TRUE DISTRIBUTION OF ISOBARIC N-GLYCANS SEPARATED BY ION MOBILITY DIRECTLY FROM FFPE COLON CANCER TISSUE BY MALDI IMAGING

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INTRODUCTION

Research studies have reported extensive alterations in protein glycosylation patterns in cancer tissues including colon cancer which is the third most common cancer in the United States. However during these studies, tissues are homogenized and the spatial information showing the localization of the glycans is lost. Mass spectrometry imaging (MSI) is an established analytical tool for biomolecular research which can accurately determine the spatial location of molecules in a tissue section. Recently, methods have been developed to determine released N-Glycans directly from both fresh frozen and FFPE tissues[1]. A major challenge in the analysis of N-glycans is the large number of isobaric glycans resulting from their complex structures with branched chains and multiple additions of fucose and sialic acid residues. Here we report the advantage of ion mobility separation to differentiate these glycans in a MALDI MSI workflow used in the research analysis of human FFPE colon cancer tissue.

METHODS

Tissue sample preparation

FFPE tissues were sectioned at 5µm and mounted on standard glass slides. After incubation for 1h at 60° C, the tissue sections were deparaffinized with two washes in xylene, and rehydrated through a series of ethanol/water solutions. This was followed by antigen retrieval in citraconic anhydride for 25 min and slides were then water washed 5 times. To release the N-glycans from their proteins, a recombinant PNGaseF (Bulldog Bio) solution was sprayed on the tissue before incubating for 2 hrs at 37° C in high humidity. A solution of MALDI matrix (α-Cyano-4-hydroxycinnamic acid) was sprayed onto the tissue.

Mass spectrometry

MSI experiment was carried out using a SYNAPT G2-S*i* HDMS mass spectrometer where the tri-wave separated ions according to their ion mobility in the gas phase.

Laser:	Nd:YAG laser (355 nm)
Pulse rate:	1000 Hz
Ionization mode:	Positivew
Spatial resolution:	90 µm (lateral)
Mass range:	m/z 500 - 3,000
IMS pressure:	2.85 mbar
Wave Height:	40 V
Wave Velocity:	800 to 200 m/s

Data management

Slides were scanned using a flatbed scanner and regions to be imaged were defined in High Definition Imaging Software (HDI) v1.4 (Waters), creating a MassLynx experiment file that was imported into a sample list. MSI data were mined using Driftscope and HDI v1.4 Software.

RESULTS

The overall MS spectrum shows strong signal for N-glycan molecules, demonstrating the efficacy of the digestion step of the methodology. Using prior knowledge of the type of glycans expected, 76 glycans were identified and mapped directly from the FFPE tissue section, ranging from a mass to charge ratio (m/z) of 771.5 up to a m/z of 2905.03 using accurate mass information.
MALDI MSI was able to distinguish the tissue morphology and determine the tumor region based on specific ions, especially sodiated N-Glycans Hex7HexNAc2 (m/z 1581.5) and Hex8HexNAc2 (m/z 1743.6) which were highly abundant in the tumor tissue whereas Hex5dHex1HexNAc5 (m/z 2012.7) and Hex5dHex1HexNAc4 (m/z 1809.6) were more abundant in the non-tumor areas (see figure 1).

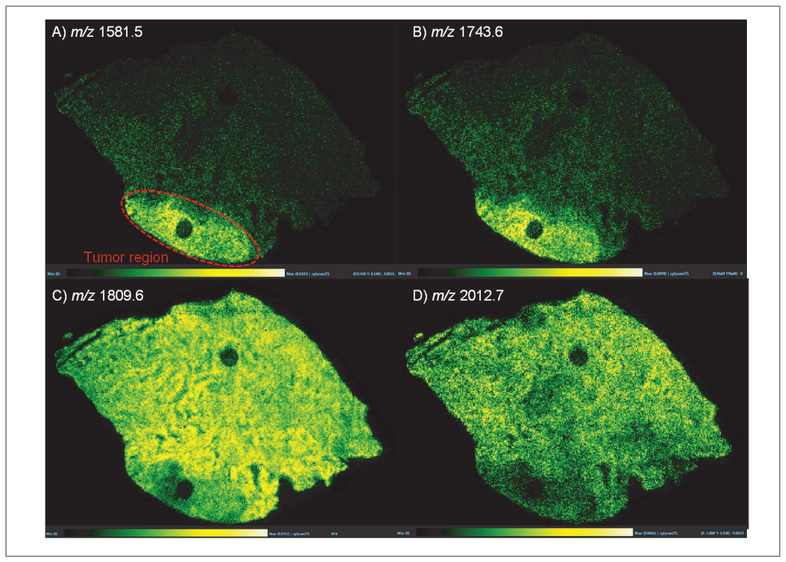

Figure 1. MALDI MSI ion images of A) m/z 1581.5 (Hex7HexNAc2 , Na^+), B) m/z 1743.6 (Hex8HexNAc2 , Na^+), C) m/z 1809.6 (Hex5dHex1HexNAc4 , Na^+) and D) m/z 2012.7 (Hex5dHex1HexNAc5 , Na^+).

When the ion mobility dimension was explored using the DriftScope software, it was clearly shown that two nested trendlines in m/z vs. drift time existed (see figure 2B).

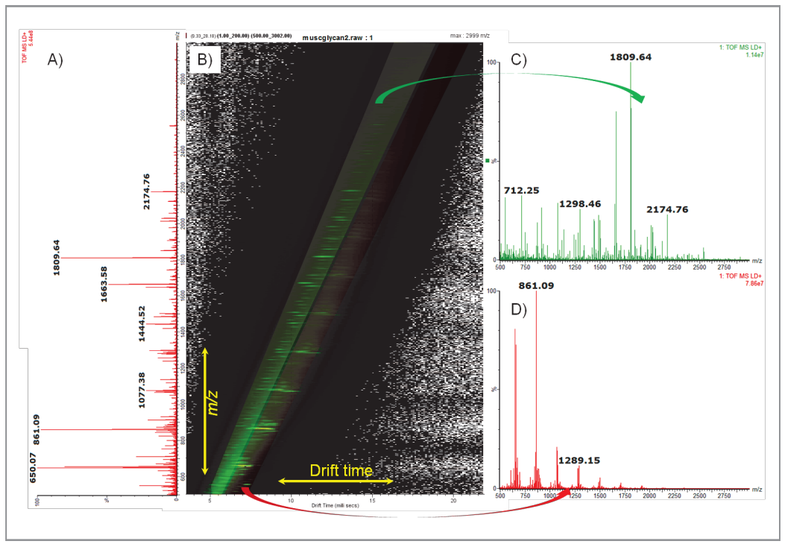

Figure 2. A) Overall MS spectrum combining all pixels; B) 2D plot m/z vs. drift time showing two trendlines; C) MS specific from fastest trendline representing the glycan species; D) MS specific from slowest trendline representing MALDI matrix clusters species.

The faster trendline corresponding to more compact conformations of the ions in the gas phase was identified to be that of the N-Glycan class of molecules (see figure 2C), whereas the slower trendline was representative of the matrix clusters ions (see figure 2D).

The IMS-MS data showed a more specific analysis of the N-Glycans than when compared to the MS data alone. In several cases, the IMS peaks were broader than the expected resolution or there were shoulder peaks, indicating that isobaric species were present.

For example, m/z 1444.5 corresponding to sodiated Hex4dHex1HexNAc3, and the IMS displayed two distinct peaks which, while not baseline separated (see figure 3B), were sufficiently separated to obtain individual ion images showing distinctly different distributions. The isobaric species with the faster drift time were evenly distributed across the healthy and tumor tissue types of the section (see figure 3C), whereas the isobaric species with the slower drift time were more abundant in the healthy regions of the tissue (see figure 3D).

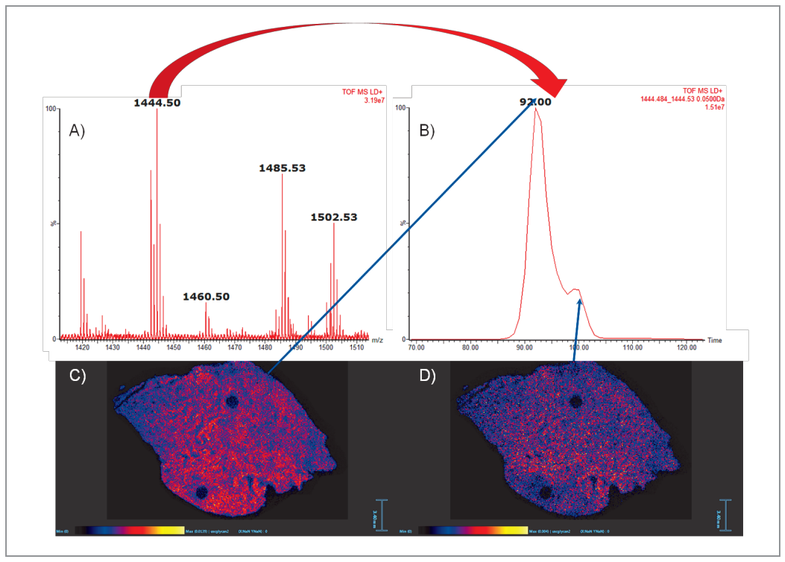

Figure 3. A) Zoomed overall MS spectrum; B) Extracted mobilogram for m/z 1444.5 showing two IMS peaks; C) ion image of m/z 1444.5 with a faster IMS; D) ion image of m/z 1444.5 with a slower IMS

CONCLUSION

- **Efficient N-Glycan release procedure applied to colonic FFPE tissue section and analysed by MALDI MSI experiment was demonstrated.**
- **IMS offers an orthogonal separation from MS alone and can be fully incorporated within MALDI MSI experiment.**
- **The glycan trendline was easily distinguished from the MALDI matrix cluster trendline, giving a more specific overall MS spectrum.**
- **A truer visualisation of isobaric N-Glycans was demonstrated , highlighting potential differences in isomer abundance in tumour vs. non tumour tissue regions.**
- **For Research use only, not for use in diagnostic procedures.**

References

1. Powers TW, Richard R. Drake et al: MALDI Imaging Mass Spectrometry Profiling of N-Glycans in Formalin-Fixed Paraffin Embedded Clinical Tissue Blocks and Tissue Microarrays, PLoS One. 2014 Sep; 9(9): e106255.

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