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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