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Classification of Immune Landscapes in Oral Squamous Cell Carcinoma

Nicole Thompson
University of the Pacific

OKU Sutro Excellence Day Project Cover Sheet

Project Title

Classification of Immune Landscapes in Oral Squamous Cell Carcinoma

Full name(s) and class year(s) of all project collaborators

Example: Jane Smith, DDS 2022; John Smith, DDS 2022

Nicole Thompson, DDS 2026

Project Category

DDS/IDS/DH - PIP Awards: Bench or Clinical Research

Enter your abstract text here (max 300 words)

Oral squamous cell carcinoma (OSCC) is an aggressive cancer with a high mortality rate. Prognostic biomarkers from the tumor immune microenviroment (TIME) are needed to determine treatment plans. We hypothesized that imaging mass cytometry (IMC) would reveal distinct TIME characteristics associated with histopathological grades. A 40-plex IMC, covering markers for tumor structure, multiple immune cells, and their signaling activity, was used to investigate the TIMEs of formalin-fixed, paraffin-embedded (FFPE) incisional oral tongue biopsies. These biopsies were sourced from 24 OSCC patients at the University of the Pacific, Arthur A. Dugoni School of Dentistry, and contained 13 well-differentiated, 10 moderately differentiated, and one poorly differentiated histological graded samples. Spatial characteristics of the tumor core, front, and stroma were identified by spatial subsetting. A multivariable predictive model with 909 IMC features accurately classified tumor histological grades (AUC: 0.88). Spatial subsetting improved intrapatient and interpatient feature reproducibility. The top predictive immune features of higher histological grade were the smaller abundance and size of stromal CD4+ memory T cells and tumor-core CD8+ memory T cells, as well as decreased interactions between regulatory CD4+ T cells and non-poliferating tumor cells at the tumor front. This study establishes a robust modeling framework for distilling complex imaging data, classifying histological grades, and uncovering sentinel characteristics of the OSCC TIME to facilitate prognostic biomarker discovery.

Classification of Immune Landscapes in Oral Squamous Cell Carcinoma

Nicole Thompson[1], Jacob Einhaus[2], Dyani Gaudilliere[3], Dorien Feyaerts[2], Julien Hedou[2], Leticia Ferreira Cabido[4], Darren Cox[4], Alice Lin[1], Makaylan Tseng[1], Karl C. Bruckman[3], David K. Lam[4], John B. Sunwoo[5], David Ojcius[1], Christian M. Schurch[6], Brice Gaudilliere[2], Xiaoyuan Han*[1].

[1] Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, USA; [2] Department of Anesthesiology, Perioperative & Pain Medicine, Stanford University School of Medicine, Stanford, CA, USA; [3] Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University School of Medicine, Stanford, CA, USA; [4] Department of Oral and Maxillofacial Surgery, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, USA; [5] Division of Head and Neck Surgery, Department of Otolaryngology, Stanford University School of Medicine, Stanford, CA, USA; [6] Department of Pathology and Neuropathology, University Hospital and Comprehensive Cancer Center Tübingen, Tübingen, Germany
*Correspondence: xhan@pacific.edu

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is an aggressive cancer with a high mortality rate. Prognostic biomarkers from the tumor immune microenvironment (TIME) are needed to determine treatment plans. We hypothesized that imaging mass cytometry (IMC) would reveal distinct TIME characteristics associated with histopathological grades.

Method: A 40-plex IMC, covering markers for tumor structure, multiple immune cells, and their signaling activity, was used to investigate the TIMEs of formalin-fixed, paraffin-embedded (FFPE) incisional oral tongue biopsies. These biopsies were sourced from 24 OSCC patients at the University of the Pacific, Arthur A. Dugoni School of Dentistry, and contained 13 well-, 10 moderate-, and 1 poor-differentiated histological graded samples. Spatial characteristics of the tumor core, front, and stroma were identified by spatial subsetting.

Results: A multivariable predictive model with 909 IMC features accurately classified tumor histological grades (AUC:0.88). Spatial subsetting improved intra-and inter-patient feature reproducibility. The top predictive immune features of higher histological grade were: 1) the smaller abundance and size of CD4+ memory T cells in the stroma; 2) fewer and smaller CD8+ memory T cells in the tumor core; and 3) decreased interactions between regulatory CD4+ T cells and non-poliferating tumor cells at the tumor front.

Conclusion: This study establishes a robust modeling framework for distilling complex imaging data, classifying histological grades, and uncovering sentinel characteristics of the OSCC TIME to facilitate prognostic biomarker discovery.

Objective

- **To develop a modeling framework identifying the spatially, phenotypically, and functionally characteristics of tumor immune microenvironment (TIME) from multiplex imaging mass cytometry (IMC) image;**
- **To identify immune features associated oral squamous cell carcinoma(OSCC) histopathological grades.**

Methods

- Twenty-four FFPE incisional oral tongue biopsies from OSCC patients were collected at the University of the Pacific, Arthur A. Dugoni School of Dentistry, and contained 13 well-, 10 moderate-, and 1 poor-differentiated histological graded samples. (**Fig1**)
- A 40-plex IMC antibody panel, covering markers for tumor structure, multiple immune cells, and their signaling activity, was created to analyze 71 regions of interest (ROIs) to reveal the functional, phenotypic, and spatial organization of TIME using a Hyperion imaging system.(**Fig1**)
- Spatial characteristics of the tumor core, front, and stroma were identified by spatial subsetting.
- A multivariable predictive model was developed using a sparse machine learning method to classify OSCC tumors by their histopathological grade.

Results

- A total of 273,408 cells were identified by the single-cell segmentation method Mesmer. Fifteen cell subsets, including 7 major populations and several subpopulations of three major populations (CD4+T cells, tumor cells, and myeloid cells), were identified by unsupervised clustering method PhenoGraph and labeled by us. (**Fig2**)
- A random forest pixel classifier was trained to derive tumor and stromal area. All cells were assigned into three zones: tumor core, tumor front (within 20 mm of the tumor border), and stroma. CD8+ T cells were the only immune cell population with consistent tumor infiltration, while other immune cells were found within the tumor boundaries. Proliferating tumor cells were more densely distributed at the tumor front compared to the tumor core, whereas MDSCs were predominantly found in the stroma. (**Fig3A**)
- Differences were observed in immune cell signaling activities between the tumor front and stroma, which were cell-type specific. Specifically, pNFkB in CD8+ T cell and pSTAT1 in M1 macrophages was higher at tumor front compared to those at stroma(**Fig3B&C**).
- A multivariable predictive model with 909 IMC features accurately classified tumor histological grades(AUC:0.88). (**Fig4B**)
- The top predictive immune features of higher histological grade were (**Fig4A**): 1) the smaller abundance and size of CD4+ memory T cells in the stroma; 2) fewer and smaller CD8+ memory T cells in the tumor core; and 3) decreased interactions between regulatory CD4+ T cells and non-poliferating tumor cells at the tumor front.

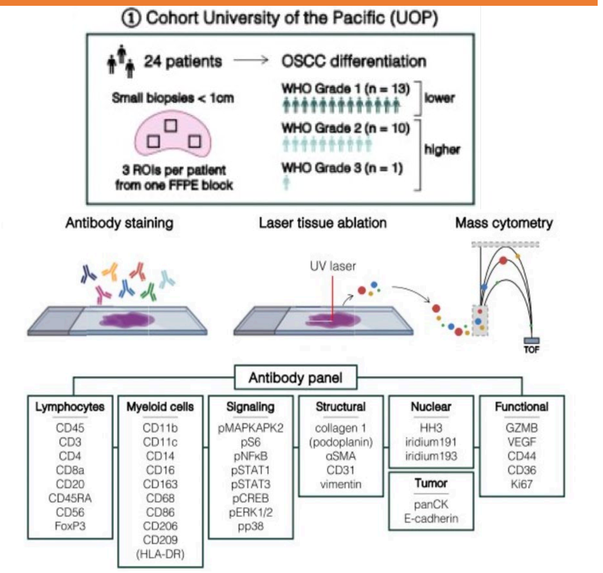


Figure 1. Experimental workflow and antibody panel.

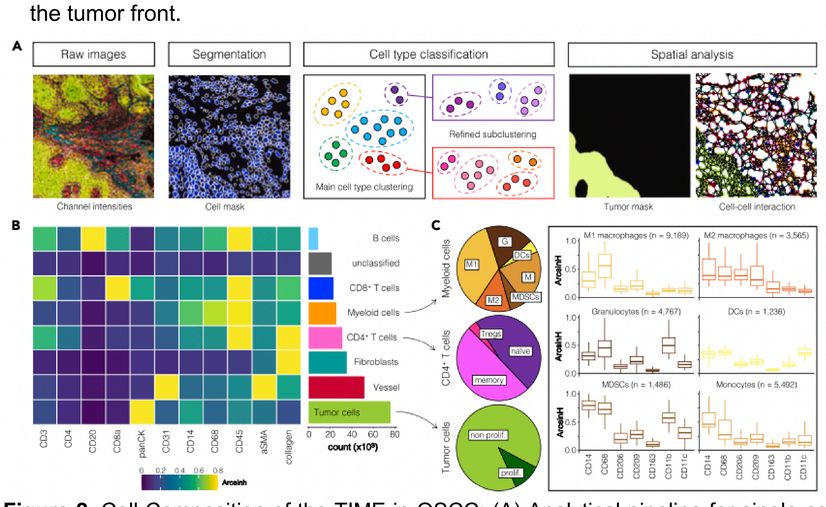
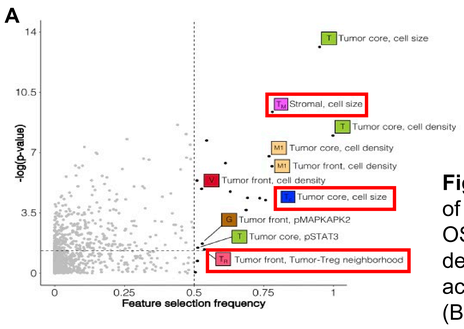


Figure 2. Cell Composition of the TIME in OSCC; (A) Analytical pipeline for single-cell and spatial feature extraction of IMC data; (B) Heatmap of mean marker expression levels of major cell subsets; (C) Relative distribution of myeloid and CD4+ cell and tumor cell subpopulations.



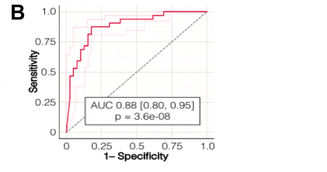


Figure 4. Integrative multivariable model of TIME features accurately classifies OSCC tumor grade; (A) Volcano plot depicting feature selection frequency across bootstrapping LASSO iterations; (B) Areas under the receiver operator curve (AUROC) plot.

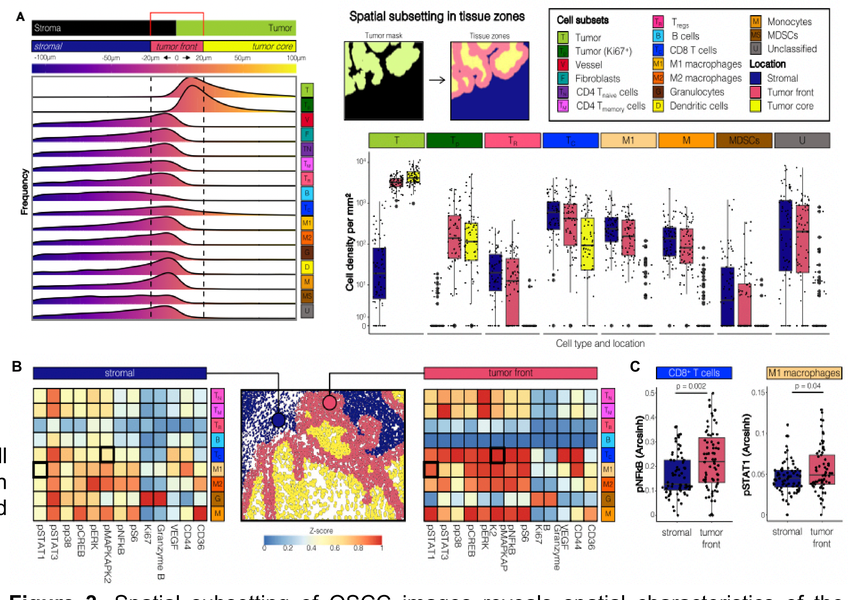


Figure 3. Spatial subsetting of OSCC images reveals spatial characteristics of the tumor front, tumor core, and stroma; (A) Spatial distribution of cell populations relative to the tumor border; (B) Heatmap of z-scored mean functional marker expression; (C) Difference in signaling activity of pNFkB in CD8+ T cells and pSTAT1 in M1 macrophages between stromal and tumor front zones.

Conclusion

This study establishes a robust modeling framework for distilling complex imaging data, classifying histological grades, and uncovering sentinel characteristics of the OSCC TIME to facilitate prognostic biomarker discovery.