Review

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Sphingolipid metabolism – an ambiguous regulator of autophagy in the brain

https://doi.org/10.1515/hsz-2018-0237
Received April 30, 2018; accepted May 25, 2018; previously published online June 15, 2018

Abstract: In mammals, the brain exhibits the highest lipid content in the body next to adipose tissue. Complex sphingolipids are characteristic compounds of neuronal membranes. Vital neural functions including information flux and transduction occur along these membranes. It is therefore not surprising that neuronal function and survival is dependent on the metabolism of these lipids. Autophagy is a critical factor for the survival of post-mitotic neurons. On the one hand, it fulfils homeostatic and waste-recycling functions and on the other hand, it constitutes an effective strategy to eliminate harmful proteins that cause neuronal death. A growing number of experimental data indicate that several sphingolipids as well as enzymes catalyzing their metabolic transformations efficiently but very differently affect neuronal autophagy and hence survival. This review attempts to elucidate the roles and mechanisms of sphingolipid metabolism with regard to the regulation of autophagy and its consequences for brain physiology and pathology.

Keywords: ceramide; gangliosides; glucosylceramide; sphingomyelin; sphingosine 1-phosphate.

Introduction

Complex glycosphingolipids (GSLs) are abundant and exhibit characteristic profiles in neuronal membranes (van Echten-Deckert and Herget, 2006). Vital neural functions including information flux and transduction occur along these membranes. It is therefore not surprising that sphingolipid metabolism is closely related to various neurological disorders (van Echten-Deckert and Herget, 2006; van Echten-Deckert and Walter, 2012; Olsen and Faergeman, 2017). During the last two decades especially, bioactive sphingolipids such as ceramide and S1P emerged as key signaling molecules with roles in diverse cellular processes (Hannun and Obeid, 2008). As part of lipid bilayers sphingolipids confer unique characteristics and fluidity to membrane domains. Together with cholesterol, sphingomyelin and gangliosides contribute to raft formation (Simons and Gerl, 2010). In addition, membrane dynamics and membrane trafficking are essentially dependent on the interconversion of sphingolipids (Zheng et al., 2006; Zhang et al., 2009). A growing number of studies document the close connection of sphingolipid metabolism and autophagy (Zheng et al., 2006; Lavieu et al., 2008; Bedia et al., 2011; Jiang and Ogretmen, 2014; Harvald et al., 2015). The latter is especially critical for the survival of post-mitotic cells with high energy demands, like neurons (Nixon, 2013). Thus suppression of basal autophagy or its loss is causative for neurodegeneration (Hara et al., 2006; Komatsu et al., 2006; Saitsu et al., 2013). There are excellent reviews showing how perturbations at different stages of the autophagy machinery are linked to defects in neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), lysosomal storage disorders (LSD) and amyotrophic lateral sclerosis (ALS) (Nixon, 2013; Menzies et al., 2015). The heavy reliance of neurons on autophagy and the global lysosomal network including the endosomal pathway becomes clear from the numerous reports mentioned in these reviews (Nixon, 2013; Menzies et al., 2015). We, therefore, attempt to review different aspects of how sphingolipid interconversion affects autophagy in neurons and also in other neural cells.

Sphingolipid metabolism

The *de novo* pathway of sphingolipid biosynthesis starts with serine and fatty acyl-CoA condensation catalyzed by the rate-limiting enzyme serine palmitoyltransferase (Figure 1). The consecutive action of 3-ketodihydrosphingosine reductase, one of six known (dihydro)ceramide

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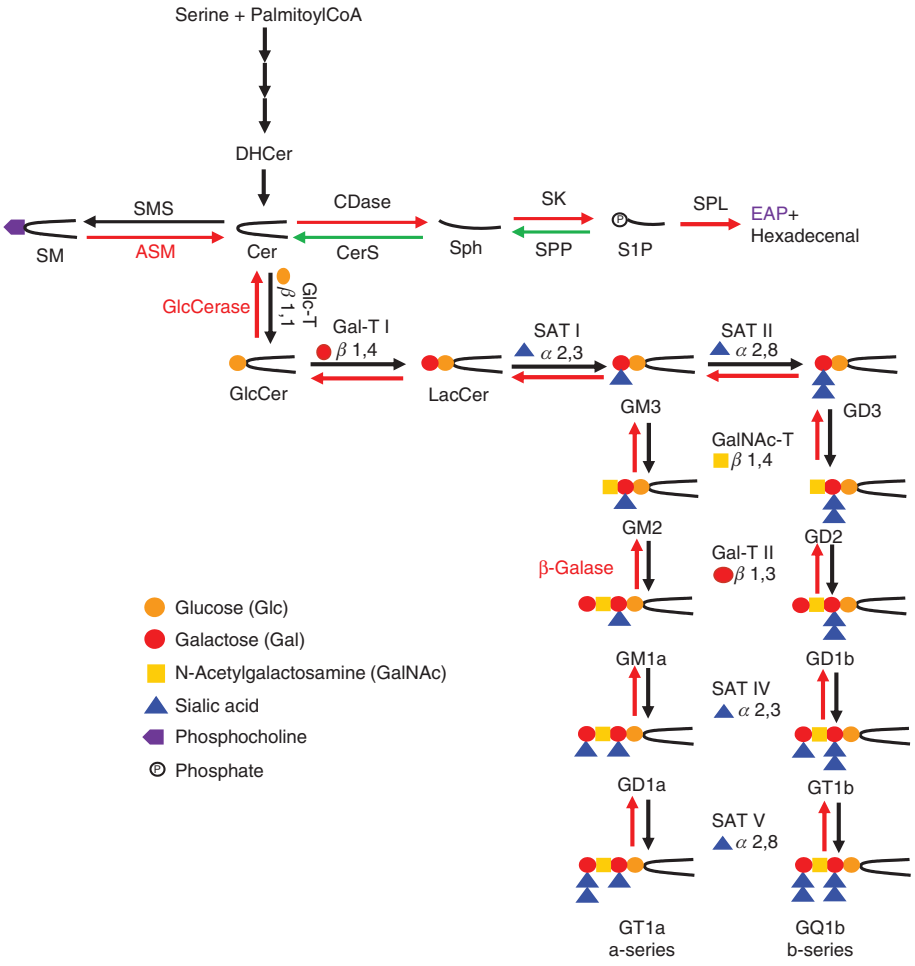

Figure 1: Scheme of central sphingolipid pathways in the mammalian brain.
Ceramide (Cer) is generated either *de novo*, starting from serine and palmitoylCoA (black arrows) or via recycling of its degradation product sphingosine 1-phosphate (S1P) (green arrows). Cer is both, the biosynthetic precursor and the degradation product of all glycosphingolipids and of sphingomyelin (SM). Degradation of ceramide occurs via hydrolysis generating a fatty acid and sphingosine (Sph), which after phosphorylation to S1P is irreversibly cleaved into ethanolamine phosphate (EAP) and hexadecenal (red arrows). Given are only degrading enzymes (red) that are mentioned in the text. Abbreviations used are (i) for lipids and their components: Cer, ceramide, N-acylsphingosine; DHCer, dihydroceramide; EAP, ethanolamine phosphate; Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; Glc, D-glucose; GlcNAc, N-acetyl-glucosamine; NeuAc, N-acetylneuraminic acid; SM, sphingomyelin; Sph, sphingosine; S1P, sphingosine 1-phosphate. The terminology used for gangliosides is that of Svennerholm (Svennerholm, 1963). (ii) for glycosyltransferases: GlcT, ceramide glucosyltransferase (GlcCer-synthase); GalT I, galactosyltransferase I (lactosyl synthase); GalT II, galactosyltransferase II (GM1a/GD1b synthase); GalNAcT, N-Acetyl-D-galactosaminyltransferase (GM2/GD2 synthase); SAT I, sialyltransferase I (GM3 synthase); SAT II, sialyltransferase II (GD3 synthase); SAT IV, sialyltransferase IV (GD1a/GT1b synthase); SAT V, sialyltransferase V (GT1a/GQ1b synthase). (iii) for other enzymes: CerS, ceramide synthases (from six different isoenzymes CerS1 is prevalent in the brain); CDase, ceramidase (acid ceramidase is one of the major glycoproteins in the brain); SK, sphingosine kinases (two known isoforms SK1 and SK2); SPP, S1P phosphatases (two known isoforms SPP1 and SPP2); SPL, S1P-lyase; SMS, sphingomyelin synthase; ASM, acid sphingomyelinase (defect in Niemann-Pick type A and B); β-Galase, β-galactosidase (defect in GM1 gangliosidosis); GlcCerase, glucocerebrosidase (defect in Gaucher disease).

synthases, and dihydroceramide desaturase generates ceramide, the membrane anchor of most sphingolipids (van Echten-Deckert and Herget, 2006). Ceramide is then either glycosylated to generate a multitude of GSLs or acquires a phosphocholine head group to form sphingomyelin (Figure 1). Alternatively, ceramide is phosphorylated to generate ceramide 1-phosphate (not shown in Figure 1). It should be mentioned that a minor part of sphingolpids is generated from dihydroceramide, indicating that glycosyltransferases and sphingomyelin synthases also accept saturated ceramides as a substrate.

Note that sialic acid containing gangliosides are highly enriched in the brain, being particularly abundant in neuronal membranes (van Echten-Deckert and

Herget, 2006; van Echten-Deckert and Walter, 2012). The degradation of GSLs is localized mainly in the lysosomes, and occurs by the stepwise action of specific acid exohydrolases, starting at the hydrophilic end of the molecule (Kolter and Sandhoff, 2010) (Figure 1). More than ten different exohydrolases are known to be involved in GSL degradation. For the degradation of GSLs with three or fewer monosaccharide residues lysosomal exohydrolases need the assistance of small glycoprotein cofactors, the sphingolipid activator proteins (SAPs) (Furst and Sandhoff, 1992). The deficiency of any one of the degrading hydrolases and or SAPs can cause an accumulation of the corresponding lipid substrate in the lysosomal compartment, leading to cellular toxicity. The so-called sphingolipidoses or gangliosidosis belong to the lysosomal lipid storage disorders (LSDs), a group of rare inherited diseases that often affect the brain as neurons are particularly sensitive to lipid accumulation (Kolter and Sandhoff, 2006; Platt et al., 2012).

The degradation of sphingomyelin to generate ceramide is catalyzed by several sphingomyelinases (Stoffel, 1999). Since the discovery of the sphingomyelin cycle and of ceramide as a bioactive signaling molecule (Hannun, 1994; Hannun and Obeid, 1995) the interest in sphingomyelinases increased continuously, especially their role in the central nervous system (CNS) (Hannun and Obeid, 2002; Ledesma et al., 2011; Ong et al., 2015).

Degradation of ceramide comprises the formation of two other bioactive sphingolipids: (i) sphingosine the product of deacylation of ceramide catalyzed by diverse ceramidases (Coant et al., 2017), and (ii) sphingosine 1-phosphate (S1P) formed upon phosphorylation of sphingosine by two different kinases (SK1 and SK2) (Maceyka et al., 2012). Final degradation of the sphingoid backbone occurs by the S1P-lyase catalyzed irreversible cleavage of S1P yielding ethanolamine phosphate and a long-chain aldehyde (Kumar and Saba, 2009). Note that S1P is also a substrate for phosphatases (SPP) (Le Stunff et al., 2002) being recycled to sphingosine (Le Stunff et al., 2007), thus starting a salvage pathway used especially by terminally differentiated cells, including neurons to rebuild complex GSLs (Gillard et al., 1998).

There are some characteristics of sphingolipid metabolism in the brain. Cellular gangliosides usually contain C18-sphingosine. However, gangliosides of the CNS also contain sphingosine with 20 carbons (Sonnino and Chigorno, 2000). They appear with the onset of neuronal differentiation and their content increases throughout the life span. From the six ceramide synthases (CerS1-6) known, CerS1 is prevalent in the brain (Ben-David and Futerman, 2010). It catalyzes the addition of stearic acid to the respective sphingoid base, generating C18-ceramide, the predominant ceramide species in the brain.

Note that from the two kinases known to generate S1P by phosphorylation of sphingosine, in the rodent brain sphingosine kinase 2 (SK2) predominates and is most active in the cerebellum followed by the cortex and brainstem (Blondeau et al., 2007). Also in human brain only SK2 was reliably detectable (Katsel et al., 2007), suggesting that sphingosine kinase 1 (SK1) plays a minor role in the CNS.

As mentioned, sphingolipid storage disorders mainly frequently affect the brain. Beside the abundancy of sphingolipids, the CNS is the cholesterol richest organ of the body (Dietschy and Turley, 2001). Thus, LSDs affecting the brain often involve the storage of sphingolipids and/or cholesterol.

Autophagy

The self-eating phenomenon was first described by Ashford and Porter in 1962 (Ashford and Porter, 1962), 6 years after the discovery of lysosomes by Christian De Duve. De Duve was the one to introduce the term *autophagy* for the process by which cells eliminate their cytoplasmic constituents via digestion in their own lysosomes (De Duve and Wattiaux, 1966). From lysosomal self-digestion, autophagy emerged to a dynamic recycling system that produces new metabolic precursors and energy thus securing survival during critical circumstances including nutritional deficiency, hypoxia and other stressful conditions (Mizushima and Komatsu, 2011). To date, three types of autophagy have been distinguished based on the different ways cellular material is transported to the lysosomes: macroautophagy, microautophagy and chaperone mediated autophagy (Mizushima and Komatsu, 2011). Here, we focus only on macroautophagy (commonly referred to as autophagy), known as the pathway for degradation of cellular organelles (Elmore et al., 2001; Iwata et al., 2006) besides the degradation of long-lived proteins (Komatsu et al., 2007). Autophagy is an evolutionarily conserved process in all eukaryotic organisms, being essential for their survival, development, differentiation and homeostasis (Levine and Klionsky, 2004). Accordingly, homologous autophagy-related genes (*ATG*) and corresponding proteins (ATG) – participate in autophagy from yeast to humans (Klionsky et al., 2003). As illustrated in Figure 2 autophagy starts with the formation of a phagophore (Mizushima et al., 2008), proposed to arise from a variety of non-ribosomal membrane sources (Menzies et al.,

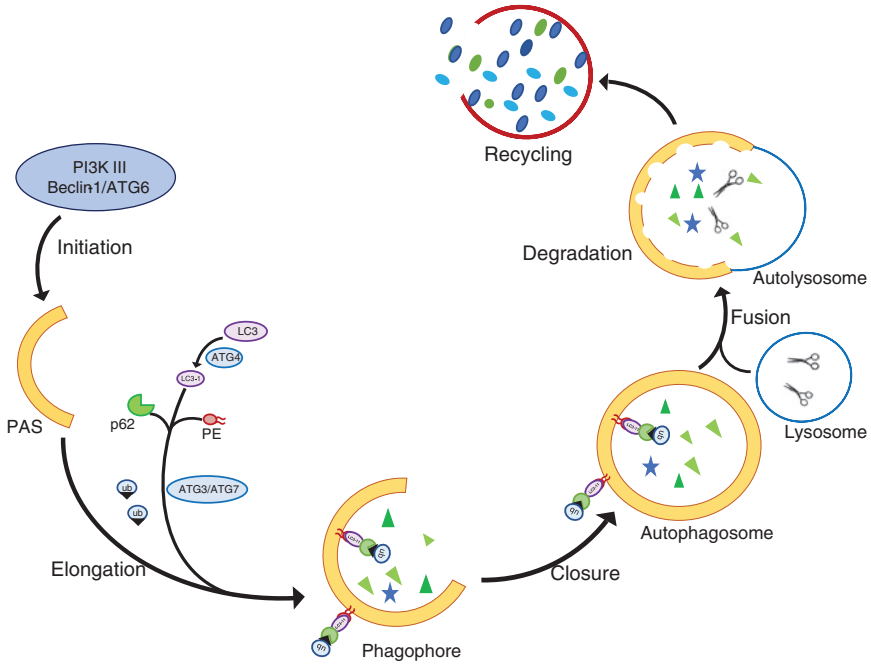

Figure 2: A simplified model of autophagy.
Autophagy starts with the formation of a phagophore at the phagophore assembly site (PAS). The phagophore then expands by recruiting degradation-prone material including ubiquitinated proteins via, e.g. the lipidated membrane-bound LC3-II and the adapter p62. The mature autophagosomes fuse with lysosomes generating autolysosomes in which degradation of the cargo occurs. Degraded material can be recycled after efflux into the cytoplasm. Abbreviations used are: ATG, proteins encoded by autophagy-related genes; LC3, microtubule associated protein light chain; PE, phosphatidylethanolamine; p62, also known as sequestosome 1; ub, ubiquitin.

2015). Subsequently this phagophore bilayer elongates to engulf the portion of cytoplasm to be removed. Closure of this growing membrane generates the autophagosome, a vesicle which fuses via its outer membrane with a lysosome to generate an autolysosome. Finally the sequestered cargo is exposed to digestion by lysosomal hydrolases and recycled (Figure 2). Initiation and regulation of the autophagy pathway requires the concerted action of about 30 evolutionarily conserved *Atgs*. Initiation of autophagy depends on a class III phosphatidylinositol-3-OH kinase (PI3K) complex formed by Beclin1/ATG6 and other proteins. Elongation and closure of the phagophore membrane requires the conversion of LC3-I (microtubule-associated protein 1 light chain, the mammalian homologue of ATG8) to its phosphatidylethanolamine (PE)-conjugated form and also conjugation of ATG5 to ATG12. ATG7 is essential for both conjugation pathways. Thus LC3 is activated by ATG7, transferred to ATG3, and conjugated to PE on the surface of elongating autophagic vacuole membranes to promote forming of the autophagosomes (Mizushima and Yoshimori, 2007).

Note that neurons are particularly reliant on autophagy (Nixon, 2013). In contrast to mitotic cells, post-mitotic neurons do not have the possibility to dilute arising cellular waste by division. In addition, the large expanses of dendritic and axonal cytoplasm constitute rather long distances that have to be covered by autophagosomes to reach the lysosomes. This might also aggravate the removal of cellular waste that accumulates over a lifetime in neurons. It is therefore not surprising that defective autophagy is often associated with neuronal dysfunction (Hara et al., 2006; Komatsu et al., 2006; Nixon, 2013; Menzies et al., 2015). Apart from homeostatic and waste-recycling tasks autophagy also constitutes an effective strategy for the elimination of aggregate-prone proteins in neurons and glial cells thus preventing neurodegeneration (Fortun et al., 2003; Ravikumar et al., 2004; Komatsu et al., 2006). There is also experimental evidence for the segregation via autophagic vacuoles of early apoptotic alterations in the neuronal cytoplasm, to prevent neuronal apoptosis (Stadelmann et al., 1999). All these findings indicate that autophagy is more than a bulk non-selective protein degradation pathway. Indeed, p62/ sequestosome 1 (SQSTM1), a common component of protein aggregates found in protein aggregation diseases of the brain like PD, AD, and Morbus Huntington (Kuusisto et al., 2001, 2002; Zatloukal et al., 2002; Nagaoka et al., 2004) was found to function as an adaptor or receptor that

selectively recruits cargo to autophagosomes (Bjorkoy et al., 2005). The p62 adaptor recognizes on the one hand ubiquitin, which decorates almost all protein aggregates and on the other hand, the LC3 family members on autophagosomes (Bjorkoy et al., 2005; Pankiv et al., 2007) (Figure 2). Note that several other proteins that are involved in the selective targeting of autophagic cargoes to autophagosomes have been described (Menzies et al., 2015). Thus, clearance of damaged mitochondria by autophagy (termed mitophagy) requires two additional proteins: PINK1 (phosphatase and tensin homologue-induced putative kinase 1) and parkin. In the absence of a mitochondrial membrane potential PINK1 accumulates on the mitochondrial outer membrane and recruits parkin, thus targeting mitochondria for degradation by autophagy (de Vries and Przedborski, 2013). Note that the PINK-parkin pathway turned out to be critical for the clearance of dysfunctional mitochondria by autophagy locally in distal neuronal axons (Ashrafi et al., 2014).

It should be mentioned that although neuronal autophagy undoubtedly is crucial for neuronal health, there is also data to suggest that autophagy in glia may be similarly important in maintaining brain health (Di Malta et al., 2012; Motori et al., 2013; Smith et al., 2013).

It thus appears that the pathogenesis of most neurodegenerative diseases is associated with compromised autophagy (Menzies et al., 2015). However, under certain circumstances autophagy can become deleterious, paradoxically even its pro-survival function (Levine and Kroemer, 2008).

Gangliosides and neuronal autophagy

As mentioned post-mitotic neurons express particularly high levels of gangliosides. These sialic acid-containing complex GSLs are involved in fundamental neurobiological processes including neuronal differentiation, synaptogenesis, synaptic transmission and memory formation (Rahmann, 1995). The neuroprotective role of gangliosides has been reported already in the early 1990s based on their neuritogenic and neurotrophic activity and on the observation that mixtures of gangliosides facilitate the repair of neuronal tissue after mechanical, biochemical or toxic injuries (Svennerholm, 1994). Especially ganglioside GM1 (see Figure 1) turned out to have a beneficial role in several neurodegenerative disorders including AD (Svennerholm et al., 2002) and PD (Wu et al., 2012). Moreover, GM1 proved to be able to pass the blood-brain barrier following intravenous administration. Although the molecular mechanism for this therapeutic effect has never been reported, GM1 is still used for the treatment of a multitude of neurological maladies (Zhu et al., 2013) including ischemic stroke (Lenzi et al., 1994) and PD (Schneider et al., 2010). A recent study explains the protective effect of GM1 against Aβ-induced neurotoxicity by its potential to enhance neuronal autophagic activity (Dai et al., 2017). However, this study does not provide any molecular mechanism except an enhanced expression of histone deacetylase 1, which is speculated to mediate an increased expression of autophagy proteins. By contrast, in a model of experimental stroke the robust protective effect of GM1 was associated with a significant inhibition of autophagy in neurons (Li et al., 2016). A possible explanation for this apparent discrepancy is the fact that neurodegenerative diseases are generally correlated with a suppressed basal autophagy (Hara et al., 2006) whereas ischemic brain injury is linked to a severely enhanced autophagic activity in the damaged brain regions (Koike et al., 2008).

Additional information regarding the ambiguous effects of gangliosides on autophagy in the brain comes from a study conducted in primary cultured astrocytes (Hwang et al., 2010b). Astrocytes are the major glial cell type in the brain and the main responders to insults under various pathological conditions including ischemia, neurodegeneration, infection and autoimmunity (Barres and Barde, 2000). It is known that under pathological circumstances gangliosides are released from damaged neurons into the extracellular space which might intensify the pathophysiological condition (Blennow et al., 1991; Michikawa et al., 2001). Hwang et al. (2010b) demonstrate that especially ganglioside GT1b and to a lesser extent GD1a and GM1 induce autophagic cell death in astrocytes. The molecular mechanism underlying this autophagic death involves several signaling components: integrity of lipid rafts, generation of reactive oxygen species (ROS), inhibition of the AKT/mTOR pathway and activation of the ERK pathway (Hwang et al., 2010b). In addition, ganglioside-induced autophagic cell death requires initiation of the NF-κB pathway which is also causative for inflammatory activation of astrocytes (Hwang et al., 2010a). The immunogenic potential of gangliosides was already detected in the early 1990s and lead to the withdrawal of GM1 from the European market (Figueras et al., 1992).

Interestingly, a direct contribution of exogenously added ganglioside GD3 to the biogenesis and maturation of autophagic vacuoles has been reported in fibroblasts (Matarrese et al., 2014). Although GD3 is not a major ganglioside in post-mitotic neurons, but abundant in the early stages of brain development (van Echten-Deckert and

Herget, 2006), this approach is to the best of our knowledge the first to suggest that gangliosides, via their molecular interaction with autophagy-associated molecules, could be recruited to autophagosomes, thus contributing to their maturation and finally to autolysosome formation (Matarrese et al., 2014).

GM1 gangliosidosis is caused by mutations in the *GLB1* gene, which affect the activity of lysosomal β-galactosidase that catalyzes cleavage of the terminal galactose residue of GM1 (see Figure 1). Thus, GM1 accumulates in the lysosomes (Okada and O'Brien, 1968) particularly in neurons due to its high abundancy there. Thus, the nervous system of GM1 gangliosidosis patients is most severely affected (Suzuki and Kamoshita, 1969). Further, in post-mitotic neurons gangliosides are generated mainly via a recycling pathway that is greatly dependent on a correct endosomal/lysosomal processing and transport. It is therefore not surprizing that autophagy has been identified as a major pathway for cellular lipid metabolism (Singh et al., 2009). However, the exact mechanism and clinical significance of perturbed autophagy in GM1 gangliosidosis is not entirely clear. Elevation of LC3-II as well as of beclin1 in the cortex and hippocampus of the respective mouse model was first interpreted as an enhancement of autophagy (Takamura et al., 2008). Additional studies revealed, however, that the increase in these two autophagy marker proteins is mTOR independent and rather indicative for an impaired autophagic flux (Boland et al., 2010).

Together these studies clearly indicate that ganglioside metabolism is definitely involved in the regulation of neuronal autophagy, but the crosstalk between gangliosides and autophagy awaits elucidation.

Glucosylceramide (GlcCer) metabolism and neuronal autophagy

GlcCer is the most abundant GSL in many cell types and the precursor of virtually all GSLs including gangliosides (see Figure 1). Further, GlcCer is a direct metabolite of the bioactive signaling lipid ceramide. It is generated by glucosylation of ceramide but can also act as a precursor for ceramide formation if its carbohydrate residue is cleaved by GlcCer hydrolases (see Figure 1). Disruption of the gene encoding the enzyme that catalyzes ceramide glucosylation demonstrated the vital role of GlcCer for embryonic development and early differentiation (Yamashita et al., 1999). On the other hand, mutations in the gene encoding glucocerebrosidase causes cytotoxic lysosomal accumulation of GlcCer (Hruska et al., 2008). This pathology, known as Gaucher disease, is the most common inherited LSD. Studies in mouse models indicate massive defects of neuronal autophagy including accumulation of ubiquitinated protein aggregates, insoluble α-synuclein and of p62 (Ward et al., 2016). In addition neurons and astrocytes of the Gaucher mouse model accumulated dysfunctional mitochondria due to impaired mitophagy (Osellame et al., 2013). The molecular mechanism underlying GlcCer-induced defects include diminished activity of lysosomal proteases, impaired autophagosome maturation and down-regulation of the master regulator of lysosomal genes, the transcription factor EB (Ward et al., 2016). A direct effect of accumulated GlcCer on autophagy is, however, not yet available.

Sphingomyelin metabolism and neuronal autophagy

Spingomyelin accounts for 10% of all mammalian cellular lipids and is often considered a hybrid phospho-sphingolipid due to its phosphocholine head group bound to ceramide. Like GlcCer it is a direct metabolite of ceramide and due to its abundancy in all cells an important source of ceramide, especially as a response to cellular stress (Hannun, 1994; Levade and Jaffrezou, 1999).

Degradation of sphingomyelin to generate ceramide is catalyzed by several sphingomyelinases that differ in subcellular localization and pH optimum (Goni and Alonso, 2002). Functional mutations of the lysosomal acid sphingomyelinase (ASM) leading to accumulation of sphingomyelin is known as Niemann-Pick disease (NP), another LSD. NP comprises type A (NPA), B (NPB) and C (NPC) of the disease. NPA and NPB are caused by mutations in the gene encoding ASM (Horinouchi et al., 1995). NPC is caused by mutations affecting proteins (NPC1 or NPC2) responsible for the cholesterol efflux from the late endosomal/lysosomal compartment (Kwon et al., 2009). Note that the molecular bases underlying this disease are closely linked to sphingomyelin metabolism (Lloyd-Evans et al., 2008). In neurons from ASM-knockout mice as well as in fibroblasts from NPA patients accumulation of sphingomyelin considerably affected autophagy leading to an accumulation of autophagosomes (Gabande-Rodriguez et al., 2014). The reason for this defective autophagy flux was due to a sphingomyelin-induced lysosomal membrane permeabilisation that leads to a release into the cytosol of lysosomal proteases and an inadequate clearance of autolysosomes (Gabande-Rodriguez et al., 2014). Of interest, this effect of accumulated sphingomyelin could be partially reduced by

inhibiting ceramide synthase with fumonisin B1. Note that in primary cultured neurons dose-response studies revealed that the generation of sphingomyelin (IC_{50} of 0.7 μM) was 10 times more sensitive to inhibition by fumonisin B1 than was glycolipid formation (IC_{50} of approximately 7 μM) (Merrill et al., 1993). Accumulation of sphingomyelin in ASM-knockout mice also caused functional deficits in the retina with the most prominent degeneration in the photoreceptor and outer nuclear layer (Wu et al., 2015). Like in neurons, these deficits were accompanied by an enhancement of the autophagosomal marker LC3-II, suggesting a block in the autophagic flux.

Note that accumulation of autophagosomal markers was often considered as excessive autophagy although it was caused by an impaired autophagic flux. A proper example highlighting the difficulties associated with the interpretation of autophagy function or dysfunction are studies regarding the role of autophagy in NPC (Pacheco and Lieberman, 2008; Elrick et al., 2012; Ordonez et al., 2012). Loss of function mutations in the NPC1 protein result in incorrect trafficking of cholesterol and accumulation of unesterified cholesterol and of sphingolipids including primarily sphingomyelin and to a lesser extent GSLs in late endosomes and lysosomes. Thus, in this disorder not only the endosomal/lysosomal system is affected but also other membrane compartments like the endoplasmic reticulum (ER), which do not receive the cholesterol required for a correct membrane composition (Pacheco and Lieberman, 2008). Accumulation of autophagosomes in degenerating Purkinje neurons in mice with mutant NPC1 suggested a cell death associated induction of autophagy (Ko et al., 2005). Improved knowledge of autophagy pathway and superior methods to measure this pathway revealed that autophagy is defective in this disease (Elrick et al., 2012; Ordonez et al., 2012). In conclusion, both, accumulation of sphingomyelin as well as an impaired lipid trafficking, contribute to the regulation of neuronal autophagy that appears to be both, induced and defective.

Ceramide metabolism and neuronal autophagy

Ceramide, a key intermediate in sphingolipid metabolism is not only the hydrophobic membrane anchor of sphingolipids but emerged as a central bioactive molecule in cellular stress response, differentiation, senescence and apoptosis, as well as in neurodegeneration, inflammation, cancer and infections (Hannun and Obeid, 2002). Its role in neuronal differentiation was reported almost two decades ago (Herget et al., 2000). There is, in addition, evidence that the hippocampal content of ceramide considerably affects neuronal growth, maturation and survival and hence behavior (Gulbins et al., 2013). The extensive implication of ceramide in the control of autophagy (Jiang and Ogretmen, 2014) implicates the question whether these effects of ceramide are correlated. Based on studies performed in diverse cell lines it has been proposed that an elevation of ceramide induces the formation of autophagosomes (Zheng et al., 2006). But is this assumption also applicable to post-mitotic neurons? A study conducted in primary cultured neurons revealed that inhibition of ceramide glucosylation enhances autophagy flux monitored by the increase of the ratio LC3II/LC3I (Shen et al., 2014). However, inhibition of GlcCer-synthase might not only lead to an increase of the amount of ceramide but might also reduce the amount of all GSLs and/or might direct ceramide either into the degradative pathway or to sphingomyelin formation (see Figure 1). Thus, diverse changes could be responsible for the enhanced autophagy flux. Nevertheless, in agreement with findings in non-neuronal cells, where ceramide-induced autophagy involved deactivation of the AKT/mTOR pathway (Scarlatti et al., 2004), the primary negative regulator of autophagy (Yang and Klionsky, 2010), inhibition of neuronal GlcCer-synthase affected the same pathway (Shen et al., 2014). Note that deactivation of AKT by ceramide occurs through the activation of PP2A (protein phosphatase 2A) which acts on PI3K-dependent kinase 2 thus precluding phosphorylation of serine 473 and hence activation of AKT (Schubert et al., 2000). Apparently, in rapidly dividing cells inhibition of the AKT-pathway often leads to autophagic cell death (Gozuacik and Kimchi, 2007) and is accompanied by an up-regulation of beclin 1 (Scarlatti et al., 2004) whereas in post-mitotic neurons it has a pro-survival effect by promoting degradation of aggregate-prone proteins (Spilman et al., 2010) most probably due to an elevated autophagic flux as well as an increase in size and number of lysosomes and late endosomes (Shen et al., 2014). Intriguingly, C2-ceramide-induced autophagy was found to be protective also in the human neuroblastoma cell line SH-SY5Y (Fan et al., 2017). Yet multiple signaling pathways were critical in C2-ceramide-induced autophagy in these cells. As described above, C2-ceramide inhibited the AKT pathway but simultaneously activated the c-Jun NH2-terminal kinase (JNK) and ERK1/2 pathways. The latter two turned out to play pro-survival roles in SH-SY5Y cells. The pro-survival role of the ERK1/2 pathway in ceramide-induced autophagy has been shown in other carcinoma cells (Zhu et al., 2014). However, the pro-survival role of JNK in C2-ceramide-induced autophagy is

rather surprising, as it was linked to cell death in other carcinoma cells (Li et al., 2009). Ceramide was shown to liberate beclin1 for autophagy induction through the JNK-mediated phosphorylation of Bcl-2 (Pattingre et al., 2009). Notably, suppressors of apoptosis such as the Bcl-2 family directly bind and control beclin1 (Pattingre et al., 2005). Of note, disruption of the beclin1/Bcl-2 complex emerged as a common mechanism in ceramide-associated autophagy that usually promotes cell death (Young et al., 2013). However, in neuroblastoma cells ceramide-induced activation of JNK did not act on the beclin1/Bcl-2 complex. One is tempted to conclude that in neurons and neuroblastoma cells ceramide-induced autophagy is protective although it is mediated by inhibition of the AKT-pathway.

More data regarding diverse mechanisms underlying ceramide-mediated autophagy in different cancer cell lines were elegantly reviewed elsewhere (Jiang and Ogretmen, 2014). Interestingly, in non-neuronal cells a growing number of studies focus on dihydroceramide (DHCer) –induced autophagy and its role on cell fate (Signorelli et al., 2009; Gagliostro et al., 2012; Siddique et al., 2013; Li et al., 2014; Cingolani et al., 2014; Rodriguez-Cuenca et al., 2015; Hernandez-Tiedra et al., 2016; Casasampere et al., 2017). Regarding the effect of DHCer on neuronal autophagy, there is to the best of our knowledge, only one report available at the present time. Although treatment of primary cultured neurons with two DHCer desaturase inhibitors reduced the enzymatic activity only by 20% after 24 h and by nearly 50% after 48 h, a series of parameters including viability, the expression of synaptic marker proteins, and the lipidome were significantly affected (Ordonez-Gutierrez et al., 2018). Although experimental evidence for an increased autophagy is rather weak, the authors speculate that accumulation of DHCer is causative for induction of autophagy (Ordonez-Gutierrez et al., 2018). On the other hand, lipophagy, a specialized form of autophagy for the degradation of lipid droplets, was correlated with DHCer desaturase activity and neurodegeneration. Consequently, in *Drosophila* depletion of DHCer desaturase caused lipophagy-mediated, activity-dependent neurodegeneration of photoreceptors (Jung et al., 2017). Similarly, impairment of lipophagy and apoptosis were induced in SH-SY5Y human neuroblastoma cells following DHCer treatment (Jung et al., 2017). Moreover, pharmacologically induced accumulation of DHCer in glioblastoma cells turned out to be cytotoxic and accompanied by increased autophagy but also by ER stress and oxidative stress (Noack et al., 2014). In conclusion, DHCer appears to be correlated with an enhanced cytotoxic autophagy in neurons and glioblastoma cells. The molecular mechanism underlying this effect is, however, not known so far.

S1P metabolism and neuronal autophagy

S1P, a catabolic intermediate of ceramide has emerged as an evolutionarily conserved bioactive lipid. In contrast to ceramide, S1P is primarily known as a survival lipid messenger due to its tissue protective properties (Olivera and Spiegel, 1993; Oskouian and Saba, 2010). Similarly, S1P has been found to induce protective autophagy, distinct from ceramide-induced autophagy-associated cell death in several malignant cell types (Li et al., 2014). Note that S1P acts either via five G-protein coupled receptors termed S1PR1-5 or as an intracellular second messenger (Spiegel and Milstien, 2003).

While the role of S1P-mediated autophagic mechanisms has been well documented in non-neuronal cells, studies on the role of S1P in neuronal autophagy are rather limited. In non-neuronal carcinoma cells S1P-mediated autophagy has not been related to the accumulation of beclin1 protein or to the suppression of AKT (Lavieu et al., 2006). However, mTOR was inhibited by increased S1P levels in cancer cells (Lavieu et al., 2006; Chang et al., 2009; Oskouian and Saba, 2010). Thus, S1P induces autophagy via inhibiting mTOR independently of AKT and PI3K. Depending on the cell type induction of autophagy by exogenous S1P requires certain functional S1P receptors including S1PR3 (Taniguchi et al., 2012) and S1PR5 (Chang et al., 2009).

Neuronal autophagy appears to be closely linked to S1P metabolism. In a search for novel regulators of autophagy in neurons the group of Andrey S. Tsvetkov found that like in non-neuronal cells SK1 that generates S1P by catalyzing sphingosine phosphorylation, enhances autophagic flux in neurons whereas S1P-degrading enzymes like SPP and S1P-lyase decrease this flux (Moruno Manchon et al., 2015). The authors suggest that activation/overexpression of SK1 triggers the biogenesis of a pre-autophagosomal beclin1-positive structure. They demonstrated that endosomes make contacts with the ER in cells undergoing autophagy and propose that endosomes, to which activated SK1 is relocated, provide the ER with S1P for the biogenesis of autophagosomes (Moruno Manchon et al., 2015). Notably, SPP and S1P-lyase are also localized to the ER, ensuring that dispensable S1P can be locally degraded to stop its signaling. Indeed, degradation of mutant huntingtin, the protein that causes Huntington's disease, can be regulated by SK1 and S1P-lyase, suggesting a key function of S1P in this neurodegenerative disease (Moruno Manchon et al., 2015). Accordingly, pharmacological inhibition of S1P-lyase with THI in striatal and cortical neurons transfected with mutant huntingtin protected them from neurotoxicity

and improved their survival (Moruno Manchon et al., 2015). In contrast to these results, we found a blocked autophagic flux in S1P-lyase deficient murine brains and also in primary cultured neurons treated with THI (Mitroi et al., 2017). The explanation for this unexpected result is the fact that depletion of S1P-lyase not only causes an increase of S1P but also a decline in phosphatidylethanolamine (PE) synthesized from ethanolamine phosphate, one of the degradation products of S1P (see also Figure 1). The abundancy of sphingolipids in neurons might explain the age-dependent decrease of PE in S1P-lyase deficient brains. Obviously PE, the lipid anchor of LC3 in phagophore membranes is essential for the maturation and closure of autophagosomes (see also Figure 2). Accordingly, in S1P-lyase deficient brain sections increased phagophore-like and reduced autolysosome-like structures were detected (Mitroi et al., 2017). The biochemical analysis showing diminished LC3-II/LC3-I ratio, and increased expression of beclin1 and p62 also suggest a blockage at the initial stages of the autophagic flux (Mitroi et al., 2017). In line with defective autophagy, we further observed the accumulation of aggregate prone proteins like amyloid precursor protein (APP) and α-synuclein in the S1P-lyase deficient brains. Moreover, treatment of neurons with PE restored the autophagy defects, followed by normalization of the neurodegenerative markers to control levels thereby providing new insights on how S1P-lyase might regulate autophagy in neurons (Mitroi et al., 2017). In contrast to other cell types, neurons are abundant in complex sphingolipids and therefore the S1P degradation product ethanolamine phosphate might be essential for the PE involved in neuronal autophagy. Our findings assume significance in the context that artificially increasing PE levels have been shown to extend the life span by increasing autophagic flux in yeast (Rockenfeller et al., 2015). Moreover, a striking scenario wherein *Leishmania* remodels the sphingolipid pathway for ethanolamine production, which otherwise is not the predominant PE synthesizing pathway in eukaryotes has been documented (Zhang et al., 2007). Remarkably, according to the findings of Moruno Manchon et al. (2015), we also found an increased expression of beclin1 in S1P-lyase deficient brains (Mitroi et al., 2017), which might be independent of the PE content but correlate with the elevated S1P in S1P-lyase deficient neurons.

From these reports, it is evident that autophagy regulation by S1P is more complicated and dynamic than it appears at first glance. In neurons manipulation of S1P-lyase, the enzyme which connects sphingolipid and phospholipid metabolism, implicates both the increase of S1P and the simultaneous decrease in PE, which both affect autophagy albeit in an antagonistic way.

Similarly, SK1 catalyzes the conversion of pro-apoptotic sphingosine to pro-survival S1P (Hannun and Obeid, 2008). In neurons especially sphingosine was shown to be involved in the regulation of membrane fusion during synaptic vesicle exocytosis by acting on different components of the SNARE complex (Darios et al., 2009). As neuronal and endocytic SNARE proteins are highly conserved in structure and function (Antonin et al., 2000) it is not surprizing that sphingoid base metabolism also regulates membrane fusion events in the endocytic pathway, which converges with the highly conserved recycling pathway of autophagy (Young et al., 2016). Thus accumulation of sphingosine, the substrate of SK1 induces the formation of enlarged dysfunctional late endosomes that impair autophagy whereas recruitment of SK1 to sphingosine-enriched endocytic vesicles and generation of S1P facilitates membrane trafficking along the endosomal pathway (Young et al., 2016).

Further, the cellular environment including basal conditions, nutritional stress or cell damage might also dictate how enzymes involved in S1P metabolism act in relation to the autophagic processes. For instance, SK2 is a key player in hypoxia-induced autophagy promoting neuronal survival (Sheng et al., 2014). SK2, a BH3-only protein (Liu et al., 2003) disrupts the beclin1/Bcl-2 complex thus releasing beclin1 for autophagy induction. Note that BH3-only proteins usually antagonize the activity of pro-survival proteins such as the Bcl-2 family (Willis and Adams, 2005). However, in mouse cortical neurons SK2-associated autophagy promotes survival and is independent of its catalytic activity. Remember, ceramide-induced and JNK-mediated disruption of the beclin1/Bcl-2 complex triggered autophagy-associated cell death in cancer cells (see previous section).

Collectively, all these studies highlight the intimate link between S1P metabolism, autophagy, and endocytic/membrane fusion events.

Conclusion and outlook

Autophagy plays a key role in brain homeostasis. There is no doubt that sphingolipid metabolism is closely involved in neuronal autophagy regulation. Yet the number of studies regarding this topic is relatively scarce but continuously increasing (Table 1). Often the role of sphingolipids appears to be ambiguous. There is plenty of work ahead of understanding the mechanisms which determine the survival or cell death function of autophagy in neurons. Generally, enhanced neuronal autophagy appears to be beneficial in post-mitotic neurons but under certain circumstances it can also be detrimental. Understanding of the exact

Table 1: Overview of the effects of sphingolipids on brain autophagy.

Sphingolipid manipulation strategy	Effect on autophagy	Proposed mechanism	Biological model	Reference
GM1 exogenously applied after Aβ treatment	Stimulated	Elevated expression of histone deacetylase I	Mouse hippocampus (*in vivo*)	Dai et al., 2017
GM1 exogenously applied following stroke	Inhibited	Unknown	Mouse brain (*in vivo*)	Li et al., 2016
GT1b, GD1a, GM1 exogenously applied	Stimulated	Generation of ROS, inhibition of the AKT/mTOR pathway, activation of the ERK pathway	Astrocytes (primary cultures)	Hwang et al., 2010b
GM1 accumulated due to β-galactosidase deficiency	Perturbed	Unknown	Hippocampus and cortex of GM1-gangliosidoses mouse model	Takamura et al., 2008; Boland et al., 2010
GlcCer accumulated due to deficient glucocerebrosidase	Impaired	Defect autophagosome maturation, down-regulation of transcription factor EB, reduced activity of lysosomal proteases	Neurons/astrocytes of Gaucher mouse model	Osellame et al., 2013; Ward et al., 2016
Sphingomyelin accumulated due to deficient ASM	Impaired	Lysosomal membrane permeabilisation, hence release of lysosomal proteases and inadequate clearance of autolysosomes	Neurons & photoreceptor cells of ASM-KO mouse model	Gabande-Rodriguez et al., 2014; Wu et al., 2015
Ceramide, increased due to inhibition of GlcCer-synthase	Stimulated	Deactivation of the AKT/mTOR pathway via activation of PPA2	Neurons (primary cultures)	Shen et al., 2014
C2 ceramide exogenously added	Stimulated	Inhibition of the AKT pathway, activation of the JNK and ERK1/2 pathways	SH-SY5Y neuroblastoma cells	Fan et al., 2017
S1P, increased by overexpression of SK1 or inhibition of SPP or S1P-lyase	Stimulated	Biogenesis of pre-autophagosomal beclin1 positive structures	Neurons (primary cultures)	Moruno Manchon et al., 2015
S1P increased due to brain targeted depletion of S1P-lyase	Impaired	Block of autophagic flux due to reduced phosphatidylethanolamine	Brains and neurons of mice with neural specific deletion of S1P-lyase	Mitroi et al., 2017

regulation of autophagy is therefore of key importance. Neuronal autophagy is, in addition, a target for therapeutic strategies of neurological disorders including neurodegenerative diseases as well as LSD. In this regard manipulating sphingolipid metabolism might represent a helpful tool to direct the autophagy activity versus neuronal health. The development of pharmacological autophagy modulators that target sphingolipid metabolism is alive, although in its infancy (Casasampere et al., 2017).

Acknowledgments: We thank the LIMES Institute for supporting S.A.

References

Antonin, W., Holroyd, C., Fasshauer, D., Pabst, S., Von Mollard, G.F., and Jahn, R. (2000). A SNARE complex mediating fusion of late endosomes defines conserved properties of SNARE structure and function. EMBO J. *19*, 6453–6464.

Ashford, T.P. and Porter, K.R. (1962). Cytoplasmic components in hepatic cell lysosomes. J. Cell Biol. *12*, 198–202.

Ashrafi, G., Schlehe, J.S., LaVoie, M.J., and Schwarz, T.L. (2014). Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. J. Cell Biol. *206*, 655–670.

Barres, B.A. and Barde, Y. (2000). Neuronal and glial cell biology. Curr. Opin. Neurobiol. *10*, 642–648.

Bedia, C., Levade, T., and Codogno, P. (2011). Regulation of autophagy by sphingolipids. Anticancer Agents Med. Chem. *11*, 844–853.

Ben-David, O. and Futerman, A.H. (2010). The role of the ceramide acyl chain length in neurodegeneration: involvement of ceramide synthases. Neuromol. Med. *12*, 341–350.

Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H., and Johansen, T. (2005). p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J. Cell Biol. *171*, 603–614.

Blennow, K., Davidsson, P., Wallin, A., Fredman, P., Gottfries, C.G., Karlsson, I., Mansson, J.E., and Svennerholm, L. (1991). Gangliosides in cerebrospinal fluid in 'probable Alzheimer's disease'. Arch. Neurol. *48*, 1032–1035.

Blondeau, N., Lai, Y., Tyndall, S., Popolo, M., Topalkara, K., Pru, J.K., Zhang, L., Kim, H., Liao, J.K., Ding, K., et al. (2007). Distribution of sphingosine kinase activity and mRNA in rodent brain. J. Neurochem. *103*, 509–517.

Boland, B., Smith, D.A., Mooney, D., Jung, S.S., Walsh, D.M., and Platt, F.M. (2010). Macroautophagy is not directly involved in the metabolism of amyloid precursor protein. J. Biol. Chem. *285*, 37415–37426.

Casasampere, M., Ordonez, Y.F., Casas, J., and Fabrias, G. (2017). Dihydroceramide desaturase inhibitors induce autophagy via dihydroceramide-dependent and independent mechanisms. Biochim. Biophys. Acta *1861*, 264–275.

Chang, C.L., Ho, M.C., Lee, P.H., Hsu, C.Y., Huang, W.P., and Lee, H. (2009). S1P(5) is required for sphingosine 1-phosphate-induced autophagy in human prostate cancer PC-3 cells. Am. J. Physiol. Cell Physiol. *297*, C451–C458.

Cingolani, F., Casasampere, M., Sanllehi, P., Casas, J., Bujons, J., and Fabrias, G. (2014). Inhibition of dihydroceramide desaturase activity by the sphingosine kinase inhibitor SKI II. J Lipid Res. *55*, 1711–1720.

Coant, N., Sakamoto, W., Mao, C., and Hannun, Y.A. (2017). Ceramidases, roles in sphingolipid metabolism and in health and disease. Adv. Biol. Regul. *63*, 122–131.

Dai, R., Zhang, S., Duan, W., Wei, R., Chen, H., Cai, W., Yang, L., and Wang, Q. (2017). Enhanced autophagy contributes to protective effects of GM1 ganglioside against Abeta1-42-induced neurotoxicity and cognitive deficits. Neurochem. Res. *42*, 2417–2426.

Darios, F., Wasser, C., Shakirzyanova, A., Giniatullin, A., Goodman, K., Munoz-Bravo, J.L., Raingo, J., Jorgacevski, J., Kreft, M., Zorec, R., et al. (2009). Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis. Neuron *62*, 683–694.

De Duve, C. and Wattiaux, R. (1966). Functions of lysosomes. Annu. Rev. Physiol. *28*, 435–492.

de Vries, R.L. and Przedborski, S. (2013). Mitophagy and Parkinson's disease: be eaten to stay healthy. Mol. Cell Neurosci. *55*, 37–43.

Di Malta, C., Fryer, J.D., Settembre, C., and Ballabio, A. (2012). Astrocyte dysfunction triggers neurodegeneration in a lysosomal storage disorder. Proc. Natl. Acad. Sci. USA *109*, E2334–2342.

Dietschy, J.M. and Turley, S.D. (2001). Cholesterol metabolism in the brain. Curr. Opin. Lipidol. *12*, 105–112.

Elmore, S.P., Qian, T., Grissom, S.F., and Lemasters, J.J. (2001). The mitochondrial permeability transition initiates autophagy in rat hepatocytes. FASEB J. *15*, 2286–2287.

Elrick, M.J., Yu, T., Chung, C., and Lieberman, A.P. (2012). Impaired proteolysis underlies autophagic dysfunction in Niemann-Pick type C disease. Hum. Mol. Genet. *21*, 4876–4887.

Fan, C., Liu, Y., Zhao, M., Zhan, R., Cui, W., Jin, H., Teng, Y., Lv, P., Zheng, L., and Huang, Y. (2017). Autophagy inhibits C2-ceramide-mediated cell death by decreasing the reactive oxygen species levels in SH-SY5Y cells. Neurosci. Lett. *651*, 198–206.

Figueras, A., Morales-Olivas, F.J., Capella, D., Palop, V., and Laporte, J.R. (1992). Bovine gangliosides and acute motor polyneuropathy. Br. Med. J. *305*, 1330–1331.

Fortun, J., Dunn, W.A., Jr., Joy, S., Li, J., and Notterpek, L. (2003). Emerging role for autophagy in the removal of aggresomes in Schwann cells. J. Neurosci. *23*, 10672–10680.

Furst, W. and Sandhoff, K. (1992). Activator proteins and topology of lysosomal sphingolipid catabolism. Biochim. Biophys. Acta *1126*, 1–16.

Gabande-Rodriguez, E., Boya, P., Labrador, V., Dotti, C.G., and Ledesma, M.D. (2014). High sphingomyelin levels induce lysosomal damage and autophagy dysfunction in Niemann Pick disease type A. Cell Death Differ. *21*, 864–875.

Gagliostro, V., Casas, J., Caretti, A., Abad, J.L., Tagliavacca, L., Ghidoni, R., Fabrias, G., and Signorelli, P. (2012). Dihydroceramide delays cell cycle G1/S transition via activation of ER stress and induction of autophagy. Int. J. Biochem. Cell Biol. *44*, 2135–2143.

Gillard, B.K., Clement, R.G., and Marcus, D.M. (1998). Variations among cell lines in the synthesis of sphingolipids in *de novo* and recycling pathways. Glycobiology *8*, 885–890.

Goni, F.M. and Alonso, A. (2002). Sphingomyelinases: enzymology and membrane activity. FEBS Lett. *531*, 38–46.

Gozuacik, D. and Kimchi, A. (2007). Autophagy and cell death. Curr. Top. Dev. Biol. *78*, 217–245.

Gulbins, E., Palmada, M., Reichel, M., Luth, A., Bohmer, C., Amato, D., Muller, C.P., Tischbirek, C.H., Groemer, T.W., Tabatabai, G., et al. (2013). Acid sphingomyelinase-ceramide system mediates effects of antidepressant drugs. Nat. Med. *19*, 934–938.

Hannun, Y.A. (1994). The sphingomyelin cycle and the second messenger function of ceramide. J. Biol. Chem. *269*, 3125–3128.

Hannun, Y.A. and Obeid, L.M. (1995). Ceramide: an intracellular signal for apoptosis. Trends Biochem. Sci. *20*, 73–77.

Hannun, Y.A. and Obeid, L.M. (2002). The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J. Biol. Chem. *277*, 25847–25850.

Hannun, Y.A. and Obeid, L.M. (2008). Principles of bioactive lipid signalling: lessons from sphingolipids. Nat. Rev. Mol. Cell Biol. *9*, 139–150.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature *441*, 885–889.

Harvald, E.B., Olsen, A.S., and Faergeman, N.J. (2015). Autophagy in the light of sphingolipid metabolism. Apoptosis *20*, 658–670.

Herget, T., Esdar, C., Oehrlein, S.A., Heinrich, M., Schutze, S., Maelicke, A., and van Echten-Deckert, G. (2000). Production of ceramides causes apoptosis during early neural differentiation *in vitro*. J. Biol. Chem. *275*, 30344–30354.

Hernandez-Tiedra, S., Fabrias, G., Davila, D., Salanueva, I.J., Casas, J., Montes, L.R., Anton, Z., Garcia-Taboada, E., Salazar-Roa, M., Lorente, M., et al. (2016). Dihydroceramide accumulation mediates cytotoxic autophagy of cancer cells via autolysosome destabilization. Autophagy *12*, 2213–2229.

Horinouchi, K., Erlich, S., Perl, D.P., Ferlinz, K., Bisgaier, C.L., Sandhoff, K., Desnick, R.J., Stewart, C.L., and Schuchman, E.H. (1995). Acid sphingomyelinase deficient mice: a model of types A and B Niemann-Pick disease. Nat. Genet. *10*, 288–293.

Hruska, K.S., LaMarca, M.E., Scott, C.R., and Sidransky, E. (2008). Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). Hum. Mutat. *29*, 567–583.

Hwang, J., Lee, H.J., Lee, W.H., and Suk, K. (2010a). NF-κB as a common signaling pathway in ganglioside-induced autophagic cell death and activation of astrocytes. J. Neuroimmunol. *226*, 66–72.

Hwang, J., Lee, S., Lee, J.T., Kwon, T.K., Kim, D.R., Kim, H., Park, H.C., and Suk, K. (2010b). Gangliosides induce autophagic cell death in astrocytes. Br. J. Pharmacol. *159*, 586–603.

Iwata, J., Ezaki, J., Komatsu, M., Yokota, S., Ueno, T., Tanida, I., Chiba, T., Tanaka, K., and Kominami, E. (2006). Excess peroxisomes are degraded by autophagic machinery in mammals. J. Biol. Chem. *281*, 4035–4041.

Jiang, W. and Ogretmen, B. (2014). Autophagy paradox and ceramide. Biochim. Biophys. Acta *1841*, 783–792.

Jung, W.H., Liu, C.C., Yu, Y.L., Chang, Y.C., Lien, W.Y., Chao, H.C., Huang, S.Y., Kuo, C.H., Ho, H.C., and Chan, C.C. (2017). Lipophagy prevents activity-dependent neurodegeneration due to dihydroceramide accumulation *in vivo*. EMBO Rep. *18*, 1150–1165.

Katsel, P., Li, C., and Haroutunian, V. (2007). Gene expression alterations in the sphingolipid metabolism pathways during progression of dementia and Alzheimer's disease: a shift toward ceramide accumulation at the earliest recognizable stages of Alzheimer's disease? Neurochem. Res. *32*, 845–856.

Klionsky, D.J., Cregg, J.M., Dunn, W.A., Jr., Emr, S.D., Sakai, Y., Sandoval, I.V., Sibirny, A., Subramani, S., Thumm, M., Veenhuis, M., et al. (2003). A unified nomenclature for yeast autophagy-related genes. Dev. Cell. *5*, 539–545.

Ko, D.C., Milenkovic, L., Beier, S.M., Manuel, H., Buchanan, J., and Scott, M.P. (2005). Cell-autonomous death of cerebellar purkinje neurons with autophagy in Niemann-Pick type C disease. PLoS Genet. *1*, 81–95.

Koike, M., Shibata, M., Tadakoshi, M., Gotoh, K., Komatsu, M., Waguri, S., Kawahara, N., Kuida, K., Nagata, S., Kominami, E., et al. (2008). Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury. Am. J. Pathol. *172*, 454–469.

Kolter, T. and Sandhoff, K. (2006). Sphingolipid metabolism diseases. Biochim. Biophys. Acta *1758*, 2057–2079.

Kolter, T. and Sandhoff, K. (2010). Lysosomal degradation of membrane lipids. FEBS Lett. *584*, 1700–1712.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E., et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature *441*, 880–884.

Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., et al. (2007). Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell *131*, 1149–1163.

Kumar, A. and Saba, J.D. (2009). Lyase to live by: sphingosine phosphate lyase as a therapeutic target. Expert Opin. Ther. Targets *13*, 1013–1025.

Kuusisto, E., Salminen, A., and Alafuzoff, I. (2001). Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. Neuroreport *12*, 2085–2090.

Kuusisto, E., Salminen, A., and Alafuzoff, I. (2002). Early accumulation of p62 in neurofibrillary tangles in Alzheimer's disease: possible role in tangle formation. Neuropathol. Appl. Neurobiol. *28*, 228–237.

Kwon, H.J., Abi-Mosleh, L., Wang, M.L., Deisenhofer, J., Goldstein, J.L., Brown, M.S., and Infante, R.E. (2009). Structure of N-terminal domain of NPC1 reveals distinct subdomains for binding and transfer of cholesterol. Cell *137*, 1213–1224.

Lavieu, G., Scarlatti, F., Sala, G., Carpentier, S., Levade, T., Ghidoni, R., Botti, J., and Codogno, P. (2006). Regulation of autophagy by sphingosine kinase 1 and its role in cell survival during nutrient starvation. J. Biol. Chem. *281*, 8518–8527.

Lavieu, G., Scarlatti, F., Sala, G., Carpentier, S., Levade, T., Ghidoni, R., Botti, J., and Codogno, P. (2008). Sphingolipids in macroautophagy. Methods Mol. Biol. *445*, 159–173.

Le Stunff, H., Galve-Roperh, I., Peterson, C., Milstien, S., and Spiegel, S. (2002). Sphingosine-1-phosphate phosphohydrolase in regulation of sphingolipid metabolism and apoptosis. J. Cell Biol. *158*, 1039–1049.

Le Stunff, H., Giussani, P., Maceyka, M., Lepine, S., Milstien, S., and Spiegel, S. (2007). Recycling of sphingosine is regulated by the concerted actions of sphingosine-1-phosphate phosphohydrolase 1 and sphingosine kinase 2. J. Biol. Chem. *282*, 34372–34380.

Ledesma, M.D., Prinetti, A., Sonnino, S., and Schuchman, E.H. (2011). Brain pathology in Niemann Pick disease type A: insights from the acid sphingomyelinase knockout mice. J. Neurochem. *116*, 779–788.

Lenzi, G.L., Grigoletto, F., Gent, M., Roberts, R.S., Walker, M.D., Easton, J.D., Carolei, A., Dorsey, F.C., Rocca, W.A., Bruno, R., et al. (1994). Early treatment of stroke with monosialoganglioside GM-1. Efficacy and safety results of the Early Stroke Trial. Stroke *25*, 1552–1558.

Levade, T. and Jaffrezou, J.P. (1999). Signalling sphingomyelinases: which, where, how and why? Biochim. Biophys. Acta *1438*, 1–17.

Levine, B. and Klionsky, D.J. (2004). Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev. Cell *6*, 463–477.

Levine, B. and Kroemer, G. (2008). Autophagy in the pathogenesis of disease. Cell *132*, 27–42.

Li, D.D., Wang, L.L., Deng, R., Tang, J., Shen, Y., Guo, J.F., Wang, Y., Xia, L.P., Feng, G.K., Liu, Q.Q., et al. (2009). The pivotal role of c-Jun NH_2-terminal kinase-mediated Beclin 1 expression during anticancer agents-induced autophagy in cancer cells. Oncogene *28*, 886–898.

Li, Y., Li, S., Qin, X., Hou, W., Dong, H., Yao, L., and Xiong, L. (2014). The pleiotropic roles of sphingolipid signaling in autophagy. Cell Death Dis. *5*, e1245.

Li, L., Tian, J., Long, M.K., Chen, Y., Lu, J., Zhou, C., and Wang, T. (2016). Protection against experimental stroke by ganglioside GM1 is associated with the inhibition of autophagy. PLoS One *11*, e0144219.

Liu, H., Toman, R.E., Goparaju, S.K., Maceyka, M., Nava, V.E., Sankala, H., Payne, S.G., Bektas, M., Ishii, I., Chun, J., et al. (2003). Sphingosine kinase type 2 is a putative BH3-only protein that induces apoptosis. J. Biol. Chem. *278*, 40330–40336.

Lloyd-Evans, E., Morgan, A.J., He, X., Smith, D.A., Elliot-Smith, E., Sillence, D.J., Churchill, G.C., Schuchman, E.H., Galione, A., and Platt, F.M. (2008). Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. Nat. Med. *14*, 1247–1255.

Maceyka, M., Harikumar, K.B., Milstien, S., and Spiegel, S. (2012). Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol. *22*, 50–60.

Matarrese, P., Garofalo, T., Manganelli, V., Gambardella, L., Marconi, M., Grasso, M., Tinari, A., Misasi, R., Malorni, W., and Sorice, M. (2014). Evidence for the involvement of GD3 ganglioside in autophagosome formation and maturation. Autophagy *10*, 750–765.

Menzies, F.M., Fleming, A., and Rubinsztein, D.C. (2015). Compromised autophagy and neurodegenerative diseases. Nat. Rev. Neurosci. *16*, 345–357.

Merrill, A.H., Jr., van Echten, G., Wang, E., and Sandhoff, K. (1993). Fumonisin B1 inhibits sphingosine (sphinganine) N-acyltransferase and de novo sphingolipid biosynthesis in cultured neurons in situ. J. Biol. Chem. *268*, 27299–27306.

Michikawa, M., Gong, J.S., Fan, Q.W., Sawamura, N., and Yanagisawa, K. (2001). A novel action of alzheimer's amyloid β-protein (Aβ): oligomeric Aβ promotes lipid release. J. Neurosci. *21*, 7226–7235.

Mitroi, D.N., Karunakaran, I., Graler, M., Saba, J.D., Ehninger, D., Ledesma, M.D., and van Echten-Deckert, G. (2017). SGPL1 (sphingosine phosphate lyase 1) modulates neuronal

autophagy via phosphatidylethanolamine production. Autophagy *13*, 885–899.

Mizushima, N. and Komatsu, M. (2011). Autophagy: renovation of cells and tissues. Cell *147*, 728–741.

Mizushima, N. and Yoshimori, T. (2007). How to interpret LC3 immunoblotting. Autophagy *3*, 542–545.

Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008). Autophagy fights disease through cellular self-digestion. Nature *451*, 1069–1075.

Moruno Manchon, J.F., Uzor, N.E., Dabaghian, Y., Furr-Stimming, E.E., Finkbeiner, S., and Tsvetkov, A.S. (2015). Cytoplasmic sphingosine-1-phosphate pathway modulates neuronal autophagy. Sci. Rep. *5*, 15213.

Motori, E., Puyal, J., Toni, N., Ghanem, A., Angeloni, C., Malaguti, M., Cantelli-Forti, G., Berninger, B., Conzelmann, K.K., Gotz, M., et al. (2013). Inflammation-induced alteration of astrocyte mitochondrial dynamics requires autophagy for mitochondrial network maintenance. Cell Metab. *18*, 844–859.

Nagaoka, U., Kim, K., Jana, N.R., Doi, H., Maruyama, M., Mitsui, K., Oyama, F., and Nukina, N. (2004). Increased expression of p62 in expanded polyglutamine-expressing cells and its association with polyglutamine inclusions. J. Neurochem. *91*, 57–68.

Nixon, R.A. (2013). The role of autophagy in neurodegenerative disease. Nat. Med. *19*, 983–997.

Noack, J., Choi, J., Richter, K., Kopp-Schneider, A., and Regnier-Vigouroux, A. (2014). A sphingosine kinase inhibitor combined with temozolomide induces glioblastoma cell death through accumulation of dihydrosphingosine and dihydroceramide, endoplasmic reticulum stress and autophagy. Cell Death Dis. *5*, e1425.

Okada, S. and O'Brien, J.S. (1968). Generalized gangliosidosis: β-galactosidase deficiency. Science *160*, 1002–1004.

Olivera, A. and Spiegel, S. (1993). Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature *365*, 557–560.

Olsen, A.S.B. and Faergeman, N.J. (2017). Sphingolipids: membrane microdomains in brain development, function and neurological diseases. Open Biol. *7*, 170069.

Ong, W.Y., Herr, D.R., Farooqui, T., Ling, E.A., and Farooqui, A.A. (2015). Role of sphingomyelinases in neurological disorders. Expert. Opin. Ther. Targets *19*, 1725–1742.

Ordonez, M.P., Roberts, E.A., Kidwell, C.U., Yuan, S.H., Plaisted, W.C., and Goldstein, L.S. (2012). Disruption and therapeutic rescue of autophagy in a human neuronal model of Niemann Pick type C1. Hum. Mol. Genet. *21*, 2651–2662.

Ordonez-Gutierrez, L., Benito-Cuesta, I., Abad, J.L., Casas, J., Fabrias, G., and Wandosell, F. (2018). Dihydroceramide desaturase 1 inhibitors reduce amyloid-β levels in primary neurons from an alzheimer's disease transgenic model. Pharm. Res. *35*, 49.

Osellame, L.D., Rahim, A.A., Hargreaves, I.P., Gegg, M.E., Richard-Londt, A., Brandner, S., Waddington, S.N., Schapira, A.H., and Duchen, M.R. (2013). Mitochondria and quality control defects in a mouse model of Gaucher disease – links to Parkinson's disease. Cell Metab. *17*, 941–953.

Oskouian, B. and Saba, J.D. (2010). Cancer treatment strategies targeting sphingolipid metabolism. Adv. Exp. Med. Biol. *688*, 185–205.

Pacheco, C.D. and Lieberman, A.P. (2008). The pathogenesis of Niemann-Pick type C disease: a role for autophagy? Expert. Rev. Mol. Med. *10*, e26.

Pankiv, S., Clausen, T.H., Lamark, T., Brech, A., Bruun, J.A., Outzen, H., Overvatn, A., Bjorkoy, G., and Johansen, T. (2007). p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J. Biol. Chem. *282*, 24131–24145.

Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X.H., Mizushima, N., Packer, M., Schneider, M.D., and Levine, B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell *122*, 927–939.

Pattingre, S., Bauvy, C., Carpentier, S., Levade, T., Levine, B., and Codogno, P. (2009). Role of JNK1-dependent Bcl-2 phosphorylation in ceramide-induced macroautophagy. J. Biol. Chem. *284*, 2719–2728.

Platt, F.M., Boland, B., and van der Spoel, A.C. (2012). The cell biology of disease: lysosomal storage disorders: the cellular impact of lysosomal dysfunction. J. Cell Biol. *199*, 723–734.

Rahmann, H. (1995). Brain gangliosides and memory formation. Behav. Brain Res. *66*, 105–116.

Ravikumar, B., Vacher, C., Berger, Z., Davies, J.E., Luo, S., Oroz, L.G., Scaravilli, F., Easton, D.F., Duden, R., O'Kane, C.J., et al. (2004). Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat. Genet. *36*, 585–595.

Rockenfeller, P., Koska, M., Pietrocola, F., Minois, N., Knittelfelder, O., Sica, V., Franz, J., Carmona-Gutierrez, D., Kroemer, G., and Madeo, F. (2015). Phosphatidylethanolamine positively regulates autophagy and longevity. Cell Death Differ. *22*, 499–508.

Rodriguez-Cuenca, S., Barbarroja, N., and Vidal-Puig, A. (2015). Dihydroceramide desaturase 1, the gatekeeper of ceramide induced lipotoxicity. Biochim. Biophys. Acta *1851*, 40–50.

Saitsu, H., Nishimura, T., Muramatsu, K., Kodera, H., Kumada, S., Sugai, K., Kasai-Yoshida, E., Sawaura, N., Nishida, H., Hoshino, A., et al. (2013). *De novo* mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat. Genet. *45*, 445–449, 449e441.

Scarlatti, F., Bauvy, C., Ventruti, A., Sala, G., Cluzeaud, F., Vandewalle, A., Ghidoni, R., and Codogno, P. (2004). Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of beclin 1. J. Biol. Chem. *279*, 18384–18391.

Schneider, J.S., Sendek, S., Daskalakis, C., and Cambi, F. (2010). GM1 ganglioside in Parkinson's disease: results of a five year open study. J. Neurol. Sci. *292*, 45–51.

Schubert, K.M., Scheid, M.P., and Duronio, V. (2000). Ceramide inhibits protein kinase B/Akt by promoting dephosphorylation of serine 473. J. Biol. Chem. *275*, 13330–13335.

Shen, W., Henry, A.G., Paumier, K.L., Li, L., Mou, K., Dunlop, J., Berger, Z., and Hirst, W.D. (2014). Inhibition of glucosylceramide synthase stimulates autophagy flux in neurons. J. Neurochem. *129*, 884–894.

Sheng, R., Zhang, T.T., Felice, V.D., Qin, T., Qin, Z.H., Smith, C.D., Sapp, E., Difiglia, M., and Waeber, C. (2014). Preconditioning stimuli induce autophagy via sphingosine kinase 2 in mouse cortical neurons. J. Biol. Chem. *289*, 20845–20857.

Siddique, M.M., Li, Y., Wang, L., Ching, J., Mal, M., Ilkayeva, O., Wu, Y.J., Bay, B.H., and Summers, S.A. (2013). Ablation of dihydroceramide desaturase 1, a therapeutic target for the treatment of metabolic diseases, simultaneously stimulates anabolic and catabolic signaling. Mol. Cell Biol. *33*, 2353–2369.

Signorelli, P., Munoz-Olaya, J.M., Gagliostro, V., Casas, J., Ghidoni, R., and Fabrias, G. (2009). Dihydroceramide intracellular increase in response to resveratrol treatment mediates autophagy in gastric cancer cells. Cancer Lett. *282*, 238–243.

Simons, K. and Gerl, M.J. (2010). Revitalizing membrane rafts: new tools and insights. Nat. Rev. Mol. Cell Biol. *11*, 688–699.

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M., and Czaja, M.J. (2009). Autophagy regulates lipid metabolism. Nature *458*, 1131–1135.

Smith, C.M., Mayer, J.A., and Duncan, I.D. (2013). Autophagy promotes oligodendrocyte survival and function following dysmyelination in a long-lived myelin mutant. J. Neurosci. *33*, 8088–8100.

Sonnino, S. and Chigorno, V. (2000). Ganglioside molecular species containing C18- and C20-sphingosine in mammalian nervous tissues and neuronal cell cultures. Biochim. Biophys. Acta *1469*, 63–77.

Spiegel, S. and Milstien, S. (2003). Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat. Rev. Mol. Cell Biol. *4*, 397–407.

Spilman, P., Podlutskaya, N., Hart, M.J., Debnath, J., Gorostiza, O., Bredesen, D., Richardson, A., Strong, R., and Galvan, V. (2010). Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-β levels in a mouse model of Alzheimer's disease. PLoS One *5*, e9979.

Stadelmann, C., Deckwerth, T.L., Srinivasan, A., Bancher, C., Bruck, W., Jellinger, K., and Lassmann, H. (1999). Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. Am. J. Pathol. *155*, 1459–1466.

Stoffel, W. (1999). Functional analysis of acid and neutral sphingomyelinases *in vitro* and *in vivo*. Chem. Phys. Lipids *102*, 107–121.

Suzuki, K. and Kamoshita, S. (1969). Chemical pathology of G-m-1-gangliosidosis (generalized gangliosidosis). J. Neuropathol. Exp. Neurol. *28*, 25–73.

Svennerholm, L. (1963). Chromatographic separation of human brain gangliosides. J. Neurochem. *10*, 613–623.

Svennerholm, L. (1994). Gangliosides – a new therapeutic agent against stroke and Alzheimer's disease. Life Sci. *55*, 2125–2134.

Svennerholm, L., Brane, G., Karlsson, I., Lekman, A., Ramstrom, I., and Wikkelso, C. (2002). Alzheimer disease – effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. Dement. Geriatr. Cogn Disord. *14*, 128–136.

Takamura, A., Higaki, K., Kajimaki, K., Otsuka, S., Ninomiya, H., Matsuda, J., Ohno, K., Suzuki, Y., and Nanba, E. (2008). Enhanced autophagy and mitochondrial aberrations in murine G(M1)-gangliosidosis. Biochem. Biophys. Res. Commun. *367*, 616–622.

Taniguchi, M., Kitatani, K., Kondo, T., Hashimoto-Nishimura, M., Asano, S., Hayashi, A., Mitsutake, S., Igarashi, Y., Umehara, H., Takeya, H., et al. (2012). Regulation of autophagy and its associated cell death by "sphingolipid rheostat": reciprocal role of ceramide and sphingosine 1-phosphate in the mammalian target of rapamycin pathway. J. Biol. Chem. *287*, 39898–39910.

van Echten-Deckert, G. and Herget, T. (2006). Sphingolipid metabolism in neural cells. Biochim. Biophys. Acta *1758*, 1978–1994.

van Echten-Deckert, G. and Walter, J. (2012). Sphingolipids: critical players in Alzheimer's disease. Prog. Lipid. Res. *51*, 378–393.

Ward, C., Martinez-Lopez, N., Otten, E.G., Carroll, B., Maetzel, D., Singh, R., Sarkar, S., and Korolchuk, V.I. (2016). Autophagy, lipophagy and lysosomal lipid storage disorders. Biochim. Biophys. Acta *1861*, 269–284.

Willis, S.N. and Adams, J.M. (2005). Life in the balance: how BH3-only proteins induce apoptosis. Curr. Opin. Cell Biol. *17*, 617–625.

Wu, G., Lu, Z.H., Kulkarni, N., and Ledeen, R.W. (2012). Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. J. Neurosci. Res. *90*, 1997–2008.

Wu, B.X., Fan, J., Boyer, N.P., Jenkins, R.W., Koutalos, Y., Hannun, Y.A., and Crosson, C.E. (2015). Lack of acid sphingomyelinase induces age-related retinal degeneration. PLoS One *10*, e0133032.

Yamashita, T., Wada, R., Sasaki, T., Deng, C., Bierfreund, U., Sandhoff, K., and Proia, R.L. (1999). A vital role for glycosphingolipid synthesis during development and differentiation. Proc. Natl. Acad. Sci. USA *96*, 9142–9147.

Yang, Z. and Klionsky, D.J. (2010). Mammalian autophagy: core molecular machinery and signaling regulation. Curr. Opin. Cell Biol. *22*, 124–131.

Young, M.M., Kester, M., and Wang, H.G. (2013). Sphingolipids: regulators of crosstalk between apoptosis and autophagy. J. Lipid. Res. *54*, 5–19.

Young, M.M., Takahashi, Y., Fox, T.E., Yun, J.K., Kester, M., and Wang, H.G. (2016). Sphingosine kinase 1 cooperates with autophagy to maintain endocytic membrane trafficking. Cell Rep. *17*, 1532–1545.

Zatloukal, K., Stumptner, C., Fuchsbichler, A., Heid, H., Schnoelzer, M., Kenner, L., Kleinert, R., Prinz, M., Aguzzi, A., and Denk, H. (2002). p62 Is a common component of cytoplasmic inclusions in protein aggregation diseases. Am. J. Pathol. *160*, 255–263.

Zhang, K., Pompey, J.M., Hsu, F.F., Key, P., Bandhuvula, P., Saba, J.D., Turk, J., and Beverley, S.M. (2007). Redirection of sphingolipid metabolism toward *de novo* synthesis of ethanolamine in Leishmania. EMBO J. *26*, 1094–1104.

Zhang, Y., Li, X., Becker, K.A., and Gulbins, E. (2009). Ceramide-enriched membrane domains – structure and function. Biochim. Biophys. Acta *1788*, 178–183.

Zheng, W., Kollmeyer, J., Symolon, H., Momin, A., Munter, E., Wang, E., Kelly, S., Allegood, J.C., Liu, Y., Peng, Q., et al. (2006). Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. Biochim. Biophys. Acta *1758*, 1864–1884.

Zhu, Y., Yang, J., Jiao, S., and Ji, T. (2013). Ganglioside-monosialic acid (GM1) prevents oxaliplatin-induced peripheral neurotoxicity in patients with gastrointestinal tumors. World J. Surg. Oncol. *11*, 19.

Zhu, W., Wang, X., Zhou, Y., and Wang, H. (2014). C2-ceramide induces cell death and protective autophagy in head and neck squamous cell carcinoma cells. Int. J. Mol. Sci. *15*, 3336–3355.