SCREENING AND SEQUENCING OF SIALYLATED GLYCOSPHINGOLIPIDS IN HUMAN GLIOBLASTOMA BY ION MOBILITY MASS SPECTROMETRY

Mirela Sarbu[1], David E. Clemmer[2], Željka Vukelić[3], Alina D. Zamfir[1,4]

[1]Department of Condensed Matter, National Institute for Research and Development in Electrochemistry and Condensed Matter, 300224 Timisoara, Romania
[2]Department of Chemistry, The College of Arts & Science, Indiana University, IN 47405-7102, Bloomington, Indiana, USA
[3]Department of Chemistry and Biochemistry, Faculty of Medicine, University of Zagreb, 10000, Croatia
[4]Department of Technical and Natural Sciences, "Aurel Vlaicu" University of Arad, 310130, Arad, Romania
e-mail: mirela.sarbu86@yahoo.co.uk

Abstract
High performance ion mobility separation mass spectrometry (IMS MS) was thoroughly optimized to allow the discovery of glioblastoma multiforme (GBM)-specific structures and the assessment of their roles as tumor markers or possible associated antigens. Ganglioside (GG) separation by IMS according to the charge state, carbohydrate chain length, degree of sialylation and ceramide composition, led to the identification of no less than 160 distinct components [1], which represents 3 folds the number of structures identified before. 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 in equal and high proportions by GD3 and GT1 forms, with a particular incidence of C24:1 fatty acids in the ceramide.

Introduction
GBM, the most widespread primary brain tumor in adults, accounts for 45.2% of malignant primary brain and central nervous system tumors. With a rapid infiltration rate into the nearby tissue, GBM has drawn a significant attention because of its poor prognosis and the limited treatment options available. In GBM, nearly all tumor cells exhibit aberrant cell-surface glycosylation patterns due to the alteration of their biosynthesis or post-synthesis modification process. Hence, the research nowadays is focused on the determination of the molecular mechanisms related to GBM tumor invasion and the discovery of innovative approaches for invasiveness suppression. Since GGs are tumor-associated antigens, we have introduced here IMS MS for the discovery of GBM-specific structures.

Experimental
GGs were extracted from a brain tumor localized in the frontotemporal cortex of the right hemisphere in a male patient, age 47. Following the surgical removal of the tumor, the histopathological analysis has shown that the tumor is GBM, grade IV. 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 40-65eV.

Results and discussion

The 2D data set of GBM GGs revealed the GG separation into mobility families based on their charge state, carbohydrate chain length, and the degree of sialylation. IMS MS offered a reliable separation, given the detection and identification in GBM of 215 ions, corresponding to no less than 160 distinct glycoforms, more than triple the number of GGs previously discriminated in GBM with no separation prior to MS. The inspection of the data has shown that GD3 species predominate in GBM, with 36% of the total number of discovered GGs. Moreover, this study has demonstrated that, in addition to GD3, GT1 species are also associated with GBM. Correlated with previous findings, these results, documenting for the first time such a high expression of GD3 and GT1 species in GBM, have a particular biological significance. The expression of GD3 fraction, actually a minor constituent of adult brain, however, with a crucial role in brain development, is markedly increased in a variety of malignant cancers, being directly connected with tumor cell proliferation, invasion and implicitly with the degree of malignancy. Moreover, in view of our present findings and the earlier connection of GT1 with highly proliferative primary and secondary brain tumors, the elevated incidence of GT1 species found here in GBM support the biomarker role of GT1 as well, along with GD3 glycoforms. The incidence of unsaturated fatty acids residues, such as C24:1 as well as of fatty acids with odd number of carbon atoms, *i.e.* C17, C19 is another characteristic pattern of GGs in glioma tumors. By the occurrence of only one mobility feature and the diagnostic fragment ions, the IMS tandem MS conducted using collision-induced dissociation (CID) disclosed for the first time the presence of GT1c(d18:1/24:1) newly proposed here as a potential GBM marker.

Conclusion

The goal of this study was the application of IMS MS to identify the ganglioside pattern uniquely developed in GBM, the most aggressive brain tumor, with the poorest prognosis among all brain cancers. IMS in combination with highly sensitive (-) nanoESI and tandem MS by CID, provided an exhaustive structural and compositional investigation of GBM gangliosides due to the advantages of the platform. The major outcome of this study is that, by IMS MS and CID MS/MS various novel species could be identified and added to the currently existing panel of glioblastoma tissue-associated structures, since the number of the GGs identified here is three times higher than ever discovered in this tumor type.

Acknowledgements

This work was supported by the Romanian National Authority for Scientific Research, UEFISCDI, through projects PN-III-P4-ID-PCE-2020-0209 granted to A.D.Z. and PN-III-P1-1.1-PD-2019-0226 granted to M.S.

References

[1] M. Sarbu, L. Petrica, D.E. Clemmer, Ž. Vukelić, A.D. Zamfir, J.A.S.M.S. 32, (2021) 1249-1257.