DISCOVERY OF GANGLIOSIDE BIOMARKERS IN BRAIN METASTASIS OF LUNG ADENOCARCINOMA BY TRAVELLING-WAVE ION MOBILITY SEPARATION MASS SPECTROMETRY

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Abstract
High performance travelling-wave ion mobility separation mass spectrometry (TWIMS MS) was thoroughly optimized to allow the discovery of brain metastasis of lung adenocarcinoma (BMLA)-specific structures and the assessment of their roles as tumor markers or possible associated antigens. Ganglioside (GG) separation by TWIMS according to the charge state, carbohydrate chain length, degree of sialylation and ceramide composition, led to the identification of no less than 151 distinct components. The detected GGs and asialo-GGs were found characterized by a high heterogeneity in their ceramide and glycan compositions, encompassing up five Neu5Ac residues. The tumor was found dominated by GM3 and GT1 forms, with a particular incidence of C26 fatty acids in the ceramide.

Introduction
Lung adenocarcinoma is a type of non-small cell lung cancer (NSCLC), which contains certain distinct malignant tissue architectural, or molecular features, and accounts for about 40% of all lung cancers. NSCLC, the leading cause of cancer death in the United States, has a high risk of brain metastasis that reportedly reaches up to 50% in brain autopsy [1]. The outcome for patients with brain metastasis is poor, with median survival time of 3–6 months [2,3]. Gangliosides (GGs), sialic acid-containing glycosphingolipids, are known to be involved in the invasive/metastatic behavior of brain tumor cells. Hence, the research nowadays is focused on the determination of the molecular mechanisms related to BMLA tumor invasion and the discovery of innovative approaches for invasiveness suppression. Since GGs are tumor-associated antigens, we introduced here TWIMS MS platform to discover possible biomarkers that can be used in the early diagnosis of the secondary tumor (metastasis cerebral).

Experimental
GGs were extracted and purified from a brain tumor localized in the cerebellar vermis of a 73-y-old male patient, previously operated for lung tumor removal. The pathohistologic examination of surgical removed tumor tissue confirmed the diagnosis of adenocarcinoma brain metastasis. The extracted GGs were dissolved in methanol to the concentration of 5 pmol/mL and infused into a Synapt G2S instrument. The signal was acquired for two minutes in the negative ion mode at 1.5kV ESI voltage and 45 V cone voltage respectively. To enhance the separation, IMS wave velocity was set at 650 m/s and IMS wave height at 40 V. MS/MS

was performed by collision-induced dissociation (CID) after mobility separation in the transfer cell, using energies between 30-35eV.

Results and discussion

The 2D data set of GGs from BMLA patients revealed their separation into mobility families based on their charge state, carbohydrate chain length, and the degree of sialylation. TWIMS MS offered a reliable separation, given the detection and identification in BMLA of 164 ions, corresponding to over 150 distinct glycoforms. NanoESI ionization followed by TWIMS separation and MS screening revealed the predominance of GM3 species, followed by the GM1, GT1 and GD1 type species with different compositions of the ceramide part. However, almost half of the total ions detected in brain metastatic tissue represent monosialylated components of GM1, GM2, GM3 and GM4 type with ceramide of variable constitutions. A special feature arising from the interpretation of the MS data is the identification of several GalNAcGalGlc-Cer species; such structures correspond to asialo GA1 and GA3. Under identical experimental conditions, TWIMS MS analysis of a normal tissue sample revealed that unlike metastatic tissue, the healthy cerebellar sample is dominated by mono- to hexasialylated structures with higher expression of GD, GT and GQ type. Observed differences in ceramide structures and altered sialylation patterns have been attributed to tumor-related changes in human carcinomas. Gangliosidic components modified by Fuc or *O*-Ac could also be detected, but in a specific pattern for adenocarcinoma, which was not found in other tissues. Most *O*-acetylated gangliosides are short GT3 type, while fucosylated components are represented by monosialo species of GM3 and GM4 structure, di- and trisialylated GT1 and GT3 exhibiting heterogeneity in their ceramide motifs. By the occurrence of only one mobility feature and the diagnostic fragment ions, the TWIMS tandem MS conducted using CID achieved a complete structural characterization of species with short oligosaccharide chains and reduced overall sialic acid content associated with brain metastasis of lung adenocarcinoma.

Conclusion

We have optimized and applied here TWIMS MS to identify the ganglioside pattern uniquely developed in BMLA. TWIMS in combination with highly sensitive (-) nanoESI and tandem MS by CID, provided an exhaustive structural and compositional investigation of BMLA gangliosides due to the advantages of the platform. The major outcome of this study is that, by TWIMS MS and CID MS/MS various novel species could be identified and added to the currently existing panel of BMLA tissue-associated structures.

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