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Sphingosine-1-phosphate signaling and biological activities in the cardiovascular system

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Abstract

The plasma lysophospholipid mediator sphingosine-1-phosphate (S1P) is produced exclusively by sphingosine kinase (SPHK) 1 and SPHK2 *in vivo*, and plays diverse biological and pathophysiological roles by acting largely through three members of the G protein-coupled S1P receptors, $S1P_1$, $S1P_2$ and $S1P_3$. $S1P_1$ expressed on endothelial cells mediates embryonic vascular maturation and maintains vascular integrity by contributing to eNOS activation, inhibiting vascular permeability and inducing endothelial cell chemotaxis via Gi-coupled mechanisms. By contrast, $S1P_2$ is expressed in high levels on vascular smooth muscle cells (VSMCs) and certain types of tumor cells, inhibiting Rac and cell migration via a $G_{12/13}$- and Rho-dependent mechanism. In rat neointimal VSMCs, $S1P_1$ is upregulated to mediate local production of platelet-derived growth factor, which is a key player in vascular remodeling. $S1P_3$ expressed on endothelial cells also mediates chemotaxis toward S1P and vasorelaxation via NO production in certain vascular bed, playing protective roles for vascular integrity. $S1P_3$ expressed on VSMCs and cardiac sinoatrial node cells mediates vasopressor and negative chronotropic effect, respectively. In addition, $S1P_3$, together with $S1P_2$ and

SPHK1, is suggested to play a protective role against acute myocardial ischemia. However, our recent work indicates that overexpressed SPHK1 is involved in cardiomyocyte degeneration and fibrosis *in vivo*, in part through S1P activation of the $S1P_3$ signaling. We also demonstrated that exogenously administered S1P accelerates neovascularization and blood flow recovery in ischemic limbs, suggesting its usefulness for angiogenic therapy. These results provide evidence for S1P receptor subtype-specific pharmacological intervention as a novel therapeutic approach to cardiovascular diseases and cancer.

1. Introduction

Sphingosine-1-phosphate (S1P) is a lysophospholipid mediator circulating in blood, which induces a wide variety of biological responses in a diversity of cell types [1-5]. These include stimulation of cell proliferation, inhibition of apoptosis and regulation of cell shape and cell motility, among others. S1P is present at the order of 10^{-7} M concentration in the plasma, largely in forms bound to plasma proteins including HDL and albumin [1]. S1P is generated by the phosphorylation of sphingosine by sphingosine kinases (SPHKs) 1 and 2, which share a conserved catalytic domain but are expressed in spaciotemporally distinct manners [6]. Deletion of either SPHK1 or SPHK2 is functionally fully compensated by the other isozyme, whereas SPHK1/SPHK2 double knockout mice are embryonic lethal, indicating that S1P is produced exclusively by SPHK's *in vivo*. The major source of plasma S1P is believed to be red blood cells and activated platelets [1, 7], which lack S1P degrading enzyme S1P lyase. In addition, extracellular SPHK1, which is derived from vascular endothelial cells and other types of cells, also likely contributes to production of plasma S1P [8].

Identification of a family of G protein-coupled cell surface receptors specific for S1P

provided strong support for the notion that most, if not all, of the diversity of biological activities of S1P are mediated through either of the S1P receptors [2-5]. However, in lower organisms, which lack S1P receptors, and in certain biological events in mammalian cells S1P and its precursors likely play regulatory roles. Indeed, a recent study demonstrated that SPHK1KO mice show defective decidua formation and decidual angiogenesis in the SPHK2 heterozygous genetic background, without a reduction in S1P levels but with marked elevations in sphingosine levels and intracellular formation of abnormal membranous structures [9]. Intracellular accumulation of sphingosine is suggested to cause inhibition of polyp growth in SPHK1KO mice in murine genetic intestinal polyposis model, in which S1P levels were not different from wild type mice [10]. An intracellular role for S1P is suggested in mast cell activation [11].

Among five S1P receptor subtypes $S1P_{1\text{-}5}$, $S1P_1$, $S1P_2$ and $S1P_3$ are widely expressed in various tissues [2-5], whereas $S1P_4$ and $S1P_5$ are expressed mainly in the immune system and the nervous system, respectively. The diversity of S1P actions depends upon their subtype-specific distinct repertoire of heterotrimeric G protein coupling, in

combination with tissue- and cell type-specific expression patterns (Fig. 1).

In this review, we focus on the cardiovascular system and angiogenesis, describing current knowledge on S1P actions and signaling mechanisms in this system, which includes novel actions of S1P that we recently identified. Understanding the cardiovascular actions of S1P provides insights into the pathophysiological roles and therapeutic significance of S1P in the cardiovascular system.

2. S1P receptor activities and signaling in the cardiovascular system revealed from gene knockout studies

$S1P_1$ is coupled exclusively via G_i to Ras-MAP kinase, phosphoinositide (PI) 3-kinase-Akt pathway and phospholipase C pathway, whereas $S1P_2$ and $S1P_3$ are coupled to multiple G proteins, i.e. G_q, $G_{12/13}$ and G_i to activate phospholipase C pathway and Rho pathway as well as the above mentioned G_i-dependent pathways [2-6].

$S1P_1$ is a major S1P receptor expressed on vascular endothelial cells, and is required for recruitment of mural cells and vascular maturation at the embryonic stage. Either

systemic disruption of $S1P_1$ gene or endothelial cell-selective knockout of $S1P_1$ resulted in embryonic lethality with a defect in vascular maturation (see below) [12], a phenotype similar to that in SPHK1/SPHK2 double KO mice [13] which essentially lack S1P, underscoring the importance of $S1P_1$ in S1P actions during embryonic phase. In addition, both $S1P_1$KO and SPHK1/SPHK2 double KO mice show defects in development of the nervous system. $S1P_1$ is also suggested to be involved in the maintenance of vascular integrity after birth, which includes inhibition of vascular permeability and endothelial cell survival [16-18].

$S1P_1$ receptors expressed on lymphocytes are responsible for their migration out of thymus and lymph nodes into the bloodstream, which is a crucial step in lymphocyte recirculation and thus immunity [14]. The immunosuppressant prodrug FTY720 is specifically phosphorylated by SPHK2 to become an active agonist for $S1P_1$, however, upon engagement to phosphorylated form of FTY720 $S1P_1$ undergoes rapid internalization and degradation through a ubiquitin-dependent process and thus strong downregulation of lymphocyte $S1P_1$ receptor [15]. This FTY720 phosphate activity as a functional $S1P_1$ antagonist leads to failure of lymphocyte egress from thymus and

peripheral lymphoid organs, and thus functional lymphopenia.

$S1P_2$ receptor is essential for morphogenesis of the heart in *Zebrafish* [19]. Although it is dispensable for murine cardiac development [20-22], $S1P_2$ receptor is required for functional integrity of the central nervous system in the weaning age and maintenance of normal structure and function of the inner ear in the adulthood [21, 22]. The latter role of $S1P_2$ appears to involve functional maintenance of the vasculature in the stria vascularis in the inner ear, in which $S1P_2$–mediated vasoconstriction controls arterial perfusion appropriately.

$S1P_1$ and $S1P_3$ are dominant S1P receptors expressed in vascular endothelial cells, whereas $S1P_2$ appears to be expressed only in endothelial cells of certain vascular beds [23]. Activation of $S1P_2$ on endothelial cell inhibits their migration and the formation of tube-like structures on Matrigel, most likely through mechanisms involving Rho-dependent inhibition of Rac and/or stimulation of the 3'-specific phosphoinositide phosphatase PTEN [23, 24]. In murine model of hypoxia-induced retinal angiogenesis, it is reported that $S1P_2$ expressed on the endothelium in the retinal vessels exerts an inhibitory effect on angiogenesis in neonatal avascular area of the retina, whereas $S1P_2$

expressed on vessels in vascular tuft invading vitreal body rather promotes pathological neo-angiogenesis, which leads to retinal hemorrhage and thus blindness [25]. The results suggest complex roles of $S1P_2$ in the regulation of pathological angiogenesis. In addition, activation of $S1P_2$ induces disruption of adherens junctions and resultantly vascular hyperpermeability [26].

$S1P_3$ receptor is abundantly expressed on vascular endothelial and medial smooth muscle cells, as well as cardiomyocytes [27]. $S1P_3$KO mice are phenotypically normal [28], however, $S1P_3$ deletion abrogates a variety of S1P effects on the cardiovascular system. These include negative chronotropic and hypertensive effects after intravenous administration of S1P *in vivo*, constriction of basilar artery, and endothelium-dependent vasodilation [27, 29]. $S1P_3$ mediates bradycardia through activation of inward rectifying G protein-activated potassium channels (GIRK1/GIRK4) via G_i in sinoatrial node cells [30], vasoconstriction through G_q–coupled Ca^{2+} mobilization and $G_{12/13}$–coupled Rho-dependent myosin light chain phosphatase inhibition in vascular smooth muscle cells (VSMCs) of certain vascular bed [27], and activation of eNOS and nitric oxide production in vascular endothelial cells through G_i- and Akt-mediated phosphorylation

of eNOS in concert with a G_q-mediated, Ca^{2+}/calmodulin-dependent activation process [31]. Being a constituent of plasma HDL, S1P is proposed to partially contribute to HDL-induced, NO-dependent vasorelaxation. However, S1P potently reduces myocardial perfusion in a manner dependent exclusively upon $S1P_3$ [32], despite the fact that HDL increases it. S1P is also vasoconstrictive for various vascular beds including kidney, mesentery and brain [33]. These observations, together with hypertensive effects of S1P, suggest that direct constrictive effects of S1P on vascular smooth muscle may dominate its endothelium-dependent dilatory effect in many vascular beds.

3. $S1P_2$–mediated inhibition of vascular smooth muscle cell migration, a possible therapeutic strategy

Regulation of VSMC migration is a potential target of vascular medicine in the near future. Upon endothelial damage, medial VSMCs are induced to migrate into the intima, which, together with bone marrow-derived vascular progenitor cells recruited to the site of endothelial lesion, undergoes phenotypic conversion to neointimal cells to contribute

to the formation of vascular occlusive lesions, including atheroma and post-angioplasty restenosis. Migration of VSMCs is induced by chemoattractants such as platelet-derived growth factor (PDGF) [34]. Chemoattractants induce chemotaxis, which is a directed cell migration toward an increasing concentration of chemoattractant. In such an environment polarized stimulation of the chemoattractant receptors at the front of the cell that faces increasing concentration of the ligand induces polarized activation of PI 3-kinase and the Rho family small GTPase Rac, resulting in directed cell movement [35]. Growth factor receptors and chemokine receptors thus mediate chemotaxis through activation of PI 3-kinase and Rac, at the site downstream of receptor tyrosine kinases and $\beta\gamma$ subunit of G_i, respectively.

PDGF, which is a potent chemoattractant for VSMCs and released from the intimal VSMCs [34], acts on its cognate receptor tyrosine kinase to induce receptor tyrosyl auto-phosphorylation, followed by activation of PI 3-kinase and Rac, all of which are essential for a chemotactic response [17, 35]. S1P stimulation of $S1P_2$ abolishes PDGF-directed chemotaxis. $S1P_2$ activation also abolishes Rac activation and Rac-dependent actin re-organization, without inhibiting PDGF receptor

autophosphorylation or PI 3-kinase stimulation [2, 17, 36]. Thus, inhibition of Rac activation is very likely a major mechanism for $S1P_2$-mediated inhibition of cell migration. $S1P_2$ by itself mediates Rho activation. Since botulinum C3 toxin, which inhibits Rho, abolishes inhibitory regulation of $S1P_2$ it is likely that Rho activation is involved in S1P inhibition of Rac activity in VSMCs (S. Takashima et al., unpublished observation).

We analyzed in detail the G protein responsible for coupling $S1P_2$ to inhibition of Rac and cell migration by studying the effects of expression of molecular probes, which include C-terminal mini-peptides of the heterotrimeric G protein α-subunits (Gα-CTs), which specifically inhibit receptor-Gα protein interaction, as well as βArk-CT, which acts as a scavenger for βγ [37]. The results indicate that, among three classes of the heterotrimeric G proteins $G_{12/13}$, G_q and G_i that $S1P_2$ activates [38], $S1P_2$-mediated inhibition of Rac and cell migration requires both $G_{12/13}$ and G_q (S. Takashima et al., unpublished observation). The $G_{12/13}$ class induces Rho activation by direct physical and functional interaction between $G\alpha_{12/13}$ and a group of Rho-guanine nucleotide exchange factors (Rho-GEFs), which includes p115RhoGEF, PDZ-RhoGEF and LARG [39], at

their conserved structural motif known as the regulator of G protein signaling domain (the RGS box). G_q was previously demonstrated to mediate Rho stimulation through mechanisms involving direct association of $G\alpha_q$ with Rho-GEFs in certain non-smooth muscle cell types. In VSMCs AT_1 angiotensin II receptor couples to G_q to induce robust phospholipase C activation, however, AT_1 by itself does not mediate either Rho stimulation or Rac inhibition (S. Takashima et al., unpublished observation). Phospholipase C or its downstream second messengers Ca^{2+} or protein kinase C are not likely to be involved in $S1P_2$-mediated Rho activation in VSMCs either. The observations indicate that G_q activation by itself is not sufficient for effective Rho activation required for inhibition of Rac and migration in VSMCs. It is unknown whether this is due to lack of the expression in VSMCs of a Rho-GEF type that G_q can associate with, or due to alternative mechanisms. It is likely that G_q exerts a permissive effect on Rho activation in cooperation with $G_{12/13}$.

There are several GPCR agonists that inhibit VSMC motility through G_s-coupled receptors and elevation in cellular cyclic AMP. These include prostaglandin E_2 (PGE_2), dopamine and adrenomedullin [40]. Different from these G_s-coupled receptors, which

suppress VSMC cell migration in the absence of a concentration gradient of the ligand [40], $S1P_2$ mediates inhibition of cell migration only in the presence of S1P concentration gradient [36]. This property of S1P is a hallmark of a chemorepellant. Certain bioactive factors including the semaphorine and the ephrine family members have recently been defined as chemorepellants for neuronal and vascular cells. These molecules act as ligands for cell surface receptors that are distinct from GPCRs. $S1P_2$ is the first example of a chemorepellant GPCR with an inhibitory activity for Rac [2].

Sanchez et al. [26] showed that $S1P_2$ inhibition of cell migration is mediated through stimulation of the 3'-specific PI phosphatase, PTEN, and in a Rho kinase-dependent manner. They suggested that PTEN stimulation by $S1P_2$ may be responsible for inhibition of cell migration, because 3'-PIs participate in cell migration through multitudes of mechanisms including actin cytoskeletal re-organization, the protein kinase Akt activation, and Rac-GEF activation. However, we observed in VSMCs that knockdown of PTEN expression by RNAi did not affect $S1P_2$–mediated inhibition of cell migration although PTEN knockdown effectively suppressed Akt activation (Sugimoto N et al., unpublished observations). The observations suggest that $S1P_2$

mediates stimulation of PTEN in VSMCs as well, but that stimulation of PTEN does not mediate inhibition of VSMC migration. These observations may suggest that $S1P_2$-mediated inhibition of cell migration depends upon different subcellular mechanisms among cell types.

A number of growth factors, hormones and cytokines have been implicated in the process of vascular restenosis, including PDGF, fibroblast growth factor (FGF), angiotensin II and transforming growth factor-β. Inhibitors of these receptors and downstream signaling pathways have been demonstrated to successfully inhibit stenosis in animal models. Among these, rapamycin, an inhibitor of mTOR which is a protein kinase located downstream of the PI 3-kinase signaling pathway, is proven to be effective in diminishing restenosis in patients when rapamycin-coated stents are implanted at stenotic sites. Selective activation of $S1P_2$ receptor in VSMCs induces inhibition of the intracellular signaling molecules Rac and Akt, leading to inhibition of VSMC migration. Inhibition of Rac and Akt may also lead to anti-proliferation and apoptosis of VSMCs. If a selective $S1P_2$ antagonist is locally administered at stenotic sites as a drug-eluting stent, it could inhibit accumulation of VSMCs into the luminal

surface by uniquely acting as both a chemorepellent and anti-proliferative agent, exerting a protective effect against vascular restenosis.

4. *In vivo* angiogenic activity of S1P and its application for angiogenic therapy in tissue ischemia

The stimulatory activities of S1P in endothelial cell proliferation, migration and capillary-like tube formation *in vitro* are mediated via $S1P_1$ and $S1P_3$ and are reported to be as potent as well characterized angiogenic peptide growth factors including vascular endothelial growth factor (VEGF) and FGF-2 [16]. S1P also stimulates angiogenesis *in vivo* via $S1P_1$ and $S1P_3$ in the Matrigel implant assay [16]. Disruption of either $S1P_1$ gene or both SPHK1 and SPHK2 genes in mice demonstrated that S1P and its receptor $S1P_1$ in vascular endothelial cells are essentially required for the recruitment process of pericytes and smooth muscle cells to the nascent capillaries and thus vascular maturation [12, 13]. A recent investigation [41] demonstrated that RNAi-mediated $S1P_1$ silencing inhibited tumor angiogenesis and tumor growth *in vivo* in an animal model of subcutaneous tumor implantation, indicating that endogenous S1P is involved in tumor

angiogenesis via $S1P_1$. It is also reported that anti-S1P antibody potently reduced tumor growth [42]. Indeed, S1P stimulates barrier integrity of endothelial cells *in vitro* in part via $S1P_1$-mediated upregulation of VE-cadherin and stimulation of intercellular adhesion [18, 44]. *In vivo*, S1P and the S1P receptor agonist FTY720-phosphate acutely suppress pulmonary edema and VEGF-induced vascular hyperpermeability [45]. In contrast, $S1P_2$ and $S1P_3$ disrupt endothelial integrity *in vitro* and increase vascular permeability *in vivo* [45, 46]. $S1P_2$ expressed in endothelial cells also potently inhibits endothelial cell migration and capillary-like tube formation [17, 23]. These observations may suggest that selective $S1P_1$ activation in combination with $S1P_2$ activation should be a most promising strategy as an angiogenic treatment.

In the light of high morbidity and mortality due to ischemic cardiovascular diseases in the developed countries, stimulating revascularization in underperfused tissues is an attractive novel therapeutic strategy [47]. To date, however, the results of clinical studies on topical and systemic administration of the angiogenic peptide growth factors, including VEGF, FGF-2 and hepatocyte growth factor, and their expression plasmids, have not been very satisfactory.

The mouse hindlimb ischemic model is one of the well-established animal models for ischemia-induced angiogenesis *in vivo* to evaluate the potential of angiogenic factors as a therapeutic agent. We recently showed for the first time that S1P is useful in stimulating angiogenesis *in vivo* in the mouse hindlimb afte ischemia [48]. The ligation and removal of unilateral femoral artery induces a 95 % reduction in the blood flow as compared to the contralateral non-ischemic limb the day after operation (day 1), as evaluated with a laser Doppler blood flow analyzer. The blood flow thereafter gradually recovers over 4 weeks. Daily intramuscular administration of S1P in hindlimb muscle dose-dependently accelerates blood flow recovery, resulting in up to twice as much blood flow at days 7 and 14 as compared to vehicle control. The stimulatory effect on the blood flow by the optimal dose of S1P is similar in the magnitude as that induced by the potent angiogenic peptide growth factor FGF-2, providing ecidence that *in vivo* angiogenic strength of S1P is comparable to that of the known angiogenic factors. S1P-induced stimulation of the blood flow recovery is accompanied by an increase in the capillary density in the ischemic hindlimb muscle, suggesting that S1P-induced stimulation of blood flow recovery is due to neovessel formation. We recently generated

SPHK1-transgenic (SPHK1-Tg) mice (see below), which exhibits a marked increase in SPHK activity and a moderate increase in the S1P level in limb muscle as well as in other tissues. In SPHK1-Tg mice, the post-ischemic blood flow recovery and angiogenesis are accelerated as compared to WT littermates. One of the drawbacks in previous trials of angiogenic therapy is local tissue edema due to vascular leakage [47]. Daily S1P injection in wild type mice does not induce an increase in vascular leakage nor does SPHK1-Tg mice exhibit an increased vascular permeability in the ischemic limb, indicating that S1P-induced neo-angiogenesis is not accompanied by vascular hyperpermeability. S1P organizes endothelial tight junctions and adherens junctions [49] and promotes vascular mural cell recruitment at least in part via upregulation of N-cadherin [12, 50]. These effects of S1P likely lead to the protection of the neovessels from hyperpermeability. Daily local administration of S1P exhibits no adverse effect in mice, in terms of general conditions, the blood cell count or the cardiovascular parameters including the heart rate and blood pressure. These data collectively suggest potential usefulness of S1P or $S1P_1$ agonist for therapeutic angiogenesis in ischemic conditions.

Recent studies showed the contribution of bone marrow-derived circulating endothelial precursor cells to the new blood vessel formation in ischemic tissues. $CD34^+$ vascular endothelial progenitors express $S1P_3$ receptor [51]. Stimulation of progenitor $S1P_3$ receptor with S1P or synthetic analog FTY720 activates the CXCR4 chemokine receptor which is essential for the effectiveness of progenitor cell therapy for angiogenesis. Therefore, S1P may stimulate angiogenesis not only by directly stimulating pre-existing endothelial cells but also through recruitment of circulating endothelial precursor cells.

5. S1P and cardiac remodeling

Cardiomyocytes express $S1P_1$, $S1P_2$ and $S1P_3$ [27]. S1P induces hypertrophy of cardiomyocytes *in vitro* via $S1P_1$ [52]. In addition, all of the three major S1P receptor subtypes are also expressed in cardiac fibroblasts which participate in cardiac hypertrophy and fibrosis, i.e. cardiac remodeling [53]. S1P reduces mortality of hypoxic cardiac myocytes *in vitro* [54]. S1P is also playing protective roles for myocardial

ischemia. For example, in an *in vivo* mouse model of myocardial ischemia/reperfusion (IR), exogenous HDL and its lipid component S1P attenuated infarct size dramatically [55]. These effects of HDL and S1P were completely absent in $S1P_3$KO mice. Deletion of both $S1P_2$ and $S1P_3$ receptors, but not either alone, in mice resulted in aggravation of acute myocardial infarction after experimental coronary artery ligation [56]. It is also reported, however, S1P potently reduces coronary perfusion [32], possibly via coronary arftery smooth muscle contraction. SPHK1 has been shown to have protective effects against IR injury in the perfused heart, and is involved in the process of ischemic preconditioning, in which mild ischemic pretreatment alleviates the extent of infarction caused by the subsequent ischemic session [57].

The heart expresses two SPHK isoforms, SPHK1 and SPHK2. Recent studies have demonstrated for various types of cells that stimulation with extracellular signals further stimulates cellular SPHK1 activity over the basal constitutive level. These include PDGF, nerve growth factor, muscarinic acetylcholine agonists, tumor necrosis factor-α, interleukin-1 and crosslinking of cell surface FcεRI or FcγRI, raising the possibility that SPHK1 and S1P are involved in certain pathological conditions. Indeed, Deutschman et

al. have reported that serum S1P level is the most reliable marker that predicts cardiovascular events to occur [58]. Overexpression of SPHK1 in cultures cells stimulates cell proliferation with accerelated G1 to S phase cell cycle progression, and in NIH3T 3 cells it resulted in cell transformation, leading to the notion that wild type SPHK1 gene is an oncogene [59].

We have generated SPHK1a-Tg mice to evaluate the effect of SPHK1a overexpression *in vivo* (N. Takuwa et al., unpublished observations). The TG mice overexpress functional SPHK1a in diverse tissues with more than 10-fold increases in the enzymatic activity. However, the TG mice of both sexes were born without malformation, showed normal development with growth curves identical to those of WT littermates, and were fertile, indicating that SPHK1a overexpression does not compromise regulated cell proliferation, differentiation or apoptosis that are required for development and reproduction. The lifespan of the TG mice are not shorter than the WT littermates. Unexpectedly, we found that the SPHK1a-Tg mice exhibited degenerative changes in cardiomyocytes and myocardial fibrosis, which aggravated with age. The SPHK1a-Tg mouse heart showed a slight but significant increase in the cardiac S1P

content, but normal sphingosine and ceramide levels. Immunohistochemical analysis of SPHK1a in the TG heart tissue revealed SPHK1a overexpression in the myocardium.

The SPHK1a-Tg mice were normotensive and have normal blood cholesterol and glucose levels. Coronary blood vessels in the Tg mice were normal. These observations indicated that cardiac fibrosis in the Tg heart is not secondary to hypertension, ischemic heart disease or other metabolic disorders. Accumulated evidence in recent investigation suggests the involvement of the Rho family GTPases, particularly Rac and Rho, in cardiac remodeling including hypertrophy and fibrosis in mice and human [60]. S1P receptor signaling induces either activation or inhibition of Rac and activation of Rho, depending on the receptor subtype [35]. We observed activation of Rac and Rho in the Tg heart as compared to WT littermates. Long term administration of an HMG CoA reductase inhibitor pitavastatin, which inhibits geranylgeranylation and thus membrane targeting of both Rac and Rho [60], strongly inhibited the development of cardiac fibrosis, consistent with the notion that the Rho family GTPases are involved in the pathogenesis of cardiac fibrosis in SPHK1a-Tg mice. Development of cardiac fibrosis is strongly suppressed in $S1P_3$KO mice, indicating that $S1P_3$ is involved in cardiac fibrosis

in the SPHK1a-Tg mice. These observations show that transgenic overexpression of SPHK1a in the mouse heart tissue leads to progressive cardiomyocyte degeneration and cardiac fibrosis, which likely involves activation of the $S1P_3$-Rho family small G protein signaling pathway as a mechanism. The relevance of these observations in transgenic mice to human cardiac fibrosis of the ischemic, hypertensive and other origins deserves further investigation.

6. Conclusion

Recent investigation has revealed an ever increasing list of previously unrecognized important roles for the S1P signaling system in homeostasis and pathophysiology of the cardiovascular system (Fig. 1). These include embryonic vascular maturation, regulation of angiogenesis and vascular integrity, vascular contraction and NO-mediated relaxation, autoregulation of vascular tone and the heart rate, as well as roles for coronary perfusion, ischemic preconditioning, protection against ischemia and myocardial remodeling. Modulation of the S1P signaling system by targeting S1P receptors and

sphingolipid-metabolizing enzymes, including SPHK1 and possibly other members, are expected to provide new therapeutic modalities for cardiovascular diseases, such as vascular occlusive diseases, tissue ischemia, and cardiac remodeling.

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Figure legend

Figure 1. S1P signaling and actions through $S1P_1$, $S1P_2$ and $S1P_3$ receptors in the cardiovascular system. S1P, which is synthesized by membrane sphingolipid metabolism and final phosphorylation of sphingosine by SPHKs, binds to $S1P_1$, $S1P_2$ and $S1P_3$ in vascular endothelial cells, VSMCs, and cardiomyocytes. These three receptors activate partially overlapping but distinct sets of signaling pathways, to exert complex effects on cardiovascular cells, which include stimulation or inhibition of cell migration and angiogenesis, a decrease or increase in vascular permeability, vascular contraction or relaxation, and protection from myocardial ischemia. SPP, sphingosine-1-phosphate phosphatase; SPL, sphingosine-1-phosphate lyase; VSM, vascular smooth muscle.

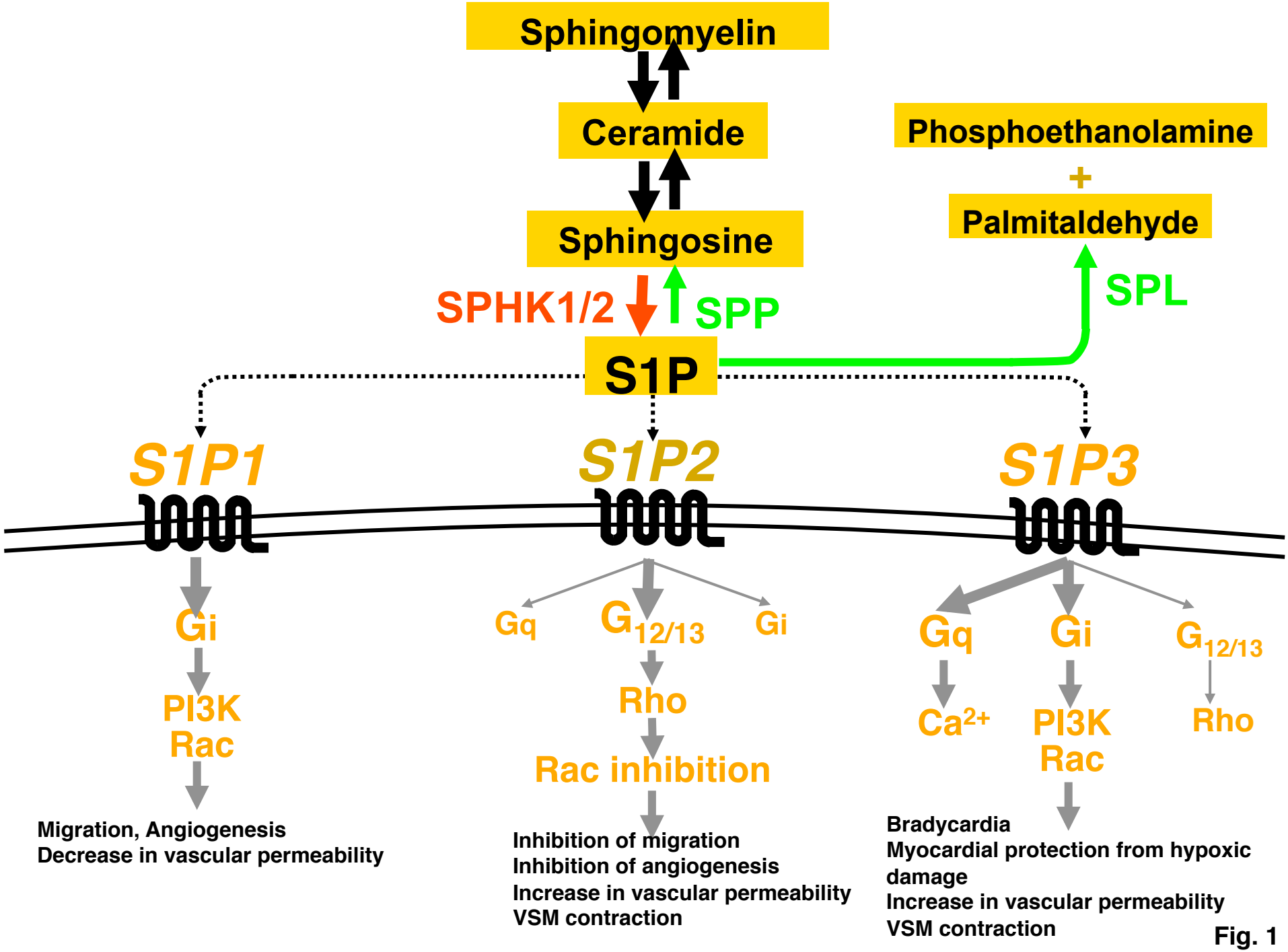
Sphingomyelin
Ceramide
Sphingosine
SPHK1/2
SPP
S1P
Phosphoethanolamine
+
Palmitaldehyde
SPL
S1P1
S1P2
S1P3
Gi
PI3K
Rac
Migration, Angiogenesis
Decrease in vascular permeability
Gq
G12/13
Gi
Rho
Rac inhibition
Inhibition of migration
Inhibition of angiogenesis
Increase in vascular permeability
VSM contraction
Gq
Ca2+
Gi
PI3K
Rac
G12/13
Rho
Bradycardia
Myocardial protection from hypoxic damage
Increase in vascular permeability
VSM contraction

Fig. 1