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Review

Review

Glucosylceramide and galactosylceramide, small glycosphingolipids with significant impact on health and disease

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Abstract

Numerous clinical observations and exploitation of cellular and animal models indicate that glucosylceramide (GlcCer) and galactosylceramide (GalCer) are involved in many physiological and pathological phenomena. In many cases, the biological importance of these monohexosylcermides has been shown indirectly as the result of studies on enzymes involved in their synthesis and degradation. Under physiological conditions, GalCer plays a key role in the maintenance of proper structure and stability of myelin and differentiation of oligodendrocytes. On the other hand, GlcCer is necessary for the proper functions of epidermis. Such an important lysosomal storage disease as Gaucher disease (GD) and a neurodegenerative disorder as Parkinson's disease are characterized by mutations in the *GBA1* gene, decreased activity of lysosomal GBA1 glucosylceramidase and accumulation of GlcCer. In contrast, another lysosomal disease, Krabbe disease, is associated with mutations in the *GALC* gene, resulting in deficiency or decreased activity of lysosomal galactosylceramidase and accumulation of GalCer and galactosylsphingosine. Little is known about the role of both monohexosylceramides in tumor progression; however, numerous studies indicate that GlcCer and GalCer play important roles in the development of multidrug-resistance by cancer cells. It was shown that GlcCer is able to provoke immune reaction and acts as a self-antigen in GD. On the other hand, GalCer was recognized as an important cellular receptor for HIV-1. Altogether, these two molecules are excellent examples of how slight differences in chemical composition and molecular conformation contribute to profound differences in their physicochemical properties and biological functions.

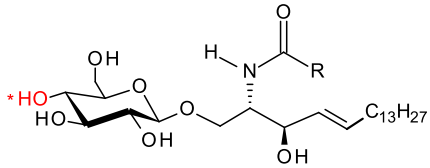
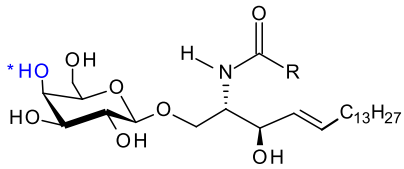
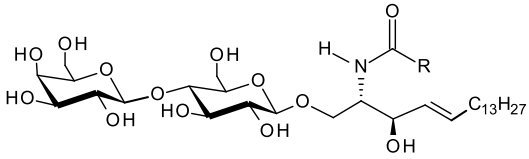
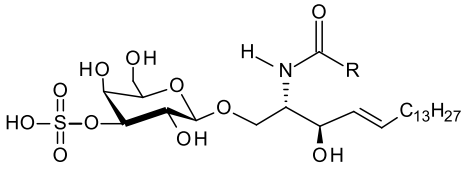
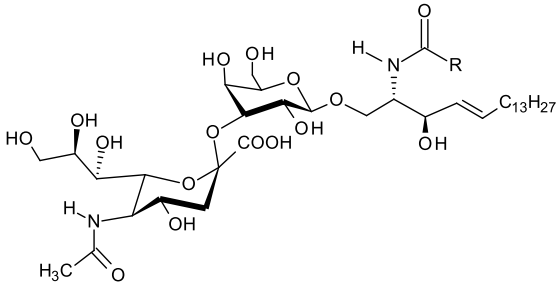
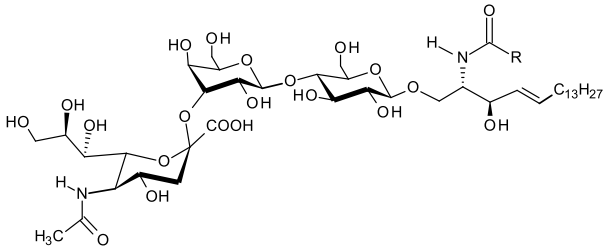
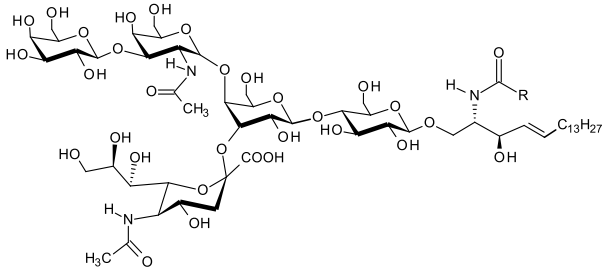
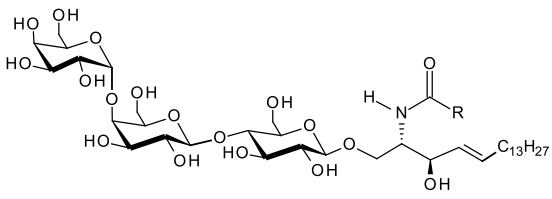
Key words: biological functions, galactosylceramide, glucosylceramide, glycosphingolipids, neurological diseases

Chemical structure, metabolism and occurrence of glucosylceramide and galactosylceramide

Glucosylceramide (GlcCer) and galactosylceramide (GalCer) are essential molecules found in three out of six kingdoms of life: animal, plant and fungus. GlcCer is a founder molecule for synthesis of hundreds of glycosphingolipids (GSLs) (Hirabayashi 2012), which are subdivided in mammals according to the sugar sequence and configuration into GSLs of ganglio-, globo-, isoglobo-, lacto- and neolacto-series (Sandhoff and Sandhoff 2018). However, GalCer may be converted only to sulphated GalCer (3-sulfo-GalCer, SM4) or sialylated GalCer (3-Neu5Ac-GalCer, GM4) or just a few galactosphingolipids (Table I) (D'Angelo et al. 2013; Schnaar and Kinoshita 2017).

GlcCer and GalCer consist, respectively, of D-glucose (Glc) and D-galactose (Gal) residue linked by a β1-1′-glycosidic bond to ceramide

Table I. Names and structures of glycosphingolipids referred in the manuscript

Full name	Abbreviation	Structure
Glucosylceramide	GlcCer	
Galactosylceramide	GalCer	
Lactosylceramide	LacCer	
3-sulfo-GalCer	SM4	
3-Neu5Ac-GalCer	GM4	
Monosialodihexosylceramide	GM3	
Monosialotetrahexosylceramide	GM1	
Globotriaosylceramide	Gb3Cer	

*The difference in the position of the hydroxyl group at the C-4 atom of Gal and Glc is pointed out by asterisk and shown, respectively, in blue and red.

(Cer) composed of D-*erythro*-sphingosine and long-chain fatty acid (Table I). Therefore, these two compounds represent very similar structures since D-galactose is an epimer of D-glucose and these two sugars differ only in the configuration at C-4. Fatty acids attached to sphingosine may vary significantly in length (C_{14}–C_{26}), with stearic acid (C_{18}) being the most abundant. The galactosylceramides are enriched in very-long-chain α-hydroxy fatty acids (C_{18}–C_{26}), whereas glucosylceramides consist of ceramides with primarily nonhydroxylated shorter chain fatty acids (usually C_{16} or C_{24}) (Schweppe et al. 2010). Various fatty acid residues have different biological roles, e.g., affecting the localization and trafficking of GSLs. The presence of monohexosylceramides with specific fatty acid residues is cell- and/or tissue-specific, which is determined by the expression of specific ceramide synthase/synthases (Levy and Futerman 2010).

Synthesis of GlcCer and GalCer starts with the generation of Cer in endoplasmic reticulum (ER) (Hannun and Obeid 2018; Ogretmen 2018). Transfer of Glc from UDP-Glc on ceramide is catalyzed by UDP-glucose: *N*-acetylsphingosine D-glucosyltransferase (EC2:4.1.80), also known under the names: glucosylceramide synthase (GCS, GlcCer synthase), ceramide glucosyltransferase (CGT) or UDP-glucose: ceramide glucosyltransferase (UGCG, GLCT-1) (Basu et al. 1968; Paul et al. 1996; Ishibashi et al. 2013) (Figure 1). The enzyme is encoded by the *UGCG* gene. The synthesis of GlcCer takes place on the cytosolic side of the Golgi apparatus (Coste et al. 1986; Futerman and Pagano 1991; Jeckel et al. 1992), as glucosylceramide synthase (GCS) (this name and acronym was chosen since it is the most frequently used), along with *O*-GlcNAc transferase (Ong et al. 2018), is the only glycosyltransferase, whose active site is located on the cytoplasmic side of the Golgi (Ichikawa et al. 1996a). GCS is localized on the pre-Golgi, and *cis*-, *medial*- and *trans*-Golgi membranes (Futerman and Pagano 1991; Jeckel et al. 1992) as well as perinuclear ER (Kohyama-Koganeya et al. 2004). It is generally accepted that GlcCer synthesized in the *cis*-Golgi is transported to the *trans*-Golgi and translocated to the luminal side, where synthesis of complex GSLs takes place (Sandhoff and Sandhoff 2018) (Figure 2). In addition to vesicular transport, the non-vesicular transport of GlcCer from the early Golgi to distal Golgi compartments is mediated by the glycolipid-binding protein FAPP2 (4-phosphate adaptor protein-2) (D'Angelo et al. 2007). However, according to Halter et al. (2007), GlcCer is synthesized in the *trans*-Golgi network (TGN). Subsequently, most GlcCer is transported back to the cytoplasmic leaflet of ER with the help of the FAPP2. There, GlcCer is translocated to the luminal side and transported again to the *trans*-Golgi apparatus, where complex GSLs are synthesized. Remaining GlcCer is transported by FAPP2 from the cytosolic surface of the TGN to the cytosolic surface of the plasma membrane or endosome. In the plasma membrane, GlcCer is translocated to the cell surface or remains on the cytoplasmic side (Halter et al. 2007; Quinn 2011).

Similar to the synthesis of GlcCer, GalCer is generated by the transfer of Gal from UDP-galactose to the same hydroxyl group at the C-1 position of the ceramide backbone by galactosylceramide synthase (EC2.4.2.62) (Morell and Radin 1969; Sprong et al. 1998), described also as ceramide galactosyltransferase or UDP-galactose:ceramide galactosyltransferase, and known under the acronyms CGT and UGT8 (Figure 1). This enzyme is encoded by the *UGT8* gene (Kapitonov and Yu 1997). However, the amino acid sequence of UGT8 shows no significant homology to GCS, which indicates different evolutionary origins of these enzymes (Ichikawa et al. 1996b). Also, the addition of Gal to ceramide moiety takes place in a different cellular compartment. GalCer is synthesized on the luminal side of the ER, as UGT8 is a type I membrane glycoprotein with its active site directed into the lumen of this organelle (Sprong et al. 1998; Sprong et al. 2003) (Figure 2). From there, GalCer is transported to the *trans*-Golgi compartment, where larger galactosphingolipids and sulfatides are synthesized.

Degradation of GlcCer to glucose and ceramide is carried out by several glucosylceramidases: GBA1, GBA2 and GBA3 (Astudillo et al. 2016) (Figure 1). GBA1 (EC3.2.1.45), also known as acid β-glucosidase, β-glucoceramidase, glucocerebrosidase, D-glucosyl-*N*-acylsphingosine glucohydrolase and GlcCerase, is a lysosomal hydrolase. GBA2 (EC3.2.1.45), i.e., bile acid β-glucosidase, is a ubiquitous nonlysosomal enzyme located on the cytosolic surface of the ER and/or Golgi apparatus. GBA3 (EC3.2.1.21), known under the names Klotho-related or KLrP, is another cytosolic glucosyl-ceramidase found in the liver, spleen, kidney and in some other tissues, but whose function is presently unclear. In the case of GalCer degradation, the only enzyme known so far is lysosomal galactosylceramidase (EC3.2.1.46), known also as β-galactocerebrosidase, and under the acronym GALC (Beier and Gorogh 2005). In both cases, ceramides that are released from GlcCer and GalCer are hydrolysed by ceramidases to sphingoid bases and fatty acids (Ogretmen 2018).

In animal cells, the main site for degradation of GlcCer and GalCer is lysosome. Monohexosyl-ceramides, after endocytosis of plasma membrane fragments, are transported to lysosome by endocytic vesicular membrane flow through early and late intraendosomal vesicles (Figure 3). Reaching the lysosomal compartment, the late endosome fuses with primary lysosome, allowing the vesicles to be transferred into this compartment. GSLs are then subsequently degraded on the surface of intralysosomal vesicles (Sandhoff and Kolter 1996; Schulze et al. 2009; Kolter and Sandhoff 2010).

Besides the enzymes, essential components of the intra-lysosomal degradation of GSLs with less than four sugar residues are sphingolipid activator proteins (SAPs): saposins (Saps) A, B, C and D and GM2-activator protein (GM2-AP) (Schulze et al. 2009). The four saposins arise by proteolytic processing of a single precursor protein, prosaposin, which is synthesized in the ER, transported to the Golgi apparatus for glycosylation and finally transported to the lysosomes. Two mechanisms were proposed to explain the role of saposins in the activation of GSL degradation. (1) Saponins facilitate the interaction between the GSLs and exohydrolases by binding, extracting and presenting the membrane localized lipids to water-soluble enzymes (Sandhoff and Kolter 1996); (2) SAPs bind directly to enzymes, not to GSLs, generating a more active enzymatic complex to hydrolyze GSLs (Fabbro and Grabowski 1991). It was found that deficiency of saposin C led to accumulation of GlcCer within the cells and resulted in a GlcCer storage disease resembling a neurologic form of Gaucher disease (GD; see *GlcCer and lysosomal* GBA1 *in GD* section), showing the crucial role of this cofactor in the metabolism of this monohexosylceramide (Kang et al. 2018). Also, the saposin A and probably saposin C are necessary components to activate the degradation of GalCer by galactosylceramidase in vivo (Harzer et al. 1997). Mice lacking, due to mutation, mature saposin A, accumulate GalCer and develop a late-onset form of chronic globoid cell leukodystrophy (Matsuda et al. 2001).

Despite structural similarities, the cellular and tissue localization of GlcCer and GalCer are different. GlcCer is present in essentially all cell types (Makita and Yamakawa 1962; Svennerholm and Svennerholm 1963; Makita 1964; Ishibashi et al. 2013), serving as a precursor for the synthesis of hundreds of different GSLs (Sandhoff and Sandhoff 2018). For example, the presence of GlcCer was analyzed

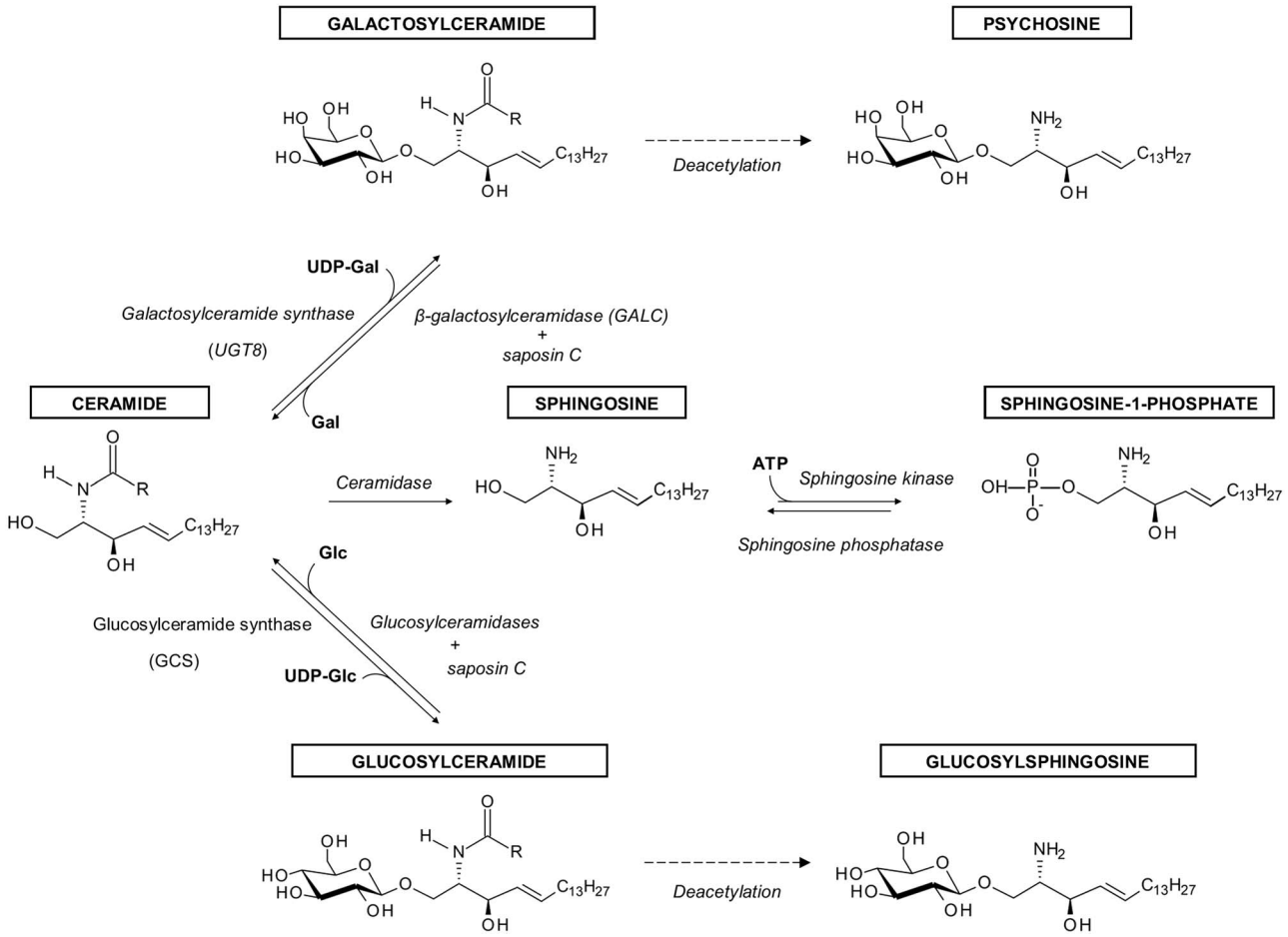


Fig. 1. The synthesis and degradation of GlcCer and GalCer and their metabolites referred to in the review.

in the mucosa of the gastrointestinal tract (Costantino-Ceccarini and Morell 1973; Breimer et al. 2012), liver (Nilsson and Svennerholm 1982) and adrenal medulla (Ariga et al. 1980). Data are also available on the expression of the *UGCG* gene in normal human tissues. The highest levels of GCS mRNA were found in the kidney, vulva, urinary bladder, stomach, pancreas and colon. Intermediate levels were seen in the prostate, lung, skin, cervix, rectum and thyroid gland, while the lowest levels of GCS mRNA were present in the breast, uterus, ovary and testis (Liu et al. 2011).

In contrast to GlcCer, GalCer is present only in a limited number of mammalian tissues/cells. GalCer is the most abundant single component of the myelin sheath (20–25% of the total lipid content) produced by oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (Norton and Autilio 1966). This monohexosylceramide is also present in larger amounts in the mucosa of the human gastrointestinal tract (Natomi et al. 1993; Breimer et al. 2012), adrenal medulla (Ariga et al. 1980), liver (Nilsson and Svennerholm 1982), testis (Vos et al. 1994) and milk (Bouhours and Bouhours 1979).

Interestingly, in nerves of protostome animals, only GlcCer and GlcCer-containing GSLs were found. This is in contrast to deuterostomes, whose myelin is rich in GalCer and 3-sulfo-GalCer (Okamura et al. 1985). These changes in GSL composition correlate with the evolution of the nervous system from loosely structured membrane-enwrapped axons to multilamellar highly structured myelin.

The analytical methods for the identification of GalCer and GlcCer

Because of the high structural similarity, identification and differentiation of GlcCer from GalCer is difficult using classical analytical lipid methods and requires either additional steps within the protocol or a simultaneous combination of several methods. Due to their amphipatic structure, GalCer and GlcCer can be separated by thin-layer chromatography (TLC) or high-performance-TLC (HP-TLC). For this purpose, borate-coated TLC plates are commonly used and separation is based on the differences in ability to form glucose-borate or galactose-borate complexes due to a different arrangement of the hydroxyl group at carbon atom 4 in these hexoses (Kean 1966). Ogawa et al. (1988) demonstrated a protocol for boron-free TLC separation using developing system (2-propanol/1.5 M ammonia solution/methyl acetate/water 75/10/5/15) which allows separation of GalCer from GlcCer in 1D chromatography. More recently, high-performance liquid chromatography of perbenzoylated monohexosylceramide derivatives was adopted for GlcCer/GalCer separation (McCluer et al. 1981). GlcCer and GalCer on HP-TLC plates can also be detected by specific antibodies (Vielhaber et al. 2001; Suchanski et al. 2018). A useful tool in the studies of GlcCer/GalCer trafficking, distribution and metabolism is radioactive or fluorescent analogues of these monohexosylceramides. Such experiments were usually carried out with short-chain fluorescent analogues of a biological active derivative of GalCer and GlcCer tagged with a

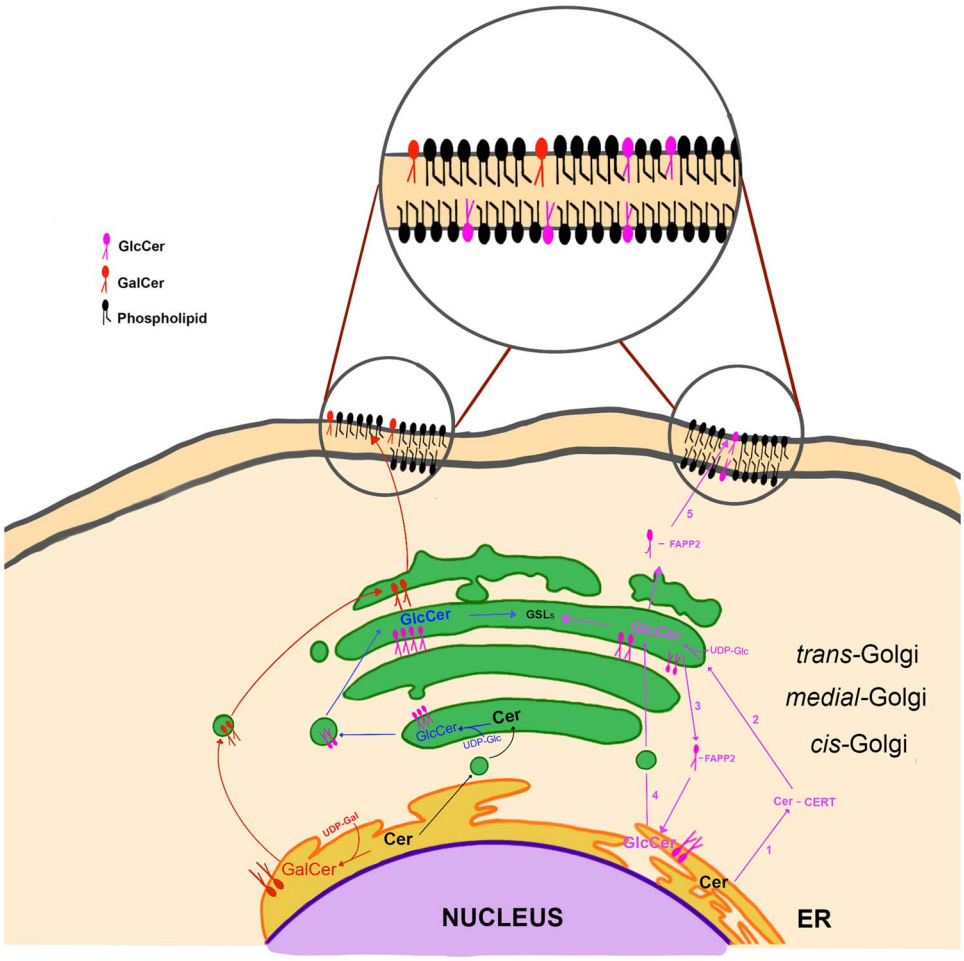


Fig. 2. Intracellular localization and transport of GlcCer and GalCer. In the "classical" model (shown in blue), GlcCer, synthesized on the cytosolic side of the *cis*-Golgi, is transported to the *trans*-Golgi and translocated to the luminal side, where synthesis of complex GSLs takes place. In the alternative model (shown in violet), ceramide is transported from the ER to the cytosol with the help of CERT (1), then GlcCer is synthesized on the cytosolic side of the *trans*-Golgi (2), from there most GlcCer is transported back to the cytoplasmic leaflet of ER with the help of the FAPP2 (3). There, GlcCer is translocated to the luminal side and transported again to the *trans*-Golgi apparatus, where complex GSLs are synthesized (4). Remaining GlcCer is transported by FAPP2 from the cytosolic surface of the *trans*-Golgi to the cytosolic surface of the plasma membrane (5). GalCer is synthesized on the luminal side of the ER (shown in red). From there, it is transported to the *trans*-Golgi compartment, where complex galactosphingolipids and sulfatides are synthesized. FAPP2—4-phosphate adaptor protein-2, CERT—ceramide transport protein.

fluorescent C-6 nitrobenzoxadiazole (C6-NBD) (Kok et al. 1995; Khiste et al. 2017).

Complete structural analysis of GSLs requires a combination of techniques to determine the composition, sequence, linkage positions, anomeric configurations of the sugars, the fatty acid and sphingoid base of the ceramide moiety. GSLs are often structurally characterized by mass spectrometry (MS) and/or nuclear magnetic resonance NMR spectroscopy. Traditional MS approaches are based on the fragmentation of organic molecules, followed by the differentiation of the resulting fragments according to their mass/charge ratio. Electrically neutral GalCer and GalCer are lipids which can be ionized (typically by electrospray) into two ion modes (negative and positive). In the negative-ion mode, cerebroside forms chlorine adducts ([M + Cl]−) (Han and Cheng 2005). In the positive ion mode, they are ionized as proton or alkaline adducts ([M + X]+, X = H, Li, Na, K), depending on the availability and affinity of the small cations (Han and Cheng 2005). GalCer and GlcCer are indistinguishable by MS analysis; therefore, their differentiation requires the use of gas-chromatography (GC) or liquid-chromatography (LC). In order to separate GalCer from GlcCer LC is used preferentially and their analysis is performed using tandem mass spectrometry (Shaner et al. 2009; Gegg et al. 2015; Hamler et al. 2017). Such analysis can often be performed without the need for the release of glycans. However, if necessary, the glycans can be released by enzymatic treatment or chemical methods. For monohexosylceramides, endoglycoceramidase I (EGCase I) can be used, which is the enzyme that catalyzes hydrolysis of the β-glycosidic linkage between oligosaccharides and ceramides in various GSLs (Albrecht et al. 2016). Chemical methods include acid hydrolysis or ozonolysis (Wang et al. 2019). For monohexosylceramides and other GSLs, quantitative release of monosaccharides is achieved by treatment with 4–6 M hydrochloric acid for 3–6 h at 100°C (Townsend 1993). The structure of monosacharide after release can be analyzed by different chromatographic and electrophoretic methods, e.g., GC-MS and its alternative, high-pH anion exchange chromatography

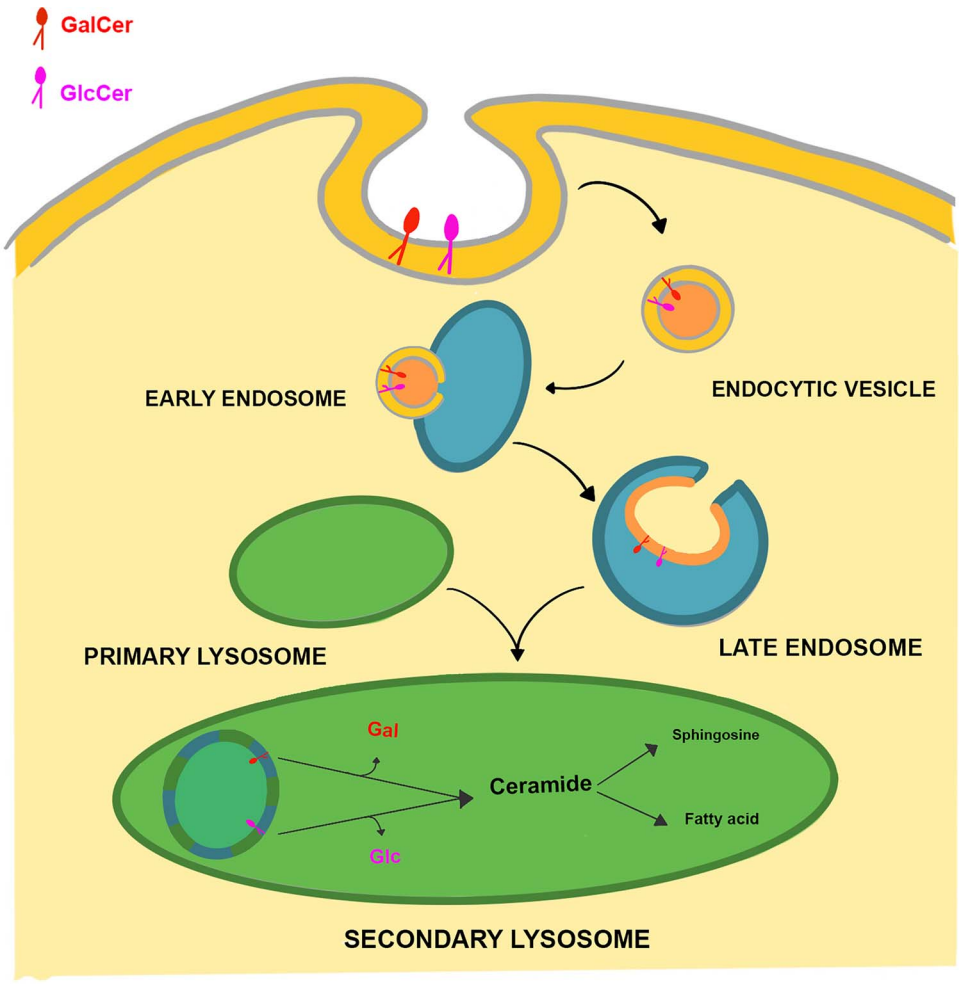


Fig. 3. Lysosomal degradation of GlcCer and GalCer. GlcCer and GalCer degradation is initiated by the invagination of the plasma membrane. During endocytosis, GSLs are assimilated as vesicles into early and late endosome. These vesicles reach the lysosome compartment after the fusion of late endosome with primary lysosome. GlcCer and GalCer are then degraded to ceramide by lysosomal enzymes, which are further hydrolyzed by ceramidase to spingoid base and fatty acid.

with pulsed amperometric detection, which has a strong anion-exchange property and does not require monosaccharide derivatization (Schnaar and Kinoshita 2017). These methods allow for simple, sensitive and specific determination of monosaccharide composition and most importantly enable the discrimination of compounds with the same chemical composition such as glucose and galactose (Corradini et al. 2013). Other popular techniques used to specify monosaccharide composition in GSLs are HPLC and high-performance capillary electrophoresis (Liu and Chan 1991) or capillary electrophoresis with laser-induced fluorescence (Rossdam et al. 2019).

Biological functions of GlcCer and GalCer

Molecular level

GSLs play a role in membrane organization regulating the fluidity of the lipid bilayer and are involved in including or excluding proteins from membrane microdomains. They also act as bioactive lipid messengers on two levels, directly affecting membranous protein functions and regulating the expression level of specific genes. For example, several receptors can be directly regulated by GSLs present in cell membranes (Zhang et al. 2019), modulating specific signaling cascades by GSL-enriched lipid rafts (Modrak et al. 2006), also called GSL-enriched microdomains or "glycosynapses" (Hakomori 2002).

So far, it has been shown that on the molecular level, GlcCer and GalCer are involved in membrane organization. They are localized in the external lipid leaflet of cell membranes and are found predominantly within tightly packed lateral domains of lipids, called membrane rafts (Thompson and Tillack 1985; Brown and Rose 1992; Morrow et al. 1992; Westerlund and Slotte 2009; Varela et al. 2016). As very-long fatty acid chains (C_{22}–C_{24} and longer) often present in such GSLs are able to penetrate far into the inner leaflet, it was proposed that such interactions allow for better interleaflet coupling and can affect association with cytosolic proteins and intracellular signaling (Skotland and Sandvig 2019). Using atomistic molecular dynamics simulations, Hall et al. (2010) demonstrated that GalCer significantly increased the thickness of raft membranes, while the average area per lipid and lipid conformational order were unchanged. They also showed that interdigitation of GalCer slows down the lateral diffusion of raft lipids and affects membrane viscosity between the two membrane leaflets, augmenting the friction between the monolayers. In addition, interdigitation of GalCer alters the lateral pressure profile, which may lead to a change in membrane protein activation (Niemela et al. 2007; Ollila et al. 2007). GalCer

has the ability to form multiple hydrogen bonds with surrounding molecules; however, it preferentially interacts with cholesterol molecules shielding them from direct contact with water (Hall et al. 2010). Similar to GalCer, GlcCer is also involved in the formation of highly ordered gel domains and increases the order of the membranous fluid phase (Varela et al. (2013). The presence of GlcCer promotes morphological alterations in lipid vesicles, which leads to the formation of flexible tubule-like structures extending from the lipid surface. However, despite tiny differences in the structures of their headgroups—stereochemical orientation of one hydroxyl group in the sugar residues—palmitoyl GalCer and palmitoyl GlcCer differ significantly in their domain forming behavior (Maunula et al. 2007). GalCer formed ordered domains which dissociated with increasing temperature, and GlcCer at the same concentration formed domains with a more cooperative dissociation behavior, but lower thermostability.

Cellular level

On the cellular level, GSLs have important functions in such cellular processes as adhesion and recognition, growth, differentiation and development, angiogenesis, inflammation and multidrug resistance in cancer cells (Hannun and Obeid 2018). Several lines of evidence suggest that GlcCer affects the proliferation potential of various cell types. Using a mouse model, it was shown that intraperitoneal injection of emulsified GlcCer and inhibition of glucosylceramidase resulted in enlargement of the liver (Datta and Radin 1988). Furthermore, the inhibition of GCS activity in renal epithelial cells decreased their proliferation rate (Shayman et al. 1991). GlcCer also affected the proliferation of Schwann cells (Yao and Yoshino 1994) and stimulated mitogenesis of murine epidermis (Marsh et al. 1995; Marchell et al. 1998) (see *GlcCer and lysosomal GBA1 in Parkinson's disease* section). On the other hand, RNA interference experiments showed that the loss of GCS expression and therefore inhibited synthesis of GlcCer resulted in enhanced apoptosis of cells in the *Drosophila melanogaster* embryo (Kohyama-Koganeya et al. 2004). These proproliferative and antiapoptotic effects are exerted not directly by GCS and GlcCer themselves, but by affecting the intracellular pool of ceramides, as increased synthesis of GlcCer decreases the level of antiproliferative ceramide and decreased synthesis of GlcCer increases the level of proapoptotic ceramide (Kohyama-Koganeya et al. 2004; Ishibashi et al. 2013). Treatment of human keratinocytes with exogenous sphingomyelinase, which is known as a potent stress inducer, first caused increased production of ceramide and therefore decreased proliferation of cells and then increased synthesis of GlcCer, which was associated with restoration of cell proliferation (Uchida et al. 2002). In the case of NIH 3T3 cells, it was shown that inhibition of GCS activity by *N*-[2-hydoxy-1-(4-morpholinymethyl)-2-phenylethyl]-decanamide (PDMP) resulted in a decreased GlcCer level and an increase in ceramide levels, which was associated with arrest of the cell cycle at G1/S and G2/M transition and decrease in the activities of two cyclin-dependent kinases, $p34^{cdc2}$ kinase and cdk2 kinase (Rani et al. 1995). Incubation of human neuroepithelial CHP-100 cells with C6-ceramide induced their apoptosis. This effect was significantly increased when cells were simultaneously treated with the same GCS inhibitor (Spinedi et al. 1998). It is now broadly accepted that ceramide is a key molecule involved in specific signaling pathways related to apoptotic and proliferative cellular responses to many stressors, including chemotherapeutics (Hannun and Obeid 2018; Ogretmen 2018) (see *Monohexosylceramides and cancer* section). However, the exact molecular mechanisms of how ceramides affect proliferation and apoptosis are unknown. It should be mentioned that the role of GCS and therefore GlcCer in the accumulation of the intercellular pool of ceramide was not confirmed by others, e.g., in Jurkat cells during apoptosis induced by CD95 (Tepper et al. 2000).

In the case of GalCer, it was found that expression of UGT8 and accumulation of GalCer in breast cancer cells increased their resistance to apoptosis induced by doxorubicin in vitro (Owczarek et al. 2013; Suchanski et al. 2018) (see *Monohexosylceramides and cancer* section).

Monohexosylcermides under physiological conditions

GalCer in myelin function and oligodendrocyte differentiation

As it was mentioned earlier, GalCer and its sulphated and sialylated analogues, 3-sulfo-GalCer (SM4) and 3-Neu5Ac-GalCer (GM4), respectively, are essential components of unique plasma membranes elaborated by oligodendrocytes (OLs) in the CNS and Schwann cells in the peripheral nervous system in the form of myelin sheaths (Norton and Cammer 1984; Jackman et al. 2009; Schnaar and Kinoshita 2017). Using knock-out mice, it was shown that these galactolipids play a role in (1) the formation of normal myelin in CNS, (2) development of normal axo-glial interactions at nodes of Ranvier and (3) promote axo-glial adhesion during myelinogenesis (Marcus and Popko 2002; Jackman et al. 2009). However, there are indications that GalCer and 3-sulfo-GalCer are not essential for the formation of myelin, but are necessary for the proper structure and stability of myelin, e.g., mice without GalCer wrap their axons in myelin sheathes that are enriched in GlcCer but non-functional due to altered structures at nodes of Ranvier (Boggs 2014). The molecular mechanisms that underlie these cellular phenomena are not fully understood. However, it has been proposed that the proper structure of multilayered myelin sheaths is dependent on the carbohydrate–carbohydrate interactions between GalCer and 3-sulfo-GalCer molecules forming domains localized on the apposed surfaces of these myelin sheaths (Coetzee et al. 1998; Boggs 2014). Furthermore, such GalCer/3-sulfo-GalCer-enriched microdomains form glycosynapses (Hakomori 2002), which are involved in signal transduction and loss of the cytoskeleton (Boggs 2014).

On the cellular level, GalCer and 3-sulfo-GalCer are also involved in terminal maturation of OLs. OL differentiation can be divided into several stages distinguished by the expression of specific cell surface markers and changes in morphology (Butts et al. 2008). In brief, the bipolar early oligodendrocyte progenitor cells (stage 1) differentiate into pro-OLs (stage 2), then, immature OLs (stage 3) and finally mature OLs (stage 4). Stage 3 immature OLs are characterized by the synthesis of GalCer and expression of 2′,3′-cyclic nucleotide 3′-phosphohydrolase and O4 marker. Interestingly, when GalCer and 3-sulfo-GalCer are synthesized and transported to plasma membrane, OL progenitors stop to proliferate and begin terminal differentiation (Bansal et al. 1999). It was shown that binding of Ranscht monoclonal antibody (R-mAb) to GalCer and 3-sulfo-GalCer present on the surface of OL progenitors inhibited their terminal differentiation, suggesting that both galactolipids are involved in the regulation of OL differentiation (Bansal and Pfeiffer 1989). However, in subsequent studies, using specific antibodies directed against 3-sulfo-GalCer or GalCer, it was found that rather

3-sulfo-GalCer, not GalCer, acted as the main inhibitory molecule in the regulation of oligodendrocyte terminal differentiation (Bansal et al. 1999). This was further confirmed using knock-out mice lacking cerebroside sulfotransferase (Hirahara et al. 2004). Using transgenic mice unable to express UGT8, Bansal et al. (1999) showed that the lack of GalCer and 3-sulfo-GalCer resulted in a 2- to 3-fold increase in the number of terminally differentiated oligodendrocytes. The authors proposed that both galactosphingolipids act as ligands for endogenous receptors generating inhibitory signals to block differentiation. However, other studies revealed that GalCer affects the differentiation of OLs indirectly as the constituent of lipid rafts present on ER membranes (Hayashi and Su 2004). Such GalCer/cholesterol microdomains are enriched in Sigma-1 receptors (Sig-1Rs) that are involved in lipid distribution in ER membranes and their transport to plasma membrane in neuronal cells (Hayashi and Su 2003a; Hayashi and Su 2003b). Moreover, it was found that in OL progenitors and myelin of developing rat brains, the amounts of lipid-raft-localized Sig-1Rs and GalCer increase as cells differentiate, and such structures remain present in myelin sheets of mature OLs (Hayashi and Su 2004). Importantly, the differentiation ability of OLs was positively affected by expression of Sig-1Rs. Altogether, these data suggest that GalCer rafts enriched with Sig-1Rs are important for OL differentiation. However, the question about the precise molecular mechanism by which GalCer affects OL differentiation remains open.

GlcCer and epidermal functions

GlcCer plays an important role in the biology of keratinocytes as well as the formation and maintenance of an epidermal permeability barrier. The outermost epidermal layer, called stratum corneum, consists of dead keratinocytes embedded with extracellular lipids such as cholesterol, ceramides and fatty acids (Elias et al. 1983; Long et al. 1985), which protect against excessive transepidermal water loss and eventual pathogen entry. These lipid components form an array of lamellar membranes derived from lamellar bodies after fusion of these organelles with the apical plasma membrane of the uppermost granular cells and subsequent release of their content into the intercellular spaces by exocytosis (Bouwstra et al. 2003; Madison 2003). The ceramides, essential components of lamellar membranes, are the result of GlcCer hydrolysis, which is stored in lamellar bodies (Holleran et al. 1993) (Figure 4). GlcCer represents only about 4% of all epidermal lipids but is one of the main components of lamellar membranes (Madison et al. 1986; Doering et al. 1999). Enzymatic hydrolysis of GlcCer takes place after the release of the contents of lamellar bodies into the intercellular domains during epidermal terminal differentiation (Gray and Yardley 1975; Elias 1981). Part of the GlcCer present in lamellar bodies is converted to acylglucosylceramide with ester-linked linoleic acid on the ω-oxygen function (Abraham et al. 1985; Hansen and Jensen 1985; Schoephoerster et al. 1985).

Jennemann et al. (2007) developed a mouse model with an epidermis-specific GCS knock-out, showing that the lack of GlcCer leads to a disrupted arrangement of the epidermal lamellar bodies, which results in excessive desquamation, uncontrolled transepidermal water loss and animal death on the fourth day after birth. Based on these results, the authors propose that the presence of GlcCer is crucial for the proper formation of lamellar bodies and subsequently ceramide-rich lamellar membranes. A recent study using a mouse model also showed that an oral intake of rice-derived GlcCer would prevent transepidermal water loss by accelerating GlcCer metabolism, which increases the amounts of ceramides in epidermis (Shimoda et al. 2012).

GlcCer and GalCer under pathological conditions

GlcCer and lysosomal GBA1 in GD

GD remains the most common lysosomal storage disorder (LSD). It is mainly caused by mutations in *GBA1* gene coding for lysosomal GBA1. Generally, the mutations greatly decrease the activity of the enzyme; however, such enzymatic deficiency is not complete, as the total absence of GBA1 is lethal for humans and mice. In the visceral GD variant (type I), mutations lead to GlcCer accumulation in lysosomes of leukocytes, primarily macrophages and antigen presenting cells (APCs) in the spleen, liver, lungs and bone marrow. The GBA1-defective macrophages accumulate GlcCer as the result of ingestion of exogenous lipids from senescent or/and apoptotic erythrocytes, leukocytes and platelets (Kattlove et al. 1969). The exact mechanisms by which GlcCer accumulation leads to GD are not known. However, there are several hypotheses about the role of excessive amounts of GlcCer in the pathogenesis of this disease. Using cellular models of GD constructed by treatment of human fibroblasts or murine RAW macrophages with inhibitor of GBA1—conduritol B epoxide (CBE), it was found that an increase in the level of GlcCer induced altered lactosylceramide (Table I) trafficking from the plasma membrane to late endosomes and lysosomes, instead of to the Golgi apparatus (Sillence et al. 2002). These data suggest that accumulation of GlcCer disrupts proper lipid transport and sorting. According to the "jamming of the endosomal system" hypothesis, accumulation of GlcCer in late endosomal membranes prevents subsequent recruitment of cholesterol and other sphingolipids, which in turn leads to a jam in the membrane transport system (Simons and Gruenberg 2000). However, the exact molecular mechanisms of this phenomenon are not known. Similarly, Hein et al. (2007), using a macrophage model of GD, showed that intracellular GlcCer accumulation caused its (as well as other GSLs) increased localization not only in lysosomes but also in other cellular compartments. In plasma membrane, GlcCer and other GSLs accumulated primarily in lipid rafts. According to the authors, aberrant localization of GSLs negatively affects different biochemical pathways, leading to macrophage activation and enhanced pro- and antiinflammatory cytokine production. This agrees with clinical observations on the prevalence of inflammatory processes in tissues of Gaucher patients. Recently, Pandey et al. (2017), based on a mouse model of GD, proposed that the mechanism connecting the accumulation of GlcCer in visceral macrophages with chronic inflammation developed in target organs. According to them, the continuous release of GlcCer from macrophages causes differentiation of B lymphocytes into plasma cells producing anti-GlcCer autoantibodies forming GlcCer-anti-GlcCer IgG immune complexes, which activate the classical pathway of complement or lead to systemic generation of C5a by C5 cleavage. Activation of C5a receptor 1 (C5aR1) by C5a on dendritic cells (DCs) up-regulates expression of co-stimulatory molecules—CD80, CD86 and CD40, which in turn activate T cells producing proinflammatory cytokines: IFNγ, IL-17, TNF, IL-1β and IL-6. Interestingly, generated C5a binds and activates C5aR1 present also on macrophages, causing increased expression of GCS, which in combination with the GBA1 deficiency, promotes the accumulation of even higher amounts of GlcCer within macrophages. Such a mechanism further propels the autoimmune response against GlcCer, as more GlcCer is released, and therefore, more

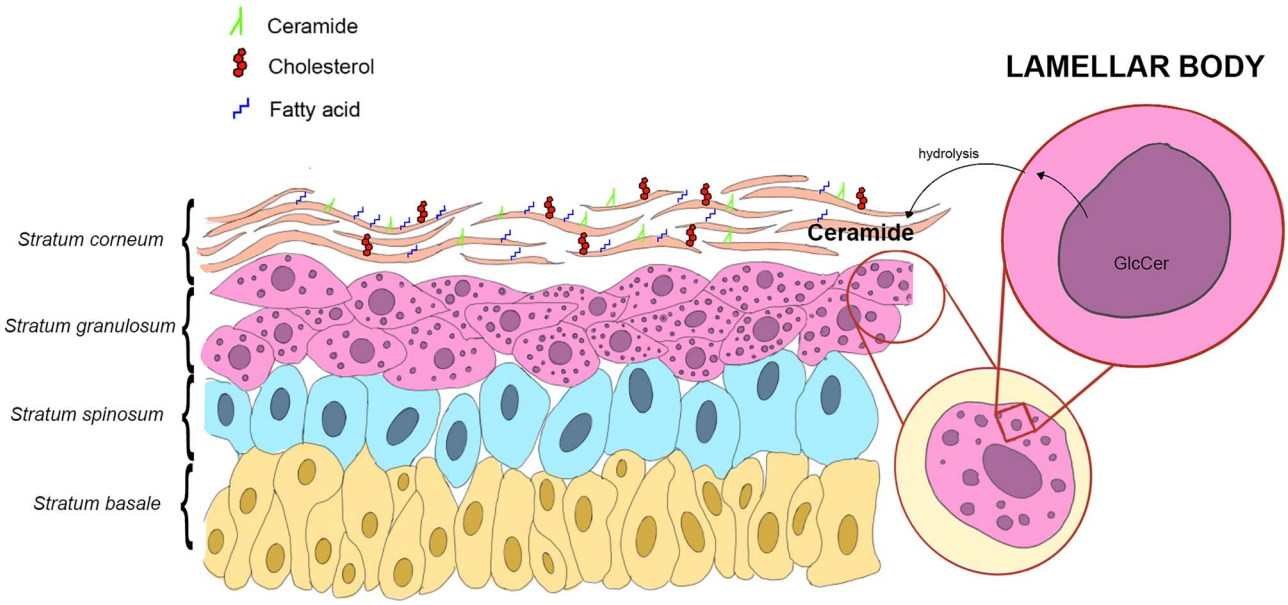


Fig. 4. GlcCer and epidermal functions. Lamellar bodies after fusion with the apical plasma membrane of the uppermost granular cells release GlcCer into the intercellular spaces by exocytosis. There, GlcCer is hydrolyzed and the released ceramide becomes a part of stratum corneum consisting of dead keratinocytes embedded with extracellular lipids such as cholesterol, ceramides and fatty acids.

anti-GlcCer antibodies are produced and more GlcCer-anti-GlcCer IgG complexes are formed. This further activates a complement pathway and promotes inflammation and tissue damage observed in GD. It should be emphasized that this model is supported by clinical data. Elevated levels of GlcCer and other GSLs in plasma membrane of Gaucher-type macrophages also affect its biophysical properties (Batta et al. 2018). It was found that such plasma membranes are characterized by decreased fluidity, leading to the enlargement and fusion of raft-like domains, which in turn highly restrict the lateral mobility of non-raft lipids and proteins. These alterations significantly affected the biological properties of Gaucher-type macrophages, as evidenced by the inhibition of clathrin-dependent endocytosis and reduction in IFN_{γ} induced STAT1 phosphorylation.

In a chemically induced model of neurological GD variant (types II and III), accumulation of GlcCer in neurons, as the result of GBA1 inhibition by CBE, makes them more sensitive to calcium-induced neurotoxicity due to increased calcium released via ryanodine receptors (RyaRs), whose activity is modulated by GlcCer (Korkotian et al. 1999; Lloyd-Evans et al. 2003). Since RyaRs are calcium channels localized in ER, the depletion of ER calcium stores makes cells susceptible to ER stress and induction of apoptosis (Kacher and Futerman 2006). Similar results were obtained with microsomes prepared from human brains of patients with type II GD (Lloyd-Evans et al. 2003). Using models of GD, it was also shown that increased amounts of GlcCer in neurons and brain tissue affected phospholipid metabolism, as GlcCer directly increased the activity of CTP:phosphocholine cytidylyltransferase. It was proposed that elevated synthesis of phosphatydylcholine affects the growth rate of neuronal cells, which is observed in GD (Bodennec et al. 2002). Similar changes in phosphatidylcholine metabolism were also observed in a chemically induced macrophage model of GD (Trajkovic-Bodennec et al. 2004). In types II and III of GD involving the CNS, accumulation of GlcCer in neuronal cells leads to the production of glucosylsphingosine (Grabowski 2012) (Figure 1). This neurotoxin induces apoptosis of neuronal cells, which in turn activates astroglial and microglial cells. As a result, the loss of neurons and subsequent neuroinflammatory effects are observed.

GlcCer and lysosomal GBA1 in Parkinson's disease

Mutations in the *GBA1* gene are one of the most common genetic risk factors for Parkinson's disease (PD) suggesting a link between PD and GD (Sidransky et al. 2009; Belarbi et al. 2020). The involvement of lysosomal GBA1 in PD pathophysiology was further supported by the discovery that this enzyme was present in Lewy bodies from brains of PD patients carrying *GBA1* mutations (Goker-Alpan et al. 2010). Lewy bodies, which contribute to PD, are protein aggregates composed mainly of α-synuclein (SNCA), a presynaptic protein regulating synaptic vesicle cycling (Spillantini et al. 1997; Taguchi et al. 2017). It was proposed that mutated GBA1 binds directly or indirectly to SNCA, which enhances aggregation of the latter or soluble SNCA oligomers trap misfolded mutant GBA1, and such complexes are converted to insoluble fibrils in Lewy bodies (Westbroek et al. 2011). Based on another model, it was suggested that misfolded mutated GBA1 does not localize in lysosomes but undergoes ubiquitination by parkin (E3 ubiquitin ligase) and subsequently ER-associated degradation. Occupation of parkin with mutated GBA1 blocks its interaction with natural substrates, which causes their accumulation to be detrimental to neuronal survival and induces apoptotic cell death (Ron et al. 2010; Westbroek et al. 2011). However, it was also shown that inhibition of GBA1 activity in neuroblastoma cells and mice subjected to treatment with CBE leads to elevated levels of SNCA and its accumulation within nigral cell bodies and astroglia (Manning-Bog et al. 2009). Recently, it was found that GlcCer and its metabolites (glucosylsphingosine, sphingosine and sphingosine-1-phosphate), which accumulate as the result of *GBA1* gene mutation in PD, are able to accelerate aggregation and induce pathologic SNCA species in neurons and other human cells (Taguchi et al. 2017).

GalCer and lysosomal galactocerebrosidase in Krabbe disease

Krabbe disease also known as globoid cell leukodystrophy or galactosylceramide lipidosis is an autosomal recessive LSD involving the white matter of the peripheral and CNSs. This disease, characterized by the loss of myelin, is caused by mutations in the *GALC* gene, resulting in deficiency and/or decreased activity of lysosomal GALC (Wenger et al. 2000), which leads to accumulation of GalCer and galactosylsphingosine (psychosine) (Figure 1) in macrophages and neural cells, especially in oligodendrocytes and Schwann cells (Won et al. 2016). According to the "psychosine hypothesis," galactosylsphingosine, not GalCer, is a highly cytotoxic compound directly linked to demyelination of the central and peripheral nervous systems. When there is a deficiency of GALC, large amounts of psychosine are produced by deacylation of accumulated GalCer by *N*-deacylase (Svennerholm et al. 1980; Kanazawa et al. 2000) and galactosylation of sphingosine by UGT8 (Cleland and Kennedy 1960). GalCer does not accumulate in the absence of GALC and therefore does not appear to be directly linked to demyelination processes, since in the absence of this enzyme, GalCer is degraded by GM1 (Table I) β-galactosidase (Won et al. 2016). Psychosine, because of its detergent-like properties, destabilizes cellular membranes, which leads to cell lysis, induces oxidative stress and mitochondrial damage, resulting in cell apoptosis, and on the cellular level causes inflammation, as well as vascular, neuronal and axonal dysfunction (Won et al. 2016).

GalCer in juvenile neuronal ceroid lipofuscinosis

Juvenile neuronal ceroid lipofuscinosis (JNCL) represents one of the genetic diseases known under the collective name of neuronal ceroid lipofuscinoses or Batten disease. It is one of the most common childhood neurodegenerative disorders. JNCL is caused by mutations in the *CLN3* gene and is characterized by abnormal accumulation of lipopigments in lysosomes of neurons (Cotman and Staropoli 2012). The loss of function by the *CLN3* gene is accompanied by massive neuronal death in the cerebrum and cerebellum. This confirms the proposal that the CLN3 protein (battenin) acts as an anti-apoptotic molecule, and the absence of which highly increases the level of the intracellular pool of pro-apoptotic ceramide (Puranam et al. 1999; El-Sitt et al. 2019).

CLN3 is a transmembrane protein characterized by the presence of a GalCer binding domain (Persaud-Sawin et al. 2004). Normal CLN3, localized in the Golgi apparatus and plasma membranes, takes part in anterograde transport of GalCer from the *trans*-Golgi to lipid rafts of plasma membrane involving early recycling endosomes (Persaud-Sawin et al. 2004; Cotman and Staropoli 2012). This proposal is supported by observations that mutations in the *CLN3* gene found in JNCL patients affect the proper structure of a GalCer binding domain, which prevents mutant CLN3 protein from normal movement between these compartments and therefore prevent delivery of GalCer to plasma membrane, which is associated with accumulation of GalCer in ER and Golgi (Rusyn et al. 2008). The absence of CLN3 protein and GalCer affects the proper composition, structure and function of lipid rafts in the Golgi and plasma membranes, which in turn leads to deregulation of ceramide levels with an end effect of increased apoptosis (Persaud-Sawin et al. 2004).

Monohexosylceramides and cancer

The presence of GlcCer and GalCer and expression of UGCG and UGT8 genes in cancer tissues. There is a lack of detailed studies comparing the presence of GlcCer in cancer cells and human tumors with corresponding normal cells and tissues. However, more information is available on the expression of the *UGCG* gene in human cancers. GCS mRNA levels were significantly higher in tumors of the rectum, small intestine, cervix and breast than in corresponding normal tissues (Liu et al. 2011). GCS mRNA expression was significantly up-regulated in metastases of breast cancer in comparison to primary tumors, benign fibroadenoma and normal mammary tissue. Also, significantly higher expression of GCS mRNA was found in Stage III tumors than Stage I and II tumors and in node-positive tumors than in node-negative tumors (Lucci et al. 1998; Liu et al. 2011). Using immunohistochemistry, it was further shown that GCS protein levels in breast cancer tissue specimens and lymph node metastases were significantly higher than in normal tissues. Furthermore, GCS expression correlated positively with ER-positive and HER2-positive metastatic breast cancer, suggesting that GCS can be a potential marker of tumor aggressiveness. However, these data have not been fully confirmed by others. Ruckhaberle et al. (2009), using transcriptome profiling, showed that the expression level of the *UGCG* gene correlated positively with positive estrogen receptor (ER) status but was inversely associated with lower histological grading, low Ki67 levels and HER2-negativity. Using the same approach, it was shown that *UGCG* is one of several genes whose elevated expression was found in metastatic tumors of clear cell renal cell cancer in comparison to primary tumors (Jones et al. 2005).

There is little information available on GalCer expression in human tumors. In studies on molecular markers in human astrocytomas and oligodendrogliomas, it was found that high amounts of GalCer were present more frequently in oligodendrogliomas than in astrocytomas (Sung et al. 1996; Popko et al. 2002). Enhanced synthesis of GalCer was found in MDR human colon cancer HT29^{col} cells derived from HT-29 G^{+} compared with parental HT-29 cells (Kok et al. 2000). However, more is known about expression of the *UGT8* gene in cancer cells and tissues. Transcriptome profiling of prostate cancer cell lines showed that metastatic cells express much higher levels of UGT8 mRNA than non-metastatic cells (Oudes et al. 2005). It was also found that elevated expression of the *UGT8* gene in breast cancer was significantly associated with ER-negativity, and therefore with a more malignant phenotype (Yang et al. 2006; Ruckhaberle et al. 2008). Furthermore, Landemaine et al. (2008) using DNA microarray analysis have shown that *UGT8* is one of six genes whose elevated expression correlated with a significantly increased risk of lung metastases in breast cancer patients. These results were verified by PCR and immunohistochemical staining using antibodies against UGT8 protein. Expression of UGT8 is significantly higher in breast cancer metastases to the lung than in corresponding primary tumors and in primary tumors of UGT8 node-positive patients than in UGT8 node-negative patients (Dziegiel et al. 2010). Also, the amounts of this enzyme in cancerous tissue correlated with higher malignancy grades. These data suggest that UGT8 could be a significant index of breast tumor malignancy and a potential marker for the prognostic evaluation of lung metastases. This proposal is supported by studies of Cao et al. (2018). In agreement with these findings, it was also shown that UGT8 and GalCer are only present in malignant "mesenchymal-like" cell lines forming metastases in nude mice (Dziegiel et al. 2010).

The role of monohexosylceramides in tumor progression. Very little is known about the role of GlcCer and GalCer in tumor progression. Inhibition of GCS activity with specific imino sugar or suppression of *UGCG* gene expression using an antisense mRNA approach

resulted in overall inhibition of ganglioside synthesis and importantly suppressed murine melanoma growth in vivo (Deng et al. 2002; Weiss et al. 2003), suggesting that GlcCer may be involved in proliferation of melanoma cells. However, based on these data, it is impossible to discriminate between the direct involvement of GlcCer in this process and its role as a precursor molecule for more complex gangliosides.

Studies on the role of GalCer in breast cancer progression suggest that this monohexosylceramide acts as an antiapoptotic molecule and its presence facilitates tumor cells to survive in the hostile microenvironment of tumors (Owczarek et al. 2013; Suchanski et al. 2018). In agreement with this hypothesis, the increased expression of UGT8 resulting in accumulation of GalCer was observed in Madin-Darby canine kidney cells in response to hyperosmotic and heat stresses (Niimura and Nagai 2008; Niimura et al. 2010). Ceramide is one of the key proapoptotic molecules (Bieberich 2004; Patwardhan et al. 2016; Ogretmen 2018), and it was originally suggested that the anti-apoptotic effects of GalCer are associated with decreased levels of ceramide as a result of increased synthesis of GalCer (Owczarek et al. 2013). However, further study revealed that cells with high or low levels of GalCer contain essentially the same amounts of ceramide, suggesting that in breast cancer cells, the key antiapoptotic molecule is GalCer itself (Suchanski et al. 2018).

The role of GlcCer and GalCer in multidrug-resistance of cancer cells. There is a lot of information available on the involvement of GlcCer in multidrug-resistance (MDR) of cancer cells. Studies on MDR breast, ovarian, colon and cervix epitheloid cancer cell lines as well as leukemic cell lines revealed that such cells are characterized by increased synthesis and accumulation of GlcCer (Lavie et al. 1996; Lavie et al. 1997; Nicholson et al. 1999; Kok et al. 2000; Morjani et al. 2001) and increased expression/activity of GCS (Liu et al. 2001). Furthermore, clinical studies demonstrated elevated levels of GlcCer in tumor specimens from breast cancer and melanoma patients who failed chemotherapy, but this marker was absent in patients who showed a clinical response (Lucci et al. 1998). In a *gain-of-function* cellular model, overexpression of the *UGCG* gene resulted in accumulation of GlcCer in MDR breast cancer MCF-7-AdrR cells, which made them even more resistant to doxorubicin, daunorubicin and actinomycin D (Liu et al. 2001). On the other hand, in *loss-of-function* cellular models, transfection of the same MCF-7-AdrR cells with antisense cDNA restored their sensitivity to anthracyclines, *Vinca* alkaloids, taxans and other anticancer drugs (Liu et al. 2000; Liu et al. 2001). In in vivo studies, such MCF-7-AdR cells with suppressed expression of the *UGCG* gene were characaterized by the siginificant loss of tumorigenicity and enhanced response to chemotherapy (Sun et al. 2010).

To explain increased resistance of GlcCer-overexpressing breast cancer cells to various anti-cancer drugs and TNF-α-induced apoptosis, it was proposed that increased glycosylation of ceramides by GCS, leading to accumulation of GlcCer, effectively decreases the intracellular pool of ceramides (Liu, Han, Giuliano, Cabot 1999; Liu, Han, Giuliano, Ichikawa, et al. 1999). Similar results were obtained in chemoresistant leukemia patients and MDR HL-60 cells, as the intracellular ceramide levels were clearly lower than in chemosensitive patients and drug-sensitive HL-60 cells, which was correlated with increased activity of GCS and sphingomyelin synthase (Itoh et al. 2003). On the other hand, inhibition of ceramide glycosylation by transfecting MDR cancer cells with GCS antisense cDNA (Liu et al. 2000) or antisense oligodeoxyribonuclotides (Liu et al. 2004) or using specific small molecule inhibitors of GCS or anticancer drugs increased the levels of ceramides, making cells more sensitive to ceramide- and drug-induced apoptosis (Lavie et al. 1997; Cabot et al. 1998; Spinedi et al. 1998; Maurer et al. 1999; Lucci, Giuliano, et al. 1999; Lucci, Han, et al. 1999; Olshefski and Ladisch 2001; Senchenkov et al. 2001). Interestingly, it was found that increased expression of the *UGCG* gene in cancer cells of breast, ovary, cervical and colon origin in response to doxorubicin treatment is dependent on the generation of ceramide (Liu et al. 2008). Therefore, it seems that ceramide is not only the substrate for GCS but also plays a role as its regulatory molecule, increasing expression of the *UGCG* gene (Abe et al. 1996; Komori et al. 2000). How the ceramide increases the expression of the *UGCG* gene is unknown; however, it was shown that signaling cascades leading to increased expression of the *UGCG* gene involve transcription factor Sp1 (Uchida et al. 2004; Liu et al. 2008). According to Liu et al. (2008), such transcriptional up-regulation of the *UGCG* gene by ceramides creates a vicious circle, since it further decreases the ceramide level and deepens the MDR phenotype of cancer cells.

However, it was also shown that overexpression of the *UGCG* gene in Jurkat cells did not affect the intracellular pool of ceramide (Tepper et al. 2000). Furthemore, in breast cancer MCF-7-AdrR cells, the inhibition of *UGCG* gene expression or inhibition of GCS activity by 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) in MDR human cervical carcinoma KB-V0.01 cells not only had no effect on the level of intracellular ceramide but also highly decreased the expression of P-glycoprotein (P-gp, MDR1) (Gouaze et al. 2005; Sun et al. 2010), which is one of the major efflux pumps, removing chemotherapy drugs from cancer cells and inducing MDR phenotype (Bradley et al. 1988; Sikic et al. 1997). On the other hand, ceramide and GlcCer upregulated the expression of the *MDR1* gene (Gouaze-Andersson et al. 2007), although the exact role of GlcCer in the regulation of *MDR1* gene expression was not evaluated. Subsequent studies confirmed that silencing of the *UGCG* gene down-regulated P-gp expression and sensitized cultured cancer cells and experimental tumors to chemotherapy (Liu, Gupta, et al. 2010). It was proposed that GCS upregulates expression of the *MDR1* gene indirectly by increasing the synthesis of globo-series GSLs, which, as components of plasma membrane, activate the cSrc and β-catenin signaling pathways, which in turn directly leads to increased expression of the *MDR1* gene. On the other hand, there are some indications that P-glycoprotein is involved in the translocation of GlcCer from the cytosolic to the luminal side of the Golgi apparatus, which increases the possibilities for synthesis of LacCer and subsequently Gb3Cer (Table I). This resulted in the negative feedback control and therefore increased the activity of GCS, which enhances synthesis of GlcCer, in this way decreasing the intracellular ceramide pool (Lala et al. 2000; Shabbits and Mayer 2002; De Rosa et al. 2004). Based on these results and others (van den Heuvel-Eibrink et al. 2001), it was proposed that expression of P-gp protein increases resistance of acute myeloid leukemia cells to ceramide-induced apoptosis by increasing the activity of GCS, which in turn decreases the level of ceramide (Turzanski et al. 2005). However, the pharmacological inhibition of GCS in T-cell leukemia cell line CCRF-CEM had no effect on expression and function of P-gp (Olshefski and Ladisch 2001).

In breast cancer MCF-7-AdR cells with inhibited expression of the *UGCG* gene, not only decreased expression of P-gp but also increased activity of caspase-3 were observed, which suggested a link between the accumulation of GlcCer, MDR phenotype and increased resistance to apoptosis. An association between the accumulation of GlcCer and decreased sensitivity of cancer cells to drug-induced apoptosis has been shown by studies on drug-resistant and

drug-sensitive human chronic myelogenous leukaemia K562 cells. It was revealed that inhibition of *UGCG* gene expression by siRNA or inhibition of GCS activity by PPMP in drug-resistant K562 correlated with down-regulation of key anti-apoptotic Bcl-2 protein and increased sensitivity to doxorubicin (Liu, Xie, et al. 2010).

In summary, it is now widely accepted that accumulation of GlcCer in cancer cells caused by overexpression of GCS attenuates the accumulation of ceramide and contributes to drug resistance in multidrug-resistant cancer cells (Ryland et al. 2011). However, despite the large amount of literature supporting the role of GCS and GlcCer in cancer cell MDR, there are some indications that their effect may be cell type and/or drug-specific (Segui et al. 2006). For example, in mouse melanoma cells, the presence of GCS did not alter their sensitivity to anticancer drugs (Veldman et al. 2003). It was also shown that inhibition of the *UGCG* gene in drug-sensitive U937 and HL-60 cells actually protects them from daunorubicin-induced apoptosis (Grazide et al. 2004). Interestingly, such treatment did not increase intracellular ceramide concentrations but instead increased GalCer levels. Furthermore, in cells enriched in exogenous GalCer, daunorubicin-induced apoptosis was significantly inhibited. Similarly, Krabbe cells with high levels of GalCer were more resistant to daunorubicin- and cytosine arabinoside-induced apoptosis than Gaucher cells with lower levels of GalCer. Therefore, these data pointed to the possible role of GalCer in MDR of cancer cells. Confirming these observations, it was found that GalCer increases the resistance of breast cancer cells to apoptosis induced by doxorubicin, suggesting that this monohexosylceramide may be involved in MDR of breast cancer cells (Owczarek et al. 2013), which supports previous findings that UGT8 expression is associated with poorer prognosis in breast tumors (Dziegiel et al. 2010). This hypothesis was supported by other studies. It was shown that GalCer expression is elevated in multidrug resistant colon cancer and ovarian cancer cells (Kok et al. 2000; Veldman et al. 2002). Also, Krabbe cells with high levels of GalCer were more resistant to daunorubicin- and cytosine arabinoside-induced apoptosis than Gaucher cells with lower levels of this monohexosylceramide (Grazide et al. 2004). Treatment of U937 and HL60 cells with inhibitors of GCS such as PDMP or PPMP protects them from-daunorubicin-induced apoptosis, which was accompanied by increased levels of GalCer (Grazide et al. 2004). In addition, cells enriched in exogenously added GalCer were significantly more resistant to apoptosis induced by this chemotherapeutic.

GlcCer as a self-antigen

Lipids, including GSLs, are able to induce cell-mediated immunity, when they are presented to the subset of T lymphocytes called Natural Killer T (NKT) cells, characterized by the expression of markers typical for T lymphocytes as well as NK cells. NKT cells are further divided into type I or invariant natural killer (iNKT) cells and type II or non-iNKT cells. iNKT cells recognize and bind lipid antigens and are characterized by a highly restricted repertoire of TCRs (Popovic et al. 2017). Lipid antigens are recognized and bound by iNKT TCRs only when they are presented by the CD1 molecules expressed by APCs (Salio et al. 2014; Kaczmarek et al. 2017). In humans, CD1 isoforms are divided into group I, represented by CD1a, CD1b, CD1c and CD1e and group II, represented by CD1d. Muroids express only CD1d (Barral and Brenner 2007), while ruminants express all CD1 proteins except CD1c (Van Rhijn et al. 2006; Thi et al. 2013). In chicken, two *CD1* genes identified so far are not similar to any mammalian genes (Salomonsen et al. 2005). As one CD1 molecule interacts with different GSLs, it is suggested that binding involved ceramide, not carbohydrate moiety. This hypothesis was directly confirmed by crystallographic data (Zajonc et al. 2003; Koch et al. 2005). Each CD1 binds a defined set of GSLs, has tissue-specific expression and presents antigens T cells with a specific repertoire of TCRs (Salio et al. 2014). It was initially thought that iNKT cells are primarily activated by exogenous glycolipid antigens with a primary α-linked monohexose such as α-galactosylceramide from *Agelas mauritanus* or bacterial glycolipids, e.g., from B*orrelia burgdorferi* and *Sphingomonas* (Kinjo and Kronenberg 2005; Kinjo et al. 2006), and therefore play an important role in microbial immunity (Cohen et al. 2009). However, it should be remembered that primary α-glycosidic linkages are not present in most microbes or in mammalian glycolipids, and importantly, iNKT cells were activated in situations where no foreign glycolipid antigens were present, such as auto-inflammation, virus infection or activation by Toll-like receptors (TLRs). Therefore, based on the data obtained from an experimental model consisting of iNKT cells activated by APCs exposed to lipopolysaccharides (LPS) or other TLR agonists, it was proposed that activation of iNKT cells requires two signals (Brigl et al. 2003). The first signal includes binding the CD1d-lipid complex by TCR. The second signal is delivered by cytokines (mainly IL-12) secreted by APCs. Subsequent studies revealed that this lipid antigen, which specifically activates iNKT cells, is most probably GlcCer, acting as a self-antigen (Brennan et al. 2011). This proposal was further supported by the following findings: (1) GlcCer accumulated in APCs in response to LPS or bacterial infection and (2) inhibition of GlcCer (but not gangliosides or LacCer) synthesis in human bone marrow-derived DCs reduced autoreactivity and iNKT cell activation after LPS addition. Recently, based on results from a mouse model, it was also proposed that GlcCer may induce an immune response acting as a self-antigen in GD (see *GlcCer and lysosomal* GBA1 *in GD* section).

Regarding GalCer, there is still a lack of evidence that β-GalCer is able to provoke an immune reaction. So far, only the α-GalCer has been shown to be an activator of NKT cells and a mediator of immune response in some cancer or virus infections (Ko et al. 2005). However, it is important to remember that α-GalCer is not produced by mammals.

GalCer as a receptor for viruses and bacteria

Even though the major HIV-1 cell receptor present on the surface of T lymphocytes is a CD4 molecule (Bour et al. 1996), CD4-negative brain-, muscle- and intestinal-derived cells and fibroblastoid cells are sensitive to virus infection (Adachi et al. 1987; Chiodi et al. 1987; Dewhurst et al. 1987; Clapham et al. 1989; Tateno et al. 1989; Fantini et al. 1991). These findings indicated that there are alternative ways for the virus to enter the cells, possibly involving another receptor (Harouse et al. 1989). Therefore, using a variety of antibodies directed against surface antigens, it was found that anti-GalCer antibodies specifically inhibited infection of CD4-negative U373-MG and SK-N-MC cells (Harouse et al. 1991). The sensitivity to HIV-1 infection was also associated with GalCer expression in human colon cancer HT-29 and Caco-2 cells (Fantini et al. 1993). Furthermore, it was shown that HIV-1 gp120 binds to mono- and polyhydroxylated forms of GalCer but not to GalCer molecules with nonhydroxylated fatty acids (Bhat et al. 1991; Fantini et al. 1993). In HT-29 cells, binding of gp120 to GalCer localized in lipid rafts caused the release of intracellular calcium and depolymerization of microtubules (Fantini et al. 2000). These results may explain why HIV-1 can affect the absorptive and secretory functions of intestinal

cells (Fantini et al. 1992; Asmuth et al. 1994). It was shown that binding of HIV-1 to GalCer is mediated by the V3 loop of HIV-1surface envelope glycoprotein gp120 (Cook et al. 1994; Yahi et al. 1995), which represents common GalCer-binding domain/structural motif present in Alzheimer β-amyloid peptide and human PrP protein (Mahfoud et al. 2002), and earlier described CLN3 protein (see *GalCer in juvenile neuronal ceroid lipofuscinosis* section).

GalCer is one of several GSLs-3-sulfo-GalCer, GM3 and LacCer (Table I)—receptors for *Helicobacter pylori* (Saitoh et al. 1991), a bacterium responsible for gastritis and duodenal ulcers.

Concluding remarks

Numerous studies unequivocally demonstrated that GlcCer and GalCer contribute to many physiological and pathological phenomena. However, in many cases, more information is available on abnormal expression and/or activities of enzymes responsible for their synthesis and/or degradation than on the involvement of these monohexosylcermides themselves. A lot of information about their biological activities is purely descriptive, meaning that in the future, the major challenge will be to carry out mechanistic studies, where the functions will be examined carefully on molecular and biological levels simultaneously. Since it is generally accepted that GSLs directly affect the functions of membrane proteins and/or regulate the expression of specific genes, the identification of specific signaling pathways and partner molecule/molecules interacting directly with GlcCer and GalCer or their metabolites as well as target genes will be required. It can be done, only by multidisciplinary research, using not only modern genetic, cell biology and biochemistry methods but also biophysical techniques. Considering the complexity of sphingolipid metabolism, such studies must be supported by the analysis of whole sphingolipidome. The elucidation of the mechanisms by which monohexosylceramides exert their biological functions is necessary in order to design effective therapeutic strategies and develop new agents with GalCer and GlcCer and their enzymes as molecular targets to treat important human diseases described in this review. For example, there are numerous reports describing GlcCer as a target for anticancer therapy to decrease the multidrug resistance of cancer cells; however, because of the lack of detailed knowledge on the action of GlcCer on the molecular level, its usefulness is questionable.

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Conflict of interest statement

None declared.

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