1. Introduction

Immune checkpoint inhibitors (ICI) have proven to be game-changing treatments for mucosal head and neck squamous cell cancer (HNSCC). Early successes with anti-PD-1/PD-L1 ICIs have produced durable responses and prolonged survival in some patients but there remains a need for better biomarkers to guide patient selection. The tumor microenvironment (TME) composition, contexture, and cellular architecture are key to understanding immune responsive and resistant HNSCC phenotypes. In this study, we designed a single-cell, multiomic spatial phenotyping experiment to characterize a HNSCC tissue from a patient with a partial response to Pembrolizumab/Nivolumab treatment. The image shown below is an H&E-stained section from the aforementioned patient. Path annotations indicate a large tumor mass, a regionally intact tonsil, as well as an Ectoepithelial Submucosal Gland, Lymphatic Ducts and normal Squamous Epithelium. In this post, we demonstrate how multiomic spatial phenotyping was used to produce a uniquely comprehensive analysis of the patient’s TME and how it may explain the patient’s partial response to ICI therapy.

2. Multiomic (RNA & Protein) Spatial Phenotyping on PhenoCycler-Fusion

The PhenoCycler®-Fusion system is a spatial biology solution that enables multiomic ultrahigh-plex imaging of whole tissues at single-cell resolution. The high-throughput coupled with in-flight data processing and file compression algorithms enables routines analyses of whole-slide tissue sections, which often contain millions of cells. The unified PhenoCycler chemistry can be used to detect both RNA and protein targets, thereby allowing thorough interrogation of complex cellular architecture are key to understanding immune responsive and resistant HNSCC phenotypes. In this study, we designed a single-cell, multiomic spatial phenotyping experiment to characterize a HNSCC tissue from a patient with a partial response to Pembrolizumab/Nivolumab treatment. The image shown below is an H&E-stained section from the aforementioned patient. Path annotations indicate a large tumor mass, a regionally intact tonsil, as well as an Ectoepithelial Submucosal Gland, Lymphatic Ducts and normal Squamous Epithelium. In this post, we demonstrate how multiomic spatial phenotyping was used to produce a uniquely comprehensive analysis of the patient’s TME and how it may explain the patient’s partial response to ICI therapy.

3. Multiomic Panel Design

The 101-plex antibody panel included comprehensive immune and metabolic markers, which enabled the discovery of substantial heterogeneity within the HNSCC tumor regions. This heterogeneity may be indicative of a partial ICI response (see Section 4).

4. 101-Plex “Cancer Hallmark” AB Panel Reveals 4 Distinct Tumor Region with Metabolic and Immune Heterogeneity

4.1 Metabolic/Stress Spatial Phenotyping Illustrates Tumor Heterogeneity

The differences in the proportions of CD8+ T cells that lie within the local enrichments of CCL22 or CCLX9 & 10 correlate with the TME heterogeneity in Tumor 3 & 4 regions.

4.2 Tumor Immune Infiltration

4.3 Localized Patterns of Chemokine Expression Leads to Distinct Chemokine-derived CD8+ T Cell Recruitment in Different Tumor Regions

5. Integrated Multiomic Spatial Phenotyping UnCOVERS Distinct Inflammatory Niches in Different Tumor Regions

5.1 Data Integration Strategy

5.2 Single-cell Metabolic and Chemokine Signatures Reveal TME Heterogeneity in Tumor Regions

5.3 Localized Patterns of Chemokine Expression Leads to Distinct Chemokine-derived CD8+ T Cell Recruitment in Different Tumor Regions

6. Multiomic Spatial Phenotyping Provides Mechanistic Insights into Partial Immune Response of HNSCC Patient

Anti-tumoral activity (Immune-responsive)

Dying Tumor Cells

Pro-tumoral activity (Immune-suppressive)

Invasive tumor cells

We used multiomic single-cell spatial phenotyping on the PhenoCycler-Fusion to provide a unique comprehensive anato-functional HNSCC tumor analysis.

- Deep spatial protein phenotyping uncovered tumor regions with heterogeneous metabolic stress and immune-infiltration profiles. Distinctively, we saw tumor regions that were highly apoptotic (T4) versus regions that were proliferating and invasive (T3).
- Subsequent RNA-based spatial phenotyping of chemokine landscapes revealed potential regulatory roles, where (1) localized patterns of CCLX9 and 10 in T4 promoted anti-tumoral activity by recruiting CD8+ T cells that release Granzyme B to induce apoptotic function, while (2) the expression of CCL22 produced the opposite effect in T3.

Collectively, these data demonstrate the power of multiomic spatial phenotyping and may, ultimately, provide information about new Spatial Biomarkers that can be used to stratify patients for ICI therapy.