Chapter 13
Glycosignaling: A General Review

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Abstract The concept of glycosignaling, in which neural cell-surface glycoconjugates form microdomains (Lipid Rafts) to facilitate the recruitment of signaling molecule components to form a transient signaling unit, is helping us understand the reason for glycoheterogeneity in the brain and is leadings to important translational efforts in medicine. In this review we first describe the origins of the concept of glycomicrodomains, how lipid heterogeneity might have relevance for the brain development, pathology and how the glycocalyx acts as a barrier in glia. After a discussion of how such microdomains are isolated and studied using modern technology such as nanoparticle labeling and molecular microscopy, we will present examples of how glycosignaling can function in such brain-specific situations as axonal growth and protein phosphorylation-mediated signaling.

Keywords Glycosphingolipids • Gangliosides • Proteoglycans • Glycoproteins • Microdomains • Lipid rafts • Neural stem cells • Quantum dots • Molecular microscopy • Protein phosphorylation

13.1 Introduction: Definition of "Glycosignaling"

The major membrane glycosphingolipids of brain contain typically up to four neutral sugars and up to five sialic acid residues within the ganglio series (GalNAcβ1–4Gal) and only trace amounts of the different core globo-series (Galα1–4-Gal), the neolactose series (Galβ1–4GlcNAc), or the lacto series (Galβ1–3GlcNAcβ1–4Gal) glycolipids (Hakomori 2008). Quantitatively dominant are the sulfated and

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non-sulfated galactosylceramides associated with the myelin sheath. Of the other glycoconjugates in brain, proteoglycans contain extensively sulfated uronic acid-GlcNAc/GalNAc repeating polymers and glycoproteins have extensively branched chains of oligosaccharides, which uniquely contain mannose and sialic acids. These are discussed extensively in other chapters. All these molecules appear to be involved in many types of cell recognition and signaling within the brain and there is increasing evidence for the importance of "glycosignaling" in all aspects of brain function.

The term "glycosignaling" was introduced by Hakomori et al. (1998) on the basis of their studies, mostly on non-CNS tissue. They described an assembly of glycosphingolipids and signal transducer molecules, which formed what they termed a "*glycosignaling domain*" (*GSD*). They ascribed to this GSD a functional role in cell interaction/adhesion, which involved second messenger signaling. An original difference from previously described sphingolipid-rich microdomains (Lipid rafts) was the absence of cholesterol and glycoprotein clusters from the GSDs. The GSL clustering could be imaged on EM using gold sol coated anti-polysialoganglioside antibody and a clustering of GSLs above a critical threshold was necessary for both antibody binding and cell adhesion. The glycosignaling concept has since been expanded to include glycoproteins and proteoglycans and the glyco-modification of lipids and proteins and many of these processes are associated with more conventional "Lipid Rafts" (Hakomori et al. 1998; Lingwood et al. 2008). Since isolated Lipid Rafts (LRs) also contain the elements of many G-protein signaling systems, there is also considerable interest in how the glycosignaling through lipid rafts (LRs) could influence the many signaling pathways critical for the functioning of the brain.

The original ideas of how glycosignaling systems might function in brain were laid out in a review by Saul Roseman (1970) which was based on many years of studying glycosyltransferases in developing chick brains. In this model, cell-surface glycosyltransferases interacted with cell surface glycolipid substrates to facilitate intercellular adhesion and recognition. This concept was refined by Hakomori/Prinetti (Hakomori 2008) whereby stimulation of GSDs by their ligands (complementary GSLs, cognins, antibodies, etc.) induces conformational changes in transducer molecules which activate positive or negative signals and then regulate such things as cell adhesion. They envisioned transducer molecules to include Src, and Ras kinase family proteins and Rho GTPases, all of which are active in brain. Evidence came from a mouse melanoma B16 cell which displays GM3-dependent adhesion to plates coated with Gg3 or anti-GM3 antibody. This resulted in enhanced c-Src phosphorylation, but there was no response when GM3 was absent or replaced with other gangliosides such as GM1 or GD1a, or asialo-glycolipids such as LacCer. From such data they concluded (Hakomori 2008) that many transducer molecules controlled cell motility and proliferation in response to various glyco-mediated stimuli. Thus, glycolipid-enriched microdomains are believed to be functionally involved in initiating or inhibiting signal transduction through interaction of their carbohydrate moiety with their ligands, hence the term "glycosignaling domain."

Mounting evidence suggests that glycosignaling operates in the heterogeneous mammalian CNS as it arises from undifferentiated neural stem cells (NSCs), with their high proliferative potential and capacity for self-renewal and multipotency (Yu and Yanagisawa 2007). Neuronal and Glial cells certainly express a sufficiently complex glycocalyx (made up from membrane-associated glycolipids, and membrane and extracellular glycoproteins and proteoglycans) to be critical participants in recognition, patterning, barrier formation and in dynamic signaling systems (Parker and Kohler 2009). A combination of a negatively charged polymer and a cluster of glycosphingolipids and glycoproteins plus heparan sulfates or a posttranslational modification to a protein could have a critical role in using glycosignaling to drive CNS development, function, and regeneration in either a positive or negative direction.

13.2 Isolation of Glycosignaling Complexes from Brain

Glycosphingolipids (GSLs) are enriched in membranes immersed in an insoluble matrix and glycosignaling complexes can be isolated by virtue of their insolubility in detergents such as 1 % Triton X-100 at 4 °C. The terms "detergent-insoluble material" (DIM) and "detergent-insoluble substrate attachment matrix" (DISAM) were originally applied to designate a special membrane compartment enriched in GSLs (Hakomori et al. 1998; Lingwood et al. 2008) but we typically now refer to all such preparations as a "Lipid Raft" (LR). For example (Dawson et al. 2012), one can extract 50 mg fresh weight of mouse cerebral hemisphere with 2 ml of 1 % Triton X-100/MES buffer–NaCI (pH 6.5) using 50 strokes of a Dounce homogenizer. Following centrifugation at $700 \times g$ to remove insoluble material such as myelin membranes, the extract is mixed with 2 ml of 1 % Triton X-100 in 80 % sucrose/MES buffer and placed in an ultracentrifugation tube. Five milliliter of 30 % sucrose/MES is layered on top of this fraction, followed by 3 ml of 5 % sucrose/MES and the samples ultracentrifuged at $39{,}000 \times g$ for 17 h. Typically, an opalescent band is seen in Fraction 4 and this band contains LR protein markers such as Flotillin-1 (Dawson et al. 2012) as well as almost all the sphingolipids. Sphingolipid modifying enzymes and other glycoproteins such a G-protein-coupled receptors may occur in this Fraction 4 or may translocate to fraction 4 after specific stimulation of the cell, for example with neurotransmitters. In brain, virtually all the gangliosides are present in the LR fraction, including those found only in fetal or pathological tissue such as GM3, GM2, GD3, and GD2. GM1 ganglioside is the cholera toxin receptor in brain but is also present in non-neural cells in a similar detergent-insoluble fraction along with virtually all other glycolipids. Lipid rafts can be visualized by transfecting cells (for example a human oligodendroglioma cell line HOG with green fluorescent protein-tagged GPI-anchored protein sequence which always localizes to Lipid Rafts (Fig. 13.1a, b)). The detergent-insolubility (and presence in insoluble LRs) of GM3 or GM1 in cells is usually interpreted to mean that these gangliosides are complexed with a cell adhesion system or

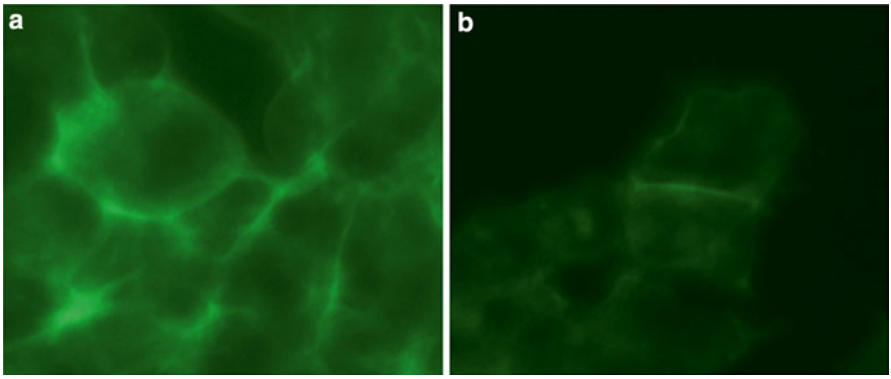

Fig. 13.1 Visualization of glycomicrodomains. GPI anchored proteins are exclusively localized to sphingolipid-enriched microdomains (LRs) and are involved in glycosignaling, for example the OMPR protein involved in forming a complex with gangliosides in regulating axonal growth (Saha et al. 2011) (as shown in Fig. 13.3). Fluorescent images were obtained after expression of LR-associated green fluorescent protein (GFP)-labeled GPI-anchored protein cDNA in neurotumor-derived HOG cells. These cells show increased concentration of GFP expression in plasma membranes (**a**), especially at adjacent cell membranes (**b**). These concentrations of GFP could represent aggregates of LRs corresponding to glycomicrodomains involved in cell adhesion and signaling

cytoskeletal system, since gangliosides are all soluble in aqueous detergent solutions such as Triton X-100.

Further evidence for LR-based glycosignaling in the brain comes from a study (Sekino-Suzuki et al. 2012) which showed that both R24 anti-GD3 antibody, and an antibody (GGR12), that specifically recognizes GD1b ganglioside, were able to induce Lyn tyrosine kinase activation in rat primary cerebellar granule cells. Immunoprecipitation studies on similar Triton X-100 LRs from these cells showed a complex containing Lyn, the transmembrane phosphoprotein Cbp and the physiological regulator of Src family kinases, Csk. The interaction of Cbp and Csk required the phosphorylation of Cbp at Tyr-314 by activated Lyn and they proposed (Sekino-Suzuki et al. 2012) that phosphorylated Cpb negatively regulated Lyn through the recruitment of Csk into LRs. Since both GD3 and GD1b contain the same Neu5Ac-(2–8)-Neu5Ac-(2–3)-Gal sequence and trigger the same response in neurons they suggest that this is the critical glycosignaling element in forming or stabilizing the LR complex. As discussed by Sonnino et al. (2013) it remains to be seen if these studies in cultured neurons have physiological significance in the brain.

13.3 Glycosignaling, Biosynthesis, and Brain Development

Notch signaling is critical for brain development and Notch is only active when glycosylated by an O-fucosylation step and the addition of GlcNAc to specific serine residues (Parker and Kohler 2009). Such glyco-posttranslational modifications

of proteins are common and are likely initiators of glycosignaling in brain development and function since their presence is essential for recognition and this recognition triggers biological events. Regulation of such glycosylation (and therefore regulation of glycosignaling) can also occur at the level of nucleotide sugar availability, glycosyltransferase activation, or secretion and is therefore both complex and dynamic.

For example, gangliosides are attached through ceramides to LR membranes but free ceramides inhibit protein kinase B (Akt) through activation of a phosphatase. When ceramide is low (as in the *Fro*/*Fro* mouse and in many tumors) (Qin et al. 2012), Akt is activated (phosphorylated) together with the mTOR, p70D6k pathway to upregulate genes such as HAS2, which cause synthesis and secretion of the extracellular matrix proteoglycan, hyaluronic acid (Qin et al. 2012). This results in accelerated metastasis of a tumor if localized or impaired bone and brain development if occurring in all tissues, as in the *Fro*/*Fro* mouse (Qin et al. 2012). Additional control of such glycosignaling can be exerted by means of the many secreted and cell surface glycosidases, sulfatases, phosphatases, etc. (Aureli et al. 2012) acting on gangliosides and other cell surface glycoconjugates. Such modifications can also be shown to affect many critical developmental signaling systems such as the Wnt, EGF, and FGF pathways (Parker and Kohler 2009). Thus, glycosignaling must be thought of as an ongoing dynamic process, which involves many glycoconjugates and can impinge on many brain functions.

13.4 The Glycocalyx as a Barrier to Glycosignaling

The glycocalyx is a negatively charged barrier in brain because of the high content of lipid- and protein-linked sialic acids, sulfated lipids, and sulfated sugar and uronic acid-containing polymers (Parker and Kohler 2009; Sonnino et al. 2012) surrounding the cells. The glycocalyx is extensively produced by glial cells in the form of chondroitin and heparan sulfates and ceramide-galactosylsulfates, but neurons also produce lesser amounts of specific types of chondroitin sulfates. Both neurons and glia produce heparan sulfates, which are extensively involved in growth regulation via glycosignaling complexes with the EGF receptor. In contrast, gangliosides are synthesized by neurons. This negative surface charge difference between neurons and glia is most dramatically demonstrated by exposing brain slices containing differentiating, integrated brain cell types to intrinsically highly fluorescent nanoparticles. These consist of a CdSe/ZnS core/shell luminescent semiconductor or "quantum dot" (QD) approximately 6–10 nm in size (the same size as a typical lysosomal hydrolase) which are coated with a stabilizing/solubilizing polymer through dihydrolipoic acid (DHLA) residues (Fig. 13.2a). This polymer can be neutral (such as polyethyleneglycol) or negatively charged (CL4) (Walters et al. 2012; Boeneman et al. 2013). The Zn on the surface of the QDs is able to bind to histidine residues (H_6) incorporated into a peptide or lipopeptide cargo such as WGDap(Palmitoyl) VKIKKP$_9$GGH$_6$ (palm-1) or any His$_6$-tagged protein. The original GDap(Palmitoyl)

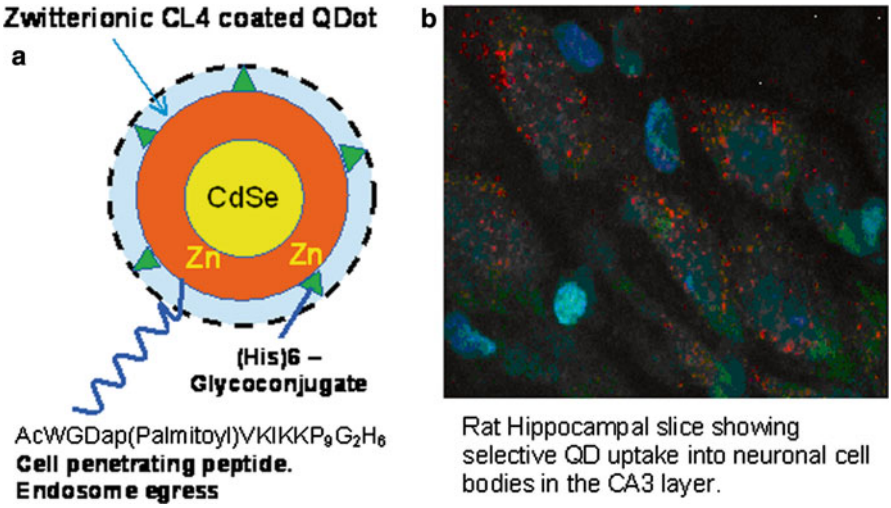

Fig. 13.2 Structure of Quantum dots showing site of attachment of His6-tagged peptides and proteins. (**a**) Quantum dot-Palm1 structure. This is a representation of a Cd/SeZnS Quantum dot coated with a dihydrolipoic acid (DHLA)-negatively charged (Zwitterionic) molecule (compact ligand 4 (CL4)). Membrane penetrating peptides such as AcWDapVKIKKP$_9$G$_2$H$_6$ or His$_6$-protein or His$_6$-glycoconjugate bind tightly to the Zn coat. These intensely fluorescent particles are 10–15 nm in diameter and can deliver various types of glycosignaling-modifying compounds to facilitate correction of brain mis-development or injury. (**b**) Confocal microscopic image showing QD delivery to hippocampal neurons. Rat hippocampal brain slices take up the highly fluorescent (625 nm) coated Quantum dots plus peptides initially into endosomes and then distribute it throughout the neuron. *Blue-staining* is DAPI (nor the intensely staining astrocyte nuclei with no QDs), *green staining* is Nissl body, neuron-specific, and *red staining* is Quantum dot–lipopeptide complexes

VKIKK sequence was based on the region of Ras-4B which is dynamically palmitoylated in vivo (Walters et al. 2012; Boeneman et al. 2013). The coating on the QD provides colloidal stability and Palm-1 uniquely allows the QDs to be taken up by cultured cells and readily exit the endosome into the soma (Walters et al. 2012; Boeneman et al. 2013). Electron microscopic images confirmed the endosomal egress only occurred in the presence of the Palm1 peptide, showing a much more dispersed cytosolic distribution of the CL4 QDs conjugated to Palm1 compared with CL4 QDs alone. Replacing a neutral polyampholyte coating (PEG) with a negatively heterocharged compact ligand, ("CL4"), was able to specifically target the palmitoylated peptide (palm-1) to neurons in the developing rat hippocampal brain slice cultures (Fig. 13.2b). There was no noticeable uptake of such Quantum dots by astrocytes, oligodendrocytes, and very little by microglia (identified by immunocytochemistry (Walters et al. 2012)). However, pretreatment of the cultures with chondroitinase ABC to reduce the surface negative charge, promoted uptake into oligodendrocytes. Thus, although neurons receive nutrients and signals by retrograde axonal transport they respond to external negatively charged glycosignals (sulfation, sialic acids, etc.), whereas glial cells are more resistant.

13.5 Glycosignaling Heterogeneity in Specific Brain Regions

Because glycoconjugates exist as both linear and branched structures and hydroxyl group modifications such as sulfation and acylation are common, the number of possible targets for glyco-binding proteins exceeds 7,000 (Lingwood et al. 2010). The importance of glycolipids to the CNS is amply demonstrated by the ability of bacteria and viruses to gain entry to cells by first binding to a specific carbohydrate sequence. Advances in technology have increased our understanding of how such glyco-recognition can be finely tuned. For example a ganglioside such as GD1a can be O-acetylated and contain either or both *N*-acetyl- and *N*-glycolyl-neuraminic acids and this affects where the GD1a is localized within the brain (Colsch et al. 2011) and which glycosignaling system it modifies.

A recent study using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (Colsch et al. 2011) showed that the qualitative localization of ganglioside species in the rat hippocampus depended both on the degree of glycosylation and type of sialylation (e.g., O-acetylation), and also the ceramide structure as defined by the presence of either sphingoid bases C18:1 or C20:1. Previous immunostaining with antibodies, lectins, or toxins to detect ganglioside molecules recognized the headgroup, but imaging mass spectrometry is able to recognize all components of a molecule, both oligosaccharide as well as ceramide core. The information obtained by molecular fragmentation produces "molecular microscopy" (Colsch et al. 2011) and enabled insights into modifications critical for glycosignaling. Thus, in the rat hippocampus, the molecular layer of the dentate gyrus (ML), is made up of three distinct layers, the inner molecular layer (IML), which contains gangliosides with C18-sphingosine exclusively, the middle molecular layer (MML), and the outer molecular layer (OML) where C20:1-sphingosine is the only sphingosine base in the gangliosides.

Intriguingly, the gangliosides which occur in embryonic development and reoccur in brain pathological conditions (GM3, GM2, GD3, and GD2) all contain predominantly C18-sphingosine and are predominantly present in cell layers made up of the pyramidal cell layer (Py) and the granular layer of the dentate gyrus (GL). In contrast, when the major mature brain gangliosides (GM1, GD1, and GT1) and GQ1s were mapped they were found to be predominantly in the substantia nigra, cerebral peduncle, hippocampus, and midbrain. However, C20:1 sphingosine GM1 was minimally present in the corpus callosum and midbrain and was mostly found in the hippocampus and the substantia nigra, Minor gangliosides GQ1 and the O-acetylated forms of GD1, GT1, and GQ1 gangliosides, contained both C18- and C20-sphingosines and their distribution was based on the degree of sialylation and acetylation of the oligosaccharide chains in the neuronal cell bodies. These are potentially exciting findings for glycosignaling (Colsch et al. 2011) since they suggest that the ceramide part of the glycosphingolipid may influence glycosignaling in the brain.

13.6 Evidence That Fatty Acids and Sphingosine Base Heterogeneity Can Affect Glycosignaling: Toxins

Although most glycolipids are typically firmly attached to membrane LR complexes (an exception being the "shedding" observed in cancer cells) the reactivity of the GSL sugars with binding proteins and antibodies does appear to be somewhat dependent upon the fatty acid composition. Most GSLs show extensive fatty acid heterogeneity (C16–24) with highly antigenic lipids such as galactosylceramides and sulfatides containing a wide range of fatty acids, about 50 % of which are alpha-hydroxylated and/or monounsaturated. This affects their reactivity, possibly because it affects their mobility in the plasma membrane lipid Raft (Lingwood 1999). A marked exception to this fatty acid heterogeneity rule are brain gangliosides which are almost exclusively C18:0 but do contain a mixture of C18 and C20 sphingoid bases. Gangliosides are very minor components of non-neural tissue and when they do occur, typically in cancerous cells, they show fatty acid heterogeneity typical of non-CNS glycolipids. So there is something unique to the fatty acid behavior of brain gangliosides, which affects the structure of microdomains (LRs) and most probably relates to some glycosignaling function. For example, fatty acid heterogeneity has been clearly shown to regulate the binding of toxins such as veratoxin to neutral glycolipids (GbOse3cer) (Lingwood 1999), so this phenomenon needs to be better investigated in brain.

13.7 Glycosignaling, Rho-GTPase, and Axonal Growth

The GalNAc transferase knockout mouse with absence of the major gangliosides appeared to have very little phenotype until Schnaar discovered that recovery from nerve injury was significantly improved and went on to show that sialidase treatment, which converted complex gangliosides to GM1, improved recovery from spinal cord injury (Mountney et al. 2010). Spinal cord injury typically results in lifelong loss of nerve function and morbidity (Barritt et al. 2006) and recent reports suggest that glycosignaling errors could contribute to this loss of function (Mountney et al. 2013). Studies have implicated the glycocalyx in inhibition of nerve injury recovery and both digestion of chondroitin sulfates and sialo-oligosaccharides has been of some therapeutic value in rat models of SC injury. Recently, Schnaar and associates (Mountney et al. 2013) showed that delivery of bacterial chondroitinase ABC or bacterial sialidase (but not the concomitant delivery of both) to the site of experimental spinal cord injuries resulted in improved spinal axon outgrowth, locomotor recovery, and cardiovascular reflex recovery.

The explanation for this is that CNS axons have the capacity to regenerate, but are inhibited from doing so by endogenous glycosylated-axon regeneration inhibitors, such as myelin-associated glycoprotein (MAG), Nogo, and oligodendrocyte-myelin glycoprotein in the myelin sheath and chondroitin sulfate proteoglycans

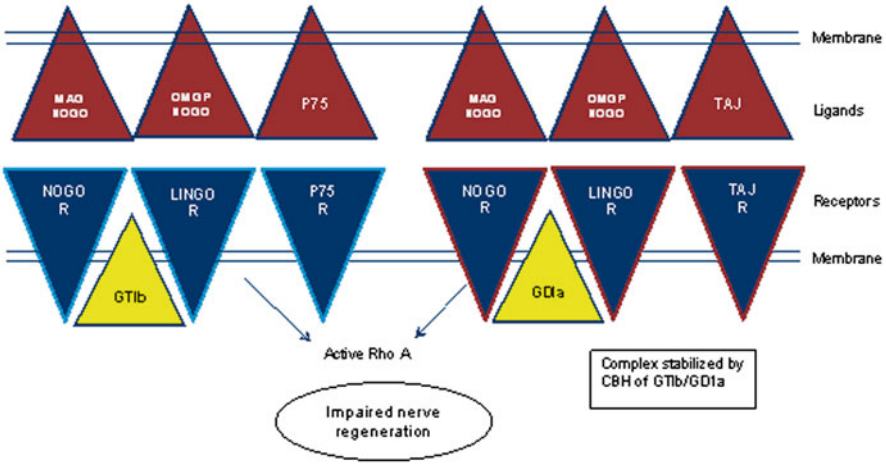

Fig. 13.3 Glycosignaling model for inhibition of nerve repair following injury. Gangliosides GT1b and GD1a (with terminal sialo 2–3 Gal) facilitate clustering of receptors for MAG, OMGP, p75 NGF, and most likely other glycoproteins to form a signaling complex which activates Rho A. In a 2-step procedure, GT1b/GD1a gangliosides associate with NgR1/LINGO-1 to form a functional receptor/co-receptor complex and this is followed by simultaneous recruitment of the transducer p75 and the myelin inhibitor Nogo-54. Once the initial GT1b/GD1a-mediated tripartite receptor/co-receptor complex is formed between NgR1/LINGO-1 and p75, the overall affinity of NgR1 for the Nogo-54 ligand is most likely enhanced and glycosignaling ensues [21]

(CSPGs) at the site of the glial scar. The glial glycoconjugates are believed to bind to complementary receptors on axons (possibly glyco sequences), thus preventing axonal regeneration. A simplified model is shown in Fig. 13.3. In this model, MAG, NOGO bind to NOGO receptors, OMGP/NOGO bind to LINGO receptors and p75/TAJ, bind to p75/TAJ receptors which are critically held together in a glycocluster by either of two gangliosides GTib/GD1a (Saha et al. 2011). Elimination of any of these components reverses some of the inhibition of nerve regeneration directed by activated RhoA. Thus, glycosignaling through this complex on the surface of the cell results in the negative signaling by RhoA inside the cell (Fig. 13.3).

13.8 Glycosignaling and Protein Phosphorylation

Yu and Yanagisawa (2007) presented a model in 2007 that described the involvement of a wide variety of glycoconjugates in signaling pathways critical for the proliferation and self-renewal of neural stem cells and which involved the regulation of protein phosphorylation. Our current view is summarized in Fig. 13.4. Such brain-derived cells the LRs are enriched in GD3 ganglioside, which activates a growth factor glycosignaling pathway, which in turn activates the Ras-MAPK pathway but not the Janus kinase (JAK-STAT) pathway. The latter is associated with

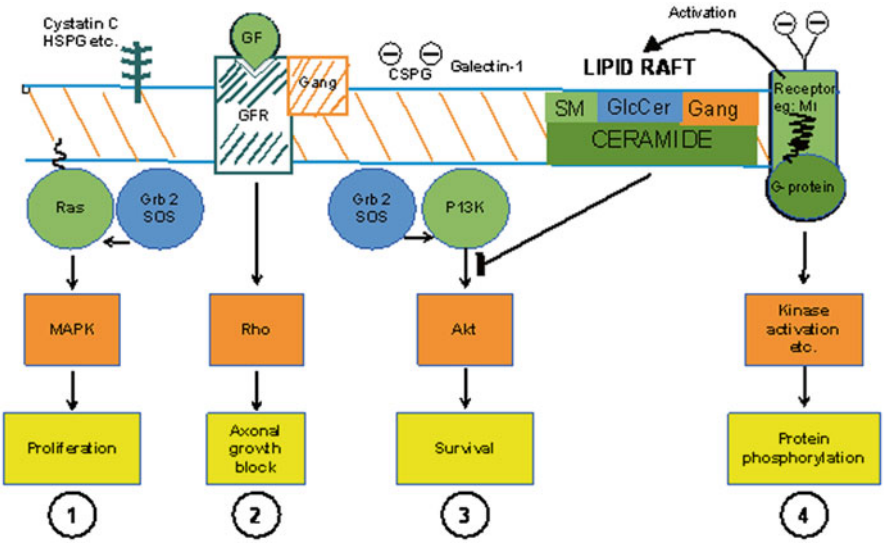

Fig. 13.4 Possible Glycosignaling pathways in Brain. (*1*) Heparan sulfate (HSPG) and chondroitin sulfate (CSPG) proteoglycans, proteins such as Cystalin C and Galectin-1, together with specific gangliosides promote a growth factor (GF) binding complex in a glycodomain which activates Ras and MAPK and promotes brain growth and development. (*2*) Activation of Rho GTPase which maintains synaptic fidelity and integrity by inhibition of axonal growth in the mature animal (see Fig. 13.3). (*3*) Activation of the phosphatidylinositol (P13K)/Akt pathway critically important for growth and development. This pathway can be negatively regulated by Ceramide (generated within a Lipid Raft whose integrity is maintained by glyco-glyco and hydrophobic interactions). Tumor cells accentuate this pathway and reduce ceramide either by glycosylation to form glucosylceramide (GlcCer) and more complex gangliosides Gang such as GD1a, increasing sphingomyelin (SM) by inhibition of sphingomyelinase or reducing de novo synthesis of ceramide. (*4*) G-Protein receptors (e.g., muscarinic M1) are activated following recruitment into Lipid Rafts where they activate palmitoylated/farnesylated modified G-proteins and promote signaling protein phosphorylation. This results in signaling under the control of complex glycosignaling pathways as discussed in the text

astrocyte differentiation and cell survival (Yu and Yanagisawa 2007). Other studies have emphasized a link between glycosignaling and protein phosphorylation, for example Shigatoxin binds to a LR glycolipid and this activates a tyrosine kinase (Katagiri et al. 1999). A similar phenomenon has been described in a neural-derived cell line, the GQ1b-dependent neuritogenesis of human neuroblastoma cell line, GOTO. This was associated with GQ1b-dependent ecto-type protein phosphorylation (Tsuji et al. 1992) of several cell surface proteins. The protein kinase inhibitor, K-252b (a non-membrane-permeable derivative of K-252a) inhibited both the GQ1b-dependent neuritogenesis as well as the GQ1b-stimulated phosphorylation, suggesting a direct coupling between the two cell events. Such events could be important in the CNS.

Another more complex glycosignaling system involves the non-sphingoglycolipid. (PhGlc) and tyrosine phosphorylation. In this study (Kaneko et al. 2011)

the novel lipid, phosphatidylglucoside (PhGlc), present in brain LRs, induced differentiation of cells as measured by the appearance of CD38 (a multifunctional ectoenzyme which acts as an NAD(+) glycohydrolase, an ADP-ribosyl cyclase, and a cyclic ADP-ribose hydrolase) and c-Myc downregulation. Reduction of endogenous cholesterol and dissociation of LRs with methyl-beta-cyclodextrin suppressed the associated tyrosine phosphorylation and signaling, suggesting that these had previously colocalized in the LR. Ligation of known components of LRs such as sphingomyelin and ganglioside GM1, with corresponding antibodies failed to induce differentiation or tyrosine phosphorylation, suggesting that PhGlc can directly glycosignal by inducing protein phosphorylation. More importantly, by using a specific anti-PhGlc antibody (Kaneko et al. 2011), they were able to show robust PhGlc staining in the two primary neurogenic regions of the adult rodent brain, the subventricular zone (SVZ) lining the lateral ventricle and the subgranular zone of the dentate gyrus. The staining pattern of PhGlc appeared to overlap that of glial fibrillary acidic protein, an adult neural stem cell marker and PhGlc expression overlapped with other proposed adult neural stem cell markers (Kaneko et al. 2011).

Thus, a glycosignaling hypothesis would propose that ceramide-enriched lipid platforms (LRs), and gangliosides contribute to receptor (for example CD38) activation to produce cADPR in response to receptor stimulation, (for example by muscarinic type 1 (M(1) agonists)). Thus, the M(1) receptor agonist, oxotremorine, should increased LR clustering on the membrane to form a complex of CD38 with LR components such as GM1, acid sphingomyelinase (ASMase), and ceramide, and the complex GSD should be abolished by LR disruptors, such as methyl-beta-cyclodextrin or filipin (Jia et al. 2008) There is experimental evidence for this. Further, fluorescence resonance energy transfer (FRET) showed the close proximity of ganglioside and CD38, and thus the formation of ceramide-enriched lipid macrodomains (LRs) appears to be crucial for agonist-induced activation of CD38 to produce cADPR. We can conclude that following translocation into lipid glycodomains transmembrane signaling through the G-protein-coupled M(1) receptor produces the second messenger cADPR (Jia et al. 2008).

However, the situation is more complex since the association of ganglioside and CD38 signaling also involves a role for sulfated glycoconjugates. This association was confirmed when the extracellular catalytic domain of CD38 was expressed as a fusion protein with maltose-binding protein, and then co-crystallized with one of its likely physiological brain ganglioside inhibitors (GT1b) in a 1:1 ratio per each asymmetric unit (Zhao et al. 2012). Three kinds of novel sulfated gangliosides structurally related to the Chol-1 (alpha-series) ganglioside GQ1b-alpha were tested and found to be potent inhibitors of the NADase activity of CD38 (Zhao et al. 2012). The disulfate of iso-GM1b was surprisingly found to be the most potent structure for both NADase inhibition and MAG-binding activity. Such sulfated gangliosides could bind to the internal sialic acid residues linked alpha2–3- to Gal as well as to siglec-dependent recognition sites with a terminal sialic acid residue. Thus, sialylated oligosaccharides binding to minor sulfated gangliosides could be a good model for carbohydrate–carbohydrate interactions at the cell membrane in the nervous system and much exciting work lies ahead in order to unravel the complex system we call “glycosignaling.”

So the role of the sphingolipid/cholesterol microdomain glycolipid or other glycoconjugate is to create an environment (the lipid raft (LR)) which recruits proteins and facilitates protein interactions leading to kinase activation and biological effects in the brain. The evidence for such a mechanism in neural stem cells involving growth factors, integrins, and the Ras-MAPK pathway has been well-summarized (Yu and Yanagisawa 2007)) and the limitations in evidence duly noted. A summary scheme is presented in Fig. 13.4. Much of the evidence supporting these ideas comes from the immune system since many pathogens target glycosphingolipids in LRs but the results have relevance for the brain. Thus lactosylceramide (LacCer, CDw17) binds to *Candida albicans* and forms membrane microdomains together with the Src family tyrosine kinase Lyn. These LacCer-enriched membrane microdomains can mediate superoxide generation, migration, and phagocytosis, indicating that LacCer functions as a Pattern Recognition Receptor in innate immunity (Nakayama et al. 2013). Other studies in non-neural cells suggest that lactosylceramide may directly activate phospholipase A2 by a translocation mechanism (Nakamura et al. 2013). Thus we may conclude that glycosignaling in the brain is most likely all about recruiting signaling partners into microdomains with associated glycoconjugate and fatty acid/sphingosine heterogeneity offering the additional binding specificities necessary for the brain to function as an amazingly complex organ.

13.9 Lysosomal Storage Modifies Glycosignaling

The failure to degrade complex carbohydrates results in lysosomal storage diseases and often the destruction of the nervous system prenatally or before adolescence. This is the most dramatic demonstration of the power of quantitatively abnormal or unique glyco-structures to disrupt brain function. Although the pathology of these diseases suggests that physical accumulation of glycoconjugates can be a primary cause of neural cell death and resultant seizures and demyelination, it has been shown that critical glycosignaling systems involving ion channels etc. may be compromised by the storage of glycoconjugates and that this contributes to the pathology of these diseases (Futerman and van Meer 2004).

Since in lysosomal storage diseases there could be perturbations in late endocytic functions leading to abnormal lipid raft composition and trafficking (Futerman and van Meer 2004) we carried out a detailed lipidomics study of a pathological lysosomal storage disease in the brain of a mouse with a typical lysosomal storage disease (San Filippo Type3a (Dawson et al. 2012)). The major gangliosides GM1, GD1a, GD1b, and GT1a were C18/20 sphingosine with C18 fatty acid (as expected) and all were exclusively present in LRs. However, although the heparan sulfate-derived storage material was not in LRs (as expected), the abnormal gangliosides (GD2, GD3, and GM2 and GM3) were all in LRs. Thus, the glyco-part could be modifying the formation of complexes, which then disrupted normal glycosignaling in the brain, leading to seizures, blindness, and loss of all cortical functions.

13.10 Future Directions for Glycosignaling in the Brain

Any system involving modification of multiple membrane glycoproteins, proteoglycans, gangliosides, sulfated oligosaccharides, and glycosylated phosphoglycerides will take a while to understand, but no one doubts that the human brain has evolved into a very complex yet capable organ, so we look forward with eager anticipation to the secrets that will be revealed by the continued improvement and application of new technology to glycosignaling.

Conflict of Interest The author declares no conflict of interest.

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