PROCEEDING

Proteome analysis for rat saliva

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Abstract : Proteome analysis is a popular method to discover biomarkers for the prevention and diagnosis of diseases. Since saliva is a non-invasively available body fluid, gathering of saliva causes minimal harm to patients. Therefore, detection of proteins for the prevention and diagnosis from the saliva sample may be the preferred method, especially for children and elderly people. However, the abundance of salivary proteins and contaminant proteins from food and mouth bacteria obscure identification of proteins present in the saliva at low concentrations. To address this problem, we developed a shotgun proteomic method using two-dimensional nano-flow LC tandem mass spectrometry. We report here that our method is able to detect proteins quantitatively even in small sample volumes of saliva. J. Med. Invest. 56 Suppl. : 224-227, December, 2009

Keywords **:** *proteome, saliva, malnutrition, biomarker*

INTRODUCTION

Proteome analysis for salivary proteins

Saliva is produced and secreted from major and minor salivary glands. The major salivary glands are the parotid, submandibular, and sublingual glands. Different salivary glands secrete different compositions of proteins. The saliva also contains proteins that originate from the plasma. Quantitative identification of salivary proteins is able to assess each protein's potential in diagnostics for human health (1-3).

Recently, the number of publications regarding the use of proteomic analysis of salivary proteins has increased. Three American groups from UCSF, UCLA and the Scripps Research Institute/University of Rochester have made a salivary proteome consortium to build a human salivary protein catalog known as the Salivary Proteome Knowledge Base, which is funded by the National Institute of Dental and Craniofacial Research (4, 5). Each group uses different analytical methods for proteomics, and they have identified different proteins, as shown in Fig. 1. At present, 914 proteins come from the parotid gland and 917 proteins from the submandibular and sublingual glands. These groups have identified 152 proteins in the parotid gland and 139 proteins in the submandibular gland commonly. A more recent report (6) identified proteins secreted from the labial minor salivary gland using three different methods. Although LC tandem mass spectrometry was eventually used in the analysis, different pretreatment methods led to the identification of different proteins. Thus, different methods for proteomic analysis identify different proteins from these samples.

Several reports have demonstrated that proteomic analysis of saliva can be used to identify biomarkers as putative peptides for diagnosis of several diseases. Whole saliva contains proteins that can be informative for detection of diseases related to oral health (7-14), in particular for oral cancer (15), head

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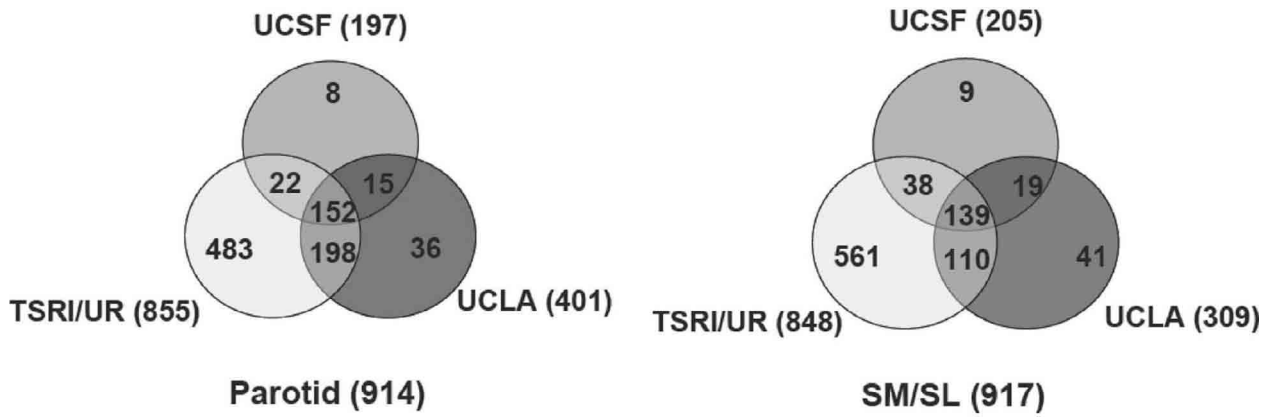

Fig. 1 The number of salivary proteins found by the three American groups at UCSF, UCLA and the Scripps Research Institute/ University of Rochester. SM/SL : submandibular and sublingual glands.

and neck squamous cell carcinoma (16), Sjögren's syndrome (17-19) and diabetes (20). The putative biomarkers for some diseases may be not directly related to proteins produced in salivary glands. These proteins may originate from the plasma. To utilize proteins as biomarkers, quantitative analysis as well as qualitative analysis is needed. For example, in type-2 diabetes (20), salivary proteins whose concentrations either increased or decreased by a factor of more than two were considered candidate proteins for biomarkers.

Development of quantitative proteomic analytical method and discovery of biomarkers in malnutrition

In considering dietary life of elderly people, progressive protein-energy malnutrition can pose a significant health threat. In that case, the malnutrition causes decreased activities of daily life, reduced immune function, and induction of bedsores. At present, serum albumin and serum prealbumin among others are used as biomarkers of malnutrition. They are examined with a blood chemistry test. Blood sampling can pose risks to elderly people. Therefore, we set out to identify malnutrition biomarkers from patients' saliva.

Although final our goal is to identify putative biomarker proteins and examine human samples for these markers by ELISA, we first needed to select them with quantitative proteomic analysis of saliva. We used saliva directly extracted from rat ducts as experimental models to begin proteomic analysis. There are three benefits of using rats as experimental animals. First, there are fewer inter-individual differences compared with humans. Second, various types of model animals are available. Third, the influence of proteins from foods and oral bacteria could be neglected since the saliva was directly sampled from the gland ducts. However, there are problems with using animal models that must be considered. There is no available database of animal salivary proteins, and sample volumes are very small. Additionally, similar to human saliva samples, rat saliva contains a variety of proteins. To resolve these problems, we relied on the benefits of mass spectrometry including miniaturization, high-sensitivity, and high-resolution. After protein detection, we used mass spectrometry to create a catalogue of rat proteins. In order to detect changes in protein concentration, quantitative proteomic analysis was needed.

We developed a shotgun proteomic method using two-dimensional nano-flow LC tandem mass spectrometry (21). In this technique, saliva proteins were digested to a peptide mixture through proteolytic processing. Then the digested peptides were analyzed with nano-flow RP-HPLC. The peptides in the MS chromatogram were further analyzed with MS spectra to get mass information and analyzed with CID spectra to get amino acid sequences. From this information, we made the database of rat salivary proteins.

MATERIALS AND METHODS

In this study, we used eight-week-old Wistar rats. The rats were fed for three weeks with a normal or low-protein diet. The low-protein diet did not contain casein. Three weeks after feeding, the animals were anaesthetized with pentobarbital. The ducts of three major salivary glands were cut, and saliva samples were collected from the cut ends.

RESULTS AND DISCUSSION

Protein catalogs were made from the rat saliva and then compared with those of humans. We identified 1076 parotid gland proteins, 815 submandibular gland proteins, and 1165 sublingual gland proteins. The number and types of detected rat proteins were similar to those from humans (Fig. 2).

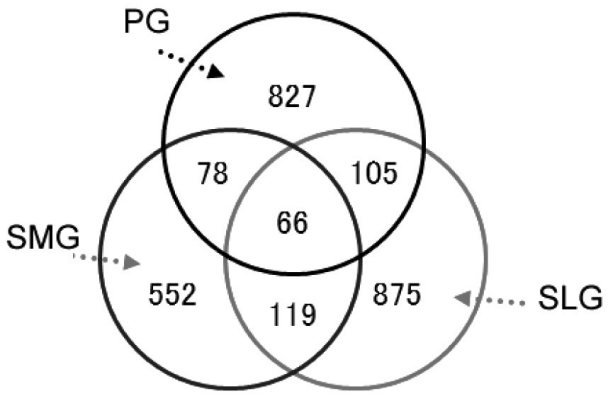

Fig. 2 The number of salivary proteins identified in the parotid (PG), submandibular (SMG) and sublingual glands (SLG) found by our proteomic analysis.

Then we used stable-isotope labeling MS to assess protein concentrations. This technique is based on isobaric labeling methods with the iTRAQ reagent, which are based on differential labeling for the N-terminal of lysine residues. iTRAQ-labeled peptides produce specific fragment ions (reporter ions), which have a different mass. Using MS/MS, relative peptide abundance can be compared via the intensity of reporter ions.

Then, relative protein concentrations were compared. When the low protein diet was compared with a normal diet, some proteins were increased and several proteins were decreased. To date, one study reported a decrease of kallikrein in saliva in rats fed a low protein diet (22). Our data supports this finding.

In conclusion, saliva collected from rat parotid, submandibular and sublingual gland ducts was analyzed using nano-flow 2DLC-MS/MS combined with the iTRAQ method. This analysis required only 10-100 microliters of rat saliva. We expect that this technology will identify candidates for salivary markers of human disease.

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