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 neo-adjuvant chemo- or radio-therapy was involved prior surgery.

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<th>Case</th>
<th>Stage</th>
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<tbody>
<tr>
<td>1</td>
<td>T2N0M0</td>
<td>2</td>
<td>T2N2M0</td>
</tr>
<tr>
<td>3</td>
<td>T2N0M0</td>
<td>4</td>
<td>T2N0M0</td>
</tr>
</tbody>
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SAMPLE PREPARATION

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

PEPTIDE IMAGING

• lipid removal: 70% ETOH, 1 min
• 70% ETOH, 1 min
• vacuum drying: 1 h
• trypsin coating: 20 µg Promega Trypsin in 50 mM NH4HCO3, incubation: 3.7°C, 18 h, humid chamber

LIPID IMAGING

no additional tissue preparation was performed

MATRIX COATING (peptides/lipids)

2.5-DHB 35mg/ml, in 50% methanol and 0.2% TFA, ImagePrep standard matrix with compounds 5
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 v1.3 software

MALDI-MSI measurements

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

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.

Conclusions

The presented study demonstrates significantly different abundances of a 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.