

Vascular sphingolipids in physiological and pathological adaptation

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1. ABSTRACT

Sphingolipids (SLs) are compounds containing a long-chain fatty alcohol amine called sphingosine which exists in cellular membranes, cytoplasm, nucleus, interstitial fluid, blood and lymphatic circulation. SLs act as essential constituents of membranes of eukaryotic cells, so the seesaw of SLs will lead to structural alteration of membranes instigating cellular functional change. SLs also act as crucial signaling molecules taking effect intracellularly or extracellularly which regulates activity of downstream molecules determining cellular adaptation to numerous stimulus. This review aims to highlight the contribution of SLs to physiological and pathophysiological remodeling of vasculature. We will first provide a short overview on metabolism, trafficking and compartmentalization of SLs. Then the regulation of SLs on reactive oxygen species (ROS) formation, vascular tone modulation, endothelial barrier integrity, apoptosis and autophagy are summarized. Finally, we will discuss how the SLs are modulated contributing to vascular development, angiogenesis and vascular remodeling in pathological situations as hypertension, atherosclerosis, and aging. The compellingly regulative

actions of SLs bring about copious therapeutic targets for potential pharmacological intervention on the diseases involving vascular maladaptation.

2. INTRODUCTION

Sphingolipids (SLs) are a batch of lipids with backbones of eighteen carbon amino-alcohol as sphingosine, phytosphingosine or dihydrosphingosine which could be further modified giving rise to large amount of bioactive metabolites with more complicated structure (1). For example, phosphorylation of the C1 hydroxyl group of sphingosine, phytosphingosine and dihydrosphingosine induce the production of sphingosine-1-phosphate (S1P), phytosphingosine-1-phosphate and dihydrosphingosine-1-phosphate, respectively. Acylation of C-2 amino groups through the action of distinct ceramide synthases brings about molecules named as ceramide (Cer), phytoceramide, or dihydroceramide which actually are a host of molecules with different length of acyl chain exerting distinct biological functions. With the use of glycosyltransferases, glycosphingolipids (GSLs) such as

glucosylceramide and lactosylceramide are produced. Cer could also be phosphorylated to generate ceramide-1-phosphate (C1P). When a phosphocholine headgroup rather than the sugar residues is added to the Cer, sphingomyelin (SM) species is synthesized.

SLs are essential structural constituents of kinds of membranes in eukaryotes. However, copious evidence have shown that the members of the SL family, centered on Cer and S1P and extended to GSLs, C1P and others, are vital bioactive molecules regulating a lot of critical cellular processes as proliferation, apoptosis, motility, differentiation, angiogenesis, stress responses, protein synthesis, carbohydrate metabolism, and intracellular trafficking (2). The regulating patterns include directly adjusting content or activity of downstream signaling factors as well as dynamically clustering with sterols forming lipid microdomains or rafts on cell membrane which function as hubs for the aggregation of receptors and signaling molecules to increase the efficiency of signaling transduction significantly (3). The lipid rafts also works for the recruitment and assembling of subunits with which the activity of enzyme such as dihydronicotinamide adenine dinucleotide phosphate (NADPH) oxidase could be enhanced prominently (4). More and more report has indicated SLs play a critical role in structural and functional adaptation of vasculature to physiological or pathological stimulus.

In this review, we will first summarize the metabolism, trafficking and compartmentalization during SLs synthesis and degradation, and then introduce the role of SLs in regulation of reactive oxygen species (ROS) production, vascular tone, endothelial barrier integrity, apoptosis, autophagy, proliferation and migration of endothelial cells (ECs) or vascular smooth muscle cells (VSMCs). Finally, regulation of SLs on vascular development, angiogenesis, and pathophysiological vascular remodeling in hypertension, atherosclerosis, magnesium deficiency, and aging will be discussed.

3. METABOLISM, TRAFFICKING AND COMPARTMENTALIZATION OF SPHINGOLIPIDS SLs

Cer lies in the center of the metabolism of SLs which is generated through pathways including de novo synthesis, sphingomyelin degradation or salvage generation from sphingosine (3). For the detail on biosynthesis, degradation and trafficking of SLs in eukaryotic cells, the readers can refer to the reviews 1, 2, 3 and 6. Briefly, the de novo synthesis of Cer begins with the condensation of serine and palmitoyl-coA upon the catalysis of serine palmitoyl transferase (SPT), then with the sequential functioning of 3-ketosphinganine reductase, dihydroceramide synthases and dihydroceramide desaturase, Cer is produced (Figure 1). The above procedures happen

on the cytosolic surface of endoplasmic reticulum (ER) and potentially in ER-associated membranes, such as the perinuclear membrane and mitochondria-associated membranes. Cer could be transported by ceramide transporter (CERT), vesicle, phosphoinositol 4-phosphate adaptor protein-2 (FAPP2) or ABC transporter from ER to inside surface of Golgi apparatus where the SM and GSLs are produced with the action of sphingomyelin synthase and glycosyltransferases, respectively. Subsequently, SM and complex GSLs are transported to the plasma membrane via vesicular trafficking where they constitute the structure of membrane. SM and GSLs together with cholesterol are major components of specialized membrane microdomains known as lipid rafts where the signaling molecules, receptor and enzyme are aggregated to ensure the effective transduction of signaling cascade. Besides, SM on cell membrane could be degraded by acid sphingomyelinase (ASM) or neutral sphingomyelinase (NSM) to produce Cer which then be further digested by ceramidase forming sphingosine. Sphingosine is phosphorylated by sphingosine kinases (Sphk) to produce S1P which then is secreted into the extracellular space by specific transporter such as spinster 2 homolog-2 (Spns2). SM might also be transferred inside cell through endosomal pathway and fuse with lysosomes. In compartment of lysosomes, SM is catalyzed by ASM to produce Cer which is further degraded by acid ceramidase to form sphingosine. Due to its amphipathic property, sphingosine could diffuse between membranes, So it leaves lysosome moving back to ER membrane and mitochondria-associated membranes for recycling as the salvage generation of Cer (1, 3, 5).

The distinct compartmentalization has been shown in the synthesis and degradation of SLs. As mentioned above, sphingosine and dihydrosphingosine could diffuse between membranes and flip between membrane leaflets for their sufficiently amphipathic structure. Catalytic sites of enzymes acting upon sphingosine are always facing the cytosolic compartment suggesting that only cytosolic sphingosine is able to be modified. Different from sphingosine, Cer is restricted to membranes, but has a relatively rapid flip rate. So after generated, Cer tends to stay in the organelle, but with the capability to bind proteins or access to enzymes on either side of bilayer (6). SM and GSLs have giant headgroups, so without the aid of specific flippases or vesicular transportation they can not leave or flip between membranes leaflets (6). S1P is soluble in a hydrophilic environment, but are unable to traverse membranes without the aid of lipid transporters which is normally restricted to the compartments where it is generated until dephosphorylated into a more hydrophobic sphingosine. Moreover, it can be exported to the extracellular space by specific transporters (6).

Cer and S1P are two series of SLs which are being thoroughly investigated and exert an important role

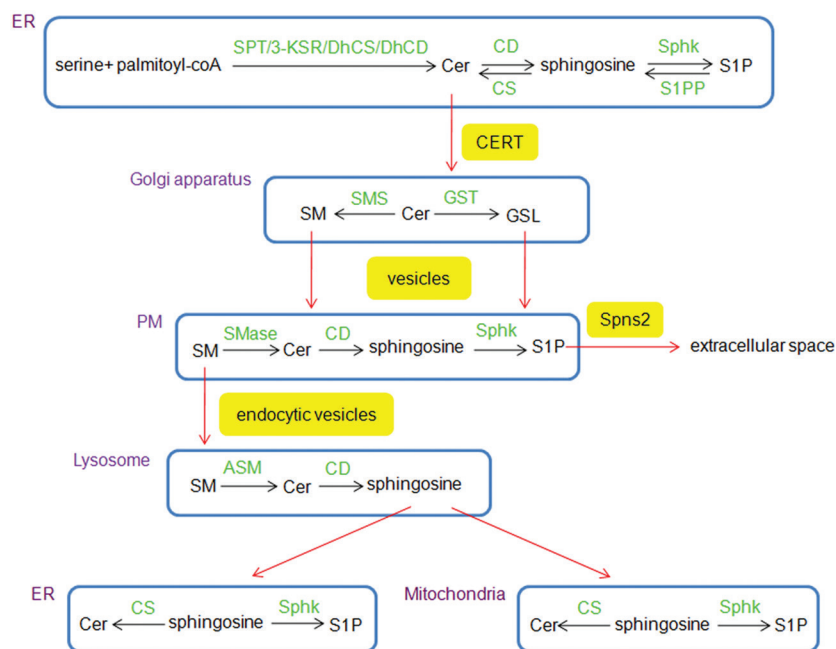


Figure 1. Metabolisms and trafficking of sphingolipids (SLs) in eukaryotic cells. SLs biosynthesis begins with the condensation of serine and palmitoyl-coA upon sequential catalysis of SPT, 3-KSR, DhCS and DhCD to produce Cer on cytosolic surface of ER. Cer can be further degraded by CD to generate sphingosine which is phosphorylated to become S1P upon the action of Sphk. Cer is transported by CERT from ER to inside surface of Golgi apparatus where SM and GSL are generated with the effect of SMS and GST, respectively. Then SM and GSL are transported to the PM via vesicular trafficking where they constitute the structure of membrane or are degraded by SMase to produce Cer which is then catalyzed into sphingosine and S1P. S1P is secreted into the extracellular space by specific transporter such as Spns2. SM might also be transported inside cell through endosomal pathway and fuse with lysosomes. In compartment of lysosomes, SM is catalyzed to produce Cer and sphingosine. Sphingosine leaves lysosome moving back to ER membrane as well as mitochondria-associated membranes to be catalyzed to generate Cer by CS or phosphorylated producing S1P. ASM, acid sphingomyelinase; CD, ceramidase; Cer, ceramide; CERT, ceramide transporter; CS, ceramide synthases; DhCD, dihydroceramide desaturase; DhCS, dihydroceramide synthases; ER, endoplasmic reticulum; GSL, glycosphingolipids; GST, glycosyltransferases; 3-KSR, 3-ketosphinganine reductase; PM, plasma membrane; SM, sphingomyelin; SMase, sphingomyelinase; SMS, sphingomyelin synthase; S1P, sphingosine-1-phosphate; Sphk, S1P kinase; Spns2, spinster 2 homolog-2. S1PP, S1P phosphatase; SPT, serine palmitoyl transferase.

in vascular adaptation. Based on the evidence that the elevation of Cer content is likely to induce cell growth arrest and apoptosis whereas S1P production is prone to elicit cell proliferation and suppress Cer-mediated apoptosis, the term of sphingolipid rheostat was proposed in 1996 (7) which means the fluctuation of the ratio of Cer and S1P partly determines the cell fate upon stimulus. For example, inhibition of Sphk leads to the decrease of S1P and increase of Cer which subsequently cause the death of cell, and vice versa. Thus, we will center on Cer and S1P to discuss the role of SLs in structural and functional remodeling of vasculature while other components of SLs with an effect on the vascular adaptation will also be referred.

4. REGULATING PATTERNS OF SLs IN VASCULAR CELLS

Cer is the key component of SLs. Extracellular stimuli such as cytokines, hormones and cell stresses promote accrual of Cer in cell resulting in cycle arrest and influencing cell differentiation, senescence, migration, adhesion and inflammatory response. Cer might exert functions intracellularly by directly binding to

and regulating activity of signaling molecules as mitogen-activated protein kinases (MAPKs), Cer-activated kinase, protein kinase C-zeta, Cer-activated serine/threonine phosphatases and phospholipases such as phospholipase A2 or D (2). Moreover, by autocrine or paracrine manner, ASM could be released reaching to the cell surface or extracellular space hydrolyzing SM there which would lead to the local production of Cer (8). Then the Cer molecules spontaneously bind together to form Cer-enriched membrane platforms where transmembrane signals are conveyed or amplified through recruitment, clustering, assembling, or integration of various signaling molecules (Figure 2).

S1P is generated due to phosphorylation of sphingosine by the action of Sphk1 and Sphk2 and after generation, it could function intracellularly as a second messenger. For example, S1P specifically binds to TNF receptor-associated factor 2 (TRAF2), a tumor necrosis factor-alpha (TNF-alpha) signaling intermediate at the amino-terminal RING domain and stimulates its E3 ligase activity necessary for lysine-63-linked polyubiquitination of RIP1, a death domain receptor-associated adaptor kinase, as well as phosphorylation and degradation

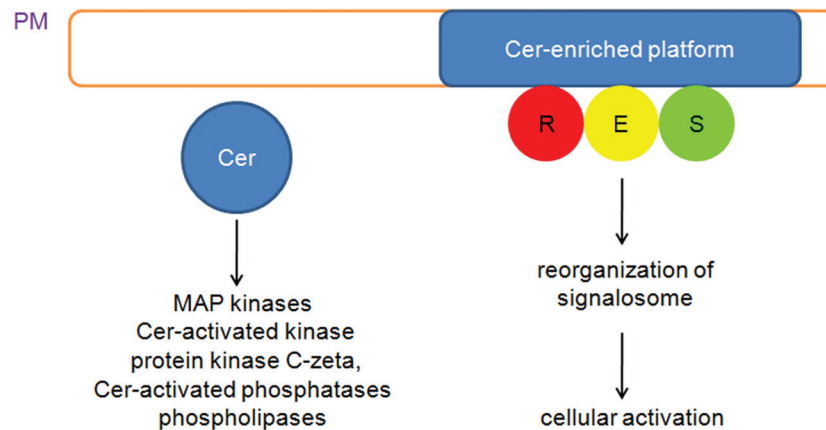


Figure 2. Regulating patterns of Cer in cells. Cer exerts functions first by directly interacting with signaling molecules as mitogen-activated protein kinases (MAPKs), Cer-activated kinase, protein kinase C-zeta, Cer-activated serine/threonine phosphatases and phospholipases such as phospholipase A2 or D to regulate activity of the molecules. Moreover, the dispersed Cer on PM can spontaneously bind together to form Cer-enriched membrane platforms where receptor (R), enzymes (E) and signaling molecules (S) are recruited, clustered and assembled to activate or amplify the transmembrane signaling cascade.

of inhibitor of NF-kappaB (IkappaB) leading to nuclear factor kappaB (NF-kappaB) activation (9). By binding to TRAF2, S1P also modulates the nuclear factor histone deacetylase HDAC1 and 2 inhibiting their catalytic activity. Moreover, after generation, S1P can be secreted outside the cell in autocrine and/or paracrine manners acting extracellularly (10). The inside-out of S1P needs specific transporters one of which, Spns2 was recently characterized (11). Extracellular S1P exerts functions mainly through five G protein coupled receptors (GPCRs) named as S1P₁₋₅ of which S1P₁, S1P₂ and S1P₃ play a significant role in adjustment of ECs and VSMCs. It has been reported that the stimulation of S1P₁₋₃ triggers G_i/phosphatidylinositol 3'-kinase (PI3K)/Akt, G_{12/13}/RhoA/Rho-associated protein kinase (ROCK) or G_q/inositol 1,4,5-triphosphate (IP3)/Ca²⁺ signaling pathways regulating vascular tone, endothelial permeability, inflammation, apoptosis, immigration and proliferation of cell (Figure 3) (10). For detailed description on specific behavior of S1P receptors, the readers could refer to reviews 10, 37, 38, 72.

The concentration of S1P in plasma is much higher than in tissue interstitial, so there is a gradient of S1P content between blood circulation and tissue which is crucial for the stimulation of S1P receptors (3). The S1P in plasma provides a stable reservoir of S1P in extracellular fluids most of which is bound to high density lipoprotein (HDL) (~65%) and albumin (~30%) (10). S1P may associate and dissociate with HDL rapidly and the beneficial property of HDL to reduce the risk of cardiovascular disease including eliciting vasodilation, endothelial cell barrier function, cardioprotection and endothelial cell migration may in part rely on its S1P chaperoning function. The molecular details of how HDL carries S1P have not yet been fully elucidated. Apolipoprotein M (ApoM) could bind to S1P, cocrystallize

with S1P and deliver S1P to its receptors which has been proved critical for S1P association with HDL in both mice and humans. In ApoM gene knocking out mice, the endothelial barrier of vasculature was damaged even though significant plasma S1P was still present associated with other plasma proteins, such as albumin (3).

5. BIOLOGICAL FUNCTION OF ACTIVE SLs IN VASCULAR CELLS

SLs regulate many biological functions of vascular cell including ROS production, vascular tone, endothelial barrier integrity, apoptosis, autophagy, proliferation and migration. We will introduce the role of SLs in all the above processes at length in the following part of the review (Figure 4).

5.1. SLs and reactive oxygen species ROS production

Redox signaling is a fundamental mechanism in cardiovascular cell biology. Various ROS components, including superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl (OH⁻), and peroxynitrite (ONOO⁻), regulate cell signaling under certain physiological or pathological conditions. One of the most important redox species in vascular cells is O₂⁻, which is unstable and short-lived for having an unpaired electron highly reactive on binding with a variety of cellular molecules, including proteins and DNA. The balance of ROS level depends on the countering effect of enzymes in charge of generation or scavenging of ROS (4).

Cer could affect mitochondrial complex assembly and stability inducing ROS production (12). Cer is a component of mitochondrial inner and outer membrane which can be formed both in the mitochondrial matrix and outer membrane. After generation, Cer interacts with

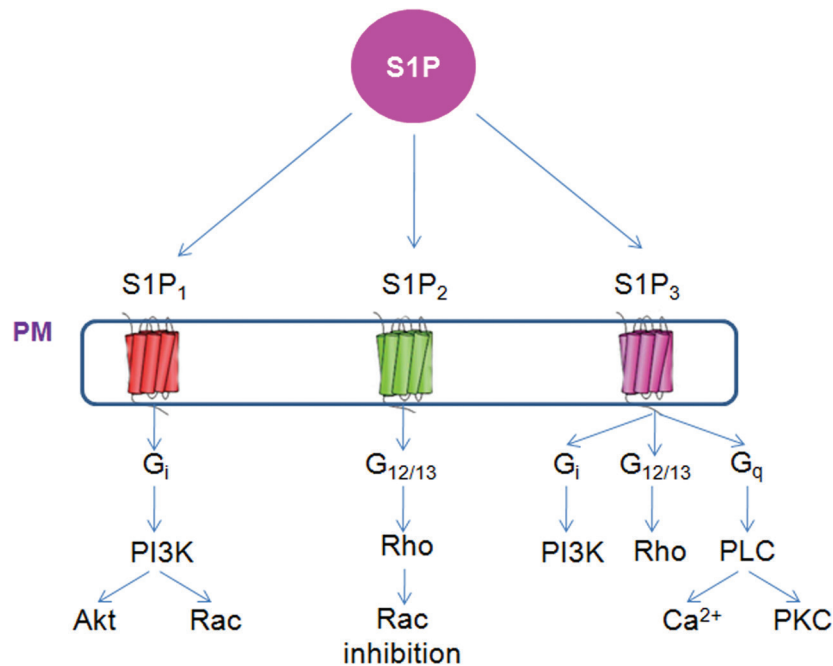


Figure 3. S1P signaling through S1P₁, S1P₂ and S1P₃ receptors in vascular cells. After secreted into the extracellular space, S1P binds to S1P₁, S1P₂ and S1P₃ on membrane of vascular endothelial cells (ECs) or smooth muscle cells (VSMCs) by which partially overlapping but distinct sets of signaling pathways are activated to mediate complex effects of S1P. PI3K, phosphatidylinositol 3'-kinase; PLC, Phospholipase C; PKC, protein kinase C.

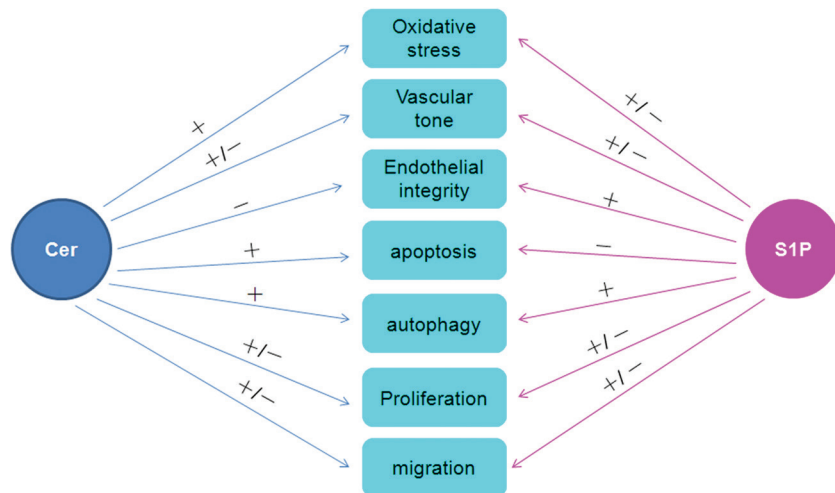


Figure 4. Regulation of Cer and S1P on multiple biological functions of vascular cells. Cer and S1P exert opposite, similar and sometime contradictory effects on reactive oxygen species (ROS) production, vascular tone, endothelial barrier integrity, apoptosis, autophagy, proliferation and migration of ECs or VSMCs. +, activation; -, inhibition.

cytochrome *c* inducing the displacement of it from the mitochondrial membrane preventing oxygen consumption. Excessive Cer could also influence electron transporter favoring the ROS generation (12). Moreover, the activity of enzymes in charge of O₂^{-•} producing depends a lot on the Cer generation and Cer-centered or enriched lipid rafts formation on cell membrane. The lipid rafts aggregation provide a platform for NOX and other NADPH oxidase subunits as well as cofactors to assemble which then

works as an active enzymatic complex. In bovine coronary arterial endothelial cell (BCAEC), upon stimulation of agonists or stresses such as Fas ligand (FasL), TNF- α , endostatin, homocysteine or radiation, subunits of NADPH oxidase as gp91phox and p47phox along with the related proteins form redox signalosomes in lipid rafts markedly increasing the activity of the enzyme and promoting O₂^{-•} production (4, 13). ASM activation itself might trigger or drive the fusion of membrane proximal

lysosomes into lipid raft on the cell membrane leading to Cer production and lipid raft clustering which contributes to endothelial dysfunction (14). TNF-related apoptosis-inducing ligand (TRAIL) induced endothelial dysfunction through death receptor 4 (DR4) also depends on the ASM stimulation and the following lysosomal movement and fusion (15). So the proper functioning of lysosomes as well as sortilin, an intracellular protein responsible for the binding and targeting of ASM to lysosomes has been proven critical for the Cer generation and $O_2^{\cdot-}$ production (16, 17). The function of other enzymes related to ROS metabolism such as superoxide dismutase (SOD), catalase and thioredoxin (TRX) also relies on the formation of lipid rafts (4). For example, H_2O_2 generated by extracellular SOD which is anchored to ECs surface via the heparin binding domain (HBD) enhances vascular endothelial growth factor (VEGF)-induced VEGF receptor 2 (VEGFR2) autophosphorylation in lipid raft, but not in other membrane area (18). In neutrophils, proteomic analysis has found catalase in lipid raft fractions playing critical roles in redox signaling by cleavage of H_2O_2 (19). There is convincing evidence that lipid raft may mediate the actions of TRX on leukocyte-EC interaction related to redox regulation during inflammation (20). However, Cer generation also displays a high sensitivity to oxidative stress (21). The decreased level of glutathione (GSH) activates NSM promoting Cer accumulation and cell apoptosis. Exposure to H_2O_2 and $ONOO^-$ activates NSM and ASM, respectively (22). The formation of Cer-centered membrane platforms in BCAEC can be reduced by SOD but increased by stimulation with $O_2^{\cdot-}$ donor or generating systems (4). H_2O_2 activated pro-survival signaling pathways, including activation of PI3K/Akt and extracellular signal-regulated kinases (ERK)1/2 relies on the lipid rafts behaviors (23). Exogenous administration of xanthine/xanthine oxidase, a $O_2^{\cdot-}$ generating system, has demonstrated leading to a dramatic increase in Cer-centered membrane platform formation on membrane of ECs (4).

The effect of S1P on ROS production varies with the kind of S1P receptor and type of vascular cells. It has been reported that $S1P_1$ mediates downregulation while $S1P_2$ mediates upregulation of oxidative stress. In human umbilical vein endothelial cells (HUVECs), high glucose induces ROS production and morphogenetic responses which is associated to decreased $S1P_1$ but increased $S1P_2$ content and could be reversed by $S1P_1$ plasmid or $S1P_2$ siRNA transfection (24). In human lung microvascular endothelial cells, hyperoxia activates Sphk1 inducing the generation of S1P which upregulates ROS synthesis mediating the dysfunction of the cell (25). In VSMCs, S1P stimulates ROS generation via $S1P_2/G_{12/13}$ pathway and the following NADPH oxidase activation promoting the expression of CYR61, a growth-factor-inducible angiogenic factor (26). The S1P induced ROS production also depends on the $S1P_2/G_{12/13}$ /Phospholipase C (PLC)-beta or $S1P_2/G_{12/13}$ /

Rho pathway facilitating VSMC migration through PI3K/phosphoinositide-dependent protein kinase-1 (PDK1)/Akt cascade as well as epidermal growth factor receptor activation (27). On the other hand, ROS abundance could influence S1P generation too. Lowering the ROS level with antioxidant stimulates Sphk activity and inhibits the degradation of Sphk which increases S1P but decreases Cer production promoting cell survival (28). However, excessive ROS reduce Sphk activity preventing S1P production which could stimulate cell apoptosis (29).

5.2. SLs and vascular tone

Vascular tone is an integrated manifestation of multiple mechanisms regulating vascular contraction and relaxation which involves endothelial nitric oxide synthase (eNOS) activation and nitric oxide (NO) production in EC, oxidative stress especially $O_2^{\cdot-}$ level, behavior of ion channels, IP3 receptor (IP3R), ryanodine receptor (RyR) and contractile apparatus. Biologically active SLs can induce both vasoconstriction and vasodilatation which may relate to the distinct action of different SLs on ECs and VSMCs as well as the specific expression pattern of S1P receptors among vascular beds (30).

The experimental evidence about effect of Cer on vascular tone seems inconsistent and contrary. As shown above, Cer enhance $O_2^{\cdot-}$ generation in ECs which neutralizes NO forming $ONOO^-$ and increasing vascular tone (4). At the same time, Cer associates with the inhibitor 2 of phosphoprotein phosphatase 2A (I2PP2A) in the cytosol, which disrupts the binding of I2PP2A with phosphoprotein phosphatase 2A (PP2A) leading to its translocation to the plasma membrane. Then the increased association between PP2A and eNOS at the plasma membrane promotes separation of an Akt-heat shock protein 90 (Hsp90)-eNOS complex reducing eNOS phosphorylation and activation as well as NO bioactivity (31). In small bovine coronary artery, C2-Cer produced a concentration-dependent decrease in KCa channel activity exerting a tonic vasoconstrictor action in coronary microcirculation (32). However, there are also quite a little reports showing vasodilating effect of Cer. C2-Cer induces vasodilation in phenylephrine (PE) precontracted thoracic aorta of rat depending on the inhibition of PE activated RhoA/ROCK pathway as well as elevations in $(Ca^{2+})_i$ (33). In isolated rat aortic rings, NSM (0.001-0.1 U/ml) and C1P, but not phosphorylcholine attenuates PE-induced contractions and $(Ca^{2+})_i$ elevations in a concentration-related manner (34). C2-Cer, C6-Cer, C16-Cer and D-sphingosine all demonstrates a dose-dependent relaxation response in endothelium-intact vessels which was reduced significantly in denuded vessels suggesting an endothelial NO-dependent mechanism may mediate those SLs regulated vascular tone (35). What's more, angiotensin II induces Cer production via AT_2 receptor compromising the vasoconstriction effect mediated by AT_1 (36).

The contribution of S1P to vascular tone is also contradictory and varies depending on the vessel size and the pattern of S1P receptors in different vascular beds. In rats, S1P has been found inducing a vasoconstriction response in small arteries such as mesenteric, cerebral and renal arteries, but having no effect on the aorta or carotid/femoral arteries (37, 38). However, there are also reports showing PE-precontracted aortic rings from rats and mice undergo vasorelaxation after S1P treatment and this effect is thought to be regulated by S1P₃ (39). The proposed mechanisms for S1P-induced regulation of vascular tone are diversified. In ECs, coupling of S1P₁ or S1P₃ to the eNOS enzyme, presumably due to G_i/PI3K/Akt signaling axis results in endothelium-dependent vasorelaxation (37). In VSMCs, S1P induces a potent contraction by stimulating S1P₂ or S1P₃ followed by activating RhoA/ROCK pathway and increasing Ca²⁺ sensitization (40). Moreover, inhibition of Sphk1 ameliorates angiotensin II-induced hypertension due to the inhibition of transmembrane Ca²⁺ entry via store-operated Ca²⁺ channel (41). The plasma S1P controls vascular tone by regulating endothelial barrier function. At low concentrations (lower than 0.1. μ M), S1P enhances the endothelial barrier integrity blocking the leakage of potential vasoconstrictors which is mediated by S1P₁ activation. At high concentrations (higher than 1 μ M), S1P acts mainly through S1P₃ increasing permeability of ECs which allows leakage of vasoconstrictors (e.g. thromboxane A2 and S1P itself) to the sub-endothelial space and induces constriction of VSMCs through thromboxane A2/prostaglandin (TP) receptors, S1P₂ and S1P₃ (42).

5.3. SLs and vascular barrier integrity

Increased endothelial permeability and vascular barrier failure are hallmarks of inflammatory responses of vessels which SLs is involved to regulate. The common view is that S1P generally augments endothelial integrity while Cer tends to promote vascular leak, and a tight balance between the two SLs components is necessary to maintain normal endothelial barrier (43).

The mechanisms for Cer to impair the endothelial barrier integrity are different among vessels. In ECs of pulmonary artery, activation of ASM to produce Cer leads to caveolin-1 accumulation in caveolae and a resultant drop in eNOS activity as well as NO production which brings about endothelial leak and edema formation (43). Nicotine exposures triggers loss of endothelial barrier in cultured cell monolayers with lung inflammation associated with increased intracellular Cer, p38 MAPK activation, and myosin light chain (MLC) phosphorylation mediated by RhoA/ROCK pathway and inhibition of myosin phosphatase target subunit 1 (MYPT1) (43). Cer could favor the endothelial dysfunction in ECs of systemic vasculature too, but through different mechanisms from pulmonary artery. Cer activates eNOS and elevates NO production in systemic ECs which cause formation of

larger paracellular gaps than in the pulmonary circulation resulting in the same end result of interstitial edema (44). The exact reasons of this divergent alteration upon Cer stimulation in pulmonary and systemic artery are still not very clear, but might relate to difference in caveolae organization and ECs subtypes. Cer can also enhance endothelial permeability by inducing apoptosis of ECs which is mediated by stress-activated protein kinase/c-Jun NH(2)-terminal kinase (SAPK/JNK) cascade, Fas-associated death domain protein complex, interleukin (IL)1 β -converting enzyme/Ced3 pathway and Bcl-2 family proteins. Furthermore, the Cer triggered inflammasome activation is also suggested contributing to the endothelial barrier dysfunction (43).

In ECs, S1P₁ signaling is predominant as compared to S1P₂ or S1P₃ signaling on the barrier formation (45). S1P stimulating S1P₁ could rearrange the endothelial cell cytoskeleton increasing stability of cell-cell contact. Rac1, a small GTPase is upregulated by S1P₁ activation mediating the effect of S1P on endothelial barrier integrity through multiple downstream targets. It increases actin polymerization at the cell periphery, recruits c-Abl protein tyrosine kinase and non-muscle MLC kinase to lipid rafts as well as EC periphery, translocates the actin-binding protein cortactin to the cellular periphery and activates p21-associated Ser/Thr kinase (PAK) inhibiting cofilin, a protein responsible for actin disassembly. All the above courses could increase cell overlap and cell-to-cell junction stabilizing and strengthening the endothelial cell barrier (43). Besides, S1P increases interaction of VE-cadherin with beta-catenin as well as induces reassignment of zona occludens protein 1 (ZO-1) to paracellular junctions, which has a stabilizing effect on endothelial tight junctions (46). The role of S1P₂ or S1P₃ signaling in endothelial barrier is relative opposite to S1P₁. The higher concentration of S1P activates S1P₂ or S1P₃ preferentially and then stimulates RhoA/ROCK pathway negating MLC phosphatase which ultimately increases the stress fiber formation and cellular contractility enhancing paracellular permeability as well as vascular leak (45).

5.4. SLs and apoptosis

Two signaling cascades had been proposed modulating apoptosis in mammalian cells called extrinsic and intrinsic signaling pathways. The former means death receptor-mediated death-inducing signaling complex (DISC) formation and the latter means mitochondrial-derived apoptosome generation. The balance of Cer and S1P plays a critical role in the modulation of cell death or survival (47).

Cer generation by stimulus such as septic shock, heat shock, lipopolysaccharide, UV, ionizing irradiation, and oxidative stress facilitates both extrinsic and intrinsic pathways of apoptosis in ECs and VSMCs. Although there are opposite reports, Cer is most

considered as an apoptosis promoter (48). It stimulates Cer-activated protein kinase (CAPK), Raf-1, ERK cascade, SAPK/JNK cascade, NF-kappaB activation and death cell receptor clustering to induce mitochondrial cytochrome c release or caspase-8 activation (43). Moreover, Cer enhance oligomerization of pro-apoptotic Bcl-2 family proteins whereas reduce expression of anti-apoptotic Bcl-2 proteins (47). The self-assembling of Cer in the mitochondrial outer membrane forming large stable channels capable of releasing cytochrome c which further interacts with apoptotic protease activating factor 1 (Apaf-1) activating several caspases and then driving cell to apoptosis (49). Cer generation is located on both upstream and downstream of IL-1beta converting enzyme (ICE)/Ced-3 family executing apoptosis (48). Finally, diverse receptors belonging to the TNF receptor superfamily and mediating apoptosis trigger ASM translocation from lysosomes to the extracellular surface of the cell membrane arousing formation of DISC and the initiation of apoptosis signaling (50).

In contrast to Cer, S1P is always regarded as a potent apoptosis preventer. Through receptor activation and downstream G-protein mediated signaling pathways or intracellular signaling cascade, S1P inhibits the cleavage of caspase-3, cytochrome c release and DNA fragmentation (51). Besides, the HDL-associated S1P in circulation promotes cell survival suppressive to the happening of apoptosis (3). S1P also prevents apoptosis by influencing mitochondria-mediated response and the expression of both Bcl-2 and Bax through PI3K/Akt pathway (51). Pretreatment of cells with S1P attenuated ethanol-induced apoptosis in rat liver sinusoidal ECs which is partially mediated by Ca^{2+} -sensitive eNOS activation and subsequent NO formation (52). A recent report showed that Sphk1 is localized in cytosol promoting growth and survival of cell whereas Sphk2 is localized in both nucleus and cytosol which is pro-apoptotic preventing DNA synthesis and cell proliferation (53). Interestingly, targeting of the pro-survival Sphk1 from cytosol to ER make it from pro-survival to pro-apoptosis indicating that differently distributed intracellular pools of S1P might participate in different metabolic and signaling pathways (53).

5.5. SLs and autophagy

Autophagy is a process by which cells keep healthy by degrading wasted or damaged materials or organelles which begins with the sequestration of cytoplasmic components in double-membrane vesicles to form autophagosomes (APs). Then the APs fuse to lysosomal compartments to produce autophagolysosomes (APLs) and the content is degraded (54). The role of autophagy in the vascular physiological or pathophysiological remodeling is relative complicated and contradictory. The common viewpoint is that basic autophagy is protective leading ECs and VSMCs to be survival by degrading damaged

intracellular material, in particular polarized mitochondria, but excessive autophagy is detrimental for it may cause autophagic death of ECs and VSMCs (54). Both Cer and S1P could induce autophagy happening, but with different consequences as S1P would promote cell survival and proliferation, whereas Cer induces growth arrest and cell death (55).

Cer promotes interaction of class III PI3K with other regulators of autophagy and inhibits Akt by activating PP2A through which the mammalian target of rapamycin (mTOR) activity is prevented, and then autophagy is upregulated (55). Cer-mediated activation of JNK and the transcription factor, c-Jun, upregulates Beclin 1, the mammalian orthologue of yeast Atg6, and LC3B, a robust marker of APs promoting autophagy (47). Several studies have implied that SLs especially Cer, are APs membrane components and SLs formed by de novo biosynthesis in the ER might be a driving force for the formation of APs vacuoles (55). In coronary artery smooth muscle cells (CASMCs) of wild-type (*Smpd1*^{+/+}) mice, 7-ketocholesterol, a major oxidation product of cholesterol found in human atherosclerotic plaques more atherogenic than cholesterol, enhances expression of LC3B and the content of both APs and APLs. In *Smpd1*^{-/-} CASMCs, such 7-ketocholesterol-induced increases in LC3B expression and APs were further augmented, but APLs formation was abolished which is due to aberrant fusion of APs with lysosomes (56). As for the linker between ASM and lysosomal fusion with APs, a mucolipin transient receptor potential channel 1 (TRPML1)/lysosomal Ca^{2+} /dynein signaling axis has been proposed. ASM deficiency inhibits the activity of TRPML1, a principle lysosomal Ca^{2+} channel, and thereby blocks the lysosomal Ca^{2+} -dependent membrane trafficking in which dynein, a multi-subunit microtubule motor protein complex, might mediate the trafficking of both lysosomes and APs promoting their meet to form APLs (56, 57). Cer also regulates cytoskeleton and microtubule assembly, which elicits autophagy by promoting vesicular trafficking in mammalian cells (47). However, overproduction of Cer or its metabolite sphingosine may increase lysosome permeability leading to impaired autophagy maturation (58). Cer could induce another type of cell death distinct from apoptosis named type II cell death which refers to that cells consume their own interior content resulting in programmed cell death (59). This type of cellular death is caspase-independent, characterized by a large number of autophagic vacuoles, early degradation of organelles, and preservation of cytoskeletal elements which is independent of apoptosis and can be rescued by autophagic inhibition (59). Recent reports have put forward a novel function of Cer for anchoring APLs with LC3B to mitochondrial membranes defining a key mechanism for the induction of lethal mitophagy (60).

S1P has been observed as a critical player for the cell survival through its induction of autophagy.

Knockdown of the ER-residing S1P phosphatase, the enzyme responsible for the degradation of S1P, augments S1P levels and induces autophagy. The accumulating S1P on ER leads to ER stress which is apt to cause autophagy (47). The class III PI3K inhibitor, 3-methyladenine, which is known to prevent APs formation, does not affect S1P induced autophagy, whereas silencing of Atg5, a protein required at a later step in the autophagic process, does inhibit S1P-induced autophagy indicating S1P regulates autophagy on specific approaches (61). It is not clear whether S1P signaling acts through or parallel to the mTOR pathway in adjusting autophagy with various paradox reports present. For instance, Overexpression of Sphk1 induces phosphorylation of two known downstream effectors of mTOR, however, down-regulation of S1P phosphorylase-1 does not affect phosphorylation of mTOR nor its downstream effectors (62). Meanwhile, several studies have described S1P as an inducer and inhibitor of autophagy through activation of the mTOR pathway via S1P₅ and S1P₃ signaling, respectively (47).

5.6. SLs and proliferation and migration

Cellular migration and proliferation are essential processes involved in not only embryogenesis but also inflammation, wound healing, tumour growth and angiogenesis.

Apart from apoptosis, Cer elicits proliferation of ECs and VSMCs as well. The determinants for the diversified effect of Cer are still unclear and might relate to the way the Cer is generated, the location where the Cer exerts function as well as characters of diseases (38). Incubation of SMC with UV-oxidized low-density lipoprotein (oxLDL) induced SM hydrolysis and a concomitant increase of intracellular Cer level (63). The effect leads to cell growth mediated by ERK1/2 activation, higher DNA synthesis and epidermal growth factor receptor (EGFR)/PI3K/Akt pathway which counteracts the apoptotic effect of oxLDL. The proliferation effect of oxLDL could be reproduced by incubating the cell with exogenous bacterial sphingomyelinase (SMase) and the cell-permeable Cer (63). Matrix metalloproteinases (MMPs) has also been shown regulating oxLDL-induced activation of the SM/Cer signaling pathway and subsequent SMC proliferation (64). Another explanation on proliferating effect of Cer is that along with the activation of SMase, ceramidase and Sphk could also be activated, and then Cer is catalyzed into S1P which is the key motivator of VSMCs proliferation (65).

S1P is a critical regulator of proliferation, motility as well as directional migration of ECs and VSMCs. Although there is an overview that S1P_{1/3} are essential in EC proliferation whereas S1P_{2/3} mainly mediates the proliferation of VSMCs, it is indeed difficult to describe the relevant receptors for specific cell proliferation as one S1P receptor can stimulate proliferation in some

cell types, but inhibit in others (38). A very similar state is observed when the relevance of S1P receptor signaling in cell migration is investigated (38). S1P is able to activate membrane-type 1 MMP in ECs representing a link between homeostasis and cell migration. However, it inhibits platelet-derived growth factor (PDGF) induced chemotaxis of human VSMCs. Moreover, the HDL associated S1P shows inhibiting effect on VSMC migration (38). Multiple evidences show that inhibitory effect of S1P on cellular proliferation and migration is mediated by S1P₂ and Rac activity as S1P₂ siRNA transfection or S1P₂ antagonist enhances cell growth and migration in ECs and VSMCs (10). So a perspective has been raised that the selective S1P₂ agonist could be used in a drug-eluting stent with its chemorepellent and anti-proliferative trait which if locally administered, might prevent vascular restenosis by inhibiting migration of VSMCs into the luminal surface (10).

5.7. Sphingosine, ceramide 1-phosphate (C1P) and glycosphingolipids (GSLs)

Sphingosine levels of cell are kept low in part due to the action of ceramide synthase and Sphk, but would change under various physiological conditions regulating a myriad of cellular functions. Sphingosine promotes apoptosis by inhibiting the function of pro-survival 14-3-3 protein which is mediated by binding to and regulating phosphorylation of dimer interface of the protein (3). Sphingosine is a physiological inhibitor of the survival signal protein kinase C and simultaneously, it upregulates caspase 3 in the cascade of apoptosis (3). Sphingosine interacts with acidic leucine-rich nuclear phosphoprotein-32A (ANP32A) increasing activity of PP2A and p38 SAPK. It enhances the gene transcription and protein expression of cyclooxygenase (COX)-2 in human ECs promoting inflammatory stress of the cell. Furthermore, sphingosine can regulate metabolic signaling pathways of cell by controlling the activity of other key enzymes such as phospholipase D (PLD) or diacylglycerol kinase (66).

C1P is a bioactive SL produced by ceramide kinase (CERK)-catalyzed phosphorylation of Cer which has mitogenic properties and inhibits apoptosis happening. It has been proven eliciting neointimal formation via cell proliferation through the regulation of the ERK1/2 protein in rat aortic VSMCs (67). Studies in the role of C1P in modulating Ca²⁺ flux have produced somewhat controversial results. In some reports C1P did not modulate (Ca²⁺)_i nor did it affect Ca²⁺ mobilization; however, others have clearly shown that C1P enhanced Ca²⁺ entering into cells through store-operated Ca²⁺ channel (68). In addition, C1P acts as an important mediator of inflammatory responses which takes place through stimulation of cytosolic phospholipase A2 followed by release of arachidonic acid and prostaglandin formation. Besides, C1P might also bind to specific plasma membrane receptors that are coupled to G_i proteins stimulating macrophage chemotaxis (68).

GSLs are mostly localized on the cell surface which are produced by sequential transferring of sugars from nucleotide sugars to Cer via the action of glycosyltransferases (1). GSLs are present in vascular cells such as ECs, VSMCs, macrophages, neutrophils, platelets, and monocytes which contribute to initiation and progress of atherosclerosis. Feeding a western diet to ApoE^{-/-} mice and normal rabbits leads to cardiac hypertrophy, extensive atherosclerosis, vascular wall thickness and stiffness accompanied by an increase in the cardiac or arterial levels of glucosylceramide and lactosylceramide. Feeding these animals D-PDMP, an inhibitor of glycosyltransferases, dose-dependently ameliorates atherosclerosis and vascular stiffness (69). OxLDL stimulates endogenous synthesis of lactosylceramide which induces NADPH oxidase activation and ROS generation resulting in the cell proliferation by stimulating GTP loading of p21ras with the kinase cascade (Raf-1, Mek2, and p44 MAPK) activated (70). In ECs, lactosylceramide mediates the TNF- α induced expression of NF- κ B and intercellular adhesion molecule (ICAM-1) via the redox-dependent transcriptional pathway. It also stimulates the expression of CD11/CD8 (Mac-1) on the surface of human neutrophils which indicates lactosylceramide contributes to the adhesion of neutrophils or monocytes to the ECs surface initiating the course of atherosclerosis (70).

6. SLs IN VASCULAR DEVELOPMENT AND VASCULAR ADAPTATION TO DISEASES

6.1. SLs and vascular development

SLs have been observed influencing differentiation, proliferation, and migration during vascular development (12). Low density lipoprotein receptor-related protein 1 (LRP1) modulates G γ -dependent S1P signaling and integrates S1P and PDGF-BB signaling pathways, which are both crucial for mural cell recruitment (12). Sphk-null mice exhibit a deficiency of S1P which severely disturbs neurogenesis, including neural tube closure, and angiogenesis causing embryonic lethality (71). A dramatic increase in apoptosis and a decrease in mitosis were also seen in the developing nervous system of Sphk-null mice (71). Vessel development may require the integration of distinct cellular activities derived from individual S1P receptors. S1P₁ and S1P₃ receptors stimulate Rac-coupled cortical actin assembly and Rho-coupled stress fiber formation, respectively, while S1P₂ mediates down-regulation of Rac activation influencing membrane ruffling, and cell migration (72). S1P₁ receptor functions within ECs to promote their interactions with VSMCs. In addition to stabilizing the vasculature, the interactions of ECs and VSMCs are required for vessel remodeling. A global deletion of the S1P₁ receptor in mice results in lethality due to severe hemorrhage for the deficient coverage of vessels by VSMCs (72). Lipid phosphate phosphatases (LPPs) are integral membrane proteins with broad substrate specificity that

dephosphorylate lipid substrates including phosphatidic acid, lysophosphatidic acid, C1P, S1P, and diacylglycerol pyrophosphate through regulating cell proliferation, cell migration, invasion and morphology which are essential for vascular development. Moreover, deficiency of LPPs is associated with elevated risk of coronary artery disease (CAD) and atherosclerotic plaques (73). Cer also plays a role in the regulation cardiovascular development which together with other SLs influences phosphorylation of ERM family (ezrin, radixin and moesin) of cytoskeletal proteins crucial for the efficient epithelial to mesenchymal transitions (EMT) in endocardial cells. S1P and Cer have been proved to stimulate the phosphorylation and dephosphorylation of ezrin, respectively (74). In addition, the mycotoxin fumonisin which is known to inhibit the activity of ceramide synthase might affect neural crest cells, migration of which is essential for proper separation of the cardiac outflow, a step prior to the formation of aorta, pulmonary and aortic arch arteries (75). Besides, inhibition of Cer generation might lead to the defect of cell membrane influencing the folate transportation which has long been realized essential for the development of the cardiovascular system (75, 76).

6.2. SLs and angiogenesis

Angiogenesis, or new blood vessel formation contain multiple phases as migration, proliferation, morphogenesis, and vascular stabilization which is critical for the growth and spread of tumor by allowing it to grow beyond a diffusion-limited size. Also, exploring a novel and effective strategy for accelerating angiogenesis is a particularly important subject for the therapy for vasoocclusive diseases (77). To date, the main therapeutic way to promote angiogenesis is by using growth factors including VEGF, fibroblast growth factor-2 (FGF-2), hepatocyte growth factor, and their expression plasmids, however, with relative severe side effects such as edema and proteinuria (77).

As a cause of cell death *in vivo*, Cer has been reported inhibiting angiogenesis through a decrease of VEGF expression in ECs (78). Ultrasound elicited microbubble sensitizes the efficiency of radiotherapy on tumor through Cer production which prevent the angiogenesis by inducing Cer-related EC apoptosis (79). Dietary glucosylceramides triggers the de novo pathway of Cer synthesis preventing growth of head or neck tumors through the inhibition of pro-angiogenic signals such as VEGF, VEGF receptor-2, and hypoxia-inducible factor-1 α (HIF-1 α) (80).

A systematic shift in sphingolipid metabolism favoring S1P over Cer by increasing Sphk1 or decreasing sphingosine phosphate phosphatase 2 (Spp2) expression raised grade of many kinds of cancer (81). On the contrary, blocking S1P production in cultured glioblastoma cells by using a highly potent and selective Sphk1 inhibitor blocks angiogenesis in cocultured ECs

without affecting VEGF secretion (81). Local injection of S1P₁ siRNA or anti-S1P antibody into established tumors inhibits S1P₁ expression on neovessels and suppresses vascular stabilization and angiogenesis, which resulted in dramatic suppression of tumor growth *in vivo* (82, 83).

In murine hindlimb ischemia, daily intramuscular administration of S1P dose-dependently stimulates blood flow recovery, resulting in up to twice as much blood flow accompanied by 1.7-fold increase in the capillary density (77). The optimal S1P effects were comparable with those obtained with FGF-2. The post-ischemic blood flow recovery and angiogenesis were accelerated in Sphk1-transgenic mice, which showed 40-fold higher Sphk1 activity and 1.8-fold higher S1P content in skeletal muscle than in wild-type mice (77). Cell therapy based on endothelial colony-forming cells (ECFCs) is a promising option for ischemic cardiovascular diseases. The angiogenic/vasculogenic activity of ECFCs depends a lot of on the up-regulation of the expression and activity of Sphk1 as well as the stimulation of S1P/S1P_{1/3} pathway (84).

6.3. SLs and vascular adaptation to hypertension

ECs dysfunction and VSMC remodeling are among others main feature of hypertension. As mentioned above, SLs take a crucial stage in the regulation of vascular contractility and growth. They also play an essential role in the initiation and progress of hypertension.

In isolated carotid arteries from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats, enhancing Cer while reducing S1P production by administration of a Sphk inhibitor (dimethylsphingosine) or exogenous application of SMase induces marked endothelium-dependent contractions in SHR vessels which were Cer dependent and involves participation of calcium-independent phospholipase A (iPLA), cyclooxygenase-1 and thromboxane synthase. Meanwhile, infusion of dimethylsphingosine increases blood pressure in SHR rats (85). The Cer level in arterial tissue and plasma of SHR is higher than that of WKY. Plasma of essential hypertension patient also has more Cer than normal person does (85, 86). Moreover, lowering blood pressure with losartan treatment reduces vascular Cer levels and Cer-mediated arterial contractions in SHR (86). Given the function of Cer to mediate ECs dysfunction and induce both vasoconstriction and vasodilation responses, it can be suspected that Cer might exert diversified functions in different phases of hypertension development.

As we have known, ECs or VSMCs express specific receptors for S1P that modulate vascular tone leading to both vasodilation and vasoconstriction responses. For now, it has been proved that the

vasodilation response is triggered by S1P with low concentrations acting on the receptors S1P₁ and S1P₃ located in ECs, via activation of eNOS. While the vasoconstriction response is induced by S1P with higher concentrations acting on the receptors S1P₂ and S1P₃ located in VSMCs (38). Nogo-B, a membrane protein of the ER, inhibits SPT thereby preventing production and autocrine of endothelial S1P as well as the following G protein-coupled receptor-dependent signaling. A latest report shows that mice lacking Nogo-B either systemically or specifically in ECs are hypotensive, resistant to angiotensin II-induced hypertension and have preserved endothelial function and nitric oxide release confirming the role of S1P in endothelium dependent vasodilation and blood pressure control (9). Other bioactive SLs are also engaged in the development of hypertension. For instance, fumonisin mycotoxicosis in pigs causes an increase in serum sphinganine and sphingosine concentrations which has relation to a decrease in mean aortic pressure and an increase in mean pulmonary arterial pressure (87).

6.4. SLs and vascular adaptation to atherosclerosis

Atherosclerosis is the foremost health killer in both developed and developing countries which is featured with leukocyte adhesion, inflammation, aggregation of low-density lipoprotein (LDL) in subendothelial space, foam cells formation, VSMC proliferation and death.

Cer and GSLs are accumulated in atherosclerotic lesions (70). At present, Cer has been regarded as a common pathway and potential therapeutic target for atherosclerosis with its interactions with lipids, inflammatory cytokines, homocysteine and MMPs (88). The atherogenic pro-inflammatory cytokines trigger ASM secretion from ECs which reaches to LDL catalyzing the hydrolysis of SM leading to Cer generation. The accrual of Cer in LDLs promotes aggregation and the following uptake of LDLs by macrophages which would resident at vasculature and differentiate into foam cells. The increased oxidized phospholipids at atherosclerotic lesions may also promote VSMC death via ASM activation (89). Plasma SM levels has been reported correlated to incidence of cardiovascular disease, while reducing plasma SM levels by inhibition of SPT with myriocin could compromise atherosclerotic lesions in murine models of atherosclerosis (89). *In vitro* studies highlight the ability of apolipoprotein C-1 (apoC-1) enriched HDLs to induce VSMC death via NSM activation. In patients, the apoC-1 content in lipoprotein remnants appears as an early marker of coronary artery disease risk. Colocalization of apoC-1, Cer, caspase-1 and -3 in regions of plaque rupture has been proved in a rabbit model of atherosclerosis (89). By modulating platelet activation and aggregation, glycolipids and sphingosine derivatives may favor thrombosis just like Cer could do by affecting tissue factor 91 or plasminogen activator inhibitor (PAI)-1120 release (90).

Sphingomyelin synthase 1 deficiency decreases SM, but dramatically increases the levels of GSLs and promotes the atherosclerosis progress (91).

The regulative effect of S1P in atherosclerosis has no agreeable viewpoint up to now and varies with the receptor types or vascular regions (38). S1P inhibits leukocyte adhesion by preventing TNF- α induced expression of E-selectin, monocyte/EC interaction and pro-inflammatory cytokine production which is mediated by S1P_{1/3} (38). The S1P analogue FTY720 dramatically reduced atherosclerotic lesion volume, macrophage, and collagen content after 20 weeks of high-cholesterol diet on ApoE^{-/-} mice (92). S1P may exert protecting function on atherosclerotic lesion by selective inhibition of toll-like receptor 2 and thrombosis (38). On the other side, S1P has pro-atherogenic potential as it is reported to promote adhesion molecule expression in ECs, stimulate release of monocyte chemotactic protein-1 (MCP-1) and IL-8, increase E-selectin via activation of NF- κ B, induce a pro-inflammatory phenotype in cultured ECs, enhance production of MMPs to degrade extracellular matrix eliciting cap thinning and plaque rupture (38). Further studies are still needed to clarify the effect of S1P on the progress of atherosclerosis as well as the underlying mechanisms.

6.5. SLs and vascular adaptation to magnesium deficiency (MgD)

Numerous recent epidemiological studies have revealed that Western populations are growing more and more deficient in daily magnesium (Mg) intake which has been linked to etiology of cardiovascular diseases. Evidence has shown that a reduction in the dietary intake of Mg, as well as low Mg content in drinking water, is a risk factor for the development of hypertension, atherosclerosis, vasospasm, sudden cardiac death, stroke, and inflammatory conditions (93).

MgD upregulates multiple enzymes related to SLs metabolism including NSM, ASM, ceramide synthase, SM synthase and SPT inducing increased synthesis and release of Cer which activates NF- κ B inducing release of mitochondrial cytochrome C, apoptosis and accumulation of several cytokines and chemokines (94). The protein kinase C- ζ isoform appears to be involved in Cer generation via the salvage pathway in Mg-deficient VSMCs (93). On the other hand, SLs is also an important regulator of (Mg²⁺)_i homeostasis. Treatment of C2-Cer, C8-Cer, C16-Cer, sphingosine, or NSM activation have been found rapidly increasing (Mg²⁺)_i of VSMCs in a concentration-dependent manner which are derived from influx of extracellular Mg²⁺. The effect is Ca²⁺ dependent mediated by phospholipase C and Ca²⁺/calmodulin/Ca²⁺-ATPase pathway (95).

6.6. SLs and vascular adaptation to aging

The incidence of cardiovascular diseases increases rapidly with age which is the major cause of

morbidity and mortality in the elderly. The cardiovascular variation related to aging include increased systolic pressure and pulse width, rise of peripheral vascular resistance, development of hypertension, greater susceptibility to atherosclerotic plaque formation, and poor autoregulation of blood flow to end organs. All these changes are ascribed to the structural and functional change of arteries including ECs dysfunction, VSMCs hypertrophy, collagen content increase and elastic structure alteration (96).

The link between SLs and aging has been fully reported and the reader may refer to the review 97 for detail. In model organisms such as yeast and *Drosophila*, the genes density of enzymes in charge of SLs metabolism is the critical determinant of longevity (97). Cer level are raised in tissues from aged rats and mice which is mainly mediated by NSM activation. ECs isolated from rat aorta has higher Cer level in old animals than in young which underlies the oxidative impairment of the cell and endothelial dysfunction (96). The upregulated SLs in aged arterial wall induces chronic low grade inflammation, driven in part by increased levels of inflammatory cytokines and oxidative stress which is considered as the fundamental basis of the onset of cardiovascular diseases (97). Encouraging evidence has been exhibited on how to lengthen life span and improve human health by modulating SLs, such as targeting rapamycin complex 1 protein kinase pathway and lowering the activity of SPT to reduce the de novo SLs biosynthesis (97).

7. CONCLUSION

SLs act as both component of multiple cellular membranes and signaling molecules exerting functions either intracellularly or extracellularly in ECs and VSMCs. The synthesis and degradation of SLs occur with significant compartmentalization which is modified by a myriad of stimulus, then regulating proliferation, differentiation, apoptosis, autophagy and senescence of cell. Vascular development, angiogenesis, as well as vascular adaptation to conditions like hypertension, atherosclerosis, MgD and aging are closely related to the fine regulation of SLs in which the balance of Cer and S1P takes a center stage. New knowledge on biology of SLs is anticipated to enhance our understanding of the mechanisms mediating vascular structural and functional remodeling. Accordingly, researches on this area would bring about new therapeutic targets for the controlling of vascular disorder in hypertension, atherosclerosis, cancer and other maladies.

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