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Bio MSC Biomedical Mass Spectrometry Center

Mass spectrometric determination of glycolipid expression and structure in brain metastasis of lung adenocarcinoma

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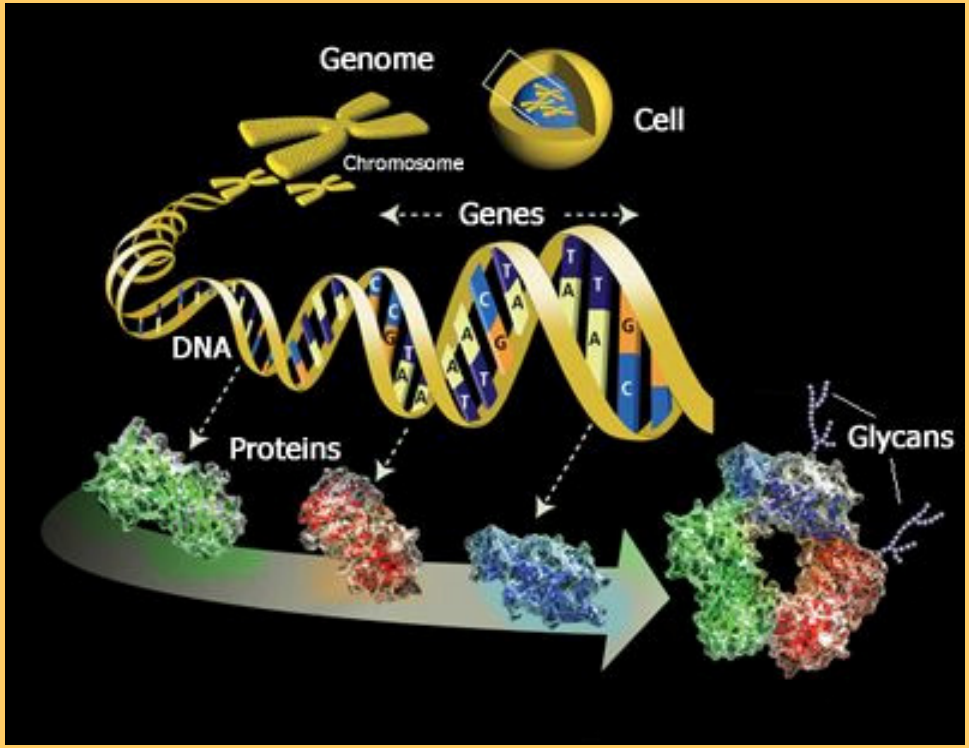
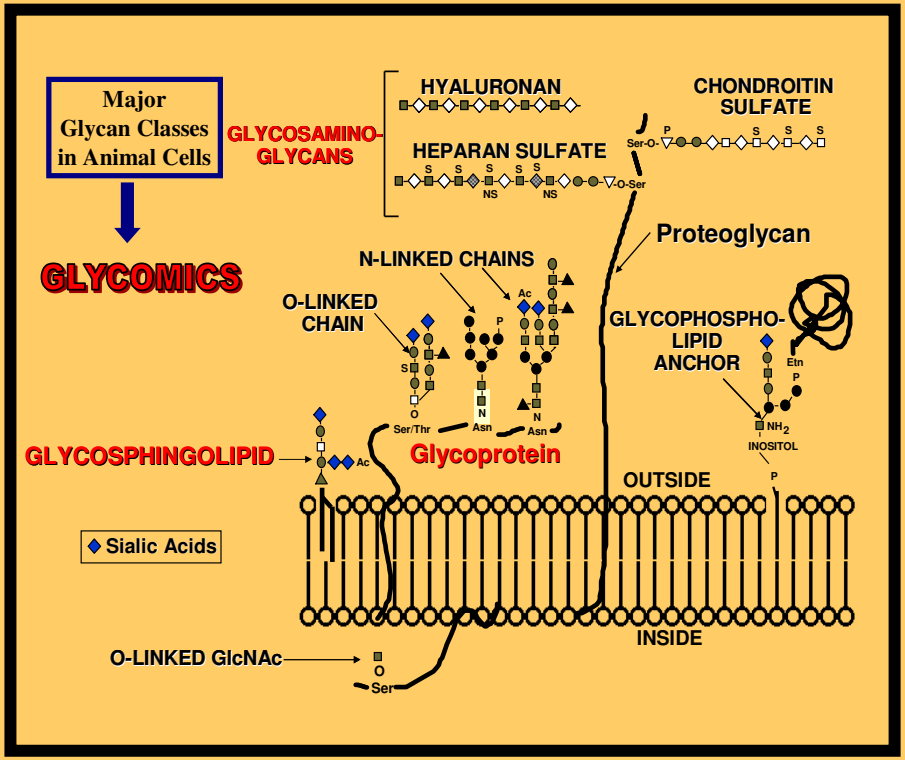
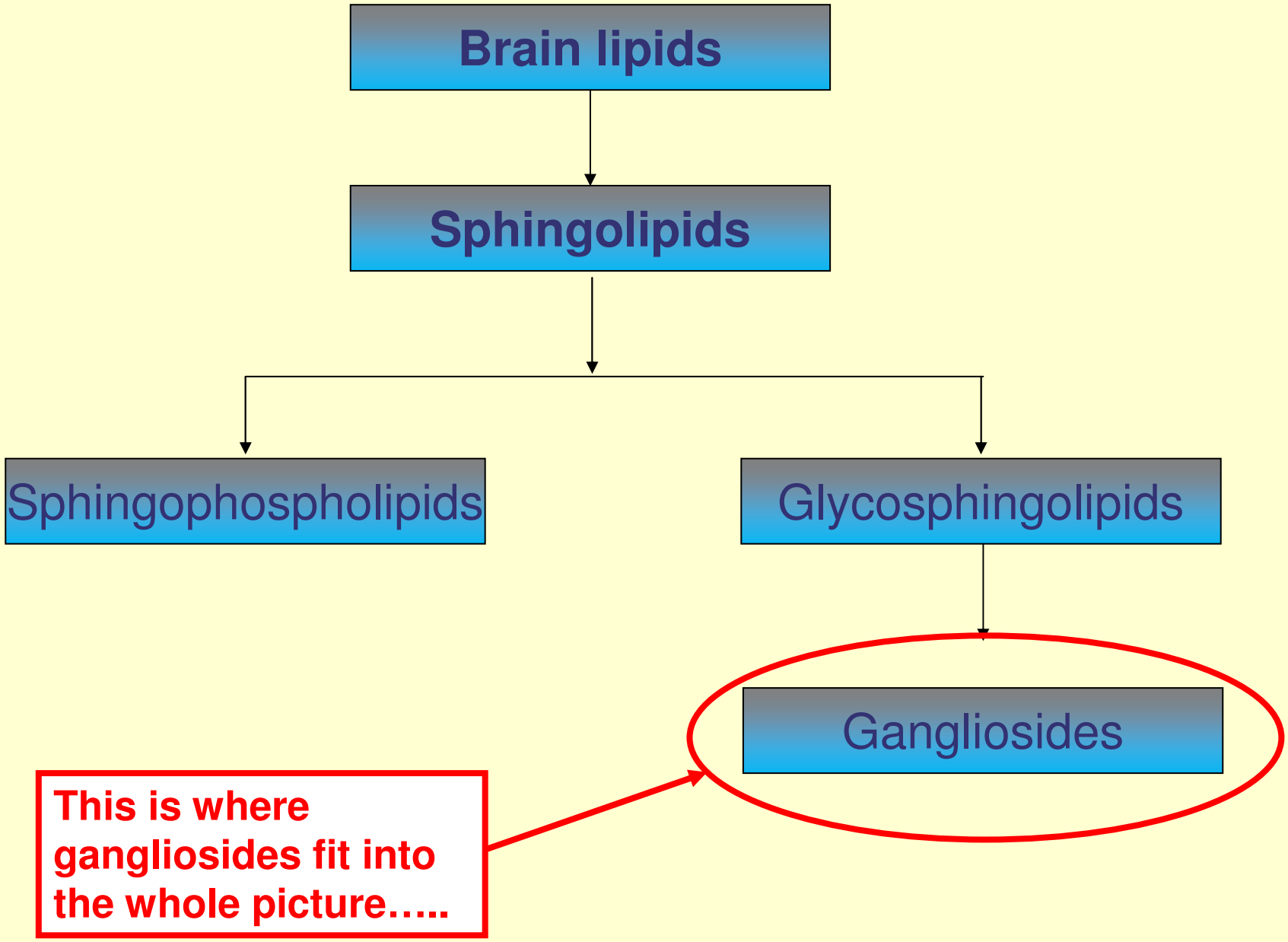
- Bucharest, 21-24 September 2010 -


Genome
Cell
Chromosome
Genes
DNA
Proteins
Glycans


Major Glycan Classes in Animal Cells
GLYCOMICS
GLYCOSAMINO-GLYCANS
HYALURONAN
HEPARAN SULFATE
CHONDROITIN SULFATE
Proteoglycan
N-LINKED CHAINS
O-LINKED CHAIN
GLYCOPHOSPHO-LIPID ANCHOR
GLYCOSPHINGOLIPID
Glycoprotein
OUTSIDE
INSIDE
Sialic Acids
O-LINKED GlcNAc


Brain lipids
Sphingolipids
Sphingophospholipids
Glycosphingolipids
Gangliosides
This is where gangliosides fit into the whole picture…..

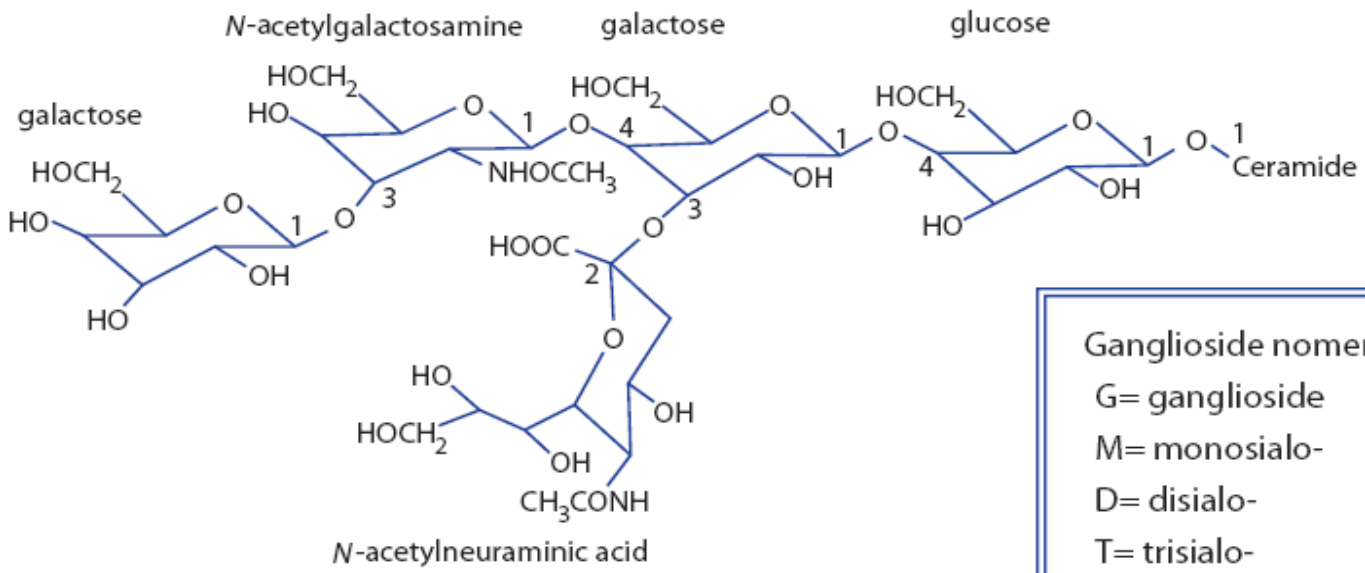
Major Classes of Glycosphingolipids

Series	Designation	Core Structure
Lacto	(LcOSe4)	Galβ3GlcNAcβ3Galβ4Glcβ1Ceramide
Lactoneo	(LcnOSe4)	Galβ4GlcNAcβ3Galβ4Glcβ1Ceramide
Globo	(GbOSe4)	GalNAcβ3Galα4Galβ4Glcβ1Ceramide
Isoglobo	(GbiOSe4)	GalNAcβ3Galα3Galβ4Glcβ1Ceramide
Ganglio	(GgOSe4)	Galβ3GalNAcβ4Galβ4Glcβ1Ceramide
Muco	(MucOSe4)	Galβ3Galβ3Galβ4Glcβ1Ceramide
Gala	(GalOSe2)	Galα4Galβ1Ceramide
Sulfatides		3-O-SulfGalβ1Ceramide

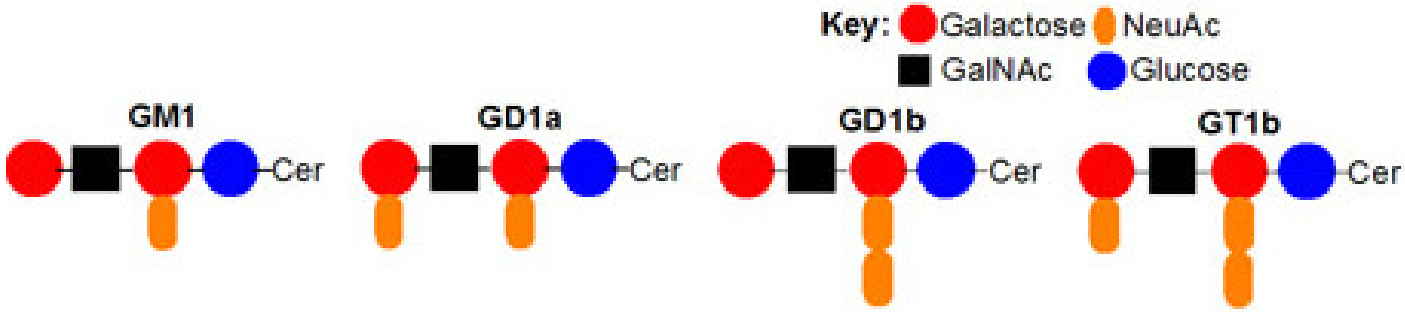
What are Gangliosides?

- A complex family of acidic glycosphingolipids
  - diverse class of glycosphingolipid due to variety of oligosaccharide species attached
- Composed of a **hydrophobic ceramide unit** that anchors the molecule to the plasma membrane and **a hydrophilic oligosaccharide chain**, to which one or more characteristic **sialic acid** groups (*i.e.*, *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid) are attached
- Several hundreds of different ganglioside species have been identified
- Common gangliosides: **GM1, GM2, GM3, GD1a, GD1b, GT1a, GT1b**
- The amounts and kinds of gangliosides in the plasma membrane change dramatically with embryonic development.
- Tumor formation induces the synthesis of a new complement of gangliosides

GM1 Ganglioside

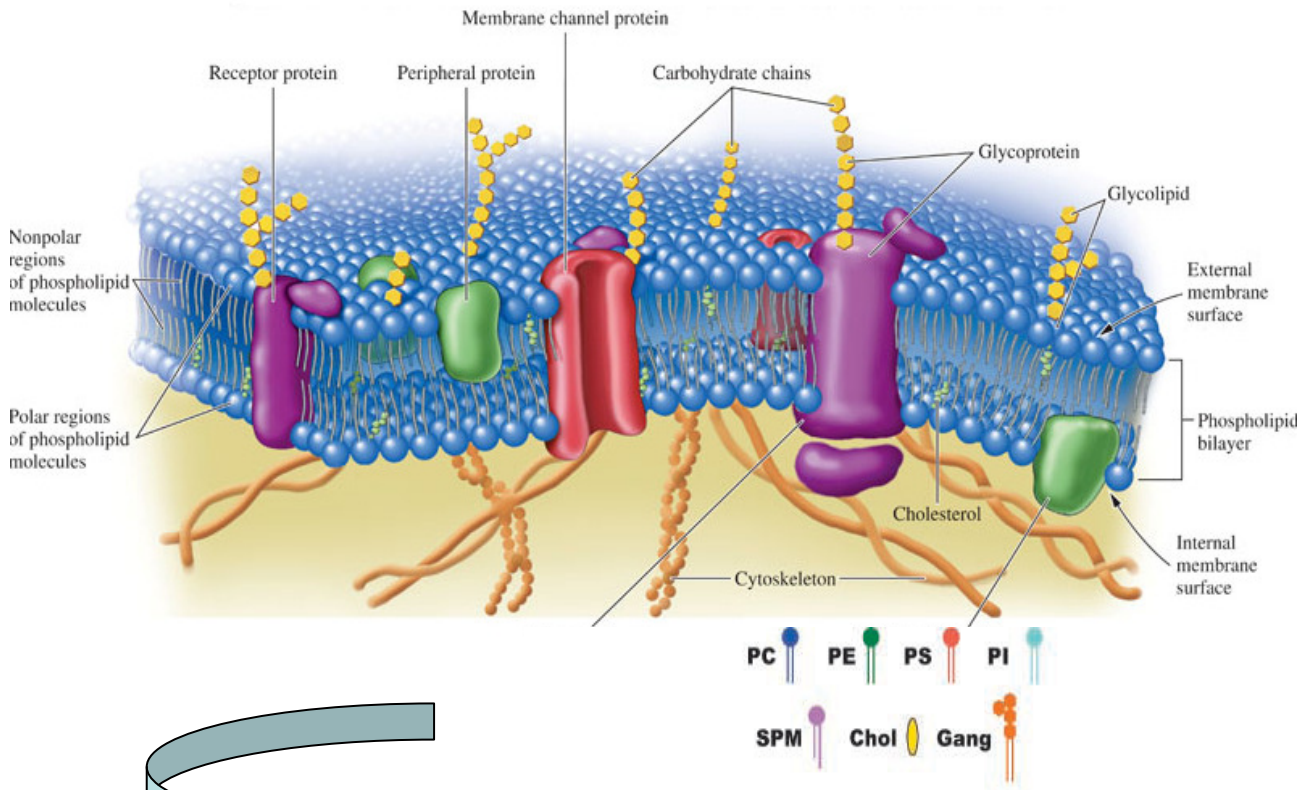


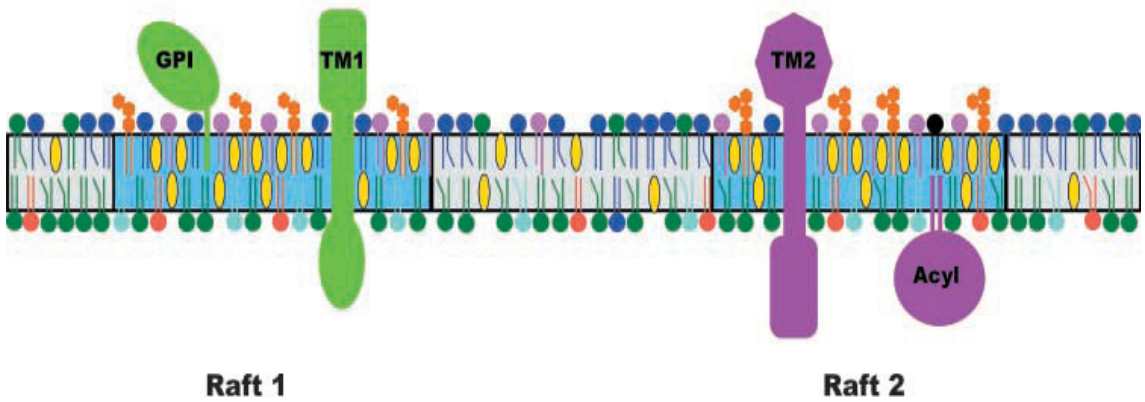
Ganglioside nomenclature:
G= ganglioside
M= monosialo-
D= disialo-
T= trisialo-
N-acetyleneuramic acid = sialic acid
1,2,3 - indicates the carbonhydrate sequence



Gangliosides in the brain…

- make up 10% of the total lipid mass of the brain, and contain most of the sialic acid within the brain
- within the Central Nervous System (CNS), are found in the highest concentrations in the cerebral cortex of the brain's grey matter
- 3 times more gangliosides in grey matter than white matter
- are found within plasma membrane of nerve cells, particularly at the nerve endings and dendrites (synaptic regions)
- important for message transmission by controlling neurotransmitter release





Lipid rafts have higher concentrations of :

- **CHOLESTEROL**
- **GLYCOSPHINGOLIPIDS**

Gangliosides in the brain…

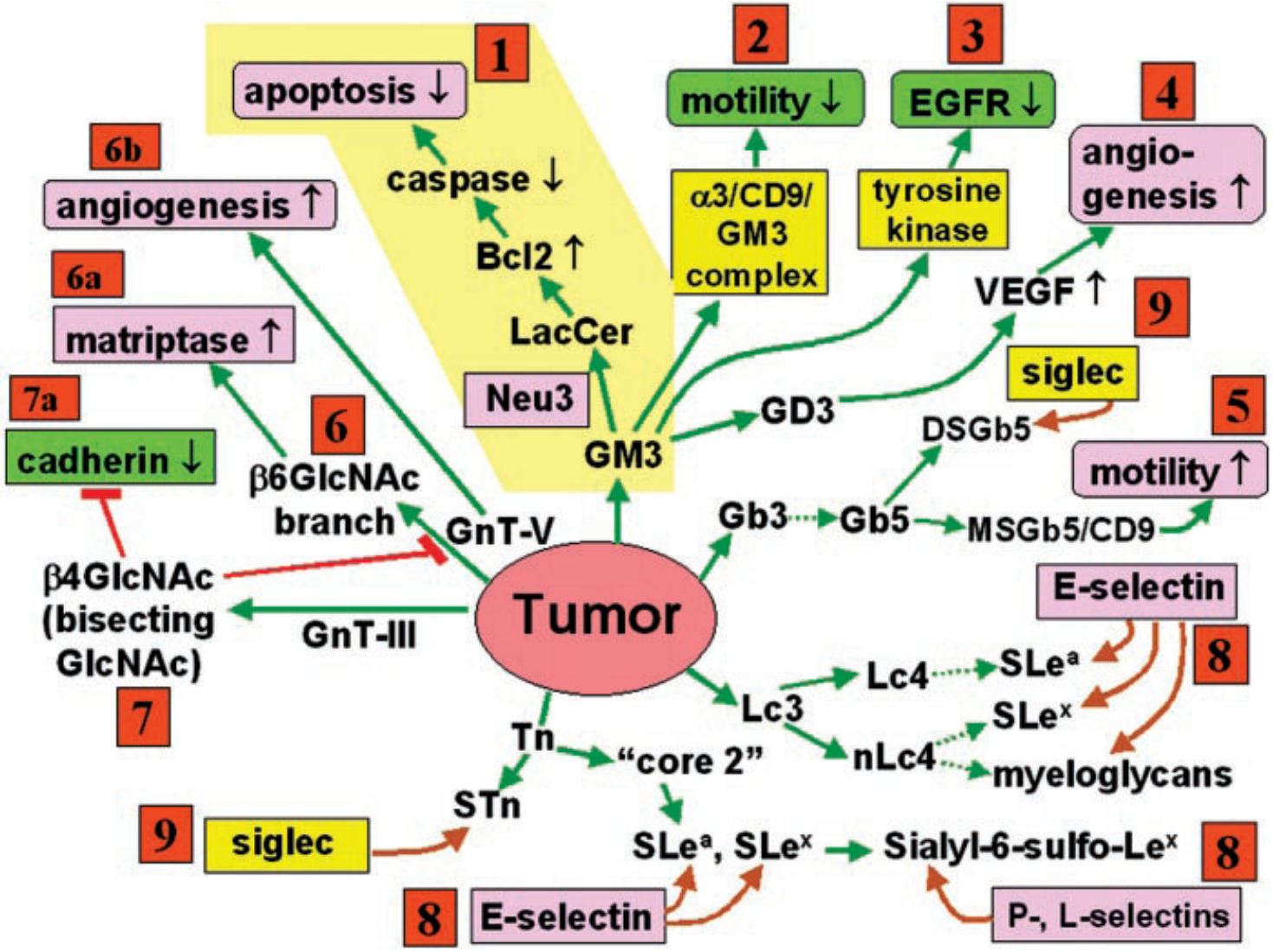
- act as modulators of various events such as cell–cell recognition, cell–substrate interaction, cell adhesion, differentiation, transformation and intracellular signaling

- participate in brain development, neuritogenesis, synaptogenesis, memory formation, aging; some promote growth of neural tissue in cell culture

- through aberrant glycosylation pathways are **involved in brain tumor invasion and metastasis**

- recently found highly important in immunology, as **target antigens** in several autoimmune neurological disorders

Gangliosides and cancer (I)



Gangliosides and cancer (II)

GD3: - promotes invasion and metastasis through angiogenesis
- the proliferative effect can be reversed by *O*- acetilation→ *O*-Ac gangliosides have antiproliferative properties : *O*-Ac GD1b (Neurostatin) was investigated as an antiproliferative agent in astroblastoma and astrocytoma

!!! GD3-based vaccines – in clinical trials in the treatment of melanoma, breast cancer and small cell lung cancer

GM2: -expressed on the cell surface of: melanoma, sarcoma, and renal cancer
- the presence of GM2 antibodies in melanoma patients appears to be associated with an improved survival rate and a longer disease-free interval

GM3: - was observed to reduce the number of brain tumor cells (ependymoma, mixt gliomas, astrocytoma, oligodendroglioma, ganglioglioma and glioblastoma multiforme) in rats but not in humans

Anti-GD2 monoclonal antibodies – in research for treatment of glioma

Lung adenocarcinoma, brain metastases and gangliosides…

- lung and breast cancer are by far the most common tumors to present with brain metastases

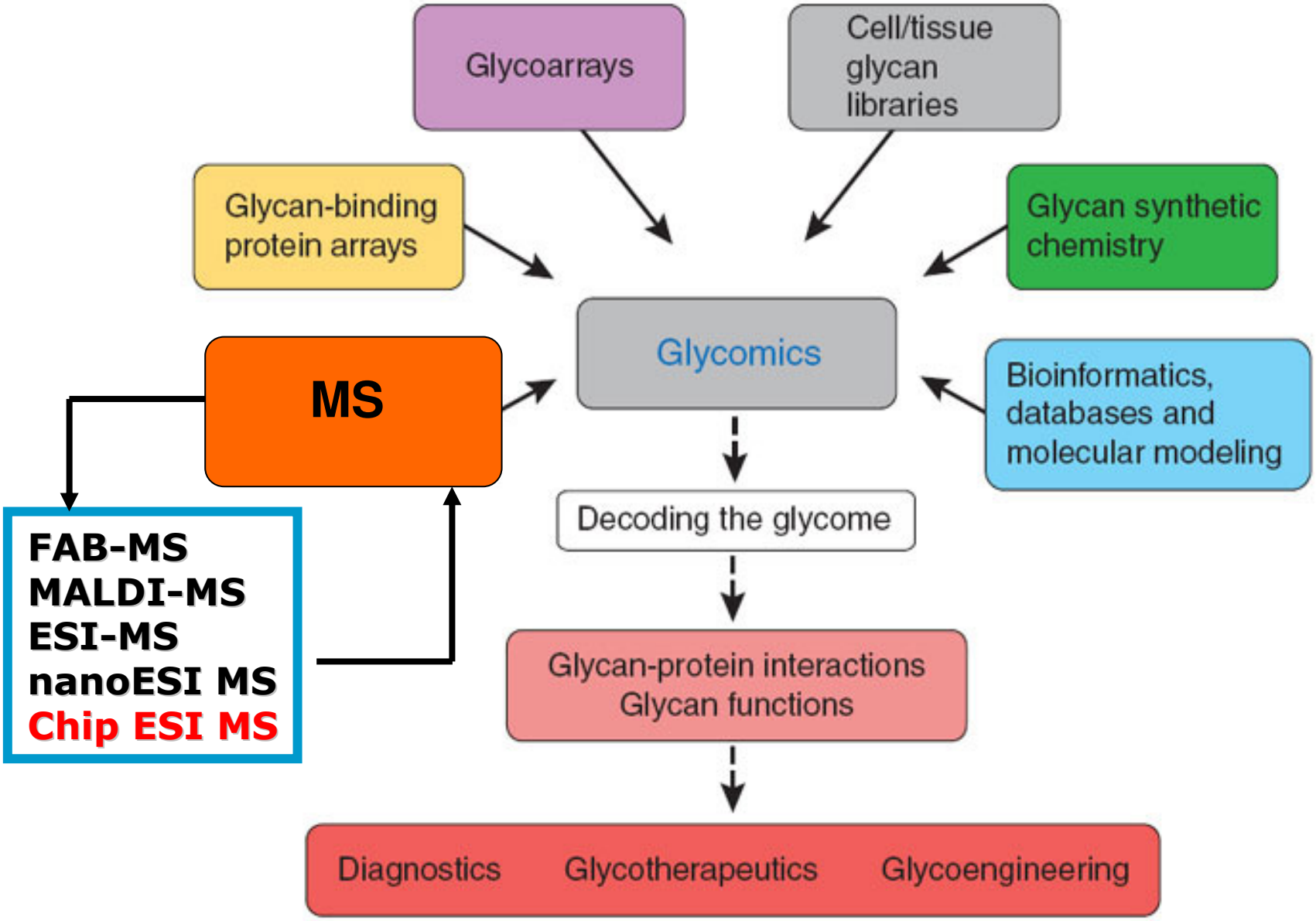
- lung cancer develops intracranial metastases relatively early and is often accompanied by neurologic symptoms on initial diagnosis

- median survival in patients with recurrent/progressive brain metastases treated with chemotherapy ranged from 3 to 6 months

- adenocarcinoma - the most frequent histological type of lung cancer which develops metastases

- anti-GM3 antibody – in treatment of metastatic melanoma

- a bidomainal fucosyl-GM1 ganglioside based vaccine for the treatment of small cell lung cancer was developed


Glycoarrays
Cell/tissue glycan libraries
Glycan-binding protein arrays
Glycan synthetic chemistry
Glycomics
MS
Bioinformatics, databases and molecular modeling
FAB-MS
MALDI-MS
ESI-MS
nanoESI MS
Chip ESI MS
Decoding the glycome
Glycan-protein interactions
Glycan functions
Diagnostics
Glycotherapeutics
Glycoengineering

Experimental

- a complex investigation of ganglioside mixture from brain metastasis of lung adenocarcinoma in parallel with ganglioside mixture from healthy brain belong both to the frontal lobe

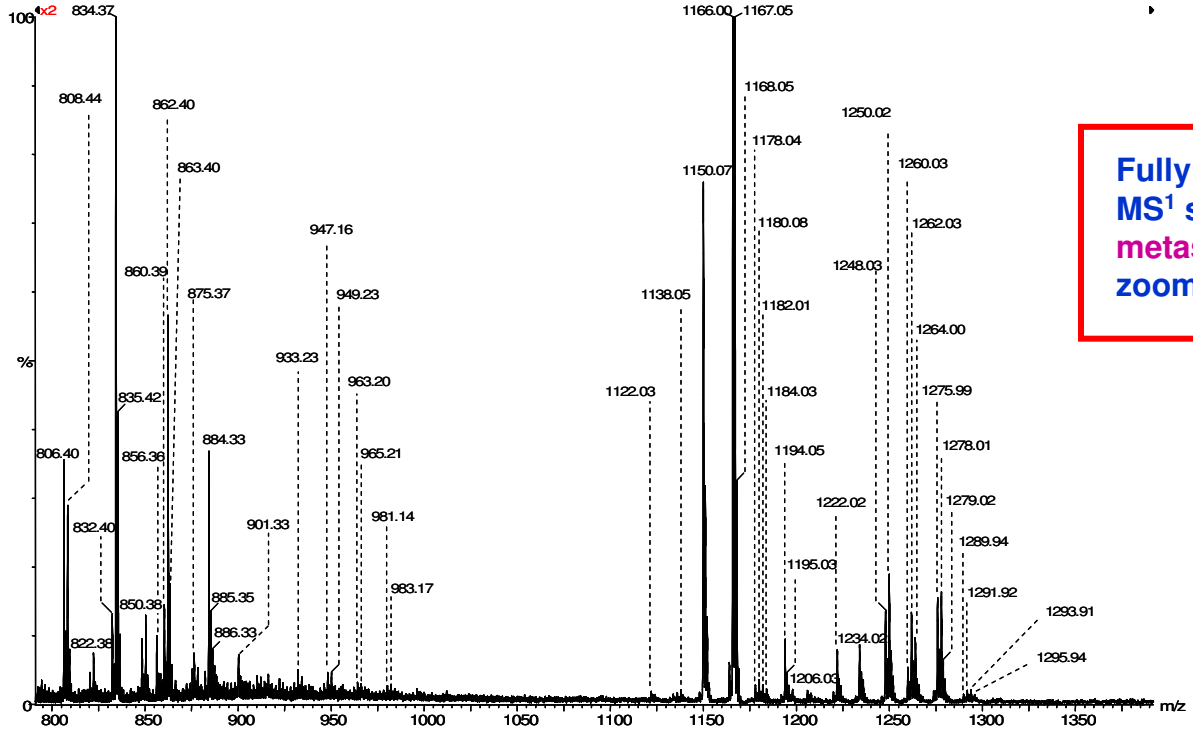
- the experimental was conducted on a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (QTOF Micro Quattro Ultima, Waters Micromass, Manchester, U.K.)

- fully automated chip-based nanoelectrospray was performed on a NanoMate robot incorporating ESI 400 Chip technology (Advion BioSciences, Ithaca, USA)

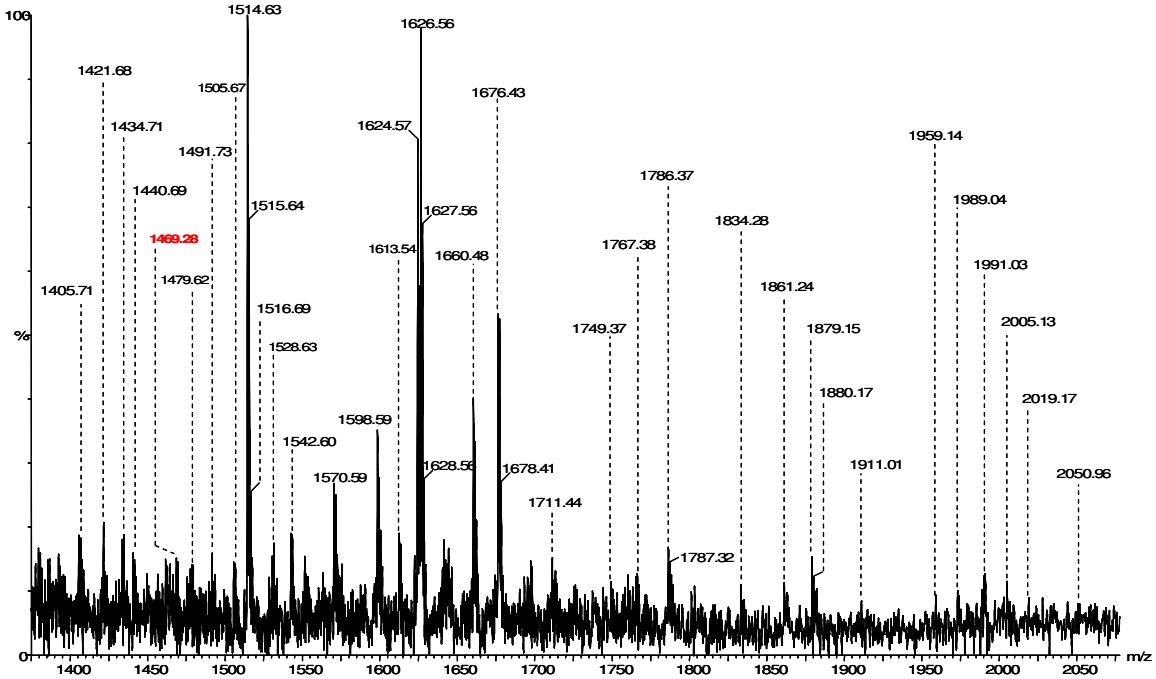
- MS^2 was carried out by collision-induced dissociation (CID) using He as the collision gas

- all mass spectra were acquired in the negative ion mode within a mass range of 100–3000 *m/z*

- nanoESI parameters were optimized to ensure a proper ionization and transfer into MS of the ganglioside components



Fully automated (-) nanoESI chip QTOF MS[1] screening of adenocarcinoma brain metastasis native ganglioside mixture; zoomed area for m/z (800-1400)



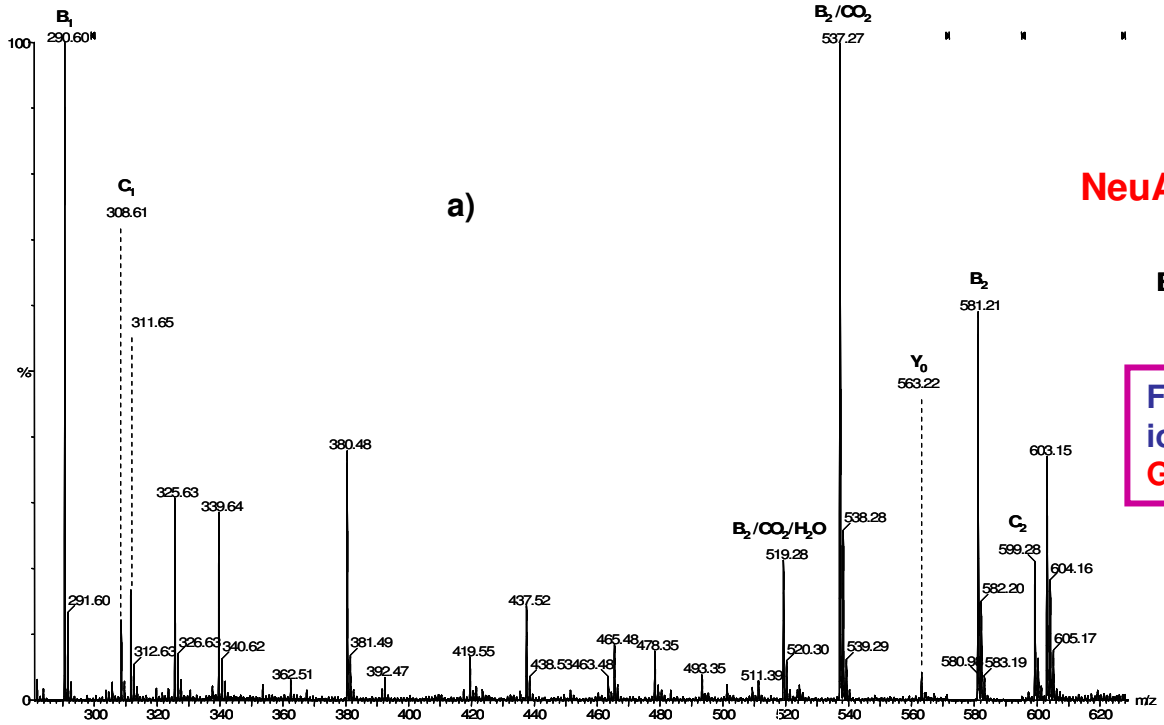
Fully automated (-) nanoESI chip QTOF MS[1] screening of adenocarcinoma brain metastasis native ganglioside mixture; zoomed area for m/z (1400-2050)

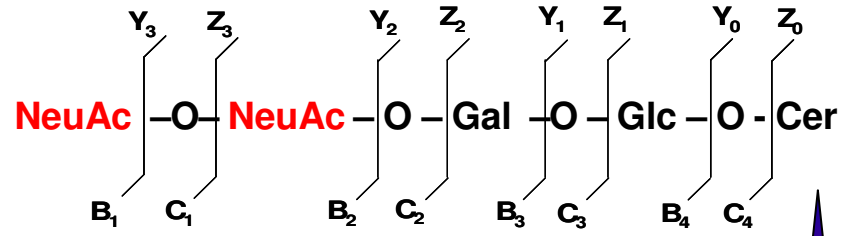
Assignment of the major ions detected in brain metastasis of lung adenocarcinoma

m/z (monoisotopic)	Molecular ion	Proposed structure
863.40	$[M-H]^-$ $(-2H_2O)$	GM4(d18:1/10:3) or GM4(d18:2/10:2)
875.37	$[M-H]^-$ $(-2H_2O)$	GM4(d18:2/11:3)
885.35	$[M-H]^-$ $(-H_2O)$	GM4(d18:1/10:1) or GM4(d18:2/10:0) or GM4(d18:0/10:2)
901.33	$[M-H]^-$	GM4(d18:1/10:2) or GM4(d18:2/10:1)
933.23	$[M-H]^-$	GM4(d18:0/12:1) or GM4(d18:1/12:0)
947.16	$[M-H]^-$ $[M+2Na-3H]^-$	O-Ac -GM4(d18:0/10:1) or O-Ac -GM4(d18:1/10:0) GM4(d18:1/10:1) or GM4(d18:0/10:2) or GM4(d18:2/10:0)
949.23	$[M-H]^-$	O-Ac -GM4(d18:0/10:0)
963.20	$[M-H]^-$ $[M-H]^-$	GM4(d18:0/14:0) O-Ac-GM4(d18:0/11:0)
965.21	$[M+2Na-3H]^-$	GM4(d18:0/11:0)
981.14	$[M-H]^-$	GA2(d18:0/10:0)
983.17	$[M+Na-2H]^-$	GM4(d18:1/14:0) or GM4(d18:0/14:1)
1122.03	$[M-H]^-$	GA2(d18:0/20:0)
1138.05	$[M-H]^-$ $(-2H_2O)$ $[M-H]^-$ $[M-H]^-$	GM4(d18:0/29:0) Fuc -GM4(d18:1/18:4) or Fuc -GM4(d18:2/18:3) Fuc -GA2(d18:0/12:0)
1150.07	$[M-H]^-$ $[M-H]^-$	GA2(d18:0/22:0) Fuc -GA2(d18:0/13:1) or Fuc -GA2(d18:1/13:0)
1166.00	$[M+Na-2H]^-$	GM4(d18:1/27:0) or GM4(d18:0/27:1)
1178.04	$[M-H]^-$ $(-H_2O)$	GM4(d18:1/31:2) or GM4(d18:2/31:1)
1180.02	$[M+Na-2H]^-$	GM4(d18:1/28:0) or GM4(d18:0/28:1)
1182.01	$[M+Na-2H]^-$	GM4(d18:0/28:0)
1184.03	$[M-H]^-$	GM4(d18:0/30:2) or GM4(d18:1/30:1) or GM4(d18:2/30:0)
1194.05	$[M-H]^-$	GM3(d18:1/19:0) or GM3(d18:0/19:1)
1206.03	$[M-H]^-$	O-Ac -GM4(d18:1/29:4) or O-Ac -GM4(d18:2/29:3) or GA2(d18:0/26:0)
1222.02	$[M-H]^-$ $(-H_2O)$ $[M-H]^-$	GM4(d18:1/34:1) or GM4(d18:2/34:0) GM3(d18:0/21:1) or GM3(d18:1/21:0)
1234.02	$[M-H]^-$	GA2(d18:0/28:0)
1248.03	$[M-H]^-$ $[M-H]^-$	GA2(d18:0/29:0) O-Ac-GA2(d18:0/26:0)
1250.02	$[M-H]^-$ $[M-H]^-$	O-Ac-GM4(d18:0/32:4) or O-Ac-GM4(d18:1/32:3) or O-Ac-GM4(d18:2/32:2) GM3(d18:1/23:0) or GM3(d18:0/23:1)
1260.03	$[M-H]^-$	GA2(d18:1/30:0) or GA2(d18:0/30:1)
1262.03	$[M-H]^-$	GA2(d18:0/30:0)
1264.00	$[M+Na-2H]^-$	GM4(d18:1/34:0) or GM4(d18:0/34:1)
1275.99	$[M-H]^-$ $[M-H]^-$ $(-H_2O)$	O-Ac-GA2(d18:0/28:0) GM3(d18:0/26:0)
1278.01	$[M-H]^-$ $[M-H]^-$	GM3(d18:1/25:0) or GM3(d18:0/25:1) O-Ac-GM3(d18:1/22:0) or O-Ac-GM3(d18:0/22:1)
1289.94	$[M-H]^-$ $[M-H]^-$	GM3(d18:0/26:2) or GM3(d18:1/26:1) or GM3(d18:2/26:0) GA2(d18:0/32:0)
1291.92	$[M-H]^-$	GM3(d18:1/26:0) or GM3(d18:0/26:1)

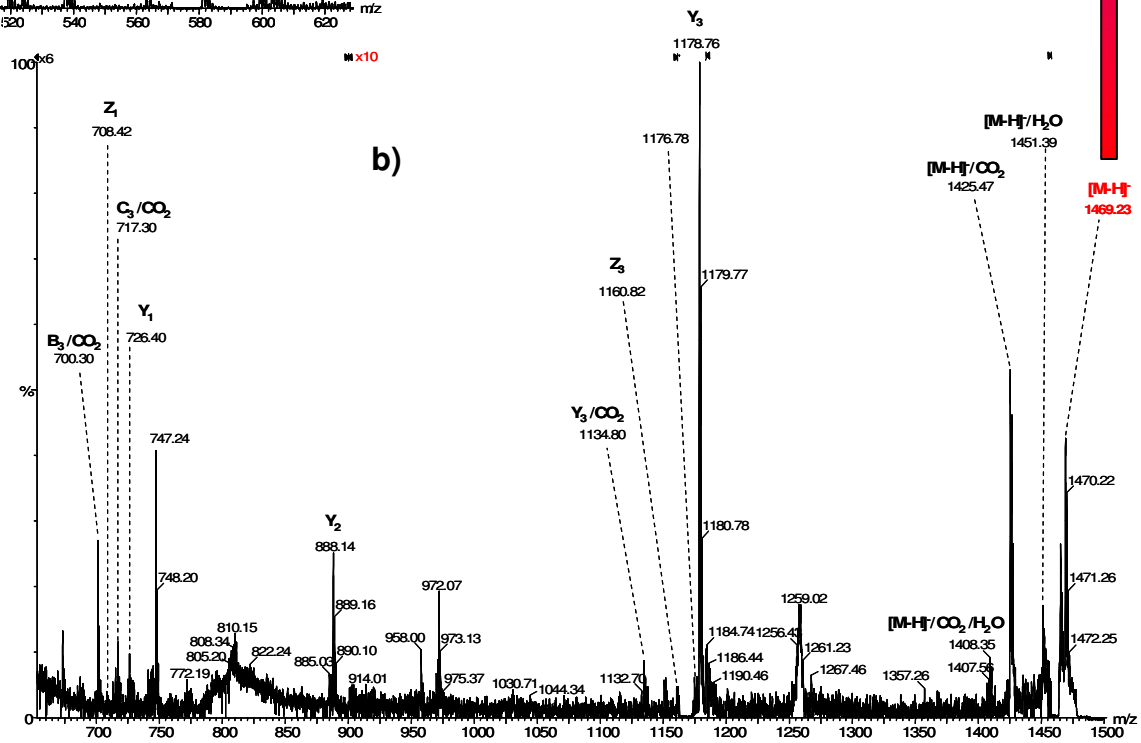
m/z (monoisotopic)	Molecular ion	Proposed structure
1293.91	$[M-H]^-$	GM3(d18:0/26:0)
1295.94	$[M-H]^-$ $[M-H]^-$	O-Ac-GA2(d18:1/30:3) or O-Ac-GA2(d18:2/30:2) or O-Ac-GA2(d18:0/30:4) GA1(d18:1/21:0) or GA1(d18:0/21:1)
1405.71	$[M+2Na-3H]^-$	GA1(d18:1/26:2) or GA1(d18:0/26:3) or GA1(d18:2/26:1)
1421.68	$[M+Na-2H]^-$ $[M-H]^-$ $[M+2Na-3H]^-$	GM3(d18:1/34:2) or GM3(d18:0/34:3) or GM3(d18:2/34:1) GA1(d18:1/30:0) or GA1(d18:0/30:1) GM3(d18:0/32:0)
1434.71	$[M+Na-2H]^-$ $[M-H]^-$	GD3(d18:1/14:1) or GD3(d18:0/14:2) or GD3(d18:2/14:0) GM1(d18:0/10:0)
1440.69	$[M-H]^-$ $(-H_2O)$ $[M-H]^-$	GM1(d18:1/12:1) or GM1(d18:0/12:2) or GM1(d18:2/12:0) GD3(d18:1/16:1) or GD3(d18:0/16:2) or GD3(d18:2/16:0)
1469.28	$[M-H]^-$ $[M-H]^-$	GM2(d18:0/24:0) Fuc-GA1(d18:0/24:0)
1491.73	$[M+2Na-3H]^-$	GA1(d18:1/32:1) or GA1(d18:0/32:2) or GA1(d18:2/32:0)
1514.63	$[M+2Na-3H]^-$ $[M-H]^-$	GD3(d18:1/18:0) or GD3(d18:0/18:1) GM1(d18:1/16:1) or GM1(d18:0/16:2) or GM1(d18:2/16:0)
1516.69	$[M+Na-2H]^-$ $[M-H]^-$	GD3(d18:1/20:2) or GD3(d18:0/20:3) or GD3(d18:2/20:1) GM1(d18:1/16:0) or GM1(d18:0/16:1)
1528.63	$[M-H]^-$	O-Ac-GM1(d18:1/14:1) or O-Ac-GM1(d18:0/14:2) or O-Ac-GM1(d18:2/14:0)
1542.60	$[M-H]^-$	GM1(d18:1/18:1) or GM1(d18:2/18:0) or GM1(d18:0/18:2)
1570.59	$[M+2Na-3H]^-$ $[M-H]^-$ $[M+Na-2H]^-$	GD3(d18:1/22:0) or GD3(d18:0/22:1) GM1(d18:1/20:1) or GM1(d18:0/20:2) or GM1(d18:2/20:0) GD3(d18:0/24:4) or GD3(d18:1/24:3) or GD3(d18:2/24:2)
1598.59	$[M+2Na-3H]^-$ $[M-H]^-$	GD3(d18:1/24:0) or GD3(d18:0/24:1) GM1(d18:0/22:2) or GM1(d18:1/22:1) or GM1(d18:2/22:0)
1613.54	$[M+Na-2H]^-$	GD2(d18:0/12:0)
1624.57	$[M+2Na-3H]^-$ $[M-H]^-$	GD3(d18:1/26:1) or GD3(d18:0/26:2) or GD3(d18:2/26:0) GD3(d18:1/29:0) or GD3(d18:0/29:1)
1626.56	$[M+2Na-3H]^-$ $[M-H]^-$	GD3(d18:0/26:1) or GD3(d18:1/26:0) GM1(d18:0/24:2) or GM1(d18:1/24:1) or GM1(d18:2/24:0)
1628.56	$[M-H]^-$	GM1(d18:0/24:1) or GM1(d18:1/24:0)
1660.48	$[M+3Na-4H]^-$	GM1(d18:1/22:3) or GM1(d18:0/22:4) or GM1(d18:2/22:2)
1676.43	$[M-H]^-$ $[M+2Na-3H]^-$	Fuc-GM2(d18:1/30:2) or Fuc-GM2(d18:0/30:3) or Fuc-GM2(d18:2/30:1) GD3(d18:1/30:3) or GD3(d18:0/30:4) or GD3(d18:2/30:2)
1711.44	$[M-H]^-$	O-Ac-GD3(d18:0/32:0)
1749.37	$[M+Na-2H]^-$ $[M-H]^-$ $[M-H]^-$ $(-H_2O)$	GM1(d18:1/31:0) or GM1(d18:0/31:1) GM1(d18:1/33:3) or GM1(d18:0/33:4) or GM1(d18:2/33:2) GM1(d18:0/34:2) or GM1(d18:1/34:1) or GM1(d18:2/34:0)

m/z (monoisotopic)	Molecular ion	Proposed structure
1767.38	$[M-H]^-$ $[M-H]^-$	O-Ac-GM1(d18:1/31:1) or O-Ac-GM1(d18:0/31:2) or O-Ac-GM1(d18:2/31:0) GM1(d18:0/34:2) or GM1(d18:1/34:1) or GM1(d18:2/34:0)
1786.37	$[M-H]^-$	O-Ac-GD2(d18:1/23:0) or O-Ac-GD2(d18:0/23:1)
1834.28	$[M-H]^-$	GT3(d18:0/23:0)
1861.24	$[M-H]^-$	Fuc -GD2(d18:0/22:0)
1879.15	$[M-H]^-$ $[M-H]^-$ $[M-H]^-$	Fuc-GD2(d18:1/24:4) or Fuc-GD2(d18:2/24:3) Fuc-GT3(d18:0/17:0) GT2(d18:0/12:2) or GT2(d18:1/12:1) or GT2(d18:2/12:0)
1911.01	$[M-H]^-$ $[M-H]^-$	Fuc-GD1(d18:0/14:0) GT2(d18:0/14:0)
1959.14	$[M-H]^-$ $[M-H]^-$ $(-2H_2O)$ $[M-H]^-$	Fuc-GT3(d18:1/23:1) or Fuc-GT3(d18:0/23:2) or Fuc-GT3(d18:2/23:0) GT2(d18:0/20:0) GT2(d18:0/18:4) or GT2(d18:1/18:3) or GT2(d18:2/18:2)
1989.04	$[M+Na-2H]^-$ $[M-H]^-$ $[M-H]^-$	GT2(d18:0/18:0) Fuc-GD1(d18:1/20:2) or Fuc-GD1(d18:0/20:3) or Fuc-GD1(d18:2/20:1) GT2(d18:0/20:3) or GT2(d18:1/20:2) or GT2(d18:2/20:1)
1991.03	$[M-H]^-$ $[M-H]^-$	GT2(d18:1/20:1) or GT2(d18:0/20:2) or GT2(d18:2/20:0) GD1(d18:0/20:2) or GD1(d18:1/20:1) or GD1(d18:2/20:0)
2005.13	$[M-H]^-$	Fuc-GT3(d18:0/26:0)
2019.17	$[M-H]^-$ $[M-H]^-$	Fuc-GD2(d18:1/34:4) or Fuc-GD2(d18:2/34:3) Fuc-GD1(d18:1/22:1) or Fuc-GD1(d18:0/22:2) or Fuc-GD1(d18:2/22:0)
2050.96	$[M-H]^-$ $[M-H]^-$ $(-H_2O)$ $[M-H]^-$	GQ3(d18:1/18:1) or GQ3(d18:0/18:2) or GQ3(d18:2/18:0) GT1(d18:1/14:1) or GT1(d18:0/14:2) or GT1(d18:2/14:0) GT2(d18:0/24:0)

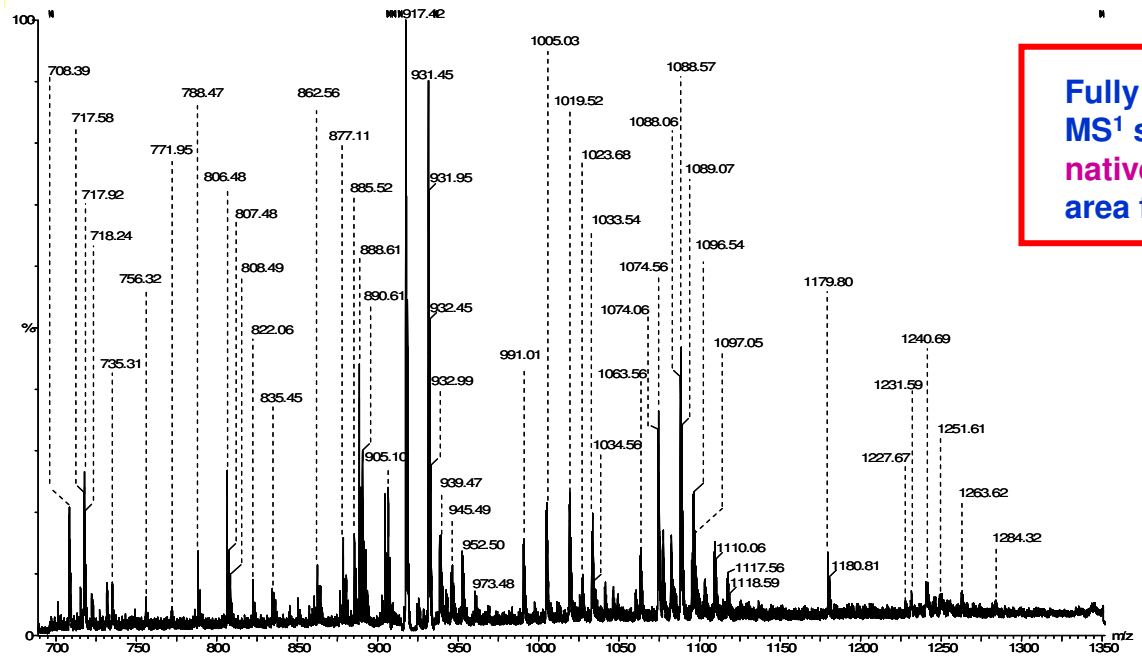




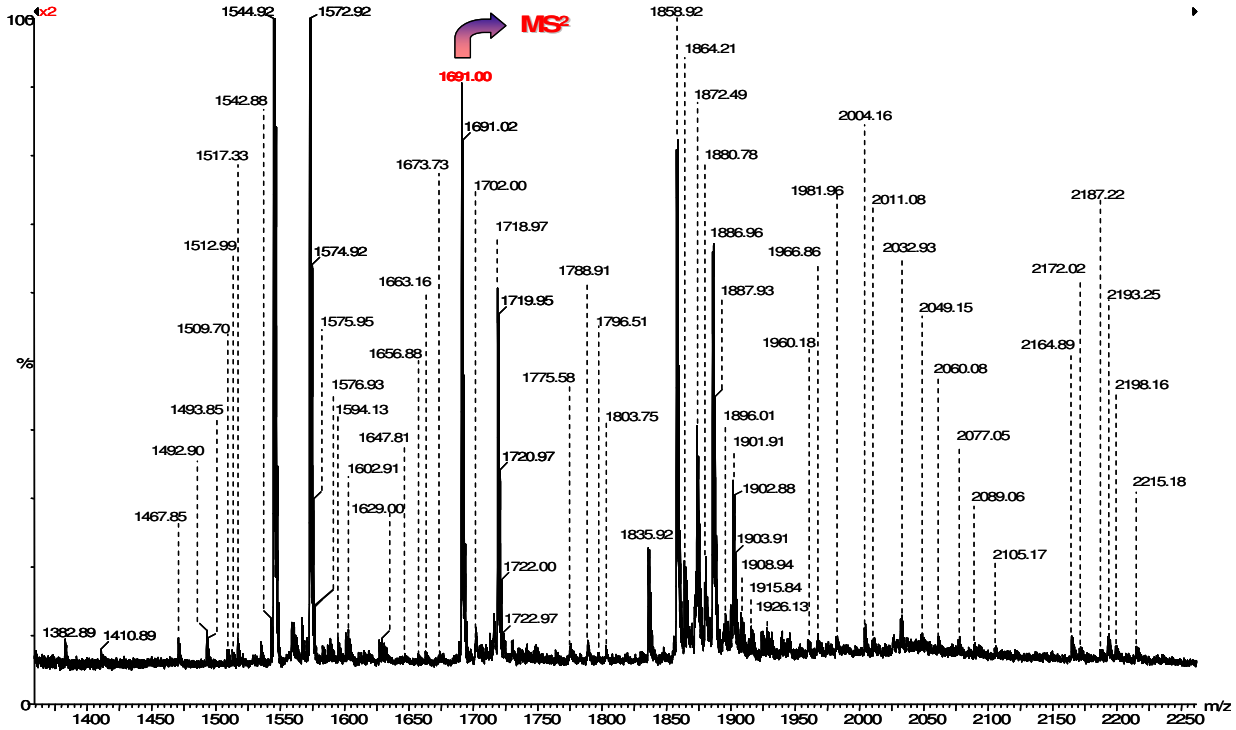
Fragmentation scheme of the [M-H]⁻ ion at *m/z* 1469.28 corresponding to GD3(d18:1/18:1)



(-) nanoESI chip QTOF MS² of the single charged ion at *m/z* 1469.28 corresponding to GD3 (d18:1/18:1) ganglioside species from adenocarcinoma brain metastasis ganglioside mixture: a) m/z (280-620); b) m/z (620-1500)



Fully automated (-) nanoESI chip QTOF MS[1] screening of normal frontal brain native ganglioside mixture; zoomed area for m/z (700-1350)



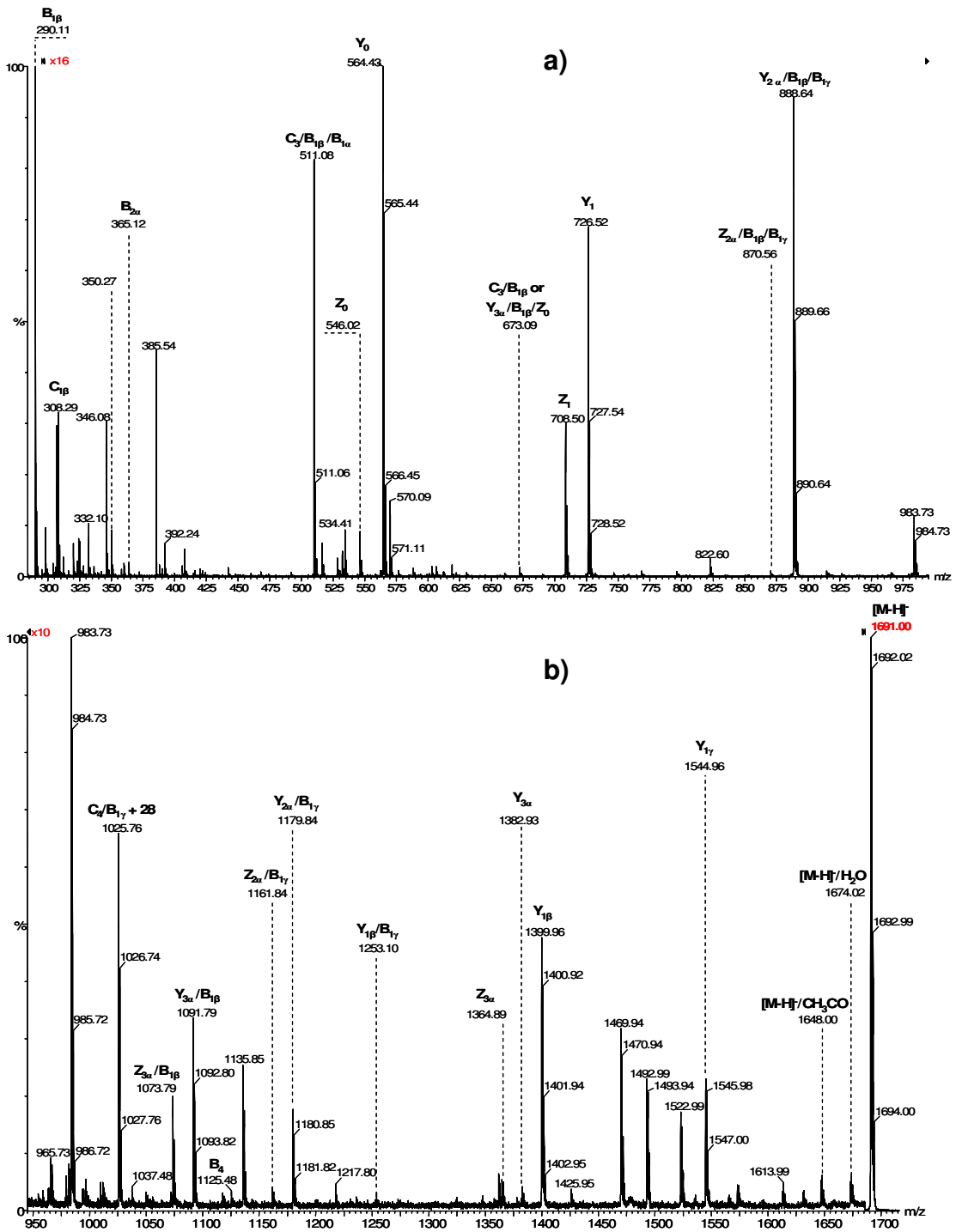
Fully automated (-) nanoESI chip QTOF MS[1] screening of normal frontal brain native ganglioside mixture; zoomed area for m/z (1350-2250)

Assignment of the major ions detected in healthy frontal brain

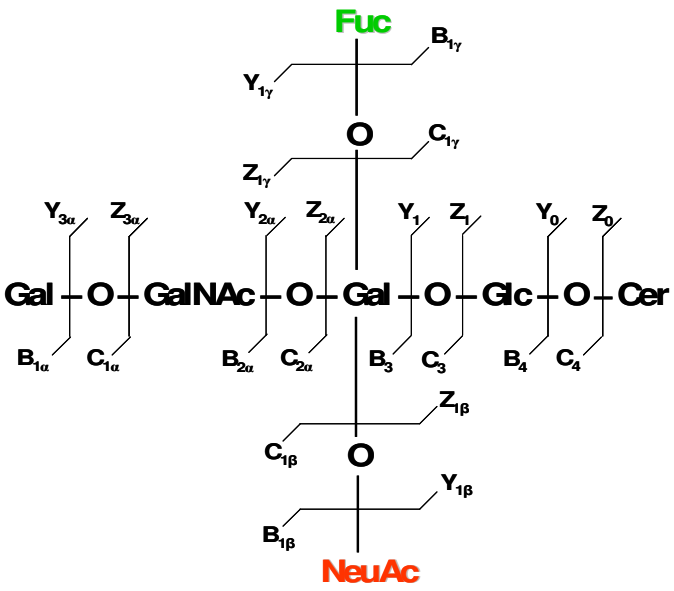
m/z (monoisotopic)	Molecular ion	Proposed structure
708.39	$[M+Na-4H]^{2-}$	GT2(d18:1/28:0)
714.42	$[M-3H]^{2-}$	O-Ac-GT2(d18:0/28:2) or O-Ac-GT2(d18:1/28:1) or O-Ac-GT2(d18:2/28:0)
717.58	$[M-3H]^{2-}$	GT1(d18:1/20:0) or GT1 (d18:0/20:1)
735.31	$[M-2H]^{2-}$	GD3 (d18:1/18:0) or GD3 (d18:0/18:1)
756.32	$[M-2H]^{2-}$	Fuc-GM2(d18:0/18:0)
771.95	$[M-2H]^{2-}$	GM1(d18:0/18:1) or GM1(d18:1/18:0)
788.47	$[M-3H]^{2-}$	O-Ac-GT1(d18:0/32:0)
822.06	$[M+Na-4H]^{2-}$	GQ1(d18:1/20:0) or GQ1(d18:0/20:1)
835.45	$[M-2H]^{2-}$	GD2(d18:1/18:1) or GD2(d18:0/18:2) or GD2(d18:2/18:0)
844.45	$[M-2H]^{2-}$	O-Ac-GD2(d18:0/16:0)
850.02	$[M-2H]^{2-}$	O-Ac-GM1(d18:0/26:0)
862.56	$[M-2H]^{2-}$ $[M-2H]^{2-}$ $[M+Na-3H]^{2-}$	Fuc-GM1 (d18:1/22:2) or Fuc-GM1 (d18:0/22:3) or Fuc-GM1 (d18:2/22:1) GD2 (d18:0/22:3) or GD2 (d18:1/22:2) or GD2 (d18:2/22:1) GD2 (d18:0/20:0)
877.11	$[M-2H]^{2-}$	O-Ac-GM1(d18:1/30:0) or O-Ac-GM1(d18:0/30:1)
885.52	$[M-2H]^{2-}$	O-Ac-GD2 (d18:1/22:0)or O-Ac-GD2 (d18:0/22:1)
890.61	$[M+Na-3H]^{2-}$ $[M-2H]^{2-}$	GD2(d18:0/24:0) GD2(d18:1/26:2) or GD2(d18:0/26:3) or GD2(d18:2/26:1)
905.10	$[M-2H]^{2-}$	O-Ac-GM1(d18:1/34:0) or O-Ac-GM1(d18:0/34:1)
917.42	$[M-2H]^{2-}$	GD1(d18:1/18:0) or GD1(d18:0/18:1)
924.96	$[M-2H]^{2-}$	GT2(d18:1/10:1) or GT2(d18:0/10:2) or GT2(d18:2/10:0)
931.45	$[M-2H]^{2-}$	GD1(d18:1/20:0) or GD1(d18:0/20:1)
939.47	$[M-2H]^{2-}$ $[M+2Na-4H]^{2-}$	GD1(t18:0/21:0) GD1 (d18:1/18:0) or GD1(d18:0/18:1)
945.49	$[M-2H]^{2-}$	GD1(d18:1/22:0)
952.50	$[M-2H]^{2-}$	O-Ac-GD1 (d18:1/20:0) or O-Ac-GD1 (d18:0/20:1)
973.48	$[M-3H]^{2-}$	GH2(d18:1/24:0) or GH2(d18:0/24:1)
991.01	$[M+Na-3H]^{2-}$	GT2(d18:1/18:2) or GT2(d18:0/18:3) or GT2(d18:2/18:3)
1005.03	$[M+Na-3H]^{2-}$	GT3(d18:0/34:0)
1019.52	$[M+Na-3H]^{2-}$	GT2 (d18:1/22:2) or GT2 (d18:0/22:3) or GT2 (d18:2/22:1)
1023.68	$[M-3H]^{2-}$	Fuc-GD1(d18:0/24:1) or Fuc- GD1(d18:1/24:0)
1033.54	$[M+2Na-3H]^{-}$	GM4 (d18:1/16:0) or GM4 (d18:0/16:1)
1041.52	$[M-2H]^{2-}$	GQ3(d18:0/20:0)
1046.44	$[M+Na-3H]^{2-}$	GT1(d18:1/14:0) or GT1(d18:0/14:1)
1049.52	$[M-2H]^{2-}$	GT1(d18:1/16:0) or GT1(d18:0/14:1)
1059.20	$[M-2H]^{2-}$	Fuc-GT2(d18:0/20:2) or Fuc- GT2(d18:1/20:1) or Fuc- GT2(d18:2/20:0)
1063.56	$[M-2H]^{2-}$	GT1 (d18:1/18:0) or GT1 (d18:0/18:1)
1074.06	$[M-2H]^{2-}$	Fuc-GT2 (d18:0/22:1) or Fuc-GT2 (d18:1/22:0)
1077.59	$[M-2H]^{2-}$	GT1 (d18:0/20:0)
1096.54	$[M-2H]^{2-}$ $[M+Na-3H]^{2-}$	O-Ac-GT1 (d18:1/20:1) or O-Ac-GT1 (d18:0/20:2) or O-Ac-GT1 (d18:2/20:0) GQ3(d18:1/28:0) or GQ3(d18:0/28:1)
1110.06	$[M-2H]^{2-}$	Fuc-GT2(d18:1/27:0) or Fuc-GT2(d18:0/27:1)
1117.56	$[M+Na-2H]^{-}$	GM3 (d18:1/12:0) or GM3 (d18:0/12:1)
1179.80	$[M-H]^{-}$	GM3 (d18:1/18:0) or GM3 (d18:0/18:1)
1227.67	$[M-H]^{-}$ $[M+Na-2H]^{-}$	GA1(d18:0/16:0) GM3 (d18:1/20:1) or GM3 (d18:0/20:2)
1231.59	$[M-H]^{-}$ $[M+Na-2H]^{-}$	GM3(d18:1/22:2) or GM3(d18:0/22:3) or GM3(d18:2/22:1) GM3 (d18:0/20:0)

m/z (monoisotopic)	Molecular ion	Proposed structure
1240.69	$[M-2H]^{2-}$	GQ2 (d18:0/34:0)
1251.61	$[M-H]^{-}$	GA1(d18:1/18:1) or GA1(d18:0/18:2) or GA1(d18:2/18:0)
1263.62	$[M-H]^{-}$ $[M-H]^{-}(-H_2O)$	GM3 (d18:1/24:0) or GM3 (d18:0/24:1) GA1(d18:1/20:0) or GA1(d18:0/20:1)
1284.32	$[M-H]^{-}$	O-Ac-GM4(d18:1/34:0) or O-Ac-GM4(d18:0/34:1)
1382.89	$[M-H]^{-}$	GM2 (d18:1/18:0) or GM2 (d18:0/18:1)
1410.89	$[M-H]^{-}$	GM2 (d18:1/20:0) or GM2 (d18:0/20:1) Fuc-GA1(d18:1/20:0) or Fuc-GA1(d18:0/20:1)
1467.85	$[M+Na-2H]^{-}$	GA1(d18:1/32:2) or GA1(d18:0/32:3) or GA1(d18:2/32:1)
1492.90	$[M-H]^{-}$	GM2(d18:1/26:1) or GM2(d18:0/26:2) or GM2(d18:2/26:0)
1509.70	$[M-H]^{-}$	Fuc-GM2(d18:1/18:1) or Fuc-GM2(d18:0/18:2) or Fuc-GM2(d18:2/18:0)
1512.99	$[M-H]^{-}$ $[M-H]^{-}$	GM1(d18:1/16:2) or GM1(d18:0/16:3) or GM1(d18:2/16:1) GD3(d18:1/21:0) or GD3(d18:0/21:1)
1517.33	$[M-H]^{-}$	Fuc-GM3(d18:0/33:2) or Fuc-GM3(d18:1/33:1) or Fuc-GM3(d18:2/33:0)
1536.65	$[M-H]^{-}$	GM2(d18:1/29:0) or GM2(d18:0/29:1)
1542.88	$[M-H]^{-}$	GM1(d18:0/18:2) or GM1(d18:1/18:1) or GM1(d18:2/18:0)
1544.92	$[M-H]^{-}$	GM1(d18:1/18:0) or GM1(d18:0/18:1)
1560.88	$[M-H]^{-}$	O-Ac-GM1(d18:0/16:0)
1566.63	$[M+Na-2H]^{-}$	GM1(d18:1/18:0) or GM1(d18:0/18:1)
1572.92	$[M-H]^{-}$	GM1 (d18:1/20:0) or GM1 (d18:0/20:1)
1589.05	$[M-H]^{-}$	GM1 (d18:0/21:0)
1594.13	$[M-H]^{-}$	Fuc-GM2(d18:0/24:2) or Fuc-GM2(d18:1/24:1) or Fuc-GM2(d18:2/24:0)
1602.91	$[M-H]^{-}$	GM1 (d18:0/22:0)
1629.00	$[M-H]^{-}$	GM1(d18:1/24:0) or GM1(d18:0/24:1)
1647.81	$[M-H]^{-}$	GD2(d18:0/16:0)
1656.88	$[M-H]^{-}$	GM1(d18:1/26:0) or GM1(d18:0/26:1)
1663.16	$[M-H]^{-}$	GD3(d18:1/32:2) or GD3(d18:0/32:3) or GD3(d18:2/32:1)
1673.73	$[M-H]^{-}$	GD2(d18:1/18:0) or GD2(d18:0/18:1)
1690.96	$[M-H]^{-}$	Fuc-GM1(d18:0/19:0)
1702.00	$[M-H]^{-}$	GD2(d18:1/20:0) or GD2(d18:0/20:1)
1709.35	$[M-H]^{-}$	O-Ac-GD3 (d18:1/32:0) or O-Ac-GD3 (d18:0/32:1)
1716.89	$[M-H]^{-}$	Fuc-GD2(d18:1/12:1) or Fuc-GD2(d18:0/12:2) or Fuc-GD2(d18:2/12:0)
1718.97	$[M-H]^{-}$	Fuc-GD2(d18:1/12:0) or Fuc-GD2(d18:0/12:1)
1729.97	$[M-H]^{-}$	GD2(d18:1/22:0) or GD2(d18:0/22:1)
1741.14	$[M-H]^{-}$	GM1(d18:1/32:0) or GM1(d18:0/32:1)
1746.20	$[M-H]^{-}$	GD2(d18:0/23:0) Fuc-GM1(d18:0/23:0)
1775.58	$[M-H]^{-}$	GD1(d18:1/14:2) or GD1(d18:2/14:1) or GD1(d18:0/14:3)
1788.91	$[M+2Na-3H]^{-}$	GT3(d18:1/16:1) or GT3(d18:0/16:2) or GT3(d18:2/16:0)
1796.51	$[M-H]^{-}(-H_2O)$ $[M-H]^{-}$	GD2(d18:1/28:0) or GD2(d18:0/28:1) Fuc-GD3(d18:1/32:0) or Fuc-GD3(d18:0/32:1)

m/z (monoisotopic)	Molecular ion	Proposed structure
1803.75	$[M-H]^{-}$ $[M+Na-2H]^{-}$	GD1(d18:1/16:2) or GD1(d18:0/16:3) or GD1(d18:2/16:1) GD1(d18:0/14:0)
1835.92	$[M-H]^{-}$	GD1(d18:1/18:0) or GD1(d18:0/18:1)
1837.64	$[M+Na-2H]^{-}$	GT3 (d18:0/22:2) or GT3 (d18:1/22:1) or GT3 (d18:2/22:0)
1858.92	$[M-H]^{-}$	Fuc-GD2(d18:1/22:0) or Fuc-GD2(d18:0/22:1)
1864.21	$[M+Na-2H]^{-}$	GD2(d18:1/30:0) or GD2(d18:0/30:1)
1872.49	$[M-H]^{-}$ $[M-H]^{-}$	GD2(d18:0/32:0) Fuc-GM1(d18:0/32:0)
1880.78	$[M-H]^{-}$	GT2(d18:1/12:0) or GT2(d18:0/12:1)
1886.96	$[M-H]^{-}$	Fuc-GT3 (d18:1/18:2) or Fuc-GT3 (d18:0/18:3) or Fuc-GT3 (d18:2/18:1)
1896.01	$[M+Na-2H]^{-}$	GT3(d18:1/26:0) or GT3(d18:0/26:1)
1901.91	$[M-H]^{-}$	GT3(d18:1/28:0) or GT3(d18:0/28:1)
1908.94	$[M-H]^{-}$	GT2(d18:1/14:0) or GT2(d18:0/14:1)
1915.84	$[M-H]^{-}$	GT3(d18:1/29:0) or GT3(d18:0/29:1)
1926.13	$[M+Na-2H]^{-}$ $[M-H]^{-}$	GT3(d18:0/28:0) GT3(d18:1/30:2) or GT3(d18:0/30:3) or GT3(d18:2/30:1)
1938.37	$[M+Na-2H]^{-}$	GD1(d18:1/24:2) or GD1(d18:0/24:3) or GD1(d18:2/24:1)
1946.16	$[M-H]^{-}$	GD1(d18:1/26:1) or GD1(d18:0/26:2) or GD1(d18:2/26:0)
1960.18	$[M-H]^{-}$	GT3(d18:0/32:0)
1966.86	$[M-H]^{-}$	GQ3(d18:1/12:1) or GQ3(d18:0/12:2) or GQ3(d18:2/12:0)
1981.96	$[M-H]^{-}$ $[M-H]^{-}$	GT3(d18:1/34:2) or GT3(d18:0/34:3) or GT3(d18:2/34:1) Fuc-GT2(d18:1/10:0) or Fuc-GT2(d18:0/10:1)
2004.16	$[M-H]^{-}$	GD1(d18:1/30:0) or GD1(d18:0/30:1)
2011. 08	$[M+Na-2H]^{-}$ $[M-H]^{-}(-2H_2O)$	GT2(d18:1/20:2) or GT2(d18:0/20:3) or GT2(d18:2/20:1) GT2(18:0/24:2) or GT2(18:1/24:1) or GT2(18:2/24:0)
2032.93	$[M-H]^{-}$ $[M+2Na-3H]^{-}$	O-Ac-GT2(d18:1/20:1) or O-Ac-GT2(d18:0/20:2) or O-Ac-GT2(d18:2/20:0) GT2(d18:1/20:2) or GT2(d18:0/20:3) or GT2(d18:2/20:1)
2049.15	$[M-H]^{-}$	GT2(d18:1/24:0) or GT2(d18:0/24:1)
2060.08	$[M-H]^{-}$	GD1(d18:1/34:0) or GD1(d18:0/34:1)
2077.05	$[M-H]^{-}$	GQ3(d18:1/20:2) or GQ3(d18:0/20:3) or GQ3(d18:2/20:1)
2089.06	$[M-H]^{-}$	O-Ac-GT2(d18:1/24:1) or O-Ac-GT2(d18:0/24:2) or O-Ac-GT2(d18:2/24:0)
2105.17	$[M-H]^{-}$ $[M-H]^{-}$	GT2(d18:1/28:0) or GT2(d18:0/28:1) Fuc-GD1(d18:1/28:0) or Fuc-GD1(d18:0/28:1)
2164.89	$[M-H]^{-}$ $[M+2Na-3H]^{-}$	O-Ac-GT1(d18:1/18:2) or O-Ac-GT1(d18:0/18:3) or O-Ac-GT1(d18:2/18:1) GT1(d18:1/18:3) or GT1(d18:2/18:2) or GT1(d18:0/18:4)
2172.02	$[M-H]^{-}$	GQ2(d18:1/12:0) or GQ2(d18:0/12:1)
2187.22	$[M-H]^{-}$	GT2(d18:0/34:2) or GT2(d18:1/34:1) or GT2(d18:2/34:0)
2193.25	$[M-H]^{-}$	GQ3(d18:1/28:0) or GQ3(d18:0/28:1)
2198.06	$[M-H]^{-}$	GQ2(d18:1/14:1) or GQ2(d18:0/14:2) or GQ2(d18:2/14:0)
2215.18	$[M-H]^{-}$	GQ3(d18:1/30:3) or GQ3(d18:0/30:4) or GQ3(d18:2/30:2)



(-) nanoESI chip QTOF MS^2 of the single charged ion at *m/z* 1690.00 corresponding to Fuc-GM1 (d18:0/19:0) ganglioside species from healthy frontal brain ganglioside mixture: a) m/z (280-980); b) m/z (950-1700)



Fragmentation scheme of the $[M-H]^-$ ion at *m/z* 1690.00 corresponding to Fuc-GM1(d18:0/19:0)

Conclusions (I)

- A comparative screening of ganglioside composition of brain metastasis in lung adenocarcinoma and healthy brain using mass spectrometry
- The method employs fully automated chip-nanoESI (NanoMate robot) for high-throughput sample infusion in the negative ion mode in combination with quadrupole time-of-flight (QTOF) MS^1 for screening and collision-induced dissociation (CID) MS^2 for fragmentation
- By MS^1 screening of the ganglioside native mixture extracted from healthy frontal brain tissue and adenocarcinoma metastasis of the frontal brain, 127 and 105 respectively, ganglioside species exhibiting a high degree of heterogeneity in the oligosaccharide core or ceramide and groups that easily cleave off such as Neu5Ac, Fuc, or *O*-Ac were detected and identified
- In adenocarcinoma brain metastasis mixture, species exhibiting short oligosaccharide chains with reduced sialic acid content (GA1, GA2, GM4, GM3, GM2, GD3, GD2, GT3, GT2, GQ3) dominate numerically
- Contrastingly, healthy frontal tissue is dominated by ganglioside species with long oligosaccharide chains with 1(GM)-6(GH) molecules of sialic acid attached to the carbohydrate portion

Conclusions (II)

- Discrimination of GD3(18:0/18:0) adenocarcinoma brain metastasis-associated species was possible by nanoESI QTOF MS^2 analysis

- The feasibility of this approach was tested for sequencing of a Fuc-GM1 species: the ion corresponding to Fuc-GM1 species having the experimentally determined M_r 1690.00 was chosen as a precursor in the MS^2 fragmentation analysis

- Within MS^2 dissociation event, a complete characterization of the oligosaccharide core including discrimination of sialylation site and also the position of the fucose residue was achieved without the need for supplementary investigation by any other method.

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