Gangliosides in Axon Stability and Regeneration

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Ronald L. Schnaar

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Abstract

Gangliosides, sialylated glycosphingolipids, are major glycans on the surfaces of vertebrate nerve cells. All mammals express the same four major brain gangliosides, GM1, GD1a, GD1b, and GT1b, which together comprise 94 % of brain gangliosides in mice and men alike. Among their functions, brain gangliosides GD1a and GT1b on neuronal axons are complementary binding partners for myelin-associated glycoprotein (MAG), which functions in axon-myelin stability and the control of axon regeneration. Human congenital disorders of ganglioside biosynthesis and related mouse genetic models reveal that complex gangliosides are required for long-term axon survival; loss of gangliosides results in paraplegia in humans and similar progressive motor neuropathy in mice. In addition to stabilizing axons, axon-myelin interactions restrict axon regeneration after injury in adults. MAG on residual myelin that persists at sites of central nervous system injury contributes to axon outgrowth inhibition by binding to gangliosides, thereby signaling axons to halt outgrowth. Modulating ganglioside structures on living nerve cells in vitro and in animal models attenuates inhibition and enhances anatomical and motor behavioral outcomes

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after axonal injury. Knowledge of ganglioside functions in the brain contributes to a more complete understanding of glycosciences in axon-related physiology and pathology.

Keywords
Axons • Brain • Gangliosides • Myelin • Myelin-associated glycoprotein • Nerve cells • Regeneration

Introduction

Every vertebrate cell is endowed with a rich and diverse surface coat of glycans with an abundance of sialic acid-terminated glycan structures. The brain is unique in that its sialome is dominated by gangliosides – sialylated glycosphingolipids (Schnaar et al. 2014). The same four structures (Fig. 1), GM1, GD1a, GD1b, and GT1b, represent the vast majority of brain gangliosides, together accounting for 94 % of total brain gangliosides in mouse and man alike. The ceramide lipid moieties of most gangliosides are firmly embedded in the extracellular leaflet of the plasma membrane, with the glycan extending 20–25 Å outward from the cell surface. Biochemical and cell biological studies indicate that gangliosides function in two characteristic modes: (i) *cis* interactions between gangliosides and proteins on their own membranes modulate cellular signaling and (ii) *trans* interactions between gangliosides on one cell and complementary binding proteins on an apposing cell regulate cell-cell interactions. Studies from a variety of disciplines, including human genetics, are beginning to reveal the functions of gangliosides in physiology and pathology.

Glycosphingolipids are biosynthesized stepwise by a suite of glycosyltransferases (Fig. 1). Ganglioside biosynthesis starts with the addition of glucose to ceramide by ceramide glucosyltransferase, the product of the *UGCG* gene. The first sialic acid of major brain gangliosides is added by GM3 synthase, the product of the *ST3GAL5* gene. Most ganglioside biosynthetic enzymes act predominantly or uniquely on glycolipids (including *ST3GAL5*, *B4GALNT1* and *ST8SIA1*), whereas others also synthesize glycoprotein glycans. This chapter summarizes genetic, biochemical, and cellular findings that reveal the biological functions of gangliosides in axon-myelin interactions.

Mutations in Ganglioside Biosynthesis Genes Cause Neural Deficits

Hereditary spastic paraplegia is a congenital disorder characterized by slowly progressing spasticity and weakness of the lower limbs, often resulting from axonal degeneration (Boukhris et al. 2013). Genetic linkage analyses traced a locus for this disorder in nine distinct family pedigrees to *B4GALNT1* (Boukhris et al. 2013;

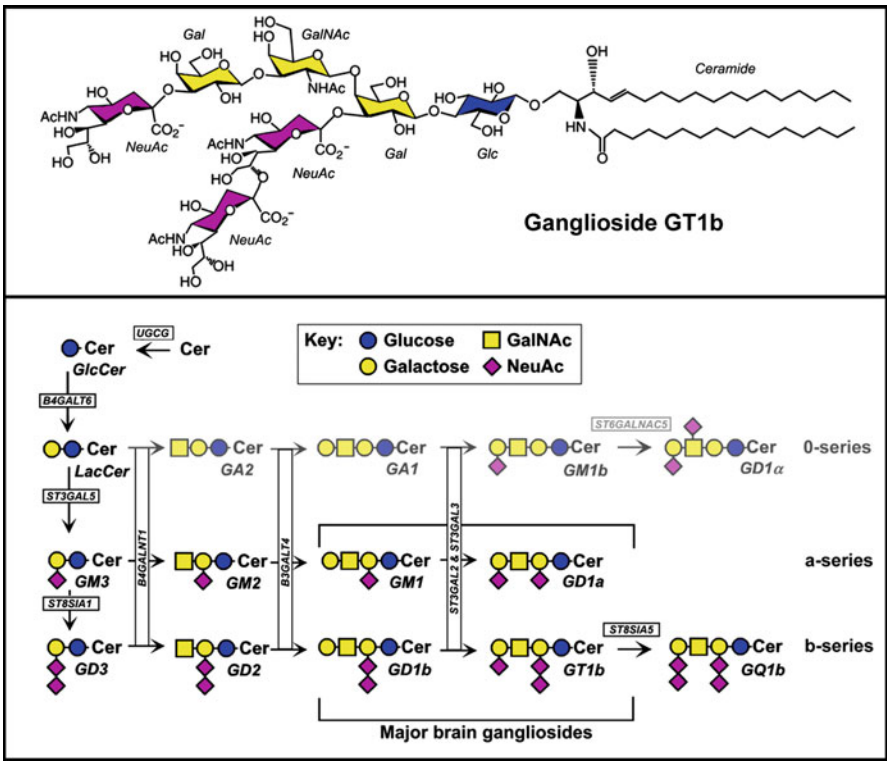

Fig. 1 *Top*: Structure of the major brain ganglioside GT1b. Other major brain gangliosides share the same neutral sugar backbone, varying only in the numbers and positions of sialic acids. *Bottom*: Brain ganglioside biosynthesis. Complex brain gangliosides are biosynthesized stepwise by the action of a suite of glycosyltransferases. The genes responsible for the expression of each glycosyltransferase are boxed. Pathways to major brain a-series and b-series gangliosides are noted, with the quantitatively minor 0-series gangliosides shown as faded structures

Harlalka et al. 2013), which encodes a glycolipid-specific N-acetylgalactosaminyl-transferase responsible for synthesis of GM2 and GD2 on the biosynthetic pathway to major brain gangliosides (Fig. 1). Based on biochemical studies of fibroblasts cultured from patients and comparable mutations in mice (see below), it is predicted that all complex brain gangliosides are diminished or absent in affected individuals, although brain ganglioside profiles have yet to be reported. Although variable and progressive lower limb spasticity and weakness primarily traced to axon degeneration are the primary symptoms that relate affected individuals in these families, each also suffers from mild to moderate intellectual disability (IQ < 70).

A more severe hereditary human disorder marked predominantly by early onset refractory epilepsy was traced by genetic linkage in three independent family pedigrees to *ST3GAL5*, which codes the sialyltransferase responsible for synthesis of GM3 (Boccuto et al. 2014; Simpson et al. 2004). In addition to severe refractory

seizures that start in the first months or years of life, each affected individual also suffers from profound motor and cognitive disability. Studies on cultured patient fibroblasts confirmed the loss of gangliosides beyond the block, although those findings are limited by the simple gangliosides of fibroblasts. Since mice compensate for the loss of this enzyme by quantitative biosynthesis of related 0-series gangliosides in the brain (see below), evaluation of brain gangliosides in affected individuals will be necessary to provide additional structure-function insight.

Mouse lines engineered to lack ganglioside biosynthetic enzymes provide further detail about ganglioside functions (Schnaar 2007). Studies of ganglioside expression in the brains of mutant mice reveal that the total brain ganglioside content remains constant despite genetic blocks in biosynthesis. For example, mutations in *B4galnt1* (see Fig. 1) result in loss of all major brain gangliosides with compensating increases in GM3 and GD3 behind the block. Likewise, mutations in *St8sia1* result in loss of all b-series gangliosides with compensating increases in a-series gangliosides. Notably, mutations in *St3gal5* block expression of GM3 and all common brain gangliosides, but this is fully compensated in mice by expression of the usually very rare 0-series gangliosides, especially GM1b and GD1α. The enzymatic mechanisms that control total ganglioside expression appear to be robust, although the mechanism of quantitative homeostasis is not known. It is also not known whether comparable quantitative compensation of total brain gangliosides occurs in human disorders of ganglioside biosynthesis.

B4galnt1-null mice, like their human counterparts, suffer from progressive hind limb weakness leading to significant motor behavioral deficits in adulthood (6–12 months of age). Histology reveals that the primary cause is progressive degeneration of central and peripheral axons. The mice have a series of deficits that point to a problem in axon-myelin interactions. Myelin, the multilayered membrane that segmentally wraps axons, has many important functions beyond insulation. Signals from myelin to axons enhance axon stability over the life of the organism, direct the membrane structure and molecular distributions at the important gaps (nodes of Ranvier) where ion channels cluster, and signal enhanced phosphorylation of axonal neurofilaments to result in increased inter-filament spacing, increased axon diameter, and enhanced axon conductivity. *B4galnt1*-null mice suffer from axon degeneration, have disrupted node of Ranvier membrane structures and molecular distributions, and have collapsed spacing of neurofilaments and reduced axon diameters. Together, these anatomical deficits are consistent with motor deficits in both mice and humans.

The molecular mechanisms responsible for these changes have been traced, in part, to the role of axonal gangliosides as receptors for myelin-associated glycoprotein (MAG) in cell-cell recognition. MAG is a sialic acid-binding lectin of the Siglec family. It is expressed only by myelinating cells – oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system – and is preferentially expressed on the innermost wrap of myelin directly across from the axon surface. Molecular recognition studies indicate that MAG binds

robustly to the terminal glycan structure of GD1a and GT1b (NeuAcα2-3Galβ1-3GalNAc), but fails to bind to GM1 and GD1b which lack that particular terminal sialic acid. *B4galnt1*-null mice, lacking that terminus, and *Mag*-null mice have similar axon-myelin anatomical and functional deficits, supporting the conclusion that MAG-ganglioside binding is important for optimal axon-myelin recognition and long-term axon health (Pan et al. 2005). Notably, these mice are also hyperactive and become seizure prone as they age, phenotypes that are not associated with axon-myelin disruption yet reflect symptoms in human congenital disorders of ganglioside biosynthesis. The mechanistic bases for these additional symptoms have yet to be determined.

St3gal5-null mice, unlike their human counterparts, do not suffer from seizures or apparent motor or cognitive deficits. As noted above, they do fully compensate loss of all normal brain gangliosides by expressing equivalent amounts of GM1b and GD1α. Mechanistically, GD1α is an excellent MAG-binding ganglioside and so could fully serve the functions of missing GD1a and GT1b. Whether other functions of gangliosides are likewise compensated in these mice and whether humans with mutations in *ST3GAL5* expresses comparable levels of 0-series gangliosides have not been determined. Relevant to these considerations is the phenotype of "GM3 only" mice, a cross between *B4galnt1* null and *St8sia1* null. These double-null mice build up GM3 behind the dual block (with no 0-series gangliosides) and suffer from early lethal audiogenic seizures. Mice that are double null for *B4galnt1* and *St3gal5* lack all gangliosides (a-, b-, and 0-series) and fail to compensate with alternative gangliosides or other sialylated brain glycolipids. These "ganglioside-null mice" were short-lived and had marked degeneration of myelinated axons, malformed nodes of Ranvier, and severe hind limb weakness. Their phenotype indicates early defects in axon-myelin interactions that are more severe than those in partial ganglioside knockout mice.

Together, genetic findings in humans and mice reveal that gangliosides (especially GD1a and GT1b) are important for axon-myelin interactions and also function in brain circuitry related to excitatory neurotransmission and seizure. Whereas the paraplegia (motor deficits) related to altered ganglioside expression may be due to the roles of GD1a and GT1b as receptors for MAG in axon-myelin interactions, the role of gangliosides in dysregulation of excitatory neurotransmission has yet to be mechanistically determined.

Gangliosides and the Control of Axon Regeneration

The long-term stability of axons is essential for lifelong nervous system function. However, the same molecular and cellular signals that ensure stability can restrict regeneration in the adult mammalian CNS. Typical CNS injuries, such as traumatic spinal cord injury, result in pinching and transection of axons which quickly reseal. Although the axons are capable of regeneration, and the neurons from which they extend remain intact, residual myelin components that persist at CNS injury sites

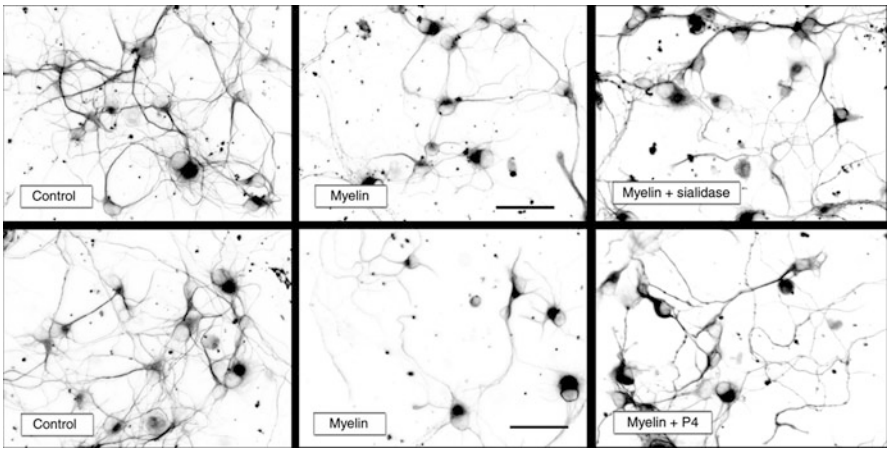

Fig. 2 Myelin inhibition of neurite outgrowth from cultured hippocampal neurons is reversed by sialidase and by the glycosphingolipid biosynthesis inhibitor P4. Hippocampal neurons from newborn rats were cultured on control surfaces or the same surfaces adsorbed with detergent-extracted myelin proteins (myelin). One hour after plating, cultures were treated with 8 mU/ml of *V. cholerae* sialidase or 1 μM of the glycosphingolipid biosynthesis inhibitor P4. After 48 h, the cultures were fixed and stained with antitubulin mAb to reveal neurites. Representative fluorescent micrographs are presented as reverse *gray* scale images to enhance clarity (bar, 100 μm) (Images used, with permission, from Mehta et al. 2007)

provide signals to the transected axons to halt outgrowth. One of the molecular inhibitors on residual myelin is MAG, and among its inhibitory receptors on axons are gangliosides (Schnaar and Lopez 2009).

Direct evidence for the function of gangliosides in axon outgrowth inhibition comes from studies performed on neurons cultured in vitro (Mehta et al. 2007). Whereas appropriately cultured neurons on control surfaces extend long neurites onto the substratum, those cultured on myelin-coated surfaces are significantly inhibited (Fig. 2). Outgrowth inhibition is partly or completely reversed by addition of sialidase, which cleaves the key terminal sialic acid from GT1b and GD1a, or by P4 (1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol), which blocks glycosphingolipid biosynthesis. MAG-mediated inhibition is also diminished in neurons from *B4galnt1*-null mice and by addition of synthetic sialoglycan mimics that block MAG-ganglioside binding. These data implicate MAG-ganglioside recognition in the control of axon outgrowth and in axon outgrowth inhibition after CNS injury.

Based on these in vitro data, a glycoscience-based strategy for enhancing axon outgrowth after CNS injury was developed (Mountney et al. 2010). Highly purified expressed bacterial sialidases were delivered via implanted pump to the site of spinal cord injuries. In multiple studies using two models of injury in the rat – brachial plexus avulsion and spinal cord contusion – delivery of sialidase significantly enhanced axon outgrowth and motor recovery. Although sialidase

may have effects on sialoglycoproteins as well as gangliosides, the data are consistent with a functional role for gangliosides in the control of axon outgrowth after injury.

Summary

Gangliosides are the major sialoglycans in the brain and are prominent molecular determinants on the surfaces of all vertebrate neurons. Human congenital disorders of ganglioside biosynthesis and related mouse genetic models, along with biochemical and cellular investigations, reveal diverse functions of gangliosides in the brain. Among these, gangliosides play important roles in axon-myelin stabilization and the control of axon regeneration via binding between gangliosides GD1a and GT1b and myelin-associated glycoprotein. These findings are consistent with human paraplegia resulting from altered ganglioside biosynthesis. However, these human disorders reveal other roles for gangliosides, including control of excitatory neurotransmission and cognition. Additional studies are needed to provide a fuller understating of the roles of ganglioside in multiple brain functions.

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