Salivary biomarkers for the detection of primary Sjögren's syndrome

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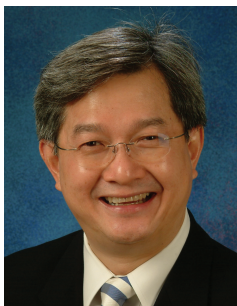
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Primary Sjögren's Syndrome (pSS) is characterized by lymphoid infiltrates, mainly in the lacrimal and salivary glands, and impaired secretory function of these moisture producing glands. Clinically, pSS occurs predominantly in perimenopausal and postmenopausal women. Owing to the nonspecificity and multiple facets of its symptoms, initial pSS diagnosis has been difficult. The refined criteria for diagnosis have facilitated the diagnosis of the established pSS, which often occurs years after the disease initiation [1]. In addition, the pathogenesis of pSS is very complex and still largely unknown. Furthermore, pSS can be associated with many other autoimmune diseases, and patients with pSS have a 20–40-fold higher risk of developing malignant lymphoma [2]. Currently treatment of pSS remains to be empiric and symptom-based [3]. It is therefore of pivotal importance to develop biomarkers for pSS, not only for prediction or early detection in order to prevent delay in diagnosis, but also for information that may help to elucidate the mechanisms of the disease, and provide possible molecular targets or rationale to optimize therapeutic treatment.

Human saliva is a mixed secretion from three pairs of major salivary glands (parotid, submandibular and sublingual) and multiple minor salivary glands lying beneath the oral mucosa. It is an attractive medium for disease diagnostics because saliva testing is simple, noninvasive and safe, especially under circumstances where it is difficult to obtain blood samples. Saliva harbors a wide spectrum of analytes, such as proteins, mRNAs, DNAs and metabolites, that may be informative for diagnosis of human diseases. To date, over 1000 distinct proteins in human whole saliva (WS) and 1100 proteins from parotid and submandibular/sublingual (SM/SL)

secretions have been identified [4–8]. A central database has been established to centralize identified saliva proteins for public access [101]. Meanwhile, by using high-density oligonucleotide microarrays, we have performed global profiling of mRNA in human WS, leading to the identification of more than 3000 human mRNAs in cell-free saliva [9]. These studies have laid the scientific foundation for human disease biomarker discovery in saliva [10]. We have also previously demonstrated proteome- and genome-wide approaches to harnessing saliva protein and mRNA signatures for human oral cancer detection [11,12].

' … saliva harbors informative protein and mRNA molecules that can discriminate pSS patients from control subjects.'

The logical vision that in pSS patients, signature biomarkers from the affected salivary parenchymal cells as well as the infiltrated lymphocytes will be shed into the lumen and secreted with saliva, combined with the inherent advantages of saliva testing and the urgent need of biomarkers for pSS, has triggered the studies on globally searching for saliva biomarkers of pSS. Indeed, saliva harbors informative protein and mRNA molecules that can discriminate pSS patients from control subjects. By using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) and two-dimensional difference gel electrophoresis (2D-DIGE), Ryu *et al*. profiled proteins in parotid saliva from pSS and control subjects. The SELDI-MS analysis revealed five proteins (11.8, 12.0, 14.3, 80.6 and 83.7 kDa) at increased levels and three proteins (17.3, 25.4, and 35.4 kDa) at decreased levels in SS samples. 2D-DIGE also identified significant increases of β-2-microglobulin, lactoferrin, immunoglobulin (Ig) κ light chain, polymeric Ig receptor, lysozyme C and cystatin C in all stages of SS. The study suggested that the salivary proteomic profile of SS is a mixture of increased inflammatory proteins and decreased acinar proteins when compared with non-SS saliva [13].

Using MS and expression microarray profiling, we have recently discovered a set of promising salivary targets that have diagnostic potential for pSS [14]. Comparative saliva proteomics indicated that 16 proteins were downregulated and 25 proteins were upregulated in patients with pSS as compared with matched controls. These proteins reflected the damage of glandular cells and inflammation of the oral cavity system in pSS. In addition, 16 peptides (10 upregulated and 6 downregulated in pSS) were found at significantly differential levels ($p < 0.05$) in WS between pSS and control groups. Using high-throughput expression microarray technology, we have identified 162 genes that are more than twofold ($p < 0.01$) and 27 that are more than threefold ($p < 0.0005$) upregulated in pSS saliva. Gene ontological analysis showed that these upregulated genes fall into the categories of autoimmune response, apoptosis and JAK-STAT cascades known to be involved in pSS pathogenesis. One of the interesting findings is that many upregulated genes in pSS are associated with activated interferon pathways. Many of the autoimmune mechanisms occurred in pSS can be induced by type I IFN indicating its important role in the pathogenesis of pSS [15]. These results support the rationale that saliva constituents reflect the underlying pathogenesis in the affected glands. We have further validated the differentially expressed salivary genes and proteins using real-time quantitative PCR (RT-qPCR) and immunoblotting, respectively. Our study also clearly suggested that WS contains more informative proteins, peptides and mRNAs than gland-specific saliva towards generating candidate biomarkers for pSS detection.

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Although promising targets have been discovered by means of salivary proteomics and transcriptomics, it is still a long journey before they can be used for clinical testing.

First and foremost, the clinical utility of these potential biomarkers requires further validation, preferably with different validation platforms and independent patient cohorts. Approval of use of a marker or set of markers for a specific disease relies on the results of large-scale multicentric clinical trials [16,17]. The eventual use of these biomarkers in clinical settings also requires

novel diagnostic devices, for example, point of care microfluidics-based diagnostic chips, for simple and high-throughput measurement of these biomarkers in patients' saliva [18,19]. In addition, the specificity of the discovered biomarkers needs to be further demonstrated.

> 'Other autoimmune disorders may complicate the clinical use of salivary biomarkers for pSS diagnosis … '

Other autoimmune disorders may complicate the clinical use of salivary biomarkers for pSS diagnosis, since patients with SS are often connected with other autoimmune diseases. For example, proteasomes are markers of cell damage and immunologic activity and have been used to distinguish pSS from various other rheumatic diseases, such as rheumatoid arthritis (RA) and systematic lupus erythematosus (SLE) [20]. Whether such cell damage and immune activity indicators are also informative in saliva remains to be demonstrated. In addition to the healthy control population, an autoimmune disease control group such as RA or SLE should be included in the study design to ensure the most discriminatory biomarkers will be attained for pSS detection.

For biomarker discovery, saliva proteomics will likely play a significant role in identifying promising targets in saliva from SS patients for the disease detection. The exquisite accuracy and sensitivity of MS measurements and sequencing capability by tandem MS ideally position the central role of MS-based proteomics in disease biomarker discovery. Specific autoantibodies (e.g., anti-Ro/SS-A and anti-La/SS-B) present in saliva may also be promising targets for disease detection [21] and the use of protein arrays for autoantibody profiling may also become a promising approach to the discovery of additional protein biomarkers for pSS.

Saliva biomarkers may also be useful for monitoring severity or progression of salivary gland dysfunction, or response to treatments. Since SS is a slowly progressive autoimmune disease, saliva analysis represents a promising approach to monitor the disease progression considering simple collection and processing of saliva samples. Comparative analysis of saliva proteomes and transcriptomes from patients with pSS, secondary SS and progressed malignant lymphoma may reveal distinct biomarkers for early detection of lymphoma within the patient population. Previous studies have indicated that the levels of certain salivary proteins in breast cancer

and oral lichen planus patients significantly changed during and after chemotherapy [22,23]. Similar studies can also be performed to monitor the SS patients' response to various treatments such as the interferon-based therapy. Salivary proteomics and genomics may be developed for evaluating the efficacy and toxicity of therapeutic treatments or classifying disease population for molecular targeted therapy.

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Website

101. UCLA Human Salivary Proteome Project www.hspp.ucla.edu

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