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Investigating Immune Profiles in Differentiated Thyroid Cancer by Multiplex Immunofluorescence

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Summer Undergraduate Research Program

Background

- Differentiated thyroid cancer (DTC) accounts for 3.8% of all cancers in the U.S., with roughly 10% of cases progressing to distant metastatic DTC, which is associated with a poor five year survival outcome despite surgery and radioactive iodine (1).
- Recently, novel immunotherapies have attracted attention as a potential therapeutic resource in cases of advanced DTC, however, the response to therapy has been variable and unpredictable (2).
- Advanced DTC has been associated with an immune suppressive circulating phenotype (3) but the intra-tumoral immune infiltrate remains to be elucidated.
- Hence, analyzing immune profiles and checkpoint expression via multiplex immunofluorescence (MxIF) in DTC would provide ***valuable prognostic and therapeutic information***.

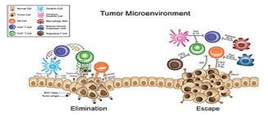

Figure 1. Proposed mechanism of immune escape

Purpose

The overall purpose of the project is to identify immune markers for DTC in order to better prognosticate the disease. In doing so, this study aims to identify and compare tumor-infiltrating immune markers with those present in the adjacent normal thyroid tissue, and collate these immune infiltrates with tumor characteristics.

Methodology

Figure 2: Staining Protocol

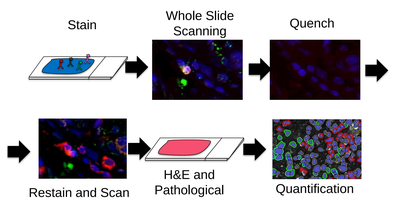

Figure 2. MxIF Protocol. Slides are deparifinized and stained cyclically across 4 rounds of staining. Once MxIF is complete, an H&E is performed for pathological review and concurrent quantification

Seventeen adult samples from patients with DTC were characterized for CD56, PD-1, PD-L1, FOXP3, CD3, CD8, CD4, CD45, CD68, CD163, INOS, HLA-DR, CD33, and CD19 using MxIF (Figure 2). Briefly standard pathological deparaffination, antigen retrieval and staining was performed. Slides were scanned, de-coverslipped and the fluorophores were quenched using an alkaline solution. Slides could then be restained to repeat the process. The data obtained was analyzed using HALO and a positive threshold was assigned based on review by a trained researcher. The minimum for positive thresholding was consistent for each antibody.

Results

In evaluating the immune profiles, important differences in the immune infiltrates between different stages of the cancer were observed. Generally, PD-1 and PD-L1 were highly expressed within the tumor, despite variability in lymphocyte infiltration, indicating the importance of PD-1 and PD-L1 as potential predictive biomarkers for the aggressiveness of thyroid cancer. Tumor from patients with distant metastases demonstrated higher T cell infiltration, T regulatory cells, macrophages and PD-L1 positive cells as compared to localized tumor.

Results

Figure 3: Immune Expression in Primary Thyroid Tumor

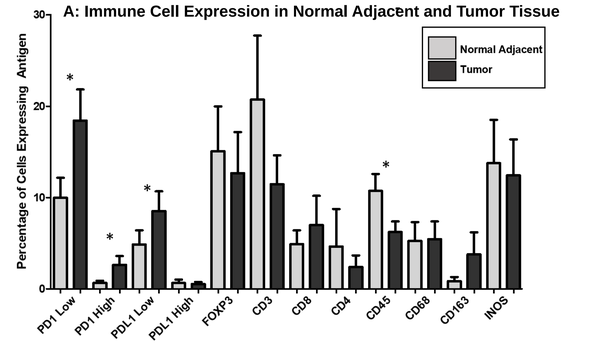

Figure 3. A. In comparing antigen expression within the tumor and adjacent normal regions, significant differences in low staining intensity PD-1, high staining intensity PD-L1, and CD45 expression were evident, as indicated by the asterisks (p<0.05). B-E. Comparing antigen expression in primary tumor of patients with distant metastasis (3), lymph node involvement (3) and low risk tumors (3)

Results

Figure 4: Colocalized Immune Infiltrates in PD-L1 High and Low Patient

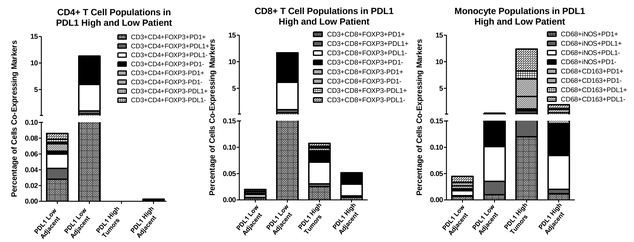

Figure 4. Illustration of the different T lymphocyte and monocyte subtypes between a high PD-L1 expressing and low PD-L1 expressing primary tumor. Cellular data was derived from colocalization information from aligned images

Results

Figure 5: Cellular Invasion Along Leading Edge of Tumor

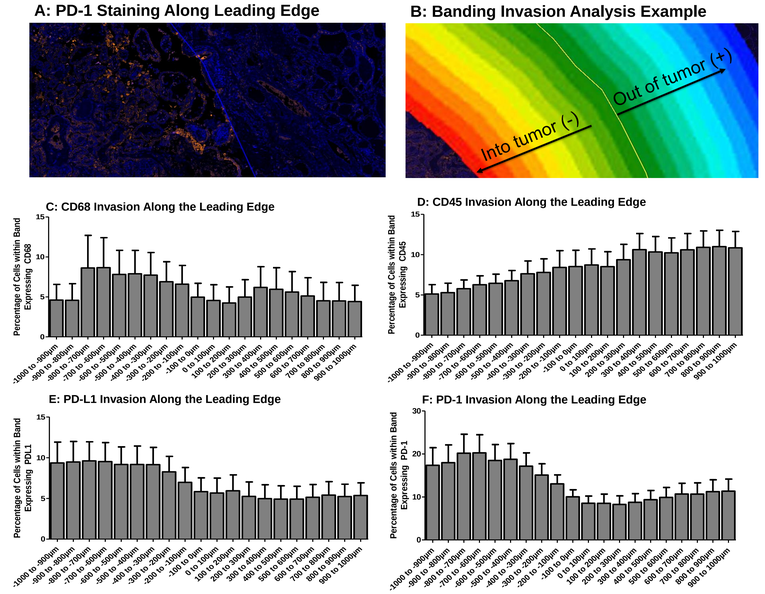

Figure 5. Cellular Invasion Along the Leading Edge of Tumor. A. Example area of PD-1 staining (Orange) of primary tumor leading edge (Blue Line). B. Example of banding strategy along leading edge. Tissue was divided into 100 um bands and the percentage of cells expressing antigens within each band was measured. C-F. Average invasion of individual antigens along the leading edge for all samples.

Conclusion and Future Directions

- Immune profiling demonstrated significant differences between tumor and adjacent healthy regions, particularly in terms of PD-1 and PD-L1 expression and lymphocyte infiltration, indicating that higher intratumor infiltration of T regulatory cells, macrophages and PD-1/PD-L1 positive cells may be associated with advanced thyroid cancer.
- Furthermore, the data demonstrates the efficacy of MxIF in gathering valuable information regarding the tumor microenvironment, which will have major implications in guiding the selection of patients for immunotherapy.
- Moving forward, we plan to investigate the immune profile of approximately 60 more samples in order to draw more accurate conclusions prior to analyzing differences in infiltrating cells based on clinical characteristics. This will then allow us to better identify immune phenotypes associated with thyroid cancer prognosis in order to personalize thyroid cancer care.

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