Predictive proteomics in head and neck squamous cell carcinoma using mass spectrometry imaging

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Introduction

Head and neck squamous cell carcinoma (HNSCC; Figure 1) has high incidence and recurrence rates. From previous studies, we observed that the morphologic pattern known as tumor budding (TB) and a grading system based on TB and cell nest size (referred to as the cellular dissociation grading; CDG) exhibited a significant association with downregulation of immune cell infiltrate (CD3, CD8, and FoxP3-positive T-cells) as well as low expression of HLA in INF- γ. These findings suggest that morphologic patterns like TB and CDG, along with tumor stroma, hold promise as potential biomarkers for immunotherapy. We have investigated HNSCC tissue heterogeneity using mass spectrometry imaging (MSI) to identify potential molecular markers for a better disease stratification.

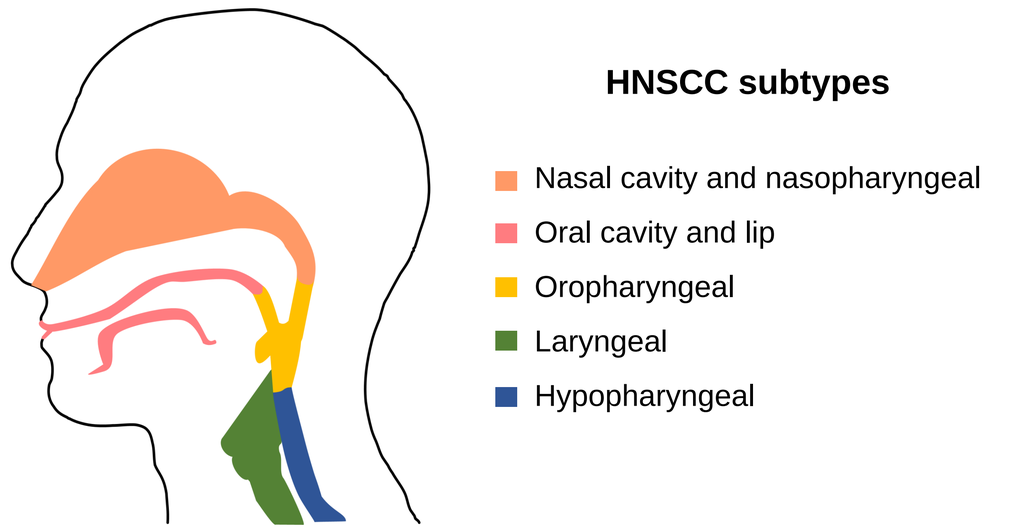

Figure 1. HNSCC subtypes according to anatomical location.

Methods

Following clinico-pathological diagnosis, next generation sequencing, and immunological evaluations, the samples (n=48, with three or four replicates per patient), assembled in four TMAs, were adhered to ITO glass slides. After deparaffinization and re-hydration, the samples were submitted to tryptic digestion, followed by matrix application using an HTX TM sprayer. The mass spectrometry proteomic data was recorded using a RapifleX MALDI-TOF instrument (Bruker) in the *m/z* range 600-3200. Following data acquisition, the tissue sections were stained using hematoxylin and eosin and scanned using a digital slide scanner (Aperio AT). Regions of interest (tumor and stroma) were annotated by a board certified pathologist. Data analysis was carried out using SCiLS Lab. Features of interest were identified from MS/MS (timsTOF, Bruker) on-tissue fragmentation.

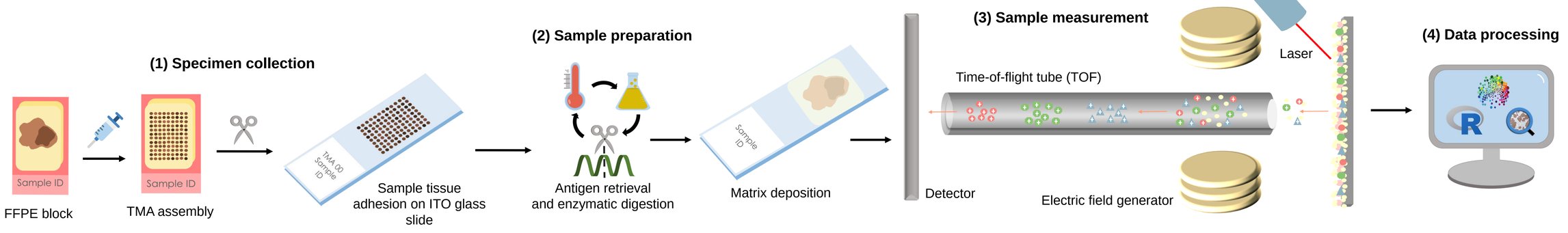

Figure 2. Sample processing to mass spectrometry imaging data acquisition workflow.

Results

Principal component analysis (Figure 4) revealed noticeable distinctions between tumor grades 3 and 1. Here; we identified molecular features from COL3A1 (Figure 5), COL1A1, and ZNF329, which were overexpressed in tumors with higher grade (Table 1).

Marked differences were also identified between carcinoma and stroma, particularly KRT5 (Figure 6).

In the stroma, we found notable proteomic differences between CD3 positive and CD3 negative samples through ROC-AUC analysis, including *m/z* 1585.8 (AUC=0.672), *m/z* 1781.9 (AUC=0.685), *m/z* 1079.7 (AUC=0.685), and *m/z* 1751.8 (AUC=0.685).

ZNF329 also presented a higher expression in tissue samples with higher tumor budding (Figure 7).

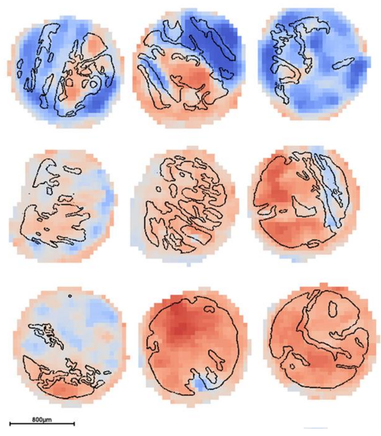

Figure 3. Principal component analysis (PCA) – Component 1 distribution. Epithelial tumor regions are annotated in black.

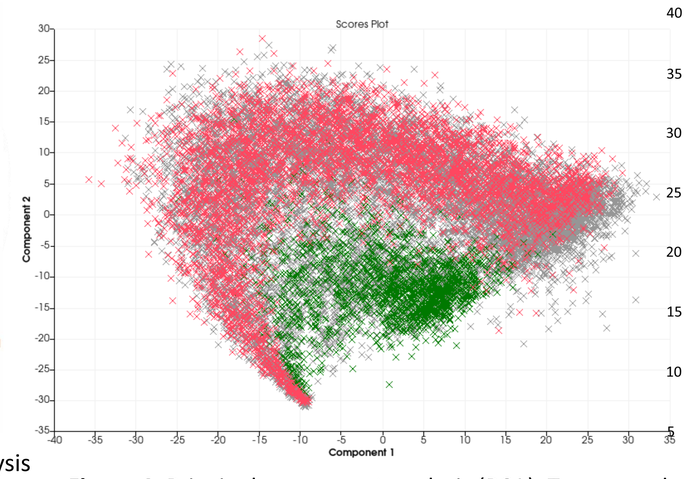

Figure 4. Principal component analysis (PCA). Tumor grade 3 (green) and tumor grade 1 (pink).

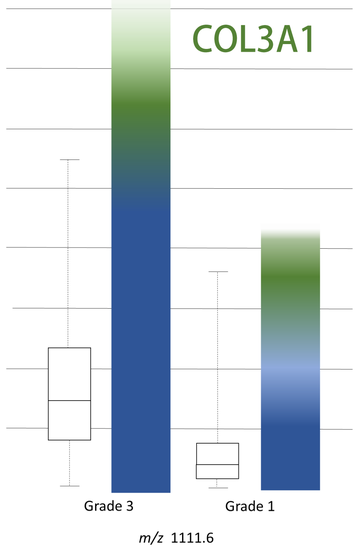

Figure 5. Intensity boxplot showing the differential expression of COL3A1 in tumors of grade 3 compared with grade 1.

m/z	Mr(calc)	Accuracy	Protein ID (MSMS)	Amino acid sequence	MASCOT score
678.32		0.813			
852.43	851.4250	0.794	Collagen alpha-1(III) chain	GAPGPQGPR	42
874.43	874.3143	0.803	ZNF329	DSSCLTK	48
1111.61	1110.5894	0.819	Collagen alpha-1(III) chain	GRPGLPGAAGAR	63
1105.57	1104.5676	0.828	Collagen alpha-1(I) chain	GVQGPPGPAGPR + Oxidation (P)	51
1116.50	1115.4843	0.803	Collagen alpha-1(I) chain	EGAPGAEGSPGR + Oxidation (P)	52
1138.55	1137.5601	0.754	Collagen alpha-1(III) chain	GLAGPPGMPGPR + Oxidation (P)	45
1303.61	1302.5953	0.770	Collagen alpha-1(III) chain	GSPGGPGAAGFPGAR + Oxidation (P)	55
1465.70	1464.6845	0.750	Collagen alpha-1(I) chain	GEPGPTGLPGPPGER + Oxidation (P)	45
1508.73		0.776	H2B1K_HUMAN *		
1546.79		0.758	Collagen alpha-1(I) chain *		

Table 1. Discriminating *m/z* features of high grade tumors, accuracy obtained by ROC-AUC calculation. MS/MS fragmentation spectra were used for identification of the parent proteins.

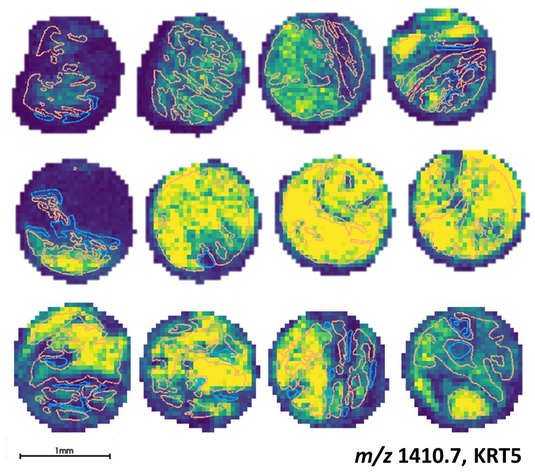

Figure 6. Expression of KRT5 was highly correlated with the carcinoma region (AUC-ROC = 0.88), which is also verified in the tissue by the expression of the peptide *m/z* 1410.7. Carcinoma regions are annotated in pink, stroma in blue.

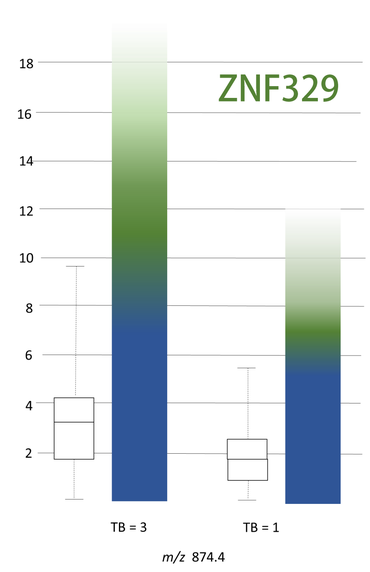

Figure 7. Intensity boxplot showing the differential expression of ZNF329 in tissue presenting tumor budding grade 3 (TB=3) compared with grade 1 (TB=1).

Conclusions

Our results demonstrate the potential of MALDI imaging as a valuable tool for aiding in the stratification and prognostication of HNSCC. Based on our current morphomolecular characterization and the proteomic data we have collected, we have identified multiple peptide signatures that can be utilized for carcinoma identification, tumor grading, and tumor budding characterization. Validation by immunohistochemistry is pending.

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