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Serial Review: Redox-Regulated Phospholipase Signal Transduction
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Sphingolipid signaling and redox regulation☆

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Abstract

Sphingolipids including ceramide and its derivatives such as ceramide-1-phosphate, glycosyl-ceramide, and sphinogosine (-1-phosphate) are now recognized as novel intracellular signal mediators for regulation of inflammation, apoptosis, proliferation, and differentiation. One of the important and regulated steps in these events is the generation of these sphingolipids via hydrolysis of sphingomyelin through the action of sphingomyelinases (SMase). Several lines of evidence suggest that reactive oxygen species (ROS; O_2^-, H_2O_2, and OH^-,) and reactive nitrogen species (RNS; NO, and $ONOO^-$) and cellular redox potential, which is mainly regulated by cellular glutathione (GSH), are tightly linked to the regulation of SMase activation. On the other hand, sphingolipids are also known to play an important role in maintaining cellular redox homeostasis through regulation of NADPH oxidase, mitochondrial integrity, and antioxidant enzymes. Therefore, this paper reviews the relationship between cellular redox and sphingolipid metabolism and its biological significance.

Keywords: Catalase; Ceramidase; Glycosylceramide; Mitochondria; NADPH oxidase; Nitric oxide synthase; Reactive nitrogen species; Reactive oxygen species; Sphingolipid; Sphingomyelinase; Superoxide dismutase

Contents

Abbreviations: A-SMase, acidic sphingomyelinase; BH_4, (6*R*)-5,6,7,8-tetrahydro-L-biopterin; DTT, dithiothreitol; eNOS, endothelial nitric oxide synthase; FAN, factor associated with N-SMase activation; GalT-2, galactosyl transferase-2; GPX, glutathione peroxidase; GSH, reduced form of glutathione; iNOS, inducible nitric oxide synthase; lyso-PAF, lyso-platelet-activating factor; nNOS, neuronal nitric oxide synthase; N-SMase, neutral sphingomyelinase; NO, nitric oxide; Phox, phagocytic NADPH oxidase; PI3K, phosphatidylinositol-3′-kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PPAR, peroxisome proliferator-activated receptor; RNS, reactive nitrogen species; ROS, reactive oxygen species; SM, sphingomyelin; SMase, sphingomyelinases; SOD, superoxide dismutase; TNF-R1, tumor necrosis factor-receptor I; TPX, thioredoxin peroxidase.

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Introduction

Under normal conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be generated as a by-product of normal metabolic processes and function as physiological signaling molecules [1,2]. However, in pathological conditions, the excessive increase in ROS, such as super oxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$), and RNS, such as nitric oxide (NO) and peroxynitrite ($ONOO^-$), by mitochondrial dysfunction, activation of xanthine, and NADPH oxidases and increased gene expression of inducible nitric oxide synthase (iNOS) can cause cell death and tissue damage [3]. Under normal conditions, the O_2^- is scavenged by superoxide dismutase (SOD) which specifically processes O_2^- and produces H_2O_2. The H_2O_2 is in turn detoxified by catalase and glutathione peroxidase (GPX) because otherwise H_2O_2 would react with transition metals to generate highly toxic hydroxyl radicals through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow {}^-OH + Fe^{3+} + {}^{\bullet}OH$). Moreover, RNS are also able to affect cellular redox homeostasis [2], such as the physiologically relevant action of NO in the activation of the guanylate cyclase and the subsequent activation of cGMP-mediated signaling cascades, whereas an excessive amount of NO can cause cell and tissue damage [3,4]. NO can scavenge O_2^- and other free radicals and inhibit the O_2^- driven Fenton reaction and lipid peroxidation. On the other hand, large amounts of NO as generated by the iNOS isoform in inflammatory disease conditions are often accompanied by a large production of ROS, and will shift NO chemistry toward indirect effects such as nitrosation, nitration, and oxidation [2,5]. The interaction of NO with molecular oxygen (O_2) or O_2^- gives rise to the formation of the potent nitrosating agent N_2O_3 and peroxynitrite ($ONOO^-$), respectively. *S*-Nitrosothiol adducts are formed by the interaction between N_2O_3 and certain protein thiol groups and evoke signaling by altering protein kinases and phosphatases, G-proteins, ion channels, protein tyrosine kinases, and redox-sensitive transcription factors [5,6].

Sphingolipids are ubiquitous constituents of membrane lipids in mammalian cells. Along with their structural role, sphingolipids have received attention due to their role as second messengers in proliferation, differentiation, apoptosis, and inflammation [7–12]. In mammalian cells, the majority of sphingolipids are colocalized with cholesterol in specific membrane domains called “lipid rafts” also known as “detergent-resistant membrane domains” due to their insoluble property in non-ionic detergents such as Triton X-100 [13,14]. These specialized membrane microdomains contain a variety of sphingolipid-metabolizing enzymes such as sphingomyelinase (SMase) [15,16], ceramidase [17], sphingosine kinase [18], and ceramide kinase [19]. Dobrowsky reported that depletion of membrane cholesterol abolished $p75^{NTR}$-dependent sphingomyelin hydrolysis and ceramide generation [13]. Due to the localization of sphingolipid-metabolizing enzyme in lipid rafts and the strong association of sphingolipids with cholesterol, cholesterol appears to affect sphingomyelin metabolism through modulation of lipid raft integrity. The lipid rafts also contain a variety of receptors and signaling enzymes, such as GTPases and kinases, and mediate receptor-mediated intracellular signaling cascades and membrane trafficking [20,21]. Therefore, it is now believed that the regulation of sphingolipid metabolism in these membrane domains may be linked to various cellular signaling events as well as cellular cholesterol levels.

Recently, it has been reported that ROS and RNS are involved in sphingolipid metabolism. For example, the depletion of cellular reduced glutathione (GSH) by increased ROS and RNS regulates enzymatic activities of SMases and ceramidase [22–25]. Conversely, sphingolipids including ceramide, sphingosine, and sphingosine-1-phosphate have the ability to regulate cellular redox homeostasis through regulation of NADPH oxidase [26], mitochondrial integrity [27], NOS [10,28,29], and antioxidant enzymes [30,31]. Therefore, in this review, we discuss the mechanisms of activation and regulation of enzymes which are involved in sphingomyelin metabolism and redox regulation.

Regulation of sphingolipid metabolism by oxidative stress

Sphingomyelin (SM) is a constituent of membrane lipids and mainly localized in the plasma membrane. At least two different subtypes of SMases are involved in SM hydrolysis which is regulated by intra- or extracellular stimuli. These are identified based on pH optima, subcellular localization, and cation dependence. The acidic, or lysosomal, sphingomyelinase (A-SMase or SMPD1) was the first sphingomyelinase to be identified and subjected to intensive investigation due to its role in ceramide generation [32,33]. Later, the neutral, membrane-bound Mg^{2+}-dependent SMases 1 and 2 (SMPD2 and 3) were cloned [34] and characterized [35,36]. Although the detailed mechanism for the activation of these SMases is still under

investigation, cellular redox potential is regarded as one of the key regulators for the activation of these enzymes.

Acid sphingomyelinase

A-SMase, which is deficient in patients affected with type A and B Niemann-Pick disease, has been known to play a role in stress signaling and apoptosis [37]. So far three types of human A-SMase have been cloned (types I, II, and III). These are generated by alternative splicing from a single transcript and only type I, which is major A-SMase species, has functional A-SMase activity [38]. A-SMase is generated from a 75-kDa proprecursor by proteolytic processing to a 72-kDa protein in ER/Golgi and further processing in endosome-lysosome to the fully active 70-kDa enzyme [39]. A-SMase is also found as an extracellular form and this secretary A-SMase is encoded from the same gene that encodes the lysosomal form of type I A-SMase but with different posttranslational modification [40].

The mechanism for A-SMase activation is not fully understood at present but several factors are identified to be involved in its activation or inhibition. These include tumor necrosis factor-receptor I (TNF-R1) [41,42], ApoC-III, an apolipoprotein [43], phosphatidylinositol-3′-kinase (PI3K)/protein kinase B (Akt) [44], and certain lipids such as mono-, di-, and triacylglycerols [45], 1,2-diacylglycerol [46], and sphingosine-1-phosphate [47]. A-SMase is also known to be regulated by thiol oxidation. Although the enzymatic activity of A-SMase is not affected by GSH, it is inhibited by DTT in a dose-dependent manner [48,49]. The inactivation by DTT may not simply be due to disulfide reduction because effects of DTT on activity were reported to be unrelated to disulfide reduction [50]. Interestingly, Qiu and co-workers [23] have shown that the C-terminal cysteine (Cys^{629}) is an unbridged free form and modification of this thiol group by dimerization, chemical modification, or deletion increases the enzymatic activity of A-SMase. Notably, restoration of the thiol group with DTT inhibits copper-mediated dimerization as well as activation of A-SMase [23]. Therefore, it is possible that oxidative modification of the C-terminal cysteine may be required for full activation of A-SMase and that certain antioxidants such as DTT may counteract by restoring the thiol group (Fig. 1).

In vitro studies have shown that NO plays a role in the regulation of A-SMase activity. NO from exogenous or endogenous sources has been known to inhibit TNF-α-mediated apoptosis via inhibition of ceramide generation [24,51]. Although the mechanism for NO-mediated inhibition of A-SMase is not fully understood, a NO-mediated increase in cGMP and the activation of cGMP-dependent protein kinase (PKG) have been suggested to be involved in the inhibition of A-SMase [52,53]. However, excessive amounts of NO are also known to activate apoptosis via activation of ceramide generation which is sensitive to A-SMase inhibitor [54]. NO-

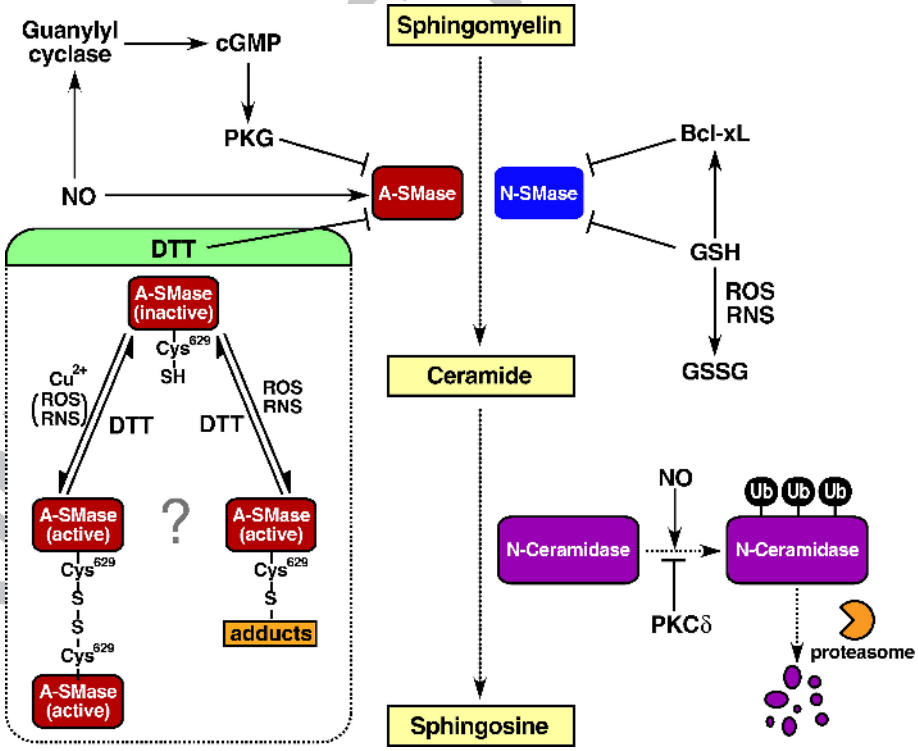

Fig. 1. The possible regulatory mechanism of sphingolipid metabolism by cellular redox potential. In mammalian cells, sphingomyelin is hydrolyzed by neutral (N-SMase) and acid sphingomyelinase (A-SMase). N-SMase is regulated by cellular level of reduced and oxidized forms of glutathione (GSH and GSSH) which are affected by antioxidant protein, Bcl-xL, and reactive oxygen or nitrogen species (ROS and RNS). Although the activity of A-SMase is not affected by GSH, it is inhibited by dithiothreitol (DTT) and cGMP-mediated pathway. The adduct formation on $cystein^{629}$ is known to enhance the activity of A-SMase. However, whether ROS and RNS are implicated in the formation of adduct on $cystein^{629}$ and whether DTT counteracts ROS/RNS-mediated adduct formation are not known at present. Ceramide accumulation is also regulated by the activity of neutral ceramidase (N-ceramidase). Nitric oxide (NO) is known to activate N-ceramidase degradation through the ubiquitine/proteasome pathway. Solid arrow and T-shaped heads represent stimulatory and inhibitory effects, respectively.

mediated interaction between A-SMase and procaspase-3 [55] suggests the possible regulation of A-SMase activity by NO through interaction with other proteins. Indeed, several protein molecules have been identified to be regulated posttranslationally by NO through *S*-nitrosylation-mediated adduct formation [56]. Therefore, posttranslational modification of A-SMase by *S*-nitrosylation, in particular on C-terminal cysteine (Cys^{629}), and its role in A-SMase activity should be an interesting investigation (Fig. 1).

Neutral sphingomyelinase

The presence of N-SMase which has neutral pH optimum (~7.4) and magnesium dependency was firstly described in 1967 by Schneider and Kennedy [57]. Later N-SMase1 was cloned and characterized in mice and humans by Tomiuk and co-workers [36]. The cloned human N-SMase1 (sphingomyelin phosphodiesterases; *smpd2*) gene is localized on chromosome 6 and encodes proteins with a predicted molecular mass of 47.6 kDa with two putative transmembrane domains at the C terminus [36]. Although it shows activity for SM hydrolysis in vitro [58], it shows no change of SM hydrolysis as compared to increased hydrolysis of 1-*O*-alkyl-lyso-phosphatidylcholine (lyso-platelet-activating factor or lyso-PAF) in N-SMase1-overexpressing cells, suggesting that the cloned enzyme is actually a lyso-PAF phospholipase C, but not N-SMase [58]. More recently, Hofmann and co-workers cloned and characterized another mammalian brain-specific magnesium-dependent N-SMase (N-SMase2; *smpd3*) from humans and mice [35]. Human N-SMase 2 gene is localized in chromosome 16 and encodes proteins of 655 amino acids, resulting in a predicted molecular mass of 71 kDa. The N terminus contains two predicted transmembrane domains, whereas the C terminus contains the putative catalytic domain. In contrast to N-SMase1, the N-SMase2 showed properties similar to the previously purified rat brain N-SMase but had no activity against lyso-PAF [35]. The primary subcellular localization of N-SMase2 was described to be in the Golgi [35]; however, later, its localization was reported to be in the plasma membrane [59].

The mechanism of N-SMase activation has been intensely studied during the past decade. In addition to bioactive lipids, such as arachidonic acid [35,36], anionic phospholipids (i.e., cardiolipin and phosphatidylglycerol) [60], and phosphatidylserine [61], N-SMase is also reported to be regulated by caspases 3 [62] and 9 [63] and TNF-R1 through FAN (factor associated with N-SMase activation) protein [64]. Upon ligation of TNF-α, TNF-R1 recruits FAN through its N-SMase activation domain (NSD) and activates N-SMase [64]. The role of FAN in N-SMase activation was further supported by other groups using FAN-deficient mice and cells which overexpressed dominant-negative FAN [65,66]. Since N-SMase1 does not exhibit sphingomyelinase activity in vivo, the increased N-SMase activity by these activators appears to be mediated by N-SMase2. However, the detailed mechanism for the activation of N-SMases2 by these activators is not known at present. Moreover, a possible role of other isoforms of N-SMase cannot be excluded. Indeed, the existence of multiple forms of N-SMase in bovine brain was demonstrated previously based on different chromatographic and biochemical properties [67].

Previously, we and other groups have observed that proinflammatory cytokines (i.e., IL-1β and TNF-α) or hypoxia induced SM hydrolysis and ceramide generation in a redox-sensitive event [12,68]. Moreover, $A\beta_{1-42}$ or its synthetic peptide $A\beta_{23-35}$, which produces pathologies of Alzheimer's disease, induces ceramide generation by activation of N-SMase in a redox-sensitive manner without altering A-SMase [69,70]. Therefore, these studies suggest that oxidative stress-mediated N-SMase activation and ceramide generation may play a key role(s) in the pathobiology of various disease conditions. However, the mechanism of regulation of N-SMase by oxidative stress is not completely known. Liu and Hannun showed that GSH, but not DTT and β-mercaptoethanol, dose dependently inhibited partially purified N-SMase activity [49]. They reported that γ-glutamylcysteine, but not the free sulfhydryl group, in GSH may function as an allosteric regulator of N-SMase. GSH was also known to regulate N-SMase activity through Bcl-xL or Bcl-2, an antiapoptotic protein, by inhibiting oxidative stress-mediated SM hydrolysis and ceramide generation [71,72]. Moreover, tyrosine kinases such as Lyn and PKCζ are also implicated in oxidative stress-mediated regulation of N-SMase activity and ceramide generation [73,74]. In addition to GSH, Takeda and associates reported that sodium nitroprusside (SNP), a NO donor, also increases cellular sphingomyelin hydrolysis and ceramide generation through activation of N-SMase [75]. However, whether NO mediates N-SMase activation through direct interaction or depletion of cellular GSH levels is not currently known (Fig. 1).

Ceramidase

In addition to the increase in ceramide levels via activation of A-SMase or N-SMase, the increased ceramide levels may also be due to concomitant inhibition of ceramidases [22]. In renal mesangial cells, NO from an exogenous donor causes a chronic upregulation of ceramide levels by activating sphingomyelinases and concomitantly inhibiting ceramidases, and particularly in the late phase ceramide generation may be responsible for the further processing of a proapoptotic signal [22]. Later, this effect was shown to be due to the action of NO on ubiquitin/proteasome-mediated proteolysis of neutral ceramidase and counterregulated by protein kinase C (PKC), especially the δ-isoform [76,77]. Therefore, neutral ceramidase may represent another novel target for interference with the cellular stress response and modulate programmed cell death, a typical feature of many inflammatory diseases (Fig. 1).

Regulation of redox potential by sphingolipids

As discussed in the preceding section, oxidative stress regulates sphingolipid metabolism to generate sphingolipid molecules which participate in intracellular signaling. On the other hand, a growing body of evidence also suggests that

certain sphingolipids, such as ceramide or its glycosyl derivatives, are able to induce cellular oxidative stress through activation of NADPH oxidase [26], mitochondrial dysfunction [27,78], and NOS [10,28,29], and/or downregulation of antioxidant enzymes [30,31]. Therefore, sphingolipid metabolism and redox homeostasis are regulated in a bidirectional manner.

Regulation of NADPH oxidase

In phagocytes, ROS are generated by a membrane-associated phagocytic NADPH oxidase (Phox; also known as NADPH oxidase-2, Nox2), with its catalytic moiety $gp91^{phox}$, which is activated by assembly with regulatory proteins such as p47phox, p67phox, and Rac [79,80]. Recently, other oxidases similar to the Phox complex have also been identified in other cell types and show different expression patterns depending on cell or tissue types [81,82]. Similar to Nox, dual oxidase (Duox) isoforms (i.e., Duox 1 and 2) include molecular mass gp91phox homologs with an N-terminal peroxidase domain in addition to the C-terminal NADPH oxidase activity.

The involvement of sphingolipids in the regulation of NADPH oxidase activity was first discussed in Gaucher disease type I [83]. Liel and co-workers reported that monocyte dysfunction in Gaucher disease type I patients is caused by suppression of NADPH oxidase-mediated superoxide generation as glucosylceramide (glucocerebroside) accumulates [83]. Recent studies by Moskwa and co-workers further support the role of glycosylceramide in the regulation of NADPH oxidase activity, in which glucosylceramide is able to inhibit NADPH oxidase in a cell-free system [84]. However, other related lipids such as lactosylceramide (CDw17) and ganglioside GD3 have been reported to upregulate NADPH oxidase activity [85–88]. The lactosylceramide-mediated activation of NADPH oxidase and ROS generation is involved in endothelial and neutrophil cell functions through regulation of endothelial cell proliferation, adhesion molecule expression, and phagocytosis [85–87]. Recently, our group also reported the involvement of lactosylceramide-mediated ROS generation in sequential activation of hRas/NFκB and inflammatory gene expression [89]. The exact mechanism of lactosylceramide-mediated activation of NADPH oxidase is not clear, but it is believed that lactosylceramide interactions with a Src family kinase (i.e., Lyn) in the lipid rafts may lead to generation of ROS through phosphatidylinositol-3-kinase-, p38 MAPK-, and PKC-dependent signal transduction pathways [90].

Along with lactosylceramide, ceramide has also been implicated in the regulation of NADPH oxidase. Recently, ceramide-mediated activation of NADPH oxidase and resultant oxidative stress were reported to be involved in endostatin-induced endothelial dysfunction [91]. The mechanism for ceramide-mediated activation of NADPH oxidase is not fully understood but involvement of ceramide-mediated activation of Rac small GTPase, a regulatory component of NADPH oxidase, was suggested recently [92]. Moreover, ceramide activates the NADPH oxidase through activation of PKCζ [93]. PKCζ may also activate $p47^{phox}$ adapter protein via phosphorylation [93], followed by translocation of activated $p47^{phox}$ to membrane to facilitate stimulus-induced binding of $p67^{phox}$ to the holo NADPH enzyme complex [26].

Mitochondrial dysfunction: mitochondrial redox regulation and apoptosis

Mitochondria play a central role in cellular metabolism. They are the site of fatty acid catabolism and the citric acid cycle, which produces NADH and $FADH_2$. These molecules transfer electrons to the respiratory chain, and finally to oxygen, a process that generates ATP. It has long been recognized that the mitochondrial electron transport chain is a site of free radical generation [94]. The two sites where this occurs are complex I (NADH-coQ reductase) and complex III (cytochrome *c* oxidase). The electron leaks from mitochondria and formation of O_2^- have been identified in normal as well as pathological conditions. Mitochondria are also known to play a central role in regulating apoptosis [95]. Mitochondria sense the catastrophic cellular changes and irreversibly commit cells to apoptosis by releasing death factors into the cytosol, such as cytochrome *c* [95], Smac 2/DIABLO [96], AIF [97], and EndoG [98].

Ceramide was reported as a regulator for the generation of ROS and activation of the mitochondrial irreversible apoptotic process. Mitochondria isolated from TNF-α-treated hepatocytes showed a higher content of ceramide, compared to control [27], and addition of C_2-ceramide to mitochondria from untreated cells increased ROS production [27]. Moreover, naturally occurring C_{16} ceramide was shown to cause an increase in ROS generation through mitochondria [78]. Ceramide may function to generate ROS from mitochondria as a consequence of cytochrome *c* release, an electron carrier of the respiration chain between complexes II and III in mitochondria [99]. Ghafourifar et al. have shown that C_2- and C_6-ceramide induce release of cytochrome *c* from isolated mitochondria [100]. Since cytochrome *c* release causes a decrease in mitochondrial oxygen consumption, mitochondrial inner transmembrane potential ($\Delta\Psi_m$), and Ca^{2+} retention and all of which lead to mitochondrial dysfunction and ROS generation [100], ceramide-mediated release of cytochrome *c* may be one of the key events in the induction of ROS generation from mitochondria (Fig. 2).

Ceramide may also affect cellular redox potential through regulation of the Bcl-2 family of proteins, which are regarded as antioxidants because they increase the GSH pool or redistribute GSH to various cellular compartments [101]. Indeed, Bcl-2 was known to prevent ROS production, GSH depletion, and cellular damage caused by lipid peroxidation [102,103] through blocking cytochrome *c* release from mitochondria [104] and/or inhibition of mitochondrial permeability transition pore opening, leading to collapse of $\Delta\Psi_m$, by opposing the effect of Bax, a component of the permeability transition pore [105,106]. Long-term treatment of human keratinocytes with C_2-ceramide induced downregulation of Bcl-2 [107]. Moreover, apoptotic DNA fragmentation following exposure to TNF-α and C_2-ceramide was also associated with downregulation of Bcl-2 mRNA in HL-60 and U-937 cells [108], suggesting the possible

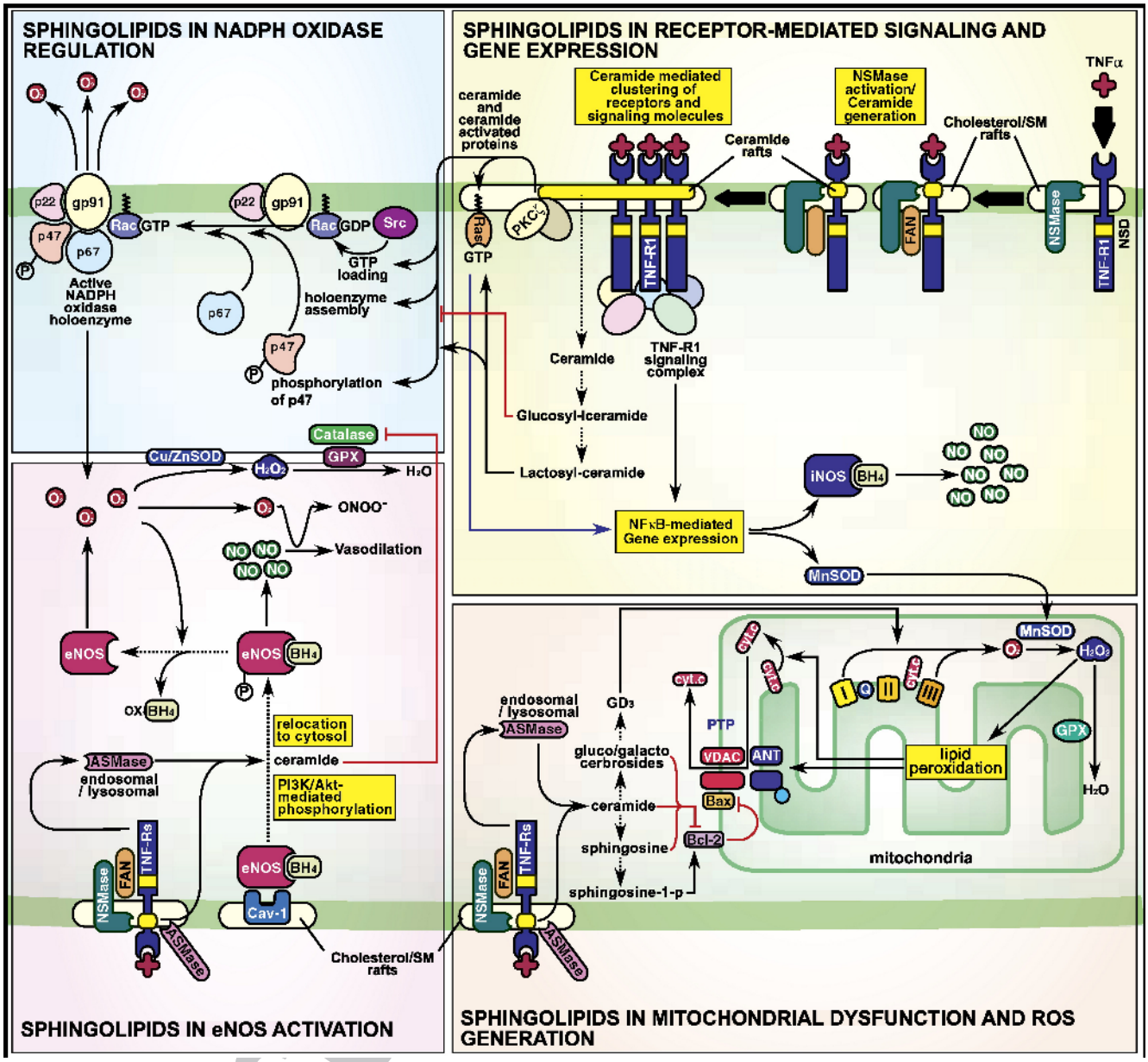

Fig. 2. The possible regulatory mechanism of cellular redox potential by sphingolipid metabolism. Ceramide is one of the key signaling mediators in receptor-mediated signaling cascades. It may activate gene expression of redox enzymes (i.e., iNOS and Mn-SOD) through receptor clustering, recruitment of signaling enzymes, and activation of ceramide-activated protein kinases (i.e., PKCζ and KSR). The ceramide-activated signaling cascades, along with its derivatives such as glucosyl-ceramide and lactosyl-ceramide, may be implicated in the regulation of NADPH oxidase activity through regulation of Rac1 GTP loading, holoenzyme assembly, and/or p47 phosphorylation. Ceramide is also implicated in the activation of eNOS through cytosolic relocation from membrane and phosphorylation by the phosphoinositide 3-phosphate kinase (PI3K)/Akt pathway. However, ceramide is also able to inhibit action of eNOS (vasodilation) through NADPH-mediated superoxide generation (O_2^-) leading to formation of peroxynitrite ($ONOO^-$) or tetrahydrobiopterin (BH_4) oxidation leading to uncoupling of eNOS. Ceramide, sphingosine, and GD3 are potential activators for mitochondrial dysfunction which leads to the production of massive amounts of O_2^-. They produce interference of electron transfer, disruption of mitochondrial inner transmembrane potential ($\Delta\Psi_m$), opening permeability transition pore (PTP), mitochondrial lipid peroxidation, and cytochrome *c* (cyto *c*) release. Ceramide is also known as a potent activator for mitochondrial Mn-SOD gene expression. The increased Mn-SOD by ceramide may be toxic depending on the level of gluthathione peroxidase (GPX) in mitochondria. The role of ceramide on the regulation of cellular GPX is not known at present, but it was known to inhibit catalase activity. Solid arrow and T-shaped heads represent stimulatory and inhibitory effects, respectively.

role for ceramide in the regulation of Bcl-2-mediated anti-oxidant activity (Fig. 2).

Ceramide was also reported to disturb the respiratory chain through direct interaction [109,110] as C_2- and C_6-ceramide treatment induced large pores in phospholipid planar membranes [111]. Interestingly, rat liver mitochondria contain free ceramide [112] and sphingolipid-metabolizing enzymes such as ceramidase [113] and ceramide synthase [114]. Thus, dynamic changes in the ceramide content of mitochondrial membranes by vesicular transport or local production could possibly regulate mitochondrial integrity and ROS generation. Moreover, ceramide can be converted into sphingosine by ceramidase

and sphingosine-1-phosphate by further action of sphingosine kinase, thus expanding the repertoire of downstream signals which might affect cell fate. Sphingosine, as a negative regulator of cell proliferation, is known to promote apoptosis [115]. Moreover, sphingosine is also involved in the down-regulation of Bcl-2 [116] and Bcl-X_L [117], increase in cytochrome *c* release [118,119], mitochondrial $\Delta\Psi$ disruption [120], and mitochondrial generation of H_2O_2 [27]. In contrast, sphingosine 1-phosphate stimulates cell growth and is thus antiapoptotic [115] through regulation of Bcl-2/Bax rheostat [121] and inhibition of cytochrome *c* release [122]. Interestingly, sphingosine kinase-overexpressing cells have decreased levels of both sphingosine and ceramide [123,124] (Fig. 2).

A recent cDNA microarray study showed that the *bcl-2* gene is downregulated in Gaucher disease, suggesting that the accumulation of either glucocerebroside or glucosylsphingosine, as a result of glucocerebrosidase deficiency, affects Bcl-2-mediated redox regulation [125]. In addition, ganglioside GD3 is also known to induce swelling of isolated mitochondria through opening permeability transition pores [126,127]; however, no such effects on mitochondrial permeability are described for other lipids such as GM1, GD1a, GM3, and GT1b. GD3 appears to interfere at the level of complex III of the electron transport chain [88] and GD3-mediated permeability transition pore opening is secondary to reactive oxygen species generation [128]. Therefore, the burst of ROS generation by GD3 could also induce opening of the permeability transition pores leading to cytochrome *c* release (Fig. 2).

Regulation of nitric oxide synthases

Since its discovery, nitric oxide (NO) has become the subject of both intense research and heated debate over its role in various biological and pathophysiological processes. Originally discovered as a mediator of vascular smooth muscle relaxation, NO has since been implicated in a wide range of physiological mechanisms ranging from lysis of tumor cells to neural transmission [129,130]. NO is a metabolic by-product of the conversion of L-arginine to L-citrulline by a class of enzymes dubbed as the nitric oxide synthases (NOS). To date, three isoforms of NOS have been identified. Neuronal NOS (nNOS or NOS1) is expressed constitutively by neurons in the brain and enteric nervous system, whereas endothelial NOS (eNOS or NOS3) exhibits constitutive expression which is confined to the endothelial cells lining the vasculature [129,131]. The third isoform of NOS is an inducible NOS (iNOS or NOS2) and as the name implies, it is expressed only in response to certain inflammatory stimuli such as bacterial products, cytokines, and lipid mediators [130,131]. Classically, NO is considered to be an activator for cGMP [132] in the regulation of cardiovascular function [133] and neurotransmission [134]. Very recently, *S*-nitrosylation, the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine, has emerged as an important mechanism for dynamic posttranslational regulation of proteins [56]. *S*-Nitrosylation thereby conveys a large part of the ubiquitous influence of nitric oxide on cellular signal transduction, and provides a mechanism for redox-based physiological regulation [56]. In addition, NO in O_2^- producing environment reacts rapidly to form the highly toxic peroxynitrite anion, which then protonates and decomposes to generate $^{\bullet}OH$ or some other potent oxidant with similar reactivity [135]. This is of particular importance in neurodegenerating disease conditions such as demyelinating disease and in ischemia and traumatic injuries associated with infiltrating peripheral mononuclear cells and the production of proinflammatory cytokines, where subsequent astrocytes and microglia-derived NO could contribute to oligodendrocyte degeneration and neuronal death [136,137] (Fig. 2).

Endothelial nitric oxide synthase

eNOS identified in endothelial cells is also expressed in cardiomyocytes [138,139]. eNOS produces NO via a complex reaction which is stimulated by Ca^{2+} and requires NADPH, along with other cofactors [138]. The role of ceramide in NO generation through eNOS was identified because ceramide affects vasorelaxation. The role of ceramide in vascular function has been extensively reviewed by Berry et al. [29]. Initial studies probing the effect of ceramide on vascular contractility demonstrated that application of cell-permeable analogs of ceramide or exogenous bacterial sphingomyelinase to preconstricted vascular segments results in concentration-dependent relaxation [140,141]. Subsequently, Jin and co-workers also reported that micromolar concentrations of ceramide (C_2-, C_6-, and C_{16}-ceramide) induce significant relaxation in a NO-dependent manner and removal of the endothelium significantly inhibited ceramide-induced relaxation [142]. Interestingly, angiotensin II type 2 receptor activation also increases intracellular concentrations of ceramide [143,144]; therefore, ceramide may contribute to some of the physiological effects of angiotensin II through stimulation of nitric oxide production [145]. Although the precise mechanism for ceramide-induced vasodilation is not fully understood, recent studies have identified phosphatidylinositol-3′-kinase and Akt as downstream candidate effectors for ceramide in eNOS activation [146]. Moreover, ceramide-mediated translocation of eNOS from plasma membrane, where it is bound to caveolin-1 as an inactive form, to the cytoplasm was also demonstrated as a crucial step in ceramide-induced synthesis of NO by eNOS [147,148]. However, there are opposing views on the role of ceramide in vasoregulation [11]. It was demonstrated that TNF-α inhibits NO-mediated endothelium-dependent vasorelaxation in small coronary arteries via sphingomyelinase activation and consequent superoxide production [149]. Indeed, ceramide was reported to inhibit endothelium-dependent vasodilation via an increase in O_2^- and a subsequent decrease in NO availability, without altering NO synthesis [145], and this impairment of endothelial function was prevented by overexpression of Cu/Zn superoxide dismutase [150]. Therefore, ceramide appears to have a bifunctional role in the regulation of NO-mediated vasoregulation through activation of eNOS-mediated NO production and/or ROS generation which lowers NO availability by generation of peroxynitrite (Fig. 2).

Interestingly, ceramide-induced ROS generation also mediates oxidation of (6*R*)-5,6,7,8-tetrahydro-L-biopterin (BH_4, a cofactor of eNOS) that leads to BH_4 deficiency [151] (Fig. 2). The deficiency of BH_4 causes an increase in uncoupled eNOS molecules which leads to the formation of O_2^- instead of NO [151,152]. This phenomenon, along with increased gene expression of eNOS [151] by ceramide, may be one of the mechanisms of ceramide-mediated impairment of endothelial function and increased oxidative stress under pathophysiological disease conditions, such as hypertension, experimental diabetes, and hypercholesterolemia, and in smokers [152].

Inducible nitric oxide synthase

In 1998, our group first reported the role of SMase and ceramide in iNOS gene expression and NO production [11]. The role of ceramide in the induction of iNOS gene expression was further supported by other groups [153,154]. Furthermore, recent studies demonstrated that a selective inhibitor of N-SMase downregulates LPS and/or Aβ-induced iNOS expression in macrophages and astrocytes [10,69]. On the other hand, neither pharmacological inhibition nor knockout of A-SMase affected the expression of iNOS [10,69,155]. These reports suggest a role of ceramide produced by N-SMase in the expression of iNOS as well as other inflammatory genes that are related to the regulation of cellular redox potential. The mechanism for initiation of the ceramide-mediated inflammatory signaling cascade is not clearly understood. Ceramide, generated by N-SMase, induced NFκB activation through activation of hRas signaling cascades [10]. Moreover, the requirement of tyrosine kinases in this reaction [154] suggests the possible action of tyrosine kinase in Ras/NFκB activation and iNOS gene induction (Fig. 2). Putative ceramide-interacting enzymes, such as Ser/Thr protein kinase (CAPK) [156], kinase suppressor of Ras (KSR) [157], phosphatase (PP) 2A and 1B [158], and PKCζ [159] either via direct interactions or indirectly through formation of specific membrane microdomains may play a role in these signaling events. Recent studies have shown that ceramide plays a role in clustering of TNF family receptors (i.e., TNF-R1, CD40, and CD95) [160–162]. Following ligation of these receptors with ligands, the ceramide produced by SMase around TNF receptors generates signaling microdomains. Since ceramide has the ability to self-aggregate [163], subsequent fusion of these small entities into larger membrane domains (ceramide rafts) [164] has been demonstrated to trigger the clustering of these receptor molecules [161,162]. The receptor clustering in the rafts induces close contact of receptors with other signaling molecules [165] and exclusion of inhibitory molecules (i.e., CD45 tyrosine phosphatase) [166], and thus stabilizes ligand-receptor-signaling protein interactions [161,162]. Therefore, the increase in ceramide and the formation of ceramide rafts may enhance inflammatory or death signaling events that are tightly related to cellular redox potential [161,162] (Fig. 2).

In addition to ceramide, its glycosylated form lactosylceramide was also demonstrated to activate iNOS and other cytokine gene expression in astrocytes and a rat spinal cord injury model [89]. The exact mechanism of this reaction is not known but inflammatory cytokine-mediated activation of phosphatidylinositol-3-phosphate kinase appears to mediate an increase in lactosylceramide via activation of galactosyl transferase-2 (GalT-2) [167]. Both ceramide [10] and lactosylceramide induce iNOS gene expression through activation of hRas/NFκB, but whether ceramide produced by N-SMase is utilized for the synthesis of lactosylceramide is not known.

Neuronal nitric oxide synthase

In contrast to eNOS and iNOS, the role of sphingolipids in the regulation of nNOS activity is relatively unknown. In neuronal cells, sphingosine treatment strongly inhibits the activity of cytosolic Ca^{2+}-independent NOS (a putative nNOS); however, treatment with ceramide, *N*-acetylsphingosine, sphingosine-1P, sphinganine, and tetradecylamine had no effect on NOS activity [28]. Increasing concentrations of calmodulin led to loss of sphingosine inhibition, suggesting that sphingosine interferes with the calmodulin-dependent activation of the enzyme by a competitive mechanism [28] but without altering the intracellular Ca^{2+} concentration [168]. These observations suggest that bioactive sphingosine plays a role in neuronal NO signaling.

Regulation of antioxidant enzymes

In mammalian cells O_2^- generated by respiration in mitochondria and by activation of NADPH-oxidase or xanthine oxidase is converted into H_2O_2 by three forms of superoxide dismutase (extracellular and intracellular CuZn- and Mn-SODs) [169]. Extracellular SOD (EC-SOD) is mainly produced by vascular muscle cells and localized between endothelium and vascular muscle cell layers where it binds to cell surface, basal membrane, and extracellular matrix [170,171]. EC-SOD was known to be a major determinant of NO bioavailability in blood vessels through inhibition of vascular peroxynitrite generation [172]. Similarly, CuZn-SOD, which is a constitutively expressed cytosolic isoform, is also involved in the regulation of vascular functions through regulation of vascular O_2^- level and peroxynitrite formation [173,174]. Notably, its overexpression is able to inhibit ceramide or lactosylceramide-mediated impairment of endothelial function or ICAM expression observed in pathological conditions [175]. Therefore, to protect NO over its entire diffusion route against ceramide-mediated ROS, normal expression of both CuZn-SOD and EC-SOD may be essential.

Mn-SOD is an inducible isoform of SOD and mainly localized in mitochondria. Because of its localization and reported lethal phenotype in null mice, Mn-SOD is considered to be the first line of defense against oxidative stress from mitochondria [31,176]. The expression or activity of Mn-SOD or both may be altered under several physiological and pathophysiological conditions. For example, Mn-SOD is particularly responsive to and upregulated by oxidative stress caused by oxidized LDL, TNF-α, or H_2O_2 [176]. Moreover, cell-permeable ceramide or bacterial sphingomyelinase also

increase the expression of Mn-SOD in various cell types such as rat primary astrocytes, rat mesangial cells, glioma, PC12 cells, skin fibroblasts [168,177], and neurons [31]. Ceramide-mediated generation of ROS and subsequent activation of redox-sensitive transcription factors such as activator protein-1 (AP-1) and NFκB may be involved in the upregulation of Mn-SOD gene expression [178,179] (Fig. 2).

Following conversion of O_2^- by SODs into H_2O_2, it is believed to play a role in various cellular signal transduction pathways associated with cellular redox [180]. In addition, in the presence of transient metals (iron or copper) it forms a hydroxyl anion which is a strong oxidant and thus participates in the pathobiology of various disease conditions. Therefore, in the absence of adequate detoxification of H_2O_2, increased activity of SOD may cause oxidative stress [30,181]. Two enzymatic systems are involved in the detoxification of H_2O_2, catalase and peroxidases [glutathione peroxidase (GPX) and thioredoxin peroxidase (TPX)]. It is not clear how ceramide regulates activity of GPX or TPX but ceramide was reported to inhibit catalase function in various cell types [30]. The mechanism for ceramide-induced inhibition of catalase is not clear at present but the inhibitory effect of ceramide on phosphatidylinositol-3-kinase has been reported to be involved in this reaction [182,183] (Fig. 2).

Peroxisomal redox is maintained by the enzyme system for production of O_2^- and H_2O_2 and the antioxidant enzyme system (Cu/Zn-SOD, Mn-SOD, catalase, and GPX) [183]. Sphingolipids may inhibit catalase activity through modulating peroxisomal function. The peroxisome is a redox-sensitive organelle where H_2O_2 produced by various oxidases is detoxified by catalase, a major peroxisomal matrix protein [182]. Drastic alteration of peroxisomal functions, as well as oxidative stress by mislocalization of catalase from peroxisomes [184], suggests that peroxisomal integrity and function are important for the regulation of catalase activity. Recently, our group reported that galactosyl-sphingosine (psychosine), a metabolites that accumulates in the brains of globoid cell leukodystrophy (GLD) [185] or Krabbe's disease [186] patients, inhibits peroxisomal functions and increases cellular free radical production [187,188]. Although the role of other sphingolipids in peroxisomal function and catalase activity has not been studied yet, the inhibitory effect of TNF-α on the expression of peroxisome proliferator-activated receptors (PPARs) [189] and catalase activity [187,188] along with the concomitant increase in ceramide levels [187,188] suggests the possible role of ceramide in peroxisome function as well as catalase activity.

Summary and conclusion

Sphingolipids including sphingosine, sphingosine-1-phosphate, ceramide, ceramide-1-phosphate, psychosine, glucosylceramide, lactosylceramide, and GD3 are known to play a key role in receptor-mediated signal cascades which regulate cell proliferation, inflammation, and endothelial function. Similarly, endogenous prooxidants such as ROS and RNS also play a key role in receptor-mediated activation of NADPH oxidase and NOS which are also involved in various aspects of cell physiological regulation. A growing body of evidence suggests that these two pathways interact with each other. Prooxidants such as ROS and RNS regulate sphingolipid metabolism through regulating the enzymes responsible for their metabolism including SMase and ceramidase. On the other hand, sphingolipids such as ceramide, lactosylceramide, and GD3 also mediate ROS and RNS generation through regulation of NADPH oxidase, NOS, and antioxidant enzymes such as Mn-SOD and catalase. Along with the physiological signaling cascades, the interaction of these two pathways may also be involved in cytotoxic or apoptotic cascades. Ceramide and other sphingolipids such as sphingosine or GD3 were initially known as potent proapoptotic agents which produce irreversible mitochondrial dysfunction and massive ROS generation. Although events that switch the roles of prooxidants and sphingolipids from physiological to proapoptotic signaling cascades are still under investigation, it is believed that cellular redox potential is a crucial factor for this transition. For example, in the CNS, oligodendrocytes, which are known to have low levels of GSH compared to astrocytes or microglia, undergo apoptotic pathway activation upon stimulation with neurotoxic substances or proinflammatory cytokines, while astrocytes and microglia undergo proliferation or inflammatory activation. Under low redox buffering states, the receptor-mediated generation of ROS and RNS may produce oxidative stress and then activate redox-sensitive SMase and ceramide generation. In this event, exclusive ceramide production may be able to induce mitochondrial dysfunction that further promotes ROS generation and apoptosis. Similarly, the interaction of ROS/RNS generation and sphingolipid metabolism may also play a crucial role in endothelial function. As discussed, ceramide exerts its role as a vasodilator through activation of eNOS. However, it may also act as a vasoconstrictor by its dual role in the activation of ROS generation when ROS are not removed due to a low redox buffering state.

During the past two decades, the regulation of sphingolipid metabolism has been under intense investigation due to the involvement of these events in the pathophysiology of various disease conditions. Here, we have discussed the interregulation of ROS/RNS generation and sphingolipid metabolism as one of the crucial factors promoting the pathological outcome. Therefore, therapeutic approaches for intervention of sphingolipid-induced pathological signal transduction pathways and the use of antioxidants may improve the efficacy of therapeutics in these disorders.

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References

[1] Boveris, A.; Chance, B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem. J.* **134:**707–716; 1973.

[2] Grisham, M. B.; Jourd'Heuil, D.; Wink, D. A. Nitric oxide: I. Physiological chemistry of nitric oxide and its metabolites:implications in inflammation. *Am. J. Physiol.* **276:**G315–G321; 1999.

[3] Endemann, D. H.; Schiffrin, E. L. Nitric oxide, oxidative excess, and vascular complications of diabetes mellitus. *Curr. Hypertens. Rep.* **6:**85–89; 2004.

[4] Arnold, W. P.; Mittal, C. K.; Katsuki, S.; Murad, F. Nitric oxide activates guanylate cyclase and increases guanosine 3′:5′-cyclic monophosphate levels in various tissue preparations. *Proc. Natl. Acad. Sci. USA* **74:**3203–3207; 1977.

[5] Pfeilschifter, J.; Eberhardt, W.; Beck, K. F. Regulation of gene expression by nitric oxide. *Pflugers Arch.* **442:**479–486; 2001.

[6] Pfeilschifter, J.; Eberhardt, W.; Huwiler, A. Nitric oxide and mechanisms of redox signalling: matrix and matrix-metabolizing enzymes as prime nitric oxide targets. *Eur. J. Pharmacol.* **429:**279–286; 2001.

[7] Pettus, B. J.; Chalfant, C. E.; Hannun, Y. A. Ceramide in apoptosis: an overview and current perspectives. *Biochim. Biophys. Acta* **1585:** 114–125; 2002.

[8] Watterson, K. R.; Ratz, P. H.; Spiegel, S. The role of sphingosine-1-phosphate in smooth muscle contraction. *Cell Signal.* **17:**289–298; 2005.

[9] Proia, R. L. Glycosphingolipid functions: insights from engineered mouse models. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* **358:**879–883; 2003.

[10] Won, J. S.; Im, Y. B.; Khan, M.; Singh, A. K.; Singh, I. The role of neutral sphingomyelinase produced ceramide in lipopolysaccharide-mediated expression of inducible nitric oxide synthase. *J. Neurochem.* **88:** 583–593; 2004.

[11] Pahan, K.; Sheikh, F. G.; Khan, M.; Namboodiri, A. M.; Singh, I. Sphingomyelinase and ceramide stimulate the expression of inducible nitric-oxide synthase in rat primary astrocytes. *J. Biol. Chem.* **273:** 2591–2600; 1998.

[12] Singh, I.; Pahan, K.; Khan, M.; Singh, A. K. Cytokine-mediated induction of ceramide production is redox-sensitive. Implications to proinflammatory cytokine-mediated apoptosis in demyelinating diseases. *J. Biol. Chem.* **273:**20354–20362; 1998.

[13] Dobrowsky, R. T. Sphingolipid signalling domains floating on rafts or buried in caves? *Cell Signal.* **12:**81–90; 2000.

[14] Gulbins, E.; Kolesnick, R. Raft ceramide in molecular medicine. *Oncogene* **22:**7070–7077; 2003.

[15] Rotolo, J. A.; Zhang, J.; Donepudi, M.; Lee, H.; Fuks, Z.; Kolesnick, R. Caspase-dependent and -independent activation of acid sphingomyelinase signaling. *J. Biol. Chem.* **280:**26425–26434; 2005.

[16] Kilkus, J.; Goswami, R.; Testai, F. D.; Dawson, G. Ceramide in rafts (detergent-insoluble fraction) mediates cell death in neurotumor cell lines. *J. Neurosci. Res.* **72:**65–75; 2003.

[17] Mitsutake, S.; Tani, M.; Okino, N.; Mori, K.; Ichinose, S.; Omori, A.; Iida, H.; Nakamura, T.; Ito, M. Purification, characterization, molecular cloning, and subcellular distribution of neutral ceramidase of rat kidney. *J. Biol. Chem.* **276:**26249–26259; 2001.

[18] Urtz, N.; Olivera, A.; Bofill-Cardona, E.; Csonga, R.; Billich, A.; Mechtcheriakova, D.; Bornancin, F.; Woisetschlager, M.; Rivera, J.; Baumruker, T. Early activation of sphingosine kinase in mast cells and recruitment to FcepsilonRI are mediated by its interaction with Lyn kinase. *Mol. Cell. Biol.* **24:**8765–8777; 2004.

[19] Hinkovska-Galcheva, V.; Boxer, L. A.; Kindzelskii, A.; Hiraoka, M.; Abe, A.; Goparju, S.; Spiegel, S.; Petty, H. R.; Shayman, J. A. Ceramide 1-phosphate, a mediator of phagocytosis. *J. Biol. Chem.* **280:** 26612–26621; 2005.

[20] Ostrom, R. S.; Insel, P. A. The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology. *Br. J. Pharmacol.* **143:**235–245; 2004.

[21] Laude, A. J.; Prior, I. A. Plasma membrane microdomains: organization, function and trafficking. *Mol. Membr. Biol.* **21:**193–205; 2004.

[22] Huwiler, A.; Pfeilschifter, J.; van den Bosch, H. Nitric oxide donors induce stress signaling via ceramide formation in rat renal mesangial cells. *J. Biol. Chem.* **274:**7190–7195; 1999.

[23] Qiu, H.; Edmunds, T.; Baker-Malcolm, J.; Karey, K. P.; Estes, S.; Schwarz, C.; Hughes, H.; Van Patten, S. M. Activation of human acid sphingomyelinase through modification or deletion of C-terminal cysteine. *J. Biol. Chem.* **278:**32744–32752; 2003.

[24] De Nadai, C.; Sestili, P.; Cantoni, O.; Lievremont, J. P.; Sciorati, C.; Barsacchi, R.; Moncada, S.; Meldolesi, J.; Clementi, E. Nitric oxide inhibits tumor necrosis factor-alpha-induced apoptosis by reducing the generation of ceramide. *Proc. Natl. Acad. Sci. USA* **97:**5480–5485; 2000.

[25] Mansat-de Mas, V.; Bezombes, C.; Quillet-Mary, A.; Bettaieb, A.; D'Orgeix, A. D.; Laurent, G.; Jaffrezou, J. P. Implication of radical oxygen species in ceramide generation, c-Jun N-terminal kinase activation and apoptosis induced by daunorubicin. *Mol. Pharmacol.* **56:**867–874; 1999.

[26] Zhang, D. X.; Zou, A. P.; Li, P. L. Ceramide-induced activation of NADPH oxidase and endothelial dysfunction in small coronary arteries. *Am. J. Physiol. Heart Circ. Physiol.* **284:**H605–H612; 2003.

[27] Garcia-Ruiz, C.; Colell, A.; Mari, M.; Morales, A.; Fernandez-Checa, J. C. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *J. Biol. Chem.* **272:**11369–11377; 1997.

[28] Viani, P.; Giussani, P.; Ferraretto, A.; Signorile, A.; Riboni, L.; Tettamanti, G. Nitric oxide production in living neurons is modulated by sphingosine: a fluorescence microscopy study. *FEBS Lett.* **506:** 185–190; 2001.

[29] Johns, D. G.; Osborn, H.; Webb, R. C. Ceramide: a novel cell signaling mechanism for vasodilation. *Biochem. Biophys. Res. Commun.* **237:** 95–97; 1997.

[30] Iwai, K.; Kondo, T.; Watanabe, M.; Yabu, T.; Kitano, T.; Taguchi, Y.; Umehara, H.; Takahashi, A.; Uchiyama, T.; Okazaki, T. Ceramide increases oxidative damage due to inhibition of catalase by caspase-3-dependent proteolysis in HL-60 cell apoptosis. *J. Biol. Chem.* **278:** 9813–9822; 2003.

[31] Macmillan-Crow, L. A.; Cruthirds, D. L. Invited review: manganese superoxide dismutase in disease. *Free Radic. Res.* **34:**325–336; 2001.

[32] Goni, F. M.; Alonso, A. Sphingomyelinases: enzymology and membrane activity. *FEBS Lett.* **531:**38–46; 2002.

[33] Levade, T.; Andrieu-Abadie, N.; Segui, B.; Auge, N.; Chatelut, M.; Jaffrezou, J. P.; Salvayre, R. Sphingomyelin-degrading pathways in human cells role in cell signalling. *Chem. Phys. Lipids* **102:**167–178; 1999.

[34] Zumbansen, M.; Stoffel, W. Neutral sphingomyelinase 1 deficiency in the mouse causes no lipid storage disease. *Mol. Cell. Biol.* **22:**3633–3638; 2002.

[35] Hofmann, K.; Tomiuk, S.; Wolff, G.; Stoffel, W. Cloning and characterization of the mammalian brain-specific, Mg2+-dependent neutral sphingomyelinase. *Proc. Natl. Acad. Sci. USA* **97:**5895–5900; 2000.

[36] Tomiuk, S.; Hofmann, K.; Nix, M.; Zumbansen, M.; Stoffel, W. Cloned mammalian neutral sphingomyelinase: functions in sphingolipid signaling? *Proc. Natl. Acad. Sci. USA* **95:**3638–3643; 1998.

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

[38] Schuchman, E. H.; Levran, O.; Pereira, L. V.; Desnick, R. J. Structural organization and complete nucleotide sequence of the gene encoding human acid sphingomyelinase (SMPD1). *Genomics* **12:**197–205; 1992.

[39] Hurwitz, R.; Ferlinz, K.; Vielhaber, G.; Moczall, H.; Sandhoff, K. Processing of human acid sphingomyelinase in normal and I-cell fibroblasts. *J. Biol. Chem.* **269:**5440–5445; 1994.

[40] Schissel, S. L.; Schuchman, E. H.; Williams, K. J.; Tabas, I. Zn2+-stimulated sphingomyelinase is secreted by many cell types and is a

product of the acid sphingomyelinase gene. *J. Biol. Chem.* **271:** 18431–18436; 1996.

[41] Itoh, N.; Nagata, S. A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J. Biol. Chem.* **268:** 10932–10937; 1993.

[42] Gulbins, E.; Grassme, H. Ceramide and cell death receptor clustering. *Biochim. Biophys. Acta* **1585:**139–145; 2002.

[43] Ahmad, T. Y.; Beaudet, A. L.; Sparrow, J. T.; Morrisett, J. D. Human lysosomal sphingomyelinase: substrate efficacy of apolipoprotein/sphingomyelin complexes. *Biochemistry* **25:**4415–4420; 1986.

[44] Bilderback, T. R.; Gazula, V. R.; Dobrowsky, R. T. Phosphoinositide 3-kinase regulates crosstalk between Trk A tyrosine kinase and p75(NTR)-dependent sphingolipid signaling pathways. *J. Neurochem.* **76:** 1540–1551; 2001.

[45] Quintern, L. E.; Sandhoff, K. Human acid sphingomyelinase from human urine. *Methods Enzymol.* **197:**536–540; 1991.

[46] Kolesnick, R. N. 1,2-Diacylglycerols but not phorbol esters stimulate sphingomyelin hydrolysis in GH3 pituitary cells. *J. Biol. Chem.* **262:** 16759–16762; 1987.

[47] Gomez-Munoz, A.; Kong, J.; Salh, B.; Steinbrecher, U. P. Sphingosine-1-phosphate inhibits acid sphingomyelinase and blocks apoptosis in macrophages. *FEBS Lett.* **539:**56–60; 2003.

[48] Rao, B. G.; Spence, M. W. Sphingomyelinase activity at pH 7.4 in human brain and a comparison to activity at pH 5.0. *J. Lipid Res.* **17:**506–515; 1976.

[49] Liu, B.; Hannun, Y. A. Inhibition of the neutral magnesium-dependent sphingomyelinase by glutathione. *J. Biol. Chem.* **272:**16281–16287; 1997.

[50] Alliegro, M. C. Effects of dithiothreitol on protein activity unrelated to thiol-disulfide exchange: for consideration in the analysis of protein function with Cleland's reagent. *Anal. Biochem.* **282:**102–106; 2000.

[51] Bulotta, S.; Barsacchi, R.; Rotiroti, D.; Borgese, N.; Clementi, E. Activation of the endothelial nitric-oxide synthase by tumor necrosis factor-alpha. A novel feedback mechanism regulating cell death. *J. Biol. Chem.* **276:**6529–6536; 2001.

[52] Falcone, S.; Perrotta, C.; De Palma, C.; Pisconti, A.; Sciorati, C.; Capobianco, A.; Rovere-Querini, P.; Manfredi, A. A.; Clementi, E. Activation of acid sphingomyelinase and its inhibition by the nitric oxide/cyclic guanosine 3′,5′-monophosphate pathway: key events in Escherichia coli-elicited apoptosis of dendritic cells. *J. Immunol.* **173:** 4452–4463; 2004.

[53] Barsacchi, R.; Perrotta, C.; Sestili, P.; Cantoni, O.; Moncada, S.; Clementi, E. Cyclic GMP-dependent inhibition of acid sphingomyelinase by nitric oxide: an early step in protection against apoptosis. *Cell Death Differ.* **9:**1248–1255; 2002.

[54] Pilane, C. M.; LaBelle, E. F. NO induced apoptosis of vascular smooth muscle cells accompanied by ceramide increase. *J. Cell. Physiol.* **199:**310–315; 2004.

[55] Matsumoto, A.; Comatas, K. E.; Liu, L.; Stamler, J. S. Screening for nitric oxide-dependent protein-protein interactions. *Science* **301:**657–661; 2003.

[56] Hess, D. T.; Matsumoto, A.; Kim, S. O.; Marshall, H. E.; Stamler, J. S. Protein S-nitrosylation: purview and parameters. *Nat. Rev. Mol. Cell. Biol.* **6:**150–166; 2005.

[57] Schneider, P. B.; Kennedy, E. P. Sphingomyelinase in normal human spleens and in spleens from subjects with Niemann-Pick disease. *J. Lipid Res.* **8:**202–209; 1967.

[58] Sawai, H.; Domae, N.; Nagan, N.; Hannun, Y. A. Function of the cloned putative neutral sphingomyelinase as lyso-platelet activating factor-phospholipase C. *J. Biol. Chem.* **274:**38131–38139; 1999.

[59] Marchesini, N.; Hannun, Y. A. Acid and neutral sphingomyelinases: roles and mechanisms of regulation. *Biochem. Cell Biol.* **82:**27–44; 2004.

[60] Marchesini, N.; Luberto, C.; Hannun, Y. A. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. *J. Biol. Chem.* **278:**13775–13783; 2003.

[61] Bernardo, K.; Krut, O.; Wiegmann, K.; Kreder, D.; Micheli, M.; Schafer, R.; Sickman, A.; Schmidt, W. E.; Schroder, J. M.; Meyer, H. E.; Sandhoff, K.; Kronke, M. Purification and characterization of a magnesium-dependent neutral sphingomyelinase from bovine brain. *J. Biol. Chem.* **275:**7641–7647; 2000.

[62] Kondo, T.; Kitano, T.; Iwai, K.; Watanabe, M.; Taguchi, Y.; Yabu, T.; Umehara, H.; Domae, N.; Uchiyama, T.; Okazaki, T. Control of ceramide-induced apoptosis by IGF-1: involvement of PI-3 kinase, caspase-3 and catalase. *Cell Death Differ.* **9:**682–692; 2002.

[63] Sumitomo, M.; Ohba, M.; Asakuma, J.; Asano, T.; Kuroki, T.; Asano, T.; Hayakawa, M. Protein kinase Cdelta amplifies ceramide formation via mitochondrial signaling in prostate cancer cells. *J. Clin. Invest.* **109:** 827–836; 2002.

[64] Adam, D.; Wiegmann, K.; Adam-Klages, S.; Ruff, A.; Kronke, M. A novel cytoplasmic domain of the p55 tumor necrosis factor receptor initiates the neutral sphingomyelinase pathway. *J. Biol. Chem.* **271:** 14617–14622; 1996.

[65] Kreder, D.; Krut, O.; Adam-Klages, S.; Wiegmann, K.; Scherer, G.; Plitz, T.; Jensen, J. M.; Proksch, E.; Steinmann, J.; Pfeffer, K.; Kronke, M. Impaired neutral sphingomyelinase activation and cutaneous barrier repair in FAN-deficient mice. *EMBO J.* **18:**2472–2479; 1999.

[66] Segui, B.; Andrieu-Abadie, N.; Adam-Klages, S.; Meilhac, O.; Kreder, D.; Garcia, V.; Bruno, A. P.; Jaffrezou, J. P.; Salvayre, R.; Kronke, M.; Levade, T. CD40 signals apoptosis through FAN-regulated activation of the sphingomyelin-ceramide pathway. *J. Biol. Chem.* **274:**37251–37258; 1999.

[67] Jung, S. Y.; Suh, J. H.; Park, H. J.; Jung, K. M.; Kim, M. Y.; Na, D. S.; Kim, D. K. Identification of multiple forms of membrane-associated neutral sphingomyelinase in bovine brain. *J. Neurochem.* **75:**1004–1014; 2000.

[68] Yoshimura, S.; Banno, Y.; Nakashima, S.; Hayashi, K.; Yamakawa, H.; Sawada, M.; Sakai, N.; Nozawa, Y. Inhibition of neutral sphingomyelinase activation and ceramide formation by glutathione in hypoxic PC12 cell death. *J. Neurochem.* **73:**675–683; 1999.

[69] Ayasolla, K.; Khan, M.; Singh, A. K.; Singh, I. Inflammatory mediator and beta-amyloid (25-35)-induced ceramide generation and iNOS expression are inhibited by vitamin E. *Free Radic. Biol. Med.* **37:** 325–338; 2004.

[70] Lee, J. T.; Xu, J.; Lee, J. M.; Ku, G.; Han, X.; Yang, D. I.; Chen, S.; Hsu, C. Y. Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J. Cell Biol.* **164:** 123–131; 2004.

[71] Okamoto, Y.; Obeid, L. M.; Hannun, Y. A. Bcl-xL interrupts oxidative activation of neutral sphingomyelinase. *FEBS Lett.* **530:**104–108; 2002.

[72] El-Assaad, W.; El-Sabban, M.; Awaraji, C.; Abboushi, N.; Dbaibo, G. S. Distinct sites of action of Bcl-2 and Bcl-xL in the ceramide pathway of apoptosis. *Biochem. J.* **336 (Pt 3):**735–741; 1998.

[73] Grazide, S.; Maestre, N.; Veldman, R. J.; Bezombes, C.; Maddens, S.; Levade, T.; Laurent, G.; Jaffrezou, J. P. Ara-C- and daunorubicin-induced recruitment of Lyn in sphingomyelinase-enriched membrane rafts. *FASEB J.* **16:**1685–1687; 2002.

[74] Bezombes, C.; de Thonel, A.; Apostolou, A.; Louat, T.; Jaffrezou, J. P.; Laurent, G.; Quillet-Mary, A. Overexpression of protein kinase Czeta confers protection against antileukemic drugs by inhibiting the redox-dependent sphingomyelinase activation. *Mol. Pharmacol.* **62:** 1446–1455; 2002.

[75] Takeda, Y.; Tashima, M.; Takahashi, A.; Uchiyama, T.; Okazaki, T. Ceramide generation in nitric oxide-induced apoptosis. Activation of magnesium-dependent neutral sphingomyelinase via caspase-3. *J. Biol. Chem.* **274:**10654–10660; 1999.

[76] Franzen, R.; Fabbro, D.; Aschrafi, A.; Pfeilschifter, J.; Huwiler, A. Nitric oxide induces degradation of the neutral ceramidase in rat renal mesangial cells and is counterregulated by protein kinase C. *J. Biol. Chem.* **277:** 46184–46190; 2002.

[77] Franzen, R.; Pfeilschifter, J.; Huwiler, A. Nitric oxide induces neutral ceramidase degradation by the ubiquitin/proteasome complex in renal mesangial cell cultures. *FEBS Lett.* **532:**441–444; 2002.

[78] Di Paola, M.; Cocco, T.; Lorusso, M. Ceramide interaction with the

respiratory chain of heart mitochondria. *Biochemistry* **39:**6660–6668; 2000.

[79] Bokoch, G. M.; Diebold, B. A. Current molecular models for NADPH oxidase regulation by Rac GTPase. *Blood* **100:**2692–2696; 2002.

[80] Nauseef, W. M. Assembly of the phagocyte NADPH oxidase. *Histochem. Cell Biol.* **122:**277–291; 2004.

[81] Lambeth, J. D. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. *Curr. Opin. Hematol.* **9:**11–17; 2002.

[82] Lambeth, J. D.; Cheng, G.; Arnold, R. S.; Edens, W. A. Novel homologs of gp91phox. *Trends Biochem. Sci.* **25:**459–461; 2000.

[83] Liel, Y.; Rudich, A.; Nagauker-Shriker, O.; Yermiyahu, T.; Levy, R. Monocyte dysfunction in patients with Gaucher disease: evidence for interference of glucocerebroside with superoxide generation. *Blood* **83:** 2646–2653; 1994.

[84] Moskwa, P.; Palicz, A.; Paclet, M. H.; Dagher, M. C.; Erdos, M.; Marodi, L.; Ligeti, E. Glucocerebroside inhibits NADPH oxidase activation in cell-free system. *Biochim. Biophys. Acta* **1688:**197–203; 2004.

[85] Arai, T.; Bhunia, A. K.; Chatterjee, S.; Bulkley, G. B. Lactosylceramide stimulates human neutrophils to upregulate Mac-1, adhere to endothelium, and generate reactive oxygen metabolites in vitro. *Circ. Res.* **82:** 540–547; 1998.

[86] Bhunia, A. K.; Han, H.; Snowden, A.; Chatterjee, S. Redox-regulated signaling by lactosylceramide in the proliferation of human aortic smooth muscle cells. *J. Biol. Chem.* **272:**15642–15649; 1997.

[87] Bhunia, A. K.; Arai, T.; Bulkley, G.; Chatterjee, S. Lactosylceramide mediates tumor necrosis factor-alpha-induced intercellular adhesion molecule-1 (ICAM-1) expression and the adhesion of neutrophil in human umbilical vein endothelial cells. *J. Biol. Chem.* **273:** 34349–34357; 1998.

[88] Garcia-Ruiz, C.; Colell, A.; Paris, R.; Fernandez-Checa, J. C. Direct interaction of GD3 ganglioside with mitochondria generates reactive oxygen species followed by mitochondrial permeability transition, cytochrome c release, and caspase activation. *FASEB J.* **14:**847–858; 2000.

[89] Pannu, R.; Won, J. S.; Khan, M.; Singh, A. K.; Singh, I. A novel role of lactosylceramide in the regulation of lipopolysaccharide/interferon-gamma-mediated inducible nitric oxide synthase gene expression: implications for neuroinflammatory diseases. *J. Neurosci.* **24:** 5942–5954; 2004.

[90] Iwabuchi, K.; Nagaoka, I. Lactosylceramide-enriched glycosphingolipid signaling domain mediates superoxide generation from human neutrophils. *Blood* **100:**1454–1464; 2002.

[91] Zhang, A. Y.; Teggatz, E. G.; Zou, A. P.; Campbell, W. B.; Li, P. L. Endostatin uncouples NO and Ca2+ response to bradykinin through enhanced O2*- production in the intact coronary endothelium. *Am. J. Physiol. Heart Circ. Physiol.* **288:**H686–H694; 2005.

[92] Yi, F.; Zhang, A. Y.; Janscha, J. L.; Li, P. L.; Zou, A. P. Homocysteine activates NADH/NADPH oxidase through ceramide-stimulated Rac GTPase activity in rat mesangial cells. *Kidney Int.* **66:**1977–1987; 2004.

[93] Reinehr, R.; Becker, S.; Eberle, A.; Grether-Beck, S.; Haussinger, D. Involvement of NADPH oxidase isoforms and SRC family kinases in CD95-dependent hepatocyte apoptosis. *J. Biol. Chem.* (in press).

[94] Das, D. K.; George, A.; Liu, X. K.; Rao, P. S. Detection of hydroxyl radical in the mitochondria of ischemic-reperfused myocardium by trapping with salicylate. *Biochem. Biophys. Res. Commun.* **165:**1004–1009; 1989.

[95] Wang, X. The expanding role of mitochondria in apoptosis. *Genes Dev.* **15:**2922–2933; 2001.

[96] Du, C.; Fang, M.; Li, Y.; Li, L.; Wang, X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* **102:**33–42; 2000.

[97] Joza, N.; Susin, S. A.; Daugas, E.; Stanford, W. L.; Cho, S. K.; Li, C. Y.; Sasaki, T.; Elia, A. J.; Cheng, H. Y.; Ravagnan, L.; Ferri, K. F.; Zamzami, N.; Wakeham, A.; Hakem, R.; Yoshida, H.; Kong, Y. Y.; Mak, T. W.; Zuniga-Pflucker, J. C.; Kroemer, G.; Penninger, J. M. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* **410:**549–554; 2001.

[98] Li, L. Y.; Luo, X.; Wang, X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* **412:**95–99; 2001.

[99] Cai, J.; Jones, D. P. Superoxide in apoptosis. Mitochondrial generation triggered by cytochrome c loss. *J. Biol. Chem.* **273:**11401–11404; 1998.

[100] Ghafourifar, P.; Klein, S. D.; Schucht, O.; Schenk, U.; Pruschy, M.; Rocha, S.; Richter, C. Ceramide induces cytochrome c release from isolated mitochondria. Importance of mitochondrial redox state. *J. Biol. Chem.* **274:**6080–6084; 1999.

[101] Voehringer, D. W.; McConkey, D. J.; McDonnell, T. J.; Brisbay, S.; Meyn, R. E. Bcl-2 expression causes redistribution of glutathione to the nucleus. *Proc. Natl. Acad. Sci. USA* **95:**2956–2960; 1998.

[102] Kane, D. J.; Sarafian, T. A.; Anton, R.; Hahn, H.; Gralla, E. B.; Valentine, J. S.; Ord, T.; Bredesen, D. E. Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* **262:**1274–1277; 1993.

[103] Voehringer, D. W. BCL-2 and glutathione: alterations in cellular redox state that regulate apoptosis sensitivity. *Free Radic. Biol. Med.* **27:** 945–950; 1999.

[104] Yang, J.; Liu, X.; Bhalla, K.; Kim, C. N.; Ibrado, A. M.; Cai, J.; Peng, T. I.; Jones, D. P.; Wang, X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* **275:**1129–1132; 1997.

[105] Marzo, I.; Brenner, C.; Zamzami, N.; Jurgensmeier, J. M.; Susin, S. A.; Vieira, H. L.; Prevost, M. C.; Xie, Z.; Matsuyama, S.; Reed, J. C.; Kroemer, G. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* **281:**2027–2031; 1998.

[106] Petit, P. X.; Lecoeur, H.; Zorn, E.; Dauguet, C.; Mignotte, B.; Gougeon, M. L. Alterations in mitochondrial structure and function are early events of dexamethasone-induced thymocyte apoptosis. *J. Cell Biol.* **130:** 157–167; 1995.

[107] Di Nardo, A.; Benassi, L.; Magnoni, C.; Cossarizza, A.; Seidenari, S.; Giannetti, A. Ceramide 2 (N-acetyl sphingosine) is associated with reduction in Bcl-2 protein levels by Western blotting and with apoptosis in cultured human keratinocytes. *Br. J. Dermatol.* **143:**491–497; 2000.

[108] Chen, M.; Quintans, J.; Fuks, Z.; Thompson, C.; Kufe, D. W.; Weichselbaum, R. R. Suppression of Bcl-2 messenger RNA production may mediate apoptosis after ionizing radiation, tumor necrosis factor alpha, and ceramide. *Cancer Res.* **55:**991–994; 1995.

[109] Gudz, T. I.; Tserng, K. Y.; Hoppel, C. L. Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. **272:**24154–24158; 1997.

[110] Schulze-Osthoff, K.; Bakker, A. C.; Vanhaesebroeck, B.; Beyaert, R.; Jacob, W. A.; Fiers, W. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J. Biol. Chem.* **267:**5317–5323; 1992.

[111] Siskind, L. J.; Colombini, M. The lipids C2- and C16-ceramide form large stable channels. Implications for apoptosis. *J. Biol. Chem.* **275:**38640–38644; 2000.

[112] Ardail, D.; Popa, I.; Alcantara, K.; Pons, A.; Zanetta, J. P.; Louisot, P.; Thomas, L.; Portoukalian, J. Occurrence of ceramides and neutral glycolipids with unusual long-chain base composition in purified rat liver mitochondria. *FEBS Lett.* **488:**160–164; 2001.

[113] El Bawab, S.; Roddy, P.; Qian, T.; Bielawska, A.; Lemasters, J. J.; Hannun, Y. A. Molecular cloning and characterization of a human mitochondrial ceramidase. *J. Biol. Chem.* **275:**21508–21513; 2000.

[114] Shimeno, H.; Soeda, S.; Sakamoto, M.; Kouchi, T.; Kowakame, T.; Kihara, T. Partial purification and characterization of sphingosine N-acyltransferase (ceramide synthase) from bovine liver mitochondrion-rich fraction. *Lipids* **33:**601–605; 1998.

[115] Cuvillier, O. Sphingosine in apoptosis signaling. *Biochim. Biophys. Acta* **1585:**153–162; 2002.

[116] Sakakura, C.; Sweeney, E. A.; Shirahama, T.; Hakomori, S.; Igarashi, Y. Suppression of bcl-2 gene expression by sphingosine in the apoptosis of human leukemic HL-60 cells during phorbol ester-induced terminal differentiation. *FEBS Lett.* **379:**177–180; 1996.

[117] Shirahama, T.; Sakakura, C.; Sweeney, E. A.; Ozawa, M.; Takemoto, M.; Nishiyama, K.; Ohi, Y.; Igarashi, Y. Sphingosine induces apoptosis

in androgen-independent human prostatic carcinoma DU-145 cells by suppression of bcl-X(L) gene expression. *FEBS Lett.* **407:**97–100; 1997.

[118] Cuvillier, O.; Edsall, L.; Spiegel, S. Involvement of sphingosine in mitochondria-dependent Fas-induced apoptosis of type II Jurkat T cells. *J. Biol. Chem.* **275:**15691–15700; 2000.

[119] Cuvillier, O.; Nava, V. E.; Murthy, S. K.; Edsall, L. C.; Levade, T.; Milstien, S.; Spiegel, S. Sphingosine generation, cytochrome c release, and activation of caspase-7 in doxorubicin-induced apoptosis of MCF7 breast adenocarcinoma cells. *Cell Death Differ.* **8:**162–171; 2001.

[120] Kagedal, K.; Zhao, M.; Svensson, I.; Brunk, U. T. Sphingosine-induced apoptosis is dependent on lysosomal proteases. *Biochem. J.* **359:** 335–343; 2001.

[121] Sauer, B.; Gonska, H.; Manggau, M.; Kim, D. S.; Schraut, C.; Schafer-Korting, M.; Kleuser, B. Sphingosine 1-phosphate is involved in cytoprotective actions of calcitriol in human fibroblasts and enhances the intracellular Bcl-2/Bax rheostat. *Pharmazie* **60:**298–304; 2005.

[122] Cuvillier, O.; Levade, T. Sphingosine 1-phosphate antagonizes apoptosis of human leukemia cells by inhibiting release of cytochrome c and Smac/DIABLO from mitochondria. *Blood* **98:**2828–2836; 2001.

[123] Edsall, L. C.; Cuvillier, O.; Twitty, S.; Spiegel, S.; Milstien, S. Sphingosine kinase expression regulates apoptosis and caspase activation in PC12 cells. *J. Neurochem.* **76:**1573–1584; 2001.

[124] Olivera, A.; Kohama, T.; Edsall, L.; Nava, V.; Cuvillier, O.; Poulton, S.; Spiegel, S. Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival. *J. Cell Biol.* **147:**545–558; 1999.

[125] Hong, Y. B.; Kim, E. Y.; Jung, S. C. Down-regulation of Bcl-2 in the fetal brain of the Gaucher disease mouse model: a possible role in the neuronal loss. *J. Hum. Genet.* **49:**349–354; 2004.

[126] Lenartowicz, E.; Bernardi, P.; Azzone, G. F. Phenylarsine oxide induces the cyclosporin A-sensitive membrane permeability transition in rat liver mitochondria. *J. Bioenerg. Biomembr.* **23:**679–688; 1991.

[127] Petronilli, V.; Costantini, P.; Scorrano, L.; Colonna, R.; Passamonti, S.; Bernardi, P. The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation-reduction state of vicinal thiols. Increase of the gating potential by oxidants and its reversal by reducing agents. *J. Biol. Chem.* **269:**16638–16642; 1994.

[128] Rippo, M. R.; Malisan, F.; Ravagnan, L.; Tomassini, B.; Condo, I.; Costantini, P.; Susin, S. A.; Rufini, A.; Todaro, M.; Kroemer, G.; Testi, R. GD3 ganglioside directly targets mitochondria in a bcl-2-controlled fashion. *FASEB J.* **14:**2047–2054; 2000.

[129] Ignarro, L. J. Nitric oxide: a unique endogenous signaling molecule in vascular biology. *Biosci. Rep.* **19:**51–71; 1999.

[130] Murad, F. Nitric oxide signaling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? *Recent Prog. Horm. Res.* **53:**43–59; discussion 59-60; 1998.

[131] Moncada, S. Nitric oxide in the vasculature: physiology and pathophysiology. *Ann. N. Y. Acad. Sci.* **811:**60–67; discussion 67-69; 1997.

[132] Schmidt, H. H.; Lohmann, S. M.; Walter, U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim. Biophys. Acta* **1178:**153–175; 1993.

[133] Lecomte, P. V.; De Mulder, P. A.; Adriaensen, H. F.; De Hert, S. G. Role of the nitric oxide-cGMP pathway in the cardiovascular effects of anesthetic agents: a review. *Acta Anaesthesiol. Belg.* **50:**105–112; 1999.

[134] Mayer, B.; Koesling, D.; Bohme, E. Characterization of nitric oxide synthase, soluble guanylyl cyclase, and Ca2+/calmodulin-stimulated cGMP phosphodiesterase as components of neuronal signal transduction. *Adv. Second Messenger Phosphoprotein Res.* **28:**111–119; 1993.

[135] Beckman, J. S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* **87:**1620–1624; 1990.

[136] Koprowski, H.; Zheng, Y. M.; Heber-Katz, E.; Fraser, N.; Rorke, L.; Fu, Z. F.; Hanlon, C.; Dietzschold, B. In vivo expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. *Proc. Natl. Acad. Sci. USA* **90:**3024–3027; 1993.

[137] Mitrovic, B.; Ignarro, L. J.; Montestruque, S.; Smoll, A.; Merrill, J. E. Nitric oxide as a potential pathological mechanism in demyelination: its differential effects on primary glial cells in vitro. *Neuroscience* **61:** 575–585; 1994.

[138] Gross, S. S.; Wolin, M. S. Nitric oxide: pathophysiological mechanisms. *Annu. Rev. Physiol.* **57:**737–769; 1995.

[139] Kelly, R. A.; Balligand, J. L.; Smith, T. W. Nitric oxide and cardiac function. *Circ. Res.* **79:**363–380; 1996.

[140] Jones, M. J.; Murray, A. W. Evidence that ceramide selectively inhibits protein kinase C-alpha translocation and modulates bradykinin activation of phospholipase D. *J. Biol. Chem.* **270:**5007–5013; 1995.

[141] Lee, J. Y.; Hannun, Y. A.; Obeid, L. M. Ceramide inactivates cellular protein kinase Calpha. *J. Biol. Chem.* **271:**13169–13174; 1996.

[142] Jin, J. S.; Tsai, C. S.; Si, X.; Webb, R. C. Endothelium dependent and independent relaxations induced by ceramide in vascular smooth muscles. *Chin. J. Physiol.* **42:**47–51; 1999.

[143] Gallinat, S.; Busche, S.; Schutze, S.; Kronke, M.; Unger, T. AT2 receptor stimulation induces generation of ceramides in PC12W cells. *FEBS Lett.* **443:**75–79; 1999.

[144] Lehtonen, J. Y.; Horiuchi, M.; Daviet, L.; Akishita, M.; Dzau, V. J. Activation of the de novo biosynthesis of sphingolipids mediates angiotensin II type 2 receptor-induced apoptosis. *J. Biol. Chem.* **274:** 16901–16906; 1999.

[145] Berry, C.; Touyz, R.; Dominiczak, A. F.; Webb, R. C.; Johns, D. G. Angiotensin receptors: signaling, vascular pathophysiology, and interactions with ceramide. *Am. J. Physiol. Heart Circ. Physiol.* **281:** H2337–H2365; 2001.

[146] Barsacchi, R.; Perrotta, C.; Bulotta, S.; Moncada, S.; Borgese, N.; Clementi, E. Activation of endothelial nitric-oxide synthase by tumor necrosis factor-alpha: a novel pathway involving sequential activation of neutral sphingomyelinase, phosphatidylinositol-3′ kinase, and Akt. *Mol. Pharmacol.* **63:**886–895; 2003.

[147] Arena, S.; Pattarozzi, A.; Thellung, S.; Villa, V.; Corsaro, A.; Massa, A.; Diana, F.; Spoto, G.; Forcella, S.; Damonte, G.; Filocamo, M.; Benatti, U.; Schettini, G.; Florioa, T. Nitric oxide production stimulated by the basic fibroblast growth factor requires the synthesis of ceramide. *Ann. N. Y. Acad. Sci.* **973:**94–104; 2002.

[148] Florio, T.; Arena, S.; Pattarozzi, A.; Thellung, S.; Corsaro, A.; Villa, V.; Massa, A.; Diana, F.; Spoto, G.; Forcella, S.; Damonte, G.; Filocamo, M.; Benatti, U.; Schettini, G. Basic fibroblast growth factor activates endothelial nitric-oxide synthase in CHO-K1 cells via the activation of ceramide synthesis. *Mol. Pharmacol.* **63:**297–310; 2003.

[149] Zhang, D. X.; Zou, A. P.; Li, P. L. Ceramide reduces endothelium-dependent vasodilation by increasing superoxide production in small bovine coronary arteries. *Circ. Res.* **88:**824–831; 2001.

[150] Didion, S. P.; Faraci, F. M. Ceramide-induced impairment of endothelial function is prevented by CuZn superoxide dismutase overexpression. *Arterioscler. Thromb. Vasc. Biol.* **25:**90–95; 2005.

[151] Li, H.; Junk, P.; Huwiler, A.; Burkhardt, C.; Wallerath, T.; Pfeilschifter, J.; Forstermann, U. Dual effect of ceramide on human endothelial cells: induction of oxidative stress and transcriptional upregulation of endothelial nitric oxide synthase. *Circulation* **106:** 2250–2256; 2002.

[152] Li, H.; Wallerath, T.; Munzel, T.; Forstermann, U. Regulation of endothelial-type NO synthase expression in pathophysiology and in response to drugs. *Nitric Oxide* **7:**149–164; 2002.

[153] Hatanaka, Y.; Fujii, J.; Fukutomi, T.; Watanabe, T.; Che, W.; Sanada, Y.; Igarashi, Y.; Taniguchi, N. Reactive oxygen species enhances the induction of inducible nitric oxide synthase by sphingomyelinase in RAW264.7 cells. *Biochim. Biophys. Acta* **1393:**203–210; 1998.

[154] Knapp, K. M.; English, B. K. Ceramide-mediated stimulation of inducible nitric oxide synthase (iNOS) and tumor necrosis factor (TNF) accumulation in murine macrophages requires tyrosine kinase activity. *J. Leukoc. Biol.* **67:**735–741; 2000.

[155] Manthey, C. L.; Schuchman, E. H. Acid sphingomyelinase-derived

ceramide is not required for inflammatory cytokine signalling in murine macrophages. *Cytokine* **10:**654–661; 1998.

[156] Mathias, S.; Dressler, K. A.; Kolesnick, R. N. Characterization of a ceramide-activated protein kinase: stimulation by tumor necrosis factor alpha. *Proc. Natl. Acad. Sci. USA* **88:**10009–10013; 1991.

[157] Zhang, Y.; Yao, B.; Delikat, S.; Bayoumy, S.; Lin, X. H.; Basu, S.; McGinley, M.; Chan-Hui, P. Y.; Lichenstein, H.; Kolesnick, R. Kinase suppressor of Ras is ceramide-activated protein kinase. *Cell* **89:**63–72; 1997.

[158] Dobrowsky, R. T.; Kamibayashi, C.; Mumby, M. C.; Hannun, Y. A. Ceramide activates heterotrimeric protein phosphatase 2A. *J. Biol. Chem.* **268:**15523–15530; 1993.

[159] Muller, G.; Ayoub, M.; Storz, P.; Rennecke, J.; Fabbro, D.; Pfizenmaier, K. PKC zeta is a molecular switch in signal transduction of TNF-alpha, bifunctionally regulated by ceramide and arachidonic acid. *EMBO J.* **14:**1961–1969; 1995.

[160] Grassme, H.; Bock, J.; Kun, J.; Gulbins, E. Clustering of CD40 ligand is required to form a functional contact with CD40. *J. Biol. Chem.* **277:**30289–30299; 2002.

[161] Grassme, H.; Jekle, A.; Riehle, A.; Schwarz, H.; Berger, J.; Sandhoff, K.; Kolesnick, R.; Gulbins, E. CD95 signaling via ceramide-rich membrane rafts. *J. Biol. Chem.* **276:**20589–20596; 2001.

[162] Grassme, H.; Jendrossek, V.; Bock, J.; Riehle, A.; Gulbins, E. Ceramide-rich membrane rafts mediate CD40 clustering. *J. Immunol.* **168:**298–307; 2002.

[163] Cremesti, A. E.; Goni, F. M.; Kolesnick, R. Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome? *FEBS Lett.* **531:**47–53; 2002.

[164] Holopainen, J. M.; Subramanian, M.; Kinnunen, P. K. Sphingomyelinase induces lipid microdomain formation in a fluid phosphatidylcholine/sphingomyelin membrane. *Biochemistry* **37:**17562–17570; 1998.

[165] Hanissian, S. H.; Geha, R. S. Jak3 is associated with CD40 and is critical for CD40 induction of gene expression in B cells. *Immunity* **6:**379–387; 1997.

[166] Veiga, M. P.; Arrondo, J. L.; Goni, F. M.; Alonso, A. Ceramides in phospholipid membranes: effects on bilayer stability and transition to nonlamellar phases. *Biophys. J.* **76:**342–350; 1999.

[167] Pannu, R.; Singh, A. K.; Singh, I. A novel role of lactosylceramide in the regulation of tumor necrosis factor alpha-mediated proliferation of rat primary astrocytes. Implications for astrogliosis following neurotrauma. *J. Biol. Chem.* **280:**13742–13751; 2005.

[168] Mattson, M. P.; Goodman, Y.; Luo, H.; Fu, W.; Furukawa, K. Activation of NF-kappaB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration. *J. Neurosci. Res.* **49:**681–697; 1997.

[169] Fridovich, I. Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* **64:**97–112; 1995.

[170] Marklund, S. L. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem. J.* **222:**649–655; 1984.

[171] Sandstrom, J.; Carlsson, L.; Marklund, S. L.; Edlund, T. The heparin-binding domain of extracellular superoxide dismutase C and formation of variants with reduced heparin affinity. *J. Biol. Chem.* **267:**18205–18209; 1992.

[172] Jung, O.; Marklund, S. L.; Geiger, H.; Pedrazzini, T.; Busse, R.; Brandes, R. P. Extracellular superoxide dismutase is a major determinant of nitric oxide bioavailability: in vivo and ex vivo evidence from ecSOD-deficient mice. *Circ. Res.* **93:**622–629; 2003.

[173] Didion, S. P.; Ryan, M. J.; Didion, L. A.; Fegan, P. E.; Sigmund, C. D.; Faraci, F. M. Increased superoxide and vascular dysfunction in CuZnSOD-deficient mice. *Circ. Res.* **91:**938–944; 2002.

[174] Fukai, T.; Galis, Z. S.; Meng, X. P.; Parthasarathy, S.; Harrison, D. G. Vascular expression of extracellular superoxide dismutase in atherosclerosis. *J. Clin. Invest.* **101:**2101–2111; 1998.

[175] Li, Y.; Huang, T. T.; Carlson, E. J.; Melov, S.; Ursell, P. C.; Olson, J. L.; Noble, L. J.; Yoshimura, M. P.; Berger, C.; Chan, P. H.; Wallace, D. C.; Epstein, C. J. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* **11:**376–381; 1995.

[176] Pahan, K.; Dobashi, K.; Ghosh, B.; Singh, I. Induction of the manganese superoxide dismutase gene by sphingomyelinase and ceramide. *J. Neurochem.* **73:**513–520; 1999.

[177] Manna, S. K.; Zhang, H. J.; Yan, T.; Oberley, L. W.; Aggarwal, B. B. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *J. Biol. Chem.* **273:**13245–13254; 1998.

[178] Reth, M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat. Immunol.* **3:**1129–1134; 2002.

[179] Rhee, S. G.; Chang, T. S.; Bae, Y. S.; Lee, S. R.; Kang, S. W. Cellular regulation by hydrogen peroxide. *J. Am. Soc. Nephrol.* **14:**S211–S215; 2003.

[180] Traber, D. L.; Adams, T., Jr.; Sziebert, L.; Stein, M.; Traber, L. Potentiation of lung vascular response to endotoxin by superoxide dismutase. *J. Appl. Physiol.* **58:**1005–1009; 1985.

[181] Kannan, R.; Jin, M.; Gamulescu, M. A.; Hinton, D. R. Ceramide-induced apoptosis: role of catalase and hepatocyte growth factor. *Free Radic. Biol. Med.* **37:**166–175; 2004.

[182] Sheikh, F. G.; Pahan, K.; Khan, M.; Barbosa, E.; Singh, I. Abnormality in catalase import into peroxisomes leads to severe neurological disorder. *Proc. Natl. Acad. Sci. USA* **95:**2961–2966; 1998.

[183] Singh, I. Peroxisomal fatty acid oxidation and cellular redox. *Methods Enzymol.* **352:**361–372; 2002.

[184] Baumgart, E.; Vanhorebeek, I.; Grabenbauer, M.; Borgers, M.; Declercq, P. E.; Fahimi, H. D.; Baes, M. Mitochondrial alterations caused by defective peroxisomal biogenesis in a mouse model for Zellweger syndrome (PEX5 knockout mouse). *Am. J. Pathol.* **159:**1477–1494; 2001.

[185] Suzuki, K. Twenty five years of the "psychosine hypothesis": a personal perspective of its history and present status. *Neurochem. Res.* **23:** 251–259; 1998.

[186] Khan, M.; Haq, E.; Giri, S.; Singh, I.; Singh, A. K. Peroxisomal participation in psychosine-mediated toxicity: implications for Krabbe's disease. *J. Neurosci. Res.* **80:**845–854; 2005.

[187] Kajita, K.; Mune, T.; Kanoh, Y.; Natsume, Y.; Ishizawa, M.; Kawai, Y.; Yasuda, K.; Sugiyama, C.; Ishizuka, T. TNFalpha reduces the expression of peroxisome proliferator-activated receptor gamma (PPARgamma) via the production of ceramide and activation of atypical PKC. *Diabetes Res. Clin. Pract.* **66 Suppl. 1:**S79–S83; 2004.

[188] Cimini, A.; Bernardo, A.; Cifone, M. G.; Di Marzio, L.; Di Loreto, S. TNFalpha downregulates PPARdelta expression in oligodendrocyte progenitor cells: implications for demyelinating diseases. *Glia* **41:**3–14; 2003.

[189] Yasmineh, W. G.; Parkin, J. L.; Caspers, J. I.; Theologides, A. Tumor necrosis factor/cachectin decreases catalase activity of rat liver. *Cancer Res.* **51:**3990–3995; 1991.

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