (ESTs) or partially sequenced cDNAs or randomly chosen cDNAs/DNAs from any library of interest can be used. The probes DNA are oligonucleotides or polymerase chain reaction (PCR) products amplified from an individual clone using specific primers or universal primers if all genes were cloned in the same vector [12].

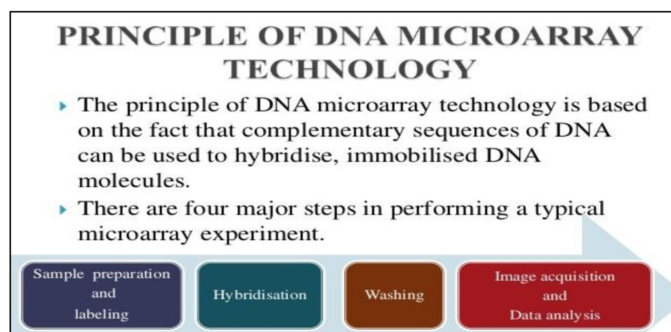

Fig 1

Preparation of target DNA: Target DNA is described as the labelled DNA which when applied to the microarray and hybridize the c DNA attached to the slide surface. The target DNA is usually created by reverse transcription of RNA into cDNA in such a way that a fluorescent nucleotide is incorporated. Preparation of target DNA includes RNA extraction from cells or tissue, conversion to cDNA and it's labelling.

Hybridization: it is the process of incubating the labelled target DNA with the probe DNA bound to the microarray substrate. Fluorescent target DNA hybridized to cDNA on the slide, and the number of immobilized fluorescence or radioactivity can be determined. Hybridization of the labelled probe is ideally linear (i.e. proportional to the amount of the probe) and sensitive (low abundance genes are detected) and specific (targets hybridize only to the desired genes in the complex probe mixture) [12].

Washing: After hybridization, the array goes through a series of washes to eliminate all unbound labelled target cDNAs. Washing breaks unstable binding of target and probe cDNAs, which may be the result of cross-hybridization. Washing is important, as incomplete washing causes deposition of salts or fluorescent materials that may cover the array partly or completely. Image

Capture: Data is captured from microarrays hybridized with the 33P-labeled target by means of a phosphor imager system. Microarray reader or scanners capture the signal intensities of all the spots on the glass slide with fluorescently labelled target. They are computer-controlled inverted scanning fluorescent microscope with a double or multiple lasers illumination system. Laser stimulates the microarrays hybridized with fluorescent targets. The emitted fluorescence is then captured by a CCD camera, non-confocal or confocal laser scanner.

Data analysis

Image processing is the first step of data analysis. Images generated by a microarray scanner represent the raw data of any microarray experiment. Computer algorithms convert the image into the numerical information that quantifies gene abundance. The second transformation, called normalization, removes non-biological influences on biological data, including unequal quantities of starting RNA, differences in labeling or detection efficiency. The third and last part of data analysis is one of the core goals of microarray analysis: to identify which gene is differentially expressed [12].

Conclusion

Microarrays made important contributions to both the basic and applied research with promise to change future practice of medicine, as well as dentistry. However, as Ingen *et al*. [16] in 2002 quoted, "although, nearly a decade has passed since first microarrays were produced, and yet we are just beginning to perceive what can be achieved with this technology," its solicitation in all disciplines of oral as well as systemic health is under development. Future will allow researchers to provide improvements in diagnosis, prevention and various techniques to provide better health management of the patients, based on randomized clinical trials.

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