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MINI-REVIEW

Simplifying complexity: genetically resculpting glycosphingolipid synthesis pathways in mice to reveal function

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Abstract Glycosphingolipids (GSLs) are a group of plasma-membrane lipids notable for their extremely diverse glycan head groups. The metabolic pathways for GSLs, including the identity of the biosynthetic enzymes needed for synthesis of their glycans, are now well understood. Many of their cellular functions, which include plasma-membrane organization, regulation of cell signaling, endocytosis, and serving as binding sites for pathogens and endogenous receptors, have also been established. However, an understanding of their functions *in vivo* had been lagging. Studies employing genetic manipulations of the GSL synthesis pathways in mice have been used to systematically reduce the large numbers and complexity of GSL glycan structures, allowing the *in vivo* functions of GSLs to be revealed from analysis of the resulting phenotypes. Findings from these studies have produced a clearer picture of the role of GSLs in mammalian physiology, which is the topic of this review.

Keywords Glycosphingolipids · Glycosyltransferases · Gangliosides · Mouse models · Gene targeting

Introduction

Glycosphingolipids (GSLs) constitute the most structurally diverse subgroup of the sphingolipid family [1]. They contain a hydrophilic glycan head group, consisting of one or more oligosaccharide residues, and a hydrophobic ceramide anchor. The GSLs reside primarily in plasma membranes, with the ceramide anchor embedded in the lipid bilayer and the oligosaccharide head group exposed to the extracellular environment.

Dedicated GSL synthesis is initiated by the transfer of either a glucose or a galactose moiety in β-linkage to the position 1 hydroxyl group of ceramide, resulting in the formation of glucosylceramide (GlcCer) or galactosylceramide (GalCer) (Fig. 1). The subsequent addition of galactose to GlcCer forms lactosylceramide (LacCer). LacCer serves as a hub in the pathway in which the substrate can be diverted into one of several pathways by the actions of distinct glycosyltransferases that lead to the formation of GSL subfamilies, with distinct core or root carbohydrate sequences, known as GSL series (Fig. 1). GalCer is also modified similarly subsequent to its synthesis, but the complexity the GalCer-based GSLs (gala series) is far less than that of the GlcCer-based GSLs.

When the oligosaccharide head groups of GSLs are modified by the addition of one or more sialic acids (usually α-2,3- or α-2,8-linked *N*-acetylneuraminic acid), the GSLs produced are termed gangliosides [2] (Fig. 1). GSL head groups can also be modified by sulfate and are called sulfatides. All told, the number of GSLs with distinct oligosaccharide structures has been estimated to be greater than 400 [1].

Some of the mechanisms by which GSLs exert their functions at a cellular and molecular level have been well defined [1, 3–5]. Their most basic function is as building blocks of the plasma membrane. In this case, GSLs, due to their ability to form liquid-ordered phases in membranes, can drive the formation of functionally and structurally discrete plasma-membrane domains [3, 6]. GSLs, through their glycan head groups, can act as binding sites for pathogens and toxins [7] and, in a similar manner, for endogenous molecules, such as myelin-associated glycoprotein, to facilitate cell-to-cell interactions [8]. Through lateral interactions with membrane proteins, such as the insulin and epidermal growth factor receptors, GSLs can modulate the strength of receptor-mediated signaling pathways [9, 10]. Finally, they can function

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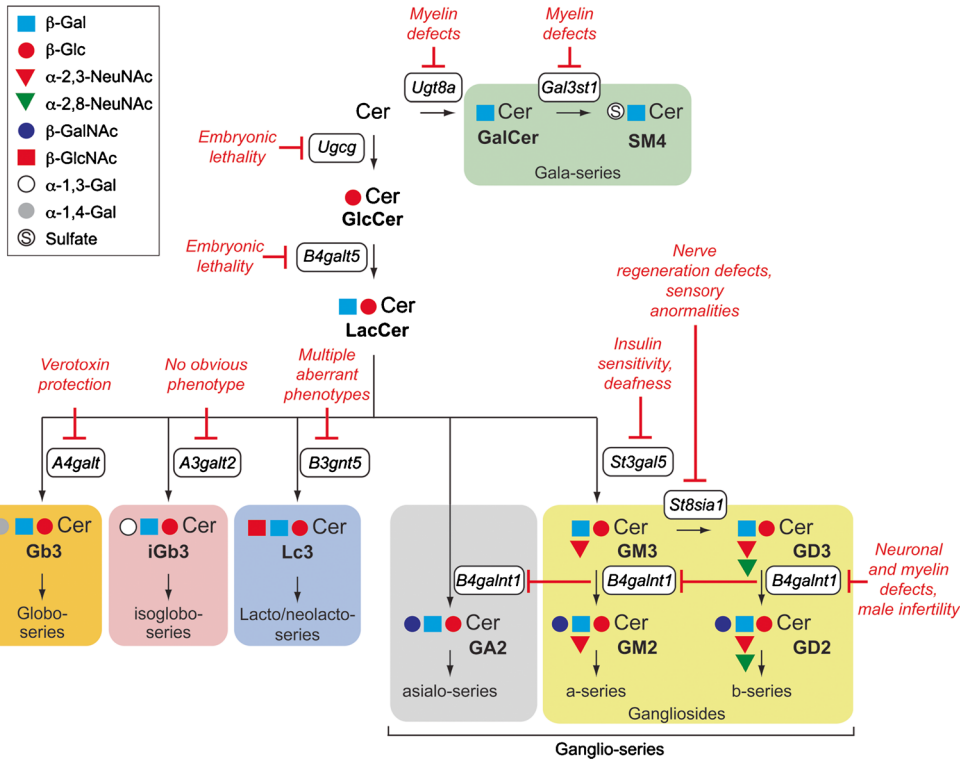

Fig. 1 GSL synthesis pathways. Genes that have been targeted are in the rounded boxes. The major mouse phenotypes caused by genetic blocks in the GSL pathways are indicated in red text. For a description of the complete pathways, see Merrill [1]. *Cer*, ceramide; *GalCer*, galactosylceramide; *GlcCer*, glucosylceramide; *LacCer*, lactosylceramide; *NeuNAc*, neuraminic acid

dynamically in the endocytic pathway by participating in the internalization of diverse cargoes [11].

This review focuses on GSL functions at a physiological level in mammals. A major challenge in this area has been to uncover their actions in the midst of the enormous structural variation of the glycan head groups that may be a source of the GSLs' functional diversity. This hurdle has been overcome by the systematic elimination of diverse GSL glycan structural groups in genetic deletion studies. Through the targeting of specific glycosyltransferases and other modifying enzymes, either singly or in combination and in a tissue-specific fashion—in effect simplifying the complexity of the GSLs—their *in vivo* functions have been elucidated from analysis of the resulting phenotypes.

Global deletion of glucosylceramide-derived GSLs (*Ugcg* knockout)

UDP-glucose:ceramide glycosyltranferase (GlcCer synthase) is the rate-limiting enzyme for GlcCer-based GSL biosynthesis. It is encoded by the *Ugcg* gene in the mouse [12, 13]. GlcCer synthase catalyzes the synthesis of GlcCer by the transfer of a glucose residue from UDP-glucose to ceramide. Once formed, GlcCer is modified by glycosyltransferases in the Golgi, ultimately giving rise to hundreds of more complex GSLs [1] (see Fig. 1).

Global deletion of the *Ugcg* gene in mice, which consequently eliminates all GlcCer-based GSLs, caused early embryonic lethality [14]. *Ugcg* knockout (KO) embryos developed until gastrulation at E6.5, forming the ectodermal, endodermal, and mesodermal layers, and with embryonic patterning. A day later, mutant embryos showed retarded growth and extensive apoptosis, primarily in the ectodermal layer, and were subsequently resorbed. These results are a vivid demonstration that GlcCer-based GSL synthesis is an absolute requirement for mammalian embryonic development [15].

Pan nervous-system deletion of GlcCer GSLs

GSLs, primarily in the form of gangliosides, are abundantly expressed in the nervous system, making up about 10 % of the total lipids and suggesting a vital role during the formation of and/or function in these tissues [5, 16]. In order to determine the importance of these lipids during the formation of a functional nervous system, two independently derived lines of mice lacking *Ugcg* in neuronal and glia cells were established by combining a floxed *Ugcg* allele and a *Cre* recombinase transgene under the control of the pan nervous system rat nestin promoter and enhancer (*NesCre*) [17, 18]. *NesCre-Ugcg* KO mice did not show evidence of embryonic lethality and appeared normal at birth. However, they developed neurologic

abnormalities over time. The severity of the phenotypes in the two lines was different and appeared to be related to the level of residual *Ugcg* expression found in the respective *NesCre-Ugcg* KO brains. The mouse line produced by Jennemann *et al*. [18], in which the levels of *Ugcg* mRNA and gangliosides in brain were nearly completely eliminated, died by 3 weeks of age, and displayed very severe neurological symptoms, including ataxia, reduced axonal branching of neurons, and disturbed myelination of peripheral nerves. The line generated in the Proia laboratory [17] showed residual *Ugcg* expression, a significant, but incomplete reduction of ganglioside levels in the brain, as well as a longer life span. These mice also displayed abnormal gait, along with a progressive loss of Purkinje cells [18].

These reports show that GlcCer-based GSL synthesis is expendable for embryonic development of the nervous system, but is required for its proper development, stability, and function after birth.

Neuronal-specific deletion of GlcCer-based GSLs

The deletion of *Ugcg* specifically in neuronal cells was achieved by generating floxed *Ugcg* mice expressing the *Cre* recombinase gene under the L7 promoter only in Purkinje cells [19]. These mice, showed Purkinje cell degeneration at about 3–4 months of age, which was characterized by axon swelling and accumulation of axonal transport cargos prior to Purkinje cell loss. Significant abnormal myelination was present, as were detached paranodal junctions (the attachment sites of neuronal axons and oligodendrocytes), implying that the neuronally synthesized GSLs were critical for these axonal-glial interactions.

In a separate study, *Ugcg* was deleted after birth using an inducible *Cre* recombinase (*Cre-ERT2*) under the control of the neuronal calcium/calmodulin-dependent kinase II α promoter (CamK). In these mice, expression of the *Ugcg* gene was deleted from the neurons of the forebrain, including the hypothalamic nuclei, which are involved in energy homeostasis regulation. *CamKCre-ERT2-Ugcg* KO mice showed progressive body-weight gain, hypometabolism, and hypothermia. This phenotype could be reversed by virus-mediated expression of *Ugcg* in the arcuate nucleus (Arc) of the hypothalamus, a key regulator of feeding and metabolism. Leptin signaling, which is essential for metabolic homeostasis, was impaired in Arc neurons from the *CamKCre-ERT2-Ugcg* KO mice. The basis for the impaired leptin responsiveness in the Arc neurons appeared to be due to a lack of GlcCer-based gangliosides, which normally directly associate with the leptin receptor on the plasma membrane and promote leptin responsiveness [20].

Oligodendrocyte-specific deletion of GlcCer-based GSLs

Ugcg was deleted specifically in oligodendrocytes by crossing floxed *Ugcg* mice with a line expressing *Cre* recombinase under the control of the myelin-associated enzyme 2′, 3′ cyclic nucleotide 3′ phosphodiesterase (*CnpCre*) [21]. The resulting oligodendrocyte-specific *CnpCre-Ugcg* KO mice did not show any myelin abnormalities, indicating that oligodendrocyte GlcCer-derived GSLs, unlike those produced by neuronal cells, are not essential for myelin structure and stabilization.

Keratinocyte-specific deletion of Glc-Cer based GSLs

Keratinocytes produce large amounts of GSLs, mainly GlcCer, accounting for almost 4 % of the total lipid content in the epidermis, which is packed into lamellar bodies and delivered to the stratum corneum for further processing to generate ceramide [22, 23]. Floxed *Ugcg* mice were crossed with a keratin K14 promoter-driven *Cre* line to produce mice lacking *Ugcg* expression in keratinocytes (*K14Cre-Ugcg* KO mice) [24]. *K14Cre-Ugcg* KO pups showed profound desquamation and epidermal dehydration and died at about 4 days of age. Their skin showed broadened epidermal layers, abnormal lamellar bodies, keratinocyte apoptosis, and inflammation. When epidermal deletion was induced after birth using a tamoxifen-inducible (TAM) *K14Cre* transgene to bypass the early lethality, *TAM-K14Cre-Ugcg* KO mice showed epidermal abnormalities similar to the *K14Cre-Ugcg* KO mice [25]. These studies supported the conclusion that *Ugcg* expression is essential for the formation of a functional epidermal permeability barrier through the generation of GlcCer, a precursor for the formation of ceramide in the skin.

Liver-specific deletion of Glc-Cer based GSLs

Liver is a central site controlling sphingolipid metabolism [26]. A liver-specific knockout of *Ugcg* was generated by crossing floxed *Ugcg* mice with mice expressing *Cre* recombinase under the control of the albumin promoter (*AlbCre-Ugcg* mice) [27]. The mice had greatly reduced GSL levels in liver and plasma, pinpointing liver as the major source of plasma GSLs. No other phenotype was detected in the *AlbCre-Ugcg* mice, even after challenge with a high-fat diet, arguing against a vital role of hepatic GlcCer synthesis in glucose control, liver steatosis, or cholesterol metabolism.

Enterocyte-specific deletion of Glc-Cer based GSLs

GSLs are highly concentrated in the columnar epithelial cells that form the villi in the intestine, known as enterocytes.

Enterocyte-specific *Ugcg*-deficient mice were generated by crossing floxed *Ugcg* mice with mice expressing *Cre* recombinase under the control of the villin promoter induced either in the embryo (using *VilCre*-expressing mice) or during adult life (using *VilCre-ERT2*-expressing mice) [28]. GlcCer-derived GSLs were profoundly absent in the intestines of the *VilCre-Ugcg* pups. Although undistinguishable from control littermates at birth, *VilCre-Ugcg* pups did not gain weight and died by postnatal day 8. *VilCre-Ugcg* pups showed normal polarization of the enterocytes, but had abnormal mucosal villi, decreased fat deposits, and a severe decrease in the ability of enterocytes to uptake lipid. Inducible *VilCre-ERT2-Ugcg* mice showed an almost complete absence of intestinal GSLs 4 days after deletion induction, which was concomitant with significant structural alterations in the villous epithelia. These mice also had decreased body weight and reduced lipid absorption by the enterocytes. These findings demonstrate that GlcCer-derived GSLs are necessary for proper intestinal function.

Deletion of lactosylceramide-based GSLs: *B4galt5* KO

LacCer is synthesized in the Golgi lumen by LacCer synthase (β-1,4-galactosyltransferase 5) via the transfer of galactose from UDP-galactose to GlcCer. *B4galt5* null mice were generated by conventional gene targeting [29] and through a large-scale gene-trap project [30]. Both gene targeting strategies led to lethality in utero. The *B4galt5*$^{-/-}$ embryos appeared normal until E6.5, showed retarded growth by E7.5, and died around E10.5. *B4galt5*$^{-/-}$ embryos showed bleeding in the extra-embryonic tissues at E7.5 [29]. The early lethality of the *B4galt5*$^{-/-}$ embryos could be rescued by wild-type extra-embryonic tissues; however, these embryos did not develop past E18.5, indicating that *B4galt5* has a direct role in later embryonic development within the embryo proper [29].

Deletion of ganglio-series GSLs: *B4galnt1* KO

GM2/GD2 synthase (β-1,4-*N*-acetyl-galactosaminyltransferase), which is encoded by *B4galnt1*, transfers *N*-acetylgalactosamine to LacCer to initiate the synthesis of ganglio-series GSLs, including the sialic acid-containing gangliosides (Fig. 1) [31]. The genetic deletion of *B4galnt1* resulted in mice lacking the normal complement of complex gangliosides and instead expressing predominantly GM3 and GD3 (Fig. 1) [32, 33]. Despite the drastic change in the ganglioside composition, *B4galnt1*$^{-/-}$ mice unexpectedly underwent largely normal brain development.

However, *B4galnt1*$^{-/-}$ mice were found to display decreased myelination of central axons, as well as demyelination of peripheral nerves [33]. As a consequence, axon degeneration occurred within the central nervous system, leading to impaired motor function [33, 34]. Nodes of Ranvier showed abnormal structures in *B4galnt1*$^{-/-}$ mice, causing defects in conduction velocity of the motor nerves [33]. One of the functions of gangliosides within the axonal membrane is as ligands for MAG displayed on oligodendrocytes to promote axon-glia interactions [35]. Indeed, *B4Galnt1* −/− mice showed a decrease in MAG expression in the brain [33] and MAG deficient mice displayed a similar phenotype as *B4Galnt1* −/− mice [35]. These findings suggest that the lack of complex ganglioside-MAG interactions was a cause of the *B4Galnt1* null phenotype and demonstrate an essential role in myelin stabilization. Another independently generated *B4galnt1*$^{-/-}$ mouse line showed subtle defects in the nervous system, with a slight reduction in the neural conduction velocity and a defect in cognitive performance [36]. These findings indicate an essential role for gangliosides in myelin stabilization and for proper neuronal function.

The discovery that *B4galnt1*$^{-/-}$ male mice are infertile revealed an important function of complex gangliosides in spermatogenesis [32, 33, 37]. The absence in the *B4galnt1*$^{-/-}$ male mice of a novel class of fucosylated, polyunsaturated GSLs with highly elongated fatty acids typically present in male germ cells was linked to their infertility [38].

Deletion of a-series gangliosides: *St3gal5* KO

GM3 synthase (CMP-sialic acid:lactosylceramide α-2,3-sialyltransferase) is encoded by *St3gal5*. The enzyme transfers sialic acid to LacCer to produce GM3, the simplest of the sialic acid-containing GlcCer-based gangliosides and a precursor for complex gangliosides [2].

St3gal5-deficient mice had normal life span [39]. Analysis of their ganglioside content demonstrated the absence of GM3 and all GM3-derived gangliosides from the a- and b- series (Fig. 1), which are the major gangliosides in the brain [2].

GM3 synthase deletion increased insulin sensitivity; that is, *St3gal5*$^{-/-}$ mice had enhanced phosphorylation of the insulin receptor in skeletal muscle after insulin binding, and better responses in glucose- and insulin-tolerance tests. Accordingly, they were also protected from insulin resistance development after a high-fat diet regimen [39].

Diabetic diet-induced obese *St3gal5*$^{-/-}$ mice were also found to be protected from a wound-healing defect. Wound healing generally involves proliferation and migration of keratinocytes, which are both mediated by the insulin receptor and insulin growth factor-1 receptor. Because diabetic obese *St3gal5*$^{-/-}$ mice indeed had activated insulin receptor and insulin growth factor-1 receptor signaling, particularly in the presence of high glucose, the wound-healing defects observed in wild-type diabetic obese mice were obviated [40].

An independently generated line of *St3gal5*$^{-/-}$ mice exhibited complete hearing loss [41]. The *St3gal5*$^{-/-}$ mice showed degeneration and disappearance of the organ of Corti, which is the hearing sensor in the cochlea. These results suggest that GM3 synthesis is essential for the early maturation of the cochlea and that it is important in development of the auditory system.

Deletion of b-series gangliosides: *St8sia1* KO

GD3, the precursor of the di-sialo b-series gangliosides (Fig. 1), is generated by the addition of sialic acid to GM3 by GD3 synthase (CMP-sialic acid: GM3 α-2,8-sialyltransferase), encoded by the *St8sia1* gene. Despite the absence of the normally prominent b-series gangliosides in the brain, the deletion of the *St8sia1* gene did not cause apparent developmental abnormalities in the nervous system [42, 43]. Adult *St8sia1*$^{-/-}$ mice were found to be viable and grossly normal.

A second independently derived line of *St8sia1*$^{-/-}$ mice exhibited reduced regeneration capacity of the hypoglossal nerve, showing fewer surviving neurons after resection of the nerve [42]. In addition, *St8sia1*$^{-/-}$ mice displayed increased sensory responses to thermal and mechanical stimuli and decreased response to subcutaneous formalin injection, which is a measure of clinical pain, suggesting GD3-based gangliosides mediate transmission of pain and might be important in the sensory system [44].

"GM3-only" mice: *St8sia1*/*B4galnt1* double knockout

St8sia1/*B4galnt1* double knockout (DKO) mice were generated by crossing animals carrying the *St8sia1* and *B4galnt1* null alleles [43]. Homozygous DKO mice were viable and expressed only GM3 as a GlcCer-based ganglioside in their brains. Strikingly, these DKO mice were extremely susceptible to sound-induced lethal seizures, and most died by 3 months of age [43]. A separate DKO line did not display any seizure-related phenotype, although these mice had decreased weight, motor dysfunction, low fear stress, and reduced sensory responses due to peripheral nerve degeneration [45]. These DKO mice also acquired severe skin lesions, possibly caused by the reduced sensory function [46]. These findings show that complex ganglioside expression is essential to maintain the normal function of the central and peripheral nervous systems, and that these functions of complex ganglioside structures cannot be replaced by the expression of the simple ganglioside GM3.

"Ganglioside-less" mice: *St3gal5*/*B4galnt1* DKO

Phenotypes seen in *B4galnt1*$^{-/-}$ or *St8sia1*/*B4galnt1* DKO mice demonstrated that expression of even simple gangliosides such as GM3 and GD3 were sufficient for the development of a functioning nervous system, although with some defects. To answer if gangliosides were truly essential for nervous-system development, the elimination of all GlcCer-based gangliosides was achieved by crossing mice carrying null mutations in the *St3gal5* and *B4galnt1* genes (Fig. 1) to create *St3gal5*/*B4galnt1* DKO mice. These DKO mice were initially viable but soon developed hindlimb weakness, tremors, and ataxia; most died within 3 months of birth [47]. As expected, and in accordance with the proposed biosynthetic pathway, these DKO mice were unable to synthesize any gangliosides of the ganglio-series and instead accumulated LacCer. DKO mice showed severe neurodegenerative symptoms, including a smaller brain and prominent areas of vacuolization in the spinal cord and brain white matter. Myelinated axons were degenerated in these mice, and showed abnormalities of paranodal junctions at the nodes of Ranvier, which suggests impairment of the axon-glia interactions. These findings indicate that GlcCer-based gangliosides are not required for early neural differentiation or morphogenesis of the brain, but are critical for promoting the formation or stabilization of functional axon-glia interactions. The absence of gangliosides ultimately leads to axonal degeneration.

Deletion of the globo-series of GSLs: *A4galt* KO

Gb3 synthase (α-1,4-galactosyltransferase), which is encoded by the *A4galt* gene, is responsible for the synthesis of Gb3 (Galα1–4Galβ1–4Glcβ1Cer) by the addition of an α-1,4-linked galactose to LacCer, initiating the production of the globo-series of GSLs. *A4galt*$^{-/-}$ mice were deficient in Gb3 and other globo-series GSLs [48]. These KO mice appeared normal and lacked any obvious phenotype. Gb3 has been reported to be a ligand for the bacterial endotoxins Shiga-like and verotoxins, which are associated with hemorrhagic colitis and hemolytic uremic syndrome. Accordingly, *A4galt*$^{-/-}$ mice were found to be protected from the effect of these toxins, clearly demonstrating that globo-series GSLs are endogenous ligands for these toxins [48].

Deletion of the isoglobo-series of GSLs: *A3galt2* KO

iGb3 synthase (α-1,3-galactosyltransferase), which is encoded by the *A3galt2* gene, initiates the formation of the isoglobo-series of GSLs with the synthesis of iGb3 (Galα1–3Galβ1–4Glcβ1Cer) from LacCer by the addition of an α-1,3-linked galactose (Fig. 1). Deletion of the *A3galt2* gene

eliminated iGb3 and the other isoglobo-series GSLs derived from iGb3 [49]. Similar to the globo-series null mice, *A3galt2*$^{-/-}$ mice did not display any obvious phenotype. Although iGb3 was thought to be an endogenous ligand for the invariant Vα14-expressing natural killer T (NKT) cells, thereby regulating their development in thymus [50], *A3galt2*$^{-/-}$ mice showed normal NKT numbers and function [49]. This result suggests that this GSL species may not be a relevant ligand for thymic NKT cells *in vivo*.

Deletion of the lacto/neolacto-series of GSLs: *B3gnt5* KO

Lc3 synthase (β-1,3-*N*-acetylglucosaminyltransferases 5), which is encoded by *B3gnt5*, produces Lc3 (GlcNAcβ1–3Galβ1–4Glcβ1Cer), initiating the lacto/neolacto-series of GSLs (Fig. 1), with the transfer of N-acetylglucosamine to LacCer. Three independent groups have reported targeting the *B3gnt5* gene in mice, with different outcomes. Genetic deletion of the *B3gnt5* gene in mice by Biellmann *et al.* [51] caused embryonic lethality at the preimplantation stage, possibly due to defects in cell-cell adhesion that contribute to embryo compaction and implantation. The line generated by Togayachi *et al.* produced viable *B3gnt5*$^{-/-}$ mice that showed a defect in B-cell signaling due to abnormal raft formation [52]. Kuan *et al.* [53] developed *B3gnt5*$^{-/-}$ mice by two different strategies. They reported that *B3gnt5*$^{-/-}$ mice showed splenomegaly and enlarged lymph nodes, and had shorter, variable life spans. They also had lower B-cell numbers, exhibited hair loss, were obese, and had reproductive defects. The reasons for the discrepancies among the phenotypes of the *B3gnt5*$^{-/-}$ mouse lines in the three reports are not apparent.

Deletion of GalCer-based GSLs: *Ugt8a* KO

Galactosylceramide synthase (UDP-galactose:ceramide galactosyltransferase), encoded by the *Ugt8a* gene, catalyzes the formation of GalCer by the attachment of a galactose residue to the ceramide backbone (Fig. 1) [13, 54]. GalCer and its sulfated derivative, GalCer-sulfate or SM4 (Fig. 1), are greatly enriched in the myelin sheath that covers axons, representing nearly 30 % of the total lipid [55]. Two independent groups generated *Ugt8a*-deficient mouse lines through conventional targeting of the *Ugt8a* gene [55, 56]. Homozygous *Ugt8a*$^{-/-}$ mice were initially viable, but were small and had shortened life spans. They also exhibited tremors, loss of locomotor activity, and disruption of nerve conduction. Surprisingly, *Ugt8a*$^{-/-}$ mice were able to form myelin sheaths lacking GalCer and its derivative SM4, but instead containing GlcCer, a GSL not normally abundant in myelin. The myelin structure of the *Ugt8a*$^{-/-}$ mice initially appeared normal, except for slightly thinner sheaths in some areas of the spinal cord. Later, the myelin became unstable and degenerated. Together, these studies demonstrated that GalCer synthesis is needed for long-term maintenance of the structure and function of the myelin.

Interestingly, restoration of oligodendrocyte *Ugt8a* expression was able to correct the aberrant phenotype in *Ugt8a*$^{-/-}$ mice, including normalizing life span, behavior, and myelin structure [57]. These results indicate that the loss of *Ugt8a* in oligodendrocytes was responsible for the myelin defects and altered behavior described for *Ugt8a*$^{-/-}$ mice.

Deletion of sulfated GalCer: *Gal3st1* KO

Gal3st1 encodes for the glycolipid-specific GalCer sulfotransferase [58], which adds a sulfate group to GalCer to form SM4 which is incorporated into myelin (Fig. 1) [59–61]. To determine the role of sulfated GSL *in vivo*, mice with a deletion of *Gal3st1* were generated [62]. *Gal3st1*$^{-/-}$ mice were normal at birth, but as they aged they developed neurological symptoms (exhibiting as weakness of the hindlimbs, tremors, and progressive ataxia) However, these mice were able to survive for more than 1 year. These mice lacked SM4, but still expressed GalCer in the brain [62]. Their axons were myelinated, but the myelin showed vacuolation, and exhibited defective paranodal junctions and abnormal ion-channel localization along the axon membrane [62–65]. These results demonstrate the important role of sulfated GalCer in the formation interactions between axons and glia.

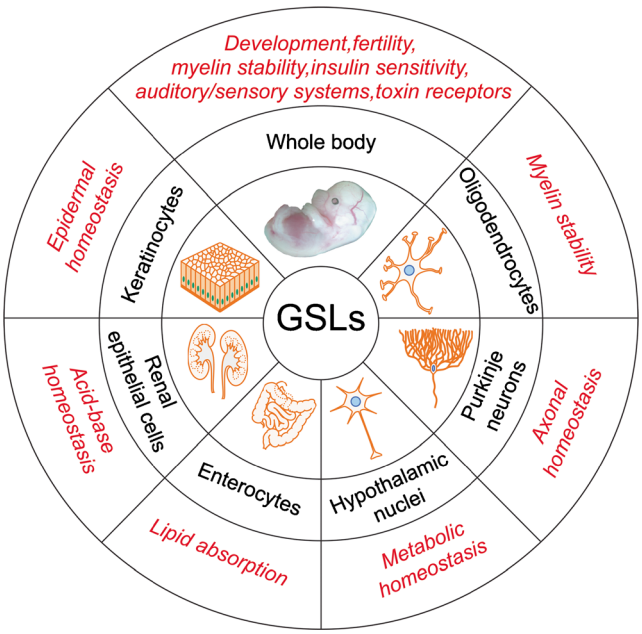

Fig. 2 *In vivo* GSL functions revealed by whole body and conditional gene deletion studies in mice

Sulfated GSLs are highly enriched in the kidney, especially at the distal nephron segments and in the renal medulla [61, 66, 67]. To determine the role of sulfated GSLs specifically in kidney, *Gal3st1* was deleted via renal epithelia-specific genetic disruption either individually to eliminate sulfated GSLs, or in combination with *Ugcg* to further remove possible compensatory anionic GlcCer-based GSLs, Mice carrying floxed *Gal3st1 and Ugcg* alleles were crossed with mice expressing the *Cre* recombinase gene under the control of the tubular epithelial cell-specific paired box gene 8 promoter (*Pax8Cre*) [68]. Kidneys from single (KO) and double KO (DKO) mice lacked the predicted sulfated GSL species in the kidney and showed generally normal kidney morphology. However, the *Pax8Cre-Gal3st1* KO and *Pax8Cre-Gal3st1*/*Ugcg* DKO mice both showed significantly lower pH and less ammonium excretion in the urine. When fed an acidic diet, the *Gal3st1*$^{-/-}$ mice exhibited decreased ammonium accumulation in the papilla, and chronic hyperchloremic acidosis. The results are consistent with the notion that sulfated GSLs in the kidney act as counterions of interstitial $NH4^{+}$, allowing its accumulation in the kidney papilla and enabling sufficient renal excretion of protons to maintain a proper blood pH [68]. The results show that sulfated GSLs are critical for physiologic acid–base homeostasis.

Conclusions and perspectives

During the period of time since the description of the first knockout mice targeting enzymes in the GSL synthesis pathway in 1996 [32, 55], we have seen a striking increase in our understanding of the roles of GSLs in physiology (Fig. 2). We have learned that they are not only fundamentally important for embryonic development, but also have key tissue-specific functions, including formation of the skin permeability barrier, acid handling in the kidney for maintenance of acid–base homeostasis, and key nervous-system functions (including in neuron-glial interaction to maintain myelin stability and neuronal control of appetite). It is likely that we have only scratched the surface of GSL functions, given the existence of hundreds of different GSL structures and that we only understand the function of a relatively very few. Furthermore, our knowledge of how GSLs act in human disease is only in its infancy. While lysosomal storage diseases in which GSL degradation pathways are disrupted have been known for many years [69], only recently have genetic diseases involving the GSL synthesis pathways been described [70–73]. A new challenge is to build on current insights into normal GSL functions (Fig. 2) to understand how GSLs play a role in the pathogenesis of other disorders. In this vein, significant progress is already being made in a number of human diseases [74–76]. These insights will eventually lead to therapeutics aimed at targeting specific GSL pathways for the treatment of these disorders.

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Conflict of interest The authors declare that they are free from conflict of interest.

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