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 transgenic tissue 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) workflow to map molecular distributions of gangliosides in mouse brains.

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 chains.

• Wild-type (WT) and β-galactosidase enzyme knockout (KO) coronal mouse brain sections (100 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 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.

• 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.

• Wild-type mice showed a 4-fold increase in GM1 content in the wild-type brain compared to knockout, which has 30% ganglioside content of wild-type. However, the ions to molecular species.

METHODS

MALDI MSI IMAGING WITH ION MOBILITY SEPARATION

• The MS/MS fragmentation of a ganglioside could result in additional fragmentation in the ion mobility spectrometer, leading to recombination in the mobility pattern obtained using MALDI MSMS. Here we show examples of structural identification of GM1 18:0 and GM1 20:0 gangliosides in mouse brain tissue.

• Gangliosides undergo dramatic changes in abundance and spatial distribution during brain development, and their accumulation caused by deficiencies in lysosomal enzymes can lead to severe neurological disorders.

• 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 that these findings will help in the development of novel therapeutic approaches for these diseases.

WILDTYPE VS. KNOCKOUT MOUSE BRAIN SECTIONS

CONCLUSIONS

• MALDI MS imaging with ion mobility separation workflow was developed to map molecular distributions of gangliosides in normal and knockout brain tissue.

• 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, and measured by MALDI MS imaging. No significant differences in other ganglioside species were found.

REFERENCES