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REVIEW

Sphingolipids and Cell Signaling: Involvement in Apoptosis and Atherogenesis

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Abstract—This review considers various functional aspects of cell sphingolipids (sphingomyelin, ceramides) and lysosphingolipids (sphingosine-1-phosphate (S1P) and sphingosine phosphorylcholine). Good evidence now exists that they are actively involved in numerous cell-signaling processes. The enzymes responsible for formation and interconversion of cell sphingolipids (sphingomyelinases, ceramidase, sphingosine kinase, S1P-lyase) exhibit high sensitivity to various stimulating factors. This determines the content of individual cell sphingolipids and therefore the mode of cell response. Special attention is paid to preferential localization of sphingolipids in the rigid plasma membrane domains (rafts) coupled to many signal proteins. The suggestion is discussed that ceramide signaling may be based on the modification of fine molecular interactions in lipid rafts, resulting in its clusterization inducing the signal transduction. The review also highlights involvement of sphingolipids in cell proliferation, apoptosis, and in processes implicated to atherosclerosis.

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Sphingomyelin (SM) is the third major membrane phospholipid (after phosphatidylcholine and phosphatidylethanolamine). For several decades, it has attracted much attention as a structural membrane element. Recent achievements in this field significantly extended our knowledge on the role of SM and its metabolites in biomembrane functioning. Good evidence now exists [1-4] that not only SM but also other sphingolipids (SPL) (ceramides, glycosphingolipids, lysosphingolipids) play an important regulatory role in crucial cell processes (figure). SPL are involved in regulation of cell proliferation, apoptosis, cell migration, adhesion, vascular tone; they play a crucial role in the development of atherosclerosis and its complications, and they are involved in cardiotoxicity induced by anthracyclines [2-12]. SPL and their derivatives mediate various biological effects of tumor necrosis factor (TNF-α); these include monocyte differentiation, fibroblast resistance to insulin, proliferation, and expression of adhesion molecules [13-15]. These diverse effects involve SPL in various processes of cell signaling.

In this review, we consider some aspects of signaling processes that involve SPL. Special attention is paid to their localization in membrane domains (rafts) and the role of SPL in some stages of apoptosis and atherogenesis.

In contrast to phosphatidylcholine, which is almost equally distributed in membranes of various cells, SPL (mainly SM) are preferentially located in brain tissues, nerve fibers, and also in vascular tissues. Such preferential location may be attributed to special mechanical resistance required by these tissues. Involvement of SM in membrane rigidity is due to combination of two saturated carbohydrate chains in its molecule and high affinity to cholesterol. There is evidence for an integrated mode of cellular metabolism of SM and cholesterol; this is confirmed by increased content of both SM and cholesterol in the same cell compartments, plasma membrane, and Golgi complex [16-18]. There are other membrane SPL

Abbreviations: ER) endoplasmic reticulum; FasL) Fas-ligand; GCS) glycosyl ceramide synthase; HDL) high density lipoproteins; LDL) low density lipoproteins; S1P) sphingosine-1-phosphate; SM) sphingomyelin; SPL) sphingolipids; SPC) sphingosine phosphocholine; TNF) tumor necrosis factor.

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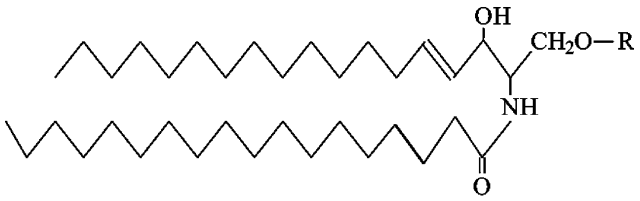


Structure of sphingolipids. R = H in ceramides, R = phosphocholine in sphingomyelin, R = sugars in glycosphingolipids

and their major proportion is preferentially located on the outer surface of plasma membrane [17]. SPL (mainly SM) have also been found in all classes of plasma lipoproteins [19].

During the last decade, unequivocal lateral distribution of SPL on the membrane surface has been firmly recognized. SPL are concentrated in recently identified microdomains known as rafts. Rafts are "islets" of more or less ordered organization of lipid molecules, which contrast to the main liquid crystal membrane liquid phase. They are characterized by detergent insolubility. Besides SPL (mainly SM), rafts concentrate the major part of membrane cholesterol and phospholipids with saturated hydrocarbon chains. Rafts are preferentially located within the outer plasma membrane layer, where they are associated with many proteins involved in cell signal transduction. So changes in raft organization represent one of the regulatory tools that may influence functioning of these proteins and, consequently, signaling processes. Caveolar rafts, or caveoles, containing the protein caveolin and organized as flask-shaped invaginations, are especially active [17, 20, 21]. Rafts and caveoles are considered as signal-initiating "platforms" of membrane surface and preferential SPL localization in them suggests involvement of these lipids in signal transduction. Numerous experimental data obtained mainly during the last decade using various cell types and animals have provided convincing evidence that SPL play a role of the second messengers regulating many important cell processes [8-11].

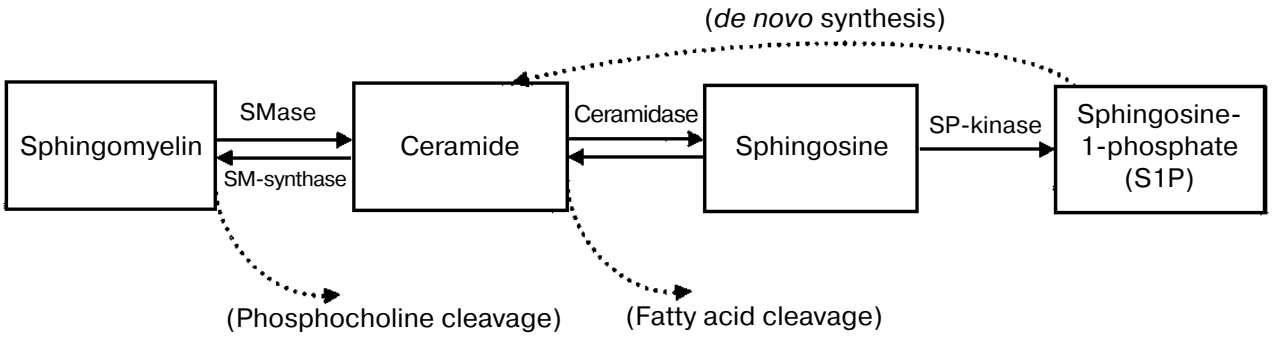
SPL may also potentiate effects of some extracellular regulators. SPL are involved in signaling processes initiated by cytokines, growth factors, stress agents, and lipoproteins [10-13]. They also act as mediators of some extracellular stimuli. The common mechanism of this phenomenon includes stimulus-induced generation of ceramide and its derivatives in the target cell. Such mechanism is supported by the fact that addition of exogenous SPL to a cell culture imitates biological effects of cytokines and growth factors. Modulation of intracellular synthesis of SPL-mediators may alter cell response to external stimuli [14, 15, 22].

Table 1 lists coupling of intracellular generation of SPL and their derivatives to the action of some effectors promoting development of pathological states.

Endothelial and smooth muscle cells are the most sensitive to various SPL-generating external stimuli. These cells respond to many stimuli by generating various SPL. Generation of ceramides, sphingosine, and sphingosine-1-phosphate (S1P) is the most frequent cell response. Increased activity of sphingomyelinase

Table 1. Pathophysiological stimulation resulting in sphingolipid generation in the cardiovascular system cells (by [22])

Cell types	Stimulus	Sphingolipid generated
Endothelial	ionizing radiation	ceramides
	lipopolysaccharide	«
	oxidized LDL	«
	interleukin-1	«
	TNF	ceramides, S1P, lactosylceramide
Smooth muscle	TNF	ceramides
	oxidized LDL	ceramides, S1P, lactosylceramide
	decreased Mg^{2+}	ceramides
	platelet derived growth factor (PDGF)	sphingosine
Platelets	thrombin	sphingosine-1-phosphate (S1P)
Monocytes/lymphocytes	Fas/FasL (apoptosis factors)	ceramides
	interleukin-2	«
	CD28	«
	oxidized LDL	«
Cardiomyocytes	ischemia/reperfusion	«
	doxorubicin	«
	TNF	sphingosine



Simplified metabolic pathway involving interconversions of the main SPL (by [22]). Abbreviations: SMase) sphingomyelinase; SM-synthase) sphingomyelin synthase; SP-kinase) sphingosine phosphokinase

Scheme 1

(SMase) accompanying formation of SPL-metabolites was found to be increased before appearance of initial stages of early atherosclerotic lesions, vascular smooth muscle cell proliferation [23, 24].

Ceramides and lyso-sphingolipids (S1P and sphingosine phosphocholine (SPC)) are the most biologically active SM-derivatives. (Biological effects of glyco-SPL have been reviewed elsewhere [3, 25].)

Ceramides are compounds possessing two hydrocarbon chains and a weakly polar head group formed by fatty acid carbonyl and sphingosine amino- and hydroxyl groups (figure). They can be formed in membranes during SM hydrolysis catalyzed by SMases cleaving the phosphocholine fragment from its molecule. Ceramides are generated in any cell types during cell stimulation and various cell responses; their formation involves acidic or neutral SMase or *de novo* synthesis [3, 14, 15, 26, 27] (Scheme 1).

Ceramides can be reutilized for SM synthesis catalyzed by SM-synthase located in the Golgi complex or plasma membrane [28, 29]. Ceramides can also be further hydrolyzed by ceramidase with formation of sphingosine. The latter may undergo re-acylation yielding ceramide or phosphorylation by sphingosine kinase yielding S1P [1, 30].

The major pathway of SM catabolism is hydrolysis to ceramide. It occurs in acidic cell compartments and involves lysosomal SMase. Subsequent ceramide cleavage is accompanied by formation of sphingosine, which can cross the lysosomal membrane. Besides lysosomal digestion, there is non-lysosomal cleavage of SM; it involves membrane-bound hydrolases with neutral or alkaline pH optima. These enzymes (also including SMases and ceramidases) may play an important role in cell signal transduction [10, 15, 31, 32].

Regulation of SPL interconversions is associated with various conditions of cell behaviors influencing SMase activity. Activation of SMase can by initiated by some cytokines, ionizing radiation, antitumor drugs, and also during apoptosis [10, 13, 33, 34]. Effects of activators can be imitated *in vitro* by addition of ceramides or exogenous SMase [35, 36]. Activation of ceramide biosynthesis can be mediated by TNF-α receptor [37]; the effect of TNF-α is characterized by cell-type specificity, and its activation of different SMases is induced in different manners [38] (Table 2).

Table 2. Effect of TNF-α on ceramide generation in cells (modified from [38])

Cells	Type of activated SMase	Duration of ceramide generation, min*
Human leukemia cells		
HL-60	neutral SMase	7.5-60
Jurkat	acidic SMase	2
U937	same	2
Human fibroblasts	neutral SMase	30
Mouse fibroblasts	acidic and neutral SMases	24**

* Time required for the "peak" of activation after treatment with TNF-α is shown.
** In hours.

Signaling pathways initiated by TNF-α, which influence hydrolysis of SM and generation of ceramides, are not completely understood. However, there is evidence that they include phosphorylation/dephosphorylation of proteins, activation of some phospholipases (including SMases) and transcription factors, and possibly involvement of reactive oxygen species formation [3, 32, 39-42].

Ceramides are also formed in the apoptotic cells in acidic SMase-independent manner; it has been suggested that other, neutral sphingomyelinases (nSMases) are involved [21]. One such enzyme, known as the isoform nSMase-1 and located in endoplasmic reticulum (ER) and/or nucleus, has been cloned [43]. Lack of SMase is associated with the genetic disease known as Niemann–Pick disease. Mice lacking SMase are used as the animal model of this disease. They are characterized by multiple cell abnormalities [44], which include deficit of SPL and cholesterol in membranes and related impairments characterized by formation of functionally active rafts. In contrast to normal animals, it is impossible to isolate rafts using standard procedures based of raft insolubility in detergents [17, 20]. The latter is attributed to low level of "rigid" lipids.

Generation and accumulation of SPL involve various pathways characterized by different subcellular localization. In many cases, it is nearly impossible to identify the preferential pathway of ceramide formation; this is due to its relation to topology with SPL metabolism, including trans-bilayer and intermembrane movement of products formed during enzymatic reactions [28, 45]. Subcellular localization of the synthesized ceramides plays an important role for selection of putative targets for their actions. This underlines diversity of ceramide effects and therefore biological responses of the cell. Cell stimulation results in activation of different SMases and various cell sites such as plasma membrane (its outer and inner surfaces and microdomains) or transiently acidified compartments. Fine and strict regulation of SPL-generating enzymes is the important aspects of formation of SPL-mediators. For example, extracellular SMase is activated by some lipids (e.g., arachidonic acid) and serine proteases, whereas stimulation of nSMase localized in ER is regulated by protein kinase C isoforms and depends on glutathione level [15, 46-48]. Coupling of SMase and ceramidase with many stimuli determines an important role of these enzymes in cell signal transduction.

In contrast to other signaling molecules, the functioning of ceramides as second messengers occurs in cell membranes. This is a characteristic feature of these SPL localized in cell membranes. Ceramide signaling in the cell is mainly associated with their effects on membrane microdomains, which are crucial for signal transduction [17, 49, 50]. Ceramide formed from the main SM pool on the external surface of plasma membrane is spontaneously transported neither within the membrane bilayer ("flip-flop") nor between lipid bilayers; it is located on the same side of membrane where it has been formed. So it seems unlikely that ceramides can function as second messengers for cytoplasmic target proteins, readily responding for various stimuli. Consequently, ceramide signaling should alter local membrane-forming properties of SPL at places of ceramide formation from SM [51, 52]. Two long saturated hydrocarbon chains of ceramide (which may also form hydrogen bonds) determine its rapid insertion into phospholipid bilayer of membranes where it is mainly segregated in rafts forming tightly packed associates with other SPL and cholesterol [17, 20, 21]. For example, in cell membranes of human *stratum corneum* the level of ceramides may reach 50% of all lipids [53]. Van Blitterswijk et al. suggested that ceramide may modify fine molecular interactions inside lipid rafts and/or between them and this results in clusterization of raft and therefore to signal transduction [51].

This suggestion was confirmed by Gulbins et al. [54]; these authors demonstrated that ceramide (generated in the raft on the outer side of the bilayer or exogenously inserted into this raft) may cause clusterization of CD95/Fas receptor followed by subsequent induction of apoptosis. Taking into consideration a low pH optimum of acidic SMase, these authors suggest that this enzyme is located in recycling endosomes and is transiently activated at low pH. After fusion with plasma membrane, ceramide generated on the endosome surface then appears on the plasma membrane outer surface, where it promotes clusterization of rafts and possibly neighboring CD95 receptors. Involvement of SMase in this process was demonstrated using hepatocytes with SMase deficiency [55]. These cells exhibited low sensitivity of CD95 receptor, which could be increased after addition of ceramide. In this model, ceramide acts as a structural component of membrane rather than second messenger [52].

Intracellular topology of ceramide formation from SM is essential for manifestation of its biological activity. This was demonstrated in a study where the authors tried to stimulate ceramide glucosylation in T-cells using glycosyl ceramide synthase (GCS) localized in Golgi complex [56]. However, this enzyme did not glucosylate ceramide accumulated during apoptosis, induced by CD95 or anti-cancer regimens. These authors believe that GCS, located at the Golgi, is topologically segregated from ceramide produced in the plasma membrane. In contrast, *de novo* synthesized ceramide was efficiently glycosylated, apparently due to favorable ceramide topology (i.e., localization in cell compartments near Golgi complex). So it was suggested that only *de novo* synthesized ceramide molecules are susceptible to GCS, whereas ceramide formed in the plasma membrane cannot be spontaneously transported to intracellular membranes. Exogenous cell-permeable short-chain ceramide analog (C2-ceramide) was effectively glycosylated. The authors explained this phenomenon by different physicochemical behavior of short-chain ceramide species and stressed

that the exogenous ceramide analogs employed in model experiments did not reflect behavior of endogenously formed ceramide. Consequently, certain precaution should be taken for explanation of ceramide effects as second messengers. For example, such precaution should be applicable for data on possibility of trans-bilayer ("flip-flop") movement of ceramide in membranes obtained using spin-labeled analogs [57].

Some other effects of ceramides have also been reported. For example, they can attenuate phenylephrine-induced vasoconstriction and elevation in intracellular calcium ions [58]. Ceramides also play a certain role in radiation and anthracycline-induced cardiotoxicity; for example, they are involved in apoptosis of cardiomyocytes induced by the antitumor antibiotic doxorubicin. This was demonstrated by blockade of SMase activation and ceramide accumulation caused by L-carnitine, preventing cardiomyocyte damage and reducing apoptosis [33]. Ceramides stimulated the expression of adhesion molecules, e.g., E-selectin. Ceramide formation induced by N-octanoyl sphingosine, bacterial SMase, or TNF-α promoted E-selectin-modulated adhesion of resting neutrophils to human endothelial cells [59].

The other targets of ceramides formed in some (unidentified) subcellular regions are serine/threonine protein kinases (including proline-directed serine/threonine ceramide-activated protein kinase), mitogen-activated protein kinases (MAPK), protein kinase C, cytosolic ceramide-activated protein phosphatase, and a transcription factor [3, 32, 41, 48]. It is suggested that ceramides activate nuclear factor κB (NF-κB), a key element of numerous biological processes [22, 38-40].

Besides ceramides other SM-metabolites, such as its lyso-derivatives acting in coordination with lysophospholipids, are involved in regulation of numerous cellular processes.

Lysosphingolipids (lyso-SPLs) are deacylated SPL-derivatives generated in membranes of stimulated cells. They are formed during reversible cleavage of ceramide to sphingosine followed by its subsequent phosphorylation (Scheme 1). Various pathways of metabolism and generation of lyso-SPLs (including S1P) are attributed to different subcellular localization of reactions determining formation of these metabolites. The most active lyso-SPLs are S1P and SPC.

Sphingosine-1-phosphate is formed in the reaction catalyzed by sphingosine kinase, which is highly active in ER. This enzyme is sensitive to stimulation by platelet derived growth factor (PDGF), phorbol ester, and other effectors. The resultant S1P may be further dephosphorylated by S1P phosphatase or cleaved by ER-bound S1P lyase [6, 9, 60].

Being a relatively water-soluble molecule, S1P can be released from the membrane into extra- or intracellular space, and the effects of S1P are different for extracellular and intracellular localization [9, 11].

Recently high biological activity of S1P has been recognized. Being released from platelets during clot formation, it determines a major portion of the chemotactic activity of blood serum [61]. *In vitro* experiments revealed an important role of S1P in angiogenesis in model systems [61, 62]. Besides stimulation of proliferation and chemotaxis of endothelial cells, S1P also stimulates formation of capillaries in a cell culture grown in a collagen gel [62]. The other study revealed that S1P did not stimulate angiogenesis in a vascular mouse cornea, but it did increase the response to basic fibroblast growth factor (bFGF) [63]. Accumulation of S1P increased the level of adhesion molecules: E-selectin and VCAM-1 (vascular cell adhesive molecules). Expression of these molecules involves activation of NF-κB and MAPK and parallel activation of protein kinase C [64]. Biological activity of S1P is believed to be controlled by coordinative regulation of sphingosine kinase and S1P lyase activities [65], which may be influenced by various factors. For example, TNF-α induced activity of sphingosine kinase and formation of S1P [38].

Recently, another active lyso-SPL regulator, **sphingosine phosphocholine (SPC)**, has been discovered. Sphingosine phosphocholine is the product of SM deacylation. It is a new signal messenger causing increased sensitivity to Ca^{2+}. Some aspects of the effects of SPC are attributed to its putative activation of NO synthase and related metabolic processes (due to easy penetration of SPC through cell membranes). SPC and S1P were found in plasma low density lipoproteins (LDL). These lipid regulators are key signaling molecules involved in stimulation of cell growth [66].

Sphingosine, S1P, and SPC are potential calcium mobilizing agonists. S1P increased intracellular Ca^{2+} release initiated by sphingosine, whereas SPC mobilized Ca^{2+} in smooth muscle and endothelial cells [7]. *In vitro* experiments on tissue sections revealed that S1P and SPC also induce constriction of micro-vessels and coronary arteries [67]. However, in coronary arteries SPC induced endothelium-dependent relaxation preceding vasoconstriction [68]. These opposite effects may be attributed to susceptibility of NO synthase to activation by ceramides or lyso-SPL. Indeed, the relaxing effect of SPC was realized via NO formation, and blockade of NO synthase blocked it [68].

Regulatory effects of lyso-SPL were manifested together with effects of lysoglycerophospholipids (especially lysophosphatidic acid). So both classes of lipids, lyso-SPL and lysoglycerophospholipids, attract much attention. Significant effects of lysophosphatidic acid and S1P (e.g., regulation of secretion, cell adhesion, and chemotaxis) are related to cell effector functions, which depend of cytoskeletal proteins [69].

Sphingolipids and lysosphingolipids in the regulation of cell proliferation. The role of SPL in malignant growth has recently been reviewed [4]. For example, hepatoma-

27 cells were characterized by higher levels of ceramides, glycosyl- and lactosylceramide, and SM than normal rat hepatocytes [70]. These SPL and also S1P and SPC may stimulate DNA synthesis in endothelial and smooth muscle cells [71]. They also promote mitogenesis induced by growth factors. Mitogen-induced proliferation of smooth muscle cells is also related to sphingosine formation; the latter involves activation of ceramidase [24]. Accumulation of these SPL accompanied by increase in MAPK activity is believed to be a key factor in cell proliferation [32].

In contrast to these effects, treatment with TNF (resulting in ceramide production) or with exogenous ceramides inhibits cell growth. In a model system of hypertensive rats, ceramides inhibited proliferation of smooth muscle cells. In these cells mitogenic response to TNF was associated with inhibition of ceramide generation and expression of sphingomyelinase mRNA [72]. In general, ceramides and sphingosine are potential inhibitors and S1P is a potential stimulator of smooth cell proliferation [62].

Lysosphingolipids and atherosclerosis. The effects of lyso-SPL on smooth muscle proliferation suggest possible involvement of these lipids in processes associated with development of atherosclerosis (see reviews [22, 24]). Recent studies also revealed that LDL and especially oxidized LDL as well as cytokines, growth factors, and ionizing radiation activate SM-ceramide pathways in vascular smooth muscle and endothelial cells (Table 1), and this may result in cell proliferation, differentiation, or apoptosis [24]. It is suggested that lyso-SPL can act as proliferation inducers partially responsible for the atherogenic effect of oxidized LDL.

Indeed, S1P activated smooth muscle cells [73], and entry of oxidized LDL into cells was accompanied by intracellular accumulation of S1P [22, 74].

Auge et al. found [73] that the mitogenic effect of LDL involves combined activation of sphingomyelinases, ceramidases, and sphingosine kinase; this results in formation of SPL metabolites including S1P (Scheme 1). Activation of neutral SMase followed by conversion of SM into ceramides and then into lyso-SPL preceded proliferation of smooth muscle cells induced by oxidized LDL. This means that at least part of the mitogenic effect of LDL is determined by S1P and SPC [12, 23, 24]. S1P generated inside cells exposed to oxidized LDL and S1P released by platelets may be involved in proliferation of cells of vascular wall; this causes its intimal thickening and stabilization of an atheroma [71]. The atheroma accumulates glyco-SPL and also ceramides generated by the secretory SMase localized in it. This SMase promotes LDL aggregation, which increases the atherogenic potential. Thus atherogenic effect of LDL is determined not only by excessive accumulation of cholesterol and reactive oxygen species in the cell, but also by generation of mitogenic signals associated with increased formation of S1P. In contrast to LDL, high density lipoproteins (HDL) decrease S1P formation in endothelial cells by inhibiting sphingosine kinase; this may represent an additional mechanism of the known anti-atherogenic effect of HDL [75].

Atherogenic effects of S1P may also be related to the increased formation of cell adhesion molecules, which play an important role in the progression of atherosclerosis. Ceramides or S1P involved in inflammatory response initiated by cytokines or oxidized LDL can increase expression of adhesion molecules and induce monocyte adhesion to vascular intima; they can also modulate aggregation and thus promote thrombus formation [64]. SPL can also influence the development of cardiovascular diseases and promote progression of heart failure and arrhythmia [21].

S1P and SPC have been found in all classes of lipoproteins [24]. However, there are contradictory data of the effect of these lipoprotein SPL on atherogenesis; the maximal content of S1P (expressed per mg protein) was found in anti-atherogenic HDL, whereas S1P content in LDL was markedly decreased during oxidation of these lipoproteins [76, 77]. Based on these data, Okajima [77] suggested that S1P may be not only the atherogenic mediator (as many researchers believe [12, 23, 24, 73-75]), but under certain conditions it may exhibit anti-atherogenic effects due to its cytoprotective action. Indeed, such a complex process as atheroma formation involves not only proliferation but also death (apoptosis) of vascular cells, promoting cell debris formation. The degree of LDL oxidation is crucial for manifestation of LDL effects: minimally or mildly oxidized LDL (with the level of oxidation products ~10 μg/ml) caused the mitogenic effect, whereas highly oxidized LDL (50-100 μg/ml) was cytotoxic [24]. Using human umbilical vein cell culture, it has been shown that addition of oxidized LDL increases cell death in the absence of serum or growth factors in the culture medium. On the other hand, HDL or purified S1P from HDL exerted cytoprotective actions. The cytoprotective actions of HDL and S1P were associated with extracellular signal-regulated kinase (ERK) activation [77]. This demonstrates that under conditions of oxidative stress (which plays an important role in the pathogenesis of atherosclerosis) S1P delivered by HDL to preformed atherosclerotic plaque (atheroma) may exert a cytoprotective effect.

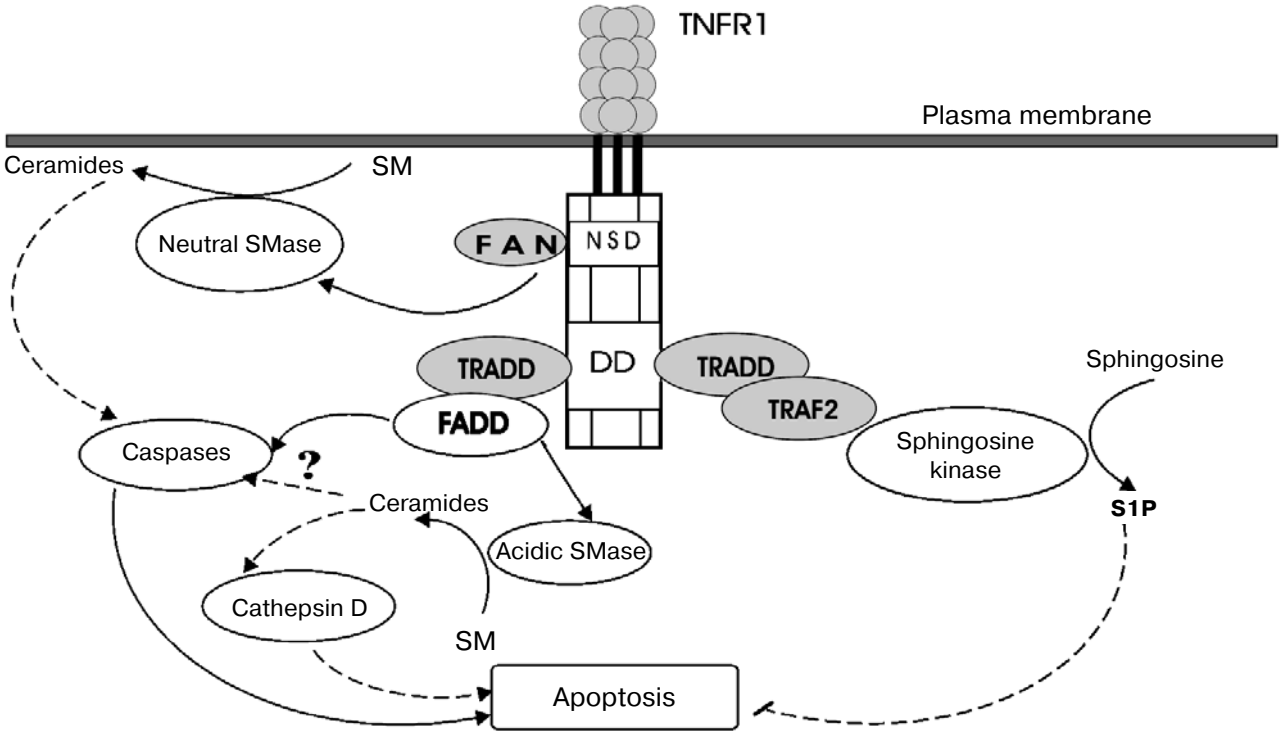
Sphingolipids and apoptotic processes. Among signaling effects of SPL, their role in apoptotic processes attracts much attention. Several studies revealed a role of ceramides as second messengers in apoptosis induced by various effectors. Stress treatments initiating apoptosis are accompanied by intracellular ceramide production, which is a characteristic manifestation of apoptosis. In response to various stress signals (e.g., cytokine attack, radiation, etc.) ceramide formed in cells from membrane SM can activate its key regulators [5, 8, 14, 38, 45].

Studies of links between ceramides and apoptosis revealed at least two phases of ceramide formation. The first rapid (and therefore hardly detected) phase is characterized by a transient increase in ceramide level; it occurs within several minutes after apoptotic stimulus in the inducer phase of apoptosis, whereas the second phase of slow constantly increasing formation is typical for the effector phase of apoptosis [78]. These diverse reactions of SPL observed on various stages of apoptosis are attributed to SPL effects on membrane microdomains crucial for signal transduction.

Apoptosis induced by TNF-α is the most studied process, employing sphingolipid-dependent cell signaling. Malagarie-Cazenave [38] proposed the following scheme of sphingolipid signaling in apoptosis (Scheme 2).

According to this scheme, interaction of TNF-α binding is accompanied by trimerization of its membrane receptor, TNFR1. This results in complex formation between the cytoplasmic part of TNFR1 (neutral sphingomyelinase domain, NSD) and the adaptor protein, FAN. One fragment of this complex can activate neutral SMase, whereas the other one is involved in activation of acidic SMase. The latter is also activated by the adaptor proteins, FADD and TRADD, which bind to DD domains of TNFR1. Ceramide formed during SMase action acts on specific proteins, caspases, inducing apoptosis [79]. Ceramide can also activate the protease cathepsin D, promoting apoptosis. The proposed mechanism is supported by the fact that in cells resistant to the cytotoxic effect of TNF, ceramide synthesis is blocked, and inherited deficiency or inhibition of SMases uncouples apoptosis. On the other hand, TRAF2 protein, the factor coupled to TNF receptor, can interact with sphingosine kinase and activate formation of S1P, which exhibits properties of the antiapoptotic mediator.

Opposite effects of ceramides and S1P on apoptosis have been considered in review [4]. They have also been demonstrated in endothelial cells, and good evidence now exists that apoptosis induced by exogenous ceramides can be prevented by activation of S1P generation [80]. Several putative mechanisms of the antiapoptotic effect of S1P have been proposed: i) activation of antiapoptotic signaling pathways (NF-κB, ERK-kinase); ii) reduction of proapoptotic Bcl-2 (Bax) protein expression (demonstrated in lymphocytes); iii) prevention of stress-induced processes in mitochondria (e.g., blockade of release of cytochrome *c*, inducing the chain of reactions of the apoptotic enzymes, caspases [79]); iv) increase in NO production [81].



Sphingolipid involvement in TNF-α receptor mediated apoptosis (modified from [38]). Abbreviations: NSD) neutral sphingomyelinase domain; FAN) factor associated with activation of neutral SMase; TNFR) tumor necrosis factor receptor; DD) death domain; TRADD) TNFR associated protein with death domain; TRAF2) TNFR associated factor 2; FADD) Fas associated protein with death domain. Dashed lines show indirect pathways

Scheme 2

In contrast to S1P, ceramides and sphingosine are considered as proapoptotic factors [78, 82, 83]. For example, lung endothelium, small intestine, and spinal cord cells of mice lacking SMase (i.e., incapable of synthesizing ceramides) were resistant to radiation-induced apoptosis, and ceramide addition induced sensitivity to apoptosis [84]. There is a relationship between apoptosis of endothelial cells exposed to the lipopolysaccharide-induced septic shock and ceramide generation [85]. However, some other data suggest that deficit or increased expression of acidic SMase in B-lymphoblasts do not influence apoptosis induced by radiation or carcinogenic preparations. The authors concluded that although activity of this enzyme promotes induction of apoptosis, it is not ultimately required for this process. Other specific features of these cell types may also be important [86].

Specific but unidentified properties of various cell types influence the apoptotic response in the presence of glucosylceramide synthase, which catalyzes ceramide glycosylation. Utilizing ceramides, this enzyme protects some cell types against apoptosis. For example, this has been demonstrated in MCF-7 cell culture under conditions of long-term treatment with adriamycin or TNF-α. However, in other cell types (Jurkat and GM95) this effect was not observed [87].

The mechanism of ceramide signaling in apoptosis remains unclear. For example, in endothelial cells signaling of sphingolipid-induced apoptosis involves inhibition of "cell survival" processes such as serine/threonine protein kinase B (PKB/Akt) [88] and/or activation of caspase cascade [79]. Szabo et al. [89] showed that apoptotic stimuli cause reorganization of rafts, which are pooled into large ceramide-enriched membrane platforms, which play specific roles in the apoptotic processes; for example, the platforms are involved in regulation of ion channels and in assembly of signaling complexes [89, 90].

Ceramide formed within the outer surface of cell membrane can induce polar redistribution and oligomerization (capping) of the apoptotic Fas receptor. This is accompanied by formation of super catalytic domain on the cytoplasmic side, which potentiates Fas-mediated apoptosis [91].

Ceramide involvement in apoptotic signal transduction may also be related to SM translocation to plasma membrane; during this translocation SM is displaced to the cytoplasmic side (due to the reverse translocation of phosphatidylserine [79]). This translocation of SM is accompanied by its hydrolysis and internal localization of forming ceramide promotes its effects on intracellular processes [20, 21, 40]. Loss of SM on the outer surface of the membrane exerts a profound influence on the physicochemical properties of plasma membrane, including fluidity of microdomains and cholesterol redistribution [52, 92]. This influences organization of rafts (and signaling associated with them) and promotes formation of membrane vesicles, loss of these vesicles, and formation of apoptotic bodies [38, 79]. Addition of exogenous SM to the apoptotic cell prevents development of these superficial changes. This demonstrates that not only ceramide formation but also translocation of SM from the membrane surface followed by its subsequent hydrolysis can be determining factors for morphological changes in the apoptotic cell [93].

Ceramide can also stimulate destabilization of mitochondrial membranes and release of apoptogenic factors. For example, increasing Bax induced membrane permeability, ceramide initiates formation of nonspecific ceramide pores, which increase outer mitochondrial membrane permeability and increase release of cytochrome *c*, which promotes caspase activation [94].

Studies of the relationship between apoptosis and ceramide formation in cells may have clinical importance, because some pathological states may be related to effects of certain apoptotic stimuli, such as abnormal production of TNF-α or its altered interaction with receptor (e.g., septic shock, Crone disease, some inflammation states).

Thus, understanding of regulatory effects of various SPL on cell processes may help development of therapeutic treatments including administration of proapoptotic SPL in oncology [4] or strategy of directed action on targets involved in sphingolipid metabolism.

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