

## Combination of ESI and MALDI-MS Imaging Enhances Analysis on Brain Gangliosides

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Recent advances in matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) have led to the direct analysis of tissue slices. The major advantage of MSI is its capability of simultaneously localizing and identifying a parent molecule and its metabolites without any labeling or any prior knowledge. MSI has been extensively employed to detect the differentiated pattern of lipids in various organs in different diseases, such as brains in Alzheimer's disease [1,2]. Poor reproducibility of MALDI MSI analysis due to the heterogeneity of the matrix-analyte crystals, hinders its use on quantitative analysis [2]. In addition, discontinuous ion flow due to quickly consumption of the samples under laser irradiation on specific site affects its ability in qualitative analysis. Although electrospray ionization mass spectrometry (ESI-MS) cannot directly be used for imaging, ESI tandem mass spectrometry (LC/ESI-MS/MS) can separate and distinguish gangliosides [3].

Zhang et al. have presented a promising workflow for qualitative, semi-quantitative and in situ analysis of gangliosides by combining the MALDI MSI and ESI-MS [4]. Following obtaining the brain from the mice, fresh-frozen murine brain sections were prepared and coated with matrix for subsequent MALDI MSI analysis. On the other hand, lipid was extracted from brain tissue by Bligh and Dyer method [5]. The gangliosides extracts were re-suspended in water for ESI-MS analysis.

For ESI-MS analysis of gangliosides, the authors firstly determine the efficiency of ganglioside extraction from brain [4]. They found that the efficiency of extraction was proportional to the length of fatty acid chain in ceramide portion, the number of saccharide residues and sialic acid, and the polarity of ganglioside. In addition, an important factor that can affect the quantitative performance of a mass detector is ion suppression. Sample matrix, coeluting compounds, and cross-talk can contribute to this effect. To bypass the phenomenon of ion suppression, the authors used UHPLC to separate lipids in brain extracts before ESI MS detection and gave reliable results. Finally, the authors employed the peak area of extracted ion in extracted ion chromatogram (XIC) for semi-quantitative analysis of the ganglioside.

For MALDI MSI analysis of gangliosides, the authors also demonstrated some improvements. As the matrix is a vital factor for MALDI MS analysis, several matrices have been tested for ganglioside analysis. DHB is a common matrix compound for lipid analysis because of its excellent signal-to-noise (S/N) ratio for the peaks of the analyte of interest [6]. 3-AQ has been shown to be better than DHB on the basis of sensitivity and resolution in negative ion mode [7]. 9-AA and nH are new developed matrices for detecting negative charged lipids and neutral oligosaccharides, respectively [8,9]. By using mouse brain extracts for MALDI-FTICR MS analysis, it was found that 3-AQ as matrix gave more gangliosides MS signals and better S/N ratio [4]. 3-AQ was then chosen as the matrix for MALDI MSI analysis of ganglioside in brain section subsequently.

The authors also found an important step during the sample preparation for MALDI MSI analysis [4]. Prior to any modified, brain sections with 3-AQ as matrix did not give any gangliosides signal but

gave much phosphatidylserines (PSs) and phosphatidylinositols (PIs) for MALDI MSI analysis of ganglioside. It has been reported that the phospholipids, such as phosphatidylcholine, would suppress other lipid signals, and a NH<sub>4</sub>Ac wash can increase the detection of ganglioside 30. Addition of ammonium sulfate to the 2,6-dihydroxyacetophenone (DHA) matrix in sample preparation can also enhance the overall spectral quality of deprotonated ions [M-H]<sup>-</sup> for all ganglioside species. Thus, they tried different solvents, including chloroform, methanol and ethanol were used to clean the tissue section. It was found that a pretreatment of cleaning by 70% EtOH for 30 s and 95% EtOH again for 30s would greatly improve the ganglioside signals. Adding ammonium formate to matrix also enhanced the signals. Based on these findings, subsequent brain tissue sections were pretreated by 70% EtOH, 95% EtOH each for 30 s and 125 mM ammonium formate was added to 3-AQ/matrix. The results demonstrated more and higher gangliosides MS signals.

The authors also examined whether these pretreatments caused any problem [4]. It was concerned whether these EtOH washing steps would remove some gangliosides from brain sections. However, no gangliosides were detected in the EtOH collected after washing, suggesting that these EtOH washing steps did not remove gangliosides from the sections. In addition, the effect of EtOH on the distribution of gangliosides was also examined. The distribution of gangliosides with or without EtOH cleanup was almost identical, suggesting that the EtOH treatment did not affect the distribution of gangliosides in the brain section.

The authors also put these modifications in a real disease situation by using amyloid precursor protein (APP) transgenic mouse, a mouse model of Alzheimer's disease (AD) [1]. They found that 20 kinds of gangliosides in cerebellum of AD mice were nearly disappeared [4]. Furthermore, most of gangliosides had lower distribution in right cerebral hemispheres of AD mice. These alteration of ganglioside distribution may be related with the damage originated from the amyloid  $\beta$  (A $\beta$ ) protein accumulation in right cerebral hemispheres, and the lack of neuronal cells in cerebellum, which may provide a new insight for bioresearch of AD.

This report demonstrated a feasible workflow to combine ESI with MALDI MSI for analysis of gangliosides in the brain. The complement of the ESI and MALDI MSI not only verified the results from each

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other but also obtained more comprehensive and accurate ganglioside distribution. This workflow is also expected to provide great help for drug distribution in the brain in the future. During the development of drugs, measurements of drug concentration in the target tissue is important to predict efficacy and safety of drug. Commonly, LC/ESI-MS/MS is employed to determine average drug concentrations but it is unable to provide any spatial information of drug in the target tissue. MSI has been recently applied to detect spatial distribution of pharmacological agents in heterogeneous targets. Results of this workflow may apply to clarifying drug distribution qualitatively and semi-quantitatively in the brain on a microscopic level, suggesting its potential to assist the improvement of drug development and translational research.

#### Conflict of Interest Statement

The author declared no competing interests.

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