

IMAGING MASS CYTOMETRY IDENTIFIES NOVEL NATURAL KILLER CELL INTERACTIONS IN THE PANCREATIC DUCTAL ADENOCARCINOMA TUMOR MICROENVIRONMENT

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Background Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of less than 10%,¹⁻³ which is due in part to its dense desmoplastic and immunosuppressive stroma, caused by cancer-associated fibroblasts (CAFs).⁴⁻⁶ T cell function has been extensively explored in many cancers; however, natural killer (NK) cells of the innate immune system are relatively understudied in PDAC. Interestingly, we have previously shown that fibroblasts express known NK cell ligands and hypothesized that CAFs interact with NK cells as a potential immunosuppressive mechanism in PDAC.⁷ To further examine immune-stromal cell interactions in the PDAC tumor microenvironment (TME), we utilized Imaging Mass Cytometry (IMC), a novel multiplex imaging system that uses up to 40 metal-conjugated antibody markers to gain information of both tissue structure and single-cell data to conduct higher order proteomic single cell analysis.⁸ This novel technology will provide a deeper understanding of the immune architecture in PDAC.

Methods We designed a 34 metal-conjugated antibody panel for known immune, epithelial, and stromal cells, and various immune synapses, cytokines/chemokines, and activation markers. IMC was performed on a human PDAC tissue microarray generated at the Lombardi Comprehensive Cancer Center. IMC image data was pre-processed, segmented, and feature extracted to generate single-cell data,⁹ and downstream analysis was performed using R. Single cells were clustered using Seurat-based clustering approaches to identify known cell populations based on the immune, epithelial, and stromal cell antibody markers. Identified cell clusters were spatially annotated on IMC images using the cytomap R package.⁹

Results IMC images and single-cell analysis preliminarily identified that NK cells are: 1) present within the PDAC TME and 2) surprisingly, co-localize with malignant PDAC cells. This has not been previously reported.

Conclusions Using IMC, we have identified potentially novel interactions between NK cells and malignant epithelial cells in PDAC. Downstream cell neighborhood analysis will reveal further details on immune-stromal interactions in the PDAC TME and identify potential cell populations in further exploit in PDAC.

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Ethics Approval This study was approved by the Lombardi Comprehensive Cancer Center's Biospecimen Use Committee (approval number: GU21-0929).

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