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REVIEW ARTICLE

The role of sphingolipids in endoplasmic reticulum stress

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(Received 3 April 2020, revised 15 May 2020, accepted 8 June 2020, available online 28 June 2020)

doi:10.1002/1873-3468.13863

Edited by Liana Silva

The endoplasmic reticulum (ER) is an important intracellular compartment in eukaryotic cells and has diverse functions, including protein synthesis, protein folding, lipid metabolism and calcium homeostasis. ER functions are disrupted by various intracellular and extracellular stimuli that cause ER stress, including the inhibition of glycosylation, disulphide bond reduction, ER calcium store depletion, impaired protein transport to the Golgi, excessive ER protein synthesis, impairment of ER-associated protein degradation and mutated ER protein expression. Distinct ER stress signalling pathways, which are known as the unfolded protein response, are deployed to maintain ER homeostasis, and a failure to reverse ER stress triggers cell death. Sphingolipids are lipids that are structurally characterized by long-chain bases, including sphingosine or dihydrosphingosine (also known as sphinganine). Sphingolipids are bioactive molecules long known to regulate various cellular processes, including cell proliferation, migration, apoptosis and cell–cell interaction. Recent studies have uncovered that specific sphingolipids are involved in ER stress. This review summarizes the roles of sphingolipids in ER stress and human diseases in the context of pathogenic events.

Keywords: acyl chain length; ceramide; disease; endoplasmic reticulum stress; sphingolipid

Sphingolipids (SLs) refer to a family of lipids that contain long-chain or sphingoid bases, which are represented by sphingosine or dihydrosphingosine (also known as sphinganine) in mammals [1,2]. SLs are some of the most important structural components of the plasma membrane, and their composition affects not only biophysical membrane properties but also the functions of proteins embedded in such membranes [3,4]. In addition, glycosphingolipids (GSLs), which are SLs that contain a carbohydrate, are involved in cell–cell or cell–pathogen interactions that influence a variety of immunologic functions [5]. Furthermore, the

Abbreviations
AD, Alzheimer's disease; AFT, activating transcription factor; Aβ, amyloid β-protein; BiP, binding immunoglobulin protein; CerS, ceramide synthases; CHOP, CAAT/enhancer-binding protein homologous protein; eIF, eukaryotic translation initiation factor; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; GalCer, galactosylceramide; GlcCer, glucosylceramide; GlcCerase, glucocerebrosidase; GSL, glycosphingolipid; HFD, high-fat diet; HNSCC, human head and neck squamous cell carcinoma; IP3R, inositol 1,4,5-triphosphate receptors; IRE1, inositol requiring enzyme 1; L-ASMase, lysosomal acid sphingomyelinase; MDA-7/IL-24, melanoma differentiation-associated gene-7/interleukin 24; NAFLD, nonalcoholic fatty liver disease; NF-B, nuclear factor kappa B; NSMase, neutral SMase; PD, Parkinson's disease; PERK, protein kinase RNA-activated-like endoplasmic reticulum kinase; RIDD, regulated inositol requiring enzyme 1-dependent decay; S1P, sphingosine-1-phosphate; S1PR, sphingosine-1-phosphate receptor; S-ASMase, secreted acid sphingomyelinase; SERCA, sarco/endoplasmic reticulum Ca2+ ATPase; SL, sphingolipid; SMase, sphingomyelinase; SphK, sphingosine kinase; SPP, sphingosine-1-phosphate phosphatase; SPTLC, serine palmitoyltransferase; XBP1, X-box binding protein 1; XBP1s, spliced isoform of XBP1.

balance between two simple SLs, ceramide and sphingosine-1-phosphate (S1P), has been proposed to impact cell fate [6,7]. SLs are regarded as critical mediators in various intracellular signalling pathways controlling fundamental processes such as cell proliferation [8], differentiation [9], apoptosis [10], inflammation [11], migration [12,13] and the cellular stress responses, which includes endoplasmic reticulum (ER) stress [14–16]. The *de novo* SL synthetic pathway begins in the ER, which is an intracellular hub for synthesis and folding of membrane and secreted proteins [17]. ER stress occurs when there is a build-up of misfolded proteins in the ER and the ER cannot handle the imbalance between the demand for protein folding and the ability to properly fold proteins [18]. ER stress due to either an increase in protein misfolding or a decrease in the capacity of the ER to manage these proteins can lead to cellular dysfunction and is linked to various human diseases, including diabetes, neurodegeneration and cancer [19,20]. In this review, we discuss the emerging link between SL and ER stress. The ER stress signalling pathways described herein are those related to SL metabolism (Fig. 1). Readers are directed to other excellent review papers for additional details about ER stress [19,21,22].

Endoplasmic reticulum stress pathway

The unfolded protein response (UPR) refers to a specific ER quality control system that is needed to maintain ER homeostasis. Because protein folding and quality checks must be highly regulated in the ER for optimal cell function, the UPR has adapted to correct misfolded proteins or, if correction is impossible, to eliminate such proteins *via* a cellular process called ER-associated protein degradation (ERAD) [21]. The ultimate goal of the UPR is to attenuate protein overload and restore ER function.

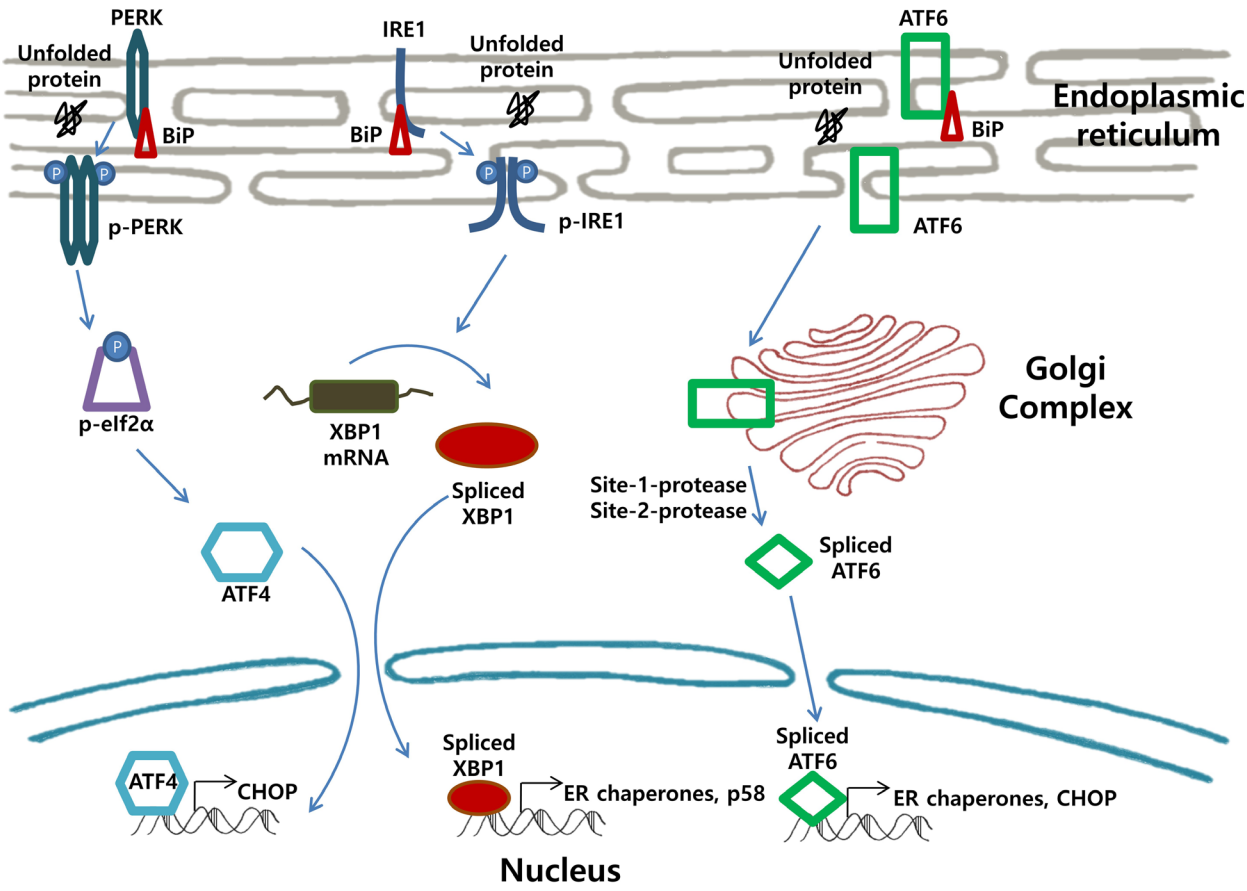


Fig. 1. Endoplasmic reticulum stress pathways. Accumulation of misfolded proteins in the ER decreases BiP binding to the three major stress sensors, IRE1, PERK and ATF6, and leads their activation. Freed PERK phosphorylates eIF2α and induces the translocation of ATF4 into the nucleus. Released IRE1 triggers a selective excision and splicing of dual stem loops within XBP1 mRNA to generate XBP1s. ATF6 that is released from BiP is trafficked from the ER to the Golgi apparatus and is cleaved by site-1 and site-2 proteases. In the nucleus, ATF4, XBP1s and cleaved ATF6 activate the transcription of UPR target genes including ER chaperones and CHOP.

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The UPR is composed of three major signalling branches: inositol requiring enzyme 1 (IRE1), protein kinase RNA-activated-like ER kinase (PERK) and activating transcription factor (AFT) 6. The ER chaperone, binding immunoglobulin protein (BiP), which is also known as glucose-regulated protein 78, is a master regulator of ER stress and functions as a negative regulator of UPR signalling. All three ER stress sensors, IRE1, PERK and ATF6, are ER-resident transmembrane proteins that are constitutively bound to BiP under normal conditions. Because BiP also binds to misfolded proteins, the accumulation of misfolded protein in the ER decreases BiP binding to the three stress sensors. Thus, an increase in misfolded protein contributes to BiP dissociation from the ER stress sensors for conversion to an active state [23]. Freed IRE1 and PERK homodimerize or oligomerize and trans-autophosphorylate to activate their downstream pathways [21,24]. Released ATF6 has an exposed ER export motif for translocation to the Golgi apparatus [21,25,26].

Inositol requiring enzyme 1 pathway

IRE1 exists as an inactive monomer until it undergoes autophosphorylation, and BiP release from IRE1 induces IRE1 oligomerization and activation. IRE1 has both a kinase domain and a ribonuclease domain. The endoribonuclease activity of IRE1 triggers the selective excision and splicing of dual stem loops within X-box binding protein 1 (XBP1) mRNA to generate a spliced isoform of XBP1 (XBP1s) [27]. XBP1s initiate the transcription of various UPR targets that are important for proper protein folding and the ERAD pathway [21]. Moreover, the ribonuclease activity of IRE1 can cleave a wide variety of transcripts that contain the consensus sequence (CUG-CAG) accompanied by a stem-loop structure through a mechanism called regulated IRE1-dependent decay (RIDD) [21,28]. The RIDD-cleaved transcripts are then subsequently degraded by exoribonucleases [28]; therefore, RIDD relieves the ER protein load *via* transcript degradation and helps to restore ER homeostasis [21].

Protein kinase RNA-activated-like ER kinase pathway

PERK belongs to a family of serine/threonine kinases, and one of its phosphorylation targets is eukaryotic translation initiation factor (eIF) 2α, which regulates the first step of protein synthesis initiation. Importantly, eIF2α promotes the binding of the initiator tRNA to 40S ribosomal subunits through the formation of an eIF2 heterotrimer [21]. PERK, once dissociated from BiP, undergoes oligomerization and is activated by trans-autophosphorylation [29]. Activated PERK phosphorylates eIF2α at serine 51, which suppresses eIF2B activity and thereby causes a global reduction in translation [21]. In contrast, eIF2α phosphorylation can also lead to more efficient translation of specific transcripts including ATF4. ATF4 can bind to the UPR element, which promotes the transcriptional modification of UPR target genes including CAAT/enhancer-binding protein homologous protein (CHOP) [30] In early UPR stages, ATF4 transcriptional activity contributes to pro-survival events, whereas in a prolonged UPR state, it induces ATP depletion, oxidative stress, and cell death *via* increased CHOP expression [21,27].

Activating the transcription factor 6 pathway

When ATF6, a transcription factor that has two isoforms, ATF6A and ATF6B, is released from BiP, it is trafficked from the ER to the Golgi apparatus where it is cleaved by the two Golgi-resident proteases, site-1 protease and site-2 protease [25]. Cleaved ATF6 is composed of a transcriptional activation domain, a DNA-binding domain and nuclear localization signals [21]. When ATF6 finally enters to nucleus, it increases the transcription of genes related to the UPR and ERAD [23]. Similar to IRE1 and PERK signalling cascades, ATF6 can also enhance the transcription of XBP1 and CHOP [21].

Protein degradation pathways during ER stress

The two main protein degradation pathways during ER stress are proteasome-mediated degradation *via* ERAD and lysosome-mediated protein degradation *via* autophagy [21]. Both cleaved ATF6 and XBP1s, which are formed during the UPR process, increase genes involved in the ERAD machinery [31]. ERAD is then responsible for the recognition and clearance of terminally misfolded proteins in the ER. These proteins are exported to the cytosol where they are disposed by the ubiquitin–proteasome pathway [32]. When the build-up of misfolded proteins cannot be properly eliminated by ERAD, autophagy is triggered as an alternative pathway to manage protein overload [32]. Autophagy is an intracellular lysosome-mediated bulk degradation pathway for recycling and eliminating worn-out proteins, protein aggregates and damaged organelles. However, it has also emerged as an essential protective mechanism during ER stress [33]. Autophagy

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sequesters targets within autophagosomes. The autophagosome then fuses with a lysosome and lysosomal hydrolases degrade the contents [21].

Sphingolipid metabolic pathways

Ceramide synthesis and degradation

The SL metabolism pathway is summarized in Fig. 2. *De novo* synthesis of ceramide starts with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase (SPTLC), which generates 3-ketosphinganine [34]. Then, 3-ketosphinganine is converted to sphinganine by 3-ketosphinganine reductase [35]. Next, dihydroceramide is formed from sphinganine *via* an N-acylation process by ceramide synthases (CerS) [36]. Six CerS have been identified in mammals, and the specificities of each CerS towards fatty acyl-CoA are different depending on the chain length [35]. Thus, each CerS generates a ceramide with a distinct acyl chain length [35,36]. Specifically, the N-acylation reaction by CerS1 and CerS2 primarily produces C18-dihydroceramide and the C22-C24-dihydroceramides, respectively [37,38]. CerS3 and CerS4 primarily produce the C26-34-dihydroceramides and the C18-C20-dihydroceramides, respectively [39–41]. CerS5 and CerS6 predominantly generate C16-dihydroceramide [39,42]. Finally, ceramide, which is located in the middle of SL metabolism, is produced from dihydroceramide by dihydroceramide desaturase and can be metabolized further to generate complex SLs [43]. Briefly, sphingomyelin is produced by sphingomyelin synthases, which transfer a phosphocholine headgroup from phosphatidylcholine to ceramide generating diacylglycerol and sphingomyelin [43]. Ceramide can also be phosphorylated by ceramide kinase

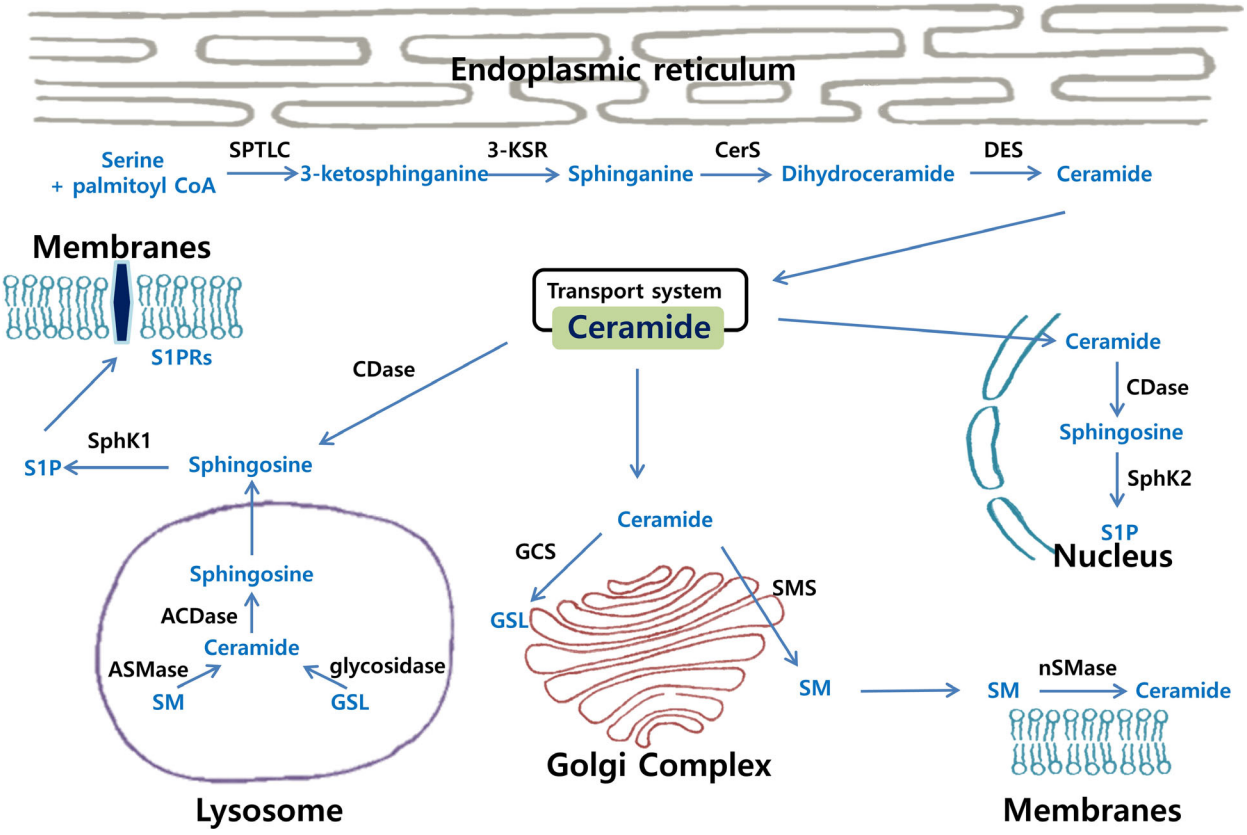


Fig. 2. Sphingolipid metabolism. The *de novo* ceramide synthesis pathway is comprised of several enzymes including serine palmitoyltransferase (SPTLC), 3-ketosphinganine reductase (3-KSR), six CerS variants and dihydroceramide desaturase (DES). Ceramide, which is the basic form of SLs, is transported out of the ER, with the transport system including ceramide transfer protein- or vesicle-mediated transport system [140,141]. Ceramide can be further metabolized into GSLs and sphingomyelin (SM) by GlcCerase and sphingomyelin synthase (SMS), respectively. Degradation of ceramide is mediated by ceramidase (CDase). Sphingosine can be phosphorylated by SphK enzymes. SphK1 is primarily located in the cytosol, and SphK2 is primarily localized in nucleus. SM can also be converted to ceramide by ASMase in the lysosomes and NSMase in the inner leaflet of plasma membranes.

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to yield ceramide-1-phosphate [44]. In addition to *de novo* ceramide synthesis, ceramide can be generated *via* sphingomyelinase (SMase)-mediated sphingomyelin hydrolysis, which is a major pathway for stress-induced ceramide generation [45]. SMase isoforms can be divided into three groups: alkaline, acid and neutral based on their optimal pH [46]. SMases exhibit specific subcellular localizations: the lysosomal acid SMase (L-ASMase) primarily localizes in the endolysosomal compartment, and the neutral SMase (NSMase) has been found in the ER and the Golgi apparatus, but also localizes in the inner leaflet of the plasma membranes [46]. Both L-ASMase and secreted ASMase (S-ASMase) are encoded by *Smpd1*, and three types of NSMase are encoded by *Smpd2, 3* and *4* [46].

Sphingosine-1-phosphate metabolism

Ceramide can also be deacylated by ceramidases to form sphingosine [47]. The structure of sphingosine is similar to sphinganine except that it contains an unsaturated hydrocarbon chain produced by the desaturation of dihydroceramide. Sphingosine can be phosphorylated by sphingosine kinases (SphK) to produce S1P, which is a critical bioactive molecule with various pathophysiological roles [48]. Two isoforms of SphK (SphK1 and SphK2) were characterized, and they were reported to display different properties. Structurally, SphK2 contains a nuclear localization signal in the N-terminal domain and a nuclear exportation signal in the C-terminal domain. However, SphK1 lacks both of these domains [48]. Thus, SphK1 is predominantly found in the cytoplasm, but it can be translocated to the plasma membrane in response to cell stimulation. S1P generated by SphK1 can not only exert intracellular effects, but also be exported outside the cell, where it can then exert 'inside-out' signalling [48,49]. In contrast, SphK2 is primarily located in the ER and can also be imported into or exported from the nucleus [48,50]. S1P degradation is mediated by two pathways: it is dephosphorylated back to sphingosine through by S1P phosphatases (SPPs) or it is irreversibly degraded by S1P lyase to phosphoethanolamine and hexadecenal [51].

'Inside-out' S1P signalling is mediated through S1P receptors (S1PR), which are G protein-coupled receptors. Five S1PRs have been reported and each one has different cell and tissue expression. Additionally, distinct G protein coupling has been characterized. $S1PR_1$ couples exclusively with the inhibitory G protein alpha subunit (Gαi), whereas $S1PR_2$ and $S1PR_3$ bind to Gαi, Gαq and Gα13. $S1PR_4$ and $S1PR_5$ couple to both Gαi and Gα13 [48,52]. Therefore, each S1PR exerts distinct intracellular functions *via* activating different downstream effectors [48,52–54].

Glycosphingolipid metabolic pathway

Glycosphingolipids are a variety of lipids that contain a ceramide backbone linked to a glycan moiety [55]. Notably, GSLs provide enormous biodiversity due to structural variations in both the ceramide and glycan components [56]. GSLs are especially important for cell–cell interactions and can act as both ligands and receptors for pathophysiological molecules [5]. GSL synthesis begins with the production of either galactosylceramide (GalCer) in the ER or glucosylceramide (GlcCer) in the Golgi apparatus [55,56]. GalCer is the precursor of sulfatides, and other GSLs are primarily produced from GlcCer [56]. Lactosylceramide is first synthesized from GlcCer and is then processed to synthesize most other GSLs (GM4, one of the gangliosides, is exceptionally derived from GalCer), which include the ganglio-, isoganglio-, lacto-, neolacto-, lactoganglio-, globo-, isoglobo-, muco-, gala-, neogala-, mollu-, arthro-, schisto- and spirometo-series [57]. The GSLs are categorized according to their carbohydrate structure and synthetic pathways [56,57]. Because two series, the ganglio- and globo-, are reported to be involved in ER stress, we will briefly review them. Gangliosides contain a common core structure (Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-ceramide), and the simplest ganglioside, which is GM3, is produced by the attachment of a sialic acid to lactosylceramide (Fig. 3) [57]. Elongation of GM3 to complex gangliosides synthesizes GM2, GM1a, GD1a and GT1a (Fig. 3). Gangliosides are classified into a-, b-, c-series gangliosides depending on the number of sialic acid(s) linked to the inner galactose residue [57]. Globosides include globotriaosylceramide (Gb3, Galα1-4Galβ1-4Glcβ1-ceramide) and globotetrahexosylceramide (Gb4, GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-ceramide) and were named since Gb4 was obtained as a globular, birefringent precipitate [58].

Sphingolipids at the centre of ER stress

The length of acyl chains dictates the type of ER stress response induced by ceramide

Endoplasmic reticulum stress is known to be propagated by ceramide. Palmitate, which is metabolized to palmitoyl-CoA, can be used as a substrate for *de novo* ceramide synthesis by SPTLC. Indeed, palmitate

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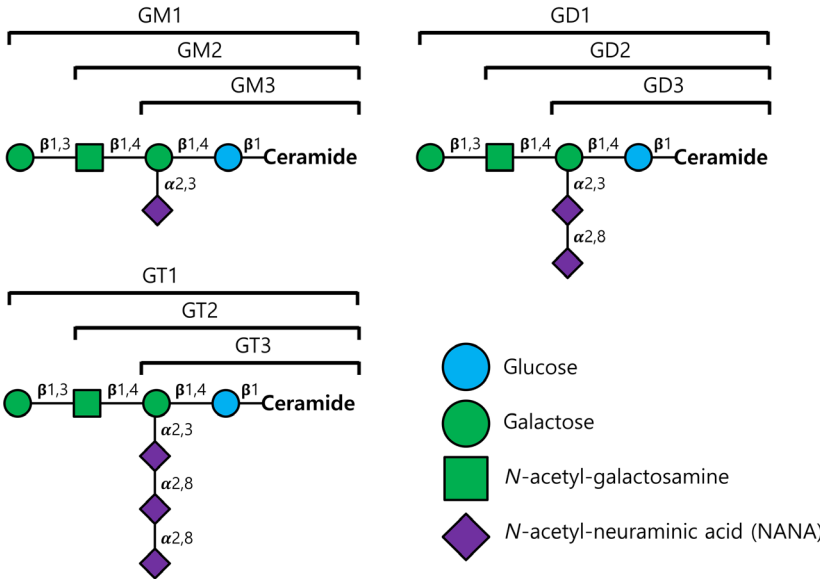


Fig. 3. Structure of gangliosides. Gangliosides contain a common core structure (Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-ceramide). GM3, the simplest ganglioside, can be modified to generate complex gangliosides. Gangliosides are classified into a-, b- and c-series gangliosides depending on the number of N-acetyl-neuraminic acid(s) linked to the inner galactose residue.

treatment increases C16- and C18-ceramide levels to induce ER stress [59]. In addition, myristate induces ER stress in intestinal epithelial cells, which requires CerS5/6 and C14-ceramide generation [60]. Ceramide generated by ASMase mediates oxidized-LDL induced ER stress in macrophage [16]. *In vivo* studies also showed similar results. Both administration of C6 ceramide and overexpression of SPTCL1-2 in the ventromedial nucleus increased ER stress in hyperthyroid rats [61]. In addition, central pharmacological inhibition of ceramide synthesis by using the SPTLC inhibitor myriocin suppressed hypothalamic ER stress, and specific downregulation of SPTLC1 exclusively in the ventromedial nucleus ameliorated ER stress and obesity and improved the metabolic health of ovariectomized rats, including a reduction in hepatic lipid content [62]. Several mechanisms for ceramide-induced ER stress have been suggested.

Endoplasmic reticulum stress can be induced by ceramide *via* cathepsin activation [63,64]. Ceramide binds to cathepsin D, which causes cleavage of active cathepsin D. ASMase deficiency [65] or inhibition of ceramide generation with desipramine reduces cathepsin D cleavage and activation [64]. LCL521, a lysosomotropic inhibitor of acid ceramidase, elevates C16-ceramide and induces autophagosome accumulation to increase ER stress *via* cathepsin B and D [63]. Ceramide generation *via* acid ceramidase inhibition or ASMase activation in the lysosomes or endolysosomes may involve cathepsin activation, which contributes to ER stress.

Ceramide generated by both the ASMase and *de novo* synthesis pathways can activate ER stress *via* CD95-PERK signalling in carcinoma cells [66]. Combined low doses of sorafenib and vorinostat, but not each treatment individually, caused ceramide generation *via* both the ASMase and *de novo* synthesis pathways. Furthermore, the treatment promoted CD95 surface localization and activation [66]. Increased ceramide levels can increase CD95 clustering to form lipid rafts; therefore, ceramide can facilitate receptor trimerization and activation in a ligand-independent manner [67]. CD95 signalling activates PERK, which is then subjected to multiple downstream regulatory signals that include both cell death and survival signals [66]. In addition, PERK can also act as an upstream signal for ceramide generation. In primary human glioblastoma, PERK inhibition blocked ceramide generation [68]. The melanoma differentiation-associated gene-7/interleukin 24 (MDA-7/IL-24) has cancer-specific cell death-inducing and tumour growth-suppressing properties in xenograft models, and MDA-7/IL-24 toxicity has been linked to alterations in ER stress signalling [68,69]. Overexpression of MDA-7/IL-24 in glioblastoma multiforme cells caused cell death that correlated with the induction of ceramide, cytosolic Ca^{2+} and oxidative stress. PERK inhibition blocked increased ceramide levels, cytosolic Ca^{2+} and oxidative stress caused by MDA-7/IL-24. Inhibition of the *de novo* ceramide synthesis pathway with myriocin or CerS6 knockdown blocked increased cytosolic Ca^{2+} and oxidative stress by MDA-7/IL-24 [68]. The ASMase pathway had similar effects, although they were less pronounced. These studies imply two potential mechanisms: (a) PERK directly or indirectly phosphorylates ASMase and/or CerS6, and (b) PERK activation *via*

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eIF2α modulates protein expression that results in elevated ER-localized ceramide activity [68]. PERK activation and ceramide generation may have significant regulatory crosstalk.

Recently, distinct roles for ceramide that depend on its acyl chain length have been reported during ER stress [70,71]. Opposite roles for CerS1-derived C18-ceramide and CerS6-derived C16-ceramide during ER stress were reported in human head and neck squamous cell carcinoma (HNSCC) cells [72]. Downregulation of CerS6/C16-ceramide activated ATF-6 after treatment with either tunicamycin or suberoylanilide hydroxamic acid, which are ER stress inducers [73]. CerS6 downregulation-induced ATF-6 activation involved the release of Ca^{2+} from the ER and Golgi membrane fragmentation. In addition, downregulation of Cer6/C_{16}-ceramide disrupted the ER/Golgi membrane network and led to the fusion of the Golgi with the ER membranes, where pro-ATF-6 is activated [73]. In contrast to the protective effect of CerS6 on ER stress in HNSCCs, CerS6-derived C16-ceramide exhibited a detrimental effect during palmitate- or high-fat diet (HFD)-induced ER stress [70,71]. Both CerS2 overexpression and the addition of very long-chain ceramide in Hep3B cells had protective effects during palmitate-induced ER stress. CerS6 overexpression and long-chain ceramide had detrimental effects [71]. Interestingly, unlike CerS6 overexpression, CerS5 overexpression, which also elevates C16-ceramide levels, did not alter the intensity of palmitate-induced ER stress in Hep3B cells [71]. The molecular mechanisms that account for the different effects of CerS5 and CerS6 during ER stress have not been elucidated. However, a recent study identified proteins that specifically interact with C16:0 SLs derived from CerS5 or CerS6. The study revealed binding to the UPR master regulator BiP by CerS6-derived C16:0 SLs, but not CerS5-derived C16:0 SLs (Fig. 4A) [74]. Therefore, different C16:0 SL binding and intracellular localization based on precursor intermediates may explain the mechanism. Similarly, a beneficial role for very long-chain sphingolipid species in ER stress has also been indicated by research on the effects of a HFD on glucose intolerance and insulin resistance [70]. Lipidomic analysis of mouse strains on HFDs revealed significant strain-specific differences in ceramide and sphingomyelin species. More specifically, C16-C22 (long-chain) ceramide increased and C > 22 (very long-chain) ceramide and sphingomyelin species decreased in the four strains that developed HFD-induced glucose intolerance. Intriguingly, the opposite pattern in SL levels was observed in BALB/c mice, which

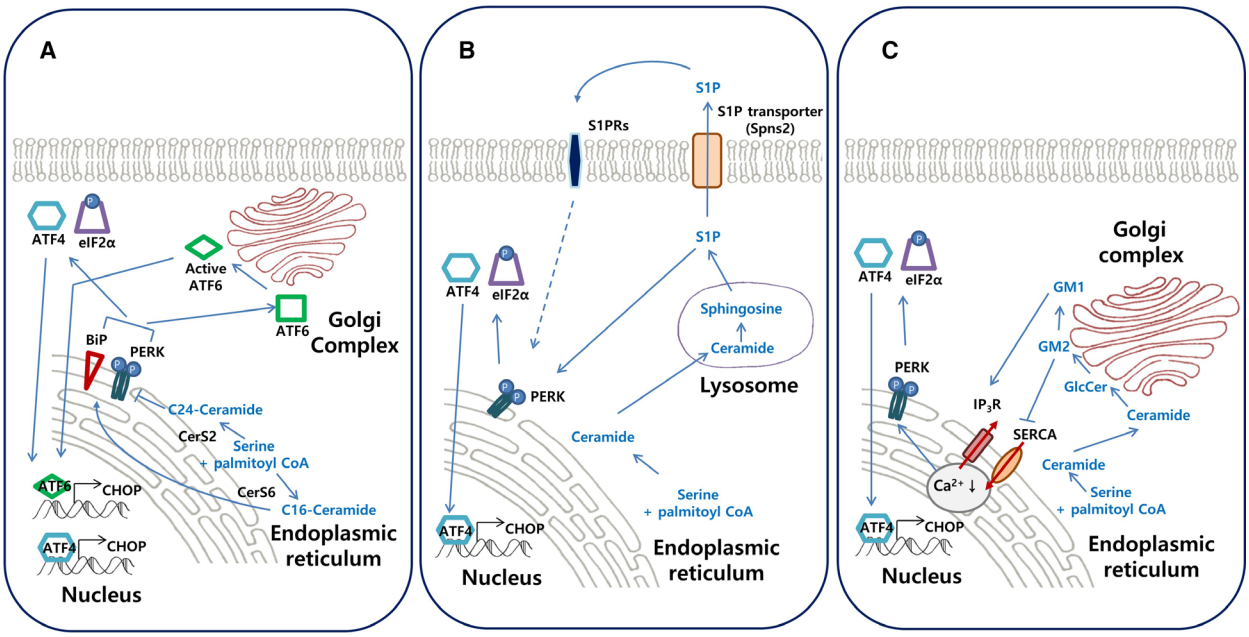


Fig. 4. Roles of SLs in ER stress. (A) C16-ceramide and C24-ceramide have opposite roles in PERK phosphorylation and ATF6 cleavage. C16-ceramide, which is generated by CerS6, may bind directly to BiP. (B) Ceramide can be degraded to sphingosine by ceramidase in the lysosomes, and sphingosine can be converted to S1P by SphK. Intracellular S1P activates ER stress signalling. However, the effects of extrinsic S1P, which is pumped out *via* a S1P transporter (Spns2, SL transporter 2), are still controversial. (C) GM1 and GM2 induce ER luminal Ca^{2+} depletion, which in turn activates PERK and the ER stress signalling pathway.

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displayed minimal deterioration in glucose tolerance and insulin action [70]. These strain-specific changes in SL acylation closely correlated with CerS2. Overexpression of CerS2 in primary mouse hepatocytes increased very long-chain ceramides, caused decreased ER stress (Fig. 4A) and contributed to a substantial improvement in insulin signal transduction despite an overall increase in ceramide abundance [70]. These data confirm that SLs have distinct roles during ER stress that depend on their acyl chain length.

Sphingosine-1-phosphate levels, localization and receptors affect ER stress

S1P levels are a balance between its synthesis by SphK1 and SphK2 and its degradation by ER-localized SPPs (SPP1 and SPP2) and S1P lyase [75]. A higher intracellular S1P concentration increases the ER stress response (Fig. 4B) [75–77]. Blocking S1P degradation, which consequently increases S1P levels, also induces ER stress. As already mentioned, S1P can be degraded by either SPPs or S1P lyase. Depletion of SPP1 in MCF7 cells induced ER stress and autophagy. Interestingly, these responses did not cause cell death, but instead they stimulated activation of the AKT survival signal in a PERK-dependent manner [75]. Similarly, the β-cells in SPP2 knockout mice displayed increased expression of ER stress markers [76]. Overexpression of sphingosine-1-phosphate lyase also protected insulin-secreting cells against cytokine toxicity *via* reduced cytokine-mediated ER stress, which consequently attenuated calcium leakage from ER stores [77]. These reports all support the framework that increased intracellular S1P levels, *via* inhibition of S1P degradation, enhance the ER stress response.

The role of S1P generation in ER stress is controversial and appears to depend on the specific SphK isoform. SphK1, which is induced by hydrogen peroxide, is linked to cerebral ischaemia–reperfusion injury *via* the induction of ER stress and activation of the nuclear factor kappa B (NF-κB) signalling pathway [78]. In contrast, a SphK1 deficiency alleviated acetaminophen-induced ER stress by altering the phosphorylation of IRE1α and PERK-eIF2α, levels of ATF4 and activation of ATF6 [79]. Taken together, S1P produced by SphK1 promotes ER stress. In contrast to SphK1, SphK2 inhibition using K145 and ABC294640 synergized with bortezomib to target myeloma by enhancing ER stress [80]. However, these results are somewhat controversial. In T-cell acute lymphoblastic leukaemia cells, SKi (a SphK1/2 inhibitor) and ROMe (a selective SphK2 inhibitor) both affected T-ALL cell viability; however, they exerted their effects through different mechanisms. The SphK2 selective inhibitor ROMe induced an autophagic cell death without affecting UPR signalling, while SKi induced apoptosis *via* an ER stress/UPR pathway [81]. Similarly, treatment in glioblastoma cells with SKI-II, which inhibits both SphK1 and SphK2, also triggered ER stress and was essential for cell death [82]. The conflicting reports may be due to either the different cell types or differences in the selective potency among SphK inhibitors. Therefore, the precise role of SphK inhibition in ER stress and autophagy still needs to be investigated with comprehensive cell and animal models using various SphK inhibitors.

In addition to the effects of S1P on ER stress, in the opposite direction, ER stress can also increase S1P levels *via* the regulation of SphK enzymes, which implicates a cyclic response. In cultured human epidermal keratinocytes, increased cellular S1P in response to ER stress, which was induced *via* SphK1, specifically interacted with heat shock protein 90s to form a signalling complex with the ER residential stress responsive proteins, IRE1α, tumour necrosis factor receptor-associated factor 2 and receptor-interacting serine/threonine-protein kinase 1, which led to NF-κB activation that stimulated the production of key antimicrobial peptide, cathelicidin [83]. Both tumour necrosis factor receptor-associated factor 2 in its SphK1-bound and SphK1-unbound states could be responsible for forming a signalling complex. In addition, this ER stress-initiated mechanism was found in both epithelial cells and macrophages, which suggests that the underlying mechanism is a universal and highly conserved response [83]. In addition to SphK1, the transcription of Sphk2, which is a major hepatic SphK isoform, is also regulated by the ER stress-mediated UPR pathways [84]. In mouse livers, Sphk2 was transcriptionally upregulated by acute ER stress through activation of ATF4 and improved perturbed hepatic glucose and fatty acid metabolism [84]. Therefore, ER stress and S1P formation can have dependent effects.

S1P can be secreted from cells to interact with five S1PRs. Information about the precise role of S1PRs in ER stress is currently limited. As mentioned above, a SphK1 deficiency protected mice from acetaminophen-induced ER stress, but supplementation with exogenous S1P significantly reversed the activation of the PERK-eIF2α-ATF4 pathway and ATF6 during ER stress [79]. In addition, both FTY720, a functional $S1PR_{1, 3–5}$ antagonist [85], and PF543, a selective SphK1 inhibitor [86], significantly ameliorated acetaminophen-induced hepatic ER stress and improved animal survival [79]. These data implicate a possible

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role of S1PRs in acetaminophen-induced hepatic ER stress. Similarly, extrinsic S1P activated $S1PR_5$ and induced autophagy by generating ER stress in PC-3 human prostate cancer cells. In contrast to these two studies, most studies report that S1PRs have insignificant effects on ER stress [87] and autophagy [75]. The S1P concentration in different experiments may affect the results and may be the cause of the experimental discrepancies. S1P at low concentrations is known to act *via* cell surface receptor activation, whereas at high concentrations that are above the optimal receptor-activating concentrations, S1P acts intracellularly [77,88]. In addition, S1PR expression depends on the cell and tissue type. Distinct S1PR expression can also result in inconsistent effects of S1PRs on ER stress. Regardless of the reason, the direct impact of each S1PR on ER stress still remains to be elucidated.

Glycosphingolipids involved in ER stress

Gangliosides at the ER membranes play key roles as regulators of the Ca^{2+} concentration in the ER since Ca^{2+} influx channels, Ca^{2+} exchange proteins and various Ca^{2+}-dependent enzymes can be altered by the level and type of GSLs that are present at the ER membrane [89,90]. The ER calcium level is tightly controlled to promote optimal protein folding by three main molecular systems: (a) molecular chaperones that bind and buffer calcium; (b) calcium importing mechanisms that increase the ER calcium concentration; and (c) channels and pores that leak or release calcium from the ER to the cytosol [91]. ER calcium release is primarily mediated by channels and pores located on the ER membrane. These include the ryanodine receptors and inositol 1,4,5-triphosphate-receptors (IP_3R). GlcCer is the precursor of most GSLs other than sulfatides, and the effect of GlcCer on ER stress is not fully understood. A lack of glucocerebrosidase (GlcCerase) results in Gaucher disease due to GlcCer accumulation. Furthermore, no UPR activation was observed in a mouse model of neuronopathic Gaucher disease that was induced by conduritol B-epoxide, which is an active site-directed inhibitor of GlcCerase [92]. UPR activation was also absent at two different stages of development in the $Gba^{flox/flox}$;Nestin-Cre mouse brain: the presymptomatic (9 days) and symptomatic stages (21 days) where GlcCerase deficiency is restricted to neural and glial progenitor cells [92,93]. However, recently UPR activation was observed in GD-derived fibroblasts, in fibroblasts derived from carriers of GD mutations, in EBV-transformed B-cells, in white blood cells derived from GD patients, and in drosophila models of GD carrier mutations, which implies a possible role of GlcCer in ER stress [94]. However, the misfolded and dysfunctional GlcCerase proteins, rather than the accumulated GlcCer, may be the major contributors to ER stress induced by GD [95].

Endoplasmic reticulum calcium-importing systems are primarily operated by sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) proteins [91], and increased GM2, one type of ganglioside, reduces the *Vmax* of SERCA to induce ER luminal Ca^{2+} depletion (Fig. 4C). In a model of GM2-gangliosidosis, calcium depletion in turn activated PERK and ER stress signalling pathways to contribute to neurodegeneration. [96]. However, the role of another ganglioside, GM1, in ER stress and calcium homeostasis is controversial. Diabetic rats that received intraperitoneal injections of GM1 exhibited reduced cerebral infarcts and improved neurological behaviour during cerebral ischaemia/reperfusion injuries by suppressing ER stress-induced cell death *via* increasing BiP and reducing CHOP expression in the ischaemic brain hemispheres [97]. However, both excessive GM1 accumulation at the ER membranes in beta-galactosidase deficient mice and GM1 loading in wild-type neurospheres activated the UPR by depleting ER Ca^{2+} stores (Fig. 4C) [98]. In addition, GM1 accumulation in glycosphingolipid-enriched microdomain fractions of mitochondria-associated ER membranes influenced the activity of IP_3R, causing a larger Ca^{2+} conductance between the ER and the mitochondria that contributed to mitochondrial apoptosis [99]. Thus, the precise role of GM1 and other GSLs in ER stress remain to be elucidated. The chemical compounds linked to ER stress and SL metabolism are summarized in Table 1.

Therapeutic potential of sphingolipid modulation in ER stress diseases

Metabolic diseases

Endoplasmic reticulum stress is a key pathogenic mechanism in metabolic diseases including fatty liver disease and diabetes. Targeting ER stress has effectively alleviated many metabolic diseases; therefore, chemical and pharmacological drugs that target individual steps of ER stress have been developed [100]. Due to the effects of SLs on ER stress, SL modulation can also be applied to ameliorate cellular dysfunction in metabolic diseases through ER stress targeting.

Altered SL acyl chain lengths have been reported in metabolic diseases [70,71,101,102]. In both alcoholic and nonalcoholic fatty livers from mice, long-chain (C16-, C18-) ceramides were elevated [71,103]. Similarly,

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Table 1. Chemical compounds that link endoplasmic reticulum stress and sphingolipid metabolism.

Cell type	Compound	Function	ER stress	Reference
Mouse myeloid-derived suppressor cells	LCL521	A lysosomotropic inhibitor of acid ceramidase C16-ceramide ↑	LC3 cleavage ↑ (autophagy ↑) IRE1α ↑	[63]
Human liver cancer cells, human renal cell carcinoma cells	Low doses of sorafenib and vorinostat	Ceramide synthesis ↑ ASMase and *de novo* synthesis ↑	p-eIF2α ↑ ATG5 ↑ Autophagy ↑ CD95 ↑	[66]
Human lung adenocarcinoma cells	Methotrexate	C16-ceramide ↑ C18-ceramide ↑ C24-ceramide ↑ C26-ceramide ↑ Sphingosine ↑	ER aggregate formation ↑	[117]
Human retinal pigment epithelial cells	Tunicamycin	C22-ceramide ↑ C24-ceramide ↑ Ceramide-1-phosphate ↑	BiP ↑ Caspase-3 ↑ Caspase-12 ↑ NF-kB1 ↑ NF-kB p65 ↑	[138]
Human T-cell acute lymphoblastic leukaemia cells	SKi	SphK1/2 inhibitor	IRE1α ↑ p-eIF2α ↑ BiP ↑ CHOP ↑	[81]
Human glioblastoma cells	SKI-II	SphK1/2 inhibitor S1P ↓ Ceramide ↓ Dihydroceramide ↑ Dihydrosphingosine ↑	BiP↑ CHOP ↑	[82]
Human myeloma cells	K145	SphK2 inhibitor S1P ↓ Ceramide ↑	p-eIF2α ↑ IRE1α ↑ ATF4 ↑ XBP-1 splicing ↑	[80]
Human prostate cancer cells	S1P	S1PR5 activation	p-eIF2α ↑ XBP-1 splicing ↑	[139]

in human alcoholic liver tissues, C14-, C16-, C18- and C20-ceramide levels were increased in concert with higher CerS1, CerS5 and CerS6 expression [104]. In several reports studying nonalcoholic fatty liver disease (NAFLD) in animal models, total hepatic ceramide levels were unchanged, but C24-ceramide was decreased and C16-ceramide was increased [71,101,102]. Recently, a critical role specifically for CerS in NAFLD progression and ER stress was reported [71]. Both CerS6 overexpression and CerS2 knockdown in Hep3B cells increased palmitate-induced ER stress, which contributed to insulin-induced gene-1 downregulation and sterol regulatory element-binding protein-1 maturation [71]. In addition, CerS2 haploinsufficiency exacerbated steatosis *via* a stronger ER stress response and increased sterol regulatory element-binding protein-1 cleavage *in vivo*. Bortezomib administration, which increased hepatic CerS2 and C24-ceramide levels, alleviated steatosis on a HFD [71]. Importantly, this study implicated CerS and its derivatives in hepatic ER stress and lipogenesis; therefore, CerS modulation is a potential therapeutic strategy for NAFLD [71]. A protective role for very long-chain SL species in the development of glucose intolerance and hepatic insulin resistance was revealed by specifically increasing very long-chain ceramide levels with CerS2 overexpression. Despite the overall increase in ceramide abundance, a substantial improvement in insulin signalling was observed in primary mouse hepatocytes. Furthermore, ER stress and gluconeogenic markers decreased [70]. In a separate study, unlike in NAFLD, inhibiting *de novo* ceramide generation did not reduce ER stress even though it was effective at improving alcohol-related steatohepatitis [105]. These studies indicate that there is a distinct role for CerS and its derivatives in ER stress linked with NAFLD and hepatic insulin resistance. Furthermore, they suggest the possible use of CerS modulators to treat these diseases.

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SMase catalyses the hydrolysis of sphingomyelin to ceramide and phosphorylcholine. In mice, ASMase was required for chronic, alcohol-induced hepatic ER stress [106]. ASMase-deficient mice exhibited decreased alcohol-induced expression of ER stress markers [106]. However, upregulation of tunicamycin-mediated ER stress markers and steatosis were similar in both WT and ASMase-deficient mice, which implicated a specific role for ASMase in alcohol-induced ER stress [106]. In addition, only exogenous ASMase, but not NSMase, induced hepatic ER stress by perturbing ER Ca^{2+} homeostasis [106]. Furthermore, imipramine, an ASMase inhibitor, also improved ethanol-induced hepatic steatosis [107]. Similarly, amitriptyline, which is another ASMase inhibitor, robustly reduced hepatic steatosis and hepatic inflammation in high-palmitic acid-containing-HFD-fed mice treated with lipopolysaccharides, while GW4869, which is a NSMase inhibitor [108], had modest effects [109]. Collectively, these studies suggest the possibility of using ASMase inhibitors as therapeutic agents for hepatic steatosis.

Endoplasmic reticulum stress plays an important role in pancreatic β-cell degeneration [20]. In diabetes, functioning β-cells in the pancreas may experience increasing and unresolvable ER stress due to compensating for neighbouring β-cells that have become dysfunctional [20]. In mice, it has been shown that even in healthy states, β-cells can experience UPR activation that is significantly greater than in other professional secretory cells; thus, β-cells are vulnerable to ER stress [20,110]. Ceramide generation has been implicated in pancreatic β-cell apoptosis and dysfunction. Indeed, blocking *de novo* ceramide synthesis attenuated both palmitate-induced pancreatic β-cell death *in vitro* [111] and islet of Langerhans apoptosis *in vivo*, which also prevented the appearance of hyperglycaemia in rats [112]. Although the mechanism linking ceramide accumulation to pancreatic β-cell dysfunction is unclear, ER stress may play a key role in the process. Ceramide accumulation and the associated decrease in ER sphingomyelin levels in response to lipotoxicity are important initiators of ER stress in β-cells [113]. Palmitate treatment in MIN6 β-cells induced ceramide synthesis and reduced ER-to-Golgi protein trafficking, which consequently caused ER stress [114]. Reduced ER-to-Golgi protein trafficking and ER stress were overcome by increasing the conversion of ceramide into GlcCer with overexpression of GlcCer synthase [114]. These results demonstrate that SLs have an important role regulating protein trafficking and ER stress in β-cells [114]. Using mass spectroscopic lipidomics and subcellular fractionation, Boslem *et al.* [113] demonstrated that palmitate pretreatment of MIN6 β-cells promoted ER remodelling of both phospholipids and SLs, but only the latter were causally linked to lipotoxic ER stress. Altered sphingomyelin metabolism specifically contributed to apoptosis and ER stress as well as ER lipid raft formation in MIN6 cells [113]. Palmitate-induced MIN6 cell apoptosis was diminished by overexpression of sphingomyelin synthase 1 and enhanced by the ER-resident NSMase *Smpd4* [113]. However, transfection of *Smpd3*, a plasma membrane-localized NSMase, did not significantly affect the apoptotic response, implicating that depleting a pool of ER-localized sphingomyelins triggers lipoapoptosis [113]. In addition, treating MIN6 cells with GW4869, a specific NSMase inhibitor [108], decreased both palmitate-induced apoptosis and ER stress, which suggests that a loss of sphingomyelin in the ER is a key event for initiating β-cell lipotoxicity [113]. Indeed, inhibition of either ASMase or NSMase improved glucose, cholesterol, triglycerides and insulin resistance in high-palmitic acid-containing HFD-fed mice [109], which suggests the potential of SMase inhibitors as therapeutic agents for diabetes.

Cancer

In cancer, the UPR plays a key role in tumour progression, metastasis, tumorigenesis and survival [115]. The highly proliferative metabolic state of cancer can activate the UPR response by disrupting the folding of ER proteins, which allows cancer cells to continue growing in nutrient-deficient environments. Furthermore, the UPR stimulates tumour cells to secrete metalloproteinases, which can increase cancer cell invasiveness [115]. Thus, the UPR can be a pro-survival response in tumour cells that act *via* adaptive mechanisms during cancer progression [116]. Although the main purpose of the UPR is to restore ER homeostasis, a persistent UPR consequently leads to cell death and ER stress is one of the main targets for anticancer agents. Several anticancer reagents including vorinostat and sorafenib are known to induce ER stress *via* ceramide synthesis [66]. In addition, the CerS6 protein, which makes C16-ceramide, was significantly elevated in several cancer cell lines treated with methotrexate, and CerS6 was required for methotrexate-induced formation of ER aggregates, which may be an ER stress indicator [117]. The anticancer effects of ceramide *via* ER stress are different, depending on its acyl chain length. C18- and C16-ceramides, which are generated by CerS1 and CerS6, respectively, played opposing pro-apoptotic and pro-survival roles in HNSCCs, and interestingly, CerS6 knockdown using small interfering RNA induced ER stress-mediated

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apoptosis, which was linked to the activation of the ATF6/CHOP arm of the UPR pathway [72]. Although expression of CerS1/C18-ceramide inhibited HNSCC xenograft growth, CerS6/C16-ceramide protected ER stress and enhanced *in vivo* tumour development and growth [72]. These results are consistent with the pro- and anti-apoptotic roles of CerS1/C18-ceramide and CerS6/C16, respectively [72]. Thus, this study elucidated the different roles of CerS1 and CerS6 in ER stress-induced apoptosis and HNSCC tumour growth [72]. Because CerS6/C16-ceramide had ER stress-inducing effects in both methotrexate-treated cancer cells [117] and palmitate-treated Hep3B cells [71], the specific effects of CerS and its derivatives in ER stress and cancer cell death might depend on the cell and cancer type. Currently, a phase I clinical trial of C6-ceramide nanoliposome to treat advanced solid tumours is underway (NCT02834611, Table 2).

Table 2. Clinical trials that tested the effects of SL modulation in nonalcoholic steatohepatitis, cancer and Parkinson's disease. This table shows the trials, which are completed or currently recruiting participants, and is based on https://clinicaltrials.gov/ct2/home.

Title	Disease	Intervention	Trial number	Phase	Duration
Nonalcoholic steatohepatitis					
Beta glucosylceramide for treatment of nonalcoholic steatohepatitis	Nonalcoholic Steatohepatitis	Beta glucosylceramide	NCT02126306	Phase 1 and 2	2006–2010
Cancer					
A phase II study of topical ceramide lipids as treatment for cutaneous breast cancer	Breast cancer	Topical ceramide cream	NCT00008320	Phase 2	2001–2009
Phase I study to evaluate the toxicity and feasibility of intratumoural injection of alpha-gal glycosphingolipids in patients with advanced or refractory solid tumours	Metastatic neoplasm	Alpha-Gal glycosphingolipid	NCT00668382	Phase 1	2007–2011
Phase I study to evaluate the toxicity and feasibility of intratumoural injection of alpha-Gal (beta-galactosidase) glycosphingolipids in patients with advanced melanoma	Metastatic melanoma	Alpha-Gal glycosphingolipid	NCT00668512	Phase 1	2007–2014
Open-label, multi-centre, dose escalation phase 1/2 study of anti-GM2 ganglioside monoclonal antibody BIW-8962 as monotherapy in subjects with previously treated multiple myeloma	Multiple myeloma	BIW-8962 (anti-GM2 monoclonal antibody)	NCT00775502	Phase 1	2008–2011
Phase I/II trial of combination of lenalidomide (Revlimid, LEN) and autologous mature dendritic cells pulsed with α-galactosyl ceramide (α-GalCer; KRN7000) in myeloma	Myeloma	KRN7000 (α-galactosylceramide)	NCT00698776	Phase 1	2008–2016
A phase I, open-label, dose escalation, safety, pharmacokinetic and pharmacodynamic study of ABC294640 in patients with advanced solid tumours	Pancreatic cancer, adult solid tumour	ABC294640 (a SphK2 inhibitor)	NCT01488513	Phase 1	2011–2015
A safety study of fingolimod with radiation and temozolomide in newly diagnosed high grade glioma	Glioblastoma, anaplastic astrocytoma	fingolimod (a functional S1PR antagonist)	NCT02490930	Phase 1	2015–2017
A study of ABC294640 (Yeliva®) alone and in combination with hydroxychloroquine sulphate in treatment of patients with advanced cholangiocarcinoma	Cholangio-carcinoma	ABC294640	NCT03377179	Phase 2	2017-in progress
Phase I study of C6 ceramide nanoliposome in patients with advanced solid tumours	Advanced solid tumour	C6 ceramide nanoliposome	NCT02834611	Phase 1	2017–in progress
A presurgical window of opportunity trial investigating the effect of imipramine on triple negative breast cancer (CTMS# 17-0037)	Breast cancer	Imipramine (an ASMase inhibitor)	NCT03122444	Phase 1	2019-in progress
Parkinson's disease					
The study of GM1 ganglioside, a potential new Parkinson's disease medication	Parkinson's disease	GM1	NCT00037830	Phase 2	1999–2010

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Table 3. Animal studies, which link between SL and ER stress in various disease models, were summarized.

Altered SL metabolic pathway	ER stress	Disease model	Reference
CerS2 haploinsufficiency	p-eIF2α ↑ p-PERK ↑ BiP ↑ CHOP ↑	Nonalcoholic fatty liver disease ↑	[71]
Hepatic CerS2 expression ↑	p-eIF2α ↓ p-PERK ↓ BiP ↓ CHOP ↓	Nonalcoholic fatty liver disease ↓	[71]
ASMase deficiency	IRE1α ↓ BiP ↓ CHOP ↓	Alcoholic fatty liver disease ↓	[106]
CerS6 expression ↑	CHOP ↓	Human head and neck squamous cell carcinoma growth ↑	[72]
SphK2 and proteasome inhibition	IRE1α ↑ p-eIF2α ↑ ATF4 ↑ XBP-1 splicing ↑ BiP ↑ CHOP ↑	Myeloma burden and myeloma-associated bone disease ↓	[80]
SPTLC inhibition	p-IRE ↓ p-PERK ↓ p-eIF2α ↓ CHOP ↓	Ovarectomy-induced obesity and metabolic abnormalities↓	[62]
Intracerebroventricular administration of C6 ceramide	p-PERK ↑ CHOP ↑	Weight gain of hyperthyroid rat ↑	[61]
SPTLC expression ↑ in the ventromedial nucleus of hypothalamus	p-eIF2α ↑ p-PERK ↑ CHOP ↑	Weight gain of hyperthyroid rat ↑	[61]

As already discussed, SphK2 inhibition using K145 and ABC294640 synergized with bortezomib to target myeloma by enhancing ER stress [80], which implied anticancer activity. Although the role of SphK isoforms in ER stress is still controversial, a phase I trial of SphK2 inhibition for solid organ cancers using ABC294640 demonstrated anticancer activity. The best response was a partial response in a patient with cholangiocarcinoma, and stable disease was observed in six patients with various other solid tumours [118]. Currently, a phase II clinical trial using ABC294640 (Yeliva®) alone and in combination with hydroxychloroquine sulphate to treat advanced cholangiocarcinoma is underway (NCT03377179, Table 2). These data suggest a potential novel strategy for the treatment of cholangiocarcinoma.

Neurologic disorders

Endoplasmic reticulum stress is known to be involved in the pathogenesis of various neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease (PD). AD is one of the most common neurodegenerative diseases and is characterized by memory loss, personality changes, cognitive decline and changes in social behaviour. AD involves the accumulation of two different protein aggregates, extracellular amyloid β-protein (Aβ) and intracellular neurofibrillary tangles composed of hyper-phosphorylated tau protein. Importantly, the UPR and ER dysfunction are hypothesized to contribute to AD-related neuronal cell death [119] Indeed, both activated ER stress and abnormal SL metabolism have been found in AD [120]. Increased ceramide levels in AD brain tissues are a result of increased ceramide generation *via de novo* synthesis and sphingomyelin hydrolysis, and these changes are accompanied by a decrease in sulfatides, GSLs and S1P [121,122]. In postmortem AD brain tissues, gene expression for ceramide synthesis, including *CerS1*, *CerS2*, *CerS4*, *SPLTC1* and *SMPD1*, increased concomitant with a total ceramide increase, and the expression of ER stress markers (BiP and CHOP) were also elevated [123,124]. Furthermore, GM1 and GM2 were increased in the lipid rafts of human AD brain tissues [125]. In the brain during AD, ceramide production can be initiated by several

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stimuli, including oxidative stress, Aβ and fatty acids, which ultimately leads to hyper-phosphorylation of the tau protein and neuronal cell death [126,127]. Because blocking ceramide generation decreased Aβ and hyper-phosphorylated tau levels in a murine model [128], ceramide may play a major role in Aβ production [129,130]. However, Aβ can also induce oxidative stress and promote ceramide accumulation [131], which suggests a vicious cycle. Because ceramide, Aβ and hyper-phosphorylated tau can all increase ER stress and regulate each other, the signalling regulation is complex and unclear. GM1 ganglioside, which can bind to Aβ to generate a GM1/Aβ complex [132], is elevated in brains with AD and is reported to enhance the cleavage of an amyloid precursor protein to increase Aβ levels by 10-fold [133]. Because GM1 can also induce the depletion of ER calcium, which then propagates ER stress and caspase activation that leads to neuronal apoptosis, GM1 modulation may be a strong therapeutic target for AD [98].

Although altered SL metabolism in PD has been reported [134], the role of SLs in PD-related ER stress is unclear. Ceramide reduced the viability of dopaminergic neuroblastoma cells and primary dopaminergic neurons in a dose-dependent manner. Furthermore, the pharmacological inhibition of SMase with three different inhibitors: desipramine (an inhibitor of ASMase [65]), GW4869 (an inhibitor of NSMase [135]) and 7c, also known as ARC39 (an inhibitor of L-ASMase and S-ASMase [136]), provided significant neuroprotection. Mechanistically, SMase inhibition attenuated ER stress and caspase-3 activation and restored the mitochondrial membrane potential [137]. This study revealed that SMase-generated ceramide and SL species have important roles in ER stress and caspase-dependent cell death of dopaminergic neurons, and SMase inhibition could provide novel drug targets for neuroprotective strategies that delay or attenuate the progressive loss of nigral dopaminergic neurons in patients with PD [137].

Unfortunately, no clinical study has proven the direct effect of SL modulation on ER stress-linked human diseases; therefore, a clinical evaluation of SL modulation in ER stress is missing. Clinical trials that tested the effects of SL modulation in nonalcoholic steatohepatitis, cancer and PD are summarized in Table 2. In addition, animal studies, which link between SL and ER stress in various disease models, are also summarized in Table 3.

Conclusions and perspectives

Endoplasmic reticulum stress plays a key role in various human diseases, including diabetes, fatty liver disease and neurodegeneration. Information about specific functions of distinct SLs in ER stress has been rapidly accumulating over the past years. Due to the different roles that individual SLs play in ER stress, ER stress modulation using SLs is expected to be a novel therapy for various human diseases. However, whether a specific SL alteration is a cause or a consequence of ER stress still needs to be determined. Future studies providing detailed mechanisms linking SLs and ER stress will be extremely important to identify precise targets within SL pathways that can be used for clinical applications.

Funding

Woo-Jae Park was supported by National Research Foundation of Korea grants funded by the Korean Government (Ministry of Education, Science and Technology) [NRF-2016R1D1A1B04930619], and Joo-Won Park was supported by grants from the National Research Foundation of Korea [NRF-2019R1F1A1057934].

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