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Ceramidases, roles in sphingolipid metabolism and in health and disease

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Abstract

Over the past three decades, extensive research has been able to determine the biologic functions for the main bioactive sphingolipids, namely ceramide, sphingosine, and sphingosine 1-phosphate (S1P)(Hannun, 1996; Hannun et al., 1986; Okazaki et al., 1989). These studies have managed to define the metabolism, regulation, and function of these bioactive sphingolipids. This emerging body of literature has also implicated bioactive sphingolipids, particularly S1P and ceramide, as key regulators of cellular homeostasis. Ceramidases have the important role of cleaving fatty acid from ceramide and producing sphingosine, thereby controlling the interconversion of these two lipids. Thus far, five human ceramidases encoded by five different genes have been identified: acid ceramidase (AC), neutral ceramidase (NC), alkaline ceramidase 1 (ACER1), alkaline ceramidase 2 (ACER2), and alkaline ceramidase 3 (ACER3). These ceramidases are classified according to their optimal pH for catalytic activity. AC, which is localized to the lysosomal compartment, has been associated with Farber's disease and is involved in the regulation of cell viability. Neutral ceramidase, which is localized to the plasma membrane and primarily expressed in the small intestine and colon, is involved in digestion, and has been implicated in colon carcinogenesis. ACER1 which can be found in the endoplasmic reticulum and is highly expressed in the skin, plays an important role in keratinocyte differentiation. ACER2, localized to the Golgi complex and highly expressed in the placenta, is involved in programmed cell death in response to DNA damage. ACER3, also localized to the endoplasmic reticulum and the Golgi complex, is ubiquitously

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expressed, and is involved in motor coordination-associated Purkinje cell degeneration. This review seeks to consolidate the current knowledge regarding these key cellular players..

Keywords

Ceramidase; Sphingolipid; Farber's disease; Sphingosine; cell proliferation; cancer

Introduction

Sphingolipids, which were initially thought to be solely structural components of cellular membranes, are now appreciated as a defining network of bioactive lipids. Sphingolipids are the second largest class of membrane lipids. (Hawthorne, 1975). They are composed of a polar head group and two non-polar tails and unlike glycerophospholipids they do not contain glycerol (Lehninger et al., 2008). The term “sphingolipids” was coined by J.L.W. Thudichum (1829–1901), a German-born physician working in London who identified sphingolipids in brain, first publishing these findings in 1884 (Thudichum, 1884). The name for this enigmatic family of lipids is taken from the Sphynx of Greek mythological fame which was well known for its love of riddles. Fortunately, through extensive research the secrets of sphingolipids have become known.

The sphingolipid backbone consists of a single molecule of a long chain amino-alcohol sphingoid base, usually sphingosine or one of its analogs, and one molecule of fatty acid attached in amide linkage to the –NH₂ on C-2. The resulting compound is a ceramide. Modifications on the C-1 of the ceramide via glycosidic or phosphodiester linkage results in different subclasses of complex sphingolipids: sphingomyelin (phosphocholine), and the glycosphingolipids (sugar). This results in a vast array of sphingolipids with distinct biologic functions (Lehninger et al., 2008).

Bioactive sphingolipids are generated via three different pathways, the *de novo* pathway, a direct hydrolytic pathway, and the salvage pathway. The *de novo* synthesis pathway begins in the cytosolic leaflet of the smooth endoplasmic reticulum (ER) with the condensation of serine and palmitoyl-CoA catalyzed by serine palmitoyltransferase (SPT) resulting in the formation of 3-keto-dihydrosphingosine. An NADPH dependent reductase converts the 3-keto-dihydrosphingosine to dihydrosphingosine (Sphinganine), and this molecule is then N-acylated by a (dihydro)ceramide synthases (CerS) to produce dihydroceramide. Oxidation of dihydroceramide by dihydroderamide desaturase generates ceramides from the corresponding dihydroceramides. Ceramides are themselves a family of closely related molecules that show structural variations, including length, desaturation, and hydroxylation of the fatty acid as well as the sphingoid base. At that point modifications on the C-1 can generate the three subclasses mentioned earlier as well as phosphorylation that generates ceramide-1-phosphate.

Ceramide synthesized in the ER can be transferred to the Golgi apparatus via the direct action of the ceramide transfer protein CERT, which couples it to sphingomyelin synthesis (Hanada, 2006, 2010). Vesicular transport delivers ceramide primarily for incorporation into the glycosphingolipids in the Golgi. Sphingomyelin and glycosphingolipids are, in turn,

transported to the plasma membrane through vesicular trafficking (D'Angelo et al., 2007). Plasma membrane sphingolipids are internalized through vesicular trafficking in the endosomal system for recycling and/or clearance through lysosomal degradation (Cheng et al., 2006; Kitatani et al., 2008).

In addition to synthesis and degradation, sphingolipids are subject to compartment-specific metabolic pathways. These pathways can generate ceramide by the action of distinct compartment-specific sphingomyelinases (Hannun and Obeid, 2008).

Ceramidases (CDases) can also hydrolyze ceramide to produce sphingosine. There are currently five known CDases genes which are distinguished by the pH required for optimal activity (acid, neutral, and alkaline). Sphingosine can then be phosphorylated by sphingosine kinases 1 and 2 to form sphingosine-1-phosphate (S1P) (Hannun and Obeid, 2011).

In the salvage pathway, sphingolipids are initially hydrolyzed mostly to sphingosine in the lysosome, which can then reenter sphingolipid metabolism via either phosphorylation by the sphingosine kinases and/or acylation by one of the six ceramide synthases.

Ceramide can also be produced by sphingomyelinase by catalyzing sphingomyelin hydrolysis. Extended studies have been especially performed on sphingomyelinase 2, the reader is referred to [Shamseddine et al](#) that discusses the roles and regulation of this enzyme and more over recent findings implicating nSMase2 in disease processes (Shamseddine et al., 2015).

Functionally, ceramide, sphingosine, S1P, ceramide 1-phosphate, and other sphingolipids have been described as bioactive molecules that regulate diverse cellular processes, including growth, differentiation, apoptosis, inflammation, invasion, migration, metabolism, and angiogenesis (Dickson and Brown, 1998; Futerman and Hannun, 2004; Hla, 2003; Spiegel and Milstien, 2003). This review will focus on the families of CDases, acting as the central enzymes converting ceramide to sphingosine.

Acid ceramidase (ASAH1)

Acid ceramidase (AC) is formally called N-acylsphingosine amidohydrolase with an Enzyme Commission number of 3.5.1.23. The first ceramidase was discovered in 1963 by Shimon Gatt in rat brain extract as an enzyme that catalyzed the hydrolysis of the amide bond of ceramide (Gatt, 1966). AC has an optimal pH of 4.5 and the enzyme catalyzes the hydrolysis of ceramide into sphingosine and a fatty acid with a preference for unsaturated ceramides with 6–16-carbon acyl chains (Mao and Obeid, 2008).

Discovery and structure

The enzyme was purified 35 years later in 1995 and identified as a heterodimer of an α (13kDa) and β (40kDa) subunits. Treating the purified enzyme with endoglycosidase H or peptido-N-glycanase F, the authors found that the molecular mass of the β subunit was reduced to ~30–35 and ~27 kDa, respectively, strongly suggesting a N-glycosylation (Bernardo et al., 1995). In contrast, no effect of glycosidase was detected on the α subunit. They also determined the apparent K_m for ceramide substrate: 149 μ M and the V_{max} : 136

nmol/mg/h (a K_m of 300 μ M and a V_{max} of 146 nmol/mg/h was proposed 35 years earlier). They also were able to produce the first antibody against AC.

The gene (*ASAH1*) was cloned in 1996 from a human fibroblast and pituitary cDNA libraries leading to a full-length cDNA of 2330-base pair (bp) predicting a 395 amino-acid protein (Bernardo et al., 1995).

The same group, 5 years later (Koch et al., 1996), showed that the biosynthesis of AC starts with a 53 kDa precursor. Then a post-transcriptional modification processes it to the 13-kDa α subunit and 40-kDa β subunit originally described within lysosomes. These authors also confirmed the presence of the glycosylation on the β subunit only.

Similar to many other enzymes, AC catalyzes the reverse reaction as well as the forward synthetic reaction as initially described. The results by Okino *et al.* (Okino et al., 2003) suggest that AC can use C12 fatty acid and sphingosine to produce ceramide. Interestingly the optimal pH for this reaction is higher than the forward reaction at 6 instead of 4.5.

AC is located in the lysosome, is ubiquitously expressed and has been found highly expressed in the heart and kidney, with lower expression in placenta, lung and skeletal muscle (Li et al., 1999).

Role of AC in Farber's disease

Mutations of AC in humans are associated with Farber's disease (OMIM #228000). This genetic disease is a very rare, autosomal recessive condition with only 80 patients have been diagnosed worldwide. It is characterized by joint deformation and the development of subcutaneous nodules. Symptoms appear a few months after birth, and the disease is progressive, often leading to death during the first few years of life (Ehlert et al., 2007). The link between AC and Farber's disease was established in 1972 by Sugitya *et al.* They cultured either skin fibroblasts or peripheral white blood cells from patients with Farber's disease, and they showed that the cells were unable to degrade radiolabeled ceramide. They also observed the accumulation of radiolabeled ceramide in lysosomes and therefore they proposed that Farber's disease is a lysosomal storage disorder (LSD) associated with a biallelic mutation of AC (Sugita et al., 1972).

Role of AC in proliferation and cancer

Realinin *et al.* found AC is specifically expressed in normal human melanocytes with higher expression in proliferative melanoma cell lines than other cancer cells (Realini et al., 2016).

AC has been reported to be involved in many other cancer types as well. In 2000 Mehta *et al.* reported increased expression of AC in primary tumor tissues and in six head and neck cancer (HNC) cell lines. AC overexpression decreased cisplatin sensitivity, and reciprocally cisplatin sensitivity was significantly increased by AC downregulation in HNC cells (Roh et al., 2016).

In 2013, Realini *et al.* suggested that AC could be involved in colon adenocarcinomas as they observed that the AC inhibitor carmofur and standard antitumoral drugs have a synergistic effect against SW403 cells (Realini et al., 2013).

In 2011 Turner *et al.* showed that prostate cancer cell lines overexpressing AC had an increased lysosomal density as well as increased autophagy. In their study, inhibition of autophagy with 3-methyladenine sensitized cells to C₆ ceramide treatment. They concluded that AC was involved in autophagy in prostate cancer cells, and that increased autophagy enhanced resistance to ceramide (Turner et al., 2011).

Interestingly in 2011 another study also suggested a role of AC in autophagy in the melanoma cell line. Bedia *et al.* showed that Dacarbazine (DTIC), a classical drug in the treatment of metastatic melanoma, induced a decrease of AC activity in human A375 melanoma cells. This effect was mediated by the activation of cathepsin B via ROS. The authors showed that this was mediated by a decrease of autophagy. Overexpression of acid ceramidase conferred resistance to DTIC, and down-regulation of acid ceramidase by siRNA sensitized the cells to DTIC (Bedia et al., 2011).

Use of AC inhibitor also suggested a role of AC in cell proliferation; as Vejselova *et al.* showed in 2016 that Ceranib-2, a small molecule acid ceramidase inhibitor, decreased viability of MCF7 cells in a dose and time dependent manner. The authors suggested that this effect was mediated by the mitochondrial apoptosis pathway through modulation of the mitochondrial membrane potential (Vejselova et al., 2016).

Meš i *et al.* also demonstrated that chemotherapeutic drugs induce apoptosis in MCF-7 cells by activating AC, suggesting AC as a molecular target for breast cancer (Mescic et al., 2015). Beckham *et al.* suggested in 2013 that acid ceramidase, through S1P, promotes nuclear export of PTEN leading to enhanced tumor formation, cell proliferation, and resistance to therapy (Beckham et al., 2013). Likewise, Cheng *et al.* showed that AC was induced by radiation, and this overexpression confers prostate cancer resistance and tumor relapse (Cheng et al., 2013). An interesting study performed by Siow *et al.* established that sphingosine kinase, in addition to producing sphingosine-1-phosphate as a signaling molecule, also consumes dihydrosphingosine to regulate ceramide synthesis. They suggested that regulators of the sphingolipid pathway are important mediators of cell stress responses (Siow et al., 2015).

Although most studies demonstrated the role of AC in cell proliferation and survival, a study by Sun *et al.* showed that AC was upregulated by Ca²⁺ in human epithelial keratinocytes (HEK) and that the upregulation of AC mediates the Ca²⁺ induced growth arrest and differentiation of HEK cells (Sun et al., 2008). Similarly, Sanger *et al.* suggested that AC is mainly associated with luminal A like breast cancers, and tumors with high AC expression among the other subtypes were also characterized by an improved prognosis (Sanger et al., 2015).

It also has been suggested that AC is highly and constitutively expressed in human alveolar macrophages. Using an inhibitor approach, the authors suggested that high AC expression is important for survival of macrophages (Monick et al., 2004). Also, Zeidan *et al.* showed that

AC is involved in the action of TNF in inflammatory pathways. These results and others suggest that AC may play a role in inflammatory regulation.

Neutral ceramidase (ASAH2, ASAH2B, ASAH2C)

Discovery and structure

Neutral ceramidase (NC) is formally named N-acylsphingosine amidohydrolase 2 (ASAH2) with an Enzyme Commission number of 3.5.1.23. The mammalian enzyme was purified initially from rat brain cells and was cloned in 2000 (Tani et al., 2000), and this defined a novel family of neutral ceramidases that were also identified at the same time from *Pseudomonas* (Kita et al., 2000). NC is a single-pass transmembrane glycoprotein. The enzyme is highly expressed in the kidney and liver, with lower expression in the brain, lung, and heart. It is highly expressed in the small intestine and colon along the brush border (Kono et al., 2006).

Investigating the physiology of NC, Kono *et al.* found that NC deficient mice, while appearing normal, were not able to degrade dietary sphingolipids. They concluded that NC regulates the levels of bioactive sphingolipid metabolites in the intestinal tract.

The initial cloning report (Tani et al., 2000) identified NC as a protein of 763 amino acids. Interestingly NC was cloned by another group as a longer protein of 782 amino acids (Hwang et al., 2005) called ASAH2B. The authors suggest that this protein was coded by the same gene but by an alternative transcription starting site.

The crystal structure of NC was recently resolved at 2.6 Å by Airola *et al.* This revealed a catalytic domain (residues 99–626), a short linker (627–641), and an immunoglobulin (IG)-like domain (642–780). The structure also revealed that the active site of human NC is composed of a narrow, 20 Å deep, hydrophobic pocket with a Zn²⁺ ion at the base. They confirmed this result by introducing arginine residues in this pocket at positions Gly124, Ala211, and Gly465 and determined that those mutations inhibit NC activity (Airola et al., 2015).

Role of NC in injury, UV damage and nutrient deprivation

NC is expressed in the brain, and Novgorodov *et al.* investigated the role of NC during traumatic brain injury with a possible role for sphingosine accumulation in mitochondria due to the activation of NC (Novgorodov et al., 2014). The authors found that NC knock out mice were partly protected from cerebral ischemia.

Further, NC has been shown to be downregulated by several stress stimuli, such as nitric oxide, in mesangial cells (Franzen et al., 2002; Franzen et al., 2001). In addition, Uchida *et al.* (2010), also found that UV-B irradiation decreased NC activity in keratinocytes, and a general ceramidase inhibitor, N-oleoylethanolamine, or specific siRNA to NC could sensitize the cells to low-dose-UVB-induced apoptosis (Uchida et al., 2010). The reader interested in DNA damage can refer to a review by Carroll *et al.* (Carroll et al., 2015).

Using a model of glycolysis inhibition via 2-deoxyglucose (2DG) and mitochondrial electron transport via antimycin A, Sundaram *et al.* showed that loss of NC protected cells from nutrient deprivation-induced necroptosis via autophagy and clearance of damaged mitochondria (Sundaram et al., 2016).

Role of NC in differentiation

NC appears to play an important role in induction of ceramide accumulation and neuronal differentiation. Tanaka *et al.*, demonstrated in 2012 that ATRA down-regulates the mRNA, protein and the enzyme activity of NC in SH-SY5Y cells (Tanaka et al., 2012). The authors found that silencing the expression of ASAH2 significantly decreased cell growth as well as neuronal differentiation phenotypes.

Role of NC in inflammation

Interestingly, Snider *et al.* demonstrated, using a DSS inflammation model, that knock out of NC resulted in paradoxical elevation of sphingosine, possibly due to induction of other CDases. This in turn resulted in S1P elevation. These results suggest that NC may protect against inflammation (Snider et al., 2012).

It was shown that PDGF up-regulates NC-like activity in rat mesangial cells (Coroneos et al., 1995) and that the enzyme was activated by interleukin-1 in rat hepatocytes (Nikolova-Karakashian et al., 1997), thus leading to a decrease in ceramide levels. Osawa *et al.* demonstrated that overexpression of NC could block TNF- α induced generation of ceramide in primary hepatocytes and protected cells from TNF- α -induced apoptosis (Osawa et al., 2005). This study also demonstrated that overexpression of NC inhibited liver injury and hepatocyte apoptosis in mice. The role of sphingolipids in inflammation has been also studied by Huang et al., they demonstrated that TGF- β increased the expression of SphK1 and S1P lyase in human lung fibroblasts and that knockdown of SphK1 or its inhibition attenuated TGF- β mediated signal transduction and the pathophysiology of lung fibrosis (Huang and Natarajan, 2015).

A role of NC in chemotherapy has been suggested by Wu *et al.* who found that gemcitabine treatment increased the levels of specific ceramides, the very long chain ceramides, in a polyoma middle T transformed murine endothelial cell line, by reducing the protein levels of NC, and that the increased ceramides may mediate gemcitabine-induced growth suppression of these cells (Wu et al., 2009).

Very recently, NC was identified as a regulator of cell survival in colon cancer cells with minimal effects on noncancerous cells. Its inhibition resulted in loss of β -catenin, a major component of pathways relevant for colon cancer development. This study also demonstrated that inhibition of NC in a xenograft model delayed tumor growth and increased ceramide. Importantly, mice lacking NC treated with the pro-carcinogen azoxymethane were protected from tumor formation (Garcia-Barros et al., 2016). These results suggest that NC may emerge as a therapeutic target in colon cancer.

Alkaline ceramidase 1 (ACER1)

Alkaline ceramidase 1 (ACER1) is formally named N-Acylsphingosine Amidohydrolases 3 with an Enzyme Commission number of 3.5.1.23.

The family of ACER enzymes was initially identified in yeast by Mao *et al.* as YPC1 and YDC1 with selective hydrolysis of phytoceramide vs dihydroceramide, respectively (Mao et al., 2000a; Mao et al., 2000b). The initial cloning of the mammalian homolog identified ACER1 as a 264-amino acid protein with a predicted molecular weight of 31 kDa. Human ACER1 is highly homologous to its mouse orthologue Acer1. Sun *et al.* showed that ACER1 hydrolyzes preferentially ceramides with an unsaturated very long acyl chain as a substrate (Sun et al., 2008). They identified the optimal pH being around 8.

ACER1 is primarily expressed in the skin in epidermal keratinocytes but not in dermal fibroblast cells. It is essential for skin homeostasis and whole-body energy homeostasis (Liakath-Ali et al., 2016). Immunofluorescence studies with over expressed ACER1 showed that the protein is expressed in the endoplasmic reticulum. Functionally, Sun et al identified a role for ACER1 in mediating the calcium-induced growth arrest and differentiation of epidermal keratinocytes.

Alkaline ceramidase 2

Alkaline ceramidase 2 (ACER2) is formally named N-Acylsphingosine Amidohydrolase 3-Like with an Enzyme Commission number of 3.5.1.23.

Discovery and structure

The initial cloning of ACER2 in 2006 showed that the enzyme is a 275-amino acid protein with a molecular weight of 31.3 kDa (Xu et al., 2006). ACER2 was localized to the Golgi complex and was highly expressed in the placenta. Using microsomes from ACER2-expressing yeast cells, in 2010, that same group (Sun et al., 2010) identified that ACER2 has seven putative transmembrane domains with its amino-terminal (N) in the lumen of the Golgi and its carboxyl-terminal (C) in the cytosol. They also described that ACER2 required Ca^{2+} for both its *in vitro* and cellular activities and that its N-terminal tail is necessary for both ACER2 activity and Golgi localization.

In 2014, Sasaki *et al.* suggested that an alkaline ceramidase, possibly ACER2, was regulated by c-Src by using sh-RNA and over-expression of c-Src in the lysate of A549 cells. The authors also suggested that this activation was independent of Ca^{2+} (Huang et al., 2015).

Role of ACER2 in MAP kinase

Huang *et al.*, in 2015 showed that serum deprivation upregulated ACER2 activity and mRNA in HeLa and HepG2 cells. It was revealed that the MAPK signaling pathway is involved in the increase of ACER2 activity following serum deprivation (Mao et al., 2001).

Role of ACER2 in DNA damage

Xu *et al.*, showed in 2016 that ACER2 mediated programmed cell death in response to DNA damage through ROS production. They showed that DNA damage induced by doxorubicin increased sphingosine levels by upregulating ACER2 in tumor cells. Knockdown of ACER2 significantly reduced the doxorubicin-induced apoptosis and necrosis of the cells (Xu et al., 2016).

Role of ACER2 in differentiation

Another study reported that ACER2 is upregulated during erythroid differentiation of K562 human erythroleukemia cells (Sun et al., 2008). Using a mouse model in which alkaline ceramidase activity was inhibited by D-MAPP, an alkaline ceramidase inhibitor, this study also suggested that alkaline ceramidase activity encoded likely by the mouse *Acer2* and/or other alkaline ceramidase genes, is important for the generation of SPH and S1P in erythrocytes and plasma S1P.

Alkaline ceramidase 3

Alkaline ceramidase 3 (ACER3) was initially called alkaline phytoceramidase with an Enzyme Commission number of 3.5.1.23.

Discovery and structure

Alkaline ceramidase 3 or ACER3 was initially cloned in 2001 by Mao *et al.* as a 267-amino acid protein with molecular weight of 31.6 kDa in 2001. It was the first alkaline ceramidase identified in mammals, and the initial report identified the enzyme by its ability to hydrolyzes phytoceramide into phytosphingosine and free fatty acid and therefore it was named initially alkaline phytoceramidase (Mao et al., 2001). The authors subsequently determined that phytoceramidase is homologous to both ACER1 and ACER2 and had several transmembrane domains, and therefore it was renamed as ACER3. ACER3 is localized in both the ER and Golgi apparatus. Northern blot analysis showed that ACER3 mRNA is ubiquitously expressed with the highest levels identified in the placenta.

Role of ACER3 in proliferation and cell cycle

Hu *et al.* in 2010 suggested that ACER3 also specifically hydrolyzes ceramides carrying unsaturated long acyl chains (ULC). They also reported that ACER3 knockdown inhibited cell proliferation and upregulated the cyclin-dependent kinase inhibitor p21(CIP1/WAF1) (Hu et al., 2010). A recent study by Chen *et al.* found a role of ACER3 in acute myeloid leukemia. ACER3 expression was found be inversely correlated with the overall survival of acute myeloid leukemia (AML) patients. The knockdown of ACER3 in human AML cells resulted in decreases of cell growth and colony formation as well as elevated apoptosis, and lower AKT signaling (Chen et al., 2016).

Role of ACER3 in Purkinje cells and balance

Using a knock out mouse model, Wang *et al.*, in 2015 suggested that ACER3 played a protective role in sphingolipid homeostasis in the brain. ACER3 deficient mice demonstrated

impairment in motor coordination and balance capabilities. This phenotype was associated with premature degeneration of Purkinje cells. Interestingly, very recently, Edvardson *et al.* suggested that homozygous mutation in ACER3 in patients was responsible for early childhood leukodystrophy (Edvardson et al., 2016). This point mutation inactivated the catalytic function of ACER3 and increased multiple classes of sphingolipids in the patients' blood.

Role of ACER3 in inflammation

More recently, Wang et al. demonstrated that loss of the mouse *Acer3* aggravates colitis and colitis-associated colorectal cancer in a murine model likely by disrupting the homeostasis of ceramides and other sphingolipids in the digestive system (Wang et al., 2016).

Conclusions

Ceramidases are a set of key enzymes that function to regulate the levels (and actions) of bioactive lipids, especially ceramide, sphingosine, and SIP. It is becoming apparent that these enzymes have roles in regulating various biological processes, such as cell proliferation, differentiation, apoptosis, and autophagy. As such, it is not surprising that mutations in certain ceramidase genes or their dysregulation through other mechanisms can lead to different diseases, such as inflammatory diseases, neurodegenerative diseases, or tumorigenesis. Therefore, studies examining sphingolipids and CDases should yield novel potential mechanisms, interventions, and novel therapeutics targeted for tumorigenesis, inflammation and other disorders.

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Abbreviations

SIP	Sphingosine-1-Phosphate
SM	Sphingomyelin
SMase	Sphingomyelinase
SPH	sphingosine
AC	acid ceramidase
NC	Neutral ceramidase
ACER	alkaline ceramidase
ER	endoplasmic reticulum
DNA	deoxyribonucleic acid
SPT	serine palmitoyltransferase

NADPH	nicotinamide adenine dinucleotide phosphate
CERT	ceramide transfer protein
kDa	kilo Dalton
bp	Base pair
LSD	lysosomal storage disorder
HNC	head and neck cancer
DTIC	dacarazine
ROS	reactive oxygen species
PTEN	phosphatase and tensin homolog
HEK	human epithelial keratinocyte
TNF	Tumor Necrosis Factor
ATRA	all trans retinoic acid
mRNA	Tumor Necrosis Factor
PDGF	Platelet-derived growth factor
Sh RNA	small hairpin ribonucleic acid
MAPK	Mitogen-activated protein kinases
ULC	unsaturated long acyl chain
AML	acute myeloid leukemia

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Figure 1. Domain organization of AC, NC, ACER1, 2, 3

The structural organization of ceramidase enzymes showing different domains and their positions. Numbers in *italic* represent the glycosylation site. NH₂: amino terminal of the protein, COOH: carboxy terminal of the protein, TM: transmembrane domain, IG-like: immunoglobulin like.

Table 1

Summary of the roles of ceramidases.

Name	Gene symbol	Cellular localization	Tissue expression	Biological functions	Related diseases
Acid ceramidase	<i>ASAH1</i>	Lysosome	heart, kidney (<i>high</i>) placenta, lung, skeletal muscle (<i>low</i>)	apoptosis, cell cycle, differentiation, cell invasion, autophagy	Farber's disease melanoma head and neck cancer breast cancer prostate cancer type II diabetes Alzheimer's disease
Neutral ceramidase	<i>ASAH2</i>	PM	small intestine, colon (<i>high</i>) kidney, liver brain, lung, heart (<i>low</i>)	apoptosis, necroptosis, cell cycle	colon cancer ischemic injury IBD
Alkaline ceramidase 1	<i>ACER1</i>	ER	skin	skin homeostasis	unknown
Alkaline ceramidase 2	<i>ACER2</i>	Golgi	placenta (<i>high</i>) ubiquitous (<i>low</i>)	apoptosis, necrosis, cell adhesion	unknown
Alkaline ceramidase 3	<i>ACER3</i>	ER/Golgi	placenta (<i>high</i>) ubiquitous	apoptosis, cell proliferation	acute myeloid leukemia colon cancer colitis leukodystrophy