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Integrated Omics Analysis of Sjogren's Syndrome

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ABSTRACT

Sjögren's syndrome (SS) is a chronic autoimmune disorder clinically characterized by dry mouth and eyes. The pathogenic mechanism of SS is inadequately understood and a long delay from the start of the symptoms to final diagnosis has been frequently observed. In this paper, we aim to provide an overview about using omics technologies to discover biomarkers for SS diagnosis and understand potential pathways underlying SS pathogenesis. Omics databases relevant to SS such as Sjögren's Syndrome Knowledge Base, Saliva Ontology and SDxMart are also discussed

Keywords: Omics; Proteomics; System Biology; Bioinformatics.

1. Introduction

Sjögren's syndrome (SS), which was first described in 1933 by the Swedish physician Henrik Sjögren [1], is a chronic autoimmune disorder clinically characterized by xerostomia and keratoconjunctivitis sicca. The disease may occur alone as primary SS or present as secondary SS, which is associated with other autoimmune diseases such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). SS has an estimated prevalence of ~ 4 million patients in the US. It primarily affects women, with a ratio of 9:1 over the occurrence in men.

Histologically, SS is characterized by infiltration of exocrine gland tissues with predominantly T lymphocytes, leading to significant reduction of saliva and tear production (dry eyes and mouth). At the molecular level, glandular epithelial cells express high levels of HLA–DR, which has led to the speculation that these cells are presenting antigen (viral antigen or autoantigen) to the invading T cells. Cytokine production follows, with interferon-γ and interleukin-2 being especially important. There is also evidence of B cell activation with autoantibody production and an increase in B cell malignancy. SS patients exhibit a 40-fold increased risk of developing lymphoma than the general population, and the most common forms are mucosa-associated lymphoid tissue (MALT) lymphomas that remain localized to the salivary glands [2-4].

2. Discovery of saliva biomarkers for SS

Current diagnosis of SS requires an invasive salivary gland tissue biopsy and a long delay from the start of the symptoms to final diagnosis has been frequently observed. There has been increasing interest in developing saliva biomarkers for simple and early diagnosis of the disease. Saliva is the secreted fluid from three pairs of major salivary glands (parotid, submandibular, and sublingual), and multiple minor salivary glands that lie beneath the oral mucosa [5]. It 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 and 1100 proteins from parotid and submandibular/sublingual secretions have been identified [6-10].

Saliva is an attractive medium for disease diagnostics because it is simple to collect and process saliva samples and particularly because salivary testing is non-invasive safe and

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inexpensive. Saliva diagnostics is especially preferred under circumstances where it is difficult to obtain blood samples or repeated sample collection is needed for monitoring a disease. The biologic fluid has been used for the survey of general health and for the diagnosis of diseases in humans, such as human immunodeficiency virus, periodontal diseases, and autoimmune diseases [5, 11-14]. In patients with SS, signature biomarkers from the affected salivary glands may be shed into the lumen and secreted with saliva, which can be identified using modern omics technology. This notion - combined with the inherent advantages of saliva testing - created the emerging interest of developing saliva biomarkers for SS diagnosis, and triggered multiple studies on globally searching for saliva biomarkers of SS.

Proteomics is a powerful approach for global study of the structure and function of all proteins expressed in a biological system. Mapping proteomes from disease tissues and body fluids, has been used to identify new disease biomarkers for clinical and diagnostic applications. By using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and two-dimensional difference gel electrophoresis, Ryu et al. profiled proteins in parotid saliva from primary SS and revealed multiple candidate protein biomarkers for SS, including β-2-microglobulin, lactoferrin, immunoglobulin kappa (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 [15]. Hu et al. found that 16 whole saliva (WS) proteins were down-regulated and 25 WS proteins were up-regulated in patients with primary SS compared with matched healthy control subjects. These proteins reflected the damage of glandular cells and inflammation of the oral cavity system in SS patients. In addition, microarray analysis revealed that 27 mRNA in saliva samples were significantly up-regulated in the primary SS patients, most of which are interferon-inducible or related to lymphocytic infiltration and antigen presentation known to be involved in the pathogenesis of primary SS [5]. A panel of these biomarkers has been successfully validated in independent patient population

Figure 1 A

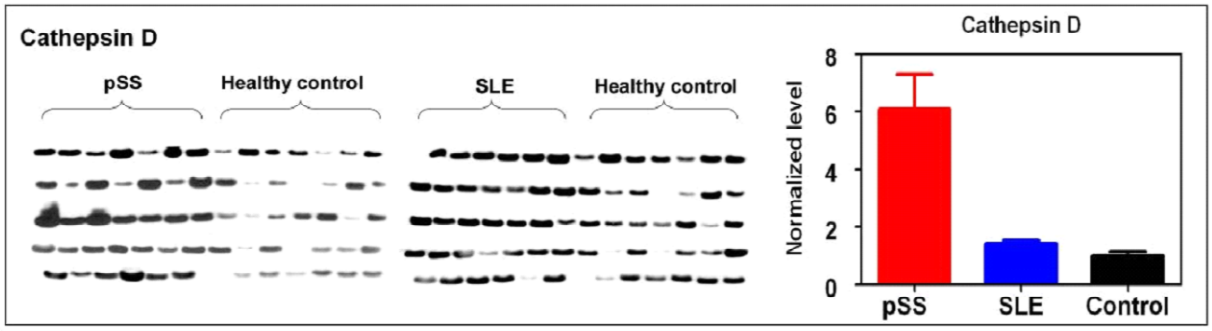

Figure 1B

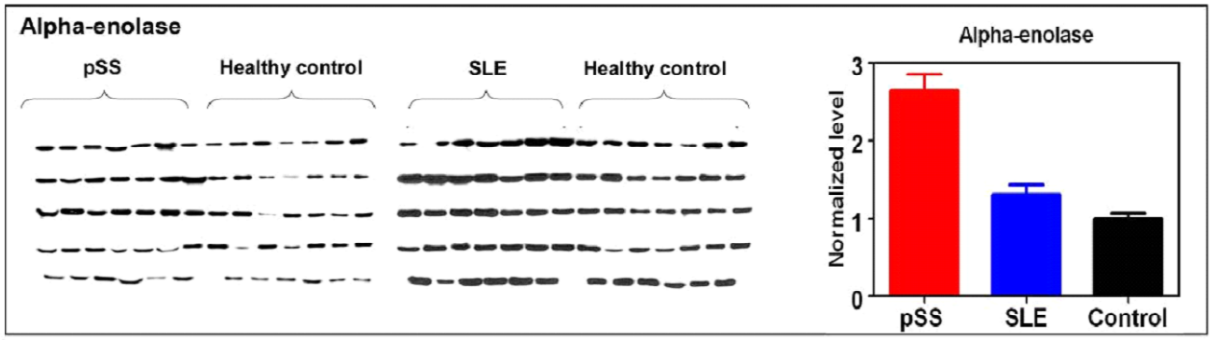

Figure 1C

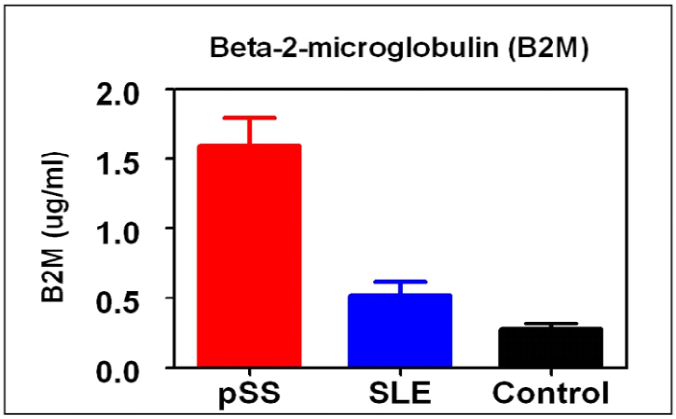

Figure 1. Validation of protein biomarkers, cathepsin D (A), alpha-enolase (B) and B2M (C), in independent patient (primary Sjögren's syndrome (SS), primary SS (pSS)) and control (systemic lupus erythematosus (SLE) and healthy control) populations (n=34 for each group). B2M was validated by ELISA and mean±SEM is plotted. Cathepsin D and alpha-enolase were validated by western blotting, and the bar figures indicate the normalized levels of cathepsin D and alpha-enolase among three groups (mean±SEM). Reprinted from [16].

suggesting that they can discriminate primary SS from both SLE and healthy controls (Figure 1). If further validated in patients with primary SS and those with sicca symptoms but no autoimmune disease, these biomarkers may lead to a simple yet highly discriminatory clinical tool for diagnosis of primary SS [16].

Rigante et al. performed the proteomic analysis of the salivary peptide complex in SS patients' salivary fluid near diagnosis and after 6 months of pharmacological therapy. The analysis revealed that clinical and functional changes of the salivary glands driven by non-steroidal antinflammatory drugs might be reflected in different proteomic patterns of the salivary fluid [17]. Using a proteomic approach, Giustil et al. characterized the WS proteins of primary SS patients and revealed a set of differentially expressed proteins in patients with primary SS, which are related to acute and chronic inflammation while some others were involved in oxidative stress injury. These findings are in line with the systemic immuno-inflammatory aspects of primary SS and open the possibility for a systematic search of diagnostic biomarkers and targets for therapeutic intervention in primary SS [18]. Peluso et al. investigated the effect of pilocarpine on the salivary peptide and protein profile in patients with primary SS and found that pilocarpine partially restored the levels and numbers of identifiable proteins in saliva from patients with primary SS. Higher levels of alpha-defensin1 and the presence of beta-defensin 2 in the saliva of patients with primary SS could be markers of oral inflammation in this patient group [19].

Using immune-response protoarrays to profile saliva autoantibodies from patients with primary SS or SLE and healthy control subjects, Hu et al. identified salivary autoantibody biomarkers that are highly specific to primary SS . A panel of 24 autoantibody biomarkers was found to be significantly over-produced in primary SS patients compared to both SLE patients and healthy individuals (Figure 2). Four saliva autoantibody biomarkers, anti-transglutaminase, anti-histone, anti-SSA, and anti-SSB, were further tested in independent primary SS (n=34), SLE (n=34), and healthy control (n=34) subjects and all were successfully validated with ELISA. This study has demonstrated the potential of a high-throughput protein microarray approach for the discovery of autoantibody biomarkers. The validated saliva autoantibody biomarkers could well discriminate patients with primary SS from both SLE patients and healthy individuals [20].

3. Systems biology analysis of Sjogren's syndrome

Hu et al. conducted a systems biology analysis of parotid gland tissues obtained from patients with primary SS, patients with primary SS/MALT lymphoma, and subjects without primary SS (non-primary SS controls) (Figure 3). The tissue samples were assessed concurrently by gene-expression microarray profiling and proteomics analysis followed by weighted gene-coexpression network analysis (WGCNA) to identify activated pathways and target genes in respective disease phenotypes. WGCNA is a systems biologic analysis method that has been successfully used to identify disease pathways and their key constituents. It basically identifies gene co-expression modules based on unsupervised clustering of microarray data and explore both gene significance (differential expression) and connectivity for each gene. Gene-coexpression modules related to primary SS or primary SS/MALT lymphoma were significantly enriched with genes known to be involved in the immune/defense response, apoptosis, cell signaling, gene regulation, and oxi-

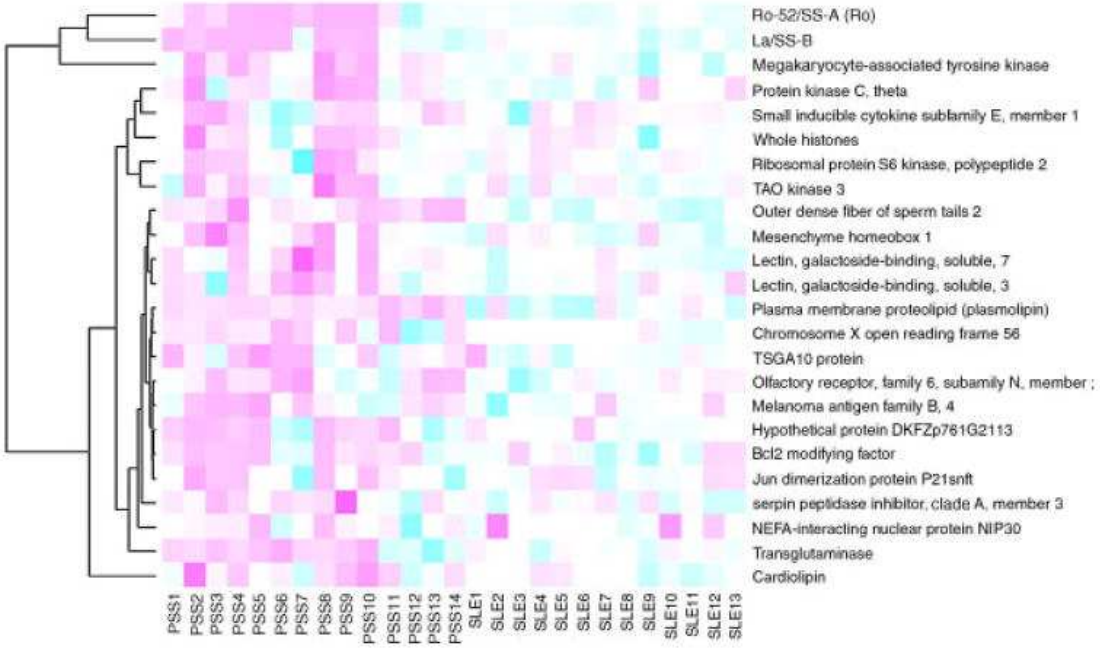

Figure 2. A heatmap of 24 saliva autoantibodies between primary Sjögren's syndrome (pSS) and SLE groups based on the protein microarray profiling. Reprinted from [20].

dative stress. Detailed functional pathway analyses indicated that primary SS-associated modules were enriched with genes involved in proteasome degradation, apoptosis, signal peptides of the class I major histocompatibility complex (MHC), complement activation, cell growth and death, and integrin-mediated cell adhesion, while primary SS/MALT lymphoma-associated modules were enriched with genes involved in translation, ribosome biogenesis and assembly, proteasome degradation, class I MHC signal peptides, the G13 signaling pathway, complement activation, and integrin -mediated cell adhesion. Combined analyses of gene expression and proteomics data implicated 6 highly connected "hub" genes for distinguishing primary SS from non-primary SS, and 8 hub genes for distinguishing primary SS/MALT lymphoma from primary SS. The identified gene modules/ pathways provide further insights into the molecular mechanisms of primary SS and primary SS/MALT lymphoma whereas the identified disease-hub genes represent promising targets for therapeutic intervention, diagnosis, and prognosis [21].

4. Bioinformatics

State-of-the-art omics technologies, including proteomics and transcriptomics, are being implemented widely in studies of human disease including SS. Bioinformatics approaches such as mining the data from multiple omics studies can provide deeper insights into the entire systems than can be obtained from any single omics study. This section introduces several bioinformatics infrastructures relevant to SS.

The Sjögren's syndrome knowledge base [22] (SSKB http://sskb.umn.ed) is a database that collects and organizes gene and protein expression data from the existing literature for comparative analysis with future gene expression and proteomic studies of SS. The SSKB is generated from PubMed using text mining of over 7,700 abstracts and listing approximately 500 potential genes/proteins. The SSKB can be used for literature reviews and literature-based validation of identified genes, functional gene enrichment studies, protein-protein interaction networks and other bioinformatics analyses.

Saliva Ontology (SALO) is a consensus-based controlled vocabulary of terms and relations dedicated to the omics domain and to saliva-related diagnostics. SALO is tested specifically in light of its capacity to meet the ontology needs for managing data derived from research on the use of a salivary marker for SS. SS-relevant portion of the ontology is to be validated through the work on annotation of representa-

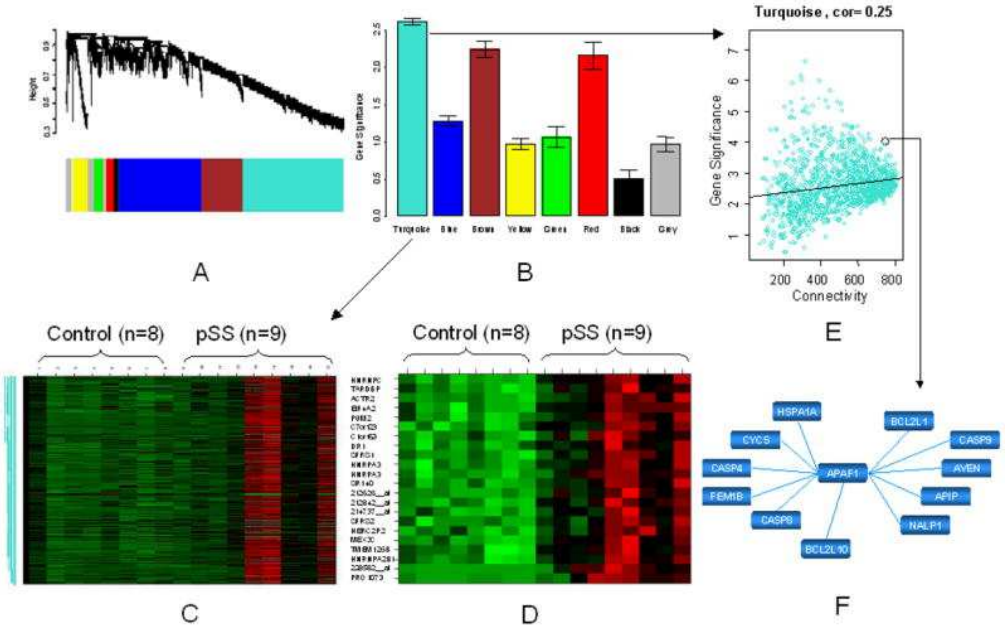

Figure 3. Module and hub gene detection based on gene expression analysis of primary SS (pSS) patients and controls. (A) Hierarchical cluster tree used for module detection. Modules correspond to branches of the tree and are assigned the same color as indicated by the color-band underneath the tree. Grey denotes genes outside proper modules; (B) Disease-related gene co-expression modules identified by WGCNA. Module significance is defined as average gene significance and the gene significance measure is defined as the absolute value of the Student T statistic for differential expression between pSS and control groups. Note that the Turquoise, Brown, and Red modules are comprised of highly differentially expressed genes (average absolute T-test > 2). (C) Heatmap of the gene expression data of the Turquoise module genes (rows) *versus* array samples (y-axis). The columns on the left hand side correspond to controls and those on the right hand side to pSS subjects. Note the Turquoise module genes tend to be highly over expressed in pSS patients. (D) Heatmap of the 22 most significant genes from the Turquoise module which can well segregate pSS and control groups. (E) Scatter plot of gene significance *versus* intramodular connectivity in the Turquoise module. While highly connected intramodular hub genes tend to be differentially expressed, connectivity (module membership) and gene significance are complementary gene screening variables. (F) One particularly promising hub gene in the Turquoise module, apoptotic peptidase activating factor 1 (APAF1), encodes a cytoplasmic protein that initiates apoptosis. Reprinted from [21].

tive research literature on the SSKB as well as our research on the salivary protein biomarkers[23].

SDxMart is a BioMart[24] data portal that hosts saliva omics data and offers access to the data by using the BioMart interface and querying environment. The SDxMart is designed to provide a variety of queries to facilitate saliva biomarker discovery including complex queries that integrate omics, clinical and functional information. The SDxMart holds data from projects of oral diseases and systemic diseases including SS. The types of omics datasets are proteomics, transcriptomics, microRNA and metabolomics[25].

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