

DEVELOPMENT AT A GLANCE

Sphingosine 1-phosphate signalling

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ABSTRACT

Sphingosine 1-phosphate (S1P) is a lipid mediator formed by the metabolism of sphingomyelin. In vertebrates, S1P is secreted into the extracellular environment and signals via G protein-coupled S1P receptors to regulate cell-cell and cell-matrix adhesion, and thereby influence cell migration, differentiation and survival. The expression and localization of S1P receptors is dynamically regulated and controls vascular development, vessel stability and immune cell trafficking. In addition, crucial events during embryogenesis, such as angiogenesis, cardiogenesis, limb development and neurogenesis, are regulated by S1P signalling. Here, and in the accompanying poster, we provide an overview of S1P signalling in development and in disease.

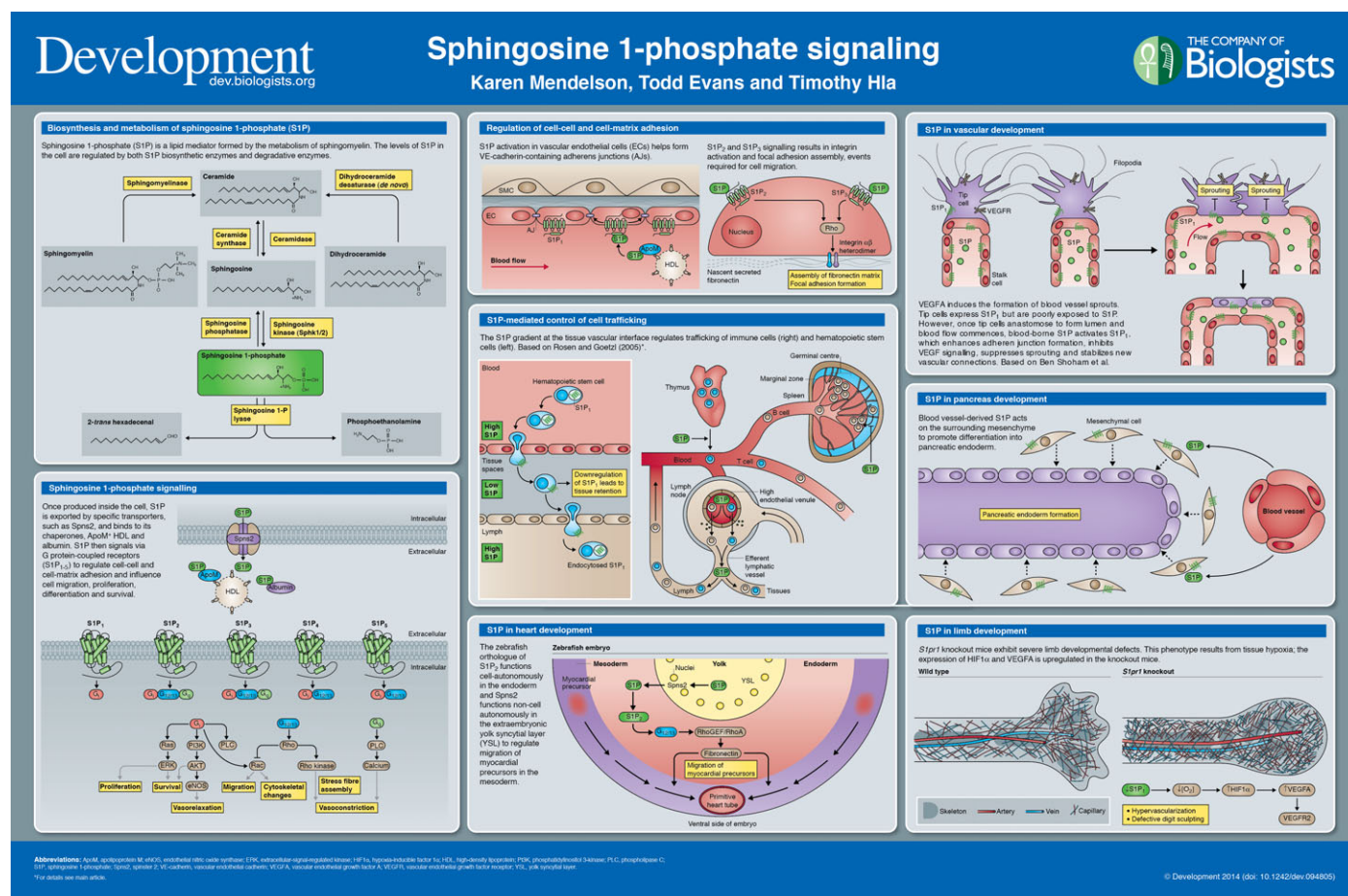
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Introduction

Sphingolipids are crucial for membrane structure and function in eukaryotes. Their metabolism leads to the formation of polar lipid mediators such as sphingosine 1-phosphate (S1P) (Saba and Hla, 2004). In vertebrates, S1P is found in the extracellular milieu and interacts with cell-surface receptors to regulate an array of cellular responses, including cell migration, differentiation and survival (Blaho and Hla, 2011; Chun et al., 2002). As such, S1P plays fundamental roles in morphogenetic mechanisms, such as collective cell migration, tissue inductive events and biomechanical signalling. In line with this, recent studies have shown that S1P signalling plays crucial roles in development, for example during angiogenesis (Gaengel et al., 2012), cardiogenesis (Kupperman et al., 2000), limb development (Chae et al., 2004a) and neurogenesis (Mizugishi et al., 2005). In this article, we summarize what is known about S1P synthesis, export, gradients, receptors and signalling. We also discuss the known roles of S1P (Ben Shoham et al., 2012) in development and explore the potential mechanisms by which this lipid mediator acts.



Synthesis and export of S1P

Sphingolipid turnover, which is initiated by the action of sphingomyelinase, leads to the formation of ceramide, which is further processed to sphingosine by the enzyme ceramidase. Sphingosine is then phosphorylated by the sphingosine kinase (Sphk) isoenzymes (Spiegel and Milstien, 2007), giving rise to S1P. The levels of S1P in the cell are regulated not only by S1P biosynthetic enzymes but also by degradative enzymes such as S1P phosphatases and S1P lyase (Blaho and Hla, 2011; Le Stunff et al., 2004; Saba and Hla, 2004). Once produced inside the cell, S1P is exported by specific transporters, such as spinster 2 (Spns2) (Kawahara et al., 2009; Osborne et al., 2008), which was recently shown to be involved in establishing a vascular S1P gradient in mice (Fukuhara et al., 2012). Other transporters, which remain uncharacterized, as well as secretion of the Sphk1 enzyme followed by extracellular biosynthesis (Hla et al., 2008), also contribute to the enrichment of S1P in the plasma of vertebrates (Venkataraman et al., 2006). Blood plasma thus contains high levels of S1P (~1 µM), whereas interstitial fluid levels of S1P are low, giving rise to a gradient of this lipid mediator (Cyster and Schwab, 2012), which plays an important role in driving S1P function.

In the plasma, S1P is bound to its chaperones, apolipoprotein M-containing high-density lipoprotein (ApoM⁺ HDL) and albumin (Christoffersen et al., 2011). Extracellular S1P can also be degraded by lysophospholipid phosphatase 3 (LPP3) into sphingosine, which can then be taken up by cells for further metabolism (Escalante-Alcalde et al., 2003; Renault et al., 2010).

S1P signalling via G protein-coupled receptors

S1P receptors are G protein-coupled receptors (GPCRs) that are closely related to lysophosphatidic acid and cannabinoid receptors. Five S1P receptors, termed S1P₁₋₅, have been described, and most cells express one or more subtypes of S1P receptor (Chun et al., 2010). All bind to S1P with high affinity and induce cellular responses.

S1P receptors exhibit unique as well as common G-protein-coupling properties. For example, S1P₁ couples exclusively to the G_{i/o} family, whereas S1P₂ is capable of coupling to G_{i/o}, to G_{12/13} as well as to the G_q family (Windh et al., 1999). This coupling results in the activation of small GTPases such as Rho, Rac and Ras (Lee et al., 2001; Ryu et al., 2002; Sugimoto et al., 2003). Further downstream effectors of S1P receptors include adenylate cyclase, PI-3-kinase, phospholipase C, protein kinase C and intracellular calcium. The transcriptional responses regulated by S1P are not well understood; however, recent work shows that Rho GTPase-coupled S1P receptors can activate the Hippo signalling pathway and its associated transcriptional effectors (Yu et al., 2012). S1P has also been suggested to function through alternative GPCR-independent pathways, such as histone deacetylases (Hait et al., 2009), TRAF2 (Alvarez et al., 2010) and prohibitin 2 (Strub et al., 2011).

The regulation of cell-cell and cell-matrix adhesion by S1P

Signalling via S1P receptors has been shown to regulate cell-cell and cell-matrix adhesive events. For example, S1P₁ receptor activation in vascular endothelial cells results in the formation of VE-cadherin-containing adherens junctions (Lee et al., 1999). In addition, heterotypic cell-cell adhesion between endothelial cells and pericytes is induced by endothelial S1P₁ signalling, owing to the activation of N-cadherin-dependent junctions (Paik et al., 2004). In addition, S1P₁ and S1P₃ receptor-dependent signalling in vascular endothelial cells results in integrin activation and focal contact assembly, events that are required for cell migration (Paik et al.,

2001). By contrast, S1P₂ receptor activation results in Rho, Rho kinase and PTEN signalling, which disrupts adherens junctions (Sanchez et al., 2005). This receptor also inhibits cell migration induced by growth factors, such as platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF), and chemokines, such as stromal-derived factor 1 (SDF1, CXCL12), C5a and CCL2 (Michaud et al., 2010; Sugimoto et al., 2003).

The effects of S1P on cell-cell and cell-matrix adhesion require dynamic changes in the actin- and microtubule-based cytoskeleton. S1P₁ induces cortical actin assembly via Rac GTPase, whereas S1P₂ regulates stress fibre assembly via Rho GTPase (Lee et al., 2001; Ryu et al., 2002; Sugimoto et al., 2003). In addition, microtubule dynamics, as revealed by imaging of the microtubule-binding protein EB1, indicates that S1P receptor activation in endothelial cells induces Rac-dependent polymerization of microtubules at the cell cortex (Paik et al., 2004). Thus, ligand-dependent activation of S1P receptors transmits a variety of intracellular signals depending on the receptor expression in a particular cell.

S1P-mediated control of cell trafficking in and out of the vascular system

The S1P gradient at the tissue-vascular interface regulates the trafficking of immune cells from lymphoid organs into the circulation (Cyster and Schwab, 2012; Pappu et al., 2007). Further analysis suggests that the cell-surface lipid phosphate phosphatase 3 (LPP3), which can degrade S1P into sphingosine, is also a major regulator of lymphocyte egress (Bréart et al., 2011). Furthermore, the S1P transporter Spns2, which is expressed primarily on lymphatic endothelial cells, is also required for efficient egress (Fukuhara et al., 2012). Sphingosine kinases in lymphatic endothelial cells are also important (Pham et al., 2010). These findings support a model whereby the uptake and metabolism of S1P from the circulation by endothelial cells, followed by polarized secretion of S1P by Spns2 across the gradient may be involved in lymphocyte trafficking. The S1P₁ receptor on the cell surface of lymphocytes is crucial for this egress (Cyster and Schwab, 2012). Antagonistic functions of retention receptors (such as the chemokine receptor CCR7) and egress-promoting receptors (S1P₁) have been shown to determine the overall migration patterns of hematopoietic cells (Pham et al., 2008). Thus, antagonism of chemokine gradients (with chemokines tending to be deposited in the tissues) with the S1P gradient (with S1P enriched in the vascular compartment) may be a general phenomenon to regulate the trafficking of hematopoietic cells (Massberg et al., 2007; Cyster and Schwab, 2012) in and out of the closed vascular system of vertebrates.

S1P signalling in endoderm and heart development

Studies in zebrafish have uncovered a key role for *s1pr2* and the S1P transporter protein *spns2* in regulating cell migration during heart development. A single missense mutation in the zebrafish orthologue of *s1pr2* causes the mutant phenotype known as miles apart (*mil*). These *mil* mutants display cardia bifida due to the failure of cardiac precursor cells to properly migrate from the anterior lateral plate mesoderm to the midline (Kupferman et al., 2000). Furthermore, two separate recessive mutations in *spns2*, known as two of hearts (*toh*) and *ko157*, phenocopy the *mil* cardiac defects (Kawahara et al., 2009; Osborne et al., 2008). *mil* functions cell autonomously in the endoderm, whereas *spns2* functions non-cell autonomously in the extraembryonic yolk syncytial layer (YSL), suggesting that S1P export from the YSL activates *s1pr2* in the endoderm to regulate migration of cardiac progenitors in the associated mesoderm.

Both *mil* and *toh* mutants also exhibit defects in the morphogenesis of the anterior endoderm. The anterior endoderm of wild-type embryos at 18 hpf forms a contiguous sheet, whereas *mil* and *toh* mutants have discontinuities in their endodermal sheet (Osborne et al., 2008). The requirement of endoderm to facilitate precardiac mesoderm migration suggests that the cardia bifida observed in *mil* and *toh* mutants results from these endodermal defects (Osborne et al., 2008). More recently, the downstream mechanism by which *s1pr2/mil* controls myocardial migration has been elucidated; *s1pr2* coupling to G₁₃ and subsequent activation of RhoGEF was shown to regulate endodermal convergence to coordinate myocardial migration (Ye and Lin, 2013). The ability of the S1P₂/G₁₃/Rho pathway to activate cell-surface integrins and fibronectin matrix assembly may be important in the endoderm to provide the correct matricellular cues for myocardial precursor cell migration towards the midline (Zhang et al., 1999).

S1P signalling has also been implicated in migration of the prechordal plate, a thickened mesodermal structure that is derived from mesendodermal cells that migrate along the midline between the ectoderm and endoderm. These cells also form the notochord. During gastrulation, cells forming the prechordal plate undergo directed migration as a coordinated cluster. Using a morpholino-based screen, *mil* suppressed defective anterior migration of the prechordal plate in *wnt11* mutant embryos, which otherwise show a reduction in E-cadherin mediated coherence of cell movement (Kai et al., 2008). In line with this role, embryos overexpressing *mil* display defects in convergence and extension movements during gastrulation, a process that simultaneously narrows the germ layers mediolaterally and elongates the embryo from head to tail (Kai et al., 2008). These studies highlight the role for S1P in mediating cell-cell cohesion during development.

S1P receptors regulate collective behaviour of endothelial cells during vascular sprouting

In mouse and zebrafish models, multiple S1P receptors (S1P₁₋₃) coordinately regulate vascular development (Kono et al., 2004; Obinata and Hla, 2012). Recent evidence, however, points towards an important role for *S1pr1* in maintaining flow-dependent vascular network stability. Global deletion of *S1pr1* causes intrauterine lethality at E12.5–14.5 due to severe haemorrhaging resulting from defective vascular maturation (Liu et al., 2000). The genetic deletion of *S1pr1* in endothelial cells in mice (*S1pr1* ECKO) results in abnormal patterning of the primary vascular plexus (Jung et al., 2012). An increased number of filopodia-bearing tip cells, branch points and dilated vessels were observed in the retinæ of these mice. Endothelial hypersprouting following *S1pr1* deletion has also been described in other vascular beds, including those of the embryonic hindbrain, neural tube, aorta and developing limbs of mice (Gaengel et al., 2012; Ben Shoham et al., 2012), and the caudal vein plexus and hindbrain vessels of zebrafish (Gaengel et al., 2012; Mendelson et al., 2013; Ben Shoham et al., 2012).

One hallmark of collective cell migration is the requirement for cell-cell cohesion, which is mediated by adherens junction proteins such as VE-cadherin in the endothelium. *S1pr1* ECKO retinal vessels display poor blood flow and vascular leakage, which induces hypoxia and vascular endothelial growth factor A (VEGFA) expression leading to increased endothelial cell sprouting (Jung et al., 2012). This vascular leakage is likely due to junction instability. Mice lacking VE-cadherin in their endothelial cells display a retinal angiogenic hypersprouting phenotype similar to that of the *S1pr1* ECKO mice (Gaengel et al., 2012). Morpholino-based studies in zebrafish have substantiated the link between *s1pr1* and VE-

cadherin signalling in regulating vascular sprouting, showing that knockdown of either *s1pr1* or *cdh5* results in similar phenotypes. Furthermore, *s1pr1* regulates VEGF signalling in human umbilical vein endothelial cells (HUVECs) *in vitro*, and in mice and zebrafish *in vivo* (Gaengel et al., 2012; Ben Shoham et al., 2012). In the developing mouse forelimb, knockout of *Vegfa* leads to a decrease in blood vessel density, whereas knockout of *S1pr1* causes increased blood vessel density (Ben Shoham et al., 2012). In developing zebrafish, *vegfa* is known to regulate intersegmental vessel sprouting and treatment of *s1pr1* morphant embryos with a chemical inhibitor against *vegfa* was shown to inhibit intersegmental vessel sprouting (Ben Shoham et al., 2012). Finally, it was shown that *S1pr1* regulates sprouting angiogenesis to prevent excessive vascular sprouting through stabilization of VE-cadherin at the cell junctions and through inhibition of VEGFR2 phosphorylation and signalling (Ben Shoham et al., 2012). *S1pr1* was also shown to be required for endothelial shear stress signalling. To preserve vascular stability, *S1pr1* can be activated in both a ligand-dependent manner and by biomechanical forces in a ligand-independent manner (Jung et al., 2012).

Vascular-dependent tissue inductive effects of S1P

S1P-derived from blood is known to regulate inductive interactions during embryonic dorsal pancreas development (Edsbacke et al., 2005). In this system, vascular-derived S1P acts on mesenchyme and/or endothelium to modulate committed endodermal cells to differentiate into pancreatic tissue. This inductive effect of S1P is independent of tissue oxygenation provided by functional vasculature. In addition, pancreatic endocrine precursor cell migration and clustering is regulated by S1P receptor signalling during both mouse and zebrafish development (Serafimidis et al., 2011). This suggests that S1P/S1P receptor signalling regulates complex tissue inductive events in development.

In addition, S1P plays a crucial role in normal limb development. *S1pr1* knockout mice exhibit several limb developmental effects such as dysmorphic limb skeletal patterning, hypervascularization and defective sculpting of digits (Chae et al., 2004a). Both global knockout and endothelial-specific knockout mice exhibit a similar phenotype. HIF1 α and VEGFA expression were upregulated in these mutants, suggesting that lack of proper oxygenation results in this phenotype. Indeed, deletion of the *Vegfr2* receptor reversed hypervascularization (Ben Shoham et al., 2012), suggesting that S1P signalling in endothelial cells can influence limb development via HIF1 α - and VEGF-dependent mechanisms.

S1P signalling in neurogenesis

S1P receptors, particularly *S1pr1*, are enriched in neuronal tissue (Chae et al., 2004b). Mice lacking *Sphk* isoenzymes exhibit severely disturbed neurogenesis. Mutant embryos at E12.5 display cell loss in the forebrain, increased numbers of apoptotic cells in the neuroepithelium of the telencephalon and diencephalon, and decreased numbers of mitotic cells in the telencephalon (Mizugishi et al., 2005). S1P treatment of neonatal brains resulted in cellular movements leading to the formation of neural folds such as sulci and gyri (Kingsbury et al., 2003; Yung et al., 2011). Furthermore, S1P signalling has been implicated in neural progenitor cell recruitment (Callihan and Hooks, 2012). In addition, widespread expression of multiple S1P receptor subtypes in neural cells, as well as the efficacy of the recently launched S1P receptor-targeted drug – Fingolimod – in not only reducing neuroinflammation but also preventing brain volume loss in humans (Kappos et al., 2010), suggests an important role for the S1P pathway in neurogenesis.

S1P and disease

Widespread expression of S1P receptors and the abundance of the ligand S1P in the circulatory systems of mammals imply its broad role in the regulation of many organ systems. However, S1P signalling was only recently therapeutically targeted in the treatment of relapsing, relapsing multiple sclerosis. Indeed, the sphingosine analogue Fingolimod, also known as FTY720, has been approved by the US Food and Drug Administration for treatment of multiple sclerosis (Cohen et al., 2010; Kappos et al., 2010). FTY720 is phosphorylated by sphingosine kinase 2 to FTY720-P, which acts initially as an agonist of *S1pr1*, *S1pr3*, *S1pr4* and *S1pr5* (Brinkmann et al., 2002; Mandala et al., 2002). However, ultimately FTY720-P acts as a functional antagonist, as binding of FTY720-P to *S1pr1* causes downregulation and degradation of the receptor and inhibits lymphocyte egress from lymphoid organs (LaMontagne et al., 2006; Oo et al., 2007). Thus, inhibition of lymphocyte trafficking into the CNS was thought to be one of the major mechanisms by which this compound achieves therapeutic efficacy. However, some adverse events associated with this compound could be due to interference with S1P signalling in other organ systems (Oo et al., 2011). Given the success of Fingolimod, numerous trials are ongoing to test additional S1P receptor modulators in various human diseases in which S1P signalling is dysregulated.

Perspectives

S1P signalling affects the collective behaviour of cells by regulating cell-matrix and cell-cell adhesion, as well as alterations to the cytoskeleton. In addition, biomechanical forces may play a crucial role in regulating S1P-dependent developmental events. For example, in the vascular system, shear stress-mediated signalling appears to cooperate with S1P ligand-dependent signalling events. It is also likely that biomechanical forces exerted by the endoderm during the collective behaviour of cells, for example, during convergence and extension movements, are involved in subsequent tissue morphogenetic events, such as heart development. It is anticipated that further developmental studies will reveal novel components of S1P signalling, as well as the fundamental importance of this lipid mediator signalling system in embryogenesis. As this signalling system is involved in human diseases, and given that therapeutic modulation of S1P receptors has just begun in the treatment of neuroinflammatory diseases, developmental insights may be useful in further application to human therapeutics.

Competing interests

The authors declare no competing financial interests.

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References

- Alvarez, S. E., Harikumar, K. B., Hait, N. C., Allegood, J., Strub, G. M., Kim, E. Y., Maceyka, M., Jiang, H., Luo, C., Kordula, T. et al. (2010). Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* **465**, 1084-1088.
- Ben Shoham, A., Malkinson, G., Krief, S., Schwartz, Y., Ely, Y., Ferrara, N., Yaniv, K. and Zelzer, E. (2012). S1P1 inhibits sprouting angiogenesis during vascular development. *Development* **139**, 3859-3869.

- Blahe, V. A. and Hla, T. (2011). Regulation of mammalian physiology, development, and disease by the sphingosine 1-phosphate and lysophosphatidic acid receptors. *Chem. Rev.* **111**, 6299-6320.
- Bréart, B., Ramos-Perez, W. D., Mendoza, A., Salous, A. K., Gobert, M., Huang, Y., Adams, R. H., Lafaille, J. J., Escalante-Alcalde, D., Morris, A. J. et al. (2011). Lipid phosphate phosphatase 3 enables efficient thymic egress. *J. Exp. Med.* **208**, 1267-1278.
- Brinkmann, V., Davis, M. D., Heise, C. E., Albert, R., Cottens, S., Hof, R., Bruns, C., Prieschl, E., Baumruker, T., Hiestand, P. et al. (2002). The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J. Biol. Chem.* **277**, 21453-21457.
- Callihan, P. and Hooks, S. B. (2012). Sphingosine-1-phosphate signaling in neural progenitors. *Methods Mol. Biol.* **874**, 193-200.
- Chae, S. S., Paik, J. H., Allende, M. L., Proia, R. L. and Hla, T. (2004a). Regulation of limb development by the sphingosine 1-phosphate receptor S1p1/EDG-1 occurs via the hypoxia/VEGF axis. *Dev. Biol.* **268**, 441-447.
- Chae, S. S., Proia, R. L. and Hla, T. (2004b). Constitutive expression of the S1P1 receptor in adult tissues. *Prostaglandins Other Lipid Mediat.* **73**, 141-150.
- Christoffersen, C., Obinata, H., Kumaraswamy, S. B., Galvani, S., Ahnström, J., Sevana, M., Egerer-Sieber, C., Müller, Y. A., Hla, T., Nielsen, L. B. et al. (2011). Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. *Proc. Natl. Acad. Sci. USA* **108**, 9613-9618.
- Chun, J., Goetzl, E. J., Hla, T., Igarashi, Y., Lynch, K. R., Moolenaar, W., Pyne, S. and Tigyi, G. (2002). International Union of Pharmacology. XXXIV. Lysophospholipid receptor nomenclature. *Pharmacol. Rev.* **54**, 265-269.
- Chun, J., Hla, T., Lynch, K. R., Spiegel, S. and Moolenaar, W. H. (2010). International Union of Basic and Clinical Pharmacology. LXXVIII. Lysophospholipid receptor nomenclature. *Pharmacol. Rev.* **62**, 579-587.
- Cohen, J. A., Barkhof, F., Comi, G., Hartung, H. P., Khatri, B. O., Montalban, X., Pelletier, J., Capra, R., Gallo, P., Izquierdo, G. et al.; TRANSFORMS Study Group (2010). Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N. Engl. J. Med.* **362**, 402-415.
- Cyster, J. G. and Schwab, S. R. (2012). Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu. Rev. Immunol.* **30**, 69-94.
- Edsagge, J., Johansson, J. K., Esni, F., Luo, Y., Radice, G. L. and Semb, H. (2005). Vascular function and sphingosine-1-phosphate regulate development of the dorsal pancreatic mesenchyme. *Development* **132**, 1085-1092.
- Escalante-Alcalde, D., Hernandez, L., Le Stunff, H., Maeda, R., Lee, H. S., Jr-Gang-Cheng, Sciorra, V. A., Daar, I., Spiegel, S., Morris, A. J. et al. (2003). The lipid phosphatase LPP3 regulates extra-embryonic vasculogenesis and axis patterning. *Development* **130**, 4623-4637.
- Fukuhara, S., Simmons, S., Kawamura, S., Inoue, A., Orba, Y., Tokudome, T., Sundén, Y., Arai, Y., Moriwaki, K., Ishida, J. et al. (2012). The sphingosine-1-phosphate transporter Spns2 expressed on endothelial cells regulates lymphocyte trafficking in mice. *J. Clin. Invest.* **122**, 1416-1426.
- Gaengel, K., Naudet, C., Hagikura, K., Laviña, B., Muhl, L., Hofmann, J. J., Ebarasi, L., Nyström, S., Rymo, S., Chen, L. L. et al. (2012). The sphingosine-1-phosphate receptor S1PR1 restricts sprouting angiogenesis by regulating the interplay between VE-cadherin and VEGFR2. *Dev. Cell* **23**, 587-599.
- Hait, N. C., Allegood, J., Maceyka, M., Strub, G. M., Harikumar, K. B., Singh, S. K., Luo, C., Marmorstein, R., Kordula, T., Milstien, S. et al. (2009). Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science* **325**, 1254-1257.
- Hla, T., Venkataraman, K. and Michaud, J. (2008). The vascular S1P gradient-cellular sources and biological significance. *Biochim. Biophys. Acta* **1781**, 477-482.
- Jung, B., Obinata, H., Galvani, S., Mendelson, K., Ding, B. S., Skoura, A., Kinzel, B., Brinkmann, V., Rafii, S., Evans, T. et al. (2012). Flow-regulated endothelial S1P receptor-1 signaling sustains vascular development. *Dev. Cell* **23**, 600-610.
- Kai, M., Heisenberg, C. P. and Tada, M. (2008). Sphingosine-1-phosphate receptors regulate individual cell behaviours underlying the directed migration of prechordal plate progenitor cells during zebrafish gastrulation. *Development* **135**, 3043-3051.
- Kappos, L., Radue, E. W., O'Connor, P., Polman, C., Hohlfeld, R., Calabresi, P., Selmaj, K., Agoropoulou, C., Leyk, M., Zhang-Auberson, L. et al.; FREEDOMS Study Group (2010). A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N. Engl. J. Med.* **362**, 387-401.
- Kawahara, A., Nishi, T., Hisano, Y., Fukui, H., Yamaguchi, A. and Mochizuki, N. (2009). The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. *Science* **323**, 524-527.
- Kingsbury, M. A., Rehen, S. K., Contos, J. J., Higgins, C. M. and Chun, J. (2003). Non-proliferative effects of lysophosphatidic acid enhance cortical growth and folding. *Nat. Neurosci.* **6**, 1292-1299.
- Kono, M., Mi, Y., Liu, Y., Sasaki, T., Allende, M. L., Wu, Y. P., Yamashita, T. and Proia, R. L. (2004). The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis. *J. Biol. Chem.* **279**, 29367-29373.
- Kupperman, E., An, S., Osborne, N., Waldron, S. and Stainier, D. Y. (2000). A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature* **406**, 192-195.
- LaMontagne, K., Littlewood-Evans, A., Schnell, C., O'Reilly, T., Wyder, L., Sanchez, T., Probst, B., Butler, J., Wood, A., Liao, G. et al. (2006). Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. *Cancer Res.* **66**, 221-231.
- Le Stunff, H., Milstien, S. and Spiegel, S. (2004). Generation and metabolism of bioactive sphingosine-1-phosphate. *J. Cell. Biochem.* **92**, 882-899.

- Lee, M. J., Thangada, S., Claffey, K. P., Ancellin, N., Liu, C. H., Kluk, M., Volpi, M., Sha'afi, R. I. and Hla, T. (1999). Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. *Cell* **99**, 301-312.
- Lee, M. J., Thangada, S., Paik, J. H., Sapkota, G. P., Ancellin, N., Chae, S. S., Wu, M., Morales-Ruiz, M., Sessa, W. C., Alessi, D. R. et al. (2001). Akt-mediated phosphorylation of the G protein-coupled receptor EDG-1 is required for endothelial cell chemotaxis. *Mol. Cell* **8**, 693-704.
- Liu, Y., Wada, R., Yamashita, T., Mi, Y., Deng, C. X., Hobson, J. P., Rosenfeldt, H. M., Nava, V. E., Chae, S. S., Lee, M. J. et al. (2000). Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J. Clin. Invest.* **106**, 951-961.
- Mandala, S., Hajdu, R., Bergstrom, J., Quackenbush, E., Xie, J., Milligan, J., Thornton, R., Shei, G. J., Card, D., Keohane, C. et al. (2002). Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* **296**, 346-349.
- Massberg, S., Schaerli, P., Knezevic-Maramica, I., Köllnberger, M., Tubo, N., Moseman, E. A., Huff, I. V., Junt, T., Wagers, A. J., Mazo, I. B. et al. (2007). Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell* **131**, 994-1008.
- Mendelson, K., Zygmunt, T., Torres-Vázquez, J., Evans, T. and Hla, T. (2013). Sphingosine 1-phosphate receptor signaling regulates proper embryonic vascular patterning. *J. Biol. Chem.* **288**, 2143-2156.
- Michaud, J., Im, D. S. and Hla, T. (2010). Inhibitory role of sphingosine 1-phosphate receptor 2 in macrophage recruitment during inflammation. *J. Immunol.* **184**, 1475-1483.
- Mizugishi, K., Yamashita, T., Olivera, A., Miller, G. F., Spiegel, S. and Proia, R. L. (2005). Essential role for sphingosine kinases in neural and vascular development. *Mol. Cell. Biol.* **25**, 11113-11121.
- Obinata, H. and Hla, T. (2012). Sphingosine 1-phosphate in coagulation and inflammation. *Semin. Immunopathol.* **34**, 73-91.
- Oo, M. L., Thangada, S., Wu, M. T., Liu, C. H., Macdonald, T. L., Lynch, K. R., Lin, C. Y. and Hla, T. (2007). Immunosuppressive and anti-angiogenic sphingosine 1-phosphate receptor-1 agonists induce ubiquitinylation and proteasomal degradation of the receptor. *J. Biol. Chem.* **282**, 9082-9089.
- Oo, M. L., Chang, S. H., Thangada, S., Wu, M. T., Rezau, K., Blaho, V., Hwang, S. I., Han, D. K. and Hla, T. (2011). Engagement of S1P₁-degradative mechanisms leads to vascular leak in mice. *J. Clin. Invest.* **121**, 2290-2300.
- Osborne, N., Brand-Arzamendi, K., Ober, E. A., Jin, S. W., Verkade, H., Holtzman, N. G., Yelon, D. and Stainier, D. Y. (2008). The spinster homolog, two of hearts, is required for sphingosine 1-phosphate signaling in zebrafish. *Curr. Biol.* **18**, 1882-1888.
- Paik, J. H., Chae Ss, Lee, M. J., Thangada, S. and Hla, T. (2001). Sphingosine 1-phosphate-induced endothelial cell migration requires the expression of EDG-1 and EDG-3 receptors and Rho-dependent activation of alpha vbeta3- and beta1-containing integrins. *J. Biol. Chem.* **276**, 11830-11837.
- Paik, J. H., Skoura, A., Chae, S. S., Cowan, A. E., Han, D. K., Proia, R. L. and Hla, T. (2004). Sphingosine 1-phosphate receptor regulation of N-cadherin mediates vascular stabilization. *Genes Dev.* **18**, 2392-2403.
- Pappu, R., Schwab, S. R., Cornelissen, I., Pereira, J. P., Regard, J. B., Xu, Y., Camerer, E., Zheng, Y. W., Huang, Y., Cyster, J. G. et al. (2007). Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* **316**, 295-298.
- Pham, T. H., Okada, T., Matloubian, M., Lo, C. G. and Cyster, J. G. (2008). S1P₁ receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity* **28**, 122-133.
- Pham, T. H., Baluk, P., Xu, Y., Grigorova, I., Bankovich, A. J., Pappu, R., Coughlin, S. R., McDonald, D. M., Schwab, S. R. and Cyster, J. G. (2010). Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J. Exp. Med.* **207**, 17-27.
- Renault, A. D., Kunwar, P. S. and Lehmann, R. (2010). Lipid phosphate phosphatase activity regulates dispersal and bilateral sorting of embryonic germ cells in *Drosophila*. *Development* **137**, 1815-1823.
- Rosen, H. and Goetzl, E. J. (2005). Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network. *Nat. Rev. Immunol.* **7**, 560-570.
- Ryu, Y., Takuwa, N., Sugimoto, N., Sakurada, S., Usui, S., Okamoto, H., Matsui, O. and Takuwa, Y. (2002). Sphingosine-1-phosphate, a platelet-derived lysophospholipid mediator, negatively regulates cellular Rac activity and cell migration in vascular smooth muscle cells. *Circ. Res.* **90**, 325-332.
- Saba, J. D. and Hla, T. (2004). Point-counterpoint of sphingosine 1-phosphate metabolism. *Circ. Res.* **94**, 724-734.
- Sanchez, T., Thangada, S., Wu, M. T., Kontos, C. D., Wu, D., Wu, H. and Hla, T. (2005). PTEN as an effector in the signaling of antimigratory G protein-coupled receptor. *Proc. Natl. Acad. Sci. USA* **102**, 4312-4317.
- Serafimidis, I., Heximer, S., Beis, D. and Gavalas, A. (2011). G protein-coupled receptor signaling and sphingosine-1-phosphate play a phylogenetically conserved role in endocrine pancreas morphogenesis. *Mol. Cell. Biol.* **31**, 4442-4453.
- Spiegel, S. and Milstien, S. (2007). Functions of the multifaceted family of sphingosine kinases and some close relatives. *J. Biol. Chem.* **282**, 2125-2129.
- Strub, G. M., Paillard, M., Liang, J., Gomez, L., Allegood, J. C., Hait, N. C., Maceyka, M., Price, M. M., Chen, Q., Simpson, D. C. et al. (2011). Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. *FASEB J.* **25**, 600-612.
- Sugimoto, N., Takuwa, N., Okamoto, H., Sakurada, S. and Takuwa, Y. (2003). Inhibitory and stimulatory regulation of Rac and cell motility by the G12/13-Rho and Gi pathways integrated downstream of a single G protein-coupled sphingosine-1-phosphate receptor isoform. *Mol. Cell. Biol.* **23**, 1534-1545.
- Venkataraman, K., Thangada, S., Michaud, J., Oo, M. L., Ai, Y., Lee, Y. M., Wu, M., Parikh, N. S., Khan, F., Proia, R. L. et al. (2006). Extracellular export of sphingosine kinase-1a contributes to the vascular S1P gradient. *Biochem. J.* **397**, 461-471.
- Windh, R. T., Lee, M. J., Hla, T., An, S., Barr, A. J. and Manning, D. R. (1999). Differential coupling of the sphingosine 1-phosphate receptors Edg-1, Edg-3, and H218/Edg-5 to the G(i), G(q), and G(12) families of heterotrimeric G proteins. *J. Biol. Chem.* **274**, 27351-27358.
- Ye, D. and Lin, F. (2013). S1pr2/Ga13 signaling controls myocardial migration by regulating endoderm convergence. *Development* **140**, 789-799.
- Yu, F. X., Zhao, B., Panupinthu, N., Jewell, J. L., Lian, I., Wang, L. H., Zhao, J., Yuan, H., Tumaneng, K., Li, H. et al. (2012). Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. *Cell* **150**, 780-791.
- Yung, Y. C., Mutoh, T., Lin, M. E., Noguchi, K., Rivera, R. R., Choi, J. W., Kingsbury, M. A. and Chun, J. (2011). Lysophosphatidic acid signaling may initiate fetal hydrocephalus. *Sci. Transl. Med.* **3**, 99ra87.
- Zhang, Q., Peyruchaud, O., French, K. J., Magnusson, M. K. and Mosher, D. F. (1999). Sphingosine 1-phosphate stimulates fibronectin matrix assembly through a Rho-dependent signal pathway. *Blood* **93**, 2984-2990.