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Quantitative proteomic analysis of microdissected oral epithelium for cancer biomarker discovery

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SUMMARY

Specific biomarkers are urgently needed for the detection and progression of oral cancer. The objective of this study was to discover cancer biomarkers from oral epithelium through utilizing high throughput quantitative proteomics approaches. Morphologically malignant, epithelial dysplasia, and adjacent normal epithelial tissues were laser capture microdissected (LCM) from 19 patients and used for proteomics analysis. Total proteins from each group were extracted, digested and then labelled with corresponding isobaric tags for relative and absolute quantitation (iTRAQ). Labelled peptides from each sample were combined and analyzed by liquid chromatography–mass spectrometry (LC–MS/MS) for protein identification and quantification. In total, 500 proteins were identified and 425 of them were quantified. When compared with adjacent normal oral epithelium, 17 and 15 proteins were consistently up-regulated or down-regulated in malignant and epithelial dysplasia, respectively. Half of these candidate biomarkers were discovered for oral cancer for the first time. Cornulin was initially confirmed in tissue protein extracts and was further validated in tissue microarray. Its presence in the saliva of oral cancer patients was also explored. Myoglobin and S100A8 were pre-validated by tissue microarray. These data demonstrated that the proteomic biomarkers discovered through this strategy are potential targets for oral cancer detection and salivary diagnostics.

Introduction

Oral cancer is the most common neoplasm of the head and neck. It may emerge as a primary lesion originating from any of the oral tissues, and can be of varied histologic types. About 90% of these malignancies are oral squamous cell carcinomas (OSCC). Approximately, 42,000 Americans are diagnosed each year with this largely preventable cancer [1–3]. Oral cancer is a major and growing problem in many parts of the globe where tobacco and alcohol are established major etiologic agents of these cancers. In addition, micronutrient deficiencies and poor oral hygiene have also been linked to increased risk [3]. In recent years, human papilloma virus (HPV) has been increasingly associated with tonsilla and pharyngeal cancers where the affected individuals are often younger and have very different risk factors [4].

Patients with oral cancer often present with symptoms at a late stage, and there is a high recurrence rate after treatment, especially in those with neck lymph node metastasis [1,2]. The delayed detection is likely a primary reason for the high morbidity and mortality rate of oral cancer patients. Meanwhile, diagnosing oral cancer at an early stage could significantly increase the 5-year survival rates [3]. Because oral cancer can spread quickly, screening of high-risk populations represents a promising way to reduce cancer incidence and mortality. As the occurrence of oral cancer rise (due to increased tobacco and alcohol abuse, and increased longevity), the need for effective early detection technologies and discriminatory biomarkers becomes more urgent [3,5]. The ideal approach for early detection should be easily performed in an out-patient set-up, which is practical and non-invasive, such as brush biopsy, tissue autofluorescence, and salivary diagnostics [6].

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Serum and biopsy tissue samples have long been applied to develop mRNA, microRNA and protein biomarkers [7] for the detection of oral cancer. Different quantitative proteomics technologies have been successfully engaged in oral cancer biomarker discovery, like two-dimensional gel electrophoresis [8] and isobaric tags for relative and absolute quantitation (iTRAQ) [9]. Although some work has been done in oral cancer biomarker discovery, however, it is still very challenging to predict which oral diseases (such as erythroplakia, leukoplakia, lichenoid and other potentially malignant mucosal) will progress to neoplasia – notably OSCC [10]. Especially, it is of profound significance to discover unique biomarkers that allow identification of high-risk oral lesions [11]. The capability to differentiate epithelial dysplasia from normal and malignant epithelial might also improve the specificity of early detection [12].

The objective of this study was to discover specific biomarkers for the detection of oral cancer. Through coupling cutting edge technologies with our unique study design, we have comprehensively analyzed the proteomics changes among morphologically malignant, epithelial dysplasia, and adjacent normal oral epithelial cells. LCM was utilized to accurately procure the specific oral epithelial cell types. Quantitative proteomics engaging iTRAQ technology were used for the biomarker discovery by comparing all the samples, simultaneously. Through quantitative analysis of oral epithelium at different stages, the progression of oral cancer was systematically discovered by analyzing the identified proteins. Potential biomarkers were selected and further pre-validated in tissue microarray and human saliva. Their utility for the detection of oral cancer was also evaluated.

Materials and methods

Analytical strategy for proteomic biomarker discovery

This study consisted of two phases. In the discovery phase, adjacent normal, epithelial dysplasia and malignant oral epithelium tissues from 19 oral cancer patients were procured by LCM (Table S1A and B). The proteins in these tissue cell samples were extracted and then digested with trypsin. The extracted peptides were labelled with iTRAQ 8-plex reagents. Follow the schematic of experimental design (Fig. 1), these labelled peptides from each sample were mixed, and fractionated into 20 fractions by strong cation exchange chromatography. Each fraction was analyzed by RPLC–MS/MS for protein identification and quantification. In the pre-validation phase, selected candidate biomarkers were tested by immunoassays, either in tissue microarray or in saliva samples.

Patients and samples

All human tissue and saliva samples were collected according to our Institutional Review Board (IRB) approved protocols (IRB#11-000592, IRB#10-000505) with informed consent from patients. The clinical profiles and demographic information of 19 oral cancer patients were listed in Supplementary Table S1A and B, which were divided into 2 groups through generally matching their demographic information. All oral epithelium were immediately embedded in the TissueTek OCT medium (Fisher Scientific, Pittsburgh, PA, USA) and frozen at −80 °C. Frozen sections were cut at 5–8 μm thicknesses. Before LCM experiments, tissues were

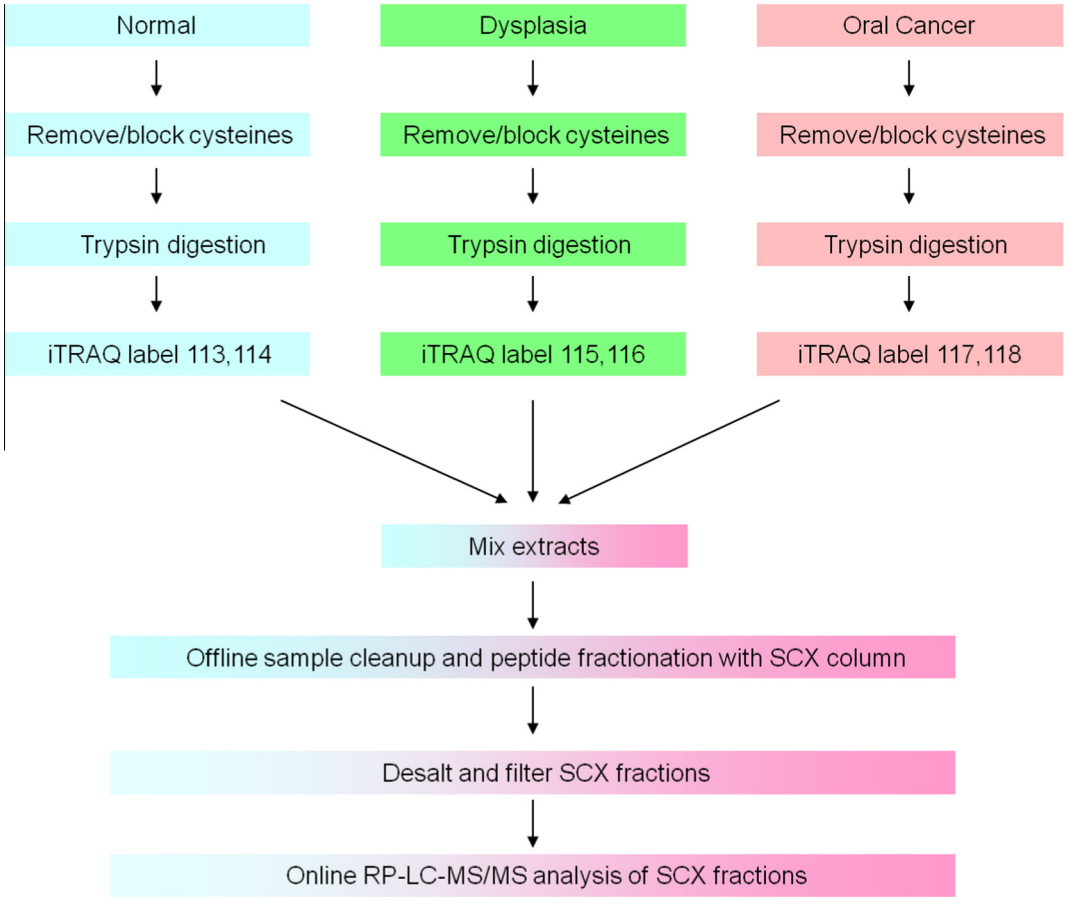

Fig. 1. Schematic diagram of the experimental design of proteomics analysis.