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Review

New insights on glucosylated lipids: Metabolism and functions

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ABSTRACT

Ceramide, cholesterol, and phosphatidic acid are major basic structures for cell membrane lipids. These lipids are modified with glucose to generate glucosylceramide (GlcCer), cholesterylglucoside (ChlGlc), and phosphatidylglucoside (PtdGlc), respectively. Glucosylation dramatically changes the functional properties of lipids. For instance, ceramide acts as a strong tumor suppressor that causes apoptosis and cell cycle arrest, while GlcCer has an opposite effect, downregulating ceramide activities. All glucosylated lipids are enriched in lipid rafts or microdomains and play fundamental roles in a variety of cellular processes. In this review, we discuss the biological functions and metabolism of these three glucosylated lipids.

1. Introduction

Glucose is the most important component for most living organisms. Hexoses—of which glucose is one example—evolved as energy sources critical for life. The brain is the most metabolically active tissue in animals and depends exclusively on glucose as its fuel source. Tumors, groups of cells that are metabolically defective, also require glucose for cell growth.

Besides being an energy source, glucose is utilized for membrane glycerolipids synthesis. Moreover, it is metabolically converted to L-serine, which serves as a precursor amino acid for the synthesis of lipids, including phosphatidyl serine/ethanolamine and glycosphingolipids (GSLs) [1]. In mammals brain, L-serine is synthesized and released into the extracellular space by astrocytes and radial glia cells but not by neurons [2,3], indicating that L-serine is essential for developing neurons.

Glucose is metabolically converted to uridine diphosphate (UDP)-glucose, which is used as a lipid head group. To date, three glucosylated lipids—glucosylceramide (GlcCer), cholesterylglucoside (ChlGlc), and phosphatidylglucoside (PtdGlc)—have been identified in mammalian cell membranes [4]. Intriguingly, these three glucosylated lipids are enriched in lipid rafts/lipid microdomains, indicating that lipid glucosylation plays fundamental roles in a variety of cellular processes. In fact, UDP-glucose ceramide glucosyltransferase (UGCG), the key enzyme in glucosylceramide synthesis, exists in essentially all animal tissues [5,6]. The high degree of UGCG gene conservation across multicellular organisms further emphasizes the biological significance of glucosylated lipids. In fact, knocking out the *Ugcg* gene in mouse and *Drosophila* results in embryonic death.

In this review, we summarize new findings of studies on the synthesis and the degradation of GlcCer and related lipids such as ChlGlc and PtdGlc. New insights are derived. We emphasize that although glucosylated lipids have simple structures, their functions are critical for cellular homeostasis and basic cellular activities. Glucosylated lipids are not simply precursor lipids for the synthesis of complex glycolipids, as previously thought.

Abbreviations: ABCA12, ATP-binding cassette transporter A12; α-GalCer, α-galactosylceramide; AMPK, AMP-activated protein kinase; BMP, bis-monoacylglycerophosphate; CBE, conduritol B epoxide; CerS, ceramide synthase; CERT, ceramide transport protein; ChlGlc, cholesterylglucoside; COPII, coat protein complex II; DIM, detergent-insoluble membrane; ER, endoplasmic reticulum; GalCer, galactosylceramide; GBA, acid β-glucosidase; GCase, glucocerebrosidase; GlcCer, glucosylceramide; GPI, glycosylphosphatidylinositol; GSL, glycosphingolipid; GTF, glycosyltransferase family; HSP, heat shock protein; iNKT cells, invariant natural killer T cells; LDs, lipid droplets; LPS, lipopolysaccharide; MS, mass spectrometry; PH, pleckstrin homology; PtdAc, phosphatidic acid; PtdCho, phosphatidylcholine; PtdEth, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdIns(4)P, phophatidylinositol-4-phosphate; PKD, polycystic kidney disease; PtdGlc, phosphatidylglucoside; RsAFP, *Raphanus sativus* antifungal peptide; SAP-C, saposin C; SMS, sphingomyelin synthase; SPT, serine palmitoyl transferase; START, steroidogenic acute regulatory protein-related lipid transfer; UDP-glucose, uridine diphosphate glucose; UGCG, UDP-glucose ceramide glucosyltransferase; VAP, vesicle-associated membrane protein-associated protein

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2. Glucosylceramide (GlcCer)

2.1. Structure of GlcCer

GlcCer is a fundamental GSL found in organisms ranging from mammals to fungi. It is composed of a hydrophilic β-linked glucose and a hydrophobic ceramide. Mammalian GlcCer mainly contains sphingosine (d18:1), a sphingoid base that has one double bond at the C4 position in a *trans* conformation, and N linked C16–C24 fatty acids (Fig. 1A). The N-acyl chain distribution varies among tissues or cell types mainly due to differences in substrate specificity and expression patterns of ceramide synthases (CerS1–6) [7]. Fig. 1A shows the acyl chain distribution of mono-hexosylceramide, which includes GlcCer and galactosylceramide (GalCer), in brain, spleen, liver, kidney, muscle, and adipose tissue of C57BL/6 mice. Do these differences in ceramide moiety link to tissue specific functions?

Brennan et al. showed that GlcCer is a self-antigen that activates invariant natural killer T (iNKT) cells [8], a subset of lymphocytes of the innate immune system that have been reported to recognize CD1d-bound glycolipid antigens such as α-galactosylceramide (α-GalCer) or isogrobotrihexosylceramide (iGb3), both containing α-linked galactose at non-reducing end [9,10]. Interestingly, activation efficiency of GlcCer depends on the composition of the N-acyl chain. For example, C24:1 GlcCer, the predominant GlcCer in spleen (Fig. 1A), effectively activates iNKT cells. This suggests that the ceramide species play an important role in conveying the biological activity of any given GlcCer.

In addition, a unique GlcCer, designated as epidermoside, was isolated from mammalian epidermis [11]. Epidermoside is composed of an amide-linked ω-hydroxy fatty acid and an ester-linked fatty acid (Fig. 1B). Ceramides are the major component of the stratum corneum, and form extracellular lamellar essential for epidermal permeability barrier [12]. Importantly, epidermal ceramides are mainly generated from GlcCer including epidermoside [13], which are packed into lamellar granule in keratinocyte and transported to extracellular lamellar [14]. ATP-binding cassette transporter A12 (ABCA12) is a keratinocyte transmembrane lipid transporter that localizes at lamellar granule [15,16]. The dysfunction of ABCA12 causes the reduction of epidermal ceramides, malformation of the epidermal lipid barrier, and ichthyosis phenotypes [15,16]. Interestingly, Mitsutake et al. suggested that ABCA12 may function as a GlcCer transporter that translocates GlcCer to the inner leaflet of lamellar granule [17]. Although there was no supporting evidence to show the GlcCer-transporting activity in vitro, they found that ABCA12-deficient keratinocyte impairs the packing of GlcCer in lamellar granule, resulting in the defect in ceramide generation [17].

Species-specific differences exist in the ceramide backbone structure of GlcCer in plants, fungi, flies, and worms (Fig. 1C). In plants, GlcCer mainly contains a *cis*-double bond at the C8 position of its sphingoid base [18] (Fig. 1C). The plant sphingolipid Δ-8-desaturase is a stereounselective enzyme that catalyzes not only *trans*- but *cis*-double bonds [19]. Plant GlcCer usually contains an α-hydroxylated fatty acid. Interestingly, oral administration of plant GlcCer improves skin barrier function by upregulating genes associated with tight junction and cornified envelope formation [20]. Fungal GlcCer has unique structural features, including two double bonds at C4 and C8 in the *trans* conformation and a methyl substituent on C9 in the sphingoid base (Fig. 1C, [21]). The gene encoding sphingolipid 9-methyltransferase is found only in fungi [22]. C16 and C18 α-hydroxylated fatty acids are linked to this characteristic sphingoid base. GlcCer of flies contains a shorter chain sphingoid base (d14:1) (Fig. 1C) [23]. In mammals, the first step in ceramide biosynthesis is the condensation of L-serine and palmitoyl-CoA to form 3-ketosphinganine by serine palmitoyl transferase (SPT). This reaction results in the formation of a sphingoid base with C18 chain length. Insect and worm biosynthesis may be different. In insect SPT, there may be a preference for dodecanoyl-CoA (C12 fatty acyl-CoA), forming a major C14 sphingoid base. C20:0 and C22:0 are major fatty acids of GlcCer in *Drosophila melanogaster* [24]. GlcCer of worms contains a C15-methyl-substituted sphingoid base (d17:1) and α-hydroxylated C20–C26 fatty acids (Fig. 1C) [25].

2.2. Biosynthesis of GlcCer

2.2.1. Structure and localization of GlcCer synthase

GlcCer is synthesized by GlcCer synthase (UDP-glucose:ceramide glucosyltransferase; UGCG, GlcT-1, CGT, or GCS, EC 2.4.1.80) from ceramide and UDP-glucose (Fig. 2A). The *ugcg* gene was first isolated by an expression cloning technique using ugcg-deficient mouse melanoma cells [26]. The gene is ubiquitously expressed in most mammalian tissues. The molecular mass of rat/mouse UGCG is approximately 38 kDa on SDS-PAGE, although the value calculated from its cDNA sequence is around 45 kDa (394 amino acid residues). No posttranslational modifications of UGCG, such as N-glycosylation, O-glycosylation, O-GlcNacylation, or acetylation, have been found. Proteome-wide quantification analysis of endogenous lysine ubiquitylation sites revealed that lysines at 44, 49, 57, 104, and 124 of UGCG are ubiquitinated in HEK293 cells [27]. However, the significance of the ubiquitination in UGCG is as yet unclear. Rat UGCG forms a dimer or oligomer with another protein [28]. Binding partners include c-Fos protein and RTN-1C, a reticulon family protein; these activate the enzymatic activity of UGCG [29,30]. These binding protein partners, however, are not essential for UGCG activity, because human UGCG expressed in *Escherichia coli* is active [26].

The 193 histidine residue of UGCG is involved in the binding of both UDP-glucose and the enzyme inhibitor o-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP) [31]. Although UGCG belongs to glycosyltransferase family (GTF) 21 [32], alignment of deduced amino acid sequences revealed that the active site motif (D1, D2, D3, and (Q/R)XXRW) in GTF2 is conserved in UGCG [33]. This enzyme is a type III membrane protein that contains a single putative transmembrane domain at its N terminus, with its C terminus located in the cytosolic face of the Golgi apparatus [5,28,34]. The active site of this enzyme faces the cytosol; thus, GlcCer is synthesized on the cytosolic surface of the Golgi apparatus (Fig. 2B). In addition to mammalian UGCG, fungal, plant, and insect UGCG also have been identified [6]. All of them share a conserved amino acid sequence and an N-terminal transmembrane domain.

Whereas mammalian UGCG mainly localizes in the Golgi apparatus, *D. melanogaster* UGCG (dGlcT-1) localizes to both the Golgi and ER [35]. Although mammals, fungi, and *Drosophila* have only one gene encoding ugcg, *Caenorhabditis elegans* has three genes, each of which encodes the active form of UGCG [36]. In plant cells, a sterol glucoside-dependent, UDP-glucose-independent GlcCer synthesis pathway is possibly present in addition to the UGCG-dependent pathway [37].

2.2.2. Ceramide transport protein (CERT) and GlcCer synthesis

For the synthesis of GlcCer and sphingomyelin, ceramide must be transported from the ER to Golgi compartments, because de novo synthesis of these sphingolipids occurs in the Golgi membranes in mammalian cells [38] (Fig. 2B). The major transport pathway between cellular organelles is the budding and fusion of membrane vesicles [39]. An alternative transport pathway involves CERT, a protein factor responsible for non-vesicular transport of ceramide [40]. CERT contains a pleckstrin homology (PH) domain that binds phophatidylinositol-4-phosphate (PtdIns(4)P), a serine repeat motif, two phenylalanines in an acidic tract (FFAT) motif that binds to vesicle-associated membrane protein-associated proteins (VAPs), the ER resident type II membrane protein, and a steroidogenic acute regulatory protein-related lipid transfer (START) domain that recognizes ceramide. CERT binds to Golgi-abundant PtdIns(4)P and to ER resident protein VAPs, then transfers ceramide from the ER to the *trans*-Golgi network at ER-Golgi membrane contact sites (Fig. 2B) [41]. Sphingomyelin synthase (SMS) 1 and 2 transfer the phosphorylcholine head group from phosphatidylcholine

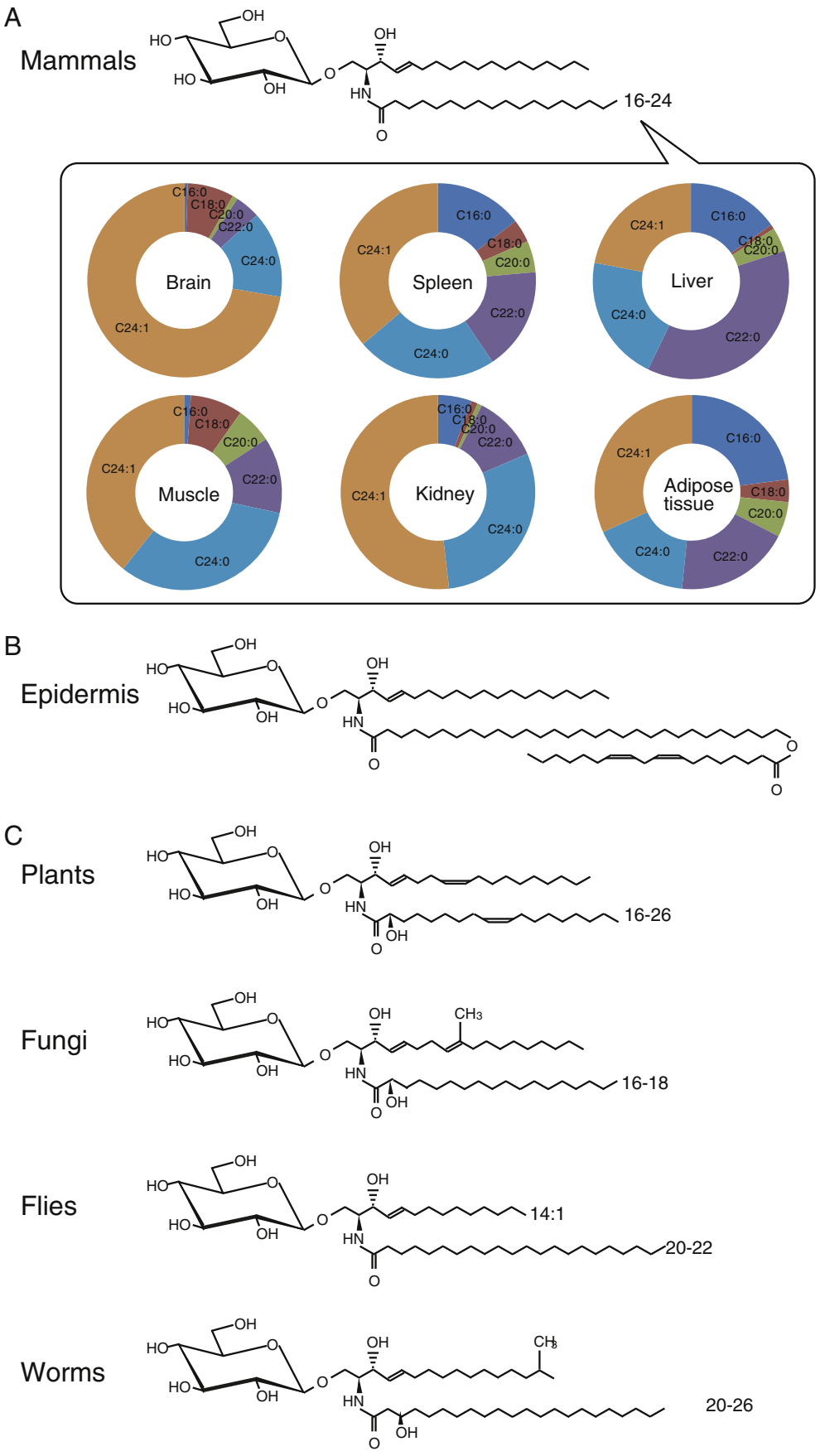

Fig. 1. Structural diversity of GlcCer. (A) Fatty acid species in GlcCer from different tissues of mammals. Sphingolipids were extracted from brain, spleen, liver, muscle, kidney, and adipose tissue of 12-week-old C57BL/6 mice, and quantified by liquid chromatography–electrospray ionization tandem mass spectrometry (LC ESI-MS/MS) analysis as described in [144]. (B) The structure of mammalian epidermal GlcCer (epidermoside). (C) The structures of plants, fungi, flies (*Drosophila*), and worms (*C. elegans*) GlcCer.

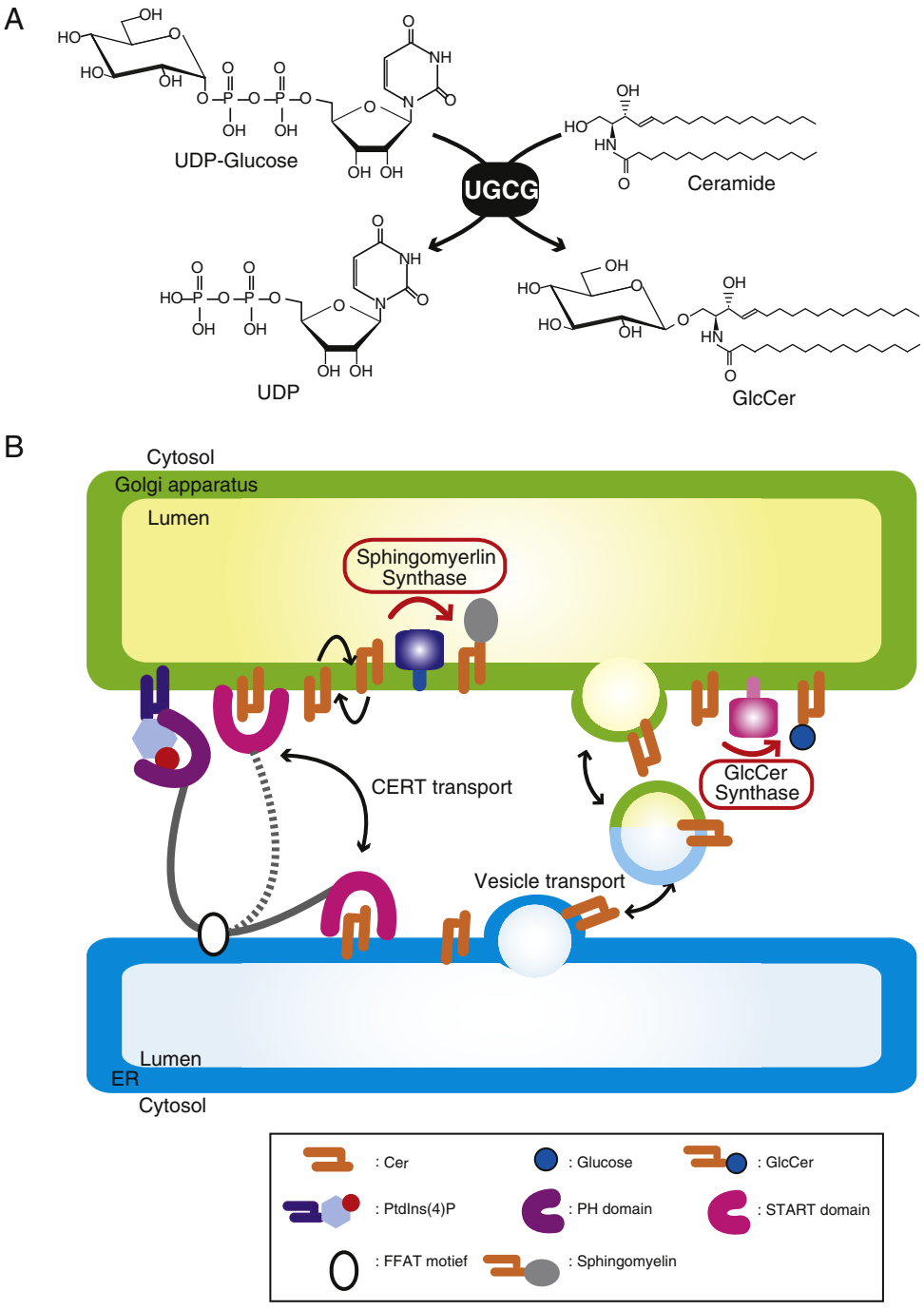

Fig. 2. Machinery of GlcCer synthesis. (A) The reaction scheme of GlcCer synthesis catalyzed by UGCG. (B) Ceramide transport pathways involved in the synthesis of GlcCer and sphingomyelin. CERT binds to PtdIns(4)P in the Golgi apparatus and transports ceramide that is destined to be used for sphingomyelin synthesis. GlcCer is synthesized at the cytosolic face of the Golgi apparatus from ceramide that is transported by a CERT-independent mechanism.

(PtdCho) to ceramide to yield sphingomyelin and diacylglycerol [42,43]. The active site of Golgi-localized SMS1 is on the luminal side of the Golgi apparatus. Once delivered to the Golgi apparatus from the ER by CERT, ceramide translocates to the lumen, possibly by spontaneous transbilayer movement. It is then utilized for sphingomyelin synthesis (Fig. 2B). In CERT-deficient mutant cells, the level of sphingomyelin, but not GlcCer, is reduced [44], supporting the role of CERT in the delivery of ceramide destined for sphingomyelin synthesis.

Ceramide required for GlcCer synthesis is thought to be transported from the ER to Golgi in a vesicle-dependent manner [45,46]. The transport of very long chain fatty acids (C22–24) containing ceramide destined for GlcCer synthesis is regulated by glycosylphosphatidylinositol (GPI) anchor molecules, important protein-anchoring lipids [47]. Cells with a defect in the GPI-anchor maturation system have decreased levels of very long chain GlcCer molecules but normal sphingomyelin levels [48]. Indeed, correctly-modified GPI anchor molecules have been proposed to act as signals for ER exit by coat protein complex II (COPII) vesicles [49]. This machinery may be involved in the transport of very long chain ceramides to the Golgi.

These observations in ceramide transport raise the question of why CERT-transported ceramide is specifically used for sphingomyelin

synthesis but not GlcCer synthesis. It has been thought that CERT mainly transports ceramide to *trans*-Golgi regions where PtdIns(4)P and SMS1 are present, while UGCG localizes at *cis*-Golgi regions or ER and is unable to utilize CERT-transported ceramide. We have suggested an alternative possibility. We found that PtdIns(4)P specifically inhibited in vitro UGCG activity in a concentration-dependent manner, while SMS activity was unaffected (manuscript in preparation). The effect of PtdIns(4)P was overridden by increasing the concentration of UDP-glucose in the reaction solution, suggesting that PtdIns(4)P may inhibit contact between the active site of UGCG and UDP-glucose (Fig. 3).

The interaction between PtdIns(4)P and the PH domain of CERT is indispensable for the transport of ceramide from ER to Golgi [50]. Therefore, CERT-transported ceramide may colocalize with PtdIns(4)P, thereby inhibiting, or interrupting, transfer of glucose from UDP-glucose to ceramide by UGCG (Fig. 3). Supporting this notion is the finding that the catalytic domains of UGCG and PtdIns(4)P both face the cytosolic side of the Golgi membrane [28,51]. It is interesting to know whether they functionally interact with each other in the Golgi membrane.

2.3. Functions of GlcCer

From in vitro and in vivo studies, important roles of UGCG in development and differentiation have been revealed. Knockout and/or knockdown of UGCG are lethal in mouse, *Drosophila*, and *C. elegans*, indicating a crucial role for GlcCer and GSLs synthesized from GlcCer during embryogenesis [35,36,52,53]. *Ugcg* knockout mice die in utero, because gastrulation is blocked by ectodermal apoptosis [53,54]. Several conditional knockout mice have been generated and carefully studied. Examination of these conditional knockout mice revealed that GlcCer synthesis has tissue-specific functions; some tissues require GlcCer synthesis, whereas others, like liver, do not. For a more detailed discussion of GlcCer function, please see the following recent review articles: [4,55]. In the present review, we focus on the role of GlcCer, as revealed by invertebrate model animals.

2.3.1. Epithelial GlcCer in cell polarity

As mentioned above, the UGCG gene is highly conserved across species. Three UGCG genes exist in *C. elegans*. Knockdown of all three UGCG genes causes growth arrest at the first larval stage [52]. It was recently identified using a global *C. elegans* tubulogenesis screening assay that UGCG is a critical enzyme for epithelial polarity [56]. GSLs mediate apical sorting and specifically help to maintain apicobasal polarity in vivo. Depletion of sugar modification genes that add sugars to GlcCer failed to generate the polarity defect phenotype, indicating that GlcCer is the glycolipid responsible for *C. elegans* tubulogenesis.

2.3.2. GlcCer in energy homeostasis

The *Drosophila* fat body is equivalent to adipose tissue and liver in mammals, and is an important tissue for energy homeostasis in this species. Since the biological system for energy metabolism and homeostasis is evolutionarily conserved, *Drosophila* has become widely used as a model for understanding the role of fat tissue in energy homeostasis [57]. UGCG expression in the *Drosophila* fat body regulates energy metabolism [24]. Overexpression of UGCG increases stored nutrition (triacylglycerol and carbohydrate) levels. Conversely, reduced expression of UGCG in the fat body causes a reduction of fat storage. GlcCer is the sole GSL of the fat body, indicating that UGCG-associated GlcCer synthesis in the fat body is responsible for regulating energy homeostasis in the whole body.

Very recently, it has been reported that UGCG in central nervous system (CNS) regulates energy homeostasis [58]. The forebrain-neuron specific deletion of UGCG in mice resulted in obesity, hypothermia, and lower sympathetic activity. Using immortalized hypothalamic neurons (N-41 cells), it has been shown that GlcCer-derived gangliosides affect proper leptin receptor signaling. Although it is not clear whether GlcCer itself plays an important role in this *regulatory system*, this study demonstrated that UGCG expression in neurons of the adult CNS regulates leptin signaling pathway and controls energy homeostasis in whole animal level.

Taken together with the *Drosophila* study, these findings demonstrate that the UGCG in central and peripheral organs is involved in control of energy homeostasis, and suggest that manipulation of UGCG activity could be a potential target for obesity therapy.

In *ob/ob* mice, an animal model of type II diabetes, GlcCer levels are increased in several tissues, such as liver and muscle. Interestingly, insulin sensitivity, glucose homeostasis, and adipocyte function are improved by treating the mice with an UGCG inhibitor, such as N-(5-adamantane-1-yl-methoxy-pentyl)-deoxynojirimycin (AMP-DNM) or N-[(1R,2R)-1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-1-hydroxy-3-(pyrrolidin-1-yl) propan-2-yl]nonanamide (Genz-123346) [59,60]. These results further

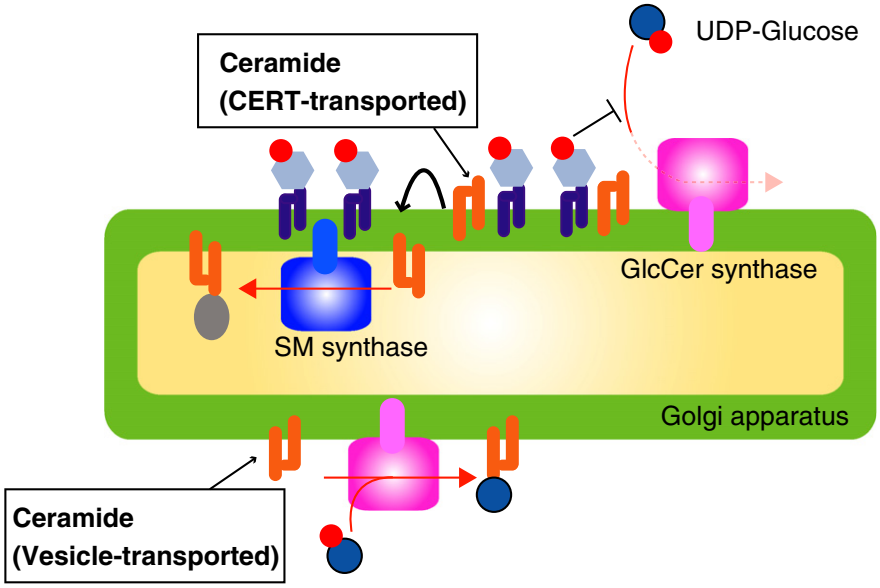

Fig. 3. Proposed mechanism explaining why CERT-transported ceramide is not used for GlcCer synthesis.

indicate that GlcCer is a conserved, key factor that regulates energy homeostasis.

Ceramide also plays a role in insulin resistance [61]. However, which is important in terms of modulator of energy homeostasis—GlcCer or ceramide? Ceramide, rather than GSLs, is required for blocking the activation of Akt/PKB in muscle [62–64]. On the other hand, adipocytes are more sensitive to glucosylated sphingolipids [59,65]. These studies demonstrate that ceramide and GlcCer have tissue-specific functions in controlling energy homeostasis.

Lipid droplets (LDs) are the major cellular organelle for the storage of neutral lipids. Until recently, LDs were thought to be biologically inert. However, LDs turn out to be extremely important for cell activities and functions. LDs exist ubiquitously in most cells and have been implicated in other cellular functions, such as protein storage and degradation [66]. LDs are believed to originate from the ER, although the exact mechanism of their biosynthesis and growth remains to be elucidated. Phosphatidylethanolamine (PtdEth) and PtdCho are major components of most LDs [67]. In addition to these lipids, sphingolipids are also present. The role of sphingolipids in LD growth, however, is still not understood.

Drosophila has become a very useful model animal in LD biology [68]. A growing number of studies on LDs using *Drosophila* have been published, providing deep and new insight into understanding human diseases, such as obesity and type II diabetes. Genome-wide RNAi screening of *Drosophila* tissue culture has revealed numerous regulators of LD biogenesis and utilization [69]. Among these are COPI trafficking proteins, which are functionally conserved and regulate LD morphology in flies and lipid utilization in mammals.

As discussed above, GlcCer exists in the LDs of the *Drosophila* fat body. *Drosophila* with high GlcCer levels in the fat body has large LDs, whereas the opposite is true for low GlcCer levels [24]. Whether GlcCer synthesis has any functional link to COPI trafficking proteins is unknown. Further studies are needed to understand how GlcCer regulates LD formation at the cytosolic surface membranes of the ER.

2.3.3. The function of fungal GlcCer

The functions of fungal GlcCer have been well investigated. Morphological transitions such as budding and germ tube formation are inhibited by GlcCer synthase inhibitors or anti-GlcCer antibodies [70,71]. Immunofluorescent microscopic analysis shows that GlcCer concentrates at the site of budding cells during cell division [71,72]. While yeasts that synthesize GlcCer can survive at higher pHs, GlcCer synthase-deficient mutants are unable to grow [73], indicating that GlcCer is involved in fungal cell growth and differentiation, especially under alkali conditions.

Fungus-specific GlcCer is also thought to be important for the action of plant defensin from *Raphanus sativus* antifungal peptides (RsAFPs) [74]. RsAFP2 inhibits the growth of pathogenic fungi such as *Candida albicans* and *Pichia pastoris*. However, GlcCer synthase-deficient mutants are resistant to RsAFP2. RsAFP2 interacts with fungus-specific GlcCer, but not with plant or mammalian GlcCer. This suggests that the ceramide moiety is important for the recognition of GlcCer by RsAFP2 (Fig. 1).

Most importantly, fungal GlcCer is required for the virulence of pathogenic fungi to be expressed. Disruption of enzymes involved in GlcCer biosynthesis reduces the pathogenicity of *C. albicans* [75] and *Cryptococcus neoformans* [76,77], which is the common species causing cryptococcosis, a life-threatening central nervous system infection [78]. Thus, targeting the metabolic pathway of fungus GlcCer has become a new therapeutic strategy for the treatment of fungal infections, because the pathway contains fungus-specific enzymes such as sphingolipid 9-methyltransferase.

2.4. Regulation of GlcCer synthase activity

2.4.1. Transcriptional regulation of ugcg

Because mammals have only one gene encoding ugcg, changes in *ugcg* mRNA expression levels are critical for determining intracellular GlcCer levels. Previous reports indicate that several compounds can alter *ugcg* mRNA expression levels. Treatment with the endotoxin lipopolysaccharide (LPS), which mimics Gram-negative infections, increases GlcCer levels and *ugcg* mRNA expression in mouse bone-marrow-derived dendritic cells (BMDCs) [8] and in liver, spleen, and kidney in Syrian hamsters [79,80]. GlcCer is a potent self-antigen that activates iNKT cells [8]. Thus, the LPS-induced accumulation of GlcCer is thought to represent an innate danger signal for microbial infections.

Polycystic kidney disease (PKD) is one of the most common genetic disorders accompanied by renal cystic growth and massive kidney enlargement [81]. GlcCer and ganglioside GM3 levels in kidney are increased in PKD patients and mouse models of PKD [82]. Since the inhibition of UGCG activity effectively prevents cystogenesis in mouse models of PKD, the accumulation of GSLs is believed to be an important factor contributing to disease progression. Although the precise mechanism is unknown, *ugcg* mRNA is overexpressed in kidney cells derived from mouse models of PKD [82].

The expression of *ugcg* mRNA is significantly increased in tumors of the breast (particularly in metastatic carcinoma), small intestine, cervix, and rectum compared to that in normal human tissues [83]. *Ugcg* overexpression is associated with drug resistance in several cancer cells and the maintenance of pluripotency in breast cancer stem cells [84,85]. Treatment of drug-sensitive cancer cells with the anticancer drug doxorubicin induces an increase in ceramide, which drives apoptosis and cell cycle arrest [86]. In drug-insensitive cancer cells, the tumor-suppressing effect of ceramide is counteracted by its rapid conversion into GlcCer, which is mediated by overexpressed UGCG [87]. Although the *ugcg* promoter region lacks TATA and CAAT boxes, it contains some transcription-factor recognizing sites such as Sp1 binding sites, which are indicators of typical housekeeping genes [88]. It also contains the motifs for AhR, NF-kB/C-Rel, AP-2, CAP, and GATA-1 binding sites [89]. In doxorubicin-treated cancer cells, Sp1-mediated transcription of *ugcg* mRNA is upregulated [90,91]. Suppression of UGCG expression by siRNA and inhibition of UGCG activity sensitize cancer cells to anticancer agents [86]. These findings suggest that UGCG could be a potential target for cancer therapy.

2.4.2. Post-translational regulation of UGCG

Mutations in presenilin 1, an essential component of the γ-secretase complex [92], are associated with early onset familial Alzheimer's disease. These mutations result in the preferential deposition of pathogenic amyloid β. Interestingly, the UGCG protein expression is reduced in mutant presenilin 1-transfected neuronal cells, but mRNA expression is unaffected [93]. Correspondingly, the amounts of GlcCer and ganglioside, which is synthesized from GlcCer, are significantly decreased in these cells. Reduction of neuroprotective ganglioside probably makes the neurons vulnerable to cellular stresses, including the deposition of amyloid β. The precise mechanism by which mutant presenilin 1 causes the reduction in UGCG protein expression is unclear. We predict that some abnormalities in the ubiquitin–proteasome pathway may be involved in this phenomenon, because UGCG has multiple ubiquitination sites in its cytoplasmic domain [27].

2.4.3. Regulation of GlcCer synthase activity by AMPK

The hydrolysis of ATP drives all energy-requiring processes in living cells. To maintain ATP at a sufficient level, eukaryotic cells have an important nutrient and energy sensor, AMP-activated protein kinase (AMPK) [94]. AMPK is a heterotrimeric serine/threonine kinase that promotes ATP-generating pathways, such as glycolysis or fatty acid oxidation, while inhibiting anabolic processes, such as biosynthesis of fatty acids, cholesterol, glycogen, and triacylglycerol under energy reducing conditions (i.e., increasing AMP/ATP or ADP/ATP ratios).

Recently, we found that AMPK also affects the biosynthesis pathway of GlcCer (manuscript in preparation). Intracellular GlcCer levels and UGCG activity were reduced by AMPK activating drugs, such as 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR)

and the anti-diabetic drug metformin. On the other hand, AMPK inhibitors or AMPK siRNA overrode the reduced GlcCer synthase activity or cellular GlcCer levels under AMPK-activating conditions, indicating that AMPK is a negative regulator of GlcCer synthesis. UGCG protein and mRNA expression were unchanged under AMPK-activating and AMPK-inhibiting conditions. It is important to understand the molecular mechanism by which GlcCer levels are controlled by AMPK.

2.5. Degradation of GlcCer

2.5.1. Mammalian GCases

GlcCer is degraded by the detachment of glucose from GlcCer by glucocerebrosidase (GCase or also called glucosylceramidase, EC3.2.1.45). In mammals, catabolism of GlcCer mainly takes place in lysosomes by acid-GCase (GBA1), with the assistance of saposin C (SAP-C), a sphingolipid activator protein, and negatively charged lipid bis-monoacylglycero-phosphate (BMP) [95]. GBA1 is sorted to lysosomes by binding with lysosomal integral membrane protein type 2 (LIMP-2) [96,97]. GBA1 is a water soluble, positively charged glycoprotein that can attach to lysosomal membranes by interacting with BMP. SAP-C activates GBA1 by mediating the interaction between the substrate and the enzyme [98,99].

A deficiency in GBA1 activity due to biallelic mutations in the *Gba1* gene leads to the most common inherited sphingolipidosis, Gaucher disease. In this patient, GlcCer accumulates in lysosomes of macrophages, causing liver and spleen enlargement, and impairment of the central nervous system [100]. Very interestingly, in addition to Gaucher disease, GBA1 mutations represent a genetic risk factor for developing insulin resistance [101] and synucleinopathies such as dementia with Lewy bodies (DLB) and Parkinson's disease [102,103]. Mazzulli et al. reported that the accumulation of GlcCer due to GBA1 dysfunction influences the formation of neurotoxic amyloid of α-synuclein in lysosomes [104].

The abnormal accumulation of GlcCer is linked to several human diseases. To counter the toxic effects of GlcCer, mammalian cells possess multiple GlcCer-degrading enzymes, in addition to GBA1, to tightly regulate intracellular GlcCer levels. GBA1 is specifically and irreversibly inhibited by conduritol B epoxide (CBE) [105]. GBA2 is a CBE-insensitive, non-lysosomal GCase that hydrolyzes not only GlcCer but also bile acid-3-O-β-glucoside [106,107]. GBA2 is a non-integral membrane protein that localizes at the cytosolic surface of ER and Golgi, where it is in close association with membranous phospholipids [108]. Based on its localization and topology, GBA2 is thought to control the amount of newly synthesized cytosolic GlcCer.

Human and mouse studies have revealed the physiological importance of GBA2. Although *Gba2* knockout mice exhibit normal bile acid metabolism, GlcCer accumulates in multiple tissues, including testis, liver, and brain [106]. Therefore, at least in these tissues, this suggests that GBA2 mainly functions as a GlcCer-degrading enzyme in vivo. Accumulation of GlcCer due to GBA2 deficiency leads to impaired male fertility as a result of aberrant sperm formation [106]. Delay of liver regeneration after partial hepatectomy has also been reported in these mice [109].

GBA2 activity is reduced in cells from *Gba1*-deficient Gaucher disease patients, suggesting that GBA2 may also be involved in the pathogenesis of Gaucher disease [108]. Moreover, *Gba2* mutations are found in some neurodegenerative disorders, such as autosomal-recessive cerebellar ataxia (ARCA) and hereditary spastic paraplegias (HSPs) [110,111]. Human genetic studies strongly suggest that dysfunction of GBA2 probably affects the progression of ARCA and HSPs [110,111].

As described above, the overexpression of UGCG confers drug resistance to cancer cells by reducing ceramide, an apoptosis-inducible lipid mediator. GBA2, on the other hand, has anticancer activity that results in the generation of ceramide at ER and promotes ER stress and apoptosis [112]. The GBA2-mediated GlcCer catabolic pathway is expected to be a fruitful avenue to focus on for new diagnostics or new therapeutics for these diseases.

GBA3, also known as Klotho-related protein (KLrP) [113], is a CBE-insensitive cytosolic GCase [114]. The function of GBA3 is largely unknown, but at least, it seems to have little influence on the manifestation of Gaucher disease [115].

Lactase phlorizin hydrolase, which is exclusively present in the plasma membrane of the small intestine, is thought to be involved in the digestion of dietary GlcCer, lactose, and other glucose-containing compounds [116]. It is a transmembrane protein with two active sites located on the luminal side [117,118].

2.5.2. Fungal GCase

As shown in Fig. 1, fungi synthesize a fungus-specific GlcCer that contains a unique sphingoid base possessing two double bounds and a methyl substitution (methyl d18:2). Correspondingly, fungi possess a fungus-specific GCase, designated as EGCrP1 [119]. This enzyme is listed in several fungal genomic databases as a homolog of endoglycoceramidase, which cleaves the ceramide-glucosidic linkage of various GSLs to release free oligosaccharide and ceramide [120–122]. Recombinant EGCrP1 specifically hydrolyzes GlcCer but not other GSLs. Disruption of egcrp1 in the pathogenic fungus *C. neoformans* leads to the accumulation of immature GlcCer, which has sphingoid bases lacking a methyl substitution and double bonds [119]. This observation indicates that EGCrP1 participates in the catabolism of GlcCer and especially functions to eliminate immature GlcCer generated as byproducts due to the broad specificity of UGCG.

Several reports have indicated that fungus-specific GlcCer possessing a methyl d18:2 sphingoid base is biologically relevant, as described above. By contrast, immature GlcCer is unable to compensate for the functions of fungus-specific GlcCer [75,77,123,124]. Ceramide maturation enzymes such as sphingolipid delta 4-desaturase and 9-methyltransferase utilize ceramide, not GlcCer, as an acceptor substrate [22,125]. Thus, once immature ceramide is converted to GlcCer, the process of maturation is terminated, resulting in the accumulation of immature GlcCer. EGCrP1 seems to convert the immature GlcCer to immature ceramide, causing the maturation of GlcCer to proceed again [119].

3. Cholesterylglucoside

In 2000, Murofushi and co-workers first isolated mammalian glucosylated cholesterol (1-O-cholesteryl-β-D-glucopyranoside, ChlGlc) from cultured TIG-3 fibroblasts [126] and gastric mucosa [127] of humans (Fig. 4). Interestingly, heat shock rapidly induces ChlGlc synthesis followed by HSF1 activation and HSP70 induction in human fibroblasts [128]. In order to decipher the molecular mechanisms of HSP70-induction by ChlGlc, it is essential to identify the enzyme responsible for ChlGlc formation. Akiyama et al. proposed that GlcCer is a glucose donor for ChlGlc synthesis [129]. Supporting this notion is the finding that UGCG-deficient GM-95 cells are unable to synthesize ChlGlc without the exogenous addition of GlcCer. ChlGlc may serve as a heat-associated signaling platform or a heat-signal molecule for HSP gene activation, but details of ChlGlc functions are as yet unknown.

4. Phosphatidylglucoside (PtdGlc)

4.1. Chemical structure and properties of PtdGlc

In 2001, Nagatsuka et al. reported the possible presence of a glucose-containing glycerolipid in human cord blood cells [130]. Later the same group found a similar glucosylated lipid in detergent-insoluble membrane (DIM) fractions of human promyelocytic leukemia cells [131]. However, the compound was in very low concentrations and its structure was alkaline-labile. Thus, it was very difficult to purify PtdGlc to determine its complete structure. In 2006, Yamazaki et al. generated a PtdGlc-specific monoclonal antibody termed DIM21 by

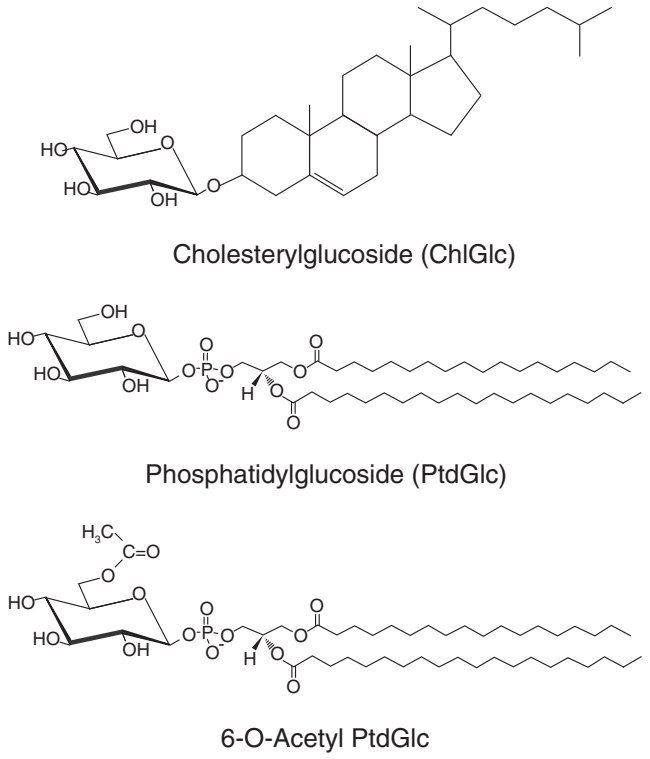

Fig. 4. Structural simplicity of ChlGlc and PtdGlc.

immunizing mice with lipid rafts isolated from HL60 cells [132]. Careful studies using synthetic derivatives of PtdGlc showed that a hydroxyl group at position 6 of the hexose ring and an ester group at *sn*−2 of arachidic acid are critical for antibody binding [133]. By using the monoclonal antibody, PtdGlc was isolated from fetal rodent brains and purified to homogeneity. Its complete structure was determined to be 1-stearyl-2-arachidoyl-*sn*-glycerol-3-phosphoryl-β-D-glucopyranoside (Fig. 4).

Acetylated PtdGlc (1-stearyl-2-arachidoyl-*sn*-glycerol-3-phosphoryl β-D-(6-O-acetyl)glucopyranoside) also exists in fetal rat brain [134] (Fig. 4). Very interestingly and importantly, PtdGlc isolated from fetal rat brain has only one fatty acid combination: the *sn*−1 and *sn*−2 chains are exclusively stearic acid (C18:0) and arachidic acid (C20:0), respectively. A single molecular species rarely occurs in natural phospholipids. Also, in mammals there are very few natural lipids that possess the C20:0 acyl chain as their major component. It is also important to note that PtdGlc from rat brain contains a PtdGlc stereoisomer with the phosphate group at the *sn*−1 position of glycerol.

4.2. Distribution

Expression of PtdGlc is developmentally regulated in the rodent brain. PtdGlc is most strongly expressed in radial glia in early stages of developing rat brains (E12.5–14.5). Neural stem cells (Type B) in the subventricular zone continuously express PtdGlc. The glycolipid is thought to be a good cell surface marker for stem cells [135].

PtdGlc is also found in various tissues of humans, indicating that it has previously unrecognized important biological roles in mammals [136]. Differentiated CD15-positive neutrophils contain PtdGlc [137]. In these cells, PtdGlc comprises C18:0/C18:0 and C18:0/C20:0 fatty acyl chains [138].

4.3. Physical properties

Examination of the thermal behavior of synthetic PtdGlc through differential scanning calorimetry (DSC) analysis shows that synthetic PtdGlc has a high main phase transition temperature of 76.4 °C [139]. This high temperature property is quite reasonable, since PtdGlc consists of saturated long-chain fatty acids. Interestingly, DSC analysis shows that PtdGlc is not miscible with sphingomyelin, even though GlcCer is. In addition, PtdGlc is poorly miscible with PtdCho. These physical properties suggest that PtdGlc forms distinct, PtdGlc-rich lipid domains on plasma membranes (please see our recent review for details [140]). Immunoelectron microscopy with DIM21 using the SDS-digested freeze fracture replica labeling method demonstrated that PtdGlc forms distinct lipid domains exclusively on the outer, not inner, leaflet of the plasma membrane of HL60 cells and A549 cells, a human alveolar epithelial cell line [139].

4.4. Possible roles of PtdGlc and its metabolites

The biological roles of PtdGlc remain to be elucidated. To this end, we used DIM21 antibody as an artificial ligand and found that adding DIM21 to cultured neural progenitor cells prepared from fetal mouse telencephalon results in the recruitment of EGF receptors into PtdGlc-rich lipid rafts, leading to the activation of EGF receptors [141]. In human neutrophils, DIM21 treatment induced Fas clustering, leading to Fas-dependent apoptotic cell death [138]. This suggests that the Fas-receptor is associated with lipid rafts composed of PtdGlc on neutrophil plasma membranes.

Although its physical properties are similar to GlcCer, PtdGlc is clearly different from GlcCer in that its acyl chain is easily cleaved by phospholipases, like PLA2, to become water-soluble lyso-PtdGlc. In mammals, the enzyme that converts GlcCer to lyso-GlcCer is yet to be identified. Lyso-PtdGlc has strong growth cone collapse activities in cultured mouse DRG neurons [4]. Like other lyso-phospholipids, such as lyso-phosphatidic acid (LPA) and lyso-PtdEth (LPE), lyso-PtdGlc also may have significant biological functions in vivo. It would also be interesting to determine whether a PtdGlc-specific phospholipase or preferential phospholipases like phosphatidylinositol (PtdIns)-specific PLC also exist.

How PtdGlc is biosynthesized remains unknown. Our preliminary studies indicated that the PtdGlc biosynthesis is UDP-glucose dependent and occurs in luminal ER membranes. Thus, the biological significance of lipid glucosylation of GlcCer and PtdGlc must be different. Future study is necessary to identify the gene responsible for glucosylation of phosphatidic acid (PtdAc).

The role of O-acetylation of PtdGlc at the C6 position of its glucose ring remains unclear. The acetylated lipid may be immunogenic and have its own biological function. The presence of an O-acetylated glucose residue may reflect the metabolic state of cells, since acetyl CoA must be incorporated into luminal ER by acetyl CoA transporter (AT-1) in the ER membrane (for details, please see review [142]).

5. Concluding remarks

All three glucosylated membrane lipids discussed in this review have conserved biological functions. It is of particular interest that GlcCer synthesis regulates the accumulation and release of stored triglycerides in the fat body of *Drosophila* and adipose tissue of mammals. The regulatory role of GlcCer in energy homeostasis is not surprising, because the basic building blocks of GlcCer synthesis, namely UDP-glucose, palmitoyl-CoA, and L-serine (derived from glucose), are directly related to energy metabolism. From this point of view, it is reasonable that UGCG can sense the energy state of a cell, since the transfer of glucose from UDP-glucose produced in the cytosol occurs in the cytosolic but not the luminal side of Golgi membranes.

In the case of GlcCer, different GlcCer species exist in different organisms. Even within the same organism, different GlcCer species exist in different tissues. These differences are due to variations in the sphingosine base and fatty acyl chain compositions of different GlcCer species. Therefore, it would be reasonable to conclude that each GlcCer species

may have specific and unique, yet far-reaching, biological functions, as proposed by Hannun and Obeid [7].

In contrast to GlcCer, PtdGlc and ChlGlc are simple in their hydrophobic moiety. PtdGlc contains only saturated fatty acids (18:0 and 20:0). Based on its physical properties, PtdGlc is thought to form specific lipid microdomains or lipid rafts on the outer leaflet of plasma membranes. Products from PtdGlc degradation are also expected to act as second messengers and/or signaling molecules functioning in cell–cell communication.

Molecular cloning of the gene that encodes the synthetic enzyme that glycosylates PtdAc to generate PtdGlc is an essential step to better understand the in vivo roles of PtdGlc. Advanced MS technology absolutely will accelerate this process, helping us to gain a more in-depth understanding of the roles of glucosylated lipids. However, one must be careful in using high-throughput approaches to identify PtdGlc and its metabolites, since the total mass number of PtdGlc is identical to that of the more abundant membrane component, PtdIns [143]. We expect that further investigations of simple monoglucosylated lipids will open a new chapter in membrane lipid biology and glycobiology.

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