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REVIEW ARTICLE

Microarrays: applications in dental research

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Revolutionary advances are underway that will dramatically change our understanding of oral 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 that nearly all clinical areas in dentistry and oral medicine will be affected by advances in molecular medicine, which in turn, promises to lead to more accurate diagnosis, effective disease monitoring, and development of targeted and specific therapies. This review provides a brief overview of microarray technologies and highlights several key examples from research efforts in dentistry and oral medicine using these powerful new tools.

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Introduction

With completion of sequencing the human genome and development of powerful microarray-based technologies, biomedical research is being revolutionized by the ability to carry out the investigations on a genome-wide scale. In general terms, microarrays refer to a variety of technical platforms in which high-density assays are performed in parallel on a solid support (Moser *et al*, 2004a). Microarrays are typically small rectangles of glass or nylon membrane to which high-density DNA or protein probes are arranged in a regular grid pattern. Samples are applied to the microarray for measurement of potentially hundreds of thousands of targets simultaneously in a single experiment.

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In dentistry, prevention, diagnosis and treatment are based on an understanding of the biologic process behind health and diseases (Kuo *et al*, 2003). The great complexity of such processes is attributable to synergism among genes. Accordingly, research aimed to improve treatment of oral diseases is likely to be most successful when the involvement of multiple genes expressed simultaneously is taken into joint consideration. Research strategies of this type, involving a large number of genes, are greatly facilitated by high-density microarrays, because they allow a 'global' view of complex biologic pathways that are differentially active under various conditions. The high-throughput nature of microarray technology also allows large volumes of data to be collected, potentially advancing our knowledge at greatly accelerated speed. Therefore, microarrays hold much promise for improving detection and an analysis of diseases in the oral cavity as well as facilitating major advances in therapeutic strategies.

In this commentary, we review the basic principles of microarray technology and the progress and potential of this methodology in dentistry.

Microarray technology

Currently, three major types of microarrays exist – tissue, protein and DNA (Moser *et al*, 2004a). Tissue microarrays immobilize small amounts of tissue from biopsies of multiple subjects on glass slides for immunohistochemical processing. Tissue microarray technology has been developed in an effort to overcome the limitations of standard histologic methods (Shergill *et al*, 2004). In protein microarrays, peptides or intact proteins are immobilized for detection by antibodies or other means. Applications of protein microarrays include assessment of enzyme–substrate, protein–protein and DNA–protein interactions. However, protein microarray technology is still in relatively early stages of development (Glökler and Angenendt, 2003). DNA microarrays are the most widely utilized application of microarray technology, where thousands to tens of thousands of datapoints may be generated in each experiment. Both genetic applications, in which hundreds of thousands of single nucleotide polymorphisms

can be genotyped, or genomic applications, in which mRNA transcripts are interrogated as a measure of gene expression, are now available and being applied in numerous biomedical conditions.

Basic principle
The human genome contains approximately 30 000 genes (Eschrich and Yeatman, 2004). In each gene, DNA exists as a double-stranded polymer containing four DNA bases tethered to a sugar-phosphate backbone. When a gene is expressed, mRNA is transcribed from DNA and serves as a template to guide the synthesis of a protein. Because an mRNA transcript is an exact copy of its complementary DNA coding region, genomic analysis of the mRNA level can be used as a measure of gene expression.

Array procedure
The most common application of microarray technology is gene expression profiling, in which mRNA levels are detected using either of two commonly available DNA microarray platforms – cDNA microarrays or oligonucleotide microarrays. cDNA microarrays are fabricated by robotic spotting of cDNA fragments onto glass slides. Samples are then tested through competitive hybridization in which reference cDNA and investigated RNA are labeled after reverse transcription with different fluorescent dyes (e.g. Cy3 for the reference cells and Cy5 for the investigated cells) and hybridized to a cDNA microarray. The slides are scanned with a laser scanning system, and two false color images are generated for each hybridization with RNA from the investigated cells and reference. Genes upregulated in the investigated cells are conventionally designated red, whereas those with decreased expression appear green, as they are more expressed in control samples. Genes with similar levels of expression in the two samples appear yellow. The overall result is the generation of a so-called 'genetic portrait' (Carinci *et al*, 2004; Francioso *et al*, 2002).

The second type of DNA microarray uses *in situ* synthesized oligonucleotide arrays fabricated using photolithographic chemistry on silicon chips. Sample RNA is processed to generate labeled transcripts that are then hybridized directly to the oligonucleotide probes. The microarray is then scanned for intensity of the signal, which is relative to the abundance of each RNA transcript. In principle, the oligonucleotide probes offer much higher specificity than probes based on amplified cDNA, because if designed properly, they can discriminate between two closely related members of a gene family (Reilly *et al*, 2004).

Microarrays have also been developed for applications such as comparative genomic hybridization (CGH) with the ability to examine segmental genomic alterations in diseased cells (Davies *et al*, 2005). CGH arrays are similar in principle to cDNA microarrays in that both the experimental and control samples are differentially labeled and compared by competitive hybridization against a wild type chromosome. Thus, cDNA and oligonucleotide microarrays investigate the differential expression levels of genes, whereas the CGH arrays detect gains or losses at the chromosome level.

Data analysis
Microarray analysis is often considered 'discovery based' rather than 'hypothesis driven', because of the potential for discovering altered expression of novel genes for which little or no prior basis to suggest a role in the disease or experimental condition examined has been considered (Albelda and Sheppard, 2000; Staudt and Brown, 2000). High-quality experiments are performed by addressing a scientific question with consistency in the execution of experimental protocols, by the use of sample sizes with as many replicates as possible, and with a plan for statistical analysis and interpretation of data.

There are three general approaches to data analysis: prognostic prediction, class discovery, and class comparison (Simon *et al*, 2002). Prognostic prediction methods are used when two or more groups of samples are analyzed, and the objective is to develop a model for prediction of class membership for new samples. This approach is commonly used in studies designed to predict various clinical outcomes. Class discovery is often applied to data sets with a heterogenous sample and the objective is to identify novel subsets of samples that may not otherwise be discernable using conventional techniques, such as morphologic or histologic criteria. Class comparison involves two or more predefined groups, such as patients and controls, for which a function is determined to find the genes that best discriminate between the groups. For each of these approaches, a number of statistical methods exist, and no common method is used for the analysis and interpretation of the complex data sets generated by microarray procedures (Slonim, 2002).

Current limitations
While microarrays are designed to give a genome-wide view of the cell on an unprecedented scale, there are some limitations that inhibit broad use of this technology outside certain research settings. One drawback is cost. Each array, depending on the platform, may cost hundreds of dollars (Kuo *et al*, 2003). Another challenge is the efficient management and analysis of the large volume of data generated by microarray approaches. However, increasingly sophisticated computational methods continue to be developed that are amenable to large data sets generated from microarray experiments (Kirmizis and Farnham, 2004). Certainly, as key disease pathways are identified, custom arrays containing relevant subsets of genes may eventually be integrated into clinical settings for more widespread use.

Dental implications

Changes in the physiologic state of cells and tissues are associated with specific changes in gene expression patterns, thus microarray analysis of oral-related diseases can provide valuable information regarding complex biologic networks. Application of microarray technologies covers a broad spectrum and includes area

such as classification of diseases based on molecular characteristics, investigation of gene functions in relation to the gene-regulatory networks, identification of putative pathways involved in congenital and developmental abnormalities, prediction of efficacy and toxicity of therapeutic regimens, and the development of drugs for targeted therapies (Kuo *et al*, 2002).

Oral cancers

Microarrays have diverse applications in oral cancers, including early diagnosis of the transformation of premalignant lesions, identification of malignancy in tissue biopsies, subclassification of histologically identified tumors, identification of biomarkers, and drug discovery (Todd and Wong, 2002). In premalignant lesions, such as leukoplakias and erythroplakias, microarrays have been used to identify genes that could serve as biomarkers for dysplastic lesions with the potential to progress to cancer (Carinci *et al*, 2005). By comparison of normal tissues with both mild and severe premalignant lesions, these studies reveal alterations in gene expression patterns related to progression toward malignancy and include genes involved in DNA repair, oxidative stress, cell-adhesion/motility, cell cycle regulation, and others. Characterization of molecular pathways that are dysregulated leading to malignant transformations can be targeted for early diagnosis and therapy.

In head and neck squamous cell carcinomas (HNSCC), DNA microarray techniques can offer molecular screening for specific cancers and the possibility of identifying candidate genes that are predictors of tumor subtypes. At least four subtypes of HNSCC have been discovered using microarray techniques with clinically distinct behaviors (Chung *et al*, 2004). Gene expression data can also provide the information about specific genes that are assumed to play a crucial role in cancer formation and importantly, suggest biomarkers for current treatment failure that may lead to recurrence or metastasis (Ginos *et al*, 2004). These studies also provide a rationale for determining and eventually selecting patients most likely to benefit from specific therapies, such as using epithelial growth factor receptor tyrosine kinase inhibitors in clinical trials (Wirth *et al*, 2005). It will soon become routine practice to use purpose-designed commercial DNA microarrays to obtain 'gene expression fingerprints' for individual tumors, with the aim of improving prognosis, and of designing individualized treatment strategies.

Periodontal diseases

The current classification of periodontal diseases abolished certain previously accepted categories, and termed the major two types as chronic periodontitis and aggressive periodontitis (Papapanou *et al*, 2004). However, these new forms are based on combinations of clinical signs and symptoms only and so lack an equivocal, pathobiology-based foundation that would discriminate them efficiently. Research aimed to focus on the biologic basis underlying these distinct entities can be greatly facilitated by microarray technology. This strategy can also aid in the identification of subtypes under a particular entity by providing a detailed description of the distinct gene expression profiles that characterize each subtype. Efforts to understand the molecular mechanisms of the complex interactions between genes, and between genes and an environment in the causation of periodontal diseases will also build on insights and thereby control on treatment outcomes (Burke, 2003).

Endodontic infections

Microarrays can complement the culture-based methods used to identify the pathogenic bacteria in infected root canals (Vianna *et al*, 2005). The prevalence of certain bacteria, particularly the fastidious anaerobes, has often been underestimated in routine cultures because of the difficulty in growing them in such conditions. The sensitivity of microarray-based approaches can allow for not only the detection of the subtle differences that are much harder to detect with conventional methods, but also of identification of novel pathogenic organisms as well as investigation of determinants of pathogenicity and interrogation of underlying host–pathogen interactions (Bryant *et al*, 2004). Such approaches will provide a more comprehensive picture of the endodontic microbiota and will prove invaluable to our understanding of the pathogenic processes with consequent improvements in diagnosis, treatment, and prevention.

Dental caries

The multifactorial nature in the causation of dental caries involves interactions between genes, diets, microorganisms and life styles. Therefore, the preventive regimens often fail to eradicate the disease. The characterization of cellular and molecular events underlying dental-tissue injury and repair is critical to our understanding of these processes (McLachlan *et al*, 2003). Microarray technology can provide a reliable tool to obtain a comprehensive picture that includes recruitment of specific cell types, cellular activation mechanisms, expression of genes resulting from interactions between different cell populations, and molecules associated with repair (McLachlan *et al*, 2005).

Temporomandibular disorders and orofacial pain

Microarrays hold much promise for an analysis of temporomandibular disorders (TMD) and orofacial pain. The diagnosis of TMD is often challenging because of its multifactorial etiology. Researchers are now studying the mediators in the disease process within the joint fluid to develop biologically based diagnostic and therapeutic approaches (Kuo *et al*, 2003). In the future, clinicians may be able to better classify and treat TMD by microarray analysis of joint aspirates. Microarrays also have a great potential in pain research for determining the network of gene regulation in different pain condition and also for producing detailed/gene expression maps in anatomical areas that process nociceptive stimuli (Reilly *et al*, 2004).

Autoimmune diseases

High-throughput microarray assays are revealing important insights into key biologic pathways that

appear to be perturbed in systemic autoimmune diseases that affect the oral cavity such as Sjögren's syndrome (Moser *et al*, 2004b). Gene expression profiling has underscored common underlying mechanisms among related diseases including Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis. For example, recent microarray studies on Sjögren's syndrome and systemic lupus erythematosus have shown that genes inducible by interferons are upregulated in patients in both of these conditions compared with healthy controls (Gottenberg *et al*, 2006; Baechler *et al*, 2003). Likewise, important differences in expression profiles may provide opportunities to stratify patients based on molecular criteria and for evaluation of targeted therapies (van der Pouw Kraan *et al*, 2004).

Developmental disorders

Microarrays may prove useful for elucidating the pathophysiologic mechanisms of congenital and developmental abnormalities of oral and craniofacial structures, such as cleft lip and palate, and mandibular prognathism (Iida and Nishimura, 2002). Analysis of the genetic pathways and exploring the dynamic interactions of genes by microarrays can help modulate the developmental process. A critical gene whose expression level is dramatically altered in a specific developmental disorder may provide a specific target for therapeutic inhibition (Kuo *et al*, 2002).

Drug development

Microarray strategies are likely to prove very useful for drug development. This comprehensive experimental approach allows for the simultaneous analysis of candidate genes that might be used as biomarkers of drug activity as well as novel indicators of therapeutic response (Raponi *et al*, 2004; Clarke *et al*, 2001). Biomarkers that enable the monitoring of drug response also have the potential to facilitate clinical evaluation of safety and efficacy of drugs in humans.

Conclusions

Improved understanding of the molecular basis of dental and oral diseases will lead to the improvements in diagnosis, treatment, and prevention. Microarrays seem set to have major impacts on the management of these diseases. Potential applications may include prediction of the behavior of diseases and the effectiveness of targeted therapies. We should take advantage of this technology to translate biologic insights into clinical applications that are feasible in dental settings.

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