Establishing the Glycomic Profile of Immune Cell Clusters in Pancreatic Ductal Adenocarcinoma Tissues Using Combined MALDI-MSI and MALDI-IHC

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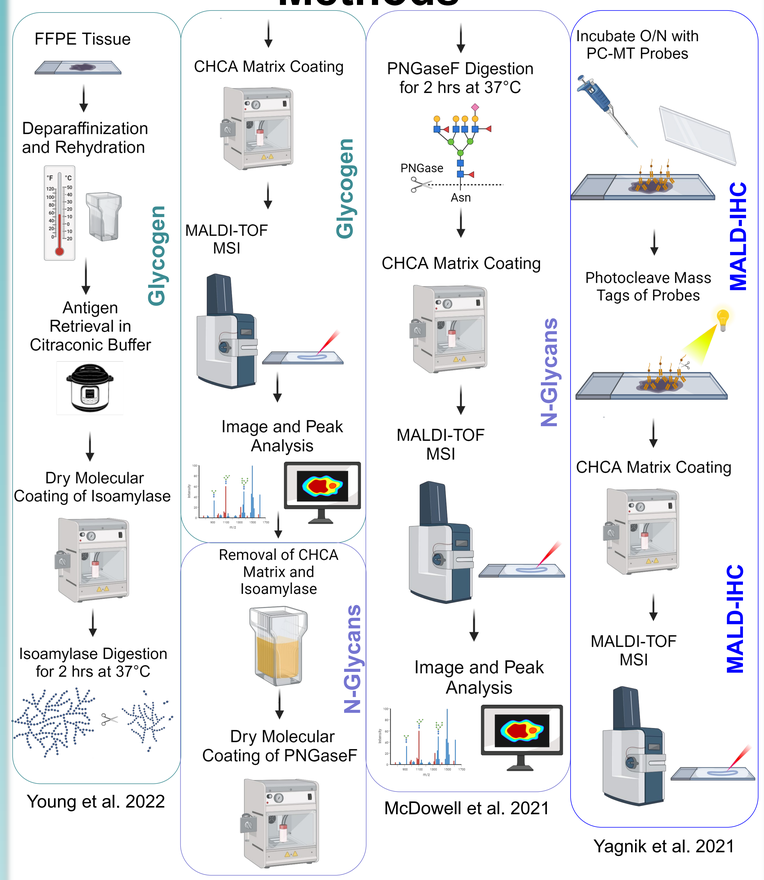
Overview

- Pancreatic cancers are targets for immunotherapies but still have limited responses.
- Immune cell clusters in PDAC tissues were assessed for detection of N-glycan by MALDI-IMS. It is hypothesized that detection of N-glycans reflects metabolic activity.
- MALDI-IHC was applied to the same tissues to define the types of immune cells present in the immune cell clusters.

Introduction

Immune Checkpoint Blockade therapy has proved incredibly effective in some solid tumor and hematologic malignancies but has shown inconsistent therapeutic potential in treating patients with **Pancreatic Ductal Adenocarcinoma** (PDAC). PDAC, predicted to be the second leading cause of cancer death by 2030, **has been previously described as having an immune quiescent tumor microenvironment but many aspects of said environment remain unknown**. Our current understanding of the PDAC immune landscape includes a lack of effector immune cells, dense desmoplastic stroma surrounding PDAC tumors, and an upregulation of immune checkpoint inhibitory signals. The exact immune cell population composition and their state of activation as determined by metabolism and displayed surface glycoproteins has yet to be elucidated. In this study, we have begun **to provide a metabolic profile of immune cell clusters (ICCs) in PDAC utilizing MALDI-MSI.** Our preliminary profile of these immune cell clusters has produced distinct glycan and glycogen signatures colocalized with the immune cell clusters. Additionally, we have optimized AmberGen's photocleavable mass tag antibody technology and executed this **MALDI-IHC technology to discretely identify different immune cell populations within the tertiary lymphoid structures**. With information from this study, we will be able to create a stratification scheme to better predict which patients diagnosed with PDAC will respond to immunotherapy and increase survival rates for this lethal cancer.

Methods



Results

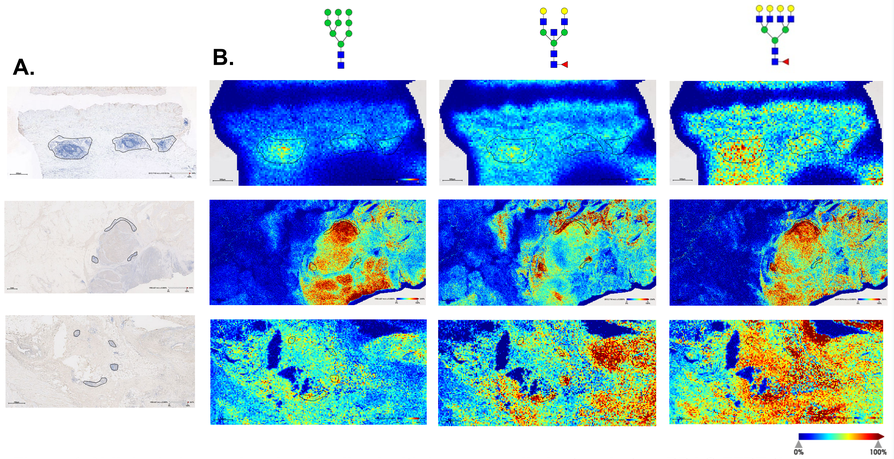


Figure 1. N-linked glycan signal colocalized with immune cell clusters in two PDAC FFPE tissue samples. **A.** Sialyl Lewis^{Y} stain identifying (ICCs) in three PDAC tissue samples. **B.** Representative images of N linked glycan signal colocalized with ICCs. Signals from the ionization of three classes of glycans including high mannose (1905 m/z), bisecting core fucosylated (2012 m/z) and tetra-antennary core fucosylated (2539 m/z) were found to be elevated and co-localized in areas with immune clusters.

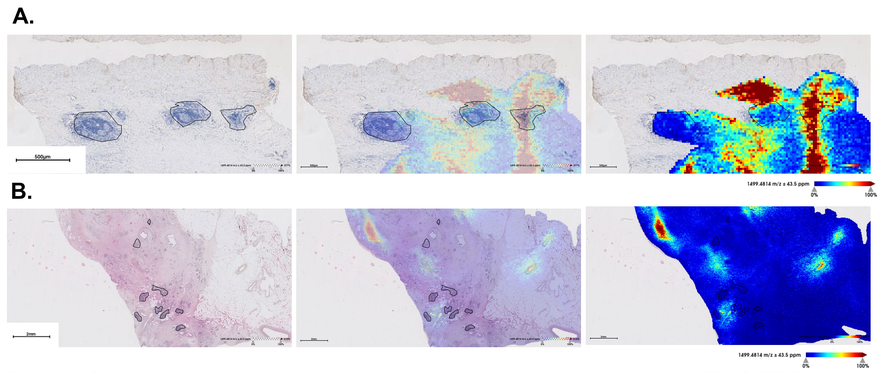


Figure 2. Glycogen signal localization compared with immune cell clusters in two PDAC FFPE tissue samples. Amylase treatment yielded glucose polymers from digested glycogen. **A. and B.** Representative images of glycogen signal (n=9 Glc) compared to ICCs. ICC's are circled.

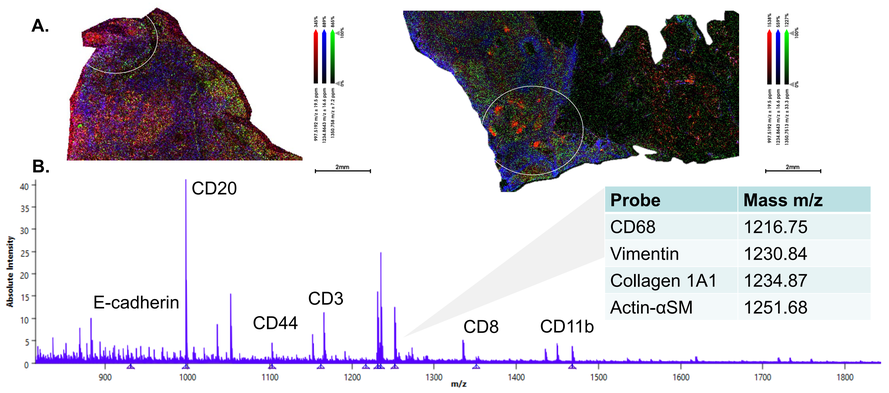


Figure 3. MALDI-IHC probe signal colocalized with immune cell clusters in two PDAC FFPE tissue samples. **A.** Representative images of PC-MT probe signal colocalized with histopathologic features of PDAC tissue. **B.** Mass spectrum annotated with antibody probes at reported masses. Antibody probes were from Ambergen, Inc.

Results

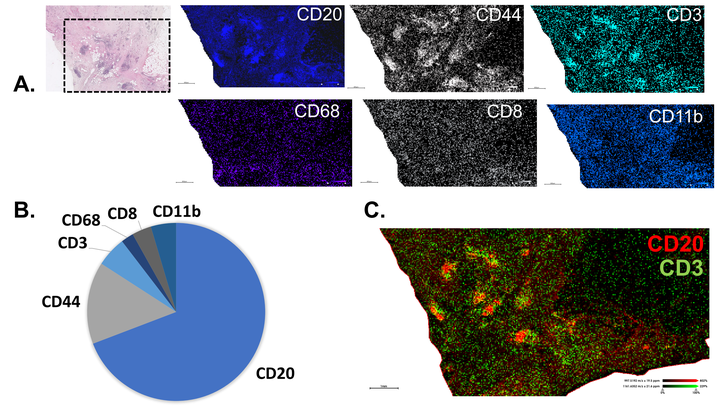


Figure 4. Immune cell composition in PDAC clusters. A. H&E stain of PDAC sample with MALDI-IHC representative images for immune cell specific probes. **B.** Pie chart displays proportion of each immune cell intensity within clusters. **C.** MALDI-IHC image displays distribution of CD20+ B cells (red) and CD3+ T cells (green) in PDAC tissue.

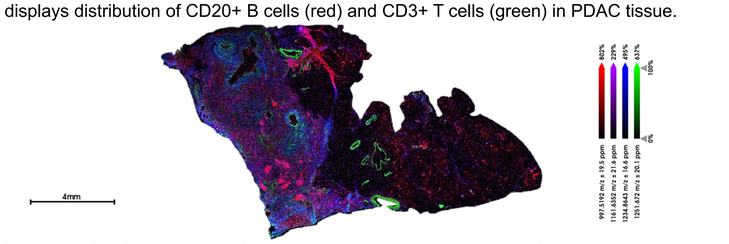


Figure 5. MALDI-IHC PDAC tissue stained with PC-MT probes. Overlay image of signals from antibody probes targeting CD20+ B cells (red), CD3+ T cells (purple), Collagen 1A1 (blue), and alpha actin Smooth Muscle (green).

Conclusions

- High mannose, core-fucosylated bisecting, and core-fucosylated tetra-antennary N-linked glycans colocalize with immune cell clusters in PDAC tissues
- Minimal glycogen detected within immune cell clusters
- MALDI-IHC staining with PC-MT probes was robust in FFPE PDAC tissues
- Immune clusters contain a heterogeneous population of innate and adaptive immune cells in PDAC tumors
- CD20+, CD44+, and CD3+ cells account for the largest proportion of cells within immune clusters in the PDAC tissues thus far analyzed.

- **FUTURE DIRECTIONS:** Evaluate more PDAC samples by combined MALDI-IHC immune cell probes and N-glycan imaging MS; Confirm immune cell cluster compositions utilizing immunofluorescence and/or MIBI-TOF.

References:

- Young, L.E.A. et al. (2022) In situ mass spectrometry imaging reveals heterogeneous glycogen stores in human normal and cancerous tissues. EMBO Mol Med. 14(11):e16029.
- McDowell, C. T.,et al. (2021). Imaging Mass Spectrometry and Lectin Analysis of N-Linked Glycans in Carbohydrate Antigen–Defined Pancreatic Cancer Tissues. Molecular & cellular proteomics, 20, 100012.
- Yagnik, G.; Liu, Z.; Rothschild, K. J.; Lim, M. J. Highly Multiplexed Immunohistochemical MALDI-MS Imaging of Biomarkers in Tissues. J. Am. Soc. Mass Spectrom. 2021, 32, 977– 988, DOI: 10.1021/jasms.0c00473

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