DISCRIMINATION OF NORMAL ORAL MUCOSA FROM ORAL CANCER
BY MASS SPECTROMETRY IMAGING OF PROTEINS AND LIPIDS

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Introduction

Head and neck squamous cell carcinomas (HNSCC) belong to oral cancers, and their etiology is connected mainly with exposure to tobacco and alcohol. Generally accepted molecular biomarkers to guide management of HNSCC patient are still missing, and determination of molecular factors discriminating between cancerous and normal mucosa for proper delineation of tumor area belongs to critical issues in the field of molecular diagnostics of HNSCC.

Experimental

CLINICAL MATERIAL

Tissue material was collected from four patients (3 males and 1 female; aged: 36-59) who underwent surgery due to head and neck squamous cell carcinoma located in tongue. In all cases no adjuvant chemoradiotherapy was involved prior surgery.

Case Stage Case Stage

1 T2N0M0 3 T2N1M0
2 T4N2M0 4 T2N0M0

SAMPLE PREPARATION

• post-operative material: frozen, stored at -80°C
• 10 µm serial sections on TDO-coated glass slides

PEPTIDE IMAGING

• lipid removal: 70% ETOH, 1 min
70% ETOH, 1 min
100% ETOH, 1 min
• vacuum drying: 1 h
• trypsin coating: 20 µg Promega Trypsin in
50 mM NH4HCO3
• incubation: 37°C, 18 h, humid chamber

LIPID IMAGING

no additional tissue preparation was performed

MATRIX COATING (peptides/lipids)

2.5-DHB mixing/mL, in 10% methanol and 0.2% TFA, ImagePrep standard matrix coating program with doubled matrix
LC-MS/MS

• Each protein digest was separated into 680 nano-LC fractions.
• Up to 10 MS/MS precursors per fraction were fragmented.
• MS/MS spectra were searched against NCBI human database using ProteinScape v3.1 software

MALDI-MSI measurements

reflection positive mode 800-4000 m/z (peptides)
300-1200 m/z (lipids)
 spectra width: 600 µm
400 shots/raster

INSTRUMENTATION

ImagePrep (Bruker Daltonik)
ultraflextrium MALDI-TOF mass spectrometer (Bruker)
EASY-nLC chromatograph (Proxeon) coupled with PROTEINER 8 fraction collector (Bruker)

COMPUTATIONAL ANALYSIS

• Coefficient of variation was used to measure molecular components’ dispersion.
• Cohen’s d value was used to estimate significance of differences in abundance of each molecular component between cancer ROIs and normal epithelium.
• Divisive K-means algorithm for spectra clustering was used to determine sub-regions in tissue preparations.
• The logistic regression technique was applied to spectra classification between cancer and normal (normal) epithelium ROIs.
• Bayesian Information Criterion (BIC) was used for model selection.

Results

Peptide and lipid domain comprised 2435 and 2108 spectra components, respectively, which represented different molecular species and their isotope envelopes.

Tissue regions corresponding to cancer and normal epithelium were delineated by an experienced pathologist after molecular image registration, and spectra from these two types of ROIs (regions of interest) were exported for further analyses.

Comparison of the size of clusters obtained during unsupervised image segmentation

The extent of clustering for molecular signatures within normal epithelium was larger than in cancerous regions. This finding may indicate that molecular dysregulation in oral cancer is less pronounced than in other cancer types.

Conclusions

The presented study demonstrates significantly different abundances of large number of cellular proteins represented by their tryptic peptides imaged by MALDI-MSI between normal and cancerous mucosa.

In contrast, differences between cancerous and normal mucosa were less obvious when corresponding ROIs were compared in respect to the subset of the analyzed lipids. Nevertheless, imaging of both proteome and lipidome components enabled discrimination of oral cancer and normal epithelium. This indicated that both molecular components of oral epithelium are potential sources of oral cancer biomarkers.

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