MASS SPECTROMETRY-BASED INSIGHTS INTO HUMAN BRAIN GANGLIOSIDE DYNAMICS IN HEALTH AND NEUROLOGICAL DISORDERS

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Abstract
Gangliosides (GGs), glycosphingolipids abundant in the central nervous system (CNS), regulate neural development and signaling, making them valuable as biomarkers and therapeutic targets. Due to their involvement in neural development and signaling, GGs are valuable indicators for the early diagnosis of CNS pathologies. We report a nanoelectrospray ionization (nanoESI) ion mobility spectrometry (IMS) mass spectrometry (MS) and collision-induced dissociation (CID) tandem MS platform for high-resolution CNS glycolipidomics. Applied to brain tissues, tumors, and cerebrospinal fluid [1-3], this method revealed tumor-associated alterations in GG composition, including increased glycoform and ceramide diversity, enhanced sialylation, and novel acetate-modified structures. These alterations underscore GG overexpression in glioblastoma and metastases as promising diagnostic markers and therapeutic targets, establishing a powerful workflow for brain tumor and neurodegenerative disease research.

Introduction
GGs are critical regulators of brain function, and their dysregulation is linked to CNS pathology. Due to their structural complexity and low abundance in fluids, sensitive analytical platforms are required for reliable detection. Our work applies advanced IMS MS and CID MS/MS to characterize GGs, discover biomarkers in glioblastoma and metastatic tumors, and profile GGs in cerebrospinal fluid.

Experimental
Extracted GGs were dissolved in methanol at a concentration of 5 pmol/mL and directly infused into a Synapt G2-S instrument. Data were acquired over two minutes in negative ion mode using a 1.5 kV ESI voltage and a 45 V cone voltage. IMS parameters were set to a wave velocity of 650 m/s, wave height of 40 V, and gas flow of 90 L/min to optimize separation. CID MS/MS was performed following mobility separation, using collision energies between 30–35 eV in the transfer cell.

Results and discussion
Systematic investigations of GGs in brain tumors revealed significant alterations in expression profiles relative to healthy brain matrices. IMS MS provided compelling evidence of the structural complexity of GG patterns in these pathological matrices, identifying: i) an increased number of glycoforms and greater ceramide chain diversity; ii) markedly elevated sialylation levels, ranging from mono- to octasialogangliosides; iii) novel GG structures bearing acetate (CH_3COO^-) modifications, suggesting potential implications in cholinergic signaling. These

findings emphasize the high sensitivity and specificity of IMS MS and CID MS/MS in detecting rare GG species and resolving structural isomers in body fluids, where such molecules occur at considerably lower abundance than in brain tissue. Several of the identified GGs may serve as promising biochemical markers for early disease detection and prognosis, and could potentially be exploited as therapeutic targets. Furthermore, the pronounced overexpression of certain glycolipids in patient biopsies, compared to healthy controls, underscores their diagnostic potential.

Conclusion

Our IMS MS and CID MS/MS workflow enables detailed structural characterization of GGs, revealing tumor-specific alterations with biomarker and therapeutic potential. This high-performance method represents a valuable tool for CNS glycolipidomics, advancing both fundamental neuroscience and clinical applications in brain tumor and neurodegenerative disease research.

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