WP248

Comparative N-glycome analysis of prostate cancer tissues using MALDI Q-TOF versus MALDI-FTICR imaging mass spectrometry workflows

Grace Grimsley, Connor A. West, Xiaowei Lu, Anand S. Mehta, Peggi M. Angel, Richard R. Drake
Medical University of South Carolina, Charleston, SC

INTRODUCTION

- Multiple changes in N-linked glycan structures are detected in the progression of prostate tumors.
- A unique set of prostate cancer tissues representing the diagnostic tumor region matched to a non-tumor inflammatory/ hyperplasia region were evaluated by N-glycan MALDI-IMS.
- Two complementary N-glycan imaging MS workflows were employed: 1) a standard PNGase F PRIME digestion, 2) a chemical amidation reaction to stabilize sialic acids, followed by PNGase F PRIME.
- The tissue pairs were analyzed on both the MALDI-FTICR and the MALDI-Q-TOF for comparative performance of both instruments

METHODS AND MATERIALS

A: Tissue Preparation

- Archived, de-identified human FFPE prostate tissues were used. Slides were heated prior to dewaxing with sequential washes of xylenes, ethanol, and water.

B: Enzyme and Matrix Application

- Stabilization of sialic acids by amidation-amidation (REF 1)
- Antigen Retrieval of tissue at pH 3.0 (REF 2)
- Apply PNGase F PRIME to slides using TM Sprayer (HTX Technologies)
- Incubate for 2 hours at 37.5° C in a humidity chamber
- CHCA matrix applied to slides with TM Sprayer

C: MALDI IMS

- Tissue sections were imaged on a MALDI FT-ICR mass spectrometer (7.0T solariX, Bruker Daltonics) in positive ion mode at a 200µm raster size.
- The same tissue sections were then imaged on a MALDI-Q-TOF mass spectrometer (timsTOF fleX, Bruker Daltonics) in positive ion mode at 40µm raster size.
- Image data were analyzed in SCiLS Lab 2020a (Bruker Daltonics), normalized to Total Ion Current (TIC).

RESULTS

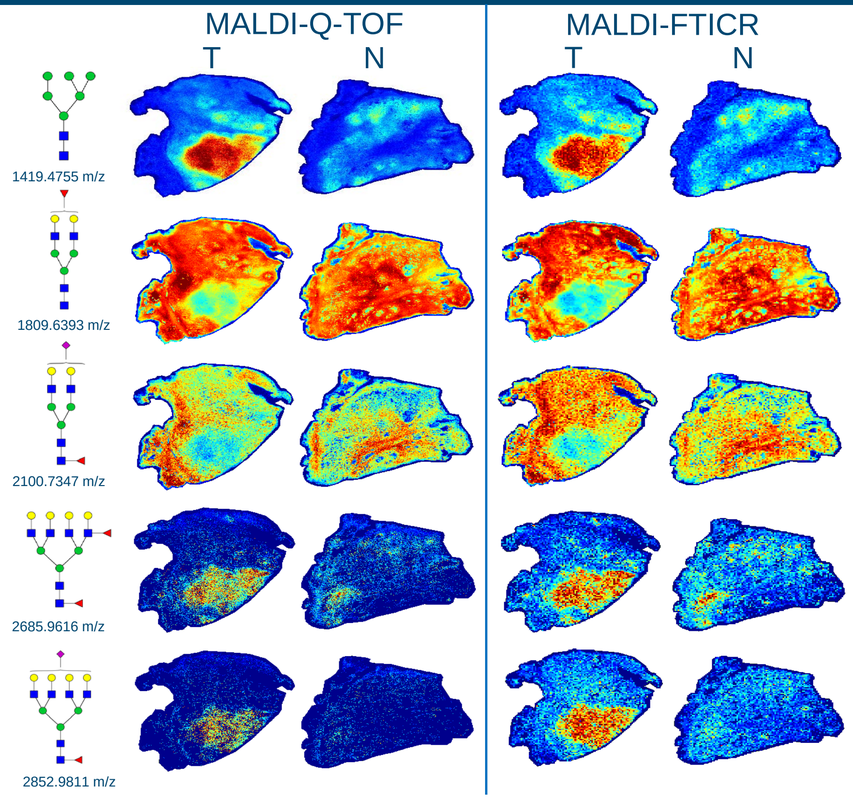

Figure 1: Glycan features of matched prostate cancer and adjacent non-tumor tissue pairs on MALDI-Q-TOF and MALDI-FTICR. Shown are the following glycans in tissue pairs of tumor (T) and non-tumor (N) done in SCiLS Lab: 1419.4755 m/z (row 1, high mannose); 1809.6393 m/z (row 2, fucosylated); 2100.7347 m/z (row 3, sialylated); 2685.9616 m/z (row 4, high mass with fucose) and 2852.9811 m/z (row 5, high mass with fucose and sialic acid). Masses are theoretical generated from GlycoWorkbench.

Table 1: List of the instrument parameters

	MALDI-Q-TOF	MADLI-FTICR
Run time	2-4 hours	6-10 hours
Raster	40 µm	150-200 µm
Number of laser shots per pixel	300	200
Number of peaks detected	80-90	100-120
Mass range of peaks detected	700-3000 m/z	700-4000 m/z

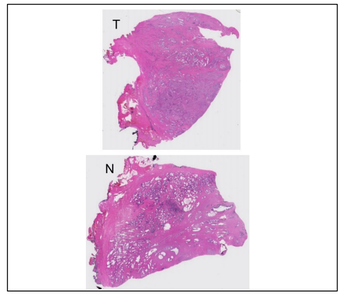

Figure 2: H&E stain of the matched prostate pair.

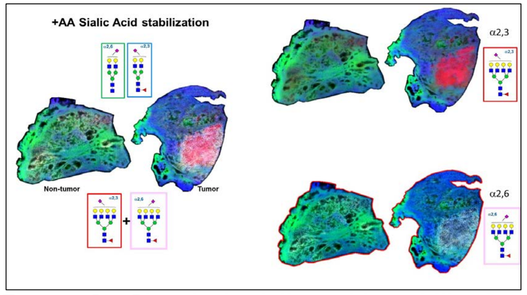

Figure 3: Chemical sialic acid stabilization analyzed on the MALDI-Q-TOF. Shown is an overlay of the α2,3 and α2,6 sialic acids. Note the improved detection of 2852.981 m/z from Figure 1.

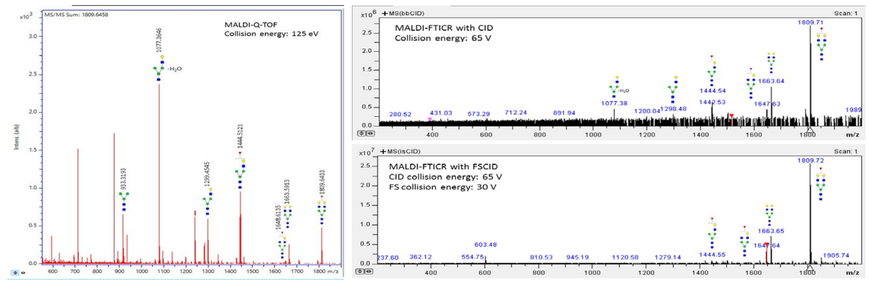

Figure 4: Spectrum of on-tissue MALDI-IMS/MS fragmentation of precursor ion 1809.6393 m/z. The spectra on the left represents the fragmentation pattern from the MALDI-Q-TOF by CID. The spectra on the upper right represents the fragmentation pattern from the MALDI-FTICR by CID. The spectra on the bottom right represents the fragmentation pattern from the MALDI-FTICR by FSCID.

CONCLUSIONS

- The image resolution and time required to obtain the data was a clear advantage of the MALDI-Q-TOF.
- Higher mass and sialylated N-glycans were more effectively detected by the MALDI-FTICR.
- There was no significant difference in the detection of the abundant bi-antennary and high mannose N-glycans.
- The stabilization of the sialic acids facilitated higher mass glycan detection of the MALDI-Q-TOF.

FUTURE DIRECTION

- Optimization of high mass N-glycans and ion mobility on MALDI-Q-TOF.
- Further optimization of MS/MS on both instruments.

REFERENCES

1. Holst et al. Anal Chem. 2016 Jun 7;88(11):5904
2. Drake et al. Curr Protoc Protein Sci. 2018 Nov;94(1):e68.