

Review

How Do Gangliosides Regulate RTKs Signaling?

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Abstract: Gangliosides, the glycosphingolipids carrying one or several sialic acid residues, are located on the outer leaflet of the plasma membrane in glycolipid-enriched microdomains, where they interact with molecules of signal transduction pathways including receptors tyrosine kinases (RTKs). The role of gangliosides in the regulation of signal transduction has been reported in many cases and in a large number of cell types. In this review, we summarize the current knowledge on the biosynthesis of gangliosides and the mechanism by which they regulate RTKs signaling.

Keywords: receptor tyrosine kinase; gangliosides; glycosylation; cell signaling; glycolipid-enriched microdomains

1. Introduction

Gangliosides are glycosphingolipids (GSL) carrying one or several sialic acid residues. According to Svennerholm, gangliosides are classified in four series (0-, a-, b-, and c-series) due to the number of sialic acid residues linked to the lactosylceramide (LacCer) (Figure 1) [1]. Normal human tissues mainly express ‘simple’ gangliosides, from 0- and a-series, whereas ‘complex’ gangliosides from b- and c-series are essentially found in developing tissues, during embryogenesis, and mainly restricted to

the nervous system of healthy adults [2]. In humans, the expression of complex gangliosides increases under pathological conditions including neurodegenerative disorders [3], immune diseases [4], and cancers [5]. For example, G_{D3} and G_{D2} are over-expressed in neuroectoderm-derived tumors such as melanoma, neuroblastoma, and breast cancer, in which they mediate cell proliferation, migration, tumor growth, and angiogenesis [6]. Gangliosides are located on the outer layer of the plasma membrane mainly in glycolipid-enriched microdomains (GEMs), also known as lipid rafts or gangliosides-rich lipid domains. As GEMs are insoluble in detergents at 4 °C, they are also known as detergent-resistant membrane domains. Together with cholesterol, transmembrane proteins, and other glycosphingolipids, gangliosides contribute to the maintenance and dynamic of the membrane organization. Notably, ganglioside-rich lipid domains are described components of caveolae [7].

Quantitative or qualitative (i.e., changes in carbohydrate moiety) modifications of gangliosides can affect GEMs architecture and functions [8]. Amongst the membrane-bound proteins associated to GEMs, many components of signal transduction pathways were identified. The role of GEMs-associated gangliosides in the regulation of signal transduction has been repeatedly reported in a variety of cell lines [9–11]. However, the molecular mechanisms sustaining these functions are poorly known. Apprehending the structural heterogeneity and the diversity of interactions between gangliosides and the other components of GEMs should therefore lead to a better understanding of the fine regulation of signal transduction. This has been eased by recent advances in structural analysis of GEMs glycolipids and by the identification of GEMs associated molecules, as reviewed herein.

2. Biosynthesis of Gangliosides

The first step of the biosynthesis of gangliosides is the transfer of a glucose residue onto ceramide (Cer) by the UDP-Glc: ceramide β -glucosyltransferase (GlcCer synthase) encoded by the *UGCG* gene (Table 1) [12]. The next step is the conversion of the glucosylceramide (GlcCer) into lactosylceramide (LacCer), the precursor of the five series of GSL, by the UDP-Gal: GlcCer β 1,4-galactosyltransferase (LacCer synthase) [13,14]. The transfer of sialic acid residue to LacCer is then catalyzed by the specific sialyltransferases ST3Gal V (G_{M3} synthase), ST8Sia I (G_{D3} synthase) and ST8Sia V (G_{T3} synthase), all being highly specific for glycolipid substrates [15]. LacCer is the only known substrate for ST3Gal V activity [16] and a loss-of-function mutation in *ST3GAL5* gene is associated with the infantile-onset symptomatic epilepsy syndrome [17]. The G_{D3} synthase ST8Sia I is highly specific for G_{M3} as acceptor substrate [18]. However, the human enzyme was also shown to resialylate its own product G_{D3} creating a chain of 3 (G_{T3}), 4 (G_{Q3}), or 5 (G_{P3}) sialic acid residues, G_{Q3} and G_{P3} being unusual structures recently described [19,20]. The human ST8Sia V exhibits a broader activity toward gangliosides, using G_{D3} , but also G_{M1b} , G_{D1a} or G_{T1b} as acceptors [21]. LacCer, G_{M3} , G_{D3} , and G_{T3} are the precursors for 0-, a-, b-, and c-series gangliosides, respectively (Figure 1). Further, monosaccharides can be transferred in a stepwise manner by the β 1,4-N-acetyl-galactosaminyltransferase I (G_{M2}/G_{D2} synthase) [22] and the β 1,3-galactosyltransferase IV (G_{M1a}/G_{D1b} synthase) [23], both acting on the four series of gangliosides [24,25]. The terminal Gal residue of the Gal β 1-3GalNAc disaccharide can be further sialylated by ST3Gal II [26,27] and ST8Sia V [21], and the GalNAc residue can be sialylated in α 2,6-linkage by the sialyltransferases ST6GalNAc III [28] or V [29] to form α -gangliosides (Figure 1).

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

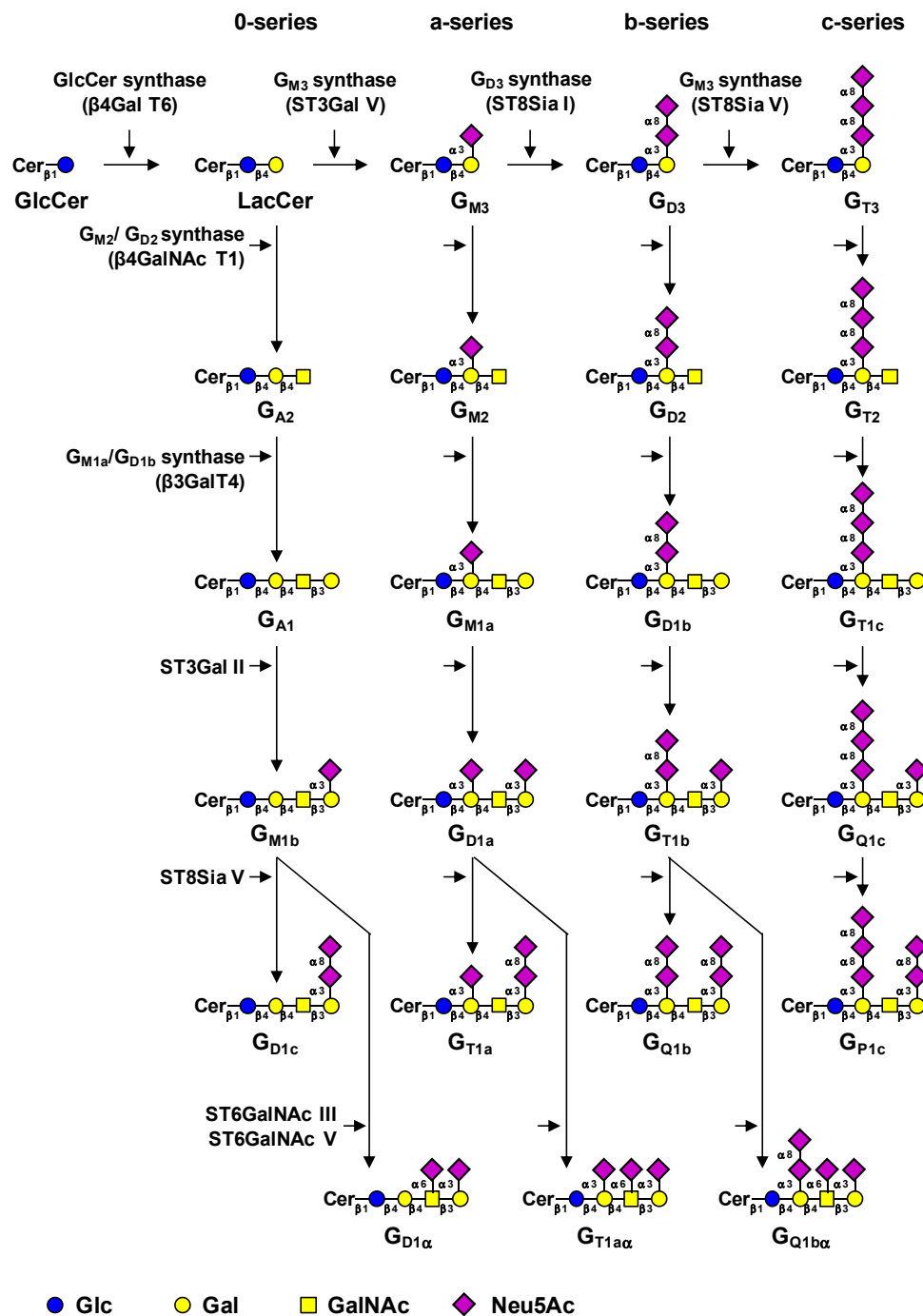
<i>Gene</i>	<i>Common name</i>	<i>Main acceptors</i>	<i>Accession #</i>	<i>Ref.</i>
UGCG	GlcCer synthase	Ceramide	NM_003358	(12)
B4GALT6	LacCer synthase	Glucosylceramide	NM_004775	(13, 14)
ST3GAL5	G _{M3} synthase	Lactosylceramide	NM_003896	(16)
ST8SIA1	G _{D3} synthase	G _{M3} , G _{D3}	NM_003034.2	(18)
ST8SIA5	G _{T3} synthase	G _{D3} , G _{M1b} , G _{D1a} , G _{T1b}	NM_013305	(21)
B4GALNACT1	G _{M2} /G _{D2} synthase	G _{A3} , G _{M3} , G _{D3} , G _{T3}	NM_001478.2	(22)
B3GALT4	G _{M1a} /G _{D1b} synthase	G _{A2} , G _{M2} , G _{D2} , G _{T2}	NM_003782.3	(23)
ST3GAL2	ST3Gal II	Galβ1-3GalNAc-R	NM_006927	(26, 27)
ST6GALNAC3	ST6GalNAc III	Neu5Acα2-3Galβ1-3GalNAc-R	NM_152996	(28)
ST6GALNAC5	ST6GalNAc V	Neu5Acα2-3Galβ1-3GalNAc-R	NM_030965.1	(29)

The first steps of gangliosides synthesis take place in the cis/median-Golgi and the later steps in the trans-Golgi and trans-Golgi network [30]. The regulation of glycosyltransferases (GT) activity is mainly achieved at the transcriptional level [31] and GT genes expression is highly tissue-specific. For example, human *B4GALNACT1* gene is essentially expressed in embryonic tissue and in adult brain, lung and testis. By contrast, *ST3GAL5* is ubiquitously expressed in human tissues [16,32,33]. GT involved in the synthesis of gangliosides can be also regulated by post translational modifications such as *N*-glycosylation, phosphorylation, and dephosphorylation. For example, protein kinases PKA and PKC can activate the G_{M2}/G_{D2} synthase while inhibiting the activity of ST3Gal II or G_{M1a}/G_{D1b} synthase [34–36].

3. Regulation of RTKs Signaling by Gangliosides

Receptor tyrosine kinases (RTKs) are key proteins involved in the control of cellular processes such as survival, proliferation, differentiation, migration and invasion. Fifty-eight RTKs have been identified in Humans. They all share a similar structural organization comprising of an extracellular domain containing the ligand-binding site, a unique transmembrane domain, and a cytoplasmic region containing the tyrosine kinase activity [37]. Usually, RTKs are activated by the binding of the ligand that induces receptor dimerization and the autophosphorylation of the intracellular domain. The role of gangliosides as modulators of signal transduction was first analyzed in the 80' by the addition of exogenous gangliosides in the medium of cultured cells [38]. However, this approach was rather limited by the unavailability of some specific gangliosides and because it not only modifies the gangliosides pattern but also increases the total amount of cell-membrane-associated gangliosides that can result in non-physiological responses [39]. From 2000, with the progress in the identification of gangliosides biosynthetic enzymes, an increasing number of papers have reported ectopic expression or antisense inhibition strategies targeting specific GT to finely analyze the role of specific gangliosides without modifying the total amount of GSLs. These different approaches have clearly demonstrated that gangliosides are fine regulators of RTKs signaling and that physio-pathological changes in cell membrane ganglioside composition result in different cellular responses [40,41] (Figure 2).

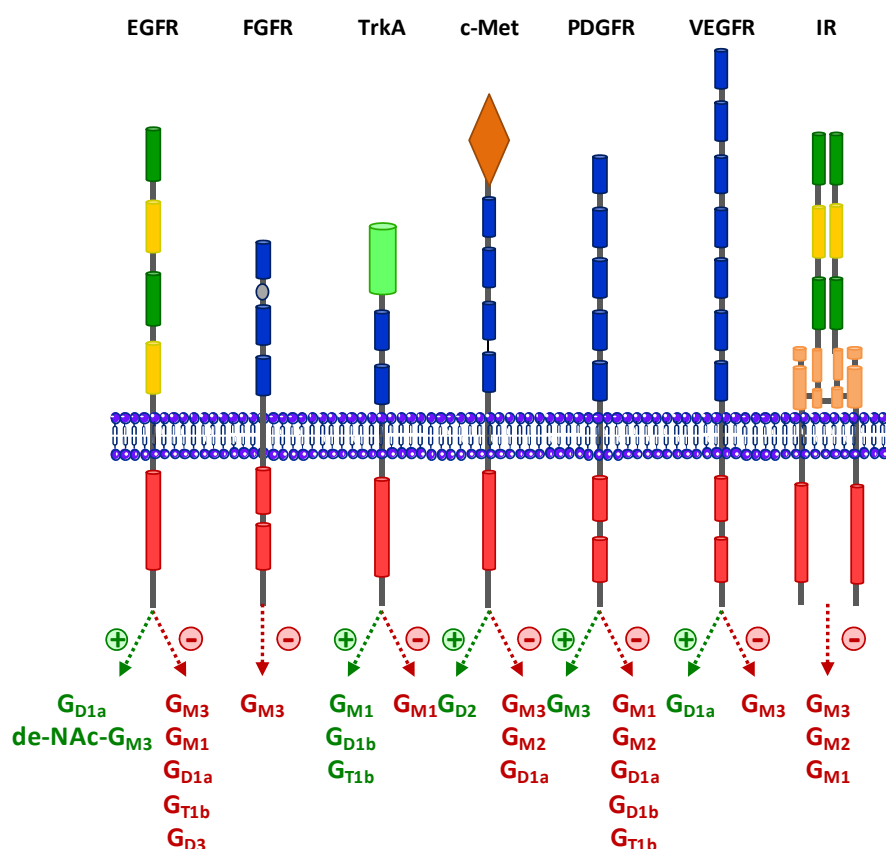
Figure 1. Biosynthesis pathway for gangliosides. Gangliosides are synthesized by the stepwise addition of monosaccharides to ceramide. The sequential action of ST3Gal V (G_{M3} synthase), ST8Sia I (G_{D3} synthase), and ST8Sia V (G_{T3} synthase) leads to the biosynthesis of the precursors of a-, b-, and c-series gangliosides, respectively. The 0-series gangliosides are directly synthesized from lactosylceramide. The code names of gangliosides are according to Svennerholm [1].



A number of growth factor receptors, including receptors for epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), hepatocyte growth factor (HGF), and insulin, were demonstrated to be regulated by gangliosides. RTKs are localized in GEMs with other lipid raft associated proteins including integrins, tetraspanins,

or plexins. Within lipid rafts, RTKs signaling can be negatively or positively regulated by gangliosides by either direct or indirect interactions [7,42]. Changes in gangliosides modify the molecular composition and the structure of glycolipid-enriched microdomains, leading to the reorganization and/or the exclusion of RTKs from GEMs [43–45]. Finally, it was also demonstrated that the crosstalk between RTKs subunits and other lipid rafts associated proteins is also regulated by gangliosides.

Figure 2. Regulation of RTKs activation by gangliosides. RTKs common structure consists in an extracellular domain containing the ligand binding site, a unique transmembrane domain and a cytoplasmic region containing the tyrosine kinase activity (in red). RTKs are activated by the binding of the ligand that induces receptor dimerization and the autophosphorylation of the intracellular domain. Gangliosides can either inhibit (red arrows) or activate (green arrows) of RTKs signaling, depending on gangliosides expression pattern, cell type, and experimental conditions. Three different mechanisms can be involved: ganglioside/ligand interactions, such as FGF/ G_{M1} interaction, the regulation of receptor dimerization as for G_{M3} with EGFR, or the regulation of RTKs activity due to the localization inside GEMs as the case for G_{M3} with Insulin receptor (IR). Adapted and updated from [7,37].



3.1. Epidermal Growth Factor Receptor (EGFR)

Several studies have shown that G_{M3} is able to bind to the extracellular domain and inhibit the kinase activity of EGFR in a variety of cell lines. The effect of gangliosides on EGF-dependent tyrosine phosphorylation of EGFR was first demonstrated in human epidermoid carcinoma cell line A431 [38].

G_{M3} added exogenously to cells in culture was shown to inhibit EGFR autophosphorylation [38,46] whereas de-N-acetyl-G_{M3} (II₃NeuNH₂LacCer) enhances serine phosphorylation independently of receptor-receptor interaction [47,48]. Similarly, depletion of G_{M3} in A431 cells by PDMP (D-threo-1-phenyl-2-decanoilamino-3-morpholino-1-propanol), which inhibits the GlcCer synthase, increased EGFR autophosphorylation upon EGF stimulation [49]. G_{M3} directly interacts with EGFR on a site distinct from the EGF-binding site [50] through direct carbohydrate-carbohydrate interactions between G_{M3} and terminal GlcNAc residues on EGFR *N*-glycans [51,52]. G_{M3} binding to EGFR is enhanced after glycosidase-treatment that exposes *N*-glycan terminal GlcNAc, whereas G_{M3} does not bind to EGFR from ManIB-knocked down cells that accumulates high mannose-type (i.e., immature form lacking terminal GlcNAc) *N*-glycans [52]. This was further confirmed using UDP-Gal 4-epimerase defective ldlID cells transfected with EGFR gene, in which high amount of terminal GlcNAc residues (that accumulate due to the lack of UDP-Gal) is correlated with an inhibitory effect of G_{M3} on EGFR [53]. G_{M3} was also shown to suppress murine hepatoma cell motility by inhibiting EGFR phosphorylation and the downstream PI3K/Akt signaling pathway [54].

More recently, it has been reported that G_{M3} and the tetraspanin tumor suppressor CD82 induce synergistic inhibition of migration Hepa1-6 cells by reducing EGFR phosphorylation [55]. By reconstituting human EGFR into proteoliposomes, it was shown that G_{M3} inhibits the structural transition from inactive EGFR to signaling EGFR dimer, by preventing the autophosphorylation of the intracellular kinase domain in response to ligand binding [56]. In parallel, stable transfection of the G_{D3} synthase in CHO-K1 cells induces cell surface expression of G_{D3} and decreases EGFR phosphorylation and Erk2 activation upon EGF stimulation [57]. Inhibition of EGFR phosphorylation and cell proliferation due to G_{M3}, G_{M1}, G_{D1a}, and G_{T1b} treatment were also reported in human neuroblastoma cells [58]. In normal human dermal fibroblasts, G_{D1a} promotes the ligand-independent EGFR dimerization and enhances EGFR-mediated activation of the mitogen-activated protein kinase (MAPK) signaling pathway [59]. Accordingly, it was also shown that EGFR phosphorylation is significantly reduced with the knockdown of ST3Gal II, the enzyme that converts G_{M1} to G_{D1a} [60].

3.2. Fibroblast Growth Factor Receptor (FGFR)

FGFR participates in many developmental, homeostatic and healing processes including neurogenesis, axon growth, differentiation, and neuronal survival [61]. The negative effect of G_{M3} on FGFR activation and tyrosine phosphorylation was first demonstrated in cultured retinal glial cells [62]. The interaction of G_{M3} with FGFR was hinted by confocal microscopy analysis in human lung embryonic fibroblast WI38, showing co-localization of G_{M3} and FGFR in the GEM fraction [63]. Moreover, G_{M3} depletion by GlcCer synthase inhibition enhances tyrosine phosphorylation of FGFR, activates PI3K/Akt pathway and increases the interactions of FGFR with $\alpha3/\alpha5/\beta1$ integrins [64]. This demonstrated that integrin-FGFR cross-talk is regulated by G_{M3} within the ganglioside-enriched microdomains.

3.3. Neurotrophins Receptors

It has been clearly demonstrated that G_{M1} ganglioside regulates neurotrophins receptors both *in vivo* and in cell cultures [65–67]. In rat pheochromocytoma PC12 cells, the addition of exogenous G_{M1} to cell culture enhances NGF/TrkA signaling and protects neuronal cells from serum deprivation-induced

apoptosis [65]. On the contrary, the over-expression of G_{M1} by the transfection of $\beta 3GalT4$ cDNA, the enzyme that converts G_{M2} in G_{M1} , inhibited NGF-induced TrkA dimerization and phosphorylation as well as the downstream pathway [68]. According to the authors, this opposite effect of G_{M1} in PC12 was due to the high concentration of G_{M1} at the plasma membrane in $\beta 3GalT4$ expressing cells that modulated membrane fluidity, impeding the NGF receptor localization within the lipid rafts [68]. In parallel, the introduction of the G_{D3} synthase gene into PC12 cells resulted in the over-expression of G_{D1b} and G_{T1b} . These gangliosides triggered a conformational change of TrkA that formed a constitutively active dimer, activating its downstream signal pathways, including Erk1/2 and PI3K/Akt, and leading to a marked enhancement of cell proliferation [69,70].

3.4. Hepatocyte Growth Factor Receptor c-Met

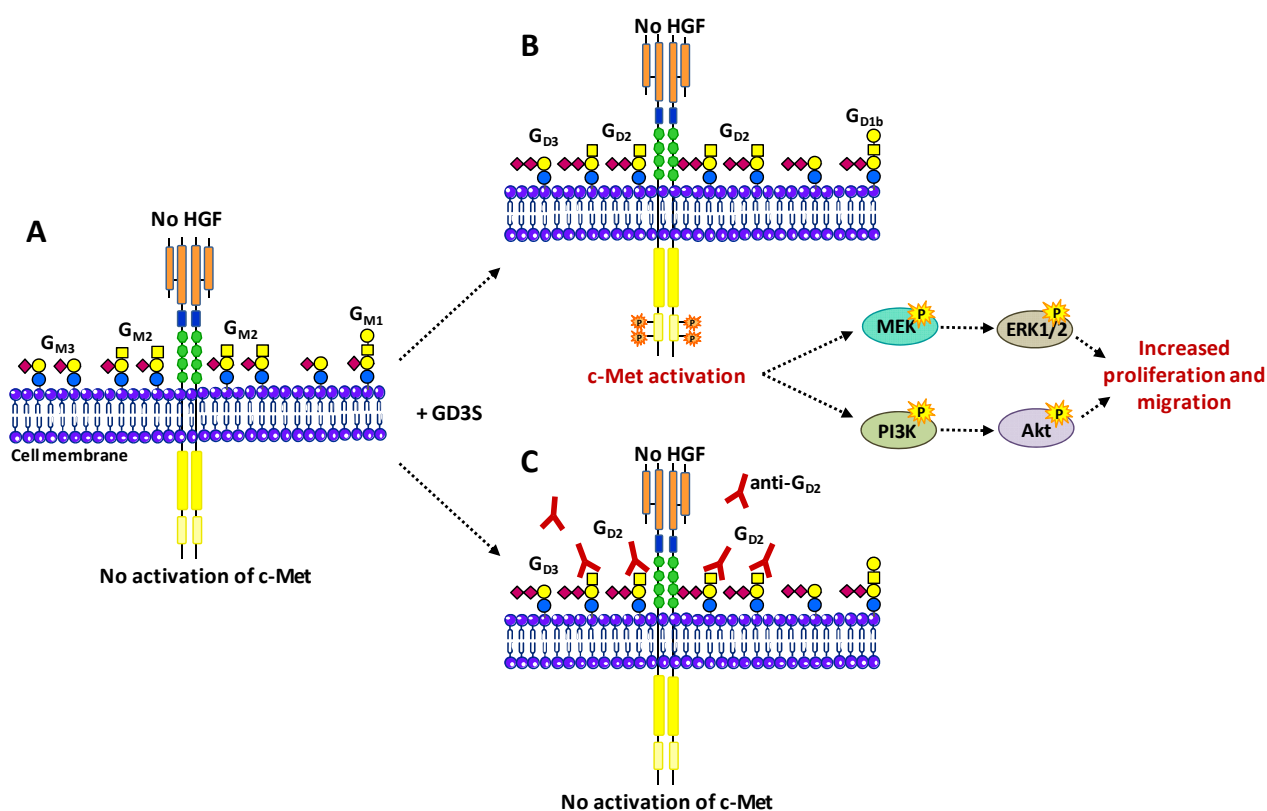
In HCV29 bladder epithelial cells, motility and growth are modulated by the expression of a-series gangliosides. In the presence of Ca^{2+} , G_{M3} , and G_{M2} form heterodimers that specifically interact with tetraspanin CD82, thus impairing the trans-phosphorylation of c-Met receptor, the recruiting of Grb2 and the activation of PI3K/Akt and MEK/Erk pathways [44,71]. Similarly, the ganglioside-dependent activation of c-Met receptor was also recently demonstrated in breast cancer cells [72]. The expression of the G_{D3} synthase in MDA-MB-231 breast cancer cells induced the cell surface accumulation of b- and c- series gangliosides including G_{D3} , G_{D2} , and G_{T3} [73,74]. Of these complex gangliosides, G_{D2} was found to be involved in the activation of c-Met, and the subsequent activation of MEK/Erk and PI3K/Akt signaling pathways, leading to enhanced cell migration and proliferation. This was shown by competition assays using anti- G_{D2} mAb that inhibited c-Met phosphorylation (Figure 3), demonstrating the role of the G_{D2} glycan moiety in c-Met activation [74]. Moreover, silencing of the G_{M2}/G_{D2} synthase ($\beta 4GalNAc$ T1) efficiently reduced both G_{D2} expression and c-Met phosphorylation. Of importance, the G_{D2} -dependent activation of c-Met occurred in the absence of HGF [72]. On the other hand, the ganglioside G_{D1a} that belongs to the a-series, was shown to inhibit HGF-induced motility and scattering of mouse osteosarcoma cell variant FBJ-LL cells through the suppression of phosphorylation of c-Met [75].

3.5. Platelet-Derived Growth Factor Receptor (PDGFR)

Various gangliosides were shown to inhibit PDGF-dependent tyrosine phosphorylation of PDGFR in several cell types including Swiss 3T3 [76], human glioma cells [77], and neuroblastoma SH-SY5Y cells [78]. Of the tested gangliosides (G_{M1} , G_{M2} , G_{M3} , G_{D1a} , G_{D1b} , G_{D3} , and G_{T1b}), only G_{M3} did not inhibit the dimerization of PDGFR [79] but could facilitate PDGF-dependent receptor activation, as an anti- G_{M3} antibody was found to inhibit PDGF receptor activation in T51B liver epithelial cells [80]. Amongst the gangliosides inhibiting PDGFR, G_{M1} was the most studied. In human glioma cells, G_{M1} treatment resulted in reduced phosphorylation of specific tyrosine residues of the cytoplasmic tail of PDGFR [81]. However, it was later shown that the cytoplasmic domain of PDGFR was not required for G_{M1} -dependent inhibition of the receptor [82]. Indeed, G_{M1} inhibition of PDGFR seems to be rather due to the exclusion of the receptor from glycolipid-enriched microdomains [83]. Recently, it was shown that the Csk binding protein PAG (Phosphoprotein Associated with Glycosphingolipid-enriched

micro-domains) [84] regulates PDGFR partitioning in caveolae and its association with SRC family protein tyrosine kinases (SFK) by controlling G_{M1} levels at the plasma membrane [85].

Figure 3. Activation of c-Met by G_{D2} ganglioside. (A) MDA-MB-231 breast cancer cells express mainly G_{M3} and G_{M2} . (B) The expression of the G_{D3} synthase induces the accumulation of b- and c-series gangliosides, mainly G_{D2} . This leads to the activation of c-Met in the absence of HGF and increases proliferation and migration through PI3K/Akt and MEK/Erk pathways. (C) Anti- G_{D2} mAb used in competition assays inhibits c-Met phosphorylation and cell proliferation [72,74].



3.6. Vascular Endothelial Growth Factor Receptor (VEGFR)

Several pieces of evidence have suggested that gangliosides also modulate tumor angiogenesis by controlling the activation of VEGF receptors FLT1 (VEGFR-1) and FLK1/KDR (VEGFR-2). It has been shown that ganglioside enrichment in human umbilical vein vascular endothelial cells (HUVEC) induces VEGFR dimerization and autophosphorylation at very low VEGF concentrations [86] and incubation of HUVEC with exogenous G_{D1a} increases VEGF-induced proliferation and migration [87]. G_{M3} is implicated in the decrease of VEGFR-2 phosphorylation and subsequent inhibition of Akt downstream signaling pathway in HUVECs [88,89]. It was also shown that G_{M3} decreases VEGF-induced VEGFR-2 activation by blocking receptor dimerization and the binding of VEGF to VEGFR-2 through a G_{M3} -specific interaction with the extracellular domain of VEGFR-2 [90]. In contrast, the elevation of the proportion of G_{M3} in CT-2A malignant mouse astrocytoma cells using G_{M2}/G_{D2} synthase shRNA reduces tumor-induced angiogenesis [91]. Moreover, the antisense inhibition of $\beta 3$ GalT4 expression in the highly angiogenic CT-2A astrocytoma cells, which mainly express G_{D1a} ,

increases G_{M3} content while reducing G_{D1a} and reduces growth, VEGF gene and protein expression, and vascularity [88]. Finally, it has been recently shown using a mass spectrometry-based approach that the soluble form of VEGFR-1 (sFLT1) binds to G_{M3} in lipid rafts on the surface of podocytes (kidney glomerular pericytes), promoting adhesion and rapid actin reorganization [92,93].

3.7. Insulin Receptor

G_{M3} has been described as a negative regulator of insulin signaling, partially responsible for insulin resistance. In 3T3-L1 adipocytes, insulin resistance induced by tumor necrosis factor (TNF) is accompanied by an increased expression of G_{M3} synthase activity and G_{M3} ganglioside [94]. The increased interaction between insulin receptor and G_{M3} leads to the dissociation of insulin receptor (IR) from caveolae [95]. Moreover, inhibition of ganglioside biosynthesis by PDMP, a specific inhibitor of the GlcCer synthase, restores insulin signaling, whereas addition of exogenous G_{M3} inhibits the IR substrate 1 (IRS-1) phosphorylation and IR signaling pathway [94,96]. Similar results were obtained with G_{M3} synthase mutant mice that show an enhanced IR phosphorylation and a heightened sensitivity to insulin [97]. In parallel, hepatic over-expression of the membrane-associated sialidase NEU3 in C57BL/6 mice reduces G_{M3} level in the liver, improving insulin sensitivity [98]. It was also demonstrated that G_{M3} interacts with a lysine residue of IR beta-subunit localized above the transmembrane domain and induces the dissociation of the IR-caveolin-1 complex, which is essential for insulin signaling [99]. Finally, G_{M1} and G_{M2} were also shown to inhibit IR phosphorylation in *in vitro* assay [100].

4. Conclusion

To conclude, it is now clear that gangliosides regulate RTKs within glycolipid-enriched microdomains either by inhibiting the dimerization and autophosphorylation of the receptors induced by specific ligands, or activating receptors signaling without ligand binding. Moreover, the activation or inhibition of RTKs is dependent on the glycan structure of gangliosides and cellular context. From a general point of view, monosialogangliosides, such as G_{M3} or G_{M1} can be considered as negative regulators of RTKs signaling whereas disialogangliosides including G_{D2} , G_{D1a} , or G_{D1b} mostly activated RTKs-mediated signal transduction. However, the molecular mechanisms by which gangliosides regulate RTKs remain poorly understood. Direct interactions between carbohydrate moiety of gangliosides and RTKs have been clearly identified as demonstrated for G_{M3} inhibition of EGFR, but direct carbohydrate-carbohydrate interactions cannot explain the different observed effects. Gangliosides regulation of RTKs also involved the reorganization of GEMs due to the change in ganglioside composition that induces the dissociation of RTKs from glycolipid-enriched microdomains, resulting in a reduced phosphorylation of the receptors as it has been demonstrated for insulin receptor. Indirect interactions with other GEMs associated transmembrane proteins including integrins and tetraspanins, can also be involved in the regulation of RTKs by gangliosides, as it has been demonstrated for c-Met receptor. In parallel, the regulation of RTKs by gangliosides is highly depending on the carbohydrate moiety of gangliosides as shown for c-Met receptor, which is activated by G_{D2} whereas G_{D3} has no effect. The fine recognition of the glycan part of gangliosides should involve membrane lectin domains, able to discriminate between subtle changes in ganglioside glycans.

The use of emergent technologies such as glycan arrays and photocrosslinking should enable to identification of such lectin domains [101,102]. Finally, changes in ganglioside composition occur in pathological conditions and are observed in a variety of cancers, mainly in neuro-ectoderm-related cancers. The understanding of the mechanisms by which gangliosides modify RTKs signaling is therefore of first importance to identify new targets in cancer therapy.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Svennerholm, L. Ganglioside designation. *Adv. Exp. Med. Biol.* **1980**, *125*, 11.
2. Yamashita, T.; Wada, R.; Sasaki, T.; Deng, C.; Bierfreund, U.; Sandhoff, K.; Proia, R.L. A vital role for glycosphingolipid synthesis during development and differentiation. *Proc. Natl. Acad. Sci. USA.* **1999**, *96*, 9142–9147.
3. Hakomori, S.I. The glycosynapse. *Proc. Natl. Acad. Sci. USA.* **2002**, *99*, 225–232.
4. Regina, T.A.; Hakomori S.I. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. *Biochim. Biophys. Acta.* **2008**, *1780*, 421–433.
5. Ariga, T.; McDonald, M.P.; Yu, R.K. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease--a review. *J. Lipid Res.* **2008**, *49*, 1157–1175.
6. Shahrizaila, N.; Yuki, N. Guillain-Barré syndrome animal model: the first proof of molecular mimicry in human autoimmune disorder. *J. Biomed. Biotechnol.* **2011**, *2011*, 829129.
7. Miljan, E.A.; Bremer, E.G. Regulation of growth factor receptors by gangliosides. *Sci. STKE.* **2002**, *2002*, re15.
8. Ohmi, Y.; Tajima, O.; Ohkawa, Y.; Mori, A.; Sugiura, Y.; Furukawa, K.; Furukawa, K. Gangliosides play pivotal roles in the regulation of complement systems and in the maintenance of integrity in nerve tissues. *Proc. Natl. Acad. Sci. USA.* **2009**, *106*, 22405–22410.
9. Birklé, S.; Zeng, G.; Gao, L.; Yu, R.K.; Aubry, J. Role of tumor-associated gangliosides in cancer progression. *Biochimie.* **2003**, *85*, 455–463.
10. Bobowski, M.; Cazet, A.; Steenackers, A.; Delannoy, P. Role of complex gangliosides in cancer progression. *Carbohydr. Chem.* **2012**, *37*, 1–20.
11. Harding, A.S.; Hancock, J.F. Using plasma membrane nanoclusters to build better signaling circuits. *Trends Cell Biol.* **2008**, *18*, 364–371.

12. Ichikawa, S.; Sakiyama, H.; Suzuki, G.; Hidari, K.I.; Hirabayashi, Y. Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of glycosphingolipid synthesis. *Proc. Natl. Acad. Sci. USA*. **1996**, *93*, 4638–4643.
13. Nomura, T.; Takizawa, M.; Aoki, J.; Arai, H.; Inoue, K.; Wakisaka, E.; Yoshizuka, N.; Imokawa, G.; Dohmae, N.; Takio, K.; *et al.* Purification, cDNA cloning, and expression of UDP-Gal: glucosylceramide beta-1,4-galactosyltransferase from rat brain. *J. Biol. Chem.* **1998**, *273*, 13570–13577.
14. Takizawa, M.; Nomura, T.; Wakisaka, E.; Yoshizuka, N.; Aoki, J.; Arai, H.; Inoue, K.; Hattori, M.; Matsuo, N. cDNA cloning and expression of human lactosylceramide synthase. *Biochim. Biophys. Acta*. **1999**, *1438*, 301–304.
15. Zeng, G.; Yu, R.K. Cloning and transcriptional regulation of genes responsible for synthesis of gangliosides. *Curr. Drug Targets*. **2008**, *9*, 317–324.
16. Ishii, A.; Ohta, M.; Watanabe, Y.; Matsuda, K.; Ishiyama, K.; Sakoe, K.; Nakamura, M.; Inokuchi, J.; Sanai, Y.; Saito, M. Expression cloning and functional characterization of human cDNA for ganglioside GM3 synthase. *J. Biol. Chem.* **1998**, *273*, 31652–31655.
17. Simpson, M.A.; Cross, H.; Proukakis, C.; Priestman, D.A.; Neville, D.C.; Reinkensmeier, G.; Wang, H.; Wiznitzer, M.; Gurtz, K.; Verganelaki, A.; *et al.* Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. *Nat. Genet.* **2004**, *36*, 1225–1229.
18. Haraguchi, M.; Yamashiro, S.; Yamamoto, A.; Furukawa, K.; Takamiya, K.; Lloyd, K.O.; Shiku, H.; Furukawa, K. Isolation of GD3 synthase gene by expression cloning of GM3 alpha-2,8-sialyltransferase cDNA using anti-GD2 monoclonal antibody. *Proc. Natl. Acad. Sci. USA*. **1994**, *91*, 10455–10459.
19. Nakayama, J.; Fukuda, M.N.; Hirabayashi, Y.; Kanamori, A.; Sasaki, K.; Nishi, T.; Fukuda, M. Expression cloning of a human GT3 synthase. GD3 and GT3 are synthesized by a single enzyme. *J. Biol. Chem.* **1996**, *271*, 3684–3691.
20. Steenackers, A.; Vanbeselaere, J.; Cazet, A.; Bobowski, M.; Rombouts, Y.; Colomb, F.; Le Bourhis, X.; Guérardel, Y.; Delannoy, P. Accumulation of unusual gangliosides G(Q3) and G(P3) in breast cancer cells expressing the G(D3) synthase. *Molecules*. **2012**, *17*, 9559–9572.
21. Kim, Y.J.; Kim, K.S.; Do, S.; Kim, C.H.; Kim, S.K.; Lee, Y.C. Molecular cloning and expression of human alpha2,8-sialyltransferase (hST8Sia V). *Biochem. Biophys. Res. Commun.* **1997**, *235*, 327–330.
22. Nagata, Y.; Yamashiro, S.; Yodoi, J.; Lloyd, K.O.; Shiku, H.; Furukawa, K. Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J. Biol. Chem.* **1992**, *267*, 12082–12089.
23. Amado, M.; Almeida, R.; Carneiro, F.; Levery, S.B.; Holmes, E.H.; Nomoto, M.; Hollingsworth, M.A.; Hassan, H.; Schwientek, T.; Nielsen, P.A.; *et al.* A family of human beta3-galactosyltransferases. Characterization of four members of a UDP-galactose: beta-N-acetylglucosamine/beta-N-acetyl-galactosamine beta-1,3-galactosyltransferase family. *J. Biol. Chem.* **1998**, *273*, 12770–12778.

24. Iber, H.; Zacharias, C.; Sandhoff, K. The c-series gangliosides GT3, GT2 and GP1c are formed in rat liver Golgi by the same set of glycosyltransferases that catalyse the biosynthesis of asialo-, a- and b-series gangliosides. *Glycobiology*. **1992**, *2*, 137–142.
25. Yamashiro, S.; Haraguchi, M.; Furukawa, K.; Takamiya, K.; Yamamoto, A.; Nagata, Y.; Lloyd, K.O.; Shiku, H.; Furukawa, K. Substrate specificity of beta 1,4-N-acetylgalactosaminyltransferase *in vitro* and in cDNA-transfected cells. GM2/GD2 synthase efficiently generates asialo-GM2 in certain cells. *J. Biol. Chem.* **1995**, *270*, 6149–6155.
26. Giordanengo, V.; Bannwarth, S.; Laffont, C.; Van Miegem, V.; Harduin-Lepers, A.; Delannoy, P.; Lefebvre, J.C. Cloning and expression of cDNA for a human Gal(beta1–3)GalNAc alpha2,3-sialyltransferase from the CEM T-cell line. *Eur. J. Biochem.* **1997**, *247*, 558–566.
27. Sturgill, E.R.; Aoki, K.; Lopez, P.H.; Colacurcio, D.; Vajn, K.; Lorenzini, I.; Majić, S.; Yang, W.H.; Heffer, M.; Tiemeyer, M.; *et al.* Biosynthesis of the major brain gangliosides GD1a and GT1b. *Glycobiology*. **2012**, *22*, 1289–1301.
28. Tsuchida, A.; Ogiso, M.; Nakamura, Y.; Kiso, M.; Furukawa, K.; Furukawa, K. Molecular cloning and expression of human ST6GalNAc III: restricted tissue distribution and substrate specificity. *J. Biochem.* **2005**, *138*, 237–243.
29. Okajima, T.; Fukumoto, S.; Ito, H.; Kiso, M.; Hirabayashi, Y.; Urano, T.; Furukawa, K. Molecular cloning of brain-specific GD1alpha synthase (ST6GalNAc V) containing CAG/Glutamine repeats. *J. Biol. Chem.* **1999**, *274*, 30557–30562.
30. Maccioni, H.J.; Daniotti, J.L.; Martina, J.A. Organization of ganglioside synthesis in the Golgi apparatus. *Biochim. Biophys. Acta*. **1999**, *1437*, 101–118.
31. Nairn, A.V.; York, W.S.; Harris, K.; Hall, E.M.; Pierce, J.M.; Moremen, K.W. Regulation of glycan structures in animal tissues: transcript profiling of glycan-related genes. *J. Biol. Chem.* **2008**, *283*, 17298–17313.
32. Hidari, J.K.; Ichikawa, S.; Furukawa, K.; Yamasaki, M.; Hirabayashi, Y. beta 1-4N-acetylgalactosaminyltransferase can synthesize both asialoglycosphingolipid GM2 and glycosphingolipid GM2 *in vitro* and *in vivo*: Isolation and characterization of a beta 1-4N-acetylgalactosaminyltransferase cDNA clone from rat ascites hepatoma cell line AH7974F. *Biochem. J.* **1994**, *303*, 957–965.
33. Fukumoto, S.; Miyazaki, H.; Goto, G.; Urano, T.; Furukawa, K.; Furukawa, K. Expression cloning of mouse cDNA of CMP-NeuAc: Lactosylceramide alpha2,3-sialyltransferase, an enzyme that imitates the synthesis of gangliosides. *J. Biol. Chem.* **1999**, *274*, 9271–9276.
34. Gu, X.; Preuss, U.; Gu, T.; Yu, R.K. Regulation of sialyltransferase activities by phosphorylation and dephosphorylation. *J. Neurochem.* **1995**, *64*, 2295–2302.
35. Bieberich, E.; Freischütz, B.; Liour, S.S.; Yu, R.K. Regulation of ganglioside metabolism by phosphorylation and dephosphorylation. *J. Neurochem.* **1998**, *71*, 972–979.
36. Yu, R.K.; Bieberich, E. Regulation of glycosyltransferases in ganglioside biosynthesis by phosphorylation and dephosphorylation. *Mol. Cell. Endocrinol.* **2001**, *177*, 19–24.
37. Lemmon, M.A.; Schlessinger, J. Cell signaling by receptor tyrosine kinases. *Cell* **2010**, *141*, 1117–1134.

38. Bremer, E.G.; Schlessinger, J.; Hakomori, S. Ganglioside-mediated modulation of cell growth. Specific effects of GM3 on tyrosine phosphorylation of the epidermal growth factor receptor. *J. Biol. Chem.* **1986**, *261*, 2434–2440.
39. Zhou, J.; Shao, H.; Cox, N.R.; Baker, H.J.; Ewald, S.J. Gangliosides enhance apoptosis of thymocytes. *Cell. Immunol.* **1998**, *183*, 90–98.
40. Bremer, E.G.; Hakomori, S.I. Gangliosides as receptor modulators. *Adv. Exp. Med. Biol.* **1984**, *174*, 381–394.
41. Hakomori, S.; Igarashi, Y. Functional role of glycosphingolipids in cell recognition and signaling. *J. Biochem.* **1995**, *118*, 1091–1103.
42. Kaucic, K.; Liu, Y.; Ladisch, S. Modulation of growth factor signaling by gangliosides: positive or negative? *Methods Enzymol.* **2006**, *417*, 168–185.
43. Lai, A.Z.; Abella, J.V.; Park, M. Crosstalk in Met receptor oncogenesis. *Trends Cell Biol.* **2009**, *19*, 542–551.
44. Park, S.Y.; Yoon, S.J.; Freire-de-Lima, L.; Kim, J.H.; Hakomori, S.I. Control of cell motility by interaction of gangliosides, tetraspanins, and epidermal growth factor receptor in A431 versus KB epidermoid tumor cells. *Carbohydr. Res.* **2009**, *344*, 1479–1486.
45. Todeschini, A.R.; Dos Santos, J.N.; Handa, K.; Hakomori, S.I. Ganglioside GM2-tetraspanin CD82 complex inhibits met and its cross-talk with integrins, providing a basis for control of cell motility through glycosynapse. *J. Biol. Chem.* **2007**, *282*, 8123–8133.
46. Rebbaa, A.; Hurh, J.; Yamamoto, H.; Kersey, D.S.; Bremer, E.G. Ganglioside GM3 inhibition of EGF receptor mediated signal transduction. *Glycobiology.* **1996**, *6*, 399–406.
47. Hanai, N.; Dohi, T.; Nores, G.A.; Hakomori, S. A novel ganglioside, de-N-acetyl-GM3 (II3NeuNH2LacCer), acting as a strong promoter for epidermal growth factor receptor kinase and as a stimulator for cell growth. *J. Biol. Chem.* **1988**, *263*, 6296–6301.
48. Zhou, Q.; Hakomori, S.; Kitamura, K.; Igarashi, Y. GM3 directly inhibits tyrosine phosphorylation and de-N-acetyl-GM3 directly enhances serine phosphorylation of epidermal growth factor receptor, independently of receptor-receptor interaction. *J. Biol. Chem.* **1994**, *269*, 1959–1965.
49. Meuillet, E.J.; Mania-Farnell, B.; George, D.; Inokuchi, J.I.; Bremer, E.G. Modulation of EGF receptor activity by changes in the GM3 content in a human epidermoid carcinoma cell line, A431. *Exp. Cell Res.* **2000**, *256*, 74–82.
50. Miljan, E.A.; Meuillet, E.J.; Mania-Farnell, B.; George, D.; Yamamoto, H.; Simon, H.G.; Bremer, E.G. Interaction of the extracellular domain of the epidermal growth factor receptor with gangliosides. *J. Biol. Chem.* **2002**, *277*, 10108–10113.
51. Yoon, S.J.; Nakayama, K.; Hikita, T.; Handa, K.; Hakomori, S.I. Epidermal growth factor receptor tyrosine kinase is modulated by GM3 interaction with N-linked GlcNAc termini of the receptor. *Proc. Natl. Acad. Sci. USA.* **2006**, *103*, 18987–18991.
52. Kawashima, N.; Yoon, S.J.; Itoh, K.; Nakayama, K. Tyrosine kinase activity of epidermal growth factor receptor is regulated by GM3 binding through carbohydrate to carbohydrate interactions. *J. Biol. Chem.* **2009**, *284*, 6147–6155.
53. Guan, F.; Handa, K.; Hakomori, S.I. Regulation of epidermal growth factor receptor through interaction of ganglioside GM3 with GlcNAc of N-linked glycan of the receptor: demonstration in IdID cells. *Neurochem. Res.* **2011**, *36*, 1645–1653.

54. Huang, X.; Li, Y.; Zhang, J.; Xu, Y.; Tian, Y.; Ma, K. Ganglioside GM3 inhibits hepatoma cell motility via down-regulating activity of EGFR and PI3K/AKT signaling pathway. *J. Cell. Biochem.* **2013**, *114*, 1616–1624.
55. Li, Y.; Huang, X.; Zhang, J.; Li, Y.; Ma, K. Synergistic inhibition of cell migration by tetraspanin CD82 and gangliosides occurs via the EGFR or cMet-activated PI3K/Akt signalling pathway. *Int. J. Biochem. Cell. Biol.* **2013**, *45*, 2349–2358.
56. Coskun, Ü.; Grzybek, M.; Drechsel, D.; Simons, K. Regulation of human EGF receptor by lipids. *Proc. Natl. Acad. Sci. USA.* **2011**, *108*, 9044–9048.
57. Zurita, A.R.; Maccioni, H.J.; Daniotti J.L. Modulation of epidermal growth factor receptor phosphorylation by endogenously expressed gangliosides. *Biochem. J.* **2001**, *355*, 465–472.
58. Mirkin, B.L.; Clark, S.H.; Zhang, C. Inhibition of human neuroblastoma cell proliferation and EGF receptor phosphorylation by gangliosides GM1, GM3, GD1A and GT1B. *Cell Prolif.* **2002**, *35*, 105–115.
59. Liu, Y.; Li, R.; Ladisch, S. Exogenous ganglioside GD1a enhances epidermal growth factor receptor binding and dimerization. *J. Biol. Chem.* **2004**, *279*, 36481–36489.
60. Yang, H.J.; Jung, K.Y.; Kwak, D.H.; Lee, S.H.; Ryu, J.S.; Kim, J.S.; Chang, K.T.; Lee, J.W.; Choo, Y.K. Inhibition of ganglioside GD1a synthesis suppresses the differentiation of human mesenchymal stem cells into osteoblasts. *Dev. Growth Differ.* **2011**, *53*, 323–332.
61. Mudò, G.; Bonomo, A.; Di Liberto, V.; Frinchi, M.; Fuxe, K.; Belluardo, N. The FGF-2/FGFRs neurotrophic system promotes neurogenesis in the adult brain. *J. Neural Transm.* **2009**, *116*, 995–1005.
62. Meuillet, E.; Cremel, G.; Dreyfus, H.; Hicks, D. Differential modulation of basic fibroblast and epidermal growth factor receptor activation by ganglioside GM3 in cultured retinal Müller glia. *Glia.* **1996**, *17*, 206–216.
63. Toledo, M.S.; Suzuki, E.; Handa, K.; Hakomori, S. Cell growth regulation through GM3-enriched microdomain (glycosynapse) in human lung embryonal fibroblast WI38 and its oncogenic transformant VA13. *J. Biol. Chem.* **2004**, *279*, 34655–34664.
64. Toledo, M.S.; Suzuki, E.; Handa, K.; Hakomori, S. Effect of ganglioside and tetraspanins in microdomains on interaction of integrins with fibroblast growth factor receptor. *J. Biol. Chem.* **2005**, *280*, 16227–16234.
65. Mutoh, T.; Tokuda, A.; Miyadai, T.; Hamaguchi, M.; Fujiki, N. Ganglioside GM1 binds to the Trk protein and regulates receptor function. *Proc. Natl. Acad. Sci. USA.* **1995**, *92*, 5087–5091.
66. Rabin, S.J.; Mocchetti, I. GM1 ganglioside activates the high affinity nerve growth factor receptor trkA. *J. Neurochem.* **1995**, *65*, 347–354.
67. Duchemin, A.M.; Ren, Q.; Mo, L.; Neff, N.H.; Hajiconstantinou, M. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. *J. Neurochem.* **2002**, *81*, 696–707.
68. Nishio, M.; Fukumoto, S.; Furukawa, K.; Ichimura, A.; Miyazaki, H.; Kusunoki, S.; Urano, T.; Furukawa, K. Overexpressed GM1 suppresses nerve growth factor (NGF) signals by modulating the intracellular localization of NGF receptors and membrane fluidity in PC12 cells. *J. Biol. Chem.* **2004**, *279*, 33368–33378.

69. Fukumoto, S.; Mutoh, T.; Hasegawa, T.; Miyazaki, H.; Okada, M.; Goto, G.; Furukawa, K.; Urano, T. GD3 synthase gene expression in PC12 cells results in the continuous activation of TrkA and ERK1/2 and enhanced proliferation. *J. Biol. Chem.* **2000**, *275*, 5832–5838.
70. Duchemin, A.M.; Ren, Q.; Neff, N.H.; Hadjiconstantinou, M. GM1-induced activation of phosphatidylinositol 3-kinase: Involvement of Trk receptors. *J. Neurochem.* **2008**, *104*, 1466–1477.
71. Todeschini, A.R.; Dos Santos, J.N.; Handa, K.; Hakomori, S.I. Ganglioside GM2/GM3 complex affixed on silica nanospheres strongly inhibits cell motility through CD82/cMet-mediated pathway. *Proc. Natl. Acad. Sci. USA.* **2008**, *105*, 1925–1930.
72. Cazet, A.; Lefebvre, J.; Adriaenssens, E.; Julien, S.; Bobowski, M.; Grigoriadis, A.; Tutt, A.; Tulasne, D.; Le Bourhis, X.; Delannoy, P. GD₃ synthase expression enhances proliferation and tumor growth of MDA-MB-231 breast cancer cells through c-Met activation. *Mol. Cancer Res.* **2010**, *8*, 1526–1535.
73. Cazet, A.; Groux-Degroote, S.; Teylaert, B.; Kwon, K.M.; Lehoux, S.; Slomianny, C.; Kim, C.H.; Le Bourhis, X.; Delannoy, P. GD3 synthase overexpression enhances proliferation and migration of MDA-MB-231 breast cancer cells. *Biol. Chem.* **2009**, *390*, 601–609.
74. Cazet, A.; Bobowski, M.; Rombouts, Y.; Lefebvre, J.; Steenackers, A.; Popa, I.; Guérardel, Y.; Le Bourhis, X.; Tulasne, D.; Delannoy, P. The ganglioside G(D2) induces the constitutive activation of c-Met in MDA-MB-231 breast cancer cells expressing the G(D3) synthase. *Glycobiology* **2012**, *22*, 806–816.
75. Hyuga, S.; Kawasaki, N.; Hyuga, M.; Ohta, M.; Shibayama, R.; Kawanishi, T.; Yamagata, S.; Yamagata, T.; Hayakawa, T. Ganglioside GD1a inhibits HGF-induced motility and scattering of cancer cells through suppression of tyrosine phosphorylation of c-Met. *Int. J. Cancer.* **2001**, *94*, 328–334.
76. Bremer, E.G.; Hakomori, S.; Bowen-Pope, D.F.; Raines, E.; Ross, R. Ganglioside-mediated modulation of cell growth, growth factor binding, and receptor phosphorylation. *J. Biol. Chem.* **1984**, *259*, 6818–6825.
77. Yates, A.J.; Saqr, H.E.; Van Brocklyn, J. Ganglioside modulation of the PDGF receptor. A model for ganglioside functions. *J. Neurooncol.* **1995**, *24*, 65–73.
78. Hynds, D.L.; Summers, M.; Van Brocklyn, J.; O'Dorisio, M.S.; Yates, A.J. Gangliosides inhibit platelet-derived growth factor-stimulated growth, receptor phosphorylation, and dimerization in neuroblastoma SH-SY5Y cells. *J. Neurochem.* **1995**, *65*, 2251–2258.
79. Van Brocklyn, J.; Bremer, E.G.; Yates, A.J. Gangliosides inhibit platelet-derived growth factor-stimulated receptor dimerization in human glioma U-1242MG and Swiss 3T3 cells. *J. Neurochem.* **1993**, *61*, 371–374.
80. Golard, A. Anti-GM3 antibodies activate calcium inflow and inhibit platelet-derived growth factor beta receptors (PDGFbetaR) in T51B rat liver epithelial cells. *Glycobiology* **1998**, *8*, 1221–1225.
81. Farooqui, T.; Kelley, T.; Coggeshall, K.M.; Rampersaud, A.A.; Yates, A.J. GM1 inhibits early signaling events mediated by PDGF receptor in cultured human glioma cells. *Anticancer Res.* **1999**, *19*, 5007–5013.
82. Oblinger, J.L.; Boardman, C.L.; Yates, A.J.; Burry, R.W. Domain-dependent modulation of PDGFRbeta by ganglioside GM1. *J. Mol. Neurosci.* **2003**, *20*, 103–114.

83. Mitsuda, T.; Furukawa, K.; Fukumoto, S.; Miyazaki, H.; Urano, T.; Furukawa, K. Overexpression of ganglioside GM1 results in the dispersion of platelet-derived growth factor receptor from glycolipid-enriched microdomains and in the suppression of cell growth signals. *J. Biol. Chem.* **2002**, *277*, 11239–11246.
84. Brdicka, T.; Pavlistova, D.; Leo, A.; Bruyns, E.; Korinek, V.; Angelisova, P.; Scherer, J.; Shevchenko, A.; Hilgert, I.; Cerný, J.; *et al.* Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. *J. Exp. Med.* **2000**, *191*, 1591–1604.
85. Veracini, L.; Simon, V.; Richard, V.; Schraven, B.; Horejsi, V.; Roche, S.; Benistant, C. The Csk-binding protein PAG regulates PDGF-induced Src mitogenic signaling via GM1. *J. Cell Biol.* **2008**, *182*, 603–614.
86. Liu, Y.; McCarthy, J.; Ladisch, S. Membrane ganglioside enrichment lowers the threshold for vascular endothelial cell angiogenic signaling. *Cancer Res.* **2006**, *66*, 10408–10414.
87. Lang, Z.; Guerrero, M.; Li, R.; Ladisch, S. Ganglioside GD1a enhances VEGF-induced endothelial cell proliferation and migration. *Biochem. Biophys. Res. Commun.* **2001**, *282*, 1031–1037.
88. Seyfried, T.N.; Mukherjee, P. Ganglioside GM3 Is Antiangiogenic in Malignant Brain Cancer. *J. Oncol.* **2010**, *2010*, 961243.
89. Mukherjee, K.; Chava, A.K.; Mandal, C.; Dey, S.N.; Kniep, B.; Chandra, S.; Mandal, C. O-acetylation of GD3 prevents its apoptotic effect and promotes survival of lymphoblasts in childhood acute lymphoblastic leukaemia. *J. Cell. Biochem.* **2008**, *105*, 724–734.
90. Chung, T.W.; Kim, S.J.; Choi, H.J.; Kim, K.J.; Kim, M.J.; Kim, S.H.; Lee, H.J.; Ko, J.H.; Lee, Y.C.; Suzuki, A.; *et al.* Ganglioside GM3 inhibits VEGF/VEGFR-2-mediated angiogenesis: direct interaction of GM3 with VEGFR-2. *Glycobiology* **2009**, *19*, 229–239.
91. Abate, L.E.; Mukherjee, P.; Seyfried, T.N. Gene-linked shift in ganglioside distribution influences growth and vascularity in a mouse astrocytoma. *J. Neurochem.* **2006**, *98*, 1973–1984.
92. Jin, J.; Sison, K.; Li, C.; Tian, R.; Wnuk, M.; Sung, H.K.; Jeansson, M.; Zhang, C.; Tucholska, M.; Jones, N.; *et al.* Soluble FLT1 binds lipid microdomains in podocytes to control cell morphology and glomerular barrier function. *Cell* **2012**, *151*, 384–399.
93. Tian, R.; Jin, J.; Taylor, L.; Larsen, B.; Quaggin, S.E.; Pawson, T. Rapid and sensitive MRM-based mass spectrometry approach for systematically exploring ganglioside-protein interactions. *Proteomics* **2013**, *13*, 1334–1338.
94. Tagami, S.; Inokuchi, J.; Kabayama, K.; Yoshimura, H.; Kitamura, F.; Uemura, S.; Ogawa, C.; Ishii, A.; Saito, M.; Ohtsuka, Y.; *et al.* Ganglioside GM3 participates in the pathological conditions of insulin resistance. *J. Biol. Chem.* **2002**, *277*, 3085–3092.
95. Sekimoto, J.; Kabayama, K.; Gohara, K.; Inokuchi, J. Dissociation of the insulin receptor from caveolae during TNF α -induced insulin resistance and its recovery by D-PDMP. *FEBS Lett.* **2012**, *586*, 191–195.
96. Kabayama, K.; Sato, T.; Kitamura, F.; Uemura, S.; Kang, B.W.; Igarashi, Y.; Inokuchi, J. TNF α -induced insulin resistance in adipocytes as a membrane microdomain disorder: Involvement of ganglioside GM3. *Glycobiology* **2005**, *15*, 21–29.

97. Yamashita, T.; Hashiramoto, A.; Haluzik, M.; Mizukami, H.; Beck, S.; Norton, A.; Kono, M.; Tsuji, S.; Daniotti, J.L.; Werth, N.; *et al.* Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc. Natl. Acad. Sci. USA*. **2003**, *100*, 3445–3449.
98. Yoshizumi, S.; Suzuki, S.; Hirai, M.; Hinokio, Y.; Yamada, T.; Yamada, T.; Tsunoda, U.; Aburatani, H.; Yamaguchi, K.; Miyagi, T.; Oka, Y. Increased hepatic expression of ganglioside-specific sialidase, NEU3, Improves insulin sensitivity and glucose tolerance in mice. *Metabolism* **2007**, *56*, 420–429.
99. Kabayama, K.; Sato, T.; Saito, K.; Loberto, N.; Prinetti, A.; Sonnino, S.; Kinjo, M.; Igarashi, Y.; Inokuchi, J. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. *Proc. Natl. Acad. Sci. USA*. **2007**, *104*, 13678–13683.
100. Sasaki, A.; Hata, K.; Suzuki, S.; Sawada, M.; Wada, T.; Yamaguchi, K.; Obinata, M.; Tateno, H.; Suzuki, H.; Miyagi, T. Overexpression of plasma membrane-associated sialidase attenuates insulin signaling in transgenic mice. *J. Biol. Chem.* **2003**, *278*, 27896–27902.
101. Rillahan, C.D.; Paulson, J.C. Glycan microarrays for decoding the glycome. *Annu. Rev. Biochem.* **2011**, *80*, 797–823.
102. Bond, M.R.; Whitman, C.M.; Kohler, J.J. Metabolically incorporated photocrosslinking sialic acid covalently captures a ganglioside-protein complex. *Mol. Biosyst.* **2010**, *6*, 1796–1799.

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