Investigating Immune Profiles in Differentiated Thyroid Cancer by Multiplex Immunofluorescence

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Background

- Differentiated thyroid cancer (DTC) accounts for 3.8% of all cancers in the U.S., with roughly 10% of cases progressing to distant metastasis, which is associated with a poor five year 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 (MIF) in DTC would provide valuable prognostic and therapeutic information.

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 correlate these immune infiltrates with tumor characteristics.

Methodology

Seventeen adult samples from patients with DTC were characterized for CD3, PD-1, PD-L1, CD3, CD8, CD4, CD45, CD68, CD163, INOS, HLA-DR, CD33, and CD144 using MIF (Figure 2).
- Briefly, standard pathological deparaffinization, antigen retrieval and staining was performed. Slides were scanned, deconvolapped and the fluorescent signals were quenched using an alkaline solution. Slides could then be rehydrated and the process was repeated. The data obtained was analyzed using HALO and a positive threshold was assigned based on review by a trained researcher. The minimum for positive thresholds 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 lymphocytic 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.

References


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