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Research paper

Aberrant ganglioside composition in glioblastoma multiforme and peritumoral tissue: A mass spectrometry characterization

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ABSTRACT

Tumor cells are characterized by aberrant glycosylation of the cell surface glycoconjugates. Gangliosides are sialylated glycosphingolipids highly abundant in neural tissue and considered as tumor markers and therapeutic targets. In this study, a detailed characterization of native ganglioside mixtures from *glioblastoma multiforme*, corresponding peritumoral tissue and healthy human brain was performed using mass spectrometry and high performance thin layer chromatography in order to elucidate their roles as tumor-associated antigens. Distinctive changes in ganglioside expression were determined in glioblastoma compared to healthy brain tissue showing 5 times lower total ganglioside content and higher abundance of simple gangliosides. Glioblastoma gangliosides were characterized by highly diverse ceramide composition with fatty acyl chains varying from 16 to 24 carbon atoms, while in normal and peritumoral tissue mostly C18 chains were found. The most abundant ganglioside in glioblastoma was GD3 (d18:1/18:0), followed by GD3 (d18:1/24:0) that was exclusively detected in glioblastoma tissue. Peritumoral tissue expressed higher abundance of GD3- and nLM1/GM1-species while lower GT1-species *vs.* normal brain. *O*-Ac-GD1, known as neurostatin, was detected in normal and peritumoral tissue, but not in glioblastoma. *O*-Ac-GD3 species were found exclusively in glioblastoma; MS structural characterization of the isomeric form possessing the *O*-acetylation at the inner sialic acid residue confirmed our previous finding that this isomer is glioma-associated. This, to our knowledge, the most detailed characterization of ganglioside composition in glioblastoma and peritumoral tissue, especially addressing the ceramide variability and *O*-acetylation of tumor-associated gangliosides, could contribute to recognition of new molecular targets for glioblastoma treatment and sub-classification.

1. Introduction

Malignant transformation is characterized by aberrant glycosylation of the cell surface glycocalix. Cell surface carbohydrates are responsible for a variety of interactions between the cell and its extracellular environment. Therefore, changes in their expression strongly affect numerous characteristics of tumor phenotype, such as cellular migration, metastasis and invasiveness [5]. Aberrant glycosylation associated with oncogenic transformation were first demonstrated with glycosphingolipids, i.e. gangliosides [6]. Gangliosides (GGs) are complex, sialic acid containing glycosphingolipids located in outer leaflet of cell-membranes, especially enriched in lipid microdomains and highly abundant in neuronal cells. They are involved in numerous processes of cellular interactions such as recognition, adhesion and signal transduction [7]. Some of these microdomains that define glycosylation-dependent adhesion and signaling are called 'glycosynapses' and may provide a micro location where tumor cells interface with host cells [8]. GGs are also actively shed from the tumor to their microenvironment which enhances tumor progression by modulating tumor-host cell interactions [9]. Many studies have indicated

Abbreviations: C, chloroform; CID, collision-induced dissociation; CNS, central nervous system; GG, ganglioside; GG-SA, ganglioside-bound sialic acid; GSL, glycosphingolipid; HPTLC, high-performance thin-layer chromatography; M, methanol; W, water; w.w., wet weight.

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Biochemical Nomenclature [4] (Gangliosides and the precursor glycosphingolipids are abbreviated according to the system of Svennerholm [1–3] and the recommendations of IUPAC-IUB Commission)

LacCer	Galβ4Glcβ1Cer
GA2	Gg_3Cer, GalNAcβ4Galβ4Glcβ1Cer
GA1	Gg_4Cer, Galβ3GalNAcβ4Galβ4Glcβ1Cer
Lc_3	GlcNAcβ3Galβ4Glcβ1Cer
Lc_4	Galβ3GlcNAcβ3Galβ4Glcβ1Cer
nLc_4	Galβ4GlcNAcβ3Galβ4Glcβ1Cer
nLc_6	Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer
Gb_3	Galα4Galβ4Glcβ1Cer
Gb_4	GalNAcβ3Galα4Galβ4Glcβ1Cer
iGb_3	Galα3Galβ4Glcβ1Cer
iGb_4	GalNAcβ3Galα3Galβ4Glcβ1Cer
GM3	II^3-α-Neu5Ac-LacCer
GD3	II^3-α-$(Neu5Ac)_2$-LacCer
GT3	II^3-α-$(Neu5Ac)_3$-LacCer
GM2	II^3-α-Neu5Ac-Gg_3Cer
GD2	II^3-α-$(Neu5Ac)_2$-Gg_3Cer
GM1a or GM1	II^3-α-Neu5Ac-Gg_4Cer
GM1b	IV^3-α-Neu5Ac-Gg_4Cer
LM1 or isoLM1 or 3'-isoLM1	IV^3-α-Neu5Ac- Lc_4Cer
nLM1 or 3'-nLM1	IV^3-α-Neu5Ac- nLc_4Cer
GD1a	IV^3-α-Neu5Ac,II^3-α-Neu5Ac-Gg_4Cer
GD1b	II^3-α-$(Neu5Ac)_2$-Gg_4Cer
LD1	IV^3-α-Neu5Ac,II^3-α-Neu5Ac-Lc_4Cer
nLD1	IV^3-α-Neu5Ac,II^3-α-Neu5Ac-nLc_4Cer
3′,8'-nLD1	II^3-α-(Neu5Ac)2-nLc4Cer
3′,6'-isoLD1	IV^3-α-Neu5Ac,III^6-α-Neu5Ac-Lc4Cer: *[NeuAc(α2-3) Gal(β1-3)(NeuAcα2−6)GlcNAc(β1-3)Gal(β1-4) Glc(β1-1′)Cer]*
GT1b	IV^3-α-Neu5Ac,II^3-α-$(Neu5Ac)_2$-Gg_4Cer
GQ1b	IV^3-α-$(Neu5Ac)_2$,II^3-α-$(Neu5Ac)_2$-Gg_4Cer

that tumor-associated GGs are a result of initial oncogenic transformation and play a key role in promoting tumor invasion and metastasis which considered them as potential targets for cancer immunotherapy [10]. GD2 ganglioside has been intensively studied for many years as a promising cancer therapy target for tumors of neuroectodermal origin, such as neuroblastoma and melanoma [11]. Nowadays, human-murine anti-GD2 monoclonal antibody is used in children therapy treatment with high-risk neuroblastoma [12].

Glioblastoma multiforme (GBM; classified by World Health Organization as grade IV astrocytoma [13]) is the most aggressive and the most common type of gliomas [14]. GBM cells are highly infiltrative and diffusely spread over long distances in the brain. Such invasive behavior of the individual cells may correspond to the neoplastic cells reacquisition of primitive migratory behavior during CNS development [15]. The prognosis for GBM patients is still very poor with median survival only 13 to max 18 months after the diagnosis [16]. Therefore, considerable effort is invested in order to find new and more effective therapeutic approaches for GBM treatment. Different manipulations in expression and activity of growth factors involved in gliomagenesis were considered as potential targets for antitumor therapy, but without significant clinical results [17]. Therefore, the urge and importance of each new attempt in finding efficient targets for GBM treatment is more than clear.

The ganglioside composition and content in different types of glioma tumors were correlated to malignancy grade and median survival time [18]. Decrease in content of complex GG species in tumors is associated to dedifferentiation processes, reflecting the GG pattern change in ontogenesis during differentiation of nervous tissue: elevation of the more complex GGs and reduction of the simple GGs species content [19]. Strong expression of simple gangliosides (GM3, GD3) detected in gliomas suggests their involvement in proliferation and dedifferentiation of high malignancy grade tumors [20]. Most recently, GD3 and GD3-synthase were found to be highly abundant in glioblastoma stem cells which supports their key role in glioblastoma tumorigenesis and considers them as potential therapeutic targets against GBM [21]. *O*-acetylation, as one of the most often sialic acid modifications which induces important physiological changes is also altered in different types of tumors [22]. Since ganglioside *O*-acetylation induces strong changes in cells behavior [23], manipulation of this modification is considered as potential therapeutic target or adjuvant therapy for glioma [24].

The use of precise and sensitive (nano)ESI methods and multistage tandem MS analysis in glycolipidomics, with specific interest in ganglioside aberrant composition in different brain tumors, has been in the focus of our research in recent years [25–29]. The aim of this study is to accomplish detailed structural and compositional characterization of gangliosides in glioblastoma in comparison to peritumoral and normal brain tissue, especially addressing the variability in ganglioside ceramide composition as well as *O*-acetylation. Special attention was given to the ganglioside expression in peritumoral tissue, since the biochemical processes taking place within this area are considered responsible for aggressive infiltration of tumor cells into the surrounding brain tissue and consequently for high incidence of GBM recidivism and poor prognosis of GBM [30]. Modern MS techniques have been complemented by the high performance thin layer chromatography (HPTLC) analysis as a supporting tool for yielding more detailed structural information of the tumor-associated gangliosides which could contribute in recognition of new molecular targets for highly needed improvement and individualization of glioblastoma therapy and glioma sub-classification.

2. Materials and methods

2.1. Glioblastoma, peritumoral tissue and normal brain tissue characterization

70 years old female patient presented with generalized seizure attack two days before surgery. Magnetic resonance (MR) of the brain showed an infiltrative tumorous lesion measuring 47 × 43 × 40 mm in the right frontal and insular region. Brain tumor sample and peritumoral tissue were obtained during the surgical procedure and stored separately in liquid nitrogen prior to analysis. The histopathological examination revealed that tumorous tissue consisted of glial fibrillary acidic protein (GFAP) and vimentin positive astroglial cells with signs of necrosis, microvascular proliferation and palisading nuclei. The proliferative index (Ki-67) was 20%. Tumor was classified as glioblastoma multiforme, Grade IV (according to the WHO classification 2016 [13]). The surgical procedure and histopathological diagnosis were performed in the Department of Neurosurgery and Clinical Department of Pathology “Ljudevit Jurak”, University Hospital Center “Sestre milosrdnice”, Zagreb, Croatia.