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Review

Cell-specific *in vivo* functions of glycosphingolipids: Lessons from genetic deletions of enzymes involved in glycosphingolipid synthesis

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ABSTRACT

Glycosphingolipids (GSLs) are believed to be involved in many cellular events including trafficking, signaling and cellular interactions. Over the past decade considerable progress was made elucidating the function of GSLs by generating and exploring animal models with GSL-deficiency. Initial studies focused on exploring the role of complex sialic acid containing GSLs (gangliosides) in neuronal tissue. Although complex gangliosides were absent, surprisingly, the phenotype observed was rather mild. In subsequent studies, several mouse models with combinations of gene-deletions encoding GSL-synthesizing enzymes were developed. The results indicated that reduction of GSL-complexity correlated with severity of phenotypes. However, in these mice, accumulation of precursor GSLs or neobiosynthesized GSL-series seemed to partly compensate the loss of GSLs. Thus, UDP-glucose:ceramide glucosyltransferase (*Ugcg*), catalyzing the basic step of the glucosylceramide-based GSL-biosynthesis, was genetically disrupted. A total systemic deletion of *Ugcg* caused early embryonic lethality. Therefore, *Ugcg* was eliminated in a cell-specific manner using the cre/loxP-system. New insights into the cellular function of GSLs were gained. It was demonstrated that neurons require GSLs for differentiation and maintenance. In keratinocytes, preservation of the skin barrier depends on GSL synthesis and in enterocytes of the small intestine GSLs are involved in endocytosis and vesicular transport.

Contents

Abbreviations: APP, amyloid precursor protein; AlbCre, albumin-cre; BCR, B cell receptor; CD19, cluster of differentiation 19; *C. elegans*, *Caenorhabditis elegans*; CoA, coenzyme A; CNP, 2′,3′-cyclic nucleotide 3′ phosphodiesterase; Cst, cerebroside sulfotransferase; EGF, epidermal growth factor; Egh, glycosyltransferase egghead; ELOVL, elongation of very long chain fatty acids; ER, endoplasmic reticulum; ERM-1, ezrin–radixin–moesin protein; GalCer, galactosylceramide; GFAP, glial fibrillary acidic protein; GlcCer, glucosylceramide; GPI, Glycosylphosphatidylinositol; GSL(s), glycosphingolipid(s); iNKT, invariant natural killer T cells; K6, keratin 6; K14, keratin 14; K16, keratin 16; K17, keratin 17; LB, lamellar body; Lyn, tyrosinkinase Lyn; MAG, myelin-associated glycoprotein; mTOR, mammalian target of rapamycin; Nes, nestin; OA, osteoarthritis; PKD, polycystic kidney disease; PNS, peripheral nervous system; PSEN1, presenilin-1; SL, sphingolipid; Spt, serine palmitoyltransferase; Sptlc2, SPT long chain base 2; UDP, uridine diphosphate; Ugcg, UDP-glucose:ceramide glucosyltransferase; Vil, villin; CNS, central nervous system; ZO-1, zona occludens protein 1.

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1. Introduction

GSLs are amphiphathic molecules and consist of a hydrophilic sugar part and a lipophilic ceramide anchor. With their ceramide anchor they are integrated in the outer leaflet of the plasma membrane of all eukaryotic cells. The ceramide contains a sphingoid base linked via amide bond to a fatty acid of different chain lengths. Synthesis of all sphingoid bases starts with the condensation of L-serine and an acyl-CoA. The fatty acid constitution can vary in its length, dependent on the cell type. To the ceramide, a phosphate group, a phosphorylcholine or a monosaccharide may be added to the hydroxyl group of the sphingoid base in position 1 resulting into ceramide-1-phosphate, sphingomyelin, and glycosphingolipids, respectively (Fig. 1). Glucosylceramide synthase-catalyzed addition of UDP-activated glucose to ceramide leads to glucosylceramide (GlcCer) on the cytoplasmic surface of the Golgi [1–3]. GlcCers may be elongated with additional carbohydrates resulting into a great variety of complex sugar moieties and GSL-series. Else, UDP-activated galactose may be added to ceramide on the luminal side of the ER [3]. Acidic glycosphingolipids such as sulfatides and gangliosides are formed by addition of sulfuric- or sialic acid residues. Whereas only comparatively few GSLs are derived from galactosylceramide (GalCer), hundreds of structurally different glycosphingolipids including gangliosides and higher sulfatides are based on the glucosylceramide core structure.

GSLs are constituents of eukaryotic cell membranes. A major portion is located exclusively on the outer leaflet of the cellular plasma membrane. GSLs influence cell adhesion [4,5] and cell differentiation [6]. Intracellularly, glycosphingolipids may be important for protein and lipid trafficking [7–9]. GSLs have been described also to be located intracellularly associated with small vesicles, tubulovesicular structures, the surface of phase-dense lipid droplets, intermediate filaments of the cytoskeleton as well as with mitochondria [10]. Their subcellular localization varies in different cell types [10]. GSLs may concentrate in lipid rich domains [11], where they are supposed to participate in signaling events [12–15], modify insulin- and EGF-receptor activities [16–19] and modulate Notch ligand activity in *Drosophila* [20]. Specific GSLs function as binding ligands of bacterial toxins [21–23] and viruses [24,25]. Moreover, they were described to be associated with or enriched in certain mammalian cancers [26–28]. Consequently, GSLs were used as target molecules in immunological approaches of tumour therapy [29–33].

GSLs are degraded in the lysosomes by soluble exo-glycosidases. A defect or mutation in genes encoding sphingolipid digesting enzymes or proteins supporting sphingolipid degradation leads to the accumulation of GSLs in lysosomal compartments. GSL storage then causes severe neurodegenerative and visceral diseases known as metachromatic leukodystrophy, GM1-gangliosidosis, Fabry-, Tay-Sachs-, Sandhoff-, Gaucher-, and Krabbe disease [34].

In the present report, we focus on the consequences from deletions of enzymes involved in GSL-synthesis. The predominant database to elucidate the functions of glycosphingolipids was obtained by *in vitro* studies in cell culture. To investigate whether or not those findings could be transferred to *in vivo* situations, great efforts were undertaken to generate animal models with various deletions of genes involved in the synthesis of glycosphingolipids.

2. Genetic inhibition of GSL-series synthesis

In mammals, enzyme-catalyzed linkage of UDP-activated glucose to ceramide results in glucosylceramide. Addition of beta-galactose to GlcCer leads to lactosylceramide (LacCer). Elongation of LacCer with further sugars results in GSL series (Fig. 1[a–d]). Hence, the addition of alpha-galactose, *N*-acetylglucosamine or *N*-acetylgalactosamine correlates with synthesis of GSLs of the globo-, lacto-, or ganglio-series, respectively. During embryonic development, a switch from globo- and lacto-series GSLs to ganglio-series GSLs has been reported [35,36]. It remains enigmatic why those GSL series, leading after elongation with multiple sugars of different linkage and anomery to a complex fingerprint-like library of hundreds of different GSL-structures, are expressed in dependence on differentiation and organs. The cell-specific GSL expression might be important for cell adhesion due to possible intercellular GSL/GSL interactions [37].

2.1. Deletion of globo-series GSLs

UDP-activated galactose can either be linked as α-galactoside at position 4 or 3 to LacCer resulting in the synthesis of globo- and isoglobo-series GSLs (Fig. 1[a] and [b]). First, mice were generated with interruption of the gene encoding the enzyme globotriaosylceramide synthase, Gb_3S (*A4galt*). Although the globo-series GSLs were completely absent in *A4galt*$^{-/-}$ mice, they reached normal age and showed no obvious phenotype [38,39]. The first synthesis-product of the globo-series, Gb_3Cer/CD77, has been reported to be the binding ligand of bacterial endotoxins such as Shiga- or verotoxins [40] causing hemorrhagic colitis [41] and hemolytic uremic syndrome [42,43]. Indeed, animals lacking Gb_3S and conse-