Localization of Immunosuppressive Cells in the Tumor Microenvironment

Background
Pancreatic ductal adenocarcinoma (PDAC) is characterized by an excessive amount of desmoplastic stroma seeded with inflammatory cells and is one of the most aggressive forms of cancer with no current specific therapies. Myeloid cells are the primary recruited effector cells during inflammation. A subset of these, consisting primarily of tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs), accumulate in tumors where they establish an inflammatory tumor microenvironment (TME) that is favorable for tumor progression. TAMs can be described as classically activated M1 types with pro-inflammatory anti-tumor functions, versus alternatively activated M2 types with immunosuppressive pro-tumor functions. The immunosuppressive functions of M2 TAMs can be exerted through release of cytokines and growth factors as well as direct recruitment of T regulatory cells (Tregs), a subset of lymphocytes responsible for immune tolerance of the system to the tumor. While the differentiation from M2 to M1 in PDAC has been shown to be associated with a worse prognosis, not much is known about PDAC TAM polarization and its potential correlation to Treg recruitment.

Methods
We have used MultiOmyx, a proprietary technology that enables visualization and characterization of multiple cell types on a single tissue section. MultiOmyx protein immunofluorescence (Pf) assay utilizes a panel of directed conjugated Cy-chromes to analyze up to 8000 cells using novel dye inactivation chemistry, enabling repeated rounds of staining and deactivation for up to 60 protein biomarkers.

Results
Using a panel of 11 antibody markers to analyze 8 stage IIB PDAC FFPE tumor-samples, we were able to identify different subtypes of tumor infiltrating lymphocytes and myeloid cell subsets. Using the pan macrophage marker CD68 in combination with either M1 marker HLA-DR or M2 marker CD163 we confirmed the presence of M1 (CD68+HLA-DR+) and M2 (CD68+CD163+) populations, the vast majority being of the M2 subtype. Moreover, we found a positive significant correlation (Pearson’s correlation p<0.05) between the presence of M2 TAMs and Tregs (CD3+CD4+FoxP3+), but not between M1 TAMs and Tregs. Using our proprietary algorithm that takes into account the staining pattern for each specific biomarker, we also examined the spatial relationship between the M2 TAM subtypes and Tregs and found M2 TAMs to be in closer proximity to Tregulatory cells than M1 TAMs.

Overview of Assay Workflow

MultiOmyx Spatial Analytics – Nearest Neighbor Analysis

In this study, utilizing MultiOmyx technology, a platform offered exclusively by NeoGenomics Laboratories, protein expression in 8 patients with pancreatic ductal adenocarcinomas were analyzed for possible correlation to known subtypes of myeloid cells and immune-suppressive T regulatory cells present in the tumor microenvironment.

When performing a MultiOmyx spatial “Nearest Neighbor Analysis”, M2 TAMs were found to be in closer proximity to Tregs compared to both the control cell population and M1 TAMs. We were able to distinguish between M2 subtypes of TAMs (CD68+CD163+) and M1 subtypes of TAMs (CD68+CD103+), the latter of which were not found to be in the close vicinity of M2 TAMs.

Conclusion

MultiOmyx spatial “Nearest Neighbor Analysis”, M2 TAMs were found to be in closer proximity to Tregs compared to both the control cell population and M1 TAMs.