Published in final edited form as:

Neuromolecular Med. 2010 December ; 12(4): 320–330. doi:10.1007/s12017-010-8120-z.

Mammalian Neutral Sphingomyelinases: Regulation and Roles in Cell Signaling Responses

Bill X. Wu, Christopher J. Clarke, and Yusuf A. Hannun*

Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425, U.S.A

Abstract

Ceramide, a bioactive lipid, has been extensively studied and identified as an essential bioactive molecule in mediating cellular signaling pathways. Sphingomyelinase (SMase), (EC 3.1.4.12) catalyzes the cleavage of the phosphodiester bond in sphingomyelin (SM) to form ceramide and phosphocholine. In mammals, three Mg^{2+} -dependent neutral SMases termed nSMase1, nSMase2 and nSMase3 have been identified. Among the three enzymes, nSMase2 is the most studied and has been implicated in multiple physiological responses including cell growth arrest, apoptosis, development and inflammation. In this review, we summarize recent findings for the cloned nSMases and discuss the insights for their roles in regulation ceramide metabolism and cellular signaling pathway.

Keywords

ceramide; bioactive lipids; neutral sphingomyelinase; Sphingolipid; SM; nSMase2

Introduction

Sphingolipids represent a major class of lipids that are important components of membranes in eukaryote cells. Since their discovery in 1876, sphingolipids were considered for a long period as inert structural components in membrane. However, intensive research on metabolism and function has found that several sphingolipids including ceramide, sphingosine, ceramide-1-phosphate and sphingosine-1-phosphate play important roles in regulating a wide range of signaling pathways (Kim et al., 2009; Morales et al., 2007; Ogretmen and Hannun, 2004; Zheng et al., 2006).

Among the bioactive species of sphingolipids, ceramide has been extensively studied and identified as an important signaling molecule in cell growth arrest, differentiation, senescence, apoptosis and inflammation (Chalfant and Spiegel, 2005; El Alwani et al., 2006; Hannun and Obeid, 2008; Modrak et al., 2006). A variety of stimuli including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, daunorubicin and other chemotherapeutics can induce the formation of ceramide (Bartke and Hannun, 2009; Nikolova-Karakashian et al., 2008) and this can occur through several pathways including the *de novo* synthetic pathway (Perry, 2002), the ceramide salvage pathway (Kitatani et al., 2008) and the hydrolysis of complex sphingolipids such as sphingomyelin (SM) (Stoffel, 1999). Sphingomyelinase (SMase) hydrolyzes the phosphodiester bond of SM yielding ceramide and phosphocholine. Several isoforms of SMases have been identified and classified by their pH optima: acid SMase, alkaline SMase, and neutral SMases (nSMases). Of these, alkaline

*To whom correspondence should be addressed: Department of Biochemistry and Molecular Biology, Medical University of South Carolina, 173 Ashley Ave., Charleston, SC 29425., Fax: 843-792-4322, hannun@musc.edu.

SMase is found in the intestinal tract and bile, and is thought to play a role in SM digestion (Duan, 2006). In contrast, acid SMase (SMPD1) comprises both a lysosomal enzyme and a secretory SMase and its mutation results in the human Niemann-Pick disease (Jenkins et al., 2009; Schuchman, 2007). The focus of this review is the group of nSMases. At present, several nSMase isoforms have been identified and suggested to play essential roles for regulating sphingolipid metabolism.

The Neutral Sphingomyelinase Family

In 1967, Scheider and colleagues first reported neutral SMase (N-SMase) activity identified in tissues from Niemann-Pick disease patients (Schneider and Kennedy, 1967); however, it was 20 years until the first N-SMase family members were cloned and identified from *Staphylococcus aureus* and *Bacillus cereus* (Coleman et al., 1986; Yamada et al., 1988). Based on homology with the bacterial SMases, the yeast N-SMase homologue, ISC1 was also identified (Sawai et al., 2000). Further, the first mammalian homologues (Fig. 1) nSMase1 (SMPD2) (Tomiuk et al., 1998) and nSMase2 (SMPD3) were identified (Hofmann et al., 2000), again based on homology to the identified bacterial SMases. More recently, the third mammalian isoform nSMase3 (SMPD4) was identified based on sequence obtained from purified bovine SMases (Krut et al., 2006). Finally, very recent studies identified N-SMase homologues in zebrafish cells (Yabu et al., 2008; Yabu et al., 2009). Notably, one of the zebrafish nSMase identified was localized to the mitochondria (Yabu et al., 2008), an important organelle for sphingolipid metabolism (Birbes et al., 2002; Futerman, 2006; Novgorodov and Guduz, 2009). This raises the possibility of additional unidentified mammalian N-SMases. Together, these enzymes comprise the N-SMase family. Notably, the existence of multiple nSMase isoforms is in agreement with studies reporting multiple N-SMase activities reported in bovine brain (Jung et al., 2000).

Overall, the homologies among the different nSMase enzymes are low. However, in all but one (nSMase3), key residues involved in magnesium binding and catalytic activity – the so-called ‘catalytic core’ residues – are strongly conserved (Clarke et al., 2006). Consequently, all the nSMases identified so far are strongly dependent on magnesium (or manganese) for their catalytic activity. This conservation suggests a common catalytic mechanism, and thus, these N-SMases are considered to belong to an extended family. The mammalian N-SMases have been suggested to play major roles in the cellular stress response (Fig. 2) for many years. In contrast, the majority of knowledge about nSMase1, –2 and –3 has been reported relatively recently. Accordingly, here we review current knowledge of the mammalian N-SMase family members. For information on yeast ISC1 and the bacterial SMases, the following recent reviews are suggested (Milhas et al., 2009; Matmati and Hannun, 2008).

Neutral Sphingomyelinase 1 (nSMase1)

In 1998, nSMase1 (SMPD2) was cloned and identified according to remote sequence similarity with bacterial SMase (Tomiuk et al., 1998). Human nSMase1 is a 423-amino acid protein with a predicted molecular weight of 47.6 kDa and shows significant homology with ISC1 at the amino acid sequence. Consistent with this, nSMase1 is also an integral membrane protein with two putative transmembrane domains at the C-terminus. Analysis of N-SMase activity *in vitro* revealed that nSMase1 was Mg^{2+} -dependent (Tomiuk et al., 1998) and mutagenesis studies identified two histidine residues, His-136 and His-272, essential for catalysis (Rodrigues-Lima et al., 2000). Enzymatic activity required reducing agents and was reversibly inhibited by reactive oxygen species (ROS) and oxidized glutathione (GSH) (Fensome et al., 2000). The enzyme is also irreversibly inactivated by peroxynitrite, a nitric oxide-derived oxidant (Josephs et al., 2002). Northern blot analysis showed that nSMase1 mRNA is ubiquitously expressed in multiple tissues as a 1.7-kb mRNA; the ubiquitous

expression pattern was further confirmed at the protein level (Tomiuk et al., 2000). The over-expressed nSMase1 colocalizes with marker proteins of the endoplasmic reticulum (ER) and the Golgi apparatus by immunocytochemistry (Tomiuk et al., 2000). Although the ER localization was further confirmed (Fensome et al., 2000; Rodrigues-Lima et al., 2000), a subsequent study in hepatoma cells found that endogenous rat nSMase1 localized mainly to the nuclear matrix. This suggests that nSMase1 may function in multiple organelles (Mizutani et al., 2001).

Role of nSMase1 in Lipid Metabolism and in Cellular Signaling

A number of reports have focused on potential signaling roles of nSMase1. Some studies have suggested that nSMase1 is important for ceramide generation in response to stress. For example, in the T cell hybridoma (3DO) cells, specific inhibition of nSMase1 by antisense RNA reduced T cell receptor mediated apoptosis (Tonnetti et al., 1999) and inhibition of nSMase1 decreased ceramide levels, IL-2 production, and MAPK activation during the T-cell receptor response. Thus, nSMase1-derived ceramide may be an important component of the T cell receptor signaling machinery. Additionally, in zebrafish embryonic cells, the inhibition of nSMase1 by an antisense oligonucleotide repressed the induction of ceramide generation, caspase-3 activation, and apoptotic cell death induced by heat stress (Yabu et al., 2008).

However, whereas nSMase1 exhibits *in vitro* SMase activity, MCF-7 cells overexpressing the protein did not show changes in SM metabolism. Instead, nSMase1 had effects on metabolism of lysoPAF, an inert metabolite of biologically active platelet-activating factor and a natural 1-alkyl 2-acetyl analog of phosphatidylcholine. Importantly, nSMase1 acts preferentially as a lysoPAF phosphodiesterase *in vitro* (Sawai et al., 1999), suggesting nSMase1 may have an alternative biochemical function. Consistent with this, stimulation of nSMase1-overexpressing cells with TNF or H₂O₂ did not elevate ceramide levels or induce apoptosis (Tomiuk et al., 1998). Moreover, in Jurkat cells, nSMase1 was not responsible for ceramide generation during the execution phase of death receptor-induced apoptosis (Tepper et al., 2001). Importantly, nSMase1 knockout (KO) mice showed no abnormality in sphingolipid metabolism or lipid storage disease (Zumbansen and Stoffel, 2002).

Taken together, these data suggest that nSMase1 may function as an *in vivo* SMase in some instances. However, with the lack of a phenotype in the nSMase1-KO mouse, the physiologic roles of nSMase1 still need to be further determined.

Neutral Sphingomyelinase 2 (nSMase2)

In 2000, the second mammalian nSMase, nSMase2, was identified based on similarity to bacterial SMase (Hofmann et al., 2000). Human nSMase2 is a 655 amino acid protein with a molecular weight of 71 kD and has a C-terminal catalytic domain, two N-terminal hydrophobic segments, with a 200-residue collagen-like triple helices between the two (Fig. 1) (Hofmann et al., 2000). In addition, nSMase2 was found to be palmitoylated in two cysteine clusters (Tani and Hannun, 2007b) with one cluster located between the two hydrophobic segments and the second cluster located in the catalytic region. Mutagenesis studies revealed that palmitoylation of nSMase2 was important for both the protein stability and the plasma membrane (PM) localization of nSMase2. Although the N-terminal hydrophobic segments of nSMase2 were originally proposed to be transmembrane domains, subsequent studies of the enzyme topology suggested that each hydrophobic segment is integrated into the membranes, but does not span the entire membrane (Tani and Hannun, 2007a). The location of the N-terminal palmitoylation cluster between the two hydrophobic segments further supports this as palmitoylation only occurs on the cytosolic side of the PM (Tani and Hannun, 2007b).

The activity of nSMase2 is optimal at neutral pH, is dependent on magnesium, and is enhanced by unsaturated fatty acids and anionic phospholipids, especially cardiolipin and phosphatidylserine (Marchesini et al., 2003). GW4869 was identified as an nSMase2 inhibitor both *in vitro* and *in vivo* and has been widely used to evaluate N-SMase roles in cellular response and physiological function. Unlike nSMase1, nSMase2 possesses *in vivo* SMase activity with overexpressing nSMase2 in MCF-7 cells showing a 40% decrease in SM levels and a concomitant 60% increase in ceramide levels (Marchesini et al., 2003); this was also observed in primary hepatocytes (Karakashian et al., 2004). Unlike the ubiquitous nSMase1, expression of nSMase2 was predominantly detected in the brain at both the protein and mRNA levels (Hofmann et al., 2000). The subcellular localization of nSMase2 has been observed in several organelles. While nSMase2 was initially localized to the Golgi in several cell lines (Hofmann et al., 2000), subsequent studies have found localization of nSMase2 predominantly at the PM. Furthermore, this PM localization could be enhanced upon stimulation (e.g. TNF- α , H₂O₂) or in confluent cells (Clarke and Hannun, 2006; Clarke et al., 2007; Levy et al., 2006; Marchesini et al., 2004). Thus, the translocation of nSMase2 from the Golgi to the PM may be important for nSMase2 functions. Interestingly, nSMase2 has also been observed in the nucleus in human airway epithelial (HAE) cells using confocal microscopy (Levy et al., 2006). However, the mechanism underlying specific localization of nSMase2 and its potential trafficking between multiple subcellular locations is unclear and needs further elucidation. Finally, nSMase2 was reported to localize to the cytosolic leaflet of the PM, suggesting the presence of an inner leaflet pool of SM on the cytosolic face of the membranes (Tani and Hannun, 2007a). Notably, this is consistent with the enrichment of the nSMase2 activating lipid phosphatidylserine in the inner leaflet of the PM (Vance and Steenbergen, 2005).

Roles of nSMase2 in Cellular Stress Responses

Since its identification, nSMase2 has emerged as a major candidate for stress-induced ceramide production and a number of anti-cancer drugs have been shown to exert effects on nSMase2. In MCF-7 cells, daunorubicin upregulated cellular N-SMase activity and ceramide levels (Ito et al., 2009). Notably, daunorubicin specifically upregulated both nSMase2 mRNA and protein levels but had no effects on nSMase1 and nSMase3. Further analysis revealed that nSMase2 was important in mediating daunorubicin-induced cell death and that several Sp1 motifs in the nSMase2 promoter region were important for transcriptional induction by daunorubicin (Ito et al., 2009). Additionally, nSMase2 overexpression enhanced cell death induced by either staurosporine or C(2)-ceramide in oligodendrocytes (Goswami et al., 2005). In aortic smooth muscle cells, apolipoprotein C-I induced N-SMase activity, ceramide levels, and apoptosis which was abolished by treatment with the nSMase2 inhibitor, GW4869 (Kolmakova et al., 2004).

Reports have also suggested that nSMase2 plays a role in stress-induced bronchial and lung injury in pulmonary diseases. For example, in HAE cells, H₂O₂ induced activation of nSMase2, ceramide generation, and apoptosis and this was inhibited by GSH. Oxidant exposure was also suggested to affect nSMase2 localization with oxidant exposure causing preferential trafficking to the PM whereas in contrast, exposure to GSH resulted in nSMase2 trafficking to the nucleus (Levy et al., 2006). A subsequent study also found that nSMase2 was required for cigarette smoke-mediated ceramide generation and apoptosis in human bronchial epithelial cells (Levy et al., 2009). As with H₂O₂, GSH also inhibited the cigarette smoke-induced effects on nSMase2 activity and apoptosis. Finally, the nSMase2 inhibitor GW4869 was shown to inhibit hypoxia-induced pulmonary vasoconstriction *in vivo* (Cogolludo et al., 2009). In isolated rat pulmonary artery smooth muscle cells, hypoxia increased ceramide content which was abrogated by GW4869. These data suggest that nSMase2 plays roles in vasoconstrictor response to hypoxia.

Roles of nSMase2 in Cell Growth and Cancer Genesis

In addition to its roles in acute stress responses, nSMase2 has also been implicated in cell growth inhibition and tumorigenesis. Three years prior to the cloning of nSMase2, a cDNA fragment CCA1- later identified as nSMase2 - was found to be upregulated in growth-arrested confluent cells. A role for nSMase2 in the regulation of cell growth was confirmed when it was found that nSMase2-overexpressing cells exhibited slower cell growth at the late exponential phase (Marchesini et al., 2003). Subsequently, it was found that upregulation of nSMase2 mRNA and protein caused a cell cycle arrest in the G0/G1 phase with consequent increases in ceramide levels, especially in the very long chain C(24:1) and C(24:0) ceramides (Marchesini et al., 2004). Conversely, siRNA downregulation of nSMase2 prevented both ceramide increases and the cell cycle arrest. Mechanistically, nSMase2 downregulation prevented dephosphorylation of the retinoblastoma protein and induction of p21/WAF1 in confluent cells, thus providing a link between nSMase2 and key regulators of cell cycle. Further, PP1, a lipid-regulated protein phosphatase, was implicated as acting downstream of nSMase2 in regulating cell motility during cell confluence (Marchesini et al., 2007). On the other hand, the antioxidants CoQ inhibited serum deprivation-induced activation of nSMase and ceramide accumulation (Navas et al., 2002). Surprisingly, while nSMase2 was reported to be distributed throughout MCF-7 cells under subconfluent condition, the protein became predominantly located at the PM in confluent, growth-arrested cells. This further supports the hypothesis that the PM is the major site of nSMase2 action. Finally, a more recent study demonstrated induction of nSMase2 mRNA and ceramide levels by retinoic acid (Somenzi et al., 2007). As retinoic acid is also known to induce G0/G1 arrest, this provides further evidence that nSMase2 is an important regulator of cell growth and the cell cycle.

More recently, nSMase2 has been implicated in cancer pathogenesis. A study of primary osteoblasts found that treatment with conditioned medium from a prostate cancer cell line (PC-3) significantly reduced nSMase2 expression (Schulze et al., 2009). As prostate cancer primarily metastasizes to bone, this study suggested that nSMase2 may play a role in cancer cell migration and osteoclast activation. Moreover, a number of mutations in the nSMase2 gene, *smpd3*, were identified in cancer (Kim et al., 2008) with nSMase2 mutations found in 5% of acute myeloid leukemias and 6% of 131 acute lymphoid leukemias. Notably, two of the reported leukemia mutations led to decreased stability of nSMase2 or mislocalization of the protein within the cell. Finally, a homozygous deletion of the *smpd3* gene was found in a murine osteosarcoma model. Taken together, these results have begun to suggest a functional role for the nSMase2/ceramide pathway in the regulation of tumorigenesis. However, considerable further studies are required to address this fully.

Role nSMase2 in Inflammation Signaling Pathway

Consistent with the stimulation of N-SMase activity by cytokines, studies have also shown that nSMase2 plays a role in the inflammatory response (Clarke and Hannun, 2006; Clarke et al., 2006; Nikolova-Karakashian et al., 2008). Among the various inflammatory stimuli, TNF- α is one of the widest studied activators of nSMase2. In MCF-7 cells, long term stimulation with TNF- α induced approximately 40% activation of overexpressed nSMase2 (Marchesini et al., 2003) while acute stimulation with TNF- α was also found to increase nSMase2 activity in a number of cell lines including A549, HUVEC, and smooth muscle cells (Clarke et al., 2007; De Palma et al., 2006; Tellier et al., 2007). In smooth muscle cells, activation of nSMase2 by TNF was reported to be downstream of furin, MT1-MMP and MMP2 whereas in A549 cells, nSMase2 activation was dependent on p38 MAPK (Clarke et al., 2007; Tellier et al., 2007). Notably, in addition to activation, TNF was also found to induce relocalization of nSMase2 to the PM in A549 lung epithelial cells and this was also

dependent on p38 MAPK as well as requiring PKC- δ (Clarke et al., 2008; Clarke et al., 2007; Tellier et al., 2007). FAN (factor associated with N-SMase activation) has been identified as an adapter protein that associates with the TNF receptor and plays a role in activating N-SMase (Adam-Klages et al., 1996). More recently, the polycomb group protein EED was found to interact with both nSMase2 and RACK1 (Philipp et al., 2010), an interaction partner of FAN (Tcherkasowa et al., 2002). Furthermore, the TNF-dependent activation of nSMase2 is completely abrogated by inhibition of EED. Thus, TNF induces formation of a TNF receptor-FAN-RACK1-EED complex at the PM resulting in activation of nSMase2. Taken together, these data confirm nSMase2 as a TNF- α -responsive enzyme.

Functionally, nSMase2 was found to be upstream of the TNF- α -stimulated expression of vascular cell adhesion molecule-1 (VCAM) and intercellular adhesion molecule-1 (ICAM) in lung epithelial cells (Clarke et al., 2007) and was important for TNF- α induced activation of endothelial nitric oxide synthase (eNOS) in human endothelial cells (De Palma et al., 2006). In smooth muscle cells, nSMase2 was important for TNF- α -mediated ERK activation, DNA synthesis, and cell proliferation (Tellier et al., 2007). Interestingly, in the latter two cases, nSMase2 appeared to function upstream of sphingosine kinase 1, as a source of ceramide for subsequent production of sphingosine-1-phosphate. More recently, nSMase2 was implicated in TNF- α induced modulation of synaptic plasticity by controlling the membrane insertion of NMDA receptors (Wheeler et al., 2009). Finally, aside from TNF- α , nSMase2 was also implicated in induction of the inducible nitric oxide synthase (iNOS) by lipopolysaccharide (LPS) in C6 rat glioma cells (Won et al., 2004).

In addition to the above reports, insight into the role of nSMase2 in aging-associated inflammation has come from the Karakashian group. Initial studies reported the activation of N-SMase by interleukin 1- β (IL-1 β) (Nikolova-Karakashian et al., 1997), and it was subsequently found that nSMase2 overexpression enhanced IL-1 β -stimulated JNK activation in primary rat hepatocytes (Karakashian et al., 2004). Moreover, the regulation of JNK phosphorylation and the ubiquitination of the IL-1 β receptor-associated kinase (IRAK) were dependent on the ceramide-activated protein phosphatase, PP2A – presumably acting downstream of nSMase2 (Karakashian et al., 2004). In further studies, the same group noted increased sensitivity to IL-1 β in aging rats. Investigating this mechanistically, it was found that hepatocytes from aged rats had increased N-SMase activity whereas expression levels of other pathways components including IL-1 β receptor I, JNK, IRAK-1, and transforming growth factor- β -activated kinase-1 were not significantly different. This suggested that enhanced N-SMase activity is a key part of the mechanism underlying IL-1 β hypersensitivity. Consistent with this, it was reported that nSMase2 overexpression in hepatocytes from young rats leads to reduced IRAK-1 degradation and increased JNK phosphorylation whereas the inhibition of N-SMase significantly abrogated the IL-1 β response in hepatocytes from aged rats. These important studies demonstrated that nSMase2 is both sufficient and required for the onset of hyperresponsive to IL-1 β during aging (Rutkute et al., 2007b). Moreover, it was also revealed that the observed increase in N-SMase activity during aging was caused by a decrease in hepatocyte GSH level (Rutkute et al., 2007a).

Taken together, these studies indicate that the N-SMases, particularly nSMase2, are important mediators of inflammatory signaling, and may have a profound role in aging associated hyperresponsiveness.

Studies of nSMase2-deficient animals

Recent insight into the functional roles of nSMase2 has come from the studies of nSMase2-deficient mice. In 2005, an nSMase2 KO mouse was generated by Stoffel and colleagues.

Interestingly, although multiple nSMases exist, disruption of nSMase2 resulted in the loss of the majority of N-SMase activity in many organs of the nSMase2 KO mouse. This confirms that nSMase2 is an important N-SMase physiologically. This was further emphasized by the complete loss of *in vitro* N-SMase activity in various tissues of a double nSMase1 nSMase2 KO mouse. The nSMase2 KO mouse was characterized by an embryonic and juvenile dwarfism phenotype (Stoffel et al., 2005). This phenotype was most strikingly manifested in the skeleton with knockout animals displaying short statured long bones and joint deformations compared to wild-type animals. Further analysis revealed that nSMase2 knockout animals had lower levels of growth hormone and decreased concentrations of serum insulin-like growth factor and this was speculated to cause the prolonged cell cycle and hypoplasia seen in the KO mice (Stoffel et al., 2005). Further study showed that the defects seen in the nSMase2 KO mouse were completely rescued via transgenic expression of nSMase2 (Stoffel et al., 2007). Importantly, these data gave essential insight into the potential functional roles of nSMase2.

The findings relating to nSMase2 regulation of the skeleton took on greater significance when, in an independent study, a mouse model of osteogenesis and dentinogenesis imperfecta (the fro/fro mouse) was attributed to a mutation of the SMPD3 gene (Aubin et al., 2005). The phenotype of the fro/fro mouse is characterized by smaller size at birth, multiple fractures in long bones and ribs, with severely undermineralized bones but normal cartilage growth. Moreover, tooth and alveolar bone abnormalities linked to hypomineralization were also observed. In 2005, the partial deletion of intron 8 and exon 9 in the *smpd3* gene was identified in the fro/fro mouse. This mutation results in the deletion of the C-terminal 33 amino acids of the nSMase2 protein. Importantly, this includes the crucial catalytic residues Asp638 and His639 which thus renders the fro/fro nSMase2 catalytically inactive. As with the nSMase2 KO mouse, only residual N-SMase activity (~10%) was detected in the brain and fibroblasts of the fro/fro mouse, further emphasizing its importance as a physiological N-SMase. Taken together, this suggests that the inactivation of nSMase2 may be at least partly responsible for fro/fro mouse phenotype.

When considered together, studies of the nSMase2 KO mouse and the fro/fro mouse present clear evidence that nSMase2 plays an important role regulating bone development and formation. This was further supported by a recent study reporting regulation of nSMase2 transcription by bone morphogenetic protein-2 (BMP-2) during osteoblasts maturation (Chae et al., 2009). However, it is important to note that the phenotypes between the fro/fro mouse and nSMase2 KO mouse are somewhat different. Unlike in the fro/fro mouse, no abnormal bone mineralization was observed in the nSMase2 KO mice; indeed, bone in the adult nSMase2 KO mice is even stronger than in the wild-type mouse (Stoffel et al., 2007). The underlying mechanisms of this discrepancy are unclear, and may be age related. This requires further investigation.

Neutral Sphingomyelinase 3 (nSMase3)

In 2000, an N-SMase protein was partially purified from bovine brain and reported as a magnesium-dependent, membrane-bound N-SMase (Bernardo et al., 2000). Subsequently, peptide sequences from the bovine brain N-SMase facilitated the identification of the third mammalian N-SMase protein, termed nSMase3 (Krut et al., 2006). The gene for nSMase3 is localized to human chromosome 2q21.1 and encodes a protein of 866 amino acids with predicted molecular mass of 97.8 kDa. Surprisingly, the amino acid sequence of nSMase3 possessed very low homology to the previously cloned nSMase1 and nSMase2. Indeed, nSMase3 was reported to be relatively highly conserved from higher to lower animals (Krut et al., 2006), suggesting it may form a distinct N-SMase family in its own right. Importantly, nSMase3 was reported to possess both *in vitro* and *in vivo* N-SMase activity, being reported

to modulate sphingolipid levels when transiently or stably expressed (Corcoran et al., 2008; Krut et al., 2006). The nSMase3 protein is a C-tail-anchored integral membrane protein containing an ER signal in the C-terminus and a proline-rich region at the N-terminus, which could potentially mediate protein-protein interactions (Fig. 1) (Corcoran et al., 2008). Northern blot analysis revealed that nSMase3 mRNA was ubiquitously expressed as a 4.6-kb mRNA with the highest expression in striated muscle and heart muscle. Finally, confocal microscopy studies reported localization of nSMase3 to both the ER and Golgi.

Role of nSMase3 in Cellular Signaling

The relatively recent cloning of nSMase3 means that there has been considerably less study on its functional roles than with the other N-SMases, with no independent confirmation of its activity yet. Thus far, though, acute stimulation of MCF-7 cells with TNF- α was reported to activate nSMase3. Notably, this was impaired by expression of dominant negative FAN suggesting that it acts downstream of FAN in the TNF signaling pathway (Krut et al., 2006). Subsequent study revealed that nSMase3 was transcriptionally induced by TNF- α and DNA damaging agents in colon cancer cells (Corcoran et al., 2008). In contrast, p53, a major genotoxic stress regulator, was observed to downregulate nSMase3 expression. Importantly, nSMase3 expression was found to be decreased in several types of primary tumors suggesting that it may play a role in regulating tumorigenesis.

N-SMases and Neuronal Disorders

In addition to the functions outlined above, the N-SMase/ceramide pathway has also been implicated in neuronal degeneration, particularly in Alzheimer's Disease (AD) (Jana et al., 2009). Amyloid- β peptide (A β) is a major constituent of senile plaques in the brains of AD patients, and a number of reports have demonstrated that A β plays roles in the pathogenesis of AD and is toxic to neurons and oligodendrocytes (Grimm et al., 2005; Yang et al., 2004). Indeed, several studies have implicated the nSMase/ceramide pathway in the development of AD through A β . This is suggested by a number of lines of evidence. First, the treatment of cultured neuronal cells with A β was reported to induce N-SMase activity and ceramide accumulation (Jana and Pahan, 2004). Additionally, the cytotoxic effects of A β were abrogated by various pharmacological inhibitors of N-SMases, including, GW4869, 3-O-Me-SM and N-acetyl-L-cysteine (Grimm et al., 2005; Ju et al., 2005; Lee et al., 2004). The use of GW4869 here suggests a role for nSMase2 and, indeed, this is consistent with the high expression of nSMase2 in brain (Hofmann et al., 2000). Importantly, the specific inhibition of nSMase2 using an antisense strategy abolished the A β effects, further supporting a role for nSMase2 in the A β response (Lee et al., 2004; Yang et al., 2004). Finally, research has also shown that the N-SMase inhibitors, GM4869, can downregulate A β levels, and that ceramide can stimulate A β biogenesis by stabilizing beta-site amyloid precursor protein-cleaving enzyme 1 (Grimm et al., 2005; Puglielli et al., 2003). This suggests that nSMases can further enhance A β level through a feedback mechanism. Importantly, there is evidence of altered sphingolipid metabolism in the brains of AD patients with both elevated ceramide and reduced SM levels being reported (He et al., 2008). Moreover, in the same report, A β induced both acid SMase and N-SMase activities in neuronal cell cultures, suggesting that both SMases might collaborate and contribute to A β signaling. Taken together, these data strongly suggest that N-SMase activation, likely the nSMase2 isoform, contributes at least partially to A β cytotoxicity and may be a potential therapeutic target for AD in the future. However, it should also be noted that all three cloned nSMases as well as acid SMase have all been detected in the central nervous system, therefore making it a complex task to elucidate the relative contribution of each SMase. Nevertheless, these data suggest that the various SMase isoforms should be the subject of active investigation relating to the pathogenesis of neuronal disorders.

Conclusions and Future Directions

In recent years, research into the SMases has taken large steps forward with the identification and cloning of the various mammalian N-SMases and the elucidation of their cellular localizations, tissue distributions, and biochemical properties. Consequently, considerable progress has begun to be made regarding the functional roles of the nSMases, particularly nSMase2, in regulating signal transduction and a variety of cell processes. However, although many studies have begun to address the important roles of N-SMases in physiological processes, there remain a large number of unanswered questions pertaining to both the regulation and roles of the various cloned nSMase proteins.

First, it will be important to identify the direct interaction between nSMase with other proteins or lipids. Although many activators and potential physiological roles of nSMases have been identified, the direct *in vivo* N-SMase activators or effectors are largely unknown. Clarification of interactions between nSMase and other molecules will lead to the discovery of missing critical pieces in the nSMase/ceramide signaling pathways.

It will also be an important goal to clarify the substrate specificity for each nSMase. Although all of the cloned nSMase proteins possess *in vitro* N-SMase activity, there is very limited information regarding their substrate preference on various molecular species of SMs (e.g. different acyl-chains). An understanding of the substrate specificity of each nSMase will be essential for the assessment of their metabolic impact on cellular sphingolipid profiles and could provide important information relating to the specific function of each N-SMase and the associated ceramide/SM species that they regulate.

In addition to specific roles of specific lipid species, the compartmentalization of lipids and associated signaling is now appreciated as an important component of signal transduction. Moreover, as the cloned nSMases have been observed in multiple organelles, it is extremely important to not only investigate the mechanisms underlying their cellular trafficking but also to consider the compartment-specific activities and functional consequences. This information will not only provide further insight into sphingolipid signaling and regulation in individual subcellular compartments but will also shed light on the physiological functions of the individual N-SMases.

Finally, there is a great need for further clarifications of both the physiological and pathological roles of the cloned nSMases. Aside from some of the studies of nSMase2 in bone homeostasis and development, the *in vivo* functions of both nSMase1 and nSMase3 are not known. Notably, unlike acid SMase knockout mice, which show SM accumulation in various tissues (Horinouchi et al., 1995; Otterbach and Stoffel, 1995), neither nSMase1 nor nSMase2 KO mice appear to demonstrate large abnormalities in SM levels (Stoffel et al., 2005). Thus, complementary and compensatory roles may exist among the different nSMases, the development of conditional KO mouse, as well as double and/or triple SMase KO mouse strains with different combination of SMases may become an essential tool for our future understanding of the physiological and pathological roles of N-SMases.

Acknowledgments

This work was supported in part by NIH grant GM43825.

Abbreviations

SMase	sphingomyelinase
-------	------------------

nSMase	neutral sphingomyelinase
SM	sphingomyelin
IL	interleukin
ER	endoplasmic reticulum
ROS	reactive oxygen species
HAE	human airway epithelial
TNF	tumor necrosis factor
GSH	glutathione
FAN	factor associated with N-SMase activation
VCAM	vascular cell adhesion molecule-1
ICAM	intercellular adhesion molecule-1
eNOS	endothelial nitric oxide synthase
LPS	lipopolysaccharide
IL-1β	Interleukin 1-beta
IRAK	IL-1 β receptor-associated kinase
KO	knockout
PM	plasma membrane
AD	Alzheimer's disease
Aβ	amyloid- β peptide

References

- Adam-Klages S, Adam D, Wiegmann K, et al. FAN, a novel WD-repeat protein, couples the p55 TNF-receptor to neutral sphingomyelinase. *Cell*. 1996; 86:937–947. [PubMed: 8808629]
- Aubin I, Adams CP, Opsahl S, et al. A deletion in the gene encoding sphingomyelin phosphodiesterase 3 (Smpd3) results in osteogenesis and dentinogenesis imperfecta in the mouse. *Nat Genet*. 2005; 37:803–805. [PubMed: 16025116]
- Bartke N, Hannun YA. Bioactive sphingolipids: metabolism and function. *J Lipid Res*. 2009; 50(Suppl):S91–96. [PubMed: 19017611]
- Bernardo K, Krut O, Wiegmann K, et al. Purification and characterization of a magnesium-dependent neutral sphingomyelinase from bovine brain. *J Biol Chem*. 2000; 275:7641–7647. [PubMed: 10713073]
- Birbes H, Bawab SE, Obeid LM, Hannun YA. Mitochondria and ceramide: intertwined roles in regulation of apoptosis. *Adv Enzyme Regul*. 2002; 42:113–129. [PubMed: 12123710]
- Chae YM, Heo SH, Kim JY, Lee JM, Ryoo HM, Cho JY. Upregulation of smpd3 via BMP2 stimulation and Runx2. *BMB Rep*. 2009; 42:86–90. [PubMed: 19250608]
- Chalfant CE, Spiegel S. Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. *J Cell Sci*. 2005; 118:4605–4612. [PubMed: 16219683]
- Clarke CJ, Guthrie JM, Hannun YA. Regulation of neutral sphingomyelinase-2 (nSMase2) by tumor necrosis factor- α involves protein kinase C- δ in lung epithelial cells. *Mol Pharmacol*. 2008; 74:1022–1032. [PubMed: 18653803]
- Clarke CJ, Hannun YA. Neutral sphingomyelinases and nSMase2: bridging the gaps. *Biochim Biophys Acta*. 2006; 1758:1893–1901. [PubMed: 16938269]

- Clarke CJ, Snook CF, Tani M, Matmati N, Marchesini N, Hannun YA. The extended family of neutral sphingomyelinases. *Biochemistry*. 2006; 45:11247–11256. [PubMed: 16981685]
- Clarke CJ, Truong TG, Hannun YA. Role for neutral sphingomyelinase-2 in tumor necrosis factor alpha-stimulated expression of vascular cell adhesion molecule-1 (VCAM) and intercellular adhesion molecule-1 (ICAM) in lung epithelial cells: p38 MAPK is an upstream regulator of nSMase2. *J Biol Chem*. 2007; 282:1384–1396. [PubMed: 17085432]
- Cogolludo A, Moreno L, Frazziano G, et al. Activation of neutral sphingomyelinase is involved in acute hypoxic pulmonary vasoconstriction. *Cardiovasc Res*. 2009; 82:296–302. [PubMed: 19088082]
- Coleman DC, Arbuthnott JP, Pomeroy HM, Birkbeck TH. Cloning and expression in *Escherichia coli* and *Staphylococcus aureus* of the beta-lysin determinant from *Staphylococcus aureus*: evidence that bacteriophage conversion of beta-lysin activity is caused by insertional inactivation of the beta-lysin determinant. *Microb Pathog*. 1986; 1:549–564. [PubMed: 3334158]
- Corcoran CA, He Q, Ponnusamy S, Ogretmen B, Huang Y, Sheikh MS. Neutral sphingomyelinase-3 is a DNA damage and nongenotoxic stress-regulated gene that is deregulated in human malignancies. *Mol Cancer Res*. 2008; 6:795–807. [PubMed: 18505924]
- De Palma C, Meacci E, Perrotta C, Bruni P, Clementi E. Endothelial nitric oxide synthase activation by tumor necrosis factor alpha through neutral sphingomyelinase 2, sphingosine kinase 1, and sphingosine 1 phosphate receptors: a novel pathway relevant to the pathophysiology of endothelium. *Arterioscler Thromb Vasc Biol*. 2006; 26:99–105. [PubMed: 16269668]
- Duan RD. Alkaline sphingomyelinase: an old enzyme with novel implications. *Biochim Biophys Acta*. 2006; 1761:281–291. [PubMed: 16631405]
- El Alwani M, Wu BX, Obeid LM, Hannun YA. Bioactive sphingolipids in the modulation of the inflammatory response. *Pharmacol Ther*. 2006; 112:171–183. [PubMed: 16759708]
- Fensome AC, Rodrigues-Lima F, Josephs M, Paterson HF, Katan M. A neutral magnesium-dependent sphingomyelinase isoform associated with intracellular membranes and reversibly inhibited by reactive oxygen species. *J Biol Chem*. 2000; 275:1128–1136. [PubMed: 10625655]
- Futerman AH. Intracellular trafficking of sphingolipids: relationship to biosynthesis. *Biochim Biophys Acta*. 2006; 1758:1885–1892. [PubMed: 16996025]
- Goswami R, Ahmed M, Kilkus J, Han T, Dawson SA, Dawson G. Differential regulation of ceramide in lipid-rich microdomains (rafts): antagonistic role of palmitoyl:protein thioesterase and neutral sphingomyelinase 2. *J Neurosci Res*. 2005; 81:208–217. [PubMed: 15929065]
- Grimm MO, Grimm HS, Patzold AJ, et al. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol*. 2005; 7:1118–1123. [PubMed: 16227967]
- Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol*. 2008; 9:139–150. [PubMed: 18216770]
- He X, Huang Y, Li B, Gong CX, Schuchman EH. Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol Aging*. 2008
- Hofmann K, Tomiuk S, Wolff G, Stoffel W. Cloning and characterization of the mammalian brain-specific, Mg²⁺-dependent neutral sphingomyelinase. *Proc Natl Acad Sci U S A*. 2000; 97:5895–5900. [PubMed: 10823942]
- Horinouchi K, Erlich S, Perl DP, et al. Acid sphingomyelinase deficient mice: a model of types A and B Niemann-Pick disease. *Nat Genet*. 1995; 10:288–293. [PubMed: 7670466]
- Ito H, Murakami M, Furuhashi A, et al. Transcriptional regulation of neutral sphingomyelinase 2 gene expression of a human breast cancer cell line, MCF-7, induced by the anti-cancer drug, daunorubicin. *Biochim Biophys Acta*. 2009; 1789:681–690. [PubMed: 19698806]
- Jana A, Hogan EL, Pahan K. Ceramide and neurodegeneration: susceptibility of neurons and oligodendrocytes to cell damage and death. *J Neurol Sci*. 2009; 278:5–15. [PubMed: 19147160]
- Jana A, Pahan K. Fibrillar amyloid-beta peptides kill human primary neurons via NADPH oxidase-mediated activation of neutral sphingomyelinase. Implications for Alzheimer's disease. *J Biol Chem*. 2004; 279:51451–51459. [PubMed: 15452132]
- Jenkins RW, Canals D, Hannun YA. Roles and Regulation of Secretory and Lysosomal Acid Sphingomyelinase. *Cell Signal*. 2009; 6:836–846. [PubMed: 19385042]

- Josephs M, Katan M, Rodrigues-Lima F. Irreversible inactivation of magnesium-dependent neutral sphingomyelinase 1 (NSM1) by peroxynitrite, a nitric oxide-derived oxidant. *FEBS Lett.* 2002; 531:329–334. [PubMed: 12417336]
- Ju TC, Chen SD, Liu CC, Yang DI. Protective effects of S-nitrosoglutathione against amyloid beta-peptide neurotoxicity. *Free Radic Biol Med.* 2005; 38:938–949. [PubMed: 15749390]
- Jung SY, Suh JH, Park HJ, et al. Identification of multiple forms of membrane-associated neutral sphingomyelinase in bovine brain. *J Neurochem.* 2000; 75:1004–1014. [PubMed: 10936181]
- Karakashian AA, Giltiay NV, Smith GM, Nikolova-Karakashian MN. Expression of neutral sphingomyelinase-2 (NSMase-2) in primary rat hepatocytes modulates IL-beta-induced JNK activation. *Faseb J.* 2004; 18:968–970. [PubMed: 15059969]
- Kim RH, Takabe K, Milstien S, Spiegel S. Export and functions of sphingosine-1-phosphate. *Biochim Biophys Acta.* 2009; 1791:692–696. [PubMed: 19268560]
- Kim WJ, Okimoto RA, Purton LE, et al. Mutations in the neutral sphingomyelinase gene SMPD3 implicate the ceramide pathway in human leukemias. *Blood.* 2008; 111:4716–4722. [PubMed: 18299447]
- Kitatani K, Idkowiak-Baldys J, Hannun YA. The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal.* 2008; 20:1010–1018. [PubMed: 18191382]
- Kolmakova A, Kwiterovich P, Virgil D, et al. Apolipoprotein C-I induces apoptosis in human aortic smooth muscle cells via recruiting neutral sphingomyelinase. *Arterioscler Thromb Vasc Biol.* 2004; 24:264–269. [PubMed: 14670935]
- Krut O, Wiegmann K, Kashkar H, Yazdanpanah B, Kronke M. Novel tumor necrosis factor-responsive mammalian neutral sphingomyelinase-3 is a C-tail-anchored protein. *J Biol Chem.* 2006; 281:13784–13793. [PubMed: 16517606]
- Lee JT, Xu J, Lee JM, et al. Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J Cell Biol.* 2004; 164:123–131. [PubMed: 14709545]
- Levy M, Castillo SS, Goldkorn T. nSMase2 activation and trafficking are modulated by oxidative stress to induce apoptosis. *Biochem Biophys Res Commun.* 2006; 344:900–905. [PubMed: 16631623]
- Levy M, Khan E, Careaga M, Goldkorn T. Neutral sphingomyelinase 2 is activated by cigarette smoke to augment ceramide-induced apoptosis in lung cell death. *Am J Physiol Lung Cell Mol Physiol.* 2009; 297:L125–133. [PubMed: 19395669]
- Marchesini N, Jones JA, Hannun YA. Confluence induced threonine41/serine45 phospho-beta-catenin dephosphorylation via ceramide-mediated activation of PP1cgamma. *Biochim Biophys Acta.* 2007; 1771:1418–1428. [PubMed: 17996206]
- Marchesini N, Luberto C, Hannun YA. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. *J Biol Chem.* 2003; 278:13775–13783. [PubMed: 12566438]
- Marchesini N, Osta W, Bielawski J, Luberto C, Obeid LM, Hannun YA. Role for mammalian neutral sphingomyelinase 2 in confluence-induced growth arrest of MCF7 cells. *J Biol Chem.* 2004; 279:25101–25111. [PubMed: 15051724]
- Matmati N, Hannun YA. Thematic review series: sphingolipids. ISC1 (inositol phosphosphingolipid-phospholipase C), the yeast homologue of neutral sphingomyelinases. *J Lipid Res.* 2008; 49:922–928. [PubMed: 18305313]
- Milhas D, Clarke CJ, Hannun YA. Sphingomyelin metabolism at the plasma membrane: Implications for bioactive sphingolipids. *FEBS Lett.* 2009
- Mizutani Y, Tamiya-Koizumi K, Nakamura N, Kobayashi M, Hirabayashi Y, Yoshida S. Nuclear localization of neutral sphingomyelinase 1: biochemical and immunocytochemical analyses. *J Cell Sci.* 2001; 114:3727–3736. [PubMed: 11707524]
- Modrak DE, Gold DV, Goldenberg DM. Sphingolipid targets in cancer therapy. *Mol Cancer Ther.* 2006; 5:200–208. [PubMed: 16505092]
- Morales A, Lee H, Goni FM, Kolesnick R, Fernandez-Checa JC. Sphingolipids and cell death. *Apoptosis.* 2007; 12:923–939. [PubMed: 17294080]

- Navas P, Fernandez-Ayala DM, Martin SF, et al. Ceramide-dependent caspase 3 activation is prevented by coenzyme Q from plasma membrane in serum-deprived cells. *Free Radic Res.* 2002; 36:369–374. [PubMed: 12069099]
- Nikolova-Karakashian M, Karakashian A, Rutkute K. Role of neutral sphingomyelinases in aging and inflammation. *Subcell Biochem.* 2008; 49:469–486. [PubMed: 18751923]
- Nikolova-Karakashian M, Morgan ET, Alexander C, Liotta DC, Merrill AH Jr. Bimodal regulation of ceramidase by interleukin-1beta. Implications for the regulation of cytochrome p450 2C11. *J Biol Chem.* 1997; 272:18718–18724. [PubMed: 9228043]
- Novgorodov SA, Gudiz TI. Ceramide and mitochondria in ischemia/reperfusion. *J Cardiovasc Pharmacol.* 2009; 53:198–208. [PubMed: 19247196]
- Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer.* 2004; 4:604–616. [PubMed: 15286740]
- Otterbach B, Stoffel W. Acid sphingomyelinase-deficient mice mimic the neurovisceral form of human lysosomal storage disease (Niemann-Pick disease). *Cell.* 1995; 81:1053–1061. [PubMed: 7600574]
- Perry DK. Serine palmitoyltransferase: role in apoptotic de novo ceramide synthesis and other stress responses. *Biochim Biophys Acta.* 2002; 1585:146–152. [PubMed: 12531548]
- Philipp S, Puchert M, Adam-Klages S, et al. The Polycomb group protein EED couples TNF receptor 1 to neutral sphingomyelinase. *Proc Natl Acad Sci U S A.* 2010; 107:1112–1117. [PubMed: 20080539]
- Puglielli L, Ellis BC, Saunders AJ, Kovacs DM. Ceramide stabilizes beta-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid beta-peptide biogenesis. *J Biol Chem.* 2003; 278:19777–19783. [PubMed: 12649271]
- Rodrigues-Lima F, Fensome AC, Josephs M, Evans J, Veldman RJ, Katan M. Structural requirements for catalysis and membrane targeting of mammalian enzymes with neutral sphingomyelinase and lysophospholipid phospholipase C activities. Analysis by chemical modification and site-directed mutagenesis. *J Biol Chem.* 2000; 275:28316–28325. [PubMed: 10871611]
- Rutkute K, Asmis RH, Nikolova-Karakashian MN. Regulation of neutral sphingomyelinase-2 by GSH: a new insight to the role of oxidative stress in aging-associated inflammation. *J Lipid Res.* 2007a; 48:2443–2452. [PubMed: 17693623]
- Rutkute K, Karakashian AA, Giltiay NV, Dobierzewska A, Nikolova-Karakashian MN. Aging in rat causes hepatic hyperresponsiveness to interleukin-1beta which is mediated by neutral sphingomyelinase-2. *Hepatology.* 2007b; 46:1166–1176. [PubMed: 17668873]
- Sawai H, Domae N, Nagan N, Hannun YA. Function of the cloned putative neutral sphingomyelinase as lyso-platelet activating factor-phospholipase C. *J Biol Chem.* 1999; 274:38131–38139. [PubMed: 10608884]
- Sawai H, Okamoto Y, Luberto C, et al. Identification of ISC1 (YER019w) as inositol phosphosphingolipid phospholipase C in *Saccharomyces cerevisiae*. *J Biol Chem.* 2000; 275:39793–39798. [PubMed: 11006294]
- Schneider PB, Kennedy EP. Sphingomyelinase in normal human spleens and in spleens from subjects with Niemann-Pick disease. *J Lipid Res.* 1967; 8:202–209. [PubMed: 4962590]
- Schuchman EH. The pathogenesis and treatment of acid sphingomyelinase-deficient Niemann-Pick disease. *J Inherit Metab Dis.* 2007; 30:654–663. [PubMed: 17632693]
- Schulze J, Albers J, Baranowsky A, et al. Osteolytic prostate cancer cells induce the expression of specific cytokines in bone-forming osteoblasts through a Stat3/5-dependent mechanism. *Bone.* 2009
- Somenzi G, Sala G, Rossetti S, Ren M, Ghidoni R, Sacchi N. Disruption of retinoic acid receptor alpha reveals the growth promoter face of retinoic acid. *PLoS One.* 2007; 2:e836. [PubMed: 17786207]
- Stoffel W. Functional analysis of acid and neutral sphingomyelinases in vitro and in vivo. *Chem Phys Lipids.* 1999; 102:107–121. [PubMed: 11001565]
- Stoffel W, Jenke B, Block B, Zumbansen M, Koebke J. Neutral sphingomyelinase 2 (smpd3) in the control of postnatal growth and development. *Proc Natl Acad Sci U S A.* 2005; 102:4554–4559. [PubMed: 15764706]

- Stoffel W, Jenke B, Holz B, et al. Neutral sphingomyelinase (SMPD3) deficiency causes a novel form of chondrodysplasia and dwarfism that is rescued by Col2A1-driven *smpd3* transgene expression. *Am J Pathol.* 2007; 171:153–161. [PubMed: 17591962]
- Tani M, Hannun YA. Analysis of membrane topology of neutral sphingomyelinase 2. *FEBS Lett.* 2007a; 581:1323–1328. [PubMed: 17349629]
- Tani M, Hannun YA. Neutral sphingomyelinase 2 is palmitoylated on multiple cysteine residues. Role of palmitoylation in subcellular localization. *J Biol Chem.* 2007b; 282:10047–10056. [PubMed: 17272284]
- Tcherkasowa AE, Adam-Klages S, Kruse ML, et al. Interaction with factor associated with neutral sphingomyelinase activation, a WD motif-containing protein, identifies receptor for activated C-kinase 1 as a novel component of the signaling pathways of the p55 TNF receptor. *J Immunol.* 2002; 169:5161–5170. [PubMed: 12391233]
- Tellier E, Negre-Salvayre A, Bocquet B, et al. Role for furin in tumor necrosis factor alpha-induced activation of the matrix metalloproteinase/sphingolipid mitogenic pathway. *Mol Cell Biol.* 2007; 27:2997–3007. [PubMed: 17283058]
- Tepper AD, Ruurs P, Borst J, van Blitterswijk WJ. Effect of overexpression of a neutral sphingomyelinase on CD95-induced ceramide production and apoptosis. *Biochem Biophys Res Commun.* 2001; 280:634–639. [PubMed: 11162568]
- Tomiuk S, Hofmann K, Nix M, Zumbansen M, Stoffel W. Cloned mammalian neutral sphingomyelinase: functions in sphingolipid signaling? *Proc Natl Acad Sci U S A.* 1998; 95:3638–3643. [PubMed: 9520418]
- Tomiuk S, Zumbansen M, Stoffel W. Characterization and subcellular localization of murine and human magnesium-dependent neutral sphingomyelinase. *J Biol Chem.* 2000; 275:5710–5717. [PubMed: 10681556]
- Tonnetti L, Veri MC, Bonvini E, D'Adamio L. A role for neutral sphingomyelinase-mediated ceramide production in T cell receptor-induced apoptosis and mitogen-activated protein kinase-mediated signal transduction. *J Exp Med.* 1999; 189:1581–1589. [PubMed: 10330437]
- Vance JE, Steenbergen R. Metabolism and functions of phosphatidylserine. *Prog Lipid Res.* 2005; 44:207–234. [PubMed: 15979148]
- Wheeler D, Knapp E, Bandaru VV, et al. Tumor necrosis factor- α -induced neutral sphingomyelinase-2 modulates synaptic plasticity by controlling the membrane insertion of NMDA receptors. *J Neurochem.* 2009; 109:1237–1249. [PubMed: 19476542]
- Won JS, Im YB, Khan M, Singh AK, Singh I. The role of neutral sphingomyelinase produced ceramide in lipopolysaccharide-mediated expression of inducible nitric oxide synthase. *J Neurochem.* 2004; 88:583–593. [PubMed: 14720208]
- Yabu T, Imamura S, Yamashita M, Okazaki T. Identification of Mg^{2+} -dependent neutral sphingomyelinase 1 as a mediator of heat stress-induced ceramide generation and apoptosis. *J Biol Chem.* 2008; 283:29971–29982. [PubMed: 18678863]
- Yabu T, Shimizu A, Yamashita M. A novel mitochondrial sphingomyelinase in zebrafish cells. *J Biol Chem.* 2009; 284:20349–20363. [PubMed: 19429680]
- Yamada A, Tsukagoshi N, Udaka S, et al. Nucleotide sequence and expression in *Escherichia coli* of the gene coding for sphingomyelinase of *Bacillus cereus*. *Eur J Biochem.* 1988; 175:213–220. [PubMed: 2841128]
- Yang DI, Yeh CH, Chen S, Xu J, Hsu CY. Neutral sphingomyelinase activation in endothelial and glial cell death induced by amyloid beta-peptide. *Neurobiol Dis.* 2004; 17:99–107. [PubMed: 15350970]
- Zheng W, Kollmeyer J, Symolon H, et al. 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.* 2006; 1758:1864–1884. [PubMed: 17052686]
- Zumbansen M, Stoffel W. Neutral sphingomyelinase 1 deficiency in the mouse causes no lipid storage disease. *Mol Cell Biol.* 2002; 22:3633–3638. [PubMed: 11997500]

Fig. 1. Schematic illustration of domains in human nSMases, nSMase1 (GenBank accession numbers O60906), nSMase2 (GenBank accession numbers Q9NY59) and nSMase3 (GenBank accession numbers NP060421).

Fig. 2. Signaling roles of nSMases. Studies on nSMases1–3 have led to the identification of many activators and potential physiological roles of nSMases.