ION MOBILITY SEPARATION OF N-GLYCANs DIRECTLY FROM FFPE COLON CANCER TISSUE

Emmanuelle Claude1; Peggi M. Angel2; Richard R. Drake2; Hernando Olivos3; Michael Batey4; James Langridge4

1Waters Corporation, Wilmslow; 2Department of Cell and Molecular Pharmacology and Experimental Therapeutics and MUSC Proteomics Center, Medical University of South Carolina, Charleston, South Carolina; 3Waters Corporation, Beverly, US

INTRODUCTION

The direct association of the genome, transcriptome, metabolome, lipoproteins and proteome with the serum glycome has revealed systems of interconnected cellular pathways. In a move towards personalised medicine, it is now becoming critical to understand disease pathogenesis, and the molecular features that accompany it in the context of the dissection of a whole system. 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 glycan is lost.

METHODS

Tissue sample preparation

FFPE tissues were sectioned at 5µm and mounted on standard glass slides. After immersion for 1hr 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 and rehydrated through a series of ethanol/water solutions.

Mass spectrometry imaging (MSI)

MSI was performed using a SYNAPT G2-S HDMS mass spectrometer where the tri positive ions were selected. The IMS data displayed two different clusters of ions, one corresponding to lower mass glycans than when compared to the MS data alone and can be fully incorporated within MALDI MS imaging studies.

RESULTS

Data management

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

The overall MS spectrum shows strong signals for N- glycans in normal colon mucosa, however, the intensity of these peaks is weaker in the CRC tissue sections. This range from a mass to charge ratio (m/z) of 770 to 2900.03 using accurate mass information. MALDI MSI was able to distinguish the tissue morphology and was able to reveal the presence of specific ions. In particular, the sodiated N-Glycan class of molecules (see figure 4C), whereas the slower drift time was representative of the matrix ions corresponding to more complex formations of the ions in the gas phase was identified to be that of the N-Glycan class of molecules (see figure 4C), whereas the slower drift time was found to be highly abundant in the non-tumor areas.

Using Ion Mobility Separation (IMS), a technique which allows for the separation of ions based on their collisional cross section, it could be clearly shown, using Driftscope software, that two nested tendencies in m/z vs. drift time were apparent in each imaging section. These signals were corresponding to more complex formations of the ions in the gas phase was identified to be that of the N-Glycan class of molecules (see figure 4C), whereas the slower drift time was found to be highly abundant in the non-tumor areas.

Using the IMS-MS data allowed for a more specific analysis of the N-Glycans than when compared to the MSI data alone. In several cases, the IMS peaks were broader than the expected resolution or there were shoulder peaks, which indicate that isobaric species were present. As IMS is a direct sampling technique, the chromatographic separation used in LC-MS is not possible. However, the IMS data can allow for quantification of isobaric species on the instrument and help to elucidate additional samples.

For example, m/z 1444.5 corresponding to sodiated Hex4Hex1HexNAc3, and the IMS data displayed two distinct peaks which, while not fully resolved (see figure 5B), were sufficiently resolved 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 5C), whereas the slower species with the slower drift time were more abundant in the healthy regions of the tissue (see figure 5D).

CONCLUSIONS

We have demonstrated an efficient N-Glycan release procedure which allows FFPE tissue sections to be analyzed by MALDI Mass Spectrometry Imaging.

Non subjective differentiation between tumour and non tumour tissue is possible using MSI.

Ion Mobility Separation offers an orthogonal separation from MS alone and can be fully incorporated within MALDI MSI experiments.

Using IMS, the glycan trendline could be easily distinguished from MALDI matrix cluster trendline, giving a more specific overall MS spectrum.

A new 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


TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS