

Article

Accumulation of Unusual Gangliosides G_{Q3} and G_{P3} in Breast Cancer Cells Expressing the G_{D3} Synthase

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Abstract: Glycosphingolipids from the ganglio-series are usually classified in four series according to the presence of 0 to 3 sialic acid residues linked to lactosylceramide. The transfer of sialic acid is catalyzed in the Golgi apparatus by specific sialyltransferases that show high specificity toward glycolipid substrates. ST8Sia I (EC 2.4.99.8, SAT-II, SIAT 8a) is the key enzyme controlling the biosynthesis of b- and c-series gangliosides. ST8Sia I is expressed at early developmental stages whereas in adult human tissues, ST8Sia I transcripts are essentially detected in brain. ST8Sia I together with b- and c-series

gangliosides are also over-expressed in neuroectoderm-derived malignant tumors such as melanoma, glioblastoma, neuroblastoma and in estrogen receptor (ER) negative breast cancer, where they play a role in cell proliferation, migration, adhesion and angiogenesis. We have stably expressed ST8Sia I in MCF-7 breast cancer cells and analyzed the glycosphingolipid composition of wild type (WT) and GD3S+ clones. As shown by mass spectrometry, MCF-7 expressed a complex pattern of neutral and sialylated glycosphingolipids from globo- and ganglio-series. WT MCF-7 cells exhibited classical monosialylated gangliosides including G_{M3} , G_{M2} , and G_{M1a} . In parallel, the expression of ST8Sia I in MCF-7 GD3S+ clones resulted in a dramatic change in ganglioside composition, with the expression of b- and c-series gangliosides as well as unusual tetra- and pentasialylated lactosylceramide derivatives G_{Q3} ($II^3Neu5Ac_4-Gg_2Cer$) and G_{P3} ($II^3Neu5Ac_5-Gg_2Cer$). This indicates that ST8Sia I is able to act as an oligosialyltransferase in a cellular context.

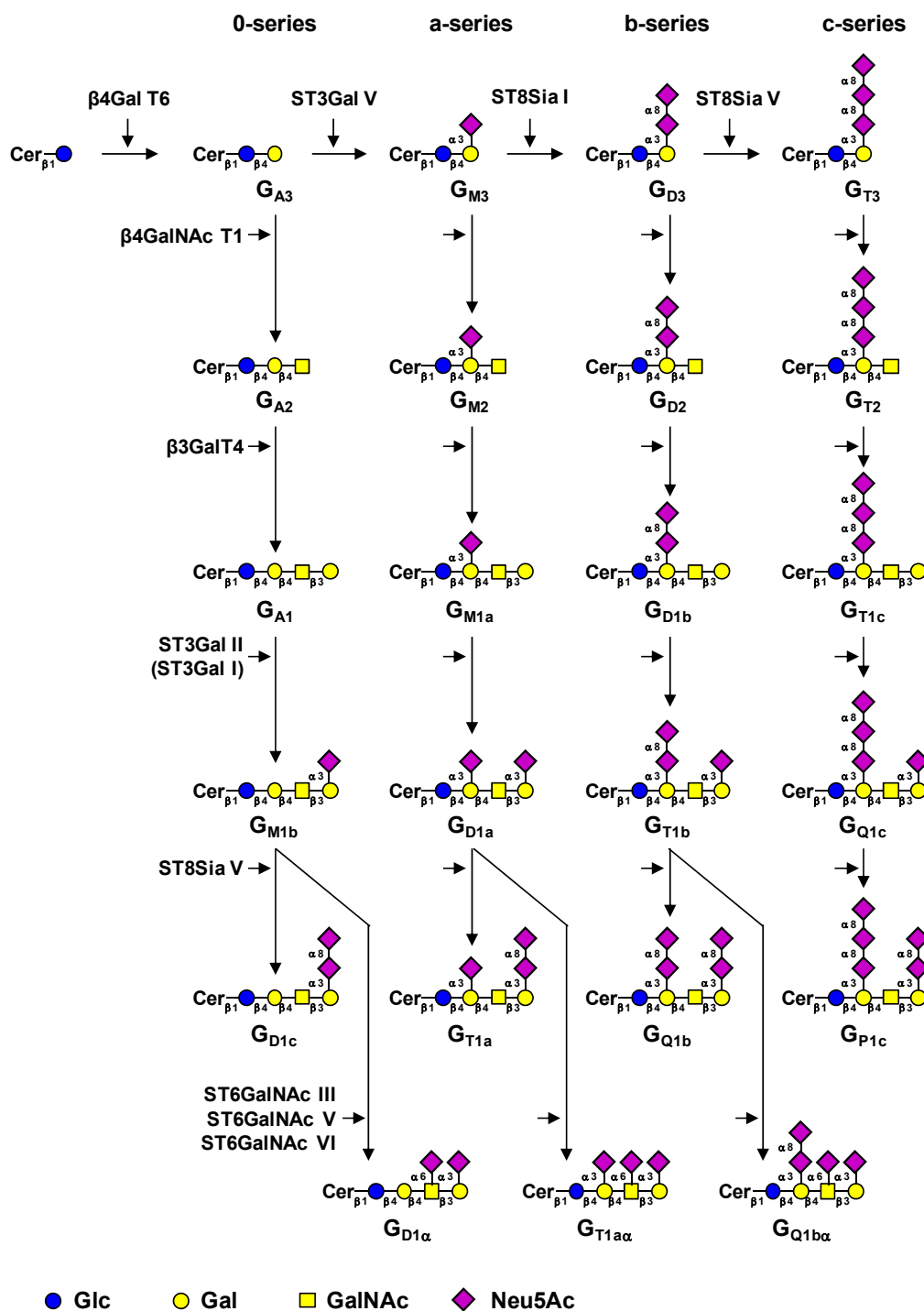
Keywords: gangliosides; G_{D3} synthase; MCF-7; unusual gangliosides

Abbreviations: BSA: Bovine Serum Albumin; Cer: ceramide; DMB: 1,2-diamino-4,5-methylenedioxybenzene; DMEM: Dulbecco's Modified Eagle's Medium; EDTA: Ethylenediaminetetraacetic Acid; DMSO: Dimethyl Sulfoxide; FBS: Fetal Bovine Serum; FITC: Fluorescein Isothiocyanate; FL-HPLC: Fluorescence Detection High Performance Liquid Chromatography; GD3S: G_{D3} synthase; GSL: glycosphingolipid; HPRT: Hypoxanthine PhosphoRibosylTransferase; HRP: horseradish peroxidase; LacCer: Lactosylceramide; mAb: monoclonal Antibody; MALDI-TOF: matrix assisted laser desorption-ionization time-of-flight; MS: Mass Spectrometry; PBS: Phosphate Buffered Saline; QPCR: Quantitative real-time Polymerase Chain Reaction; SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; ST3Gal V: G_{M3} synthase; ST8Sia I: G_{D3} synthase; ST8Sia V: G_{T3} synthase; WT: Wild Type.

1. Introduction

Glycosphingolipids (GSL) from the ganglio-series are classified in four series according to the presence of 0 to 3 sialic acid residues linked to lactosylceramide ($Gal\beta 1-4Glc-Cer$, LacCer) [1]. The transfer of sialic acid to LacCer is catalyzed in the Golgi apparatus by specific sialyltransferases (namely ST3Gal V, ST8Sia I and ST8Sia V) that show high specificity toward glycolipid substrates [2]. LacCer, G_{M3} ($Neu5Ac\alpha 2-3Gal\beta 1-4Glc-Cer$, $II^3Neu5Ac_1-Gg_2Cer$), G_{D3} ($Neu5Ac\alpha 2-8Neu5Ac\alpha 2-3Gal\beta 1-4Glc-Cer$, $II^3Neu5Ac_2-Gg_2Cer$) and G_{T3} ($Neu5Ac\alpha 2-8Neu5Ac\alpha 2-8Neu5Ac\alpha 2-3Gal\beta 1-4Glc-Cer$, $II^3Neu5Ac_3-Gg_2Cer$) are therefore the precursors for 0-, a-, b- and c-series gangliosides and the biosynthesis of these compounds determine the relative proportion of gangliosides in each series (Figure 1). Elongation of the precursors can then occur by the sequential action of *N*-acetylgalactosaminyltransferase ($\beta 4GalNAc$ T1), galactosyltransferase ($\beta 3Gal$ T4) and sialyltransferases (ST3Gal I, ST3Gal II and ST8Sia V), α -gangliosides deriving from the action of ST6GalNAc III, V or VI on G_{M1b} , G_{D1a} or G_{T1b} (Table 1).

Figure 1. Biosynthesis pathway for gangliosides. The code names of gangliosides are according to Svennerholm [1]. Cer: ceramide.



The sialyltransferase ST8Sia I (EC 2.4.99.8, SAT-II, SIAT 8a) is the only enzyme known to catalyze the transfer of a sialic acid residue onto G_{M3} through an $\alpha 2,8$ -linkage to synthesize G_{D3} . ST8Sia I and G_{D3} are expressed in fetal tissues at an early developmental stage [3,4] where they play a key role in cell-cell interaction, cell differentiation and proliferation [5], whereas in adult human tissues, ST8Sia I is essentially detected in the brain [6]. ST8Sia I and G_{D3} have been also shown to be over-expressed in neuroectoderm-derived malignant tumors such as melanoma, glioblastoma and neuroblastoma, and in estrogen receptor negative breast cancer [7–10].

Table 1. Glycosyltransferases involved in gangliosides biosynthesis. R = G_{A3}, G_{M3}, G_{D3} or G_{T3}.

Gene	Common name	Main acceptor(s)	Accession #	Reference
<i>UGCG</i>	GlcCer synthase	Ceramide	NM_003358	[18]
<i>B4GALT6</i>	LacCer synthase	Glucosylceramide	NM_004775	[19,20]
<i>ST3GAL5</i>	G _{M3} synthase	Lactosylceramide	NM_003896	[21]
<i>ST8SIA1</i>	G _{D3} synthase	G _{M3} , G _{D3}	NM_003034.2	[11–13]
<i>ST8SIA5</i>	G _{T3} synthase	G _{D3} , G _{M1b} , G _{D1a} , G _{T1b}	NM_013305	[17]
<i>B4GALNACT1</i>	G _{M2} /G _{D2} synthase	G _{A3} , G _{M3} , G _{D3} , G _{T3}	NM_001478.2	[22–24]
<i>B3GALT4</i>	G _{M1a} /G _{D1b} synthase	G _{A2} , G _{M2} , G _{D2} , G _{T2}	NM_003782.3	[23,25]
<i>ST3GAL1</i>	ST3Gal I	Galβ1-3GalNAcβ1-4-R	NM_003033	[26]
<i>ST3GAL2</i>	ST3Gal II	Galβ1-3GalNAcβ1-4-R	NM_006927	[27]
<i>ST6GALNAC3</i>	ST6GalNAc III	Neu5Acα2-3Galβ1-3GalNAcβ1-4-R	NM_152996	[28]
<i>ST6GALNAC5</i>	ST6GalNAc V	Neu5Acα2-3Galβ1-3GalNAcβ1-4-R	NM_030965.1	[29]
<i>ST6GALNAC6</i>	ST6GalNAc VI	Neu5Acα2-3Galβ1-3GalNAcβ1-4-R	NM_013443.3	[29]

The human ST8Sia I cDNA was simultaneously isolated by expression cloning by three research groups [11–13]. The *ST8SIA1* gene is located on chromosome 12, in p12.1-p11.2 and consists of five coding exons spanning over 135 kbp of genomic DNA [14]. ST8Sia I cDNA encodes a 341 amino acid membrane-bound Golgi enzyme with a 12 amino-acid cytoplasmic tail, a transmembrane domain of about 20 residues and a catalytic domain containing the conserved Sialyl motifs involved in substrate binding and transfer [15].

Whereas ST8Sia I mainly sialylates G_{M3}, Nakayama and co-workers have underlined its ability to synthesize G_{T3} from G_{D3} [6]. ST8Sia I was also shown to use G_{M1b}, G_{D1a} or G_{T1b} as acceptor substrates to synthesize G_{D1c}, G_{T1a} or G_{Q1b}, respectively, both *in vitro* and *in vivo* [16]. However, the α2,8-sialyltransferase ST8Sia V is a much better candidate for G_{T1a}/G_{Q1b} synthase activity [17] and no ST8Sia V activity was detected toward G_{M3}. Consequently, ST8Sia I is considered as the only G_{D3} synthase (GD3S) that controls the biosynthesis of gangliosides from the b- and c-series.

By stable transfection of the full-length cDNA of human G_{D3} synthase, we have isolated cellular clones deriving from MCF-7 breast cancer cells that constitutively express GD3S together with b- and c-series gangliosides. Here, we show by mass spectrometry and HPLC analysis that clones that express a high level of GD3S also accumulate unusual tetra- and pentasialylated derivatives of LacCer, G_{Q3} (II³Neu5Ac₄-Gg₂Cer) and G_{P3} (II³Neu5Ac₅-Gg₂Cer).

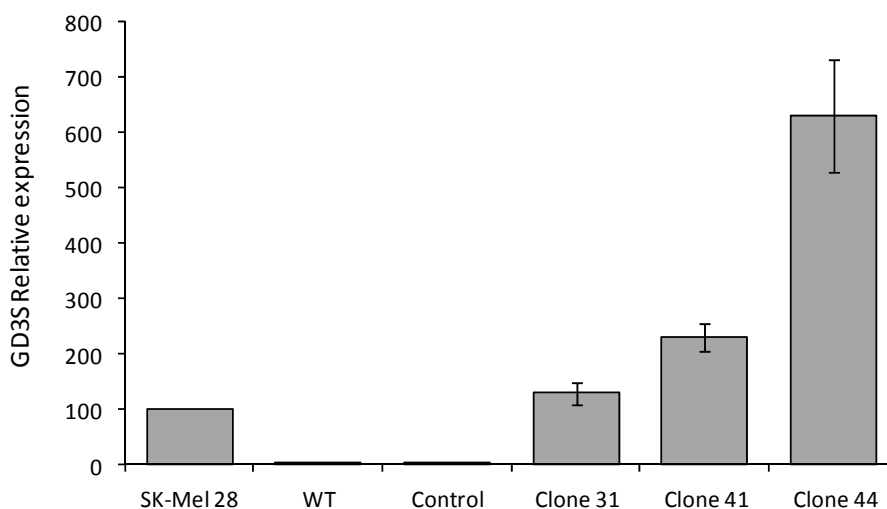
2. Results and Discussion

2.1. Analysis of ST8Sia I Expression by QPCR in Control and GD3S+ MCF-7 Clones

MCF-7 cells were transfected with the pcDNA3-GD3S expression vector containing the full-length cDNA of human GD3S or the empty pcDNA3 vector as control. Transfected cells were cultured 21 days in the presence of 1 mg/mL G418. Individual G418-resistant colonies were isolated by limiting dilution cloning. Forty-four clones were obtained and analyzed for the expression of GD3S. As previously shown [30], QPCR analysis of GD3S expression (Figure 2) indicates that GD3S mRNA is expressed at a very low level in wild-type and control (empty vector transfected) MCF-7 cells compared to SK-Mel 28 melanoma cells used as positive control [31]. Within the forty-four analyzed clones,

three GD3S+ clones (clone #31, #41 and #44) were selected according to the high expression of GD3S compared to SK-Mel 28 (1.3-fold, 2.3-fold and 6.3-fold, respectively) (Figure 2).

Figure 2. QPCR analysis of GD3S expression in control and GD3S+ MCF-7 clones. Quantification of GD3S expression was performed by the method described by Livak and Schmittgen [32] and normalized to HPRT. The expression of GD3S in MCF-7 cells was relative to SK-Mel 28, which was regarded as 100%.



2.2. Flow Cytometry Analysis of Gangliosides Expression in MCF-7 GD3S+ Clones

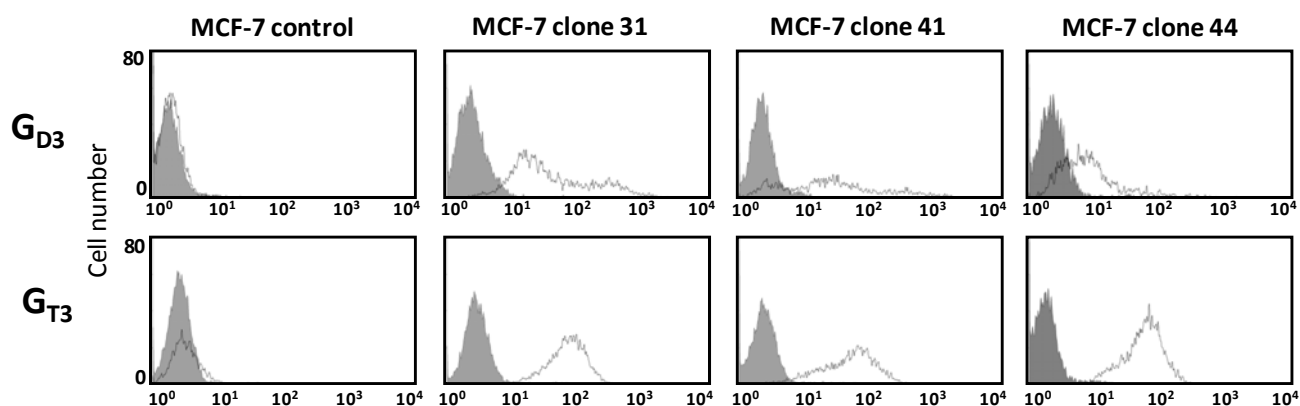
The pattern of gangliosides was monitored in the three selected MCF-7 GD3S+ clones (clone #31, #41, #44) by flow cytometry using anti-G_{D3} R24 and anti-G_{T3} A2B5 mAbs. As shown in Figure 3, the three GD3S+ clones expressed G_{D3} and G_{T3} whereas wild-type and control (empty vector transfected) MCF-7 cells did not express complex gangliosides. G_{T3} is expressed at a similar level in the three GD3S+ clones but a decrease of G_{D3} is observed in clone #44 compared to clone #31 and #41 whereas the expression level of GD3S was 4.8-fold or 2.7-fold higher in clone #44 compared to clone #31 and #41, respectively (Figure 2). Control cells showed no change in the ganglioside profile compared with wild-type MCF-7 (data not shown).

2.3. MS Analysis of Gangliosides in MCF-7 and GD3S+ Clones

Glycolipids were extracted from cells, purified by reverse phase chromatography and permethylated prior to MS analysis. Mass spectrometry analysis established that glycolipid profiles of MCF-7 WT and GD3S+ clones were characterized by complex patterns of neutral and sialylated glycosphingolipids from globo- and ganglio-series. Profiles of all cell lines were dominated by two signals at *m/z* 1460 and 1572 both identified based on their MALDI-TOF/TOF fragmentation patterns (data not shown) and in agreement with previously published analyses [33] as mixtures of G_{b4} and G_{A1} differing by the nature of their lipid moieties (d18:1-16:0 or d18:1-24:0). Along these two major components, MS and MS/MS analyses permitted us to identify other minor neutral GSLs including LacCer and G_{b3} (Figure 4). The comparison of MS profiles did not show any significant difference in neutral GSLs content between MCF-7 WT and GD3+ clones. On the contrary, the content in sialylated

glycolipids varied among the different cell lines. MCF-7 WT cells exhibited monosialylated gangliosides including G_{M3} , G_{M2} , and G_{M1} . The only disialylated GSL observed in MCF-7 WT cells was G_{D1} at m/z 2182 and 2194. Its sequence analysis by MALDI-TOF/TOF typified it as G_{D1a} , thus lacking disialylated motif (data not shown). $GD3^+$ clones did not show GSLs from the $G2$ and $G1$ families but synthesized instead a family of unusual highly sialylated lactosylceramide derivatives substituted by up to 5 Neu5Ac residues tentatively identified as G_{D3} , G_{T3} , G_{Q3} and G_{P3} . The structure of these four compounds was confirmed by MALDI-TOF/TOF sequencing.

Figure 3. Flow cytometry analysis of gangliosides in pcDNA3-GD3S transfected MCF-7 cells. Immunodetection of G_{D3} and G_{T3} was performed using anti- G_{D3} R24 (dilution 1:100) and anti- G_{T3} A2B5 (dilution 1:10) mAbs. The gray peaks correspond to the negative controls (incubation with secondary antibody alone, anti-mouse IgM or anti-mouse IgG labeled with Alexa 488).



The Glycolipid profile of SK-Mel 28 cells was also analyzed in respect to the presence of polysialylated lactosylceramide derivatives. Contrarily to MCF-7 WT cells, GSLs extracted from SK-Mel 28 cells did not contain globo-series but were dominated by ganglio-series, which induces much higher overall sialic acid content (data not shown). In particular, disialylated G_{D3} appears to be the major component along with monosialylated G_{M3} and trisialylated G_{T3} . However, the tetra- and pentasialylated gangliosides synthesized by MCF-7 $GD3^+$ clones were not detected in SK-Mel 28.

Figure 4. Comparison of MS profiles of permethylated glycosphingolipids purified from MCF-7 WT and $GD3^+$ clone #44. All GSLs are present as $d18:1/C16:0$ (Cer*) and $d18:1/C24:0$ (Cer**) isomers. ○, Gal; ●, Glc; ■, GalNAc; ◆, Neu5Ac.

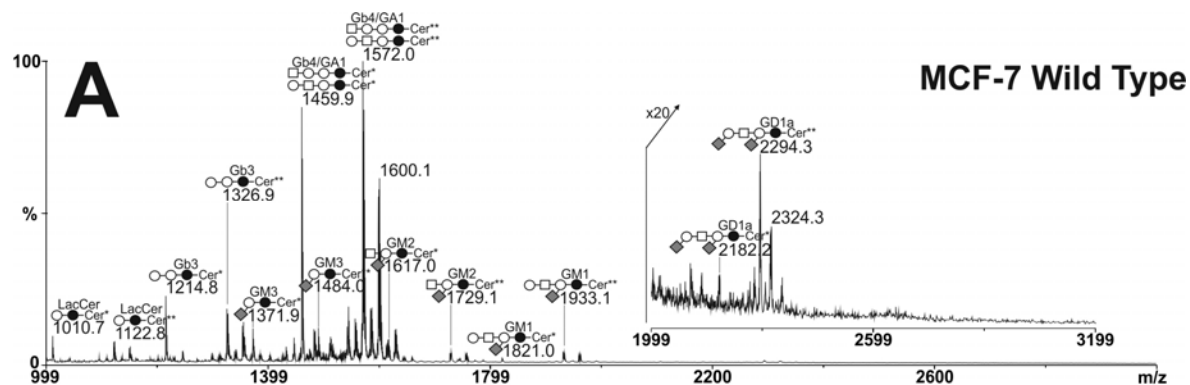
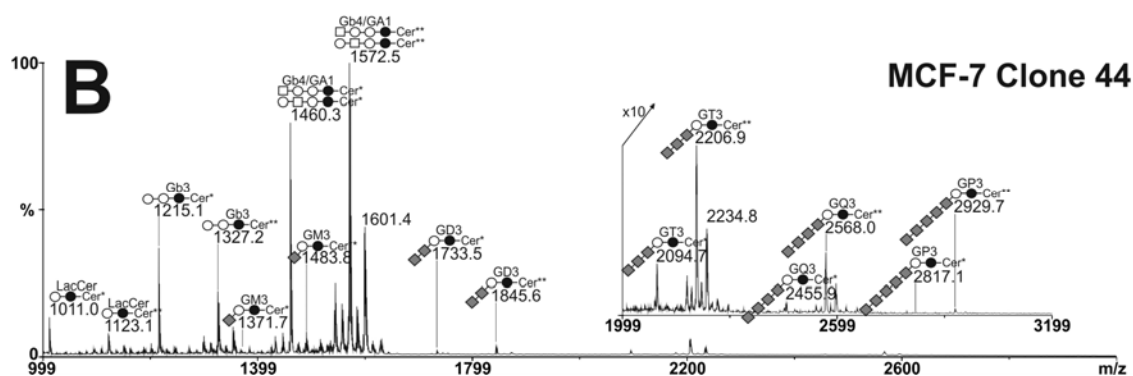
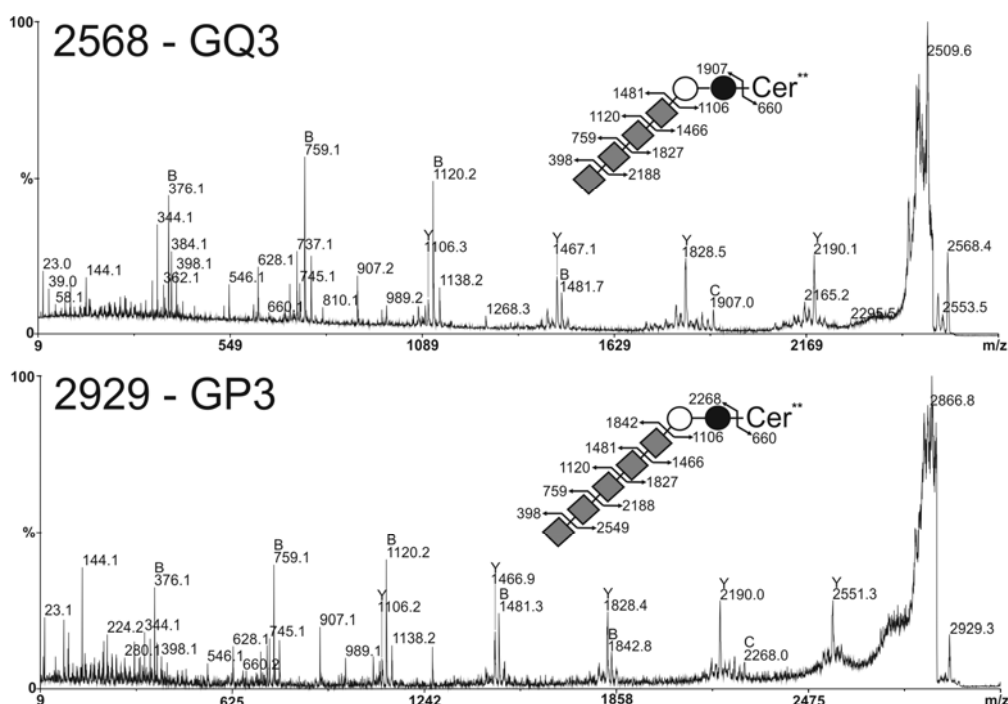


Figure 4. Cont.



We illustrate the sequence analyses of unusual G_{Q3} and G_{P3} in Figure 5. These two molecules differing in the presence of a single Neu5Ac residue were structurally related. They shared the fragmentation pattern of a linear stretch of four sialic acid residues in terminal non-reducing position as $[M+Na]^+$ B-ions at m/z 398, 759, 1120 and 1481 and a linear sequence of Sia_4Hex_2Cer at reducing end as $[M+Na]^+$ Y-ions at m/z 1106, 1466, 1827 and 2188. G_{P3} showed additional B and Y ions at m/z 1842 and 2551 typifying a linear Sia_5 sequence. Altogether, these data established that MCF-7 GD3+ clone #44 synthesizes an unusual family of oligosialylated lactosylceramide derivatives presented from 2 to 5 Neu5Ac residues. The disappearance of G_{M2} , G_{M1} and G_{D1a} in MCF-7 GD3+ clone #44 could be explained by the depletion of G_{M3} substrate caused by the over-expression of the GD3S+ that competes with β -4GalNAcT1 for the use of G_{M3} substrate.

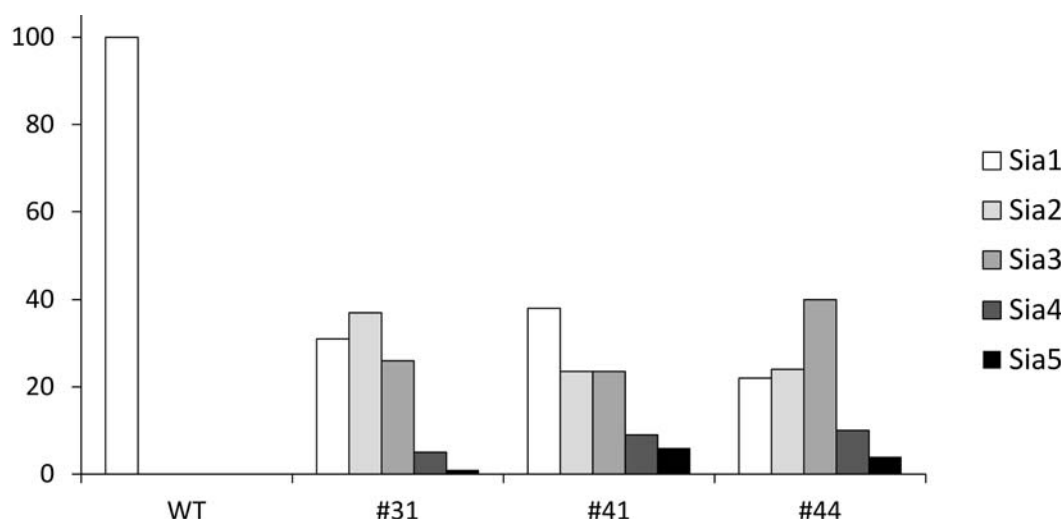
Figure 5. MS/MS sequencing of permethylated glycosphingolipids. Analysis of G_{Q3} at m/z 2568 (A) and G_{P3} at m/z 2929 (B) with ceramide moieties d18:1/C24:0 (Cer**). All fragments are observed as $[M+Na]^+$ adducts except those at m/z at 376 and 660 observed as $[M+H]^+$ adducts. Fragment ions were annotated according to nomenclature of Domon and Costello [34]. \circ , Gal; \bullet , Glc; \blacksquare , GalNAc; \blacklozenge , Neu5Ac.



2.4. Quantification of Polysialylation Associated with Gangliosides by HPLC

Because mass spectrometry does not provide reliable quantitative data, we quantified the extent of oligosialylation modifications in GSLs induced by the overexpression of G_{D3} in MCF-7 by screening all three GD3S+ clones (#31, #41 and #44) (Figure 6). To do that, oligosialylated motifs were released from purified GSLs by mild hydrolysis according to optimized procedures [35] and labeled by DMB before separation and quantification by FL-HPLC [36]. The analysis of relative quantifications of sialic acid chains in GSLs of all MCF-7 GD3S+ clones compared to MCF-7 WT demonstrated a sharp increase of sialylation oligomerization degree, up to five residues, in accordance with mass spectrometry analysis. In MCF-7 WT, no oligosialylation could be observed, in accordance with the sole identification of G_{D1a} by MS. In contrast, all GD3S+ clones contained about 30% of Sia₂ and Sia₃ motifs, up to 10% of Sia₄ and up to 5% of Sia₅. Although clones presented similar GSL oligosialylation profiles, small scale quantitative differences could be observed between the three clones, with a prevalence of higher DP values for clone #44 compared to clones #31 and #41.

Figure 6. Relative quantification of oligosialylation sequence on GSL of MCF-7 WT GD3S+ clones #31, clone #41 and clone #44 by FL-HPLC after DMB derivation.



3. Experimental Section

3.1. Antibodies and Reagents

Anti- G_{D3} R24 mAb was purchased from Abcam (Cambridge, UK) and anti- G_{T3} A2B5 mAb was kindly provided by Pr. Jacques Portoukalian (Depart. of Transplantation and Clinical Immunology, Claude Bernard University and Edouard Herriot Hospital, Lyon, France). FITC-conjugated sheep anti-mouse IgG was from GE Healthcare (Templemars, France). FITC-conjugated anti-mouse IgM was purchased from Molecular Probes (Invitrogen, Carlsbad, CA, USA).

3.2. Cell Culture

The breast cancer cell line MCF-7 and the melanoma cell line SK-Mel 28 were obtained from the American Type Cell Culture Collection. Cell culture reagents were purchased from Lonza

(Levallois-Perret, France). Cells were routinely grown in monolayer and maintained at 37 °C in an atmosphere of 5% CO₂, in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 units/mL penicillin-streptomycin. GD3S positive (GD3S+) MCF-7 clones were obtained by stable transfection of the pcDNA3-GD3S expression vector encoding the full-length human G_{D3} synthase [37] as previously described [33]. Individual resistant colonies were isolated by limit dilution. Three positive clones (#31, #41 and #44), expressing different levels of GD3S were used for further study. Control cells (empty vector transfected) and GD3S+ clones were cultured in the presence of 1 mg/mL G418 (Invitrogen, Cergy-Pontoise, France).

3.3. Quantitative Real-Time-PCR (QPCR) Analysis of G_{D3} Synthase

Total RNA was extracted using the Nucleospin RNA II kit (Macherey Nagel, Hoerd, France), quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA) and the purity of the preparation was checked by ratio of the absorbance at 260 and 280 nm. The cDNA was synthesized using 2 µg of RNA (GE Healthcare). PCR primers for GD3S and Hypoxanthine PhosphoRibosylTransferase (HPRT) were previously described [30,38] and synthesized by Eurogentec (Seraing, Belgium). PCR reactions (25 µL) were performed using 2X SYBR[®] Green Universal QPCR Master Mix (Stratagene, Amsterdam, The Netherlands), with 2 µL of cDNA solution and 300 nM final concentration of each primer. PCR conditions were as follows: 95 °C for 30 s, 51 °C for 45 s, 72 °C for 30 s (40 cycles). Assays were performed in triplicate and GD3S transcript expression level was normalized to HPRT using the $2^{-\Delta\Delta C_t}$ method described by Livak and Schmittgen [32]. Serial dilutions of the appropriate positive control cDNA sample were used to create standard curves for relative quantification and negative control reactions were performed by replacing cDNA templates by sterile water.

3.4. Analysis of Cell Surface Ganglioside by Flow Cytometry

Cells were washed in cold PBS and detached by EDTA 2 mM. Cells were incubated at 4 °C during 1 h with anti-G_{D3} R24 (1:100) and anti-G_{T3} A2B5 (1:10), diluted in phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (PBS-BSA) (Sigma-Aldrich). After washing with PBS-BSA, cells were incubated on ice during 1 h with Alexa Fluor 488 anti-IgG or anti-IgM (1:500). After two washes in PBS-BSA, cells were analyzed by flow cytometry (FACScalibur, Becton Dickinson). Control experiments were performed using secondary antibody alone.

3.5. Extraction and Preparation of Glycolipids

Twenty dishes (10 cm diameter) of cultured cells were washed twice with ice-cold PBS and cells were scraped and sonicated on ice in 200 µL of water. The resulting material was dried under vacuum and sequentially extracted by CHCl₃/CH₃OH (2:1, v/v), CHCl₃/CH₃OH (1:1, v/v) and CHCl₃/CH₃OH/H₂O (1:2:0.8, v/v/v). Supernatants were pooled, dried and subjected to a mild saponification in 0.1 M NaOH in CHCl₃/CH₃OH (1:1) at 37 °C for 2 h and then evaporated to dryness [39]. Samples were reconstituted in CH₃OH/H₂O (1:1, v/v) and applied to a reverse phase C₁₈

cartridge (Waters, Milford, MA, USA) equilibrated in the same solvent. After washing with CH₃OH/H₂O (1:1, v/v), GSLs were eluted by CH₃OH, CHCl₃/CH₃OH (1:1, v/v) and CHCl₃/CH₃OH (2:1, v/v).

3.6. Mass Spectrometry Analysis of GSL

Prior to mass spectrometry analysis, GSL were permethylated according to Ciucanu and Kerek [40]. Briefly, compounds were incubated 2 h in a suspension of 200 mg/mL NaOH in dry DMSO (300 µL) and CH₃I (200 µL). The methylated derivatives were extracted in CHCl₃ and washed several times with water. The reagents were evaporated and the sample was dissolved in CHCl₃ in the appropriate dilution. MALDI-MS and MS/MS analyses of permethylated GSL were performed on 4800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) mass spectrometer, operated in the reflectron mode. For MS acquisition, 5 µL of diluted permethylated samples in CHCl₃ were mixed with 5 µL of 2,5-dihydroxybenzoic acid matrix solution (10 mg/mL dissolved in CHCl₃/CH₃OH (1:1, v/v)). The mixtures (2 µL) were then spotted on the target plate and air dried. MS survey data comprises a total of 50 sub-spectra of 1500 laser shots. Peaks observed in the MS spectra were selected for further MS/MS. CID MS/MS data comprises a total of 100 sub-spectra of 3000 laser shots. Two or more spectra can be combined post-acquisition with mass tolerance set at 0.1 Da to improve S/N ratio. The potential difference between the source acceleration voltage and the collision cell was set to 1 kV and argon was used as collision gas.

3.7. Analysis of Oligo-Sialylated Sequences by HPLC

In order to minimize internal fragmentation of polysialylated sequences, sialylated glycan samples were directly coupled to 1,2-diamino-4,5-methylenedioxybenzene (DMB) without prior mild hydrolysis [35]. Samples were incubated for 2.5 h at 50 °C in 50 µL of a DMB reagent solution (2.7 mM DMB, 9 mM sodium hydrosulfite, and 0.5 mM β-mercaptoethanol in 20 mM TFA). 10 µL of 1 M NaOH was then added and the reaction mixtures further incubated in the dark at room temperature for 1 h. Samples were stored at 4 °C before analysis. DMB-derivatized sialic acid oligomers were separated on a HPLC apparatus fitted with a CarboPac PA-100 column (Dionex). CarboPac column was eluted at 1 mL/min with a concentration gradient of 2 to 32% of 1 M NaNO₃ in water. Elution was monitored by an on line fluorescence detector set at wavelengths of 373 nm for excitation and 448 nm for emission.

4. Conclusions

ST8Sia I is the only sialyltransferase able to transfer a sialic acid residue onto G_{M3} to synthesize G_{D3} and is therefore considered as the G_{D3} synthase. The animal ST8Sia family can be divided in three groups according to the capacity to carry out poly-, oligo- and mono-α2,8-sialylation and phylogenetic analyses have clearly associated ST8Sia I to the group of mono-α2,8-sialyltransferase [41]. However, by expression cloning of the human G_{T3} synthase, Nakayama and co-workers have underlined the ability of ST8Sia I to synthesize G_{T3} from G_{D3} [6]. Here, we show, for the first time, that ST8Sia I is able to synthesize unusual highly sialylated lactosylceramide derivatives substituted by up to

5 Neu5Ac residues and identified as G_{Q3} (II³Neu5Ac₄-Gg₂Cer) and G_{P3} (II³Neu5Ac₅-Gg₂Cer), showing that this enzyme can act as an oligosialyltransferase.

In humans, the *ST8SIA1* gene is located on chromosome 12, in p12.1-p11.2 and consists of five coding exons spanning over 135 kbp of genomic DNA [14]. Two initiation codons on the first exon lead to two protein isoforms of 356 or 341 amino acids that differ in their N-terminal part. However, the relative capacity of each isoform to transfer more than two sialic acid residues has not been evaluated. By *in vitro* sialyltransferase assay, a recombinant soluble form of the human G_{D3} synthase was shown to synthesize, after a long period of incubation, higher polysialogangliosides, which presumably have more than three sialic acid residues but these compounds were not characterized [6].

From a general point of view, the occurrence of oligosialylation associated with glycolipids has been rarely reported so far. One exception is the recent identification of polysialogangliosides containing α 2,8-linked polyNeu5Ac with DPs ranging from 2 to at least 16 in sea urchin sperm head [42]. However, to our knowledge, tetra- and pentasialylated lactosylceramide derivatives have never been described in human tissues and cells. Although SK-Mel 28 cells that constitutively express high levels of GD3S, synthesize high quantities of G_{D3} and G_{T3}, we were not able to detect these unusual tetra- and pentasialylated gangliosides in this cell line. Indeed, in our cellular model, these unusual structures were obtained after transfection of GD3S cDNA and may not exist in natural conditions, and the depletion of G_{M3} in GD3S+ MCF-7 clones could explain that GD3S used other gangliosides, such as G_{T3} or G_{Q3}, as acceptor substrates. Nevertheless, one may thus expect that revisiting the structure of human gangliosides with recent high sensitivity mass spectrometry techniques will uncover oligosialylated glycolipids in cancerous cell lines and in normal or pathological tissues.

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Sample Availability: Not available.

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