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Review

Regulation of vascular physiology and pathology by the $S1P_2$ receptor subtype

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Sphingosine-1-phosphate (S1P) is now recognized as a lipid mediator that acts via G-protein-coupled receptors. S1P receptors couple to various heterotrimeric G-proteins and regulate downstream targets and ultimately cell behaviour. The prototypical $S1P_1$ receptor is known to couple to G_i and regulates angiogenesis, vascular development, and immune cell trafficking. In this review, we focus our attention on the $S1P_2$ receptor, which has a unique G-protein-coupling property in that it preferentially activates the $G_{12/13}$ pathway. Recent studies indicate that the $S1P_2$ receptor regulates critical intracellular signalling pathways, such as Rho GTPase, the phosphatase PTEN, and VE-cadherin-based adherens junctions. Analysis of mutant mice has revealed the critical role of this receptor in inner ear physiology, heart and vascular development, vascular remodelling, and vascular tone, permeability, and angiogenesis in vertebrates. These studies suggest that selective modulation of $S1P_2$ receptor function by pharmacological tools may be useful in a variety of pathological conditions.

1. Introduction

Sphingosine-1-phosphate (S1P), a bioactive lysophospholipid, is now established as a lipid mediator with a broad spectrum of biological activities. In the early 1990s, S1P was discovered to regulate calcium release from intracellular stores, cell proliferation, and cell shape change in Swiss 3T3 fibroblasts.[1,2] Indeed, over the ensuing decade and a half, S1P was reported to mediate a wide variety of biological responses, including cell growth, survival, cell trafficking, and cytoskeleton rearrangements.[3–5] S1P and other phosphorylated long-chain sphingoid bases have also been detected in lower organisms such as yeasts, flies, and worms, which underscore the significance of this lipid as an evolutionarily conserved signalling mediator.[6–8] A major development in the field that preceded widespread interest was the identification of the EDG family of G-protein-coupled receptors (GPCRs) as S1P receptors.[9–11] These are the $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors that regulate diverse downstream signalling properties due to coupling to distinct heterotrimeric G-proteins.[9,11–13]

The ligand S1P is produced from sphingomyelin metabolism. *De novo* synthesis of sphingolipids is initiated at the cytosolic face of the endoplasmic reticulum (ER) with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase, ultimately resulting in the formation of ceramide. Ceramide, the common backbone of sphingolipid metabolism, is formed primarily by hydrolysis of the membrane phospholipid sphingomyelin. It can be further metabolized by ceramidase to produce sphingosine, which is phosphorylated by sphingosine kinases (SphKs) to generate S1P. Once formed, S1P can be dephosphorylated to sphingosine by specific phosphatases or be irreversibly degraded into hexadecanal and phosphoethanolamine by S1P lyase[14,15] (*Figure 1*). Although the significance of S1P as an intracellular second messenger has not been unequivocally established, many studies in different cell types have demonstrated that intracellular S1P action may be involved in the promotion of cell survival and proliferation.[16–21] Notwithstanding the essential role of the S1P metabolic pathway in cell physiology, much remains to be learnt about the intracellular function of S1P as a second messenger. The identification of intracellular S1P-binding proteins and/or signalling targets could enhance further progress in this area.

The concentration of S1P in plasma is estimated to be between 0.1 and 0.6 μM, mostly associated with HDL and albumin. In sharp contrast, tissue S1P levels are low.[22,23] Therefore, a large concentration gradient of S1P is maintained between vascular (plasma) and extravascular compartments (i.e. interstitial fluid) in mammals. Previous studies have suggested that platelets are the major source of S1P.[24] However, it was recently reported that a major source of plasma S1P is red blood cells.[25–27] Work from our laboratory suggests that vascular endothelium also

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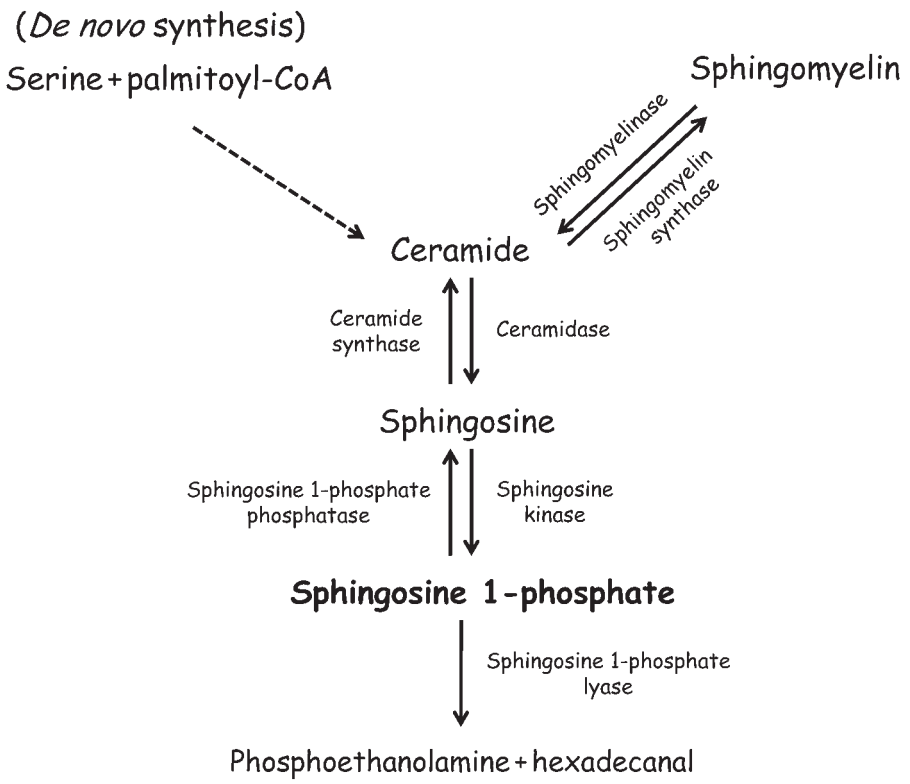

Figure 1 Sphingosine-1-phosphate (S1P) metabolism. *De novo* synthesis of sphingolipids produces ceramide and sphingomyelin. Sphingomyelinase, ceramidase, and sphingosine kinase are required to produce S1P. The degradation of S1P is achieved by the enzymes S1P phosphatase and S1P lyase.

contributes to plasma S1P.[28] Thus, erythrocytes and endothelial cells may maintain the S1P gradient, which is physiologically important for immune cell trafficking.

Given the increased realization of the physiological importance of S1P as a lipid mediator, much research has been focused on the characterization of specific S1P receptor subtypes and their signalling properties, biological actions, and roles in physiology and disease. Much has been learnt about the $S1P_1$ receptor, the prototypical S1P receptor, that regulates vascular development, immune cell trafficking, angiogenesis, and other functions. In contrast, less information is available about $S1P_2$ receptor, which signals in an opposite manner than that of the $S1P_1$ receptor. In this review, we will focus on the physiological and pathological processes that S1P regulates via the $S1P_2$ receptor in the cardiovascular system, underscoring the therapeutic potential of the S1P/$S1P_2$ receptor axis in vascular disease.

2. Sphingosine-1-phosphate receptor 2 signalling *in vitro*

S1P interacts with high affinity with the S1P family ($S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$) of GPCRs that trigger multiple downstream signalling processes by coupling to distinct heterotrimeric G-proteins.[11,29–31] The first member of this family of receptors to be identified was the G_i-coupled $S1P_1$ receptor, whereas the $S1P_2$ and $S1P_3$ receptors couple to G_i as well as G_q and $G_{12/13}$ proteins.[29,32–34] Similarly, the $S1P_4$ receptor associates with G_i and $G_{12/13}$ proteins, whereas the $S1P_5$ receptor couples only to G_i and G_{12} proteins.[35]

Indeed, the S1P2 receptor [a.k.a. H218, AGR16, Edg-5, LP(B2)] was originally identified as a novel cDNA clone from a rat smooth muscle cDNA library encoding a putative GPCR with high sequence similarity to the $S1P_1$ receptor.[36] Furthermore, S1P is an extracellular high-affinity ligand ($K_d = 16–27$ nM).[37–39] This receptor has a wide tissue distribution.[36,40–44] It couples to G_i, G_q, as well as $G_{12/13}$ heterotrimeric G-proteins even though its coupling efficiency to the $G_{12/13}$ pathway is particularly strong.[29,45] Thus, $S1P_2$ receptor coupling to diverse heterotrimeric G-proteins suggests the activation of multiple downstream signalling pathways, leading to profound effects on the physiology of different cell types, tissues, organs, and finally functional systems.[46] Several reports indicate that the $S1P_2$ receptor is able to activate the MAP kinase protein ERK and mediate S1P-induced cell proliferation and survival in a pertussis toxin- and Ras-dependent manner suggesting the participation of G_i heterotrimeric G-protein. Indeed, $S1P_2$ receptor expression in HTC4 hepatoma cells, CHO, and C6 glioma cells leads to ERK phosphorylation and immediate-early induction of c-Jun and c-Fos proto-oncogenes.[37,47–49]

In addition, the $S1P_2$ receptor has been reported to trigger phospholipase C (PLC) activation and downstream Ca^{2+} release in response to coupling to G_q heterotrimeric G-protein.[37,38,49–54] Moreover, the $S1P_2$ receptor induces phospholipase D (PLD) activity in epithelial cells as well as muscle cells and facilitates C2C12 myoblast differentiation into myotubes by coupling S1P to PLD activation.[55,56] Although the $S1P_2$ receptor has been shown not to couple to G_s heterotrimeric G-protein, it is able to activate adenylate cyclase and greatly enhance increases in intracellular concentration of cAMP by a mechanism that is not well

defined yet.[29,37,38] A recent study using RNAi and pharmacological inhibitors suggests that S1P acts through the $S1P_2$ receptor and couples to G_{13} heterotrimeric G-protein to stimulate cAMP synthesis.[57] More importantly, the $S1P_2$ receptor reportedly stimulates other members of the MAPK family such as *JNK* and *p38 MAPK* that are well known to play diverse roles in broad physiological functions such as cell stress, pro-inflammatory cytokine production, and apoptosis.[37,58,59] It is also important to recognize that dissection of the $S1P_2$ receptor downstream signalling pathway in such a fashion may represent an oversimplification. However, it becomes apparent that the complex character of the $S1P_2$ receptor is able to trigger both pro-survival as well as pro-apoptotic signalling pathways in different aspects of the cellular physiology. This raises an interesting question as to whether $S1P_2$ receptor function is differentially regulated in a stressor-specific manner, from physiological to pathophysiological conditions. (*Figure 2*).

In addition to G_i and G_q heterotrimeric G-proteins, the $S1P_2$ receptor can also couple to $G_{12/13}$ and activate Rho small GTPase.[29,37,47,60,61] In fact, this receptor isoform couples to the $G_{12/13}$/Rho pathway strongly, compared with other receptor isoforms. Northern blot analysis revealed the expression of $S1P_2$ receptor transcript in rat hepatocytes, in which S1P decreased DNA synthesis induced by hepatocyte growth factor. The inhibitory effect of S1P on hepatocyte proliferation was attenuated by inactivation of small GTPase Rho with C3 exotoxin and also by JTE-013, an $S1P_2$ receptor antagonist. Therefore, through small GTPase activation, the $S1P_2$ receptor could act as a negative regulator of tissue repair and remodelling.[62] Moreover, $S1P_2$ receptor expression in CHO cells leads to complete inhibition of PI3-kinase-dependent Rac activation which is essential for chemotactic and migratory events. Mechanistically, evidence supports the notion that the $S1P_2$ receptor effect on Rac inhibition involves stimulation of a GTPase-activating protein for Rac, rather than inhibition of Rac-guanine nucleotide exchange protein. The $S1P_2$ receptor actions were mimicked by expression of V14Rho (dominant active form) and were abolished by C3 toxin (RhoA inhibitor) and N19Rho (dominant-negative form).[60,63] S1P induced inhibition and activation, respectively, of GTP-Rac and GTP-RhoA in B16 melanoma cells, which were abrogated by JTE-013.[64] Several reports demonstrate that S1P induces significant increase in the amounts of GTP-RhoA in $S1P_2$ receptor-expressing cells through $G_{12/13}$, thereby inhibiting Rac, cortical actin assembly, and cell migration.[60,63,65–68] On the other hand, a recent report also showed that suppression of the $S1P_2$ receptor function by either RNAi tools or JTE-013 completely blocked S1P augmentation of fibroblast chemotaxis to fibronectin, in human lung fibroblasts. S1P-stimulated Rho activation and focal adhesion kinase phosphorylation were also significantly inhibited by the $S1P_2$ receptor antagonist JTE-013.[69] The reason for such discrepancies is not clear, although different cell lines used in these studies may be one of the causes. Indeed, many cell types including vascular smooth muscle, endothelial cells, and fibroblasts express more than a single type of S1P receptors. In addition, a further insight into the mechanistic details behind the crosstalk between the $S1P_2$ receptor and other receptors or co-activators that determine migratory events is necessary to understand the regulation of migration by S1P/$S1P_2$ receptor axis. It is evident that an integration of the S1P/$S1P_2$ receptor-positive and -negative signals on the small GTPases activation and consequent downstream effectors is an essential determinant for the regulation of cell chemotaxis by S1P.

Furthermore, $S1P_2$ receptor expression in endothelial cells markedly inhibited S1P-induced migration and protein kinase B/Akt phosphorylation. The anti-migratory role of the $S1P_2$ receptor implicates the involvement of p160-ROCK which is well-characterized downstream target of small GTPase Rho.[65] Studies by Takashima *et al*. further clarified the anti-migratory action of the $S1P_2$ receptor in vascular smooth muscle cells. These observations suggest that $S1P_2$ receptor-dependent small GTPase Rho activation is dependent on both $G_{12/13}$ and G_q heterotrimeric proteins, indicating that G_q may well facilitate Rho GTPase activation in cooperation with $G_{12/13}$, whereas PLC and its downstream second messengers (Ca^{2+}, PKC) are likely not involved in the $S1P_2$ receptor anti-migratory role in vascular smooth muscle cells.[68] In addition, it has been reported that the $S1P_2$ receptor interacts with and actively regulates the tumour suppressor gene *PTEN* (a PIP_3 phosphatase) as a necessary downstream effector in the anti-migratory response, in vascular endothelial cells, as well as mouse embryonic fibroblasts.[65,70] Recent studies suggest that $S1P_2$ receptor expression is markedly induced in cultured senescent vascular endothelial cells. Importantly, suppression of $S1P_2$ receptor expression or expression of dominant-negative PTEN phosphatase greatly rescues the S1P-mediated Rac activation, wound-healing, and chemotactic responses in senescent endothelial cells, suggesting that $S1P_2$ receptor-dependent PTEN activation might be implicated in vascular dysfunction.[70] However, a recent study by Malchinkhuu *et al*. and Lepley *et al*. indicates that $S1P_2$ receptor stimulation leads to the inhibition of glioma cell migration through Rho-GTPase signalling pathways regardless of PTEN expression, suggesting that a further mechanistic understanding of $S1P_2$ receptor-dependent inhibition of glioma cell migration is required to clarify the function of the receptor during critical events of tumourigenicity such as invasion and metastasis.[71]

In addition, activation of the $S1P_2$ receptor resulted in RhoA GTPase-dependent increase in myosin light chain

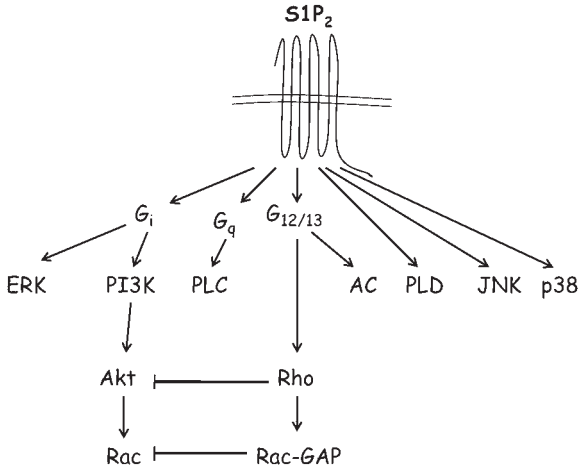

Figure 2 Signal transduction of the $S1P_2$ receptor. The plasma membrane-localized $S1P_2$ receptor couples to several heterotrimeric G-proteins as indicated. However, coupling to $G_{12/13}$ is most prominent. Downstream of Rho, the Rac GTPase is inhibited.

phosphorylation and prominent stress fibre formation.[37,60,72] Ectopic expression of the $S1P_2$ receptor in vascular endothelial cells stimulates the assembly of stress fibres, phosphorylation, and disruption of VE-cadherin-based junctions, leading to increased paracellular permeability, whereas JTE-013 significantly improved the barrier integrity.[73] Considering the multifaceted signalling mechanisms involved in regulation of vascular permeability by S1P receptors, it is critical to better our understanding of the events that promote $S1P_2$ receptor activation in diverse capillary beds throughout the vascular system, leading to compromised endothelial barrier protection. In C2C12 myoblasts, S1P acting through the $S1P_2$ receptor reduces serum-induced cell proliferation and enhances the expression of myogenic differentiation markers such as myosin heavy chain, thus promoting myogenic differentiation, whereas it greatly stimulates contraction of coronary artery smooth muscle cells.[37,52,74–76] The functional complexity and ambiguity of the $S1P_2$ receptor in different cell types of the cardiovascular system could result in spatial and temporal profiles of activation, mobilized signalling networks through crosstalk with other S1P receptors or plasma membrane adaptor proteins, silencing or activation of downstream target genes under different conditions, thus $S1P_2$ receptor activation could have a substantial affect on the physiology of the cell. Nevertheless, further study of $S1P_2$ receptor activation and regulation is anticipated, as advancements in the development of potent and specific $S1P_2$ receptor antagonists might have great implications in cardiovascular development and pathology.

3. Sphingosine-1-phosphate receptor 2 in cardiovascular system development

S1P receptors regulate important physiological functions of the vascular system, such as vascular morphogenesis and maturation, cardiac function and remodelling, vascular permeability, and tumour angiogenesis.[77–84] Recent studies with the use of genetic and pharmacological tools, from worms to rodents, suggest that the $S1P_2$ receptor is implicated in cardiovascular system function, in health and disease (*Table 1*).

Indeed, mutations in the zebrafish gene *miles-apart* (*Mil*), an *S1p2* ortholog, results in cardiac developmental defects (cardia bifida phenotype, two laterally positioned hearts) due to defective migration of the heart precursors to the midline, revealing an important function of the $S1P_2$ receptor in zebrafish heart organogenesis.[85] Importantly, when mutant cells were transplanted into a wild-type embryo, they could migrate normally, whereas wild-type cells in a mutant embryo fail to migrate, suggesting that the *S1p2* receptor may be involved in generating an environment permissive for heart tube formation in the mesoderm. In agreement with this observation, a recent report shows further evidence that cell–matrix interaction driven by *Mil* is required for proper myocardial migration.[86] Given these results, it would be interesting to know the role of the enzymes of sphingolipid metabolism such as SphKs, phosphatases, and lyase in the development a zebrafish circulatory system and heart morphogenesis. The ability to alter the sphingolipid levels and receptor expression during and/or after the establishment of the vascular system in zebrafish could provide better understanding of the mechanisms involved in vascular endothelial cell migration and morphogenesis.

Mice that lack the $S1P_2$ receptor are viable. Although embryos null for the $S1P_2$ receptor do not show defects in the development of the vascular system, when embryos are null for both $S1P_1$ and $S1P_2$ receptors, they die between E10.5 and E12.5 compared with singe-null embryos for $S1P_1$ that die between E12.5 and E14.5.[87] Indeed, substantial haemorrhage was evident suggesting that vascular abnormalities were a cause of death. In addition, detailed examination of the head region revealed that in the double-null embryos, the capillary network was less developed and contained fewer branches when compared with the wild-type embryos and the single-null *S1p1* mice. Furthermore, when mice lack both $S1P_2$ and $S1P_3$ receptors, 50% of the embryos die after E13.5, whereas mice null for $S1P_3$ receptor are viable and fertile. Importantly, double-null embryos that had survived showed severe bleeding phenotype, indicating that a vascular defect was the main reason for the embryonic lethality.[54,87] However, bleeding phenotype was not observed in the single-null *S1p2* or *S1p3* embryos. Embryos deficient for both $S1P_2$ and $S1P_3$ receptors began to haemorrhage around E13.5, whereas free red blood cells and oedema were evident in subcutaneous areas. The dorsal aortas of

Table 1 Roles for $S1P_2$ receptor in development and disease of the cardiovascular system

Model system	Function and pathophysiology
Miles-apart gene mutations (Zebrafish)	Cardiac defects (cardia bifida)[85]
$S1p1^{-/-}S1p2^{-/-}$ mice	Haemorrhage at E11.5, capillary network underdeveloped in mouse embryo[87]
$S1p2^{-/-}S1p3^{-/-}$ mice	Haemorrhage at E13.5, red blood cells and oedema in subcutaneous areas, endothelial cells with thin cell body[87]
$S1p1^{-/-}S1p2^{-/-}S1p3^{-/-}$ mice	Haemorrhage at E10.5, vascular remodelling defects in the embryo head[87]
$S1p2^{-/-}$ mice	Hearing loss, vascular remodelling defects in Stria vascularis structure of the inner ear[87]
$S1p2^{-/-}$ mice	Increased regional blood flow, decreased vascular resistance[99]
$S1p2^{-/-}$ mice	Enhanced revascularization of the hypoxic mouse retina[97]
$S1p2^{-/-}$ mice	Increased neointimal lesions development in mouse artery[102]
$S1p2^{-/-}S1p3^{-/-}$ mice	Increased infarct size upon myocardial ischaemia–reperfusion injury[100]
JTE-013 antagonist	Increased angiogenesis in Matrigel mouse implants[96]
JTE-013 antagonist	Inhibition of H_2O_2-induced lung oedema[73]

S1p2/*S1p3* double-null embryos appeared to be covered normally by vascular smooth muscle cells, suggesting that the $S1P_2$ receptor is dispensable for vascular maturation, unlike the $S1P_1$ receptor that is essential for mural–endothelial cell interaction and consequent vascular stabilization.[79,80] Microscopic examination of the microvessels revealed that endothelial cells had abnormally thin and occasionally fractured cell bodies, whereas endothelial cell junctions appeared normal.[87] Finally, embryos lacking all three receptors die between E10.5 and E11.5 due to abnormal bleeding and severe vascular remodelling defects in the head.[87] These observations suggest that S1P receptor signalling and function is redundant since $S1P_1$ and $S1P_3$ receptors may play a compensatory role when the $S1P_2$ receptor is dysfunctional. Clearly, $S1P_2$ receptor diverse signalling and regulation of cytoskeletal dynamics greatly contributes to the establishment and maintenance of a mature vascular system during mouse embryonic development.

Although *S1p2* null adult mice do not present any major abnormalities, the litter sizes produced are decreased, suggesting that females that are null for the $S1P_2$ receptor could have fertility defects that compromise their ability to mate or reproduce.[54,87] Interestingly, $S1P_1$ and $S1P_2$ receptors are expressed in decidual endothelial cells, suggesting a role for the receptors in uterine mesometrial angiogenesis during the implantation phase of early gestation.[88] In addition, *S1p2* null mice in the C57/Bl6 genetic background exhibit spontaneous, sporadic, and occasionally fatal seizures between 3 and 7 weeks of age. Excitability of the neocortical pyramidal neurons was shown to be altered, whereas the molecular mechanism behind this phenomenon is unknown.[89] Moreover, *S1p2* null mice are profoundly deaf and show vestibular impairment due to multiple inner ear abnormalities. Mice deficient for both $S1P_2$ and $S1P_3$ receptors develop additional inner ear pathologies.[90–92] The $S1P_2$ receptor is highly expressed in the cochlea and loss of the gene leads to degeneration of sensory hair cells and the spiral ganglion neurons. Indeed, one of the very early events during the progress of the pathology is the formation of an abnormal capillary basal lamina in stria vascularis structure, with dilated and chaotically oriented microvessels. In addition, the $S1P_2$ receptor antagonist JTE-013 blocked the S1P-induced vasoconstriction of the spiral modiolar artery, which supplies blood directly to the stria vascularis.[90] However, it is worth mentioning that studies by Salomone *et al.*[93] indicate that the $S1P_2$ receptor antagonist JTE-013 does not appear to be highly selective in rodents. These reports suggest that the $S1P_2$ receptor is essential for mouse inner ear vascular structure maintenance and provide a means for S1P-related therapeutic application in degenerative and noise-induced hearing loss. Although the detailed cellular and molecular mechanisms that trigger these phenotypes are still underexplored, these studies indicate that the $S1P_2$ receptor positively regulates the formation and maintenance of the mouse vascular network during normal development.

4. Sphingosine-1-phosphate receptor 2 in cardiovascular pathology

It is well established that $S1P_1$ receptor is a major S1P receptor expressed on vascular endothelial and smooth muscle cells, whereas it regulates physiological as well as pathological effects on vascular homeostasis, which include endothelium vasorelaxation, smooth muscle contraction, enhancement of tumour angiogenesis, and blood vessel maturation during embryonic development.[79,81,94,95] In sharp contrast, the role of the $S1P_2$ receptor in vascular pathophysiology has just begun to be unravelled.

Indeed, $S1P_2$ receptor expression in vascular endothelial cells inhibits migration in a Rho GTPase-dependent manner, suggesting that the $S1P_2$ receptor could potentially play an antiangiogenic and/or angiostatic role during tumour formation and progression.[65] Furthermore, inhibition of the $S1P_2$ receptor by JTE-013 antagonist substantially augmented S1P stimulation of migration and tube-like morphogenesis of mouse vascular endothelial cells. In accordance with these *in vitro* findings, the $S1P_2$ receptor was expressed in vascularized Matrigel plugs *in vivo*, whereas inhibition of the receptor function greatly enhanced S1P-driven angiogenic processes in the Matrigel mouse implants.[96] These data are in apparent contradiction with the requirement for the $S1P_2$ receptor in mouse embryogenesis and inner ear development.[87,90] These findings suggest that $S1P_2$ may regulate the vasculature in a context-dependent manner.

We recently reported that activation of the $S1P_2$ receptor on endothelial cells results in disruption of VE-cadherin-based junctions and increased vascular paracellular permeability. In addition, JTE-013 treatment of primary endothelial cells enhanced cortical actin assembly and adherens junctions formation, improving S1P-driven endothelium barrier integrity.[73] Moreover, when rat lungs were perfused with the pharmacologic inhibitor JTE-013, H_2O_2-induced lung oedema was markedly inhibited, as measured by a substantial decrease in the rate of lung wet weight gain after H_2O_2 treatment. These *ex vivo* experiments indicate that blockade of $S1P_2$ receptor activation in endothelial cells under stress conditions could have applications in the treatment of pulmonary oedema or other permeability disorders linked to vascular injury. In the mouse model of ischaemia-driven retinal pathological angiogenesis, it is reported that $S1P_2$ receptor expression is induced in the course of the hypoxia-triggered vascular injury. Hypoxic mouse retinas that lack the $S1P_2$ receptor present significantly decreased inflammatory cell infiltration and substantially enhanced revascularization of the retina tissue, indicating that the $S1P_2$ receptor activates inflammatory pathways that facilitate vascular permeability and pathological angiogenesis under ischaemic conditions.[97] Mechanistically, expression of the receptor in vascular endothelial cells induces the expression of the pro-inflammatory molecule cyclooxygenase-2 and downregulates endothelial nitric oxide synthase with major implications in vascular inflammation and congestion. In agreement with this report, a recent study suggests that the humanized monoclonal antibody that selectively binds S1P significantly reduced macrophage influx into ischaemic retina and strongly suppressed oxygen-induced ischaemic retinopathy and choroidal neovascularization.[98] Although this result needs to be confirmed and the mechanism of inhibition of S1P action by the monoclonal antibody needs to be clarified, this intriguing report suggests the functional role of the $S1P_2$ receptor in pathologic vascular neovascularization. The idea that stress conditions such as hypoxia, oxidative stress, or

vascular injury could activate $S1P_2$ receptor-driven pro-inflammatory pathways and potentiate vascular permeability, pathological neo-angiogenesis, and finally vascular dysfunction remains to be addressed. Although significant progress is being made in dissecting $S1P_2$ receptor signalling in endothelial, smooth muscle cells, and cardiomyocytes, the fundamental questions about the factors that regulate expression and localization of the $S1P_2$ receptor have yet to be fully addressed.

A recent report implicates the $S1P_2$ receptor as an important mediator of normal vascular haemodynamics.[99] Although mice deficient for the $S1P_2$ receptor have no blood pressure abnormalities, loss of the receptor leads to significant elevation of regional blood flow and decrease in vascular resistance in response to α-adrenergic stimulation, which clearly indicates that the $S1P_2$ receptor plays an important physiological role in modulating vascular tone.[99] Indeed, $S1P_2$ receptor-driven vasoconstriction could explain the formation of greatly dilated capillaries in $S1P_2$ receptor-deficient stria vascularis structure of the inner ear as well as the improved vascular tone in $S1P_2$ receptor null ischaemic retinas.[90] In any event, it is apparent that $S1P_2$ receptor activation can strongly affect vascular homeostasis in different vascular beds through complex signalling pathways that involve cytoskeleton rearrangements and downstream gene regulation in both vascular smooth muscle and endothelial cells.

In a recent report, the role of S1P in cardiomyocyte survival following *in vivo* myocardial ischaemia–reperfusion (I/R) injury was examined. Indeed, infarct size following I/R was significantly increased only in mice deficient for both $S1P_2$ and $S1P_3$ receptors. In addition, activation of Akt in response to I/R was markedly attenuated in double-null mouse hearts, suggesting that $S1P_2$ and $S1P_3$ receptors together potentiate Akt activation, cardiomyocyte survival, and finally cardioprotection upon ischaemia.[100] A previous report showed that $S1P_2$ receptor activation inhibits Akt activation in endothelial cells via coupling to the PTEN phosphatase, indicating that $S1P_2$ receptor-mediated Akt inhibition depends on diverse subcellular mechanisms in different cell types.[65,70] Furthermore, in response to acute balloon injury of the rat carotid artery, $S1P_2$ receptor expression was increased at 7–10 days post-injury, whereas inhibition of the $S1P_2$ receptor with JTE-013 potentiated S1P-induced proliferation and reduced the expression of differentiation marker genes such as smooth muscle α-actin in rat aortic smooth muscle cells (SMCs).[101] More importantly, when neointimal lesion formation was induced in mice by ligation of the left carotid artery, large neointimal lesions developed in $S1P_2$ receptor-deficient mouse arteries.[102] In addition, $S1P_2$ receptor null arteries showed a significant increase in both medial and intimal SMC replication. Furthermore, S1P failed to increase Rho-GTPase activation in $S1P_2$ receptor-deficient SMCs, thus leading to significant increase in SMC migration.[102] These observations suggest that activation of the $S1P_2$ receptor suppresses SMC growth and migration in arteries, whereas these studies show strong evidence for the implication of the $S1P_2$ receptor in neointima formation and development of atherosclerotic disease. Therefore, it can be concluded that $S1P_2$ receptor activation may play an important role in the pathogenesis of various cardiovascular diseases.

5. Conclusions

Recent findings using genetic and pharmacological approaches have led to new knowledge of $S1P_2$ receptor function in development and pathology of the cardiovascular system. Indeed, $S1P_2$ receptor activation is required for proper establishment and maturation of the mouse embryonic vascular system and the structure of the stria vascularis in the inner ear. More importantly, considerable progress has been made in uncovering the functional role of the receptor in vascular injury, from myocardial remodelling and neointima formation to vascular permeability, pathological neovascularization, and tumour angiogenesis. However, our current understanding of $S1P_2$ receptor regulation and complex downstream pathway activation in the vasculature remains rather preliminary—detailed mechanistic insights are necessary to better assess whether and how regulation of the receptor could become a potential novel therapeutic approach for cardiovascular disease.

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