Distinguishing Dendritic Cell Subtypes in the Tumor Microenvironment Using MultiOmyx™

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Background and Results

Background: Dendritic cells (DCs) are key initiators and regulators of the innate and adaptive immune responses. An emerging interest in cancer therapies is the capability to activate endogenous DCs to induce antigen specific T cell responses and thereby generate DC-based immunotherapies. Understanding the function and diversity of DC subsets in the tumor environment will help improve therapies developed for cancer treatment. DC subpopulations have been recognized in humans and categorized based on their phenotype and functional criteria. These DC subsets are classified based on biomarker expressions and include CD123+ plasmacytoid dendritic cells (pDCs), CD141+Clec9a+CD11c+HLADR+ classical dendritic cells (cDCs), and CD11c+HLADR+DC-SIGN monocyte derived dendritic cells (moDCs). However, difficulty of obtaining large number of these cells from cancer patients for exploratory purpose has been a challenge.

Methods: To help understand the complexity of distinct subsets of DCs, their spatial distribution within the tumor microenvironment (TME), and correlation with other immune cells, multiplex immunohistochemistry using a panel of antibodies broad enough to differentiate and characterize multiple DC subsets and T cell populations will be used. MultiOmyx, a novel hyperplexed multi "omic" technology, enables visualization and characterization of multiple biomarkers across multiple assays on a single 4 µm tissue section. MultiOmyx protein immunofluorescence (IF) assays utilize a pair of directly conjugated Cyanine dye-labeled (Cy3, Cy5) antibodies per round of staining. Each round of staining is imaged and followed by novel dye inactivation chemistry, enabling repeated rounds of staining and deactivation for up to 60 protein biomarkers. Biomarkers including CD11c, CD123, CD141, Clec9a, DC-LAMP, DC-SIGN, HLADR, CD14, CD68, CD163, CD3, CD4, CD40, and tumor segmenation marker SOX10 were used from a single 4 µm FFPE section in order to identify different subsets of DCs in tumor tissue from patients with Melanoma, a cancer type in which immunotherapeutic treatment has had a transformative effect and became the dominant therapeutic approach.

Summary: MultiOmyx image analyses using NeoGenomics' proprietary deep-learning algorithms for immune cell classification was performed. Dendritic cell subsets along with other immune subsets found in the tumor microenvironment of 10 melanoma resection samples were evaluated for immune cell density. A tri-pearson correlation analysis with the different immune cell subsets showed a positive trend between DCs and T cells in patient tumor samples. Interestingly, no correlation between plamacytoid dendritic cells (pDCs) and macrophages were observed. Although these results did not reach statistical significance with this sample set, the observed results were not so unexpected since the presence of antigen-presenting DCs in the tumor environment has been shown to recruit and activate T cells.

MultiOmyx Panel and Multiplexing Setup – MultiOmyx™

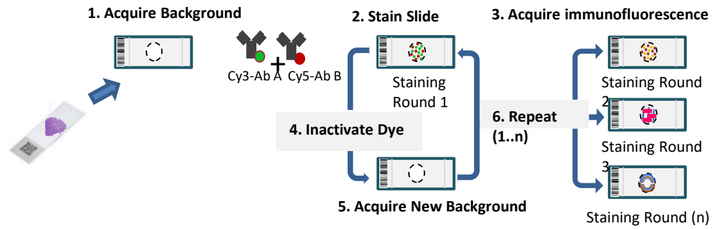

Figure 1. MultiOmyx Assay Workflow. A single 4 µm section from each FFPE tumor sample was multiplex IF stained. For each round of staining, conjugated fluorescent antibodies were applied, followed by image acquisition of stained slides. The dye was erased, enabling a subsequent round of staining with another pair of fluorescent antibodies.

Table 1. List of co-expression markers for immune subsets

Co-expression	Immune phenotypes
CD3+	T cells
CD3+CD8+	Cytotoxic T cells
CD3+CD4+	T helper cells
CD68+	Macrophage
CD68+CD163+	M2 Macrophage
CD123+ HLADR+	pDCs
CD123+ HLADR+CD40+	CD40 expressing pDCs
CD123+ HLADR+DC-LAMP+	Activated pDCs
CD11c+Clec9a+CD141+HLADR+	cDCs
CD11c+Clec9a+CD141+CD40+	CD40 expressing cDCs
CD11c+Clec9a+CD141+DC-LAMP	Activated cDCs
CD14+CD11c+DC-SIGN+HLADR+	Monocyte derived DCs (moDCs)

MultiOmyx Images – Immune cells in melanoma tumors

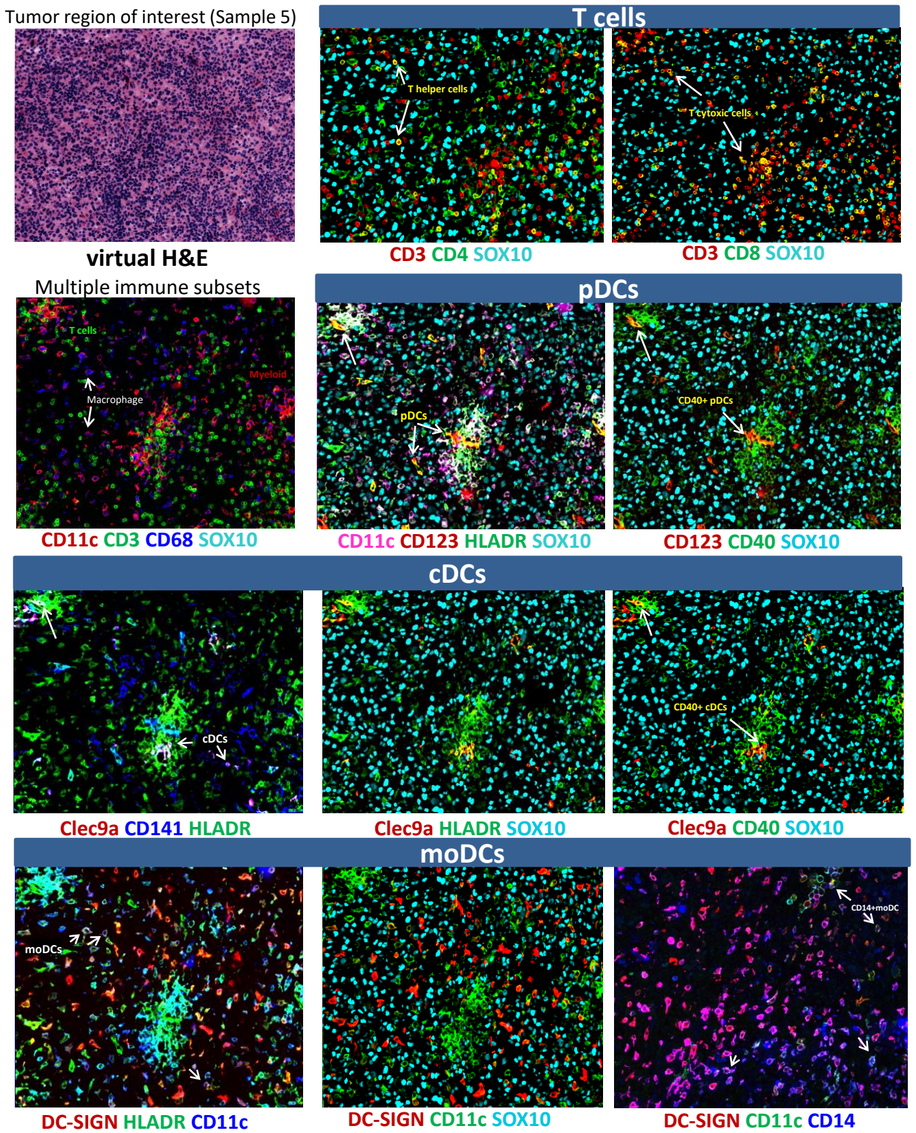

Figure 2. Multiplexed overlay images of a region of interest from melanoma. Multiple immune subsets can be identified in one single field of view. A representative field of view from sample 5 shows a pathology annotated region of interest that contains tumor and immune cells. This field of view shows multiple DC subset that include pDCs, cDCs and moDCs as indicated by co-expressing markers expressed by the individual cells as well as other immune cells including T cells and macrophages. Sample 9 was used to show a representative image of CD14+moDCs.

Interaction of Dendritic Cells with Immune Subsets in the Tumor Microenvironment

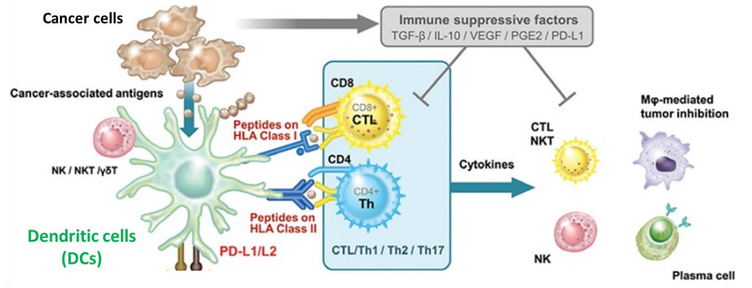

Figure 3. Dendritic cell interaction with T cells in the cancer microenviroment. Dendritic cells process and present cancer-associated antigens to prime lymphocyte populations such as effector (CD8) and helper (CD4) T cells resulting in activation and recruitment of other immune subsets. Image obtained from Shimoidaira et al., Immunome Research, 2016.

Immune Cell Density Heat Map and Correlation Table

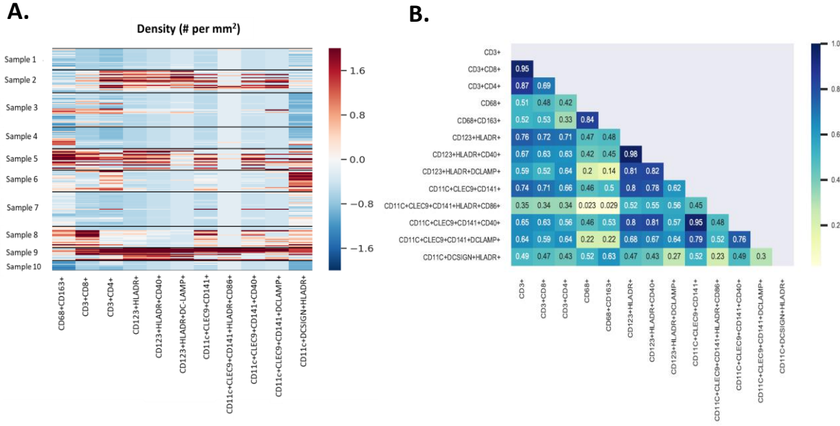

Figure 4. MultiOmyx Analytics. Density heat map for multiple immune subsets: each column represents samples with highest density in red and lowest density in blue for that immune subset (A.). Ccorrelations were evaluated for multiple DC subsets and T helpers (CD3+CD4+), T cytotoxic cells (CD3+CD8+), macrophages (CD68+), and M2 macrophages (CD68+CD163+) (B) .

Key Findings

- We evaluated the cell density of multiple DC subsets and observed a positive trend between activated DCs and T cells found in melanoma tissue samples.
- Analysis of DC subsets can provide a better understanding of DC mediated priming of tumor- specific T cells and immune activation.
- MultiOmyx multiplex IHC and image analysis using Neogenomic's proprietary deep learning algorithms can be a useful exploratory tool for DC-based immunotherapy along with other types of cancer treatments.

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