Chapter 14

Meta-Analysis Approach for the Identification of Molecular Networks Related to Infections of the Oral Cavity

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ABSTRACT

Chronic periodontitis is the most common infection of the oral cavity. Understanding how and why bacteria enter host cells, and how barrier cells respond to limit their impact, provides a biological basis of infection in the mixed bacterial-human ecosystem of the oral cavity. In addition, elucidation of the underlying shared pathogenic mechanisms of complex diseases like diabetes and oral infections can lead to new insight into the involvement of genes in increased susceptibility of patients with oral infections to complex systemic diseases and vice versa. Transcriptional profiling, statistical and ontology tools are used to uncover and dissect genes and pathways of human gingival epithelial cells that are modulated upon interaction with the periodontal pathogens. Affymetrix microarrays are applied to search the gene expression underlying infection with oral bacteria and identify distinct classes of up- and down-regulated genes during this process. The developed meta-analysis approach can help to extract sets of genes related to oral infection and interaction networks by integrating and combining quantitative gene expression data using statistical approaches. By means of overrepresentation analysis, the authors discovered molecular networks related to immune systems responses.

BACKGROUND

Humans are hosts of a complex population of microorganisms that colonize the oral mucosal membranes. More than 700 species of bacteria can inhabit the oral cavity (Aas et al., 2005). A subset of oral microorganisms, including F. nucleatum, Aggregatibacter (Actinobacillus) actinomycetemcomitans, and P. gingivalis, can invade host cells (Han et al., 2000; Handfield et
al., 2005; Lamont & Jenkinson 1998; Tribble et al., 2006).

Although it is well established that the bacterial inhabitants of the subgingival crevice are direct precursors of periodontal disease, the oral microorganisms form a spectrum ranging from commensals, such as Streptococcus gordonii, to aggressive pathogens, such as P. gingivalis. Other species like F. nucleatum, are frequently found in individuals with good oral health but are also able to contribute to disease (Avila-Campos et al., 1999; Dzink et al. 1988, Feng & Weinberg 2006).

Periodontitis is a common chronic inflammatory disease which may lead to tooth loss.

Inflammation involves production of reactive oxygen species (Chapple & Matthews 2007), raised levels of proinflammatory cytokines (Graves 2008), and elevated concentrations of matrix metalloproteinases (Sorsa et al., 2006) within periodontal tissues and fluid. Some of the genes that encode the molecular components involved in the inflammatory process might confer susceptibility to both periodontitis and rheumatoid arthritis (De Pablo et al., 2009; Nilsson & Kopp, 2008), resulting in an association between the two diseases. Furthermore, periodontal infections have been associated with systemic inflammation and risk for atherosclerosis and vascular disease (Desvarieux et al., 2004).

The goal of periodontal therapy is complete regeneration of the tissues lost as a result of periodontitis. Clinical and radiographic measurements are applied for the diagnosis of periodontitis but offer little information regarding the pathogenesis of the disease. Therefore, the comparison of diseased to healthy gingival tissue transcriptomes provide a valuable scientific tool that may reveal insights of the pathobiology of periodontitis (Demmer et al., 2008). The interdependency between host and pathogen in an infectious disease involves changes in gene expression. Clinical studies have investigated the similarities in cytokine profiles and gene expression profiles in blood cells between patients with and those without periodontitis.

**MICROARRAY ANALYSIS TO STUDY ORAL INFECTION**

Microarray analysis allows the simultaneous investigation of thousands of genes. The use of microarrays has become the method of choice for gene expression analysis of host-pathogen interaction (McGuire & Glass, 2005). Transcriptional profiling using microarrays provided a way to monitor host cell responses to microorganisms on a global scale (Cummings & Relman 2000; Mans 2006) and revealed differentially expressed up-regulated and down-regulated genes in oral infection.

Innate immune factors, have been found to be differentially regulated in host cells infected with pathogenic organisms compared to the regulation in uninfected controls (Handfield et al., 2005; Jenner, & Young 2005; Mans et al., 2006). Transcriptional profiling has been used to uncover and dissect genes and pathways of human gingival epithelial cells that are modulated upon interaction with periodontal pathogens like *Actinobacillus actinomycetemcomitans* and *Porphryomonas gingivalis*. Demmer et al. (2008) examined gene expression signatures in healthy and diseased human gingival tissues by using the Affymetrix platform (AffymetrixU133Plus2.0 arrays). Handfield et al. (2005) analyzed the gene expression of human immortalized gingival keratinocytes (HIGK) cells with transcriptional profiling by using Affymetrix microarrays to define the biological pathways of HIGK cells modulated by the oral pathogenic *Porphryomonas gingivalis* and *Aggregatibacter* (formerly actinobacillus) *actinomycetemcomitans*. The authors analyzed the gene expression underlying infection and identified up- and down-regulated genes between infected with either the oral pathogenic *P. gingivalis* (Pg) or *A. actinomycetemcomitans* (Aa) and non infected HIGK cells. In another study (E-GEOD-10526) HIGK cells were infected with either the oral pathogen *P. gingivalis* (Pg) or an isogenic mutant for SerB (Hasegawa et al.,