MOLECULAR IMAGING OF GANGLIOSIDES TO INVESTIGATE LYSOSOMAL STORAGE DISEASES USING MASS SPECTROMETRY IMAGING WITH ION MOBILITY SEPARATION

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OVERVIEW

- **GM1 and GM2 gangliosidosis are autosomal recessive lysosomal storage diseases characterized by accumulation of gangliosides in the central nervous system (CNS).**
- **Ganglioside content in the CNS has been measured by liquid chromatography (LC) mass spectrometry (MS).**
- **Spatial distributions of gangliosides in a diseased, normal, and treated tissues can provide complementary information to LC-MS measurement to develop new therapeutic approaches.**
- **Here we present, matrix-assisted laser desorption/ ionization (MALDI) mass spectrometry imaging (MSI) with ion mobility separation (IMS) workflow to map molecular distributions of gangliosides in mouse brain.**

INTRODUCTION

Gangliosides are glycosphingolipids containing a ceramide base and a carbohydrate chain containing at least one sialic acid. In addition to variability in the number and position of sialic acid residues attached to carbohydrates, gangliosides exhibit heterogeneity due to chain length and degree of saturation of fatty acyl group in the ceramide base.

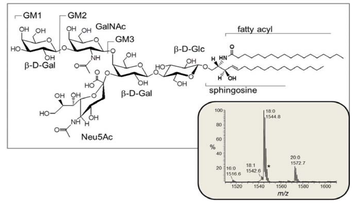

Figure 1. Molecular structure of GM gangliosides. The inset shows MALDI mass spectrum of gangliosides from brain.

Gangliosides undergo dramatic changes in abundance and spatial distribution during brain development, and their accumulation caused by specific enzyme defects can lead to various neurological diseases.

GM1 and GM2 gangliosidosis are autosomal recessive lysosomal storage diseases resulting from depletion of β-galactosidase or β-hexosaminidase, respectively.

In this work, we used a β-galactosidase double knockout (KO) mouse model and MALDI imaging workflow to map spatial distribution of GM1 and GM2 gangliosides in KO brain tissue. We anticipate this molecular mapping approach will lead to a better understanding of the effectiveness of gene therapy treatment and will help facilitate moving preclinical studies forward with a greater biological insight.

METHODS

- **Wild type (WT) and β-galactosidase enzyme knockout (KO) coronal mouse brain sections (10 µm thick) were thaw-mounted onto a microscope glass slide, dehydrated for 15 minutes in a vacuum chamber, and coated with CMBT (5-chloro-2-mercapto-benzothiazole) matrix using TM-Sprayer (HTX technologies LLC, Carrboro, NC).**
- **MALDI MSI was acquired using a SYNAPT G2-S*i* MALDI mass spectrometer (Waters Corporation, Milford, MA) with traveling wave ion mobility separation. Mass spectra were collected at a spatial resolution of 60 µm between the *m/z* 200 to 2500 range in the negative ion mode. IMS travelling wave height and velocity were set at 40 v and 440 m/s.**

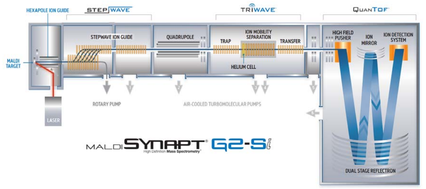

Figure 2. Schematic of SYNAPT-G2Si mass spectrometer with ion mobility separation and MALDI ion source.

- **The MS/MS fragmentation of a ganglioside was carried out by collisional induced dissociation in transfer cell using collision voltage of 90 eV. Assignment of fragment ions was done manually using values from literature and in-silico fragmentation (lipidmaps.org).**
- **Post-acquisition processing, e.g., analysis on region of interest (ROI), was performed using High Definition Imaging (HDI) software (version 1.4, Waters Corporation, Wilmslow, UK). Quantitative analysis of gangliosides was carried out by normalizing with matrix ion peaks.**

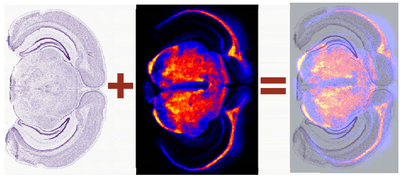

Figure 3. HDI 1.4 was employed for multimodal imaging workflow for correlation of MS image with histological image from mouse brain atlas. HDI also supports multimodal analysis of MALDI image with desorption electrospray ionization (DESI) imaging and other imaging modalities.

MALDI MS IMAGING WITH ION MOBILITY SEPARATION

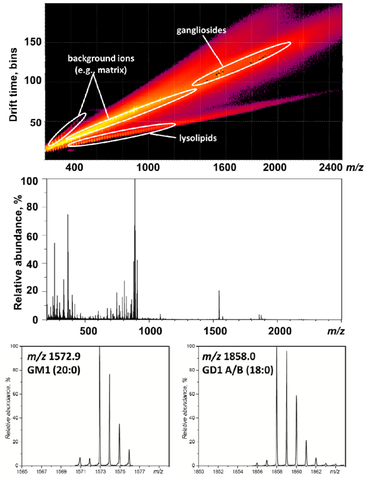

Figure 4. MALDI MSI detected various gangliosides and other lipids in mouse brain tissue. Ion mobility separation helped to separate out background ions from matrix ion peaks, giving a better S/N ratio for gangliosides and other lipid ions of interest.

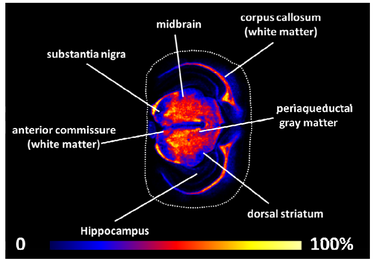

Figure 5. MALDI MS image generated by mapping ion intensities of lipid species, e.g., sulfatide 24:1 in wildtype mouse, shows its spatial distribution in various parts of the mouse brain.

WILDTYPE VS. KNOCKOUT MOUSE BRAIN SECTIONS

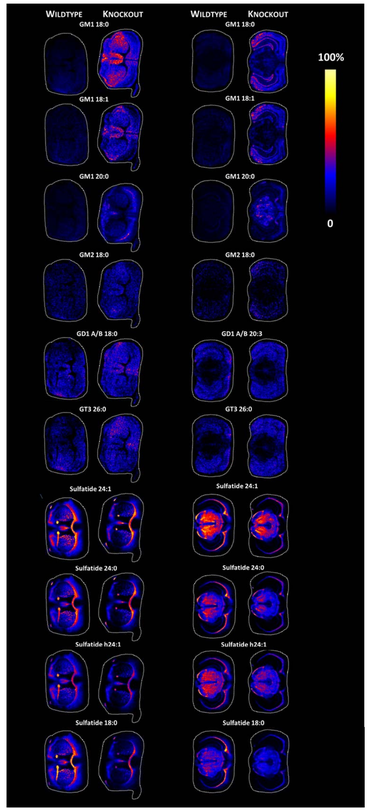

Figure 6. β-galactosidase knockout mouse brain showed specific spatial accumulation of GM1. GM1 with different fatty acid chain lengths on the ceramide base (i.e. 18:0, 20:0) showed different tissue distributions. Spatial distribution of other lipids, e.g., sulfatides were also imaged. Two brain sections were 100µm apart.

CONFIRMATION OF GANGLIOSIDES BY TANDEM MS

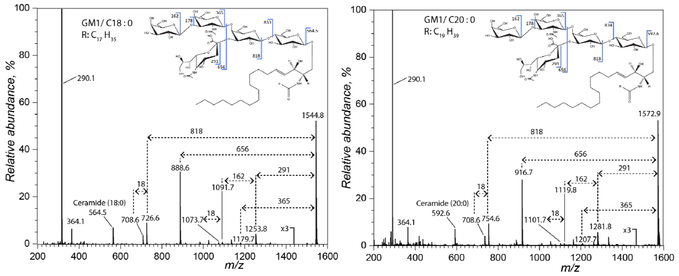

Figure 7. Molecular identification of gangliosides species in the mouse brain was aided by structural fragmentation pattern obtained using MALDI MSMS. Here we show examples of structural identification of GM1 18:0 and GM1 20:0 lipids by obtaining ganglioside fragmentation pattern, as well as, ceramide group 18:0 and 20:0 fragments.

QUANTITATION OF GANGLIOSIDES

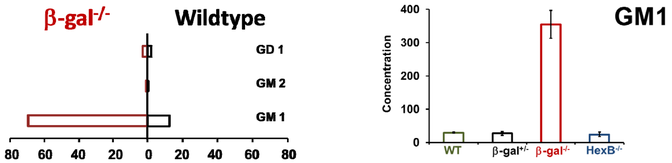

Figure 8. (a) β-galactosidase knockout mouse brain showed ~4 fold increase of GM1 relative to wild-type, with no significant changes in GM2. (b) LC-MS/MS of tissue homogenates (n=3) were in good agreement with the MALDI imaging result even though MALDI provides information on a specific section of tissue, while LC-MS gives information for the entire brain concentration.

CONCLUSIONS

- **MALDI MS imaging with ion mobility separation workflow was developed to map molecular distributions of gangliosides and other related lipids in the mouse brain to study autosomal recessive lysosomal storage diseases.**
- **Ion mobility separation, with reproducible ion drift times, can be utilized to filter and reduce chemical noise and matrix interferences improving S/N of molecules and leading up to tissue maps with better molecular specificity.**
- **High mass accuracy of ions (unit ppm), isotopic pattern, structural analysis by tandem MS helped assign the ions to molecular species.**
- **A fold-change between GM1 ganglioside was measured in knockout model, which is characteristic of β-galactosidase deficiency, was measured by MALDI and LC-MS/MS. No significant differences in other ganglioside species were found.**

References

1. Yu, R.K., Tsai, Y., Ariga, T., Yanagisawa, M., Structures, biosynthesis, and function of gangliosides – An overview, J Oleo Sci. (2011) 60, 537-544.
2. Domon, B., and Costello, C.E. A systematic nomenclature for carbohydrate fragmentation in FAB MS/MS spectra of glyconjugates, Glycoconjugate J. (1988) 5, 397-409.

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