

Review

The pleiotropic roles of sphingolipid signaling in autophagy

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The autophagic process involves encompassing damaged proteins and organelles within double- or multi-membraned structures and delivering these molecules to the lytic compartments of vacuoles. Sphingolipids (SLs), which are ubiquitous membrane lipids in eukaryotes, participate in the generation of various membrane structures, including rafts, caveolae, and cytosolic vesicles. SLs are a complex family of molecules that have a growing number of members, including ceramide, sphingosine-1-phosphate, and dihydroceramide, which have been associated with the essential cellular process of autophagy. This review highlights recent studies focusing on the regulation and function of SL-associated autophagy and its role in cell fate, diseases, and therapeutic interventions.

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Subject Category: Cancer Metabolism

Facts

- Autophagy is generally considered to be a cell survival mechanism and may also contribute to cell death, depending on its various biological contexts.
- Sphingolipids were first discovered in brain extracts in 1876, and within a century of intensive research, the chemical structures of thousands of individual sphingolipids had been elucidated.
- Sphingolipid-associated autophagy contributes to a range of diseases, including neurodegeneration, tumors, metabolic disorders, and heart diseases.

- Sphingolipid-mediated signals that have long been known to induce apoptosis are now also recognized to cause autophagy. Are sphingolipid-associated apoptosis and autophagy interlinked?
- How does sphingolipid-associated autophagy occur and to what extent do alterations in autophagy contribute to disease phenotype?
- What drugs or compounds that target sphingolipid metabolism are attractive candidates for therapeutic development?

Open Questions

- Sphingolipids are abundant lipid components of eukaryotic plasma membranes that function in a wide range of biological processes. Are sphingolipids involved in the composition of autophagic membranes?
- Why do perturbations of sphingolipid metabolism by chemotherapy and nutrition starvation trigger lethal autophagy and protective autophagy, respectively?

Autophagy is generally considered to be a cell survival mechanism, and this process also contributes to cell death in several situations.¹ Sphingolipids (SLs) are ubiquitous components of membrane structures, and renewed interest in SLs has focused on SL-induced intracellular and extracellular signaling.² Accumulating evidence indicates that a direct link exists between SL metabolism and autophagy. Moreover, studies have uncovered that a dynamic balance among SL metabolites is significant in cell fate determination.^{3,4} Here we review the key aspects of bioactive SLs that have emerged as important effectors in regulating the autophagic pathway, mediating the cross talk between apoptosis and autophagy,

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Abbreviations: 4-HPR, 4-hydroxy (phenyl) retinamide; aCDase, acid ceramidase; Atg, autophagy-related gene; CDase, ceramidase; Cer, ceramide; CerS, Cer synthase; dhCer, dihydroceramide; ER, endoplasmic reticulum; GCase, glucocerebrosidase; LC3, microtubule-associated protein light chain 3; mTOR, mammalian target of rapamycin; NPC, Niemann-Pick disease type C; PI3K, phosphatidylinositol 3-kinase; S1P, sphingosine-1-phosphate; SL, sphingolipid; SMase, sphingomyelinase; SphK, sphingosine kinase

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and determining the associated cell fate. A deeper understanding of the relationship between SL metabolism and autophagy explains how intact or impaired SL-related autophagic pathways are involved in the malfunctions associated with neurodegeneration, cancer, and other diseases. We also discuss how autophagy-regulating drugs work via cells' SL metabolism and the methods that could be used to monitor SL-related autophagy. Present and future investigations regarding SL-related autophagy will help to develop novel treatment strategies to control autophagy-related diseases.

The SL Family

SLs represent a significant class of lipids that contain a backbone of sphingoid bases and are ubiquitous constituents of membranes in eukaryotes. SLs were first discovered in brain extracts in 1876,³ and within a century of intensive research, the chemical structures of thousands of individual SLs had been elucidated.⁴ The SL metabolic pathway displays an intricate network of reactions that result in the formation of multiple SLs, including ceramide (Cer), dihydroceramide (dhCer), and sphingosine-1-phosphate (S1P).⁵

SLs are produced in at least three distinct ways (Figure 1). First, a substantial portion of SLs are derived from *de novo* biosynthesis in most organisms,^{2,6} with the condensation of serine and palmitoyl-CoA catalyzed by serine palmitoyl transferase to generate dehydrosphinganine. Dehydrosphinganine is subsequently reduced to form dihydrosphingosine (sphinganine), which is then *N*-acylated by dhCer synthase to produce dhCer or Cer. Second, through hydrolysis via a

complex lipid-turnover pathway,^{2,6} several specific hydrolases are involved in the turnover process, including sphingomyelinases (SMases) and glucocerebrosidase (GCase). In addition to the above two sources, Cer is also recycled.^{6,7} In this pathway, sphingosine is recycled into Cer via Cer synthases (CerS); therefore, this pathway is also called the salvage pathway. The cellular metabolic homeostasis of the sphingolipidome is often achieved through the coordination of the biosynthesis and removal of SL species, which requires a delicate balance between *de novo* biosynthesis, turnover, and recycling.⁸

SLs are found in cellular membranes, lipoproteins, and other lipid-rich structures; however, they are synthesized in the endoplasmic reticulum (ER) and Golgi apparatus. The scheme in Figure 1 depicts the subcellular localization of SL biosynthesis, turnover, and recycling. In addition, SLs may be incorporated into other intracellular compartments, such as mitochondria or autophagosomes.³ The turnover and recycling of SLs also occur at several intracellular locations, including the endosome, phagosome, and lysosome.³ Thus, SLs travel between organelles, and this transport occurs via either transport vesicles or transfer proteins.⁹ CERT (Cer transfer protein) and FAPP2 (four-phosphate adaptor protein 2) have proved to be two critical SL-trafficking proteins that regulate the trafficking of SLs to specific compartments within cells.^{10,11} Lipids are increasingly implicated in the control of the membrane remodeling and vesicle transport that underlie the biogenesis of autophagosomes.¹² However, our knowledge of whether and how SL trafficking is associated with the autophagic process remains incomplete.

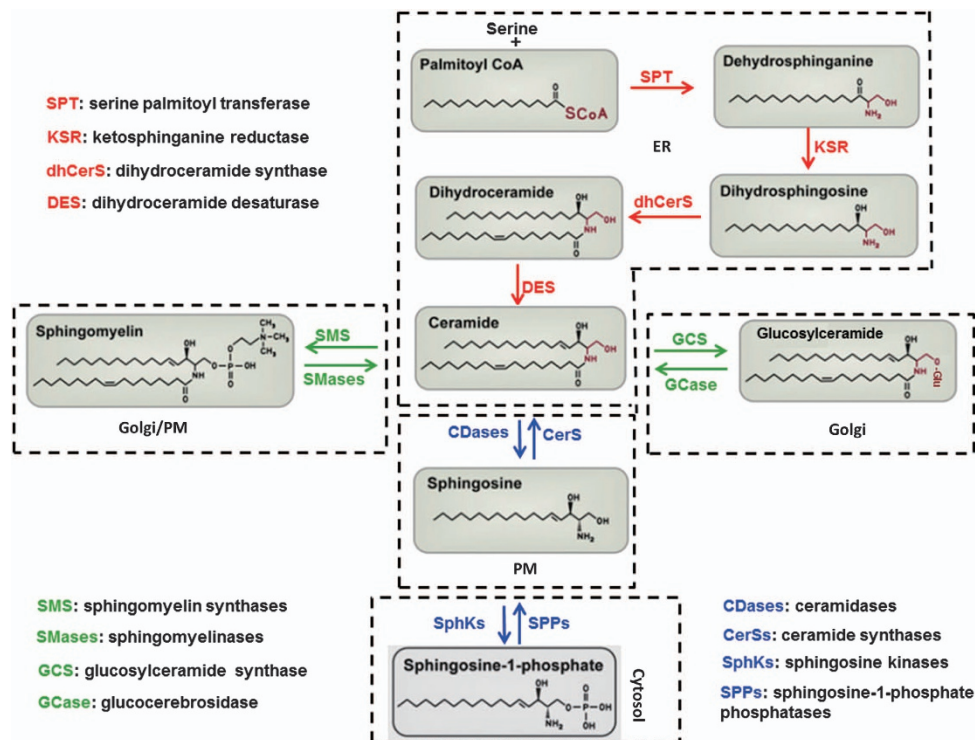


Figure 1 Cer formation through the *de novo*, turnover, or salvage pathway. Red arrows: *de novo* pathway; green arrows: turnover pathway; blue arrows: salvage pathway. PM, plasma membrane

SLs are considered to primarily have roles as components of membranes and other biological structures. However, several SL metabolites, including Cer, dhCer, and S1P, have drawn attention as bioactive signaling molecules that mediate cell growth, differentiation, senescence, apoptosis, and autophagy.^{4,5}

SLs Involved in the Autophagic Pathway

'Autophagy,' which is derived from Greek and means 'to eat oneself,' ensures the synthesis, degradation, and recycling of cellular components in eukaryotic cells ranging from yeasts to mammals.^{13–15} At present, the following three modes of autophagy have been identified: macroautophagy (which is commonly called 'autophagy,' including in this review), microautophagy, and chaperone-mediated autophagy. During the autophagic process, nonspecific or targeted cytoplasmic constituents are delivered to and degraded in the lysosome via these autophagic pathways.^{16,17} The formation of the autophagosome is a multistep process that includes the biogenesis of the isolation membrane, followed by its elongation and closure.¹⁸ Most autophagy-related genes (Atgs) contribute to autophagosome formation, and many are well conserved from yeasts to mammals.^{19,20}

SLs are abundant lipid components of eukaryotic plasma membranes that function in a wide range of biological processes,^{21,22} which make SLs strong candidates as possible autophagosomal lipids. Several studies have implied that SLs, and especially Cer, are autophagosomal membrane components.^{23,24} In addition, SLs formed by *de novo* biosynthesis in the ER might be a driving force for the formation of the autophagosomal vacuole, in what has been referred to as the 'membrane extension' step,²⁵ which occurs after many of the associated autophagosomal proteins have been recruited. Because the enzymes required for *de novo* Cer biosynthesis reside in the ER,²⁶ it is possible that they might be recruited into autophagosomes and perhaps continue to produce SLs there.

The best-characterized pathway regulating autophagy includes a class I phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR), which act to inhibit autophagy. Although how autophagy is suppressed by these molecular signals remains to be revealed, mTOR complex 1 (mTORC1) is known to phosphorylate the autophagy regulatory complex, ULK1 (containing unc-51-like kinase 1), the mammalian Atg13 protein, and focal adhesion kinase-interacting protein of 200 kD (FIP200).^{27,28} A class III PI3K is needed for the activation of autophagy.^{4,16} SLs participate in cell survival and cell death signaling pathways.^{29,30} Complex autophagy regulation might be mediated by SLs or SL metabolism. Indeed, different bioactive SL species have been shown to mediate distinct autophagic pathways, described as protective autophagy and autophagy-associated cell death (Figure 2), which have opposing functions in cellular life-or-death decisions.^{3,4,31}

Cer, which is known to induce cell cycle arrest and has been implicated in important physiological roles in cell differentiation, senescence, migration, adhesion, and inflammatory responses, is the key intermediate in SL metabolism.^{6,32} Exogenous Cer-related autophagy was shown to trigger

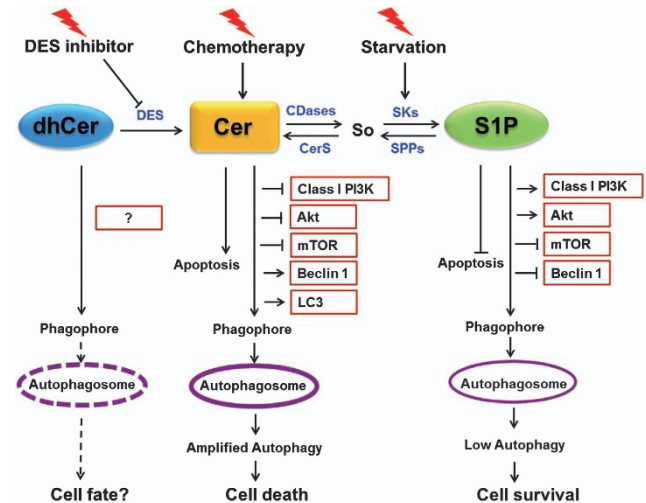


Figure 2 Model of SL-related autophagy and its consequences on cell fate. CerSs, ceramide synthases; DES, dihydroceramide desaturase; So, sphingosine; SPPs, sphingosine-1-phosphate phosphatases

autophagy-associated cell death, which occurs in several malignant cell types, including colon cancer, breast cancer, cervical cancer, nasopharyngeal cancer, and glioma cells.^{3,33–37} In addition to exogenous Cer, endogenous Cer species are critical for the induction of autophagy. Tamoxifen, an estrogen receptor antagonist used to treat several types of breast cancer, has been shown to induce autophagy-associated cell death by increasing endogenous Cer levels.³³ Exogenous or endogenous Cer induces autophagy through several mechanisms. For example, Cer has been found to stimulate autophagy by regulating classic or atypical autophagic pathways and signals.⁴ Via these signals, class I PI3K and Akt negatively regulate autophagy, but a class III PI3K is needed for the activation of autophagy. Cer was found to promote the interaction of class III PI3K with other regulators of autophagy³⁸ and to inhibit Akt by activating phosphoprotein phosphatase 2A.^{33,39,40} Moreover, exogenously added Cer and the accumulation of endogenous Cer due to treatment with certain chemotherapeutic drugs, such as tamoxifen, inhibit the mTOR signaling pathway,³³ which has a central role in inducing autophagy and increasing the expression of Beclin1,³³ an upstream regulator of autophagy.⁴¹ Simultaneously, Cer promotes the dissociation of Beclin1 from the Beclin1/Bcl-2 complex.^{34,42,43} A short-chain Cer analog has also been associated with the induction of autophagy-associated cell death by increasing the transcription of the BH3-only protein, a mitochondria-associated protein that induces nonapoptotic cell death.³⁵ A recent study showed that Cer directly interacts with microtubule-associated protein light chain 3 (LC3) on mitochondrial membranes to induce deadly autophagy via an increase in intracellular mitophagy.⁴⁴

S1P has emerged as a cell-proliferative lipid messenger. S1P has been found to induce survival-mediated or protective autophagy under nutrient starvation, distinct from Cer-associated autophagy-associated cell death.^{45,46} In contrast to Cer-related autophagy, S1P-mediated autophagy has not been shown to be related to the accumulation of Beclin1 protein or to the suppression of class I PI3K or Akt.⁴⁵

However, mTOR was still inhibited by increased S1P levels,^{45–47} which suggests that S1P induces autophagy via inhibiting mTOR and is independent of the class I PI3K signal.

dhCer has been used as a negative control for Cer treatments to induce autophagy^{33,34,48} because dhCer has long been thought to be biologically inactive. However, dhCer was recently identified as a novel SL-based mediator of autophagy.^{3,49,50} dhCer induces both protective autophagy and autophagy-associated cell death. Endogenously added dhCer induced a transient, early increase in dhCer levels via inhibition of dhCer desaturase, such as XM462, which successively promoted autophagy and reduced etoposide toxicity in gastric and colon cancer cells.^{49,51} However, anticancer agents such as fenretinide (4-hydroxy (phenyl) retinamide or 4-HPR)⁵⁰ and gamma tocotrienol (a lipophilic antioxidant of vitamin E)⁵² induced cancer cell death through the elevation of intracellular dhCer levels. Interestingly, none of the reported Cer-mediated autophagic pathways is induced by both Cer and dhCer.³³ Intriguingly, the accumulation of dhCer may serve as an additional 'switch' to regulate cell fate; however, the biological activity of dhCer remains controversial and unclear.

In addition to the major SLs that induce autophagy, gangliosides and several rare SLs are also involved in autophagy-related cell fate decisions. Gangliosides induce autophagy-associated cell death in both isolated astrocytes and astrocytoma cells.⁵³ An increase in the amount of LC3-II and an accumulation of autophagic vacuoles were observed in cells that underwent ganglioside treatment.^{53,54} Cer methylaminoethyl phosphonate and sphingadienes are two rare SLs that promote autophagy-associated cell death through the downregulation of PI3K/Akt and the activation of Beclin1, similar to Cer.^{55,56}

Overall, although Cer, dhCer, and S1P are all able to induce autophagy, the effects of S1P-mediated autophagy are markedly mild compared with the effects elicited by Cer. dhCer-related autophagic effects are moderate, and their intensity level lies between the levels caused by Cer and S1P. Treatment with chemotherapeutic agents often promotes the biosynthesis of intracellular Cer and leads to autophagy-associated cell death; however, elevation in S1P levels is typically a cell response to nutrition starvation and mediates cytoprotective autophagy. Furthermore, cells maintain a dynamic equilibrium between the levels and the effects of Cer, dhCer, and S1P. The conversion of Cer to S1P simultaneously accumulates the survival effects and removes the death signals. This observation has led to the concept of a so-called 'SL rheostat' or 'SL biostat,' based on the relative amounts and reciprocal roles of these antagonistic metabolites, which are critical in guiding the destiny of cells.^{12,57,58}

Possible SL Involvement in the Cross Talk Between Autophagy and Apoptosis

Apoptosis and autophagy have been recognized as two principal forms of programmed cell death. Apoptosis generally initiates cell death, whereas autophagy is primarily a protective process for the cell that may also contribute to cell death.⁵⁹ There is a sophisticated and not yet fully understood association between autophagy-related cell death and

survival that depends on various biological situations.¹ In certain cellular contexts, autophagy functions as a stress response to suppress apoptosis and promote cell survival.^{16,60} However, in other cases, autophagy may serve as a mechanism for caspase-dependent or -independent cell death.^{61,62}

Certain SLs are now thought to elicit autophagy; however, they have also been known to induce apoptosis. Cer is a well-established inducer of apoptosis via activation of the mitochondrial pathway. In a number of different cell types, increased Cer levels arrest cell growth and promote cell apoptosis.^{63–66} In contrast to Cer, S1P facilitates cell survival and suppresses cell apoptosis.^{67–69} dhCer has also been suggested to exhibit antiapoptotic effects because the lipid inhibits Cer channel assembly in isolated mitochondria.⁷⁰ In addition, other SL metabolites, such as sphingosine and ganglioside, are involved in the modulation of apoptosis and autophagy.⁷¹ SL-associated apoptosis and autophagy are interlinked. The conversion of Cer to S1P switches the cell fate from apoptosis to autophagy-induced survival.^{57,58} Several exogenous stimuli, and particularly the activation of the enzymes that interconvert Cer and S1P, such as sphingosine kinases, lead to an increase in S1P levels, a corresponding reduction in Cer levels, and the antagonistic effects of Cer.⁶⁸ In addition, dhCer has been proposed to exhibit antiapoptotic effects to promote cell survival during hypoxia through the induction of autophagy, while also serving as a lipid reserve for the rapid production of Cer to address cellular damage on reperfusion.⁷² dhCer serves as a unique regulator of cell fate, controlling the 'switch' between cytoprotective autophagy and Cer-mediated apoptosis in response to stress. Intriguingly, Cer promotes both apoptosis and autophagy through the inhibition of class I PI3K and Akt signaling or the mTOR pathway.^{33,40,73,74} Furthermore, suppressors of apoptosis, such as the Bcl-2 family, also directly bind and control Beclin1.⁷⁵ Disruption of the Beclin1–Bcl-2 complex has also emerged as a common mechanism in Cer-associated autophagy.⁷¹ In addition to altering the balance between Beclin1 and Bcl-2 protein levels, endogenous Cer liberates Beclin1 for autophagy induction through the JNK-mediated phosphorylation of Bcl-2.³⁴ Notably, Bcl-2 has emerged as a critical regulator of the Cer-mediated cross talk between autophagy and apoptosis.

SL-Associated Autophagy and Diseases

Although the primary function of autophagy is to aid in adaptation to cellular stress under adverse conditions by enabling cells to degrade cytosolic proteins and organelles to generate a supply of essential nutrients, autophagy has also emerged in the pathological process of many disorders, including neurodegeneration, tumors, immunity responses, and heart diseases.¹⁶ SL metabolism-associated autophagy also contributes to a range of diseases.³¹

Sphingolipidoses are a collection of more than 40 genetically distinct disorders caused by inherited deficiencies of lysosomal hydrolytic activities or lipid transport. These deficiencies result in intracellular accumulations of cholesterol and lipids in the endosomal/lysosomal network, usually leading to signs of neurodegeneration.^{76,77} The biochemical

abnormalities in sphingolipidoses are complex and lead to the accumulation of SL metabolites by diverse pathways (Table 1).^{78,79}

Niemann–Pick disease type C (NPC) is a complex neurodegenerative sphingolipidosis characterized by the accumulation of unesterified cholesterol, SLs, and complex gangliosides in late endosomes and lysosomes.⁸⁰ The disease is caused by mutations in either the *NPC1* or the *NPC2* gene,^{81,82} which disturbs not only the regular transport of endocytosed lipids but also the autophagic flux, leading to an accumulation of autophagic vacuoles in cells.^{83,84} *NPC1* and *NPC2*^{81,82} are genes whose protein products mediate proper intracellular lipid transport through pathways that are incompletely understood. The brains of afflicted humans and NPC-deficient animal models are marked by a loss of neurons.^{85,86} Histological studies and cell culture experiments have shown that defective lysosomal degradation of autophagosomes may contribute to the abnormal autophagic flux.^{84,87} The deficiency present in NPC promotes both the generation of autophagosomes and the impairment of autophagic flux. An imbalance between induction and flux through the autophagic pathway contributes to cell stress and neuronal loss in NPC.⁷⁷

Gaucher disease (GD), a kind of sphingolipidosis, is characterized by the accumulation of glucosylceramide or glucosylsphingosine in the lysosomes of the cells of the monocyte/macrophage system.^{88,89} In GD, the point mutations within the *GBA* (glucosidase, beta, acid) gene lead to the production of acid β -glucosidase with functional, kinetic, trafficking, and/or stability defects and a resultant decrease in lysosomal function and increase in the accumulation of glucosylceramide and glucosylsphingosine.⁷⁶ GD can be classed into three subsets, based on the age of onset and the presence of central nervous system abnormalities. Type 1 is known as the non-neuronopathic form, and types 2 and 3 are differentiated from type 1 by neurodegeneration of the central nervous system with either rapid or chronic progression. Vaccaro *et al.*⁸⁹ reported that mutation of saposin (Sap) C, and not a direct *GBA* gene mutation, was associated with the type 3 or type 1 phenotype in cases of GD. The decrease in/absence of Sap C affected GCase intracellular localization, resulting in lysosomal lipid accumulation and enhanced

autophagy. This study suggested that Sap C might have a role in intralysosomal SL transport, as indicated by the accumulation of glucosylceramide and Cer in the lysosomes of the Sap C-mutant cells. In addition, deficient degradation of autophagic substrates in cells in GD can lead to an increased risk of Parkinson disease.⁹⁰ For NPC, GD, and other sphingolipidoses,^{76,91} the relevance of the impairment of autophagic flux to disease pathogenesis remains poorly defined. In addition, the extent to which the accumulation of autophagic substrates contributes to neuron dysfunction needs to be determined in future studies.

Dysregulated SL metabolism occurs in numerous cancers and has been shown to contribute to cancer progression and chemoresistance.^{92,93} Several species of SLs have been shown to have aberrant expression or metabolism in cancer cells.⁹⁴ The tumor suppressor Cer and the tumor promoter S1P are generally recognized to trigger autophagy; however, Cer and S1P have different outcomes regarding cell death and survival.²⁹ Nevertheless, recent studies have suggested that *de novo*-generated Cer-associated autophagy can be lethal as well as protective for cells.⁹² For example, Beljansky *et al.*⁹⁵ determined that enhancement of Cer levels through treatment with a SphK 2-selective inhibitor suppresses tumor growth by leading to autophagy-associated cell death. Conversely, Park *et al.*⁹⁶ suggested that Cer-CD95-PERK signaling promotes cell death via the cascade activation of caspase proteins; however, gene silencing of *Atg5* further strengthened the cell death process, indicating that the autophagy was protective. The contradictory activities may correspond to variations among Cers in their carbon chain lengths, double bond numbers, subcellular distributions, and versatile targets. In addition, Cer is metabolized into S1P by SphK 1 or SphK 2, which are associated with protective autophagy and other biological behaviors, including survival, infiltration, angiogenesis, metastasis, and resistance to anticancer drugs in cancer cells.⁹²

Intact and impaired SL-related autophagy has significant implications for many other diseases and pathological processes. For example, CerS 5-mediated autophagy has recently been implicated in lipotoxic cardiomyopathy and hypertrophy.⁹⁷ In addition, Kdo 2-lipid A induced substantial alterations in SL metabolism and composition in RAW264.7 cells, a mouse macrophage-like cell line. These changes apparently promote the *de novo* SL biosynthesis that is required for autophagosome generation, which is suggested to have an essential role in the processes of the innate immune response.²⁴ Cer also mediates the augmentation of interleukin-1(IL-1) levels and the release of tumor necrosis factor (TNF) alpha that are induced by toll-like receptor 4, and this process may contribute to the enhanced inflammatory response in metabolic diseases, including obesity and diabetes, which are characterized by dyslipidemia.⁹⁸

Overall, the aberrations in the bioactive SLs that mediate autophagy have been associated with diverse pathological conditions, including neurodegeneration, carcinogenesis, metabolic diseases, and inflammatory responses. Understanding the specific mechanism connecting SL-related autophagy and these diseases has substantial implications for revealing these diseases' biochemical characteristics and for designing and developing new therapeutic strategies.

Table 1 SL-associated autophagy in SLs

Disease	Gene mutations	Accumulated SL substrates	Autophagic status	Ref
Niemann–Pick disease type C	<i>NPC1</i> <i>NPC2</i>	Sphingomyelin Glucosylceramide Lactosylceramide Sphingosine	Accumulated autophagosomes and ubiquitinated proteins; impaired autophagic flux	76–87
Gaucher disease	<i>GBA</i> <i>Sap C</i>	Glucosylceramide Glucosylsphingosine	Accumulated autophagosomes and autophagic substrates; deficient degradation of autolysosome cargo	76,88–91
Fabry disease	<i>GLA</i>	Globotriaosylceramide	Induction of autophagy; impaired autophagic flux	76,91

Abbreviations: ref, references; SL, sphingolipids

Compounds and Drugs that Might Trigger Autophagy-Associated Cell Death Through Regulation of SL Metabolism

Many human disease states may be caused by the aberrant regulation of SL metabolism-associated autophagy; therefore, drugs and compounds that target SL metabolism, including chemotherapeutic drugs, sphingomimetics, and enzyme inhibitors, are attractive candidates for therapeutic development (Table 2).

Many chemotherapeutic drugs elicit autophagy and exert their anticancer properties by increasing the intracellular

levels of SLs. A large number of malignant cells often acquire genetic deletions or mutations that render them resistant to the classic apoptotic cell death that is induced by many anticancer therapies, which ultimately results in poor prognosis.^{99,100} In these cases, the use of autophagy-inducing drugs offers an alternative way to induce cell death in apoptosis-resistant tumors.^{99,100} As reviewed in the present paper, one of the major results of the chemotherapy-mediated increase in the levels of intracellular SLs, such as Cer and dhCer, is cell death induction by autophagy in various human cancer cells.

Table 2 Summary of antitumor compounds that might regulate autophagy through SL metabolism

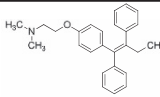
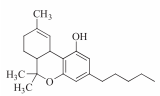
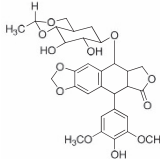
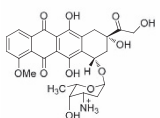
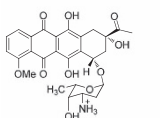
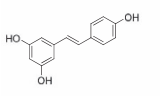
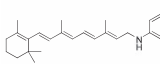
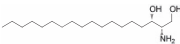
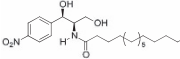
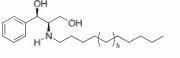
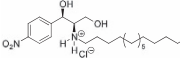
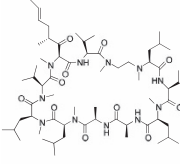
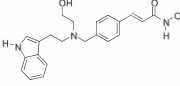
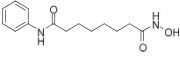
Category	Name	Structure	Targeted SLs or enzymes and related SL side chains	Autophagic status	Ref
Chemo therapy	Tamoxifen		Cer	Accumulated autophagic Vacuoles	33
	Arsenic trioxide	O=As-O-As=O	Cer	Increased autophagy-associated cell death	101, 102
	Δ^9 -Tetrahydrocannabinol		Cer	Increased number of autophagosomes	103
	Etoposide		SphK/Cer	Accumulated autophagosomes/ autolysosomes	61, 104
	Doxorubicin		SphK	Switch from protective autophagy to apoptosis	105
	Daunorubicin		SMase	Induction of a high level of protective autophagy	106, 107
	Resveratrol		dhCer	Accumulated vesicles with distinct double membranes	49, 108
4-HPR		dhCer	Increased numbers of lysosomes/ autophagosomes	52, 109–112	
			(C16-dhCer)		
			(C18-dhCer)		

Table 2 (Continued)

Sphingomimetics	Safingol		S1P	Increased presence of small and large single membrane-bound vesicles and double membrane-bound autophagosomes	115, 116
Enzyme inhibitors	B13		aCDase	ND	118, 123–124
	LCL385		aCDase	ND	118, 125
	LCL204		aCDase	ND	118, 126
	PSC 833		aCDase	Formation of acridine orange-stained acidic vesicles and an increase in LC3 expression	120
Histone deacetylase inhibitors	LAQ824		Cer	ND	130
	SAHA		Cer	Many huge vacuoles in the cytoplasm and the presence of double- or multiple-membraned vesicles; The larger vacuoles contain multiple cellular organelles	131

Abbreviations: ND, not determined; ref, reference; SL, sphingolipids

Autophagy is initiated by tamoxifen, an antagonist of the estrogen receptor, leading to an increase in endogenous Cer levels that acts against breast cancer cells.³³ Arsenic trioxide, a potent antineoplastic agent, has been used clinically for the treatment of certain types of leukemia. Arsenic trioxide was shown to induce the biosynthesis of Cer in both acute promyelocytic leukemia and adult T-cell leukemia cells, and the cytotoxic effects were attributed to the induction of both apoptosis and autophagy.^{101,102} Δ^9 -Tetrahydrocannabinol,

the major active component of marijuana, was reported to induce the activation of autophagy-mediated apoptotic cell death by increasing *de novo* Cer biosynthesis.¹⁰³ Moreover, SL metabolism may be involved in the regulation of autophagy by other chemotherapeutic drugs, including, but not limited to, etoposide,^{61,104} doxorubicin,¹⁰⁵ and daunorubicin.^{106,107}

Resveratrol is a natural polyphenolic phytoalexin produced by grapes and other berries that exerts its cancer-preventive properties in several animal models and exhibits its potent

anticancer activities in leukemia and many solid tumors. The capacity to trigger dhCer biosynthesis contributes to resveratrol-induced autophagy-associated cell death. Puissant *et al.*¹⁰⁸ found that resveratrol initiates autophagy in chronic myelogenous leukemia cells through the AMP-activated protein kinase (AMPK)-dependent induction of autophagy and that p62 is also required for resveratrol-mediated autophagy. Whether other polyphenolic compounds, such as curcumin, which has two phenolic hydroxyl groups; genistein, which has three phenolic hydroxyl groups; and quercetin, which has four phenolic hydroxyl groups, are implicated in SL metabolism and SL-mediated autophagy remains to be determined.

In clinical trials, 4-HPR has been encouraging as a chemopreventive and therapeutic agent for breast cancer.^{109,110} The promise of 4-HPR as a therapeutic agent is enhanced by its unique ability to activate the autophagy-associated cell death pathway when the apoptotic pathway is deregulated, which is a characteristic that is not shared by other chemotherapeutic agents, including cisplatin and etoposide.¹⁰⁹ Furthermore, the accumulation of dhCer following treatment with 4-HPR was found to induce cell cycle arrest in neuroblastoma cells.^{111,112}

SL metabolism is an exploitable target for the discovery of novel chemotherapeutics, and this target has been validated through the development of several sphingomimetics.^{8,113,114} Safingol, a synthetic sphinganine, triggers autophagy in several tumor cell types by the suppression of class I PI3K and Akt signaling.¹¹⁵ Safingol has been assessed in phase I clinical trials alone or in combination with cisplatin for adults with advanced solid tumors and for children with neuroblastoma. Safingol treatment was shown to trigger a dose-dependent decrease in S1P levels, and safingol is a promising representative drug whose primary mechanism for promoting tumor cell death is autophagy.¹¹⁶

Targeting enzymes in SL metabolism is another attractive avenue to provide therapeutic benefits in SL-related human diseases.^{117,118} Acid SMases (aSMases) and acid ceramidases (aCDases) are crucial enzymes that modulate the synthesis and degradation of Cer in SL metabolism.^{2,6} aCDase overexpression has been observed in cancer cell lines and primary tumors and contributes to resistance to chemotherapy and radiation.^{119–121} The consequence of aCDase overexpression is the ability to convert Cer, which is often produced as a proapoptotic response to stress, to sphingosine, which can then be converted to S1P.¹²² In addition to the ability to metabolize the Cer produced in response to stress, Turner *et al.*¹¹⁹ found that prostate cancer cell lines overexpressing aCDase also have an increased lysosomal density and increased levels of autophagy. Morad *et al.*¹²⁰ identified an analog of cyclosporin A, PSC 833 (Valspodar, Novartis Pharma AG, Basel, Switzerland), a second-generation, non-immunosuppressive P-glycoprotein antagonist that inhibits aCDase and generates autophagy-associated cytotoxicity in pancreatic cancer cells. B13 was found to be a potent CDase inhibitor that induced colon cancer cell death¹²³ and the inhibition of colon tumor growth in a xenograft model.¹²⁴ Furthermore, LCL385, a B13 analog, enhanced the sensitivity of prostate cancer cells to radiation and inhibited tumor growth in a nude mouse model.¹²⁵

Another novel B13 analog, LCL204, has been shown to overcome the resistance of head and neck squamous carcinoma cells to Fas-induced cell death in both *in vitro* and *in vivo* experiments.¹²⁶ Most aCDase inhibitors are lysosomotropic agents and cause lysosomal destabilization and a change in SL metabolism enzymes in the lysosomal compartment.¹¹⁸

aSMase hydrolyzes sphingomyelin into Cer and phosphorylcholine. For the most part, aSMase seems to reside in classic lysosomes, where it mediates the catabolism of sphingomyelin.¹¹⁷ aSMase activity is essential for lysosomal stability and the survival of cancer cells, as well as for the multidrug-resistant phenotype. Because of their ability to inhibit autophagic flux, certain aSMase inhibitors, such as chloroquine, siramesine, and clomipramine, are currently being tested as anticancer agents in several clinical trials and laboratory studies.¹²⁷ aSMase was also found to be a target for the treatment of Niemann–Pick disease type A (NPA), which is caused by loss-of-function mutations in the aSMase gene and is a lysosomal storage disorder leading to neurodegeneration. Fibroblasts from NPA patients and aSMase-knockout mouse brains show similar autophagolysosome accumulation and impaired autophagy.¹²⁸ A recent study has suggested that the control of lysosome trafficking and fusion by aSMase is essential to normal autophagic flux in coronary arterial smooth muscle cells and has a protective role in atherosclerosis.¹²⁹

Other chemotherapeutic treatments, such as several histone deacetylase inhibitors, were also demonstrated to lead to leukemic cell death by enhancing Cer production via the degradation of sphingomyelin.¹³⁰ One of these histone deacetylase inhibitors is suberoylanilide hydroxamic acid, which was demonstrated to trigger autophagy-associated cell death in apoptosis-deficient tumor cells, such as chondrosarcoma cells.¹³¹

Conclusion

Numerous well-developed and convenient experimental methods and techniques that can be used to detect autophagy in different species systems exist.^{132,133} To establish that an autophagic response is occurring in SL-related autophagy, multiple methods have been used, including cellular ultrastructure studies by transmission electron microscopy,^{132,133} an LC3-puncta formation assay combined with immunoblots for autophagic cargoes (for example, p62 and NBR1), and the detection of autophagy-related molecules.^{132,134} In addition, performing a large-scale and comprehensive sphingolipidomic analysis has been challenging in the study of SL-associated autophagy.^{135,136} Both nets and hooks are indispensable tools to allow efficient ‘fishing’ for multiple intermediates and products directly from the sophisticated process of SL metabolism.

Because there is an obvious overlap between the subcellular localization of enzymes needed for SL biosynthesis and autophagosome formation, SLs have been increasingly implicated in the composition of the phagophore and the autophagosomal membrane.

Autophagy-associated cell death and cell survival represent the so-called ‘Yin–Yang’ regulatory mechanism. The balance

has been exemplified by interconvertible SLs that produce opposite autophagic effects on cells that were termed as 'SL rheostat' or 'SL biostat'. In addition, the increase in intracellular Cer levels usually results from chemotherapy and often stimulates autophagy-associated cell death, although this increase has also been found to be cytoprotective. In contrast, starvation treatment triggers protective autophagy via increasing S1P levels. However, the underlying mechanisms by which chemotherapy and nutrient deprivation mediate different regulation are still largely unknown.

In addition to autophagy, SLs have long been identified to induce apoptosis. SLs mediate the cross talk between apoptosis and autophagy because certain SLs share several common kinase signaling pathways that regulate cell fate that affect both apoptosis and autophagy or execute a seesaw type of regulation of key molecules.

Increasing new knowledge on the biochemistry and cell biology of SL-mediated autophagy is beneficial for deepening our understanding of SL-associated diseases, including neurodegeneration, cancer pathogenesis, and inflammatory responses. An imbalance between induction of and flux through the autophagic pathway contributes to cell stress and neuronal loss in NPC. Intact and impaired SL-related autophagy has significant implications for the development of malignant tumors.

In addition, the current understanding of the effects of multiple SLs on the modulation of various autophagy-related cell fates has primarily arisen from exogenous SLs or pharmacological perturbations of SL metabolism. The identification of SL-mediated cytoprotective autophagy that can be shifted to cell death may provide a novel strategy for cancer therapy, including chemotherapy, sphingomimetics, SL metabolic enzyme inhibitors, and histone deacetylase inhibitors.

Conflict of Interest

The authors declare no conflict of interest.

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