ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 3, pp. 235-249. © Pleiades Publishing, Ltd., 2009.
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REVIEW

Ganglioside GM3 and Its Biological Functions

N. V. Prokazova*, N. N. Samovilova, E. V. Gracheva, and N. K. Golovanova

Institute of Experimental Cardiology, Russian Cardiology Research Center, Russian Ministry of Health, 3-ya Cherepkovskaya ul. 15a, 121552 Moscow, Russia; fax: (495) 414-6699; E-mail: prokazova@cardio.ru

Received July 4, 2008
Revision received September 18, 2008

Abstract—Metabolism, topology, and possible mechanisms for regulation of the ganglioside GM3 content in the cell are reviewed. Under consideration are biological functions of GM3, such as involvement in cell differentiation, proliferation, oncogenesis, and apoptosis.

DOI: 10.1134/S0006297909030018

Key words: ganglioside GM3, GM3 synthase, metabolism, cell differentiation, proliferation, oncogenesis, apoptosis

The GM3 ganglioside is the first and simplest member in the metabolic series of a glycosphingolipid family containing sialic acids (N-acetyl- and N-glycolyl-neuraminic acids and their O-acyl derivatives) (Scheme 1). Gangliosides make up a minor proportion (1-2%) of total cell lipids, except those in tissues of the central nervous system. GM3 is the main ganglioside in the majority of extraneural tissues of vertebrata, and its contents are varied (Table 1) [1-23]. GM3 is a metabolic precursor of more complex natural gangliosides and, thus, determines their contents in cells and tissues. GM3 is one of the essential components of plasma membrane rafts. Therefore, the regulatory mechanisms of metabolism and biological functions of this ganglioside are actively studied.

Abbreviations: CDK, cyclin-dependent kinase; Cer, ceramide; CRE, cAMP-responsive element; CREB, CRE-binding protein; DIM, detergent-insoluble material; EGF, epidermal growth factor; ERK, extracellularly regulated protein kinase; FAK, focal adhesion kinase; FGF, fibroblast growth factor; Gal, galactose; GEM, ganglioside-enriched microdomains; Glc, glucose; GPI, glycosyl phosphatidyl inositol; LacCer, lactosylceramide; MAPK, mitogen-activated protein kinase; Neu3, plasma membrane sialidase; NeuAc (SA), N-acetylneuraminic (sialic) acid; PDGF, platelet-derived growth factor; PDMP, D-1-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PI3K, phosphatidylinositol-3 kinase; PKA, PKB/Akt, and PKC, protein kinase A, B/Akt, and C, respectively; PPPP, D-1-threo-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol; SAP, saposin; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

* To whom correspondence should be addressed.

METABOLISM OF GM3

Biosynthesis. Structure of carbohydrate chains of gangliosides is determined by specificity of glycosyl transferases relative to lipid substrates, as well as their location in different compartments of the Golgi apparatus where glycosylation occurs. Therefore, only some strictly determined oligosaccharide sequences are found in the carbohydrate chains of gangliosides of higher animals and humans [24-26] (Scheme 2). Among these sequences, lactose Galβ1-4Glc, which is always bound with ceramide (lactosylceramide, LacCer), is the main glycolipid core in all gangliosides of the lacto- and ganglioseries. Upon sialylation of lactosylceramide, i.e. formation of GM3, the carbohydrate chain is growing further with production of gangliosides of a-, b-, and c-series (we shall further call them "gangliosides", Scheme 2) [27].

GM3 synthase (ST I, ST3Gal V, CMP-NeuAc:lactosylceramide-α2,3-sialyl transferase; EC 2.4.99.9), which catalyzes the transfer of NeuAc from CMP-NeuAc onto the terminal galactose residue of lactosylceramide, is a key enzyme of ganglioside biosynthesis (Scheme 2). Similarly to all mammalian sialyl transferases, GM3 synthase belongs to the type II transmembrane glycoproteins located in the Golgi apparatus [28, 29].

Due to organization of its genome, GM3 synthase is unique in the ST3Gal subfamily of known sialyl transferases [28]. Note that this sialyl transferase and sialyl transferase of the GD3 ganglioside are highly specific to their lipid substrates, while other sialyl transferases can sialylate a number of lipid substrates.

Table 1. GM3 ganglioside contents in some human tissues

Tissue	Gangliosides	GM3, % of the total ganglioside content	Method of GM3 determination	References
Liver	214-220*	90-91.6	TLC, colorimetry	[1-3]
Kidneys	30-60*	74	same	[1, 4]
Spleen	200-300*	–	–"–	[1, 5]
Placenta	100-200*	–	–"–	[1, 6]
Muscles			TLC and	[1, 7, 8]
skeletal muscles	50-80*	70	immunostaining	
heart	–	50		
Skin	30-35*	–	TLC, colorimetry	[1, 9]
Skin fibroblasts	6 μg/10^6 cells	77	same	[10]
Adipose tissue	10-15*	80-90	–"–	[1, 11]
Aorta	46*	82	–"–	[12, 13]
Blood serum	6-8 nmol/ml**	57-67	–"–	[14]
Blood cells			TLC, colorimetry	[15-18]
erythrocytes	1.3 μg/mg protein	14	and HPLC	
platelets	0.6 μg/mg protein	90	same	
lymphocytes	–	72	–"–	
Thyroid gland	120*	48	–"–	[1, 19]
Adrenal medulla	0.2 mg/g wet tissue	55.8	–"–	[20]
Milk	10 mg/liter**	27	TLC, colorimetry	[21, 22]
Brain			same	[1, 23]
cortex	3000-3500*	–		
white matter	1000*	4.1		
gray matter	–	3.8		

Note: –, indicates lack of data.
*, nmol of lipid-bound sialic acid (SA) per g wet tissue.
**, lipid-bound SA quantity.

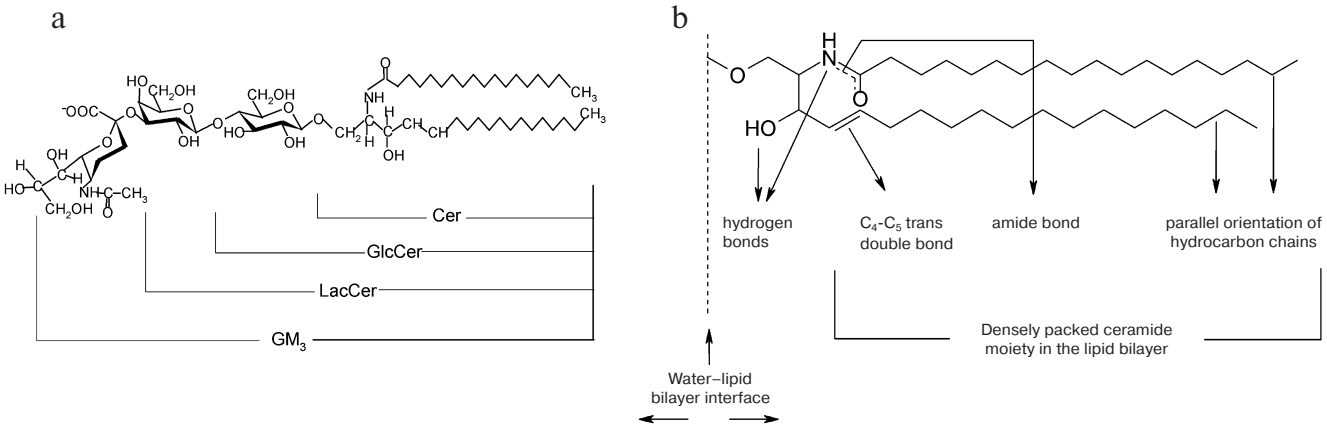
Because of extremely low contents of sialyl transferases, it is difficult to isolate sufficient amounts from tissues of mammals and other higher animals by classical approaches to determine amino acid sequence or prepare specific antibodies to these enzymes. A few micrograms of GM3 synthase was isolated from rat liver and brain by affinity chromatography using immobilized lactosylceramide and antibodies to the partially purified enzyme [30, 31].

Until recently, there were no data on the GM3 synthase activities in human tissues. The human GM3 synthase activity was mainly studied in human myeloid leukemic cell line HL60 and in some other cells (K562, KG-1, ML-1, HO, U937), in tumor cells (see section "Cell differentiation"), and in fibroblasts upon some passages in culture [32]. Recently, a high activity of GM3 synthase was detected in intact monocytes of human peripheral blood [33].

Progress in molecular cloning of genes encoding sialyl transferases, including ganglioside-synthesizing ones, resulted in prediction of amino acid sequences of these enzymes and investigation of the regulatory mechanism for their expression. Now cDNA of GM3 synthase has been isolated from some objects and its gene expression is shown to depend on the type of tissue, and it dominates in brain, muscles, testes, and placenta [34-39].

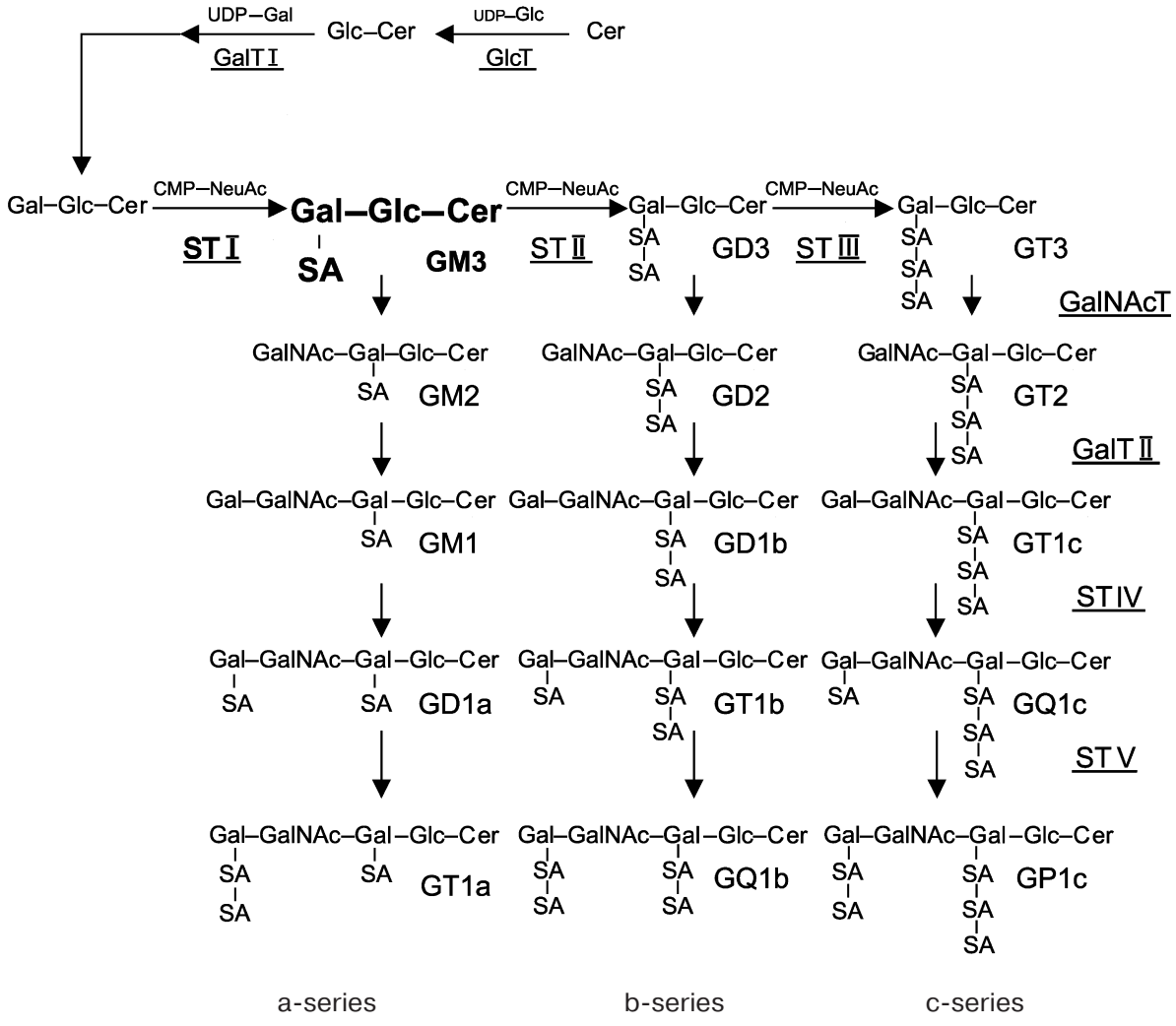
Successful cloning of the gene encoding GM3 synthase makes it possible to prepare the recombinant enzyme both as full sequence and fragments. Recently, polyclonal antibodies to a cloned fragment of human GM3 synthase were obtained in our laboratory to provide for studies on the location of GM3 synthase in human aortal intima in atherosclerosis. The amino acid sequence of this fragment is minimally like the amino acid sequences of other sialyl transferases [40], and polyclonal antibodies to such recombinant protein can recognize GM3 synthase among other sialyl transferases, which in addition to specific amino acid sequences contain highly conservative regions.

Studies on genomic structure of human GM3 synthase gene and on functioning of its promoters are neces-



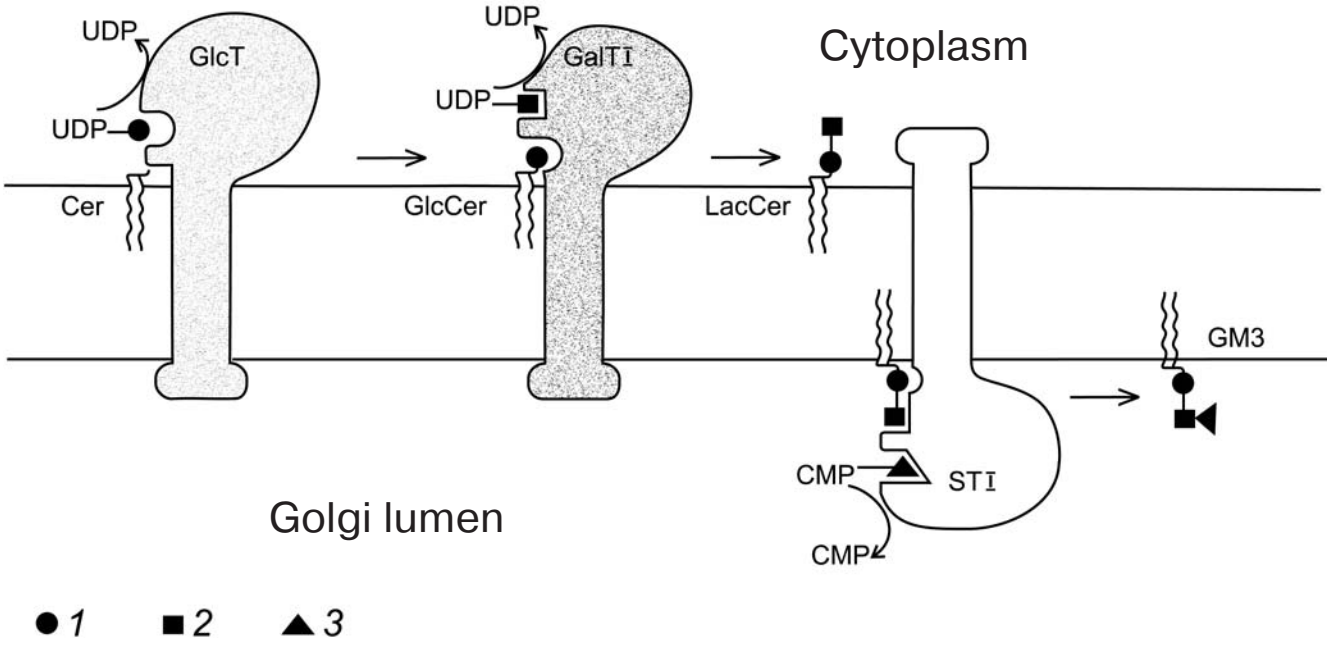
Structure of ganglioside GM3 (a); functional groups of ceramide, which determine the conformation of the GM3 molecule in a membrane (b)

Scheme 1



Biosynthesis of a-, b-, and c-series of gangliosides. Cer, ceramide; SA, sialic acid; ST I, GM3 synthase; ST II, GD3 synthase; ST III, GT3 synthase; ST IV, GD1a synthase; ST V, GT1a synthase; GlcT, GlcCer synthase; GalT I, LacCer synthase; GalT II, GM1 synthase; GalNAcT, N-acetylgalactosylaminyl transferase, GM2 synthase

Scheme 2



Model of the initial glycosylation in Golgi apparatus: *1-3*) glucose, galactose, and sialic acid, respectively

Scheme 3

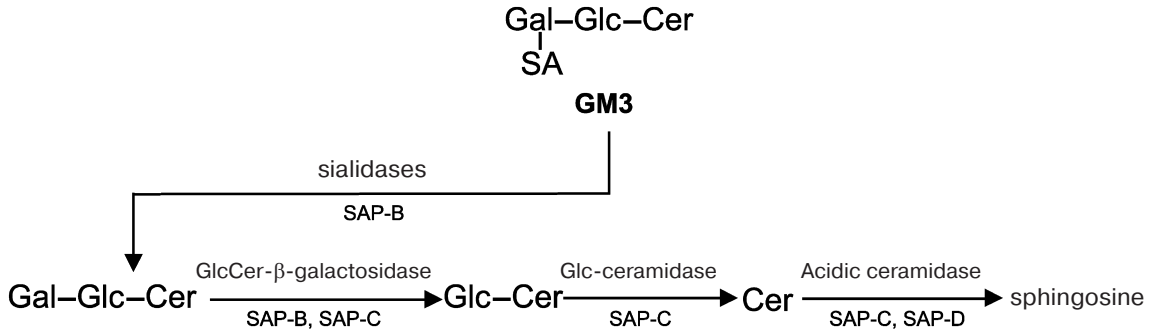
sary for understanding mechanisms regulating GM3 contents in tissues and cells. The gene encoding human GM3 synthase is located on the second chromosome and consists of nine exons containing the coding region in exons 4-9 [41]. No conventional transcriptional repeats have been found in the nucleotide sequence of the 5′-flanking region [41]. Functional analysis of the 5′-flanking region by the transient express method revealed that the −177 to −83 region from the transcription initiating site functions as the core promoter essential for transcriptional activation of the GM3 synthase gene in cells of human neuroblastoma and hepatoma. In HL60 cells, the −143 to −83 region contains CREB binding site in position −143 and functions as phorbol ester inducible promoter. Thus, an increase in the GM3 synthase expression induced by phorbol ester and the resulting increase in the GM3 content in the cell are mediated by CREB binding with the promoter region of the gene [42, 43].

Several signal pathways (PKA, PKC, stress-activated protein kinase/c-Jun N-terminal kinase, and p38 MAPK) can activate CREB binding to CRE on DNA and regulate the transcription phosphorylation at Ser133 residue [44]. Thus, not only phorbol ester can influence on expression of the GM3 synthase gene, but also other more physiological effectors.

The 5′-flanking fragment of the human GM3 synthase gene has also been shown to contain binding sites for eight transcription factors [45]. The 5′-flanking fragment of the mouse gene DNA was found to possess regulatory elements containing binding sites for Sp-1 and AP2 transcription factors, which enhance the basal activity of the GM3 synthase gene promoter [46].

GM3 synthase knockout mice were recently obtained [47, 48]. These mice had no disorders in vitally important functions. This might be because GM2 but not GM3 is the main ganglioside in all mouse tissues except nervous tissue [49]. Nevertheless, these mice manifested an increased sensitivity to insulin [47]. Simpson et al. [50] identified an autosomal recessive infantile-onset symptomatic epilepsy syndrome associated with mutation in SIAT9, which resulted in the termination of the GM3 synthase enzyme. Biochemical analysis of plasma glycosphingolipids confirmed that affected individuals lack GM3 and its biosynthetic derivatives but show increase in lactosylceramide.

Intracellular location of GM3 synthase is a factor regulating synthesis of GM3 and higher gangliosides (Scheme 3) [51]. There is now no doubt that the location of GM3 synthase is specific for the majority of cells and tissues and can envelop both endoplasmic reticulum and the Golgi apparatus [52]. This must be taken into account because subcellular location of glycosyl transferases determines the direction of ganglioside synthesis in various tissues. Thus, based on studies of two GM3 synthase isoforms and on literature data on location of the GM3 synthesis, Berselli et al. [39] proposed that a short isoform of GM3 synthase located in the "proximal" (*cis/medial/trans*) Golgi apparatus should be mainly involved in synthesis of simple gangliosides GM3 and GD3. A longer isoform of GM3 synthase located in the



Degradation of GM3 ganglioside. Cleavage of GM3 to lactosylceramide and sialic acid by lysosomal membrane sialidase is stimulated by SAP-B; the subsequent hydrolysis of galactose by galactosylceramide β-galactosidase or GM1 β-galactosidase needs the presence of SAP-B or -C. Finally, a stepwise hydrolysis of the hydrophilic group from GM3 leads to ceramide which is cleaved in the presence of SAP-D to sphingosine and fatty acid

Scheme 4

late Golgi apparatus (distal, *trans*-net) is mainly involved in synthesis from GM3 of more complex gangliosides including GD1a, CT1a, GT1b, and GQ1b. The lactosylceramide transformation into GM3 in the late Golgi apparatus was also shown in another work [53].

The NH_2-terminal domains of glycosyl transferases are involved in formation of multienzyme complexes of GM3 synthase with GD3, GM2, and GD2 sialyl transferases [54]. The location of the glycosyl transferase complexes depends on relative activities of the enzymes involved in formation of such complexes. Thus, down-regulation of GD3 synthase by a specific antisense RNA in CHO-K1 cells resulted in changes in sublocation of this multienzyme complex in the Golgi apparatus and in increase in sialylation of lactosylceramide. Thus, topology of glycolipid synthesis is adapted to functional state of the cells.

Degradation. The first step of ganglioside degradation is the hydrolysis of sialic acids, which is catalyzed by sialidases of lysosomal and plasma membranes (Neu3) [55-58]. Substrates of these sialidases are gangliosides with a sialylated terminal galactose residue of both lacto- and ganglio-series and also gangliosides with polysialic chains, whereas gangliosides GM1 and GM2 with a sialic acid residue at the carbohydrate chain branching are not hydrolyzed [57]. Neu3 plays an important role in regulation of cell surface functions through modulation of ganglioside contents in the plasma membrane [25, 59, 60].

Sialidase of lysosomal membranes is distinct from Neu3 [55]. This enzyme hydrolyzes sialic acid from the terminal galactose residue and also from that located at the carbohydrate chain branching of gangliosides and glycoproteins [57]. Just this sialidase is responsible for ganglioside metabolism in acidic compartments of mammalian cells such as endosomes and lysosomes. These compartments also contain the whole set of lysosomal glycosidases that successively remove sugar residues from the non-reducing end of glycolipid substrates [25] (Scheme 4). Ganglioside transport from plasma membrane into lysosomes (endocytosis vesicular flow) is an important stage of ganglioside degradation. Glycosphingolipids become a moiety of lysosomal membranes, and their degradation requires the presence of glycoprotein cofactors, the so-called sphingolipid-activating proteins, or saposins (SAP).

ORGANIZATION OF GM3 IN THE CELL MEMBRANE

The great variety of biological functions of gangliosides is provided by their physical properties and functional association with signal-transducing molecules (receptor and non-receptor tyrosine kinases, cellular antigens, receptors, adhesion molecules) in lipid ganglioside-enriched microdomains (GEM), which are also termed rafts. These small and very dynamic structures are cholesterol–sphingolipid–protein complexes responsible for signal transduction, membrane transport, cell adhesion, etc. [61]. Specific properties of the ganglioside structure are favorable for formation of segregated ganglioside domains in the phospholipid bilayer (Scheme 1b). The carbohydrate moiety of the ganglioside molecule occupies large areas on the plane of the membrane outer leaflet, whereas predominantly saturated acyl chains interact with acyl chains of phospholipids and occupy spaces between cholesterol and phospholipids. Thus, gangliosides can play an active and paramount role in formation and/or stabilization of lipid membrane domains in cells [62, 63]. In particular, molecules of GM3 and other gangliosides are distributed as clusters on the surface of human peripheral blood lymphocytes. This has been shown by immunoelectron microscopy using highly specific antibodies to gangliosides [64].

Due to clustering, gangliosides in mixtures with phospholipids and cholesterol within artificial membranes are insoluble in aqueous solutions of detergents [65]. Experimental data confirm the crucial role of cholesterol in formation of lipid clusters resistant to detergents. Interaction of cholesterol with fatty acid chains of phospholipids and gangliosides promotes their dense packing and makes their mixture insoluble in detergents. This allows researchers to isolate clusters and analyze their composition. Thus, treatment with a detergent of hamster fibroblasts (BHK cells) detached from a glass surface resulted in preparation of a detergent insoluble GM3-enriched ganglioside fraction, or the so-called detergent-insoluble material (DIM) which contain glycosyl phosphatidylinositol (GPI)-anchored proteins, the Src-family, and other types of protein kinases. The composition of this fraction was different from the composition of caveolae. Immunoprecipitation with antibodies to GM3 resulted in co-precipitation of GM3 with CD4, Src, RhoA, and FAK of T-lymphocytes and melanoma B16 cells. These examples indicate that GM3 clusters are related with receptors and signaling molecules [64]. Note that GM3 is the main ganglioside in monocytes and lymphocytes [66].

Activation of T-lymphocytes by CD28 engagement and some other antigens is associated with clustering of membranes and redistribution of kinase-enriched rafts. This process leads to more complete and stable tyrosine phosphorylation of several substrates and consumption of Lck kinases. At the time, immunoelectron microscopy using gold-labeled monoclonal antibodies to GM3 has shown the presence of GM3-enriched domains on plasma membranes of the $CD4^+$- and $CD8^+$-subpopulations of T-lymphocytes [67, 68]. DIM isolated from lymphocytes contains all CD4 of the cell and a 20-fold higher amount of GM3 than the total cell lysate [69]. Moreover, formation of the CD4 and $p56^{lck}$ complex in the GM3-enriched domains is necessary for redistribution of such kinases, as $p56^{lck}$ and PI3K, and also of the antigen-1 related with lymphocyte function (LFA) [70-72]. A tight association was also found in membranes of T-lymphocytes between GM3 and Zap-70 kinases of the Syk-family [73] and caspase-8 [74].

In plasma membrane of NS20Y-cells GM3 is co-located with a neurotrophic factor prosaposin. Additional experiments revealed that this complex was located in DIM and was a component of a prosaposin receptor involved in cell differentiation [75].

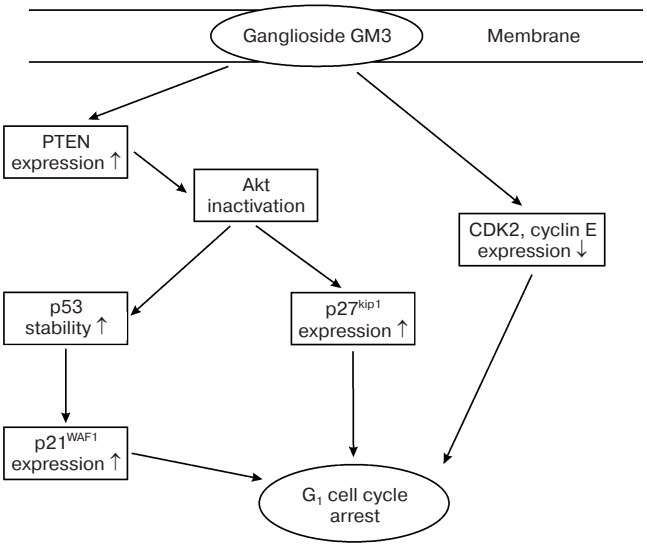
It was shown by scanning confocal microscopy of T-lymphoblasts that binding of the HIV gp120 protein recruited CXCR4 (fusin) into GM3-enriched microdomains of plasma membranes. This suggests that GM3 and chemokine receptors CXCR4 are components of a multi-molecular fusion complex critical for HIV-1 entry [76].

Note that the main function of GEM in signal transduction is to concentrate receptors (to ensure their interaction with ligands) and signaling molecules on the membrane to maximally use the effect of the ligand–receptor binding during the signal transduction and to prevent undesirable "talks" between different signaling pathways.

BIOLOGICAL FUNCTIONS OF GM3

As mentioned above, GM3 is involved in regulation of various processes including cell proliferation and differentiation, apoptosis, embryogenesis, oncogenesis, etc.

GM3 in cell proliferation and oncogenesis. The finding of the involvement of GM3 in control of cell population growth was an important contribution to investigation of the functional role of this ganglioside. GM3 and to a lesser extent GM1, but not GD1a or globoside, influence stimulation of cell proliferation by fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), strongly decreasing tyrosine phosphorylation of their receptors [77]. Comparative studies of glycosphingolipid composition of actively proliferating and contact inhibited cells indicated that biosynthesis of GM3 sharply increased on the stage of cell contact formation [78-80]. The cell loss of sensitivity to contact inhibition as a result of multiple *in vitro* passages or upon viral transformation



Mechanism of inhibition of colorectal cancer cell proliferation by GM3 ganglioside. Ganglioside GM3 inhibits proliferation of the tumor cells due to increasing (↑) or decreasing (↓) expression of factors regulating the cell cycle. The ganglioside GM3 significantly increases the expression of PTEN, which modulates tumor cell survival through inhibition of PI3K/PKB/Akt pathway and enhancement of p53 protein stability. The GM3-caused induction of p53 leads to transcriptional activation of the *p21WAF1* gene. The PTEN-caused inhibition of the signal transduction via PI3K/PKB/Akt induces $p27^{kip1}$ expression

Scheme 5

was associated with an increase in their content of lactosylceramide and in a twofold decrease in the GM3 level, whereas galactosyl lactosylceramide disappeared. Moreover, as a result of Neu3 activation, GM3 is rapidly catabolized to lactosylceramide on the plasma membrane of actively proliferating fibroblasts, and this abolishes the inhibiting effect of GM3 on the tyrosine kinase activity of epidermal growth factor (EGF) and triggers pre-replicative mechanisms [81]. On formation of a cell monolayer, the GM3 ganglioside catabolism seems to be retarded. This leads to its accumulation, inhibition of the EGF mitogenic effect, and, as a result, suppression of cell growth.

GM3 can also influence proliferation by another mechanism: this ganglioside can inhibit the cell population growth by prolonging the G_1-phase of the cell cycle in fibroblast cultures (3T3 and NIL) [82-84] (Scheme 5).

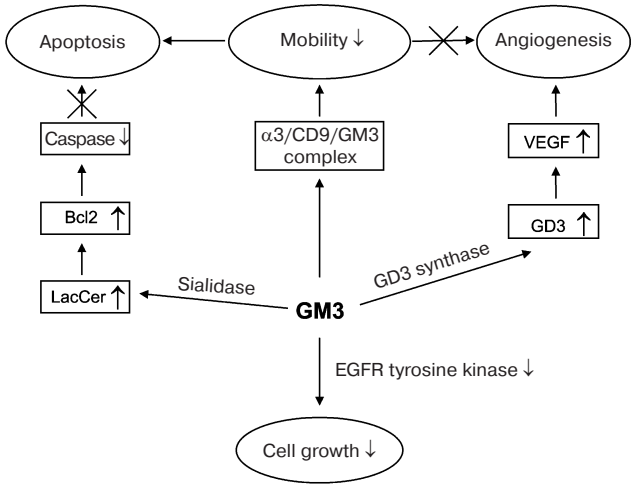
Data on the ability of GM3 to inhibit the growth of tumor cells and tumor development are presented in the works [80, 85-96]; this ability is associated with a fundamental property of this ganglioside to suppress tyrosine phosphorylation of growth factor receptors in membranes of tumor cells (Table 2 and Scheme 6).

Increased expression of the EGF receptor is specific for many tumors [97]. GM3 disrupted dimerization and disturbed autophosphorylation of EGF receptor, which is required for its activation and transduction of the intracellular signal in cells of human epidermoid carcinoma [98]. GM3, caveolin-1, and tetraspanin CD82 in GEM of these cells enable formation of a complex between EGF receptor and PKC_α which phosphorylated the EGF receptor at Thr654 with resulting receptor internalization. Destruction of the membrane by methyl-β-cyclodextrin leads to dissociation of the EGF receptor/GM3/caveolin/CD82/PKC_α complex and prevents the inhibitory effect of PKC_α on the EGF receptor phosphorylation. This observation indicates an interaction between caveolin-1, CD82, and the ganglioside with EGF receptor and PKC_α within undestroyed cholesterol-enriched GEM.

EGF receptor can be activated not only due to binding with the ligand but also through interaction (“talks”)

Table 2. GM3 in oncogenesis

Tumor type	Mode of changing GM3 cell content	Effect on tumor	References
KB and A431 human epidermoid carcinoma cells	exogenous addition	suppression of proliferation	[80, 85, 86]
Human glioblastoma cells	the same	the same	[87]
Rat 9L gliosarcoma cells challenged into mice	–"–	increase in symptom-free survival of mice	[87]
Primary human brain tumors ependymomas combined gliomas astrocytomas oligodendrogliomas gangliogliomas	–"–	suppression of proliferation	[87, 88]
Human urinary bladder cancer cells (T24 and KK-47)	–"–	decrease in invasive potential	[89]
The SCC12 human epidermoid carcinoma cells	increase in GM3 degradation by transfecting with cDNA of neuraminidase Neu3	activation of EGFR-induced proliferation	[90, 91]
The same	increase in GM3 content by inhibition of higher ganglioside synthesis by treatment with antisense oligodeoxynucleotides	activation of urokinase-caused proliferation	[91]
Meningeal gliomatosis of rats	intrathecal addition	increase in animals’ lifespan	[88]
Cells of urinary bladder cancer	cell lines with high and low GM3 content, treatment of the cells with PPPP, exogenous addition	decrease in cell mobility, invasiveness, and metastasizing	[92-94]



Involvement of GM3 in cell growth, mobility, and angiogenesis. These processes are positively (↑) or negatively (↓) regulated through changes in GM3 content

Scheme 6

with other signaling pathway components, e.g. integrins. The integrin-dependent activation of EGF receptor contributes to regulation of cell proliferation, survival, migration, and invasiveness. The accumulation of GM3 in carcinoma cells caused by their treatment with antisense oligonucleotides preventing synthesis of higher gangliosides disturbed the alternative activation of the EGF receptor induced by the cross-interaction with β1-integrin. A decrease in GM3 content by transfection of the cells with cDNA of Neu3 enhanced the EGF receptor association with β1-integrin phosphorylated on tyrosine and prompted cell proliferation. Cell adhesion to the matrix induces formation of macromolecular complexes of EGF receptor, β1-integrin, Src kinase, an adapter protein, and the transmembrane protein caveolin-1 acting as a scaffolding protein on aggregation of growth factor receptors. This complex can be isolated within DIM. Upon increase in the GM3 content in the cell, caveolin-1 exits DIM, and this disturbs the phosphorylation and activation of receptors, and, as a result, the transduction of adhesion and proliferation signals [90, 97].

The same authors have recently reported that, by contrast, the ganglioside GM3 in the presence of urokinase increased proliferation of carcinoma cells via activation of kinase, which phosphorylated serine and threonine residues of the urokinase receptor with an intermediate phosphorylation of PI3K and PKC_ζ and their activation (Scheme 7) [91].

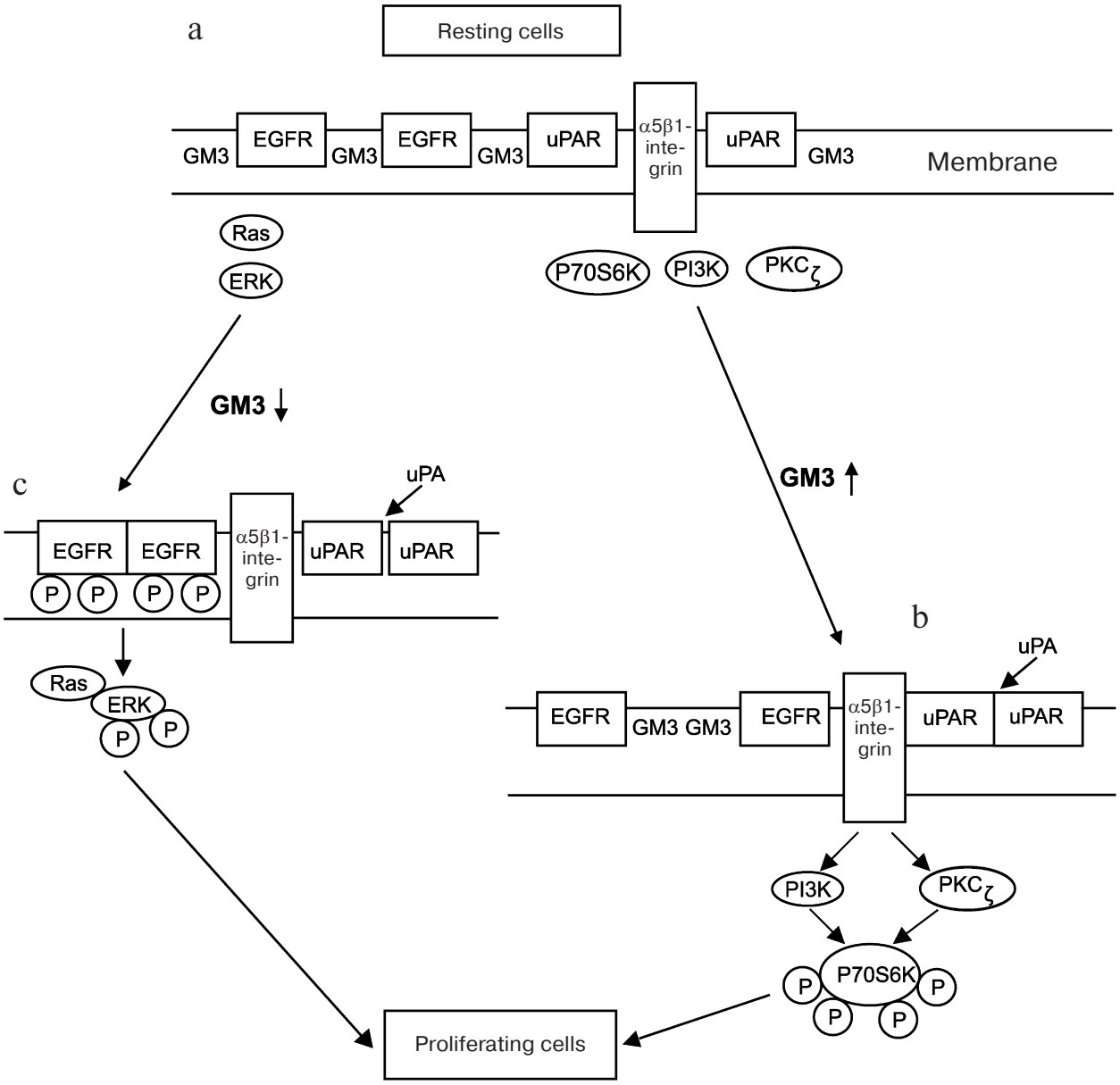
GM3 also suppressed the activation of G-protein-coupled receptors, which activate phosphoinositide-specific phospholipase C. In a culture of rat glioma cells, the effect of endothelin-1 inversely depended on the GM3 content, and this suggested that GM3 acted as a physiological modulator of signal transduction from endothelin-1 in the glial cells [99].

Gangliosides play important roles in metastasizing and angiogenesis of tumors [100] (Scheme 6). GM3 inhibits invasiveness of tumor cells by promoting the interaction of integrin with anti-metastatic membrane proteins CD9 and CD82 and greatly enhancing the cell adhesion to the intercellular matrix along with their loss of invasiveness [101, 102]. Addition of exogenous GM3 lowered the invasive potential of urinary bladder tumors. Suppression of GM3 synthesis by treatment of the cells with D-1-threo-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol (PPPP, an inhibitor of glucosylceramide synthesis) enhanced the cell mobility and invasiveness.

Membrane domains of large intestine and urinary bladder cancer cells contain α3-integrin/CD9/GM3 complexes, which strengthen the cell adhesion to the extracellular matrix (Scheme 6). On the contrary, complexes of CD9 with complex gangliosides pronouncedly increase mobility and invasiveness.

Fragments of surface membranes shed by tumor cells influence the adjacent endothelial cells of the tumor carrier's microvessels and can induce generation of new vessels, or angiogenesis. Gangliosides are present in the shed membrane vesicles and can influence cell proliferation and angiogenesis induced by angiogenic factors [103]. The growth of endothelial cells can be modulated depending on the relative contents of GM3 and complex gangliosides in the cell microenvironment [104, 105]. Addition of GM3 into the cultural medium inhibited proliferation of endothelial cells, and this inhibition was abolished after the cells were transferred into a GM3-free medium. However, a mixture of GM3, GD3, and GM2 could either induce or suppress the growth of endothelium depending on the ratio of these gangliosides. In the presence of GM3 but not of complex gangliosides the ability of endothelial cells to bind fibronectin and I and IV type collagens, which is necessary for cell proliferation, was sharply decreased. Generation of new vessels in the rabbit eye cornea stimulated by prostaglandin E1 and FGF could be modulated or even blocked by changing the GM3 and GD3 ratio [105].

In cultured human umbilical vein endothelial cells, GM3 suppressed phosphorylation of VEGF receptor and of PKB/Akt involved in signal transduction from this receptor [106, 107]. GM3 also effectively inhibited the pro-angiogenic *in vivo* effect of VEGF in a subcutaneous implant of matrigel and suppressed the VEGF-induced *in vitro* migration of human umbilical vein endothelial cells. Suppression of GM3 biosynthesis by an inhibitor of glucosyl transferase increased cell proliferation and phosphorylation of VEGF receptor and of PKB/Akt. The effect of the inhibitor was reversible: exogenous introduction of GM3 re-suppressed the growth of vessels in the matrigel. The authors concluded that GM3 could be pos-



Hypothetical mechanism of GM3-dependent cell proliferation triggered by urokinase (uPA). The urokinase receptor (uPAR) is bound with α5β1-integrins, and this allows uPA to induce cross-activation of the signaling pathway through EGF receptor (tyrosine kinase) and stimulate cell proliferation via the ERK-dependent (c) and ERK-independent (b) pathways. The cells can proliferate independently of the increase (b) or decrease (c) in their content of the ganglioside GM3. But the proliferation mechanisms are different. As discriminated from resting cells (a), increase in GM3 content (b) is associated with activation in the cells of PI3K, PKC, and p70S6K, and cell proliferation begins in spite of suppression of the ERK-mediated signaling pathway. The cleavage of GM3 and decrease in its content in cells (c) is associated with activation of the ERK-mediated signaling pathway, which also results in production of proliferating cell phenotype

Scheme 7

sibly used as a therapeutic agent for down-regulation of tumor angiogenesis.

After introduction into mouse brain, experimental cancer EPEN cells, which mainly express GM3 formed a poorly vascularized solid tumor with intensive necrosis [108]. And these cells transfected with cDNA of N-acetylgalactosaminyl transferase (the key enzyme of synthesis of complex gangliosides) expressed GM2, GM1, and GD1a and formed richly vascularized solid tumors with less pronounced necrosis. These results indicate once more that the ratio of GM3 to complex gangliosides determines their influence on tumor growth and angiogenesis.

Gabri et al. [109, 110] have described a vaccine against melanoma, which was prepared by hydrophobic

conjugation of GM3 with a complex of external membrane proteins from *Neisseria meningitides* (GM3/VSSP). Preimmunization inhibited tumor formation and increased the lifetime in mice challenged with melanoma B16 cells expressing GM3. The vaccine did not influence the lifetime of mice challenged with mammary gland carcinoma F3II cells not expressing GM3. Preclinical data suggest a possibility of using this vaccine in patients with melanoma to induce antitumor immunity.

GM3 is involved in the regulation of expression of tumor necrosis factor-α (TNF-α), which is a multifunctional cytokine with a well-established role in immunity modulation and inflammation and a known inducer of insulin resistance [111] and apoptosis [112, 113] (Table 3 [114-121]). Expression of this cytokine in adipocytes and melanoma B16 cells changed in parallel with changes in GM3 content [113, 114]. Thus, the GM3 content increased twofold and the expression of TNF-α mRNA increased threefold in melanoma cells stably transfected with LacCer synthase cDNA. On the contrary, the down-regulation of LacCer synthase (by transfection with antisense cDNA) in these cells manifested a decrease in the expression of TNF-α mRNA concurrently with a decrease in GM3 content (Scheme 6). An increase in GM3 level by addition of the ganglioside into the culture medium of B16 cells and transfected cells of the mouse melanoma pronouncedly increased the expression of TNF-α, whereas suppression of GM3 synthesis in these cells by inhibitor of glucosylceramide synthesis, D-1-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), decreased expression of this cytokine. Inhibition of GM3 synthase in melanoma B16 cells with small interfering RNAs also lowered both GM3 content and expression of TNF-α mRNA. GM3 increased phosphorylation of PI3K/PKB/Akt in both B16 cells and transfected melanoma cells, whereas suppression of PKB/Akt expression by a specific inhibitor affected the regulatory effect of GM3 on TNF-α expression. Thus, the influence of GM3 on TNF-α expression is mediated by its action on signal transduction via protein kinase PI3K/PKB/Akt cell survival kinase (Table 3) [117, 122].

Similar results were obtained when human renal carcinoma cell line ACNH mainly containing GM3 were transfected with cDNA of Neu3 or treated by IL-6. This cytokine of inflammatory and immune processes activated Neu3, decreased the GM3 content along with increase in the lactosylceramide content, and thus prevented apoptosis and increased the cell mobility (Scheme 6) [123, 124].

Due to its influence on apoptosis, GM3 plays an important role in development of the nervous system in higher animals [118, 119] (Table 3). Moreover, it was recently found that in plasma membranes of human fibroblasts GM3 could be cleaved by Neu3 and other glycohydrolases to ceramide, which is the main mediator of apoptosis [124]. Thus, the role of GM3 in apoptotic processes is not univalent: on one hand, it suppresses effect of apoptotic cytokines, and on the other hand, it causes apoptosis due to inhibition of cell proliferation.

Cell adhesion. It is now established that adhesion of melanoma B16 cells on endothelial cells is caused by a specific interaction between GM3 expressed on the melanoma cells and lactosylceramide on the endothelial cell surface [125]. The authors of this work found that the adhesion degree to the endothelial cells or to lactosylceramide-coated plates was different for four types of B16 cells, depended on the GM3 expression level on their surface, and, possibly, was a mechanism of formation of metastatic foci by the melanoma cells.

A mutant cell line with a high content of GM3 was prepared from parental cells of mouse mammary carcinoma with a high content of lactosylceramide but no GM3 [126]. In contrast to the parent line cells, the mutant cells showed clear adhesion to fibronectin, although both types of the cells express the same quantity of fibronectin receptors. During incubation in a GM3-containing medium, adhesiveness of the parental cells markedly increased, and this suggested a role of GM3 in functioning of integrin receptors. This ability of GM3 is crucial for cell adhesion, as interaction of cytoskeleton with extracellular matrix proteins fibronectin and laminin through integrin receptors occurs during the first stages of adhesion and is terminated by formation of focal adhesion with involvement of kinases, among which focal adhesion kinase (FAK) plays the main role, and by transduction of the cell adhesion signal through the kinase cascade.

Subfractions of DIM differing from caveolea and enriched with GM3 and signal transduction mediators (c-Src, RhoA, and FAK) were isolated from melanoma cells [127, 128]. As the cells were capable of adhesion and signal transduction from the integrin receptor after destruction of the caveolea by filipin or nistatin, and because incubation of the melanoma cells with synthetic GM3 analogs destroying microdomains decreased adhesiveness of the tumor cells and suppressed the FAK and c-Src activities, the authors concluded that the adhesion occurs at via GM3-enriched DIM [129].

Adhesion of artificial membranes containing GM3, sphingomyelin, and c-Src to Petri dishes coated with asialo-GM2 or antibodies to GM3 resulted in the GM3-dependent phosphorylation of c-Src, which was not observed in the absence of GM3 or its replacement by other gangliosides [130]. Based on these studies, GM3 was concluded to interact with growth factor receptors coupled with integrins and also with tetraspanins modulating their functions and promoting changes in cell adhesion and mobility [131]. Moreover, GM3 within GEM realizes cell adhesion via carbohydrate–carbohydrate or lectin-like interactions resulting in activation or inhibition of signal transduction. The authors consider the carbohydrate–carbohydrate adhesion of GM3-

Table 3. Influence of GM3 on expression and effects of cytokines and on apoptosis

Object	Treatment	Biochemical effect	Physiological effect	References
Mouse adipocytes	tTNF-α	increase in GM3 content, decrease in insulin receptor expression	insulin resistance	[114]
	PPPP + TNF-α	decrease in GM3 expression, increase in insulin receptor expression	absence of insulin resistance	
Adipose tissue of diabetic Zucker fa/fa rats and ob/ob mice		increase in GM3 synthase mRNA expression, increase in TNF-α content	obesity, insulin resistance	[114]
Proliferating glomerular mesangial cells of diabetic rats	high level of glucose, PDMP, TGF-β1	decrease in GM3 content, decrease in K_m of GM3 synthase	proliferation	[115, 116]
	high level of glucose or TGF-β1 + exogenous GM3		decrease in proliferation	[116]
Monocyte-derived human dendritic cells	exogenous GM3 and GD3	suppression of CD1a, CD54, CD80, and CD40 expression	suppression of differentiation to dendritic cells, decrease in survival, and induction of apoptosis	[117]
Nerve cells-precursors	intraventricular administration of GM3		decrease in proliferation, induction of apoptosis	[118]
Cell line HT22 of mouse hippocampus	exogenous glutamate	increase in GM3 content	induction of apoptosis	[119]
	exogenous GM3	the same	the same	[119]
	transfection of cells with GM3 synthase cDNA	overexpression of GM3 synthase	–"–	[119]
Zebra fish embryo	microinjection of GM3 synthase mRNA	overexpression of GM3 synthase, inhibition of production of reactive oxygen species and of entrance of extracellular calcium into cells	apoptosis of nerve cells	[119]
	RNA-interference	decrease in GM3 synthase expression	prevention of glutamate-induced apoptosis of nerve cells	[119]
GM3-deficient lines of tumor cells (3LL Lewis lung carcinoma cell line)	transfection of cells with GM3 synthase cDNA ("GM3-reconstruction" of cells)	synthesis of GM3, suppression of activities of caspase -3 and caspase-9, increase in content of Bcl-2	prevention of apoptotic effect of chemotherapeutic agents	[120, 121]

expressing cells, so-called glycosynapses, as a self-dependent type of adhesion, which occurs in synergy with carbohydrate–protein or protein–protein adhesion types [132].

Thus, data on the role of GM3 in cell adhesion indicate that this ganglioside can increase interaction of cells with one another and with the extracellular matrix, and this is consistent with data on the involvement of GM3 in suppression of cell mobility, invasiveness, and apoptosis.

Cell differentiation. A tumor mitogen and classical activator of PKC 12-O-tetradecanoylphorbol-13-acetate (phorbol ester) induced differentiation of human polypotent leukemic cells (HL60, K562, U937, etc.) to monocyte/macrophage phenotype, and this differentiation was associated by a tenfold increase in the GM3 content and activation of GM3 synthase [133-139]. Recently, Kim et al. [42] showed that GM3 synthase gene has promoter region complementary to the nuclear factor CREB,

which in the HL60 cells was activated downstream from PKC. Thus, phorbol ester activated PKC that finally increased GM3 synthesis [42].

The involvement of GM3 in monocyte/macrophage differentiation is proved by some findings:

– first, GM3 itself can induce monocyte/macrophage differentiation of HL60 cells, and accumulation of GM3 in the plasma membrane leads to changes in such features of the cell surface that are essential for differentiation, e.g. adhesiveness, decrease in the sensitivity to growth factors, or activation of cytokine receptors [134, 140];

– second, the differentiation to a granulocyte type caused by treatment with dimethyl sulfoxide or retinoic acid leads to accumulation of sialosylparagloboside [141]. This ganglioside as in the case of GM3 was able to initiate HL60 cell differentiation in to granulocyte phenotype when it was added into the culture medium;

– and, finally, Zeng et al. [142] found that differentiation of HL60 cells to monocyte/macrophage phenotype could be obtained by regulation of ganglioside synthesis to increase the GM3 content using antisense oligodeoxynucleotides to sequences of GM2 and GD3 synthases for down-regulation of the more complex ganglioside synthesis.

Differentiation of human peripheral blood monocytes to macrophages is associated with an increase in activities of sialidases which desialylate protein and lipid substrates (Neu1 and Neu3, respectively) [143]. Thus, it seems that the sialylation degree of monocyte-derived macrophages is controlled by differentiation factors.

The differentiation to the monocyte/macrophage phenotype is now shown to be associated with an increase in the GM3 content in monocytes differentiating to macrophages under conditions close to physiological ones, i.e. in the absence of mitogens such as phorbol ester, etc. [144]. Moreover, such differentiation processes seem to occur also within atherosclerotic lesions of human vessels, which display a significantly higher accumulation of GM3 than normal tissues and an increased expression of GM3 synthase in resident macrophages generated from blood monocytes [12, 145].

Thus, GM3, which is the main ganglioside in the majority of mammalian cells, is involved in such processes as embryogenesis, differentiation, cell adhesion, mobility, apoptosis, and cell death. Being a precursor of all types of higher cellular gangliosides, GM3 contributes to their functioning because their expression depends on intensity of GM3 synthesis. Therefore, the great attention given during recent years to studies on metabolism of this simplest ganglioside is not surprising. However, the answers to many questions are still unclear, especially in the field of causal–consequence relationships between changes in biosynthesis and degradation and the cell processes associated with these changes. Even partial answers to these questions will be an important contribution to understanding of mechanisms of the above-mentioned biological processes.

This work is financially supported by the Russian Foundation for Basic Research (grant No. 06-04-48277).

REFERENCES

1. Svennerholm, L. (1980) in *Structure and Function of Gangliosides* (Svennerholm, L., ed.) Plenum Press, New York-London, pp. 533-540.
2. Seyfried, T. N., Ando, S., and Yu, R. K. (1978) *J. Lipid Res.*, **19**, 538-543.
3. Nilsson, O., and Svennerholm, L. (1982) *J. Lipid Res.*, **23**, 327-334.
4. Rauvala, H. (1976) *Biochim. Biophys. Acta*, **424**, 284-295.
5. Vanier, V. T. (1983) *Biochim. Biophys. Acta*, **750**, 178-184.
6. Dyatlovitskaya, E. V., Kryukova, E. V., Mikhalev, I. I., Somova, O. G., Kogtev, L. S., and Bergelson, L. D. (1987) *Biokhimiya*, **7**, 1226-1229.
7. Muthing, J., and Cacic, M. (1997) *Glycoconj. J.*, **14**, 19-28.
8. Cacic, M., Muthing, J., Kracun, I., Neumann, U., and Weber-Schurholz, S. (1994) *Glycoconj. J.*, **11**, 477-485.
9. Dawson, G., Matalon, R., and Dorfman, A. (1972) *J. Biol. Chem.*, **247**, 5944-5950.
10. Komleva, A. V., Prokazova, N. V., Gabrielyan, N. D., Mukhin, D. N., Kashnikova, L. N., Zvezdina, N. D., Martinova, L. N., and Panasyuk, A. F. (1995) *Clin. Exp. Rheum.*, **13**, 581-588.
11. Ohashi, M. (1979) *Lipids*, **14**, 52-57.
12. Bobryshev, Y. V., Lord, R. S., Golovanova, N. K., Gracheva, E. V., Zvezdina, N. D., Sadovskaya, V. L., and Prokazova, N. V. (1997) *Biochim. Biophys. Acta*, **1361**, 287-294.
13. Prokazova, N. V., Orekhov, A. N., Mukhin, D. N., Mikhailenko, I. A., Kogtev, L. S., Sadovskaya, V. L., Golovanova, N. K., and Bergelson, L. D. (1987) *Eur. J. Biochem.*, **167**, 349-352.
14. Negroni, E., Chigorno, V., Tettamanti, G., and Sonnino, S. (1996) *Glycoconj. J.*, **13**, 347-352.
15. Watanabe, K., Powell, M. E., and Hakomori, S. I. (1979) *J. Biol. Chem.*, **254**, 8223-8229.
16. Ferroni, P., Lenti, L., Martini, F., Ciatti, F., Pontieri, G. M., and Gazzaniga, P. P. (1997) *Thromb. Haemost.*, **77**, 548-554.
17. Martini, F., Riondino, S., Pignatelli, P., Gazzaniga, P. P., Ferroni, P., and Lenti, L. (2002) *Biochim. Biophys. Acta*, **1583**, 297-304.
18. Kiguchi, K., Henning-Chubb, C. B., and Huberman, E. (1990) *J. Biochem. (Tokyo)*, **107**, 8-14.
19. Svennerholm, L. (1985) *Biochim. Biophys. Acta*, **835**, 231-235.
20. Ariga, T., Ando, S., Takahashi, A., and Miyatake, T. (1980) *Biochim. Biophys. Acta*, **618**, 480-485.
21. Bode, L., Beermann, C., Mank, M., Kohn, G., and Boehm, G. (2004) *J. Nutr.*, **134**, 3016-3020.
22. Pan, X., and Izumi, T. (2000) *Early Hum. Dev.*, **57**, 25-31.
23. Urban, P. F., Harth, S., Freysz, L., and Dreyfus, H. (1980) *Adv. Exp. Med. Biol.*, **125**, 149-157.
24. Yu, R. K. (1994) *Progr. Brain Res.*, **101**, 31-44.

25. Sandhoff, K., and Kolter, T. (2003) *Philos. Trans. Roy. Soc. Lond. B*, **358**, 847-861.
26. Holthuis, J. C. M., Pomorski, T., Raggers, R. J., Sprong, H., and van Meer, G. (2001) *Physiol. Rev.*, **81**, 1689-1723.
27. Kolter, T., Proia, R. L., and Sandhoff, K. (2002) *J. Biol. Chem.*, **277**, 25859-25862.
28. Harduin-Lepers, A., Mollicone, R., Delannoy, P., and Oriol, R. (2005) *Glycobiology*, **15**, 805-817.
29. Lloyd, K. O., and Furukawa, K. (1998) *Glycoconj. J.*, **15**, 627-636.
30. Melkerson-Watson, L. J., and Sweeley, C. C. (1991) *J. Biol. Chem.*, **266**, 4448-4457.
31. Preuss, U., Gu, X., Gu, T., and Yu, R. K. (1993) *J. Biol. Chem.*, **268**, 26273-26278.
32. Swamy, M. J., and Sweeley, C. C. (1989) *Biochem. Biophys. Res. Commun.*, **162**, 1188-1193.
33. Gracheva, E. V., Samovilova, N. N., Golovanova, N. K., Andreeva, E. P., Andrianova, I. V., and Prokazova, N. V. (2007) *Biochemistry (Moscow)*, **72**, 772-777.
34. Kono, M., Takashima, S., Liu, H., Inoue, M., Kojima, N., Young-Choon, L., Hamamoto, T., and Tsuji, S. (1998) *Biochem. Biophys. Res. Commun.*, **253**, 170-175.
35. Ishii, A., Ohta, M., Watanabe, Y., Matsuda, K., Ishiyama, K., Sakoe, K., Nakamura, M., Inokuchi, J., Sanai, Y., and Saito, M. (1998) *J. Biol. Chem.*, **273**, 31652-31655.
36. Kapitonov, D., Bieberich, E., and Yu, R. K. (1999) *Glycoconj. J.*, **16**, 337-350.
37. Fukumoto, S., Miyazaki, H., Goto, G., Urano, T., Furukawa, K., and Furukawa, K. (1999) *J. Biol. Chem.*, **274**, 9271-9276.
38. Stern, C. A., Braverman, T. R., and Tiemeyer, M. (2000) *Glycobiology*, **10**, 365-374.
39. Berselli, P., Zava, S., Sottocornola, E., Milani, S., Berra, B., and Colombo, I. (2006) *Biochim. Biophys. Acta*, **1759**, 348-358.
40. Golovanova, N. K., Samovilova, N. N., Gracheva, E. V., Peklo, M. M., Vlasik, T. N., Sobolev, A. Yu., Yurchenko, Yu. V., and Prokazova, N. V. (2004) *Biochemistry (Moscow)*, **69**, 275-280.
41. Kim, K. W., Kim, S. W., Min, K. S., Kim, C. H., and Lee, Y. C. (2001) *Gene*, **273**, 163-171.
42. Kim, S. W., Lee, S. H., Kim, K. S., Kim, C. H., Choo, Y. K., and Lee, Y. C. (2002) *Biochim. Biophys. Acta*, **1578**, 84-89.
43. Choi, H. J., Chung, T. W., Kang, N. Y., Kim, K. S., Lee, Y. C., and Kim, C. H. (2003) *FEBS Lett.*, **555**, 204-208.
44. Chung, T. W., Choi, H. J., Lee, Y. C., and Kim, C. H. (2005) *Glycobiology*, **15**, 233-244.
45. Zeng, G., Gao, L., Xia, T., Tencomnao, T., and Yu, R. K. (2003) *Biochim. Biophys. Acta*, **1625**, 30-35.
46. Xia, T., Zeng, G., Gao, L., and Yu, R. K. (2005) *Gene*, **351**, 109-118.
47. Yamashita, T., Hashiramoto, A., Haluzik, M., Mizukami, H., Beck, S., Norton, A., Kono, M., Tsuji, S., Daniotti, J. L., Werth, N., Sandhoff, R., Sandhoff, K., and Proia, R. L. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 3445-3449.
48. Shevchuk, N. A., Hathout, Y., Epifano, O., Su, Y., Liu, Y., Sutherland, M., and Ladisch, S. (2007) *Biochim. Biophys. Acta*, **1771**, 1226-1234.
49. Shaposhnikova, G. I., Prokazova, N. V., Buznikov, G. A., Zvezdina, N. D., Teplitz, N. A., and Bergelson, L. D. (1984) *Eur. J. Biochem.*, **140**, 567-570.
50. Simpson, M. A., Cross, H., Proukakis, C., Priestman, D. A., Neville, D. C., Reinkensmeier, G., Wang, H., Wiznitzer, M., Gurtz, K., Verganelaki, A., Pryde, A., Patton, M. A., Dwek, R. A., Butters, T. D., Platt, F. M., and Crosby, A. H. (2004) *Nat. Genet.*, **36**, 1225-1229.
51. Maccioni, H. J., Daniotti, J. L., and Martina, J. A. (1999) *Biochim. Biophys. Acta*, **1437**, 101-118.
52. Yu, R. K., Bieberich, E., Xia, T., and Zeng, G. (2004) *J. Lipid Res.*, **45**, 783-793.
53. Allende, M. L., Li, J., Darling, D. S., Worth, C. A., and Young, W. W. (2000) *Glycobiology*, **10**, 1025-1032.
54. Uliana, A. S., Crespo, P. M., Martina, J. A., Daniotti, J. L., and Maccioni, H. J. (2006) *J. Biol. Chem.*, **281**, 32852-32860.
55. Miyagi, T., Wada, T., Yamaguchi, K., and Hata, K. (2004) *Glycoconj. J.*, **20**, 189-198.
56. Ha, K. T., Lee, Y. C., Cho, S. H., Kim, J. K., and Kim, C. H. (2004) *Mol. Cells*, **17**, 267-273.
57. Miyagi, T., Wada, T., Iwamatsu, A., Hata, K., Yoshikawa, Y., Tokuyama, S., and Sawada, M. (1999) *J. Biol. Chem.*, **274**, 5004-5011.
58. Hasegawa, T., Yamaguchi, K., Wada, T., Takeda, A., Itoyama, Y., and Miyagi, T. (2000) *J. Biol. Chem.*, **275**, 8007-8015.
59. Wang, Y., Yamaguchi, K., Wada, T., Hata, K., Zhao, X., Fujimoto, T., and Miyagi, T. (2002) *J. Biol. Chem.*, **277**, 26252-26259.
60. Kalka, D., von Reitzenstein, C., Kopitz, J., and Cantz, M. (2001) *Biochem. Biophys. Res. Commun.*, **283**, 989-993.
61. Hooper, N. M. (1998) *Curr. Biol.*, **8**, R114-R116.
62. Sonnino, S., Mauri, L., Chigorno, V., and Prinetti, A. (2006) *Glycobiology*, **18**, 1698-2663.
63. Tettamanti, G., Masserini, M., Giuliani, A., and Pagani, A. (1988) *Ann. Ist. Super. Sanita*, **24**, 23-31.
64. Sorice, M., Longo, A., Garofalo, T., Mattei, V., Misasi, R., and Pavan, A. (2004) *Glycoconj. J.*, **20**, 63-70.
65. Hakomori, S. I. (2000) *Glycoconj. J.*, **17**, 143-151.
66. Kiguchi, K., Henning-Chubb, C. B., and Huberman, E. (1990) *J. Biochem. (Tokyo)*, **107**, 8-14.
67. Offner, H., Thieme, T., and Vandenbark, A. A. (1987) *J. Immunol.*, **139**, 3295-3305.
68. Sorice, M., Pavan, A., Misasi, R., Sansolini, T., Garofalo, T., Lenti, L., Pontieri, G. M., Frati, L., and Torrisi, M. R. (1995) *Scand. J. Immunol.*, **41**, 148-156.
69. Sorice, M., Parolini, I., Sansolini, T., Garofalo, T., Dolo, V., Sargiacomo, M., Tai, T., Reschle, C., Torrisi, M. R., and Pavan, A. (1997) *J. Lipid Res.*, **38**, 969-980.
70. Garofalo, T., Sorice, M., Misasi, R., Cinque, B., Giammatteo, M., Pontieri, G. M., Cifone, M. G., and Pavan, A. (1998) *J. Biol. Chem.*, **273**, 35153-35160.
71. Garofalo, T., Sorice, M., Misasi, R., Cinque, B., Mattei, V., Pontieri, G. M., Cifone, M. G., and Pavan, A. (2002) *J. Lipid Res.*, **43**, 971-978.
72. Barbat, C., Trucy, M., Sorice, M., Garofalo, T., Manganelli, V., Fischer, A., and Mazerolles, F. (2007) *Biochem. J.*, **402**, 471-481.
73. Garofalo, T., Lenti, L., Longo, A., Misasi, R., Mattei, V., Pontieri, G. M., Pavan, A., and Sorice, M. (2002) *J. Biol. Chem.*, **277**, 11233-11238.
74. Garofalo, T., Misasi, R., Mattei, V., Giammarioli, A. M., Malorni, W., Pontieri, G. M., Pavan, A., and Sorice, M. (2003) *J. Biol. Chem.*, **278**, 8309-8315.

75. Misasi, R., Sorice, M., Garofalo, T., Griggi, T., and Campana, W. M. (1998) *J. Neurochem.*, **71**, 2313-2321.
76. Sorice, M., Garofalo, T., Misasi, R., Longo, A., Mattei, V., Sale, P., Dolo, V., Gradini, R., and Pavan, A. (2001) *FEBS Lett.*, **506**, 55-60.
77. Bremer, E. G., Schlessinger, J., and Hakomori, S. (1986) *J. Biol. Chem.*, **261**, 2434-2440.
78. Hakomori, S. (1970) *Proc. Natl. Acad. Sci. USA*, **67**, 1741-1747.
79. Hakomori, S. (1981) *Annu. Rev. Biochem.*, **50**, 733-764.
80. Hakomori, S. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 10231-10233.
81. Usuki, S., Lyu, S. C., and Sweeley, C. C. (1988) *J. Biol. Chem.*, **263**, 6847-6853.
82. Laine, R. A., and Hakomori, S. (1973) *Biochem. Biophys. Res. Commun.*, **54**, 1039-1045.
83. Choi, H. J., Chung, T. W., Kang, S. K., Lee, Y. C., Ko, J. H., Kim, J. G., and Kim, C. H. (2006) *Glycobiology*, **16**, 573-583.
84. Uemura, S., Feng, F., Kume, M., Yamada, K., Kabayama, K., Nishimura, S., Igarashi, Y., and Inokuchi, J. (2007) *Glycobiology*, **17**, 568-577.
85. Song, W. X., Vacca, M. F., Welti, R., and Rintoul, D. A. (1990) *J. Biol. Chem.*, **265**, 12059-12066.
86. Rebbaa, A., Hurh, J., Yamamoto, H., Kersey, D. S., and Bremer, E. G. (1996) *Glycobiology*, **6**, 399-406.
87. Noll, E. N., Lin, J., Nakatsuji, Y., Miller, R. H., and Black, P. M. (2001) *Exp. Neurol.*, **168**, 300-309.
88. Fujimoto, Y., Izumoto, S., Suzuki, T., Kinoshita, M., Kagawa, N., Wada, K., Hashimoto, N., Maruno, M., Nakatsuji, Y., and Yoshiminem, T. (2005) *Neurooncol.*, **71**, 99-106.
89. Kawamura, S., Ohyama, C., Watanabe, R., Satoh, M., Saito, S., Hoshi, S., Gasa, S., and Orikasa, S. (2001) *Int. J. Cancer*, **94**, 343-347.
90. Wang, X. Q., Sun, P., and Paller, A. S. (2003) *J. Biol. Chem.*, **278**, 48770-48778.
91. Wang, X. Q., Sun, P., Go, L., Koti, V., Fliman, M., and Paller, A. S. (2006) *J. Invest. Dermatol.*, **6**, 2687-2696.
92. Mitsuzuka, K., Handa, K., Satoh, M., Arai, Y., and Hakomori, S. (2005) *J. Biol. Chem.*, **280**, 35545-35553.
93. Satoh, M., Ito A., Nojiri, H., Handa, K., Numahata, K., Ohyama, C., Saito, S., Hoshi, S., and Hakomori, S. I. (2001) *Int. J. Oncol.*, **19**, 723-731.
94. Watanabe, R., Ohyama, C., Aoki, H., Takahashi, T., Satoh, M., Saito, S., Hoshi, S., Ishii, A., Saito, M., and Arai, Y. (2002) *Cancer Res.*, **62**, 3850-3854.
95. Zebda, N., Pedron, S., Rebbaa, A., Portoukalian, J., and Berthier-Vergnes, O. (1995) *FEBS Lett.*, **362**, 161-164.
96. Hanai, N., Nores, G. A., MacLeod, C., Torres-Mendez, C. R., and Hakomori, S. (1988) *J. Biol. Chem.*, **263**, 10915-10921.
97. Yoon, S.-J., Nakayama, K., Hikita, T., Handa, K., and Hakomori, S. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 18987-18991.
98. Wang, X. Q., Yan, Q., Sun, P., Liu, J. W., Go, L., McDaniel, S. M., and Paller, A. S. (2007) *Cancer Res.*, **67**, 9986-9995.
99. Bassi, R., Viani, P., Giussani, P., Riboni, L., and Tettamanti, G. (2001) *FEBS Lett.*, **507**, 101-104.
100. Dyatlovitskaya, E. V., and Kandyba, A. G. (2006) *Biochemistry (Moscow)*, **71**, 347-353.
101. Kawakami, Y., Kawakami, K., Steelant, W. F., Ono, M., Baek, R. C., Handa, K., Withers, D. A., and Hakomori, S. (2002) *J. Biol. Chem.*, **277**, 34349-34358.
102. Ono, M., Handa, K., Sonnino, S., Withers, D. A., Nagai, H., and Hakomori, S. (2001) *Biochemistry*, **40**, 6414-6421.
103. Alessandri, G., Cornaglia-Ferraris, P., and Gullino, P. M. (1997) *Acta Oncol.*, **36**, 383-387.
104. Alessandri, G., de Cristan, G., Ziche, M., Cappa, A. P., and Gullino, P. M. (1992) *J. Cell Physiol.*, **151**, 23-28.
105. Ziche, M., Morbidelli, L., Alessandri, G., and Gullino, P. M. (1992) *Lab. Invest.*, **67**, 711-715.
106. Manfredi, M. G., Lim, S., Claffey, K. P., and Seyfried, T. N. (1999) *Cancer Res.*, **59**, 5392-5397.
107. Mukherjee, P., Faber, A. C., Shelton, L. M., Baek, R. C., Chiles, T. C., and Seyfried, T. N. (2008) *J. Lipid Res.*, **49**, 929-938.
108. Abate, L. E., Mukherjee, P., and Seyfried, T. N. (2006) *J. Neurochem.*, **98**, 1973-1984.
109. Gabri, M. R., Ripoll, G. V., Alonso, D. F., and Gomez, D. E. (2002) *J. Cancer Res. Clin. Oncol.*, **128**, 669-677.
110. Gabri, M. R., Mazorra, Z., Ripoll, G. V., Mesa, C., Fernandez, L. E., Gomez, D. E., and Alonso, D. F. (2006) *Clin. Cancer Res.*, **12**, 7092-7098.
111. Ryden, M., and Arner, P. (2007) *J. Intern. Med.*, **262**, 431-438.
112. Nagata, S. (1996) *Adv. Exp. Med. Biol.*, **406**, 119-124.
113. Nachbur, U., Kassahn, D., Yousefi, S., Legler, D. F., and Brunner, T. (2006) *Blood*, **107**, 2790-2796.
114. Inokuchi, J. (2006) *Biol. Pharm. Bull.*, **29**, 1532-1537.
115. Rho, Y. I., Kwak, D. H., Lee, S., Ahn, S. H., Baek, S. H., Song, J. H., Choo, Y. K., Choi, B. K., and Jung, K. Y. (2004) *Life Sci.*, **75**, 51-62.
116. Kwak, D. H., Lee, S., Kim, S. J., Ahn, S. H., Song, J. H., Choo, Y. K., Choi, B. K., and Jung, K. Y. (2005) *Life Sci.*, **77**, 2540-2551.
117. Peguet-Navarro, J., Sportouch, M., Popa, I., Berthier, O., Schmitt, D., and Portoukalian, J. (2003) *J. Immunol.*, **170**, 3488-3494.
118. Nakatsuji, Y., and Miller, R. H. (2001) *Exp. Neurol.*, **168**, 290-299.
119. Sohn, H., Kim, Y. S., Kim, H. T., Kim, C.-H., Cho, E. W., Kang, H. Y., Kim, N. S., Kim, C.-H., Ryu, S. E., Lee, J. H., and Ko, J. H. (2006) *FASEB J.*, **20**, 1248-1250.
120. Azuma, Y., Sato, H., Higai, K., and Matsumoto, K. (2007) *Biol. Pharm. Bull.*, **30**, 1680-1684.
121. Noguchi, M., Kabayama, K., Uemura, S., Kang, B. W., Saito, M., Igarashi, Y., and Inokuchi, J. (2006) *Glycobiology*, **16**, 641-650.
122. Wang, P., Wu, P., Zhang, J., Sato, T., Yamagata, S., and Yamagata, T. (2007) *Biochem. Biophys. Res. Commun.*, **356**, 438-443.
123. Ueno, S., Saito, S., Wada, T., Yamaguchi, K., Satoh, M., Arai, Y., and Miyagi, T. (2006) *J. Biol. Chem.*, **281**, 7756-7764.
124. Valaperta, R., Chigorno, V., Basso, L., Prinetti, A., Bresciani, R., Preti, A., Miyagi, T., and Sonnino, S. (2006) *FASEB J.*, **20**, 1227-1229.
125. Kojima, N., Shiota, M., Sadahira, Y., Handa, K., and Hakomori, S. (1992) *J. Biol. Chem.*, **267**, 17264-17270.
126. Zheng, M., Fang, H., Tsuruoka, T., Tsuji, T., Sasaki, T., and Hakomori, S. (1993) *J. Biol. Chem.*, **268**, 2217-2222.
127. Kasahara, K., and Sanai, Y. (2000) *Glycoconj. J.*, **17**, 153-162.

128. Iwabuchi, K., Handa, K., and Hakomori, S. (1998) *J. Biol. Chem.*, **273**, 33766-33773.
129. Zhang, Y., Iwabuchi, K., Nunomura, S., and Hakomori, S. (2000) *Biochemistry*, **39**, 2459-2468.
130. Iwabuchi, K., Zhang, Y., Handa, K., Withers, D. A., Sinay, P., and Hakomori, S. (2000) *J. Biol. Chem.*, **275**, 15174-15181.
131. Hakomori, S. (2000) *Glycoconj. J.*, **17**, 627-647.
132. Hakomori, S. (2004) *Glycoconj. J.*, **21**, 125-137.
133. Momoi, T., and Yokota, J. (1983) *J. Natl. Cancer Inst.*, **70**, 229-236.
134. Nojiri, H., Takaku, F., Terui, Y., Miura, Y., and Saito, M. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 782-786.
135. Nojiri, H., Takaku, F., Tetsuka, T., Motoyoshi, K., Miura, Y., and Saito, M. (1984) *Blood*, **64**, 534-541.
136. Momoi, T., Shinmoto, M., Kasuya, J., Senoo, H., and Suzuki, Y. (1986) *J. Biol. Chem.*, **261**, 16270-16273.
137. Saito, M. (1989) *Develop. Growth Differ.*, **31**, 509-522.
138. Xia, X. J., Gu, X. B., Sartorelli, A. C., Yu, R. K., and Sartorelli, A. C. (1989) *J. Lipid Res.*, **30**, 181-188.
139. Nakamura, M., Kirito, K., Yamanoi, J., Wainai, T., Nojiri, H., and Saito, M. (1991) *Cancer Res.*, **51**, 1940-1945.
140. Nakamura, M., Ogino, H., Nojiri, H., Kitagawa, S., and Saito, M. (1989) *Biochem. Biophys. Res. Commun.*, **161**, 782-789.
141. Nojiri, H., Kitagawa, S., Nakamura, M., Kirito, K., Enomoto, Y., and Saito, M. (1988) *J. Biol. Chem.*, **263**, 7443-7446.
142. Zeng, G., Ariga, T., Gu, X. B., and Yu, R. K. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 8670-8674.
143. Stamatos, N. M., Liang, F., Nan, X., Landry, K., Cross, A. S., Wang, L. X., and Pshezhetsky, A. V. (2005) *FEBS J.*, **272**, 2545-2556.
144. Bobryshev, Y. V., Golovanova, N. K., Tran, D., Samovilova, N. N., Gracheva, E. V., Efremov, E. E., Sobolev, A. Y., Yurchenko, Y. V., Lord, R. S., Cao, W., Lu, J., Saito, M., and Prokazova, N. V. (2006) *Atherosclerosis*, **184**, 63-71.
145. Bobryshev, Y. V., Lord, R. S., Golovanova, N. K., Gracheva, E. V., Zvezdina, N. D., and Prokazova, N. V. (2001) *Biochim. Biophys. Acta*, **1535**, 87-99.