

Analyzing the Tumor Immune Microenvironments of the ROC1 Oral Cavity and Flank Tumor Models with tSNE

Shawn Nguyen¹, Andrea Hernandez¹, Gemalene M. Sunga¹, Neeraja Dharmaraj, Ph.D.,¹ and Simon Young¹, DDS, MD, Ph.D., FACS

1. Katz Department of Oral & Maxillofacial Surgery, University of Texas Health Science Center at Houston, School of Dentistry, Houston, Texas, USA.

Objectives: Conventional treatments for head and neck squamous cell carcinomas (HNSCC), the sixth most common cancer worldwide, are surgery, radiotherapy, chemotherapy, and targeted therapy. Patients given these treatments have a five-year survival rate of 40-50% because of high local, regional, and distant recurrence rates. Immunotherapy is an attractive alternative that boosts one's immune system to fight cancer. Less than 20% of recurrent/metastatic HNSCC cases respond to immunotherapy requiring a comprehensive understanding of the immunosuppressive tumor immune microenvironment (TIME). In this study, the Rangel Oral Cancer 1 (ROC1) preclinical tumor model was used to identify specific immune cell populations in the TIME by flow cytometry. Specifically, we will utilize an automatic tool, t-Distributed Stochastic Neighbor Embedding (tSNE), to analyze these populations. tSNE helps in clustering and visualizing complex data sets. Additionally, it avoids human bias encountered by traditional methods of flow cytometry analysis. We will also determine TIME differences in ROC1 orthotopic (oral cavity) and heterotopic (flank) tumor models.

Experimental Methods: Previously obtained flow cytometry data from ROC1 oral cavity and flank preclinical models was used for analysis. A series of steps and plugins (manual gating, downsampling, and tSNE) in the FlowJo software were employed to identify immune cell populations. Statistical analysis was performed using unpaired t-tests to compare the TIMEs.

Results: We found significant differences between the preclinical models in the number of CD8+T-cells, NK cells, G-MDSCs, macrophages, DCs, and cDC2s, and the functional markers of CD3+ T-cells, CD8+ T-cells, CD4+ T-cells, NK cells, G-MDSCs, DCs, and cDC2s.

Conclusion: After using tSNE to analyze these models' TIMEs, we found that the ROC1 flank model's TIME was more immunosuppressive than the oral cavity's. Understanding these differences is essential for consideration in future study design and providing a rationale for the use of either model in HNSCC.

This study was supported by the UTSD Student Research Program and NIDCR R01-DE030140.