Establishment of the Full Glycan and Collagen Biomarker Profile for Prostate Cancer Tissue Samples Representing a Broad Spectrum of Histopathologies Using MALDI-IMS

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MUSC Medical University of South Carolina | Hollings Cancer Center, An NCI-Designated Cancer Center

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BACKGROUND

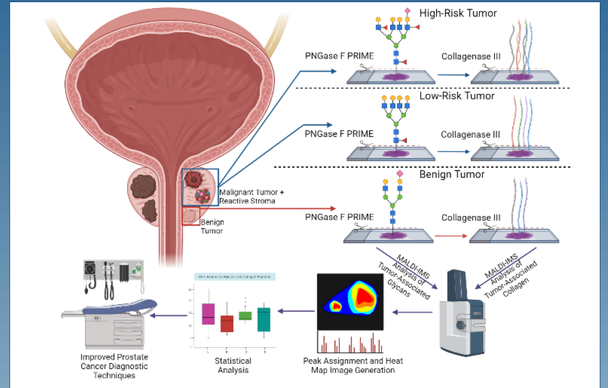
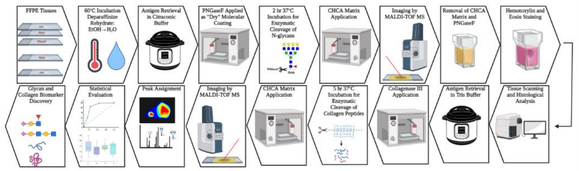


Figure 1: Prostate Cancer is characterized by changes in glycosylation and the emergence of reactive stroma. This study aims to analyze these changes via MALDI-IMS to improve Prostate Cancer diagnostic techniques. Created with BioRender.com

METHODS



Tumor Gleason Score	Number of Tissues	Number of Different Histopathologies				
Gleason 4	45	Glomeruloid: 4	Cribriform: 20	Fused: 20	PFG: 11	IDC: 2
Gleason 5	38	Solid: 26		Single: 17		Necrosis: 3
Other Malignant	8	IDC: 6		Gleason 3: 1		Ductal: 1
Benign	40					

Figure 2: (Top) Tissue preparation and analysis workflow. Created with BioRender.com. Each tissue was processed for N-glycan MALDI-IMS by digestion with PNGase F PRIME. Following N-glycan and matrix removal, collagenase digestion and further MALDI-IMS detection of ECM peptides was performed. Data was processed using SCiLS and Microsoft Excel. H&E Staining was performed for histological analysis. (Bottom) Chart detailing the histopathological distribution of tumors within the cohort. Total number of tissues is 112

N-GLYCAN DISTRIBUTION IN GLEASON 4 COHORT

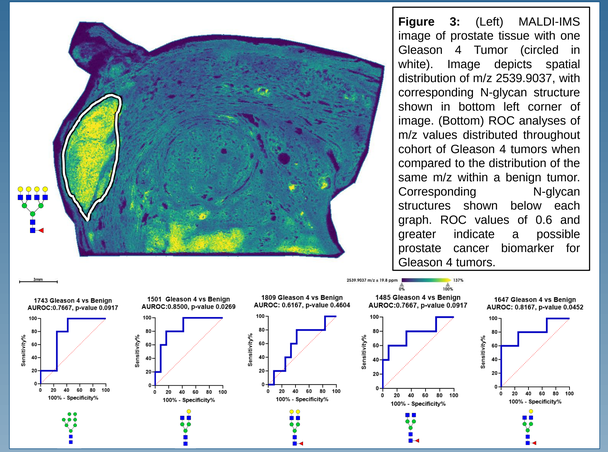


Figure 3: (Left) MALDI-IMS image of prostate tissue with one Gleason 4 Tumor (circled in white). Image depicts spatial distribution of m/z 2539.9037, with corresponding N-glycan structure shown in bottom left corner of image. (Bottom) ROC analyses of m/z values distributed throughout cohort of Gleason 4 tumors when compared to the distribution of the same m/z within a benign tumor. Corresponding N-glycan structures shown below each graph. ROC values of 0.6 and greater indicate a possible prostate cancer biomarker for Gleason 4 tumors.

N-GLYCAN DISTRIBUTION IN GLEASON 5 COHORT

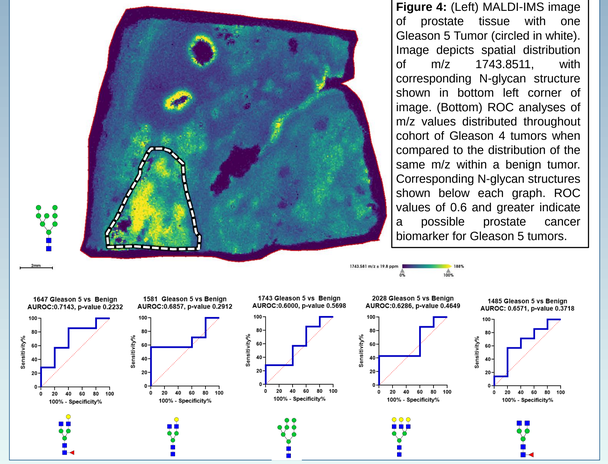


Figure 4: (Left) MALDI-IMS image of prostate tissue with one Gleason 5 Tumor (circled in white). Image depicts spatial distribution of m/z 1743.8511, with corresponding N-glycan structure shown in bottom left corner of image. (Bottom) ROC analyses of m/z values distributed throughout cohort of Gleason 4 tumors when compared to the distribution of the same m/z within a benign tumor. Corresponding N-glycan structures shown below each graph. ROC values of 0.6 and greater indicate a possible prostate cancer biomarker for Gleason 5 tumors.

STROMA-ASSOCIATED N-GLYCANS

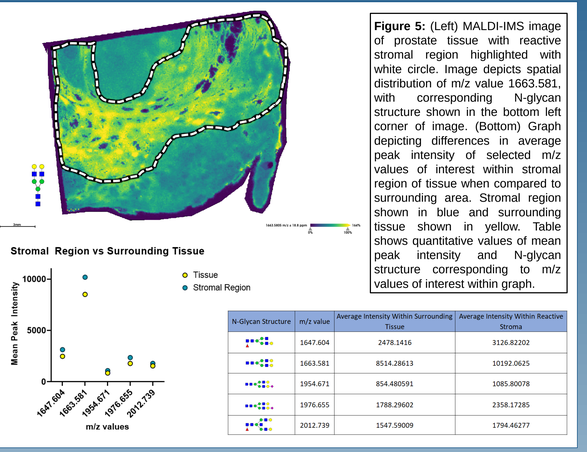


Figure 5: (Left) MALDI-IMS image of prostate tissue with reactive stromal region highlighted with white circle. Image depicts spatial distribution of m/z value 1663.581, with corresponding N-glycan structure shown in the bottom left corner of image. (Bottom) Graph depicting differences in average peak intensity of selected m/z values of interest within stromal region of tissue when compared to surrounding area. Stromal region shown in blue and surrounding tissue shown in yellow. Table shows quantitative values of mean peak intensity and N-glycan structure corresponding to m/z values of interest within graph.

N-Glycan Structure	m/z value	Average Intensity Within Surrounding Tissue	Average Intensity Within Reactive Stroma
	1647.604	2478.1416	3126.82202
	1663.581	8514.28613	10192.0625
	1954.671	854.480591	1085.80078
	1976.655	1788.29602	2358.17285
	2012.739	1547.59009	1794.46277

N-GLYCAN DISTRIBUTION IN OTHER MALIGNANCIES

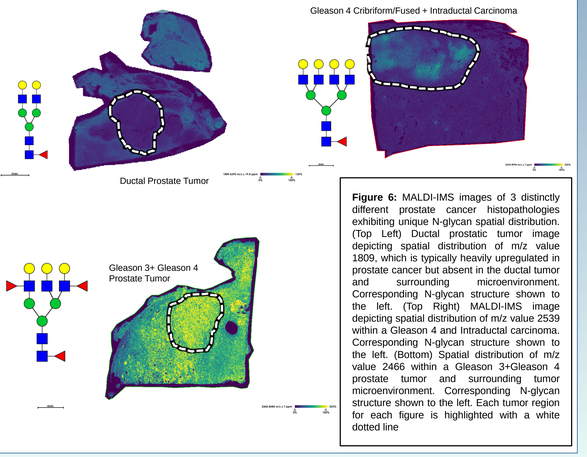


Figure 6: MALDI-IMS images of 3 distinctly different prostate cancer histopathologies exhibiting unique N-glycan spatial distribution. (Top Left) Ductal prostatic tumor image depicting spatial distribution of m/z value 1809, which is typically heavily upregulated in prostate cancer but absent in the ductal tumor and surrounding microenvironment. Corresponding N-glycan structure shown to the left. (Top Right) MALDI-IMS image depicting spatial distribution of m/z value 2539 within a Gleason 4 and Intraductal carcinoma. Corresponding N-glycan structure shown to the left. (Bottom) Spatial distribution of m/z value 2466 within a Gleason 3+Gleason 4 prostate tumor and surrounding tumor microenvironment. Corresponding N-glycan structure shown to the left. Each tumor region for each figure is highlighted with a white dotted line

COLLAGEN ANALYSIS OF COHORT

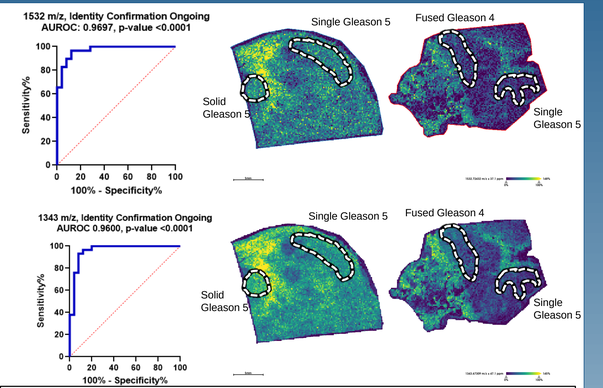


Figure 7: Collagenase III digestion of 2 tissues, each containing Gleason 4 and Gleason 5 tumors (labeled on images). ROC analysis with AUROC values shown to the left of each tissue corresponding to detected collagen m/z value. Confirmation of peptide identities is ongoing.

CONCLUSION

- A multi-enzymatic approach was used to understand the N-glycan and collagen composition of prostatic tumors
- Major tumor-associated N-glycans fall within the high-mannose and pauci-mannose categories
- Multiple branched N-glycan species with different combinations of fucose and sialic acid constituents were also associated with tumor incidence and progression
- A subset of branched N-glycans were associated with higher intensities in tumors with higher Gleason scores
- Additional proteomic studies are ongoing with the goal of evaluating identity and prevalence of ECM and collagen peptide distribution in correlation with different tumor subtypes
- Analyses to evaluate N-glycan isomer distributions for fucosylated and sialylated N-glycan species are also ongoing

REFERENCES

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