

Sphingosine-1-phosphate: the Swiss army knife of sphingolipid signaling

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Abstract The sphingolipid metabolite sphingosine-1-phosphate (S1P) and the kinases that produce it have emerged as critical regulators of numerous fundamental biological processes important for health and disease. Activation of sphingosine kinases (SphKs) by a variety of agonists increases intracellular S1P, which in turn can be secreted out of the cell and bind to and signal through S1P receptors (S1PRs) in an autocrine and/or paracrine manner. Recent studies suggest that this “inside-out” signaling by S1P may play a role in many human diseases. As the roles of the S1PRs in cell and organismal physiology are discussed elsewhere in this volume, we focus this review mainly on recent reports showing how SphKs are activated and S1P reaches its receptors, the role of SphKs and S1P in regulating sphingolipid homeostasis, and the potential importance of the SphK/S1P axis as a therapeutic target in human diseases.—Maceyka, M., S. Milstien, and S. Spiegel. Sphingosine-1-phosphate: the Swiss army knife of sphingolipid signaling. *J. Lipid Res.* 2009. 50: S272–S276.

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Sphingolipids are ubiquitous components of all membranes in eukaryotic cells. Analogous to the lipid signaling molecules derived from metabolism of glycerolipids, sphingolipids produce bioactive sphingolipid metabolites such as sphingosine, sphingosine-1-phosphate (S1P), ceramide, and ceramide-1-phosphate that have key roles in regulating many important physiological and pathological functions (1). Signal-induced activation of several types of sphingomyelinases generates ceramide in a variety of compartments within the cell. Deacylation of ceramide by ceramidases yields sphingosine, the most common sphingoid base in mammals. Sphingoid bases can be recycled into complex sphingolipids or phosphorylated by one of two

sphingosine kinase isozymes (SphK1 and SphK2) to form S1P. There are two pathways of S1P degradation: reversible dephosphorylation to sphingosine by nonspecific phosphatases, including lysosomal and lipid-specific phosphatases, and by two S1P-specific phosphatases, SPP1 and SPP2; and irreversible cleavage by S1P lyase (SPL), which can lead to the formation of phosphatidylethanolamine. The latter is the only pathway for degradation of sphingoid bases in mammalian cells.

Ceramide, sphingosine, and S1P are readily interconvertible, which is of great significance not only because they are potent signaling molecules, but they also regulate cell growth and survival in different manners. Ceramide and sphingosine are important regulatory components of stress responses, typically inducing growth arrest and apoptosis (1). Conversely, S1P inhibits apoptosis and promotes proliferation (2). Thus, the dynamic balance between S1P and its precursors, ceramide and sphingosine, and their consequent regulation of opposing signaling pathways is an important factor that determines cell fate (3). Although many of the effects of S1P result from its action as a ligand for a family of five G protein-coupled receptors, denoted S1P₁₋₅, there is some evidence indicating that S1P can also act through still not yet well-characterized intracellular targets (2).

COULD S1P BE A CENTRAL REGULATOR OF SPHINGOLIPID METABOLISM?

Many studies have demonstrated an inverse correlation between SphK1 activity and ceramide levels. A potential mechanism for the association of SphK1 activity and ceramide is provided by the observation that overexpression of SphK1 also increased dihydrosphingosine (sphinganine) and dihydro-S1P, suggesting that SphK1-generated S1P may negatively regulate CerS(s) activity, leading to a buildup of

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Abbreviations: S1P, sphingosine-1-phosphate; S1PR, S1P receptor; SphK, sphingosine kinase.

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its substrate, dihydrosphingosine (4). Indeed, it was recently shown that S1P inhibits CerS2 activity *in vitro* by interacting with two residues that are part of an S1PR-like motif found only in CerS2 and not in other CerS (5). However, CerS2 does not efficiently utilize C16-ceramide as a substrate, and it is predominantly this ceramide species that is most reduced when SphK1 is overexpressed (4).

Several recent reports have examined the effect of specifically targeting SphK1 and production of S1P in cancer cells on ceramide-mediated apoptosis induced by anti-cancer drugs. Treatment of human K562 chronic myeloid leukemia cells with the Bcr-Abl tyrosine kinase inhibitor imatinib induced apoptosis mediated by increased ceramide, mainly C18-ceramide, which is generated by CerS1 (6). There were no changes in expression or activity of CerS1 in imatinib-resistant K562 cells. Rather, there were significant increases in expression of SphK1 and generation of S1P. Similarly, SphK1 activity was markedly depressed in imatinib-sensitive LAMA84 chronic myeloid leukemia cells concomitant with increased ceramide and decreased S1P. Overcoming imatinib resistance by combination therapy also decreased SphK1 activity in these cells (7). Several SphK inhibitors have been shown to reduce tumorigenesis in xenograft models (8, 9). Because of the different actions of SphK1 and SphK2 (see later discussion), a specific inhibitor of SphK1 should be useful for cancer therapeutics, yet most of the SphK inhibitors identified so far, including F-12509a and B-5354c isolated from marine bacteria and chemical inhibitors identified by medium throughput screening, inhibit SphK1 and SphK2. However, we recently described a water-soluble and potent isozyme-specific SphK1 inhibitor, SK1-I, that markedly decreased S1P levels while increasing ceramide levels in U937 and Jurkat human leukemia cells (10). SK1-I markedly reduced growth of U937 acute myelocytic leukemia xenograft tumors and potently induced apoptosis of leukemic blasts isolated from patients with acute myelogenous leukemia but did not significantly affect normal peripheral blood mononuclear leukocytes (10). This study provides proof of concept that a specific SphK1 inhibitor might be a useful new adjunct to the current therapeutic armamentarium for human cancer.

OPPOSING ROLES OF SPHK1 AND SPHK2 IN SPHINGOLIPID METABOLISM?

In contrast to SphK1 and consistent with its ability to enhance apoptosis, SphK2 expression increases proapoptotic ceramide levels (4). Recent studies have provided a plausible explanation for how S1P produced from SphK2, but not from SphK1, can be converted to ceramide. An unusual pathway in mammalian cells for the salvage of sphingosine back into ceramide has been uncovered that requires its phosphorylation by SphK2 (but not SphK1) and then dephosphorylation by SPP1 (but not SPP2) before it can be reacylated to ceramide by CerS (11). Similarly, renal mesangial cells isolated from SphK2-, but not SphK1-knockout mice, had increased levels of sphingosine (12).

This seemingly futile cycle has previously been demonstrated to occur in yeast (13), suggesting that this is an evolutionarily conserved pathway.

ACTIVATION OF SPHKS

In the decade since their initial characterization and cloning, much has been learned about the two ubiquitously expressed SphK isoenzymes, SphK1 and SphK2. The necessity for expression of two similar enzymes that catalyze the phosphorylation of sphingosine to produce the same product, S1P has now been clarified by the demonstration that mice with knockouts of either SphK1 or SphK2 have nearly normal S1P levels and no severe phenotypes, while the double SphK1/SphK2 knockout is embryonically lethal due to severe effects on brain and cardiovascular development and embryos are devoid of S1P (14). Thus, production of S1P is vital for life and SphK1 and SphK2 can have compensatory, overlapping, or even different physiological functions, depending on the cell or tissue where they are expressed.

While numerous stimuli, including growth factors, hormones, chemokines, and cytokines, have been shown to activate SphK1, much less is still known about SphK2. In many cases, activation of SphK1 is biphasic with a rapid increase in enzymatic activity within a few minutes followed by an increase in SphK1 protein levels over the next 24–48 h. As purified and recombinant SphKs are highly active, access to their lipophilic and membrane-associated substrate sphingosine is likely an important *in vivo* regulatory mechanism. Indeed, Pitson et al. (15) have shown that SphK1 is phosphorylated on Ser225 (human SphK1) by ERK1/2, which is necessary for its activation and translocation to the plasma membrane, perhaps by exposing a putative binding site for the membrane lipid phosphatidylserine (16). Subsequently, SphK1 was found to have a calmodulin binding site between residues 191–206, which was necessary for agonist-induced translocation to the plasma membrane but not required for activation (17). Recently, it was shown that protein phosphatase 2A negatively correlated with Ser225 phosphorylation and SphK1 membrane localization, suggesting that it may regulate SphK1 translocation (18). SphK1 has also been shown to interact with numerous other proteins that may contribute to its translocation and regulation.

The first clues to the mechanism of agonist-mediated SphK2 activation were provided by the observations that EGF and an activator of protein kinase C induce rapid phosphorylation of SphK2, which was markedly reduced by inhibition of MEK1/ERK pathway (19). Moreover, down-regulation of ERK1 expression prevented EGF-induced phosphorylation of SphK2 and inhibited migration of MDA-MB-453 human breast-cancer cells toward EGF (19). To date, there are only a few examples of cells in which both SphK1 and SphK2 are activated in response to agonist stimulation. In rodent mast cells, crosslinking of FcεRI, the high-affinity receptor for IgE, stimulates SphK1 and SphK2, increasing production and secretion of S1P

(20). Recent results suggest that differential formation of SIP by SphK1 and SphK2 has distinct and important actions in mast cells (21, 22).

“INSIDE-OUT” SIGNALING TO S1PRS

There is no doubt that the most important actions of SIP are mediated by binding to its specific receptors. For example, SIP₁ is required for trafficking of lymphocytes and hematopoietic progenitors through blood, lymph, and peripheral tissues (23, 24) and also is critical for angiogenesis and vascular maturation. SIP₂ is required for development of vestibular and hearing functions (25). More details on specific functions of S1PRs will be reviewed extensively elsewhere in this volume.

However, as mentioned above, many cells that produce SIP by activation of SphK1 also secrete it, which can then activate receptors present on neighboring cells (paracrine) or the same cell (autocrine). In this inside-out signaling, activation of S1PRs is required for complete agonist actions. This was first suggested by the observation that spatially restricted formation of SIP and activation of cell surface SIP₁ receptors were essential for PDGF-directed cell movement (26). This paradigm has now been extended to many other growth factors, hormones, and cytokines, and only a few recent examples are discussed here. For example, SphK1 and activation of SIP₃ or SIP₁ are required for migratory responses of cancer cells toward another serum-borne lysophospholipid, lysophosphatidic acid (27), and toward heregulin (28), respectively. This is consistent with the notion that activation of SIP₁ or SIP₃, in contrast to SIP₂, promotes directed cell movement. Recently, we demonstrated that filamin A, an actin-cross-linking protein involved in cell movement, is a bona fide SphK1-interacting protein that is required for heregulin-induced activation of SphK1, migration, lamellipodia formation, activation of PAK1, and subsequent filamin A phosphorylation (28). Moreover, filamin A links SphK1 and production of SIP and SIP₁ (but not SIP₂) with PAK1 to locally influence the dynamics of the actin cytoskeletal at lamellipodia that promote cell movement (28).

eNOS activation and NO production induced by TNF- α also occurs through SIP generation and stimulation of its receptors leading to activation of Akt to confer cytoprotection from excitotoxic and neurotoxic agents (29). Intriguingly, lipopolysaccharide and thrombin, known to increase mouse lung microvascular permeability, also induced a delayed activation of SphK1 that was coupled to activation of SIP₁ to reverse microvessel leakiness (30). Interestingly, the SphK1/SIP/SIP₃ axis is a downstream component of protease activated receptor-1 (PAR1) signaling that regulates amplification of inflammation in sepsis syndrome. Importantly, inhibition of dendritic cell PAR1/SphK1/SIP/SIP₃ signaling in the lymphatics could attenuate systemic inflammation by containing inflammation to draining lymph nodes downstream of severe inflammation or infection (31).

A critical question that arises from the many examples of inside-out signaling is, what is the mechanism for export

of SIP from cells? A few studies have begun to provide evidence for the involvement of ATP-binding cassette (ABC) family of transporters. Release of SIP from mast cells activated by Fc ϵ R1 crosslinking was mediated by ABCB1 and not by ABCB1 (32). Similarly, it was suggested that thrombin-stimulated SIP secretion from platelets is also mediated by an ABC transporter (33, 34), and in astrocytes, the ABCA1 transporter is critical for release of SIP (35). Taken together, these studies suggest that members of the large family of ABC transporters might be important for export of SIP out of cells.

THE SIP GRADIENT

Mammals maintain an SIP gradient between blood, lymph, and tissues: SIP levels in plasma are near μ M levels, lymph fluid levels are 4- to 5-fold lower, while interstitial fluid levels are roughly 1,000-fold less (36). This gradient is of substantial importance for the trafficking and maturation of hematopoietic cells, including T-, B-, and dendritic cells, as well as regulation of vascular permeability and tone. Evidence from knockout mice has shown that SphK1 and SphK2 contribute to the plasma levels of SIP, though SphK1 plays a larger role (21). Platelets were originally suggested to be the major source of SIP in plasma, as these cells release large amounts of SIP upon activation. However, it was recently shown that depletion of platelets in mice by genetic or pharmacologic means had only a small effect on circulating plasma levels (36, 37). Using an elegant conditional gene deletion approach, Pappu et al. (36) were able to specifically ablate SphK1 and SphK2 in hematopoietic cells, vascular endothelium, and liver. Intriguingly, these mice survived into adulthood even though they had no detectable SIP in either plasma or lymph. Lethal irradiation followed by wild-type bone-marrow reconstitution restored normal SIP levels in plasma, but not in lymph. Conversely, plasma SIP levels in lethally irradiated wild-type mice were not restored upon bone-marrow reconstitution with SphK-deficient bone marrow, although lymph levels remained unaffected. Moreover, the plasma levels of SIP in SphK-deficient mice could be restored by transfusion of an equivalent of 20% wild-type erythrocytes, suggesting that these cells might be the main source of circulating SIP. Thus, the authors concluded that plasma and lymph SIP levels are maintained by different cell types, with circulating SIP being mainly contributed by erythrocytes (36). Indeed, another study demonstrated that SIP is released from isolated erythrocytes, but not platelets or lymphocytes, in a plasma-dependent manner (38). It is still not clear whether erythrocytes produce SIP or just store and release it. Moreover, studies with ABC receptor knockout mice showed that neither ABCB1, ABCA1, nor ABCA7 contribute to plasma SIP levels (39). Nevertheless, these studies are consistent with recent findings in humans that plasma SIP levels positively correlate with erythrocyte levels (40). It has also recently been suggested that the vascular endothelium, in addition to the hematopoietic system, may also contribute to plasma SIP (37) and that ABCA1

and ABCG1 might be involved in SIP release from endothelial cells (39). Thus, it is likely that multiple sources contribute to maintaining the high levels of SIP in blood. Moreover, these levels of SIP can change in pathological conditions and diseases, such as anaphylaxis (21), stress (41), and inflammation-induced cancer (42).

ROLES OF SIP IN HEALTH AND DISEASE

Many of the initial studies on the roles of SIP in disease focused on the growth promoting and angiogenic properties of SIP in cancer. This area of research has grown with the observations that SphK1 expression is up-regulated in many types of cancers. With the discovery of FTY720, a potent immunosuppressive prodrug that is phosphorylated in vivo to a SIP mimetic, there has been an explosion of research on SIP regulation of immune cell trafficking and the role of S1PRs in immunosuppression, transplantation, multiple sclerosis, atherosclerosis, allergy, autoimmunity, and anaphylaxis. There is also some evidence for SphK1-dependent stimulation of cyclooxygenase-2 expression in late pregnancy and for physiological relevance of SphK activation and SIP release on uterine smooth muscle during gestation (43).

Recent studies with animal models of human diseases have begun to utilize SphK1 knockout mice to confirm the importance of SphK1. Treatment of mice with dextran sulfate sodium produces a model of colitis or inflammatory bowel disease. Previous studies implicated SphK1 and SIP production in implicated in induction of cyclooxygenase-2 by TNF- α (44), important components of inflammatory pathways. SphK1^{-/-} mice treated with dextran sulfate sodium had significantly less blood loss, weight loss, and colon abnormalities than wild-type mice and had no systemic inflammatory responses (45). These findings suggest that inhibition of SphK1 might be a novel means not only to target tumorigenesis but also to decrease systemic and local inflammation in inflammatory bowel disease.

While SIP is known to play an important role in the lung in stabilizing interendothelial junctions to prevent microvessel leakiness (46), whether this SIP is produced by SphK1, SphK2, or both was not known. However, it was recently demonstrated that treatment of mice with lipopolysaccharide increased lung microvascular permeability followed by activation of SphK1 that was coupled to restoration of normal permeability (30). Lipopolysaccharide markedly enhanced pulmonary edema in SphK2 knockout mice, compared with wild-type mice. These results together with earlier studies also demonstrated that the SIP₁ receptor was required for normal endothelial barrier function and indicate a crucial role for SIP inside-out signaling in the regulation of endothelial barrier homeostasis (30).

An emerging new area of interest is the role of SphK1 and SIP in virology. Human cytomegalovirus exploits a variety of cellular signaling pathways to promote its own replication. Human cytomegalovirus infection increased SphK1 expression and activity and also increased levels of dihydro-SIP and ceramide. Both de novo ceramide

synthesis and SphK1 were necessary for virus gene expression and virus growth, suggesting that host cell sphingolipids are dynamically regulated upon herpes virus infection in a manner that impacts virus replication (47). ■

REFERENCES

1. Hannun, Y. A., and L. M. Obeid. 2008. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* **9**: 139–150.
2. Spiegel, S., and S. Milstien. 2003. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev. Mol. Cell Biol.* **4**: 397–407.
3. Cuvillier, O., G. Pirianov, B. Kleuser, P. G. Vanek, O. A. Coso, S. Gutkind, and S. Spiegel. 1996. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature*. **381**: 800–803.
4. Maceyka, M., H. Sankala, N. C. Hait, H. Le Stunff, H. Liu, R. Toman, C. Collier, M. Zhang, L. Satin, A. H. Merrill, Jr., et al. 2005. Sphk1 and Sphk2: sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism. *J. Biol. Chem.* **280**: 37118–37129.
5. Laviad, E. L., L. Albee, I. Pankova-Kholmyansky, S. Epstein, H. Park, A. H. Merrill, Jr., and A. H. Futerman. 2008. Characterization of ceramide synthase 2: tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate. *J. Biol. Chem.* **283**: 5677–5684.
6. Baran, Y., A. Salas, C. E. Senkal, U. Gunduz, J. Bielawski, L. M. Obeid, and B. Ogretmen. 2007. Alterations of ceramide/sphingosine 1-phosphate rheostat involved in the regulation of resistance to imatinib-induced apoptosis in K562 human chronic myeloid leukemia cells. *J. Biol. Chem.* **282**: 10922–10934.
7. Bonhoure, E., A. Lauret, D. J. Barnes, C. Martin, B. Malavaud, T. Kohama, J. V. Melo, and O. Cuvillier. 2008. Sphingosine kinase-1 is a downstream regulator of imatinib-induced apoptosis in chronic myeloid leukemia cells. *Leukemia*. **22**: 971–979.
8. French, K. J., R. S. Schrecengost, B. D. Lee, Y. Zhuang, S. N. Smith, J. L. Eberly, J. K. Yun, and C. D. Smith. 2003. Discovery and evaluation of inhibitors of human sphingosine kinase. *Cancer Res.* **63**: 5962–5969.
9. Pchejetski, D., N. Doumerc, M. Golzio, M. Naymark, J. Teissie, T. Kohama, J. Waxman, B. Malavaud, and O. Cuvillier. 2008. Chemosensitizing effects of sphingosine kinase-1 inhibition in prostate cancer cell and animal models. *Mol. Cancer Ther.* **7**: 1836–1845.
10. Paugh, S. W., B. S. Paugh, M. Rahmani, D. Kapitonov, J. A. Almenara, T. Kordula, S. Milstien, J. K. Adams, R. E. Zipkin, S. Grant, et al. 2008. A selective sphingosine kinase 1 inhibitor integrates multiple molecular therapeutic targets in human leukemia. *Blood*. **112**: 1382–1391.
11. Le Stunff, H., P. Giussani, M. Maceyka, S. Lepine, S. Milstien, and S. Spiegel. 2007. Recycling of sphingosine is regulated by the concerted actions of sphingosine-1-phosphate phosphohydrolase 1 and sphingosine kinase 2. *J. Biol. Chem.* **282**: 34373–34380.
12. Hofmann, L. P., S. Ren, S. Schwalm, J. Pfeilschifter, and A. Huwiler. 2008. Sphingosine kinase 1 and 2 regulate the capacity of mesangial cells to resist apoptotic stimuli in an opposing manner. *Biol. Chem.* **389**: 1399–1407.
13. Funato, K., R. Lombardi, B. Vallée, and H. Riezman. 2003. Lcb4p is a key regulator of ceramide synthesis from exogenous long chain sphingoid base in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **278**: 7325–7334.
14. Mizugishi, K., T. Yamashita, A. Olivera, G. F. Miller, S. Spiegel, and R. L. Proia. 2005. Essential role for sphingosine kinases in neural and vascular development. *Mol. Cell Biol.* **25**: 11113–11121.
15. Pitson, S. M., P. Xia, T. M. Leclercq, P. A. Moretti, J. R. Zebol, H. E. Lynn, B. W. Wattenberg, and M. A. Vadas. 2005. Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling. *J. Exp. Med.* **201**: 49–54.
16. Stahelin, R. V., J. H. Hwang, J. H. Kim, Z. Y. Park, K. R. Johnson, L. M. Obeid, and W. Cho. 2005. The mechanism of membrane targeting of human sphingosine kinase 1. *J. Biol. Chem.* **280**: 43030–43038.
17. Sutherland, C. M., P. A. Moretti, N. M. Hewitt, C. J. Bagley, M. A. Vadas, and S. M. Pitson. 2006. The calmodulin-binding site of sphingosine kinase and its role in agonist-dependent translocation of sphingosine kinase 1 to the plasma membrane. *J. Biol. Chem.* **281**: 11693–11701.
18. Barr, R. K., H. E. Lynn, P. A. Moretti, Y. Khew-Goodall, and S. M. Pitson. 2008. Deactivation of sphingosine kinase 1 by protein phosphatase 2A. *J. Biol. Chem.* **283**: 34994–35002.

19. Hait, N. C., A. Bellamy, S. Milstien, T. Kordula, and S. Spiegel. 2007. Sphingosine kinase type 2 activation by ERK-mediated phosphorylation. *J. Biol. Chem.* **282**: 12058–12065.
20. Olivera, A., N. Urtz, K. Mizugishi, Y. Yamashita, A. M. Gilfillan, Y. Furumoto, H. Gu, R. L. Proia, T. Baumruker, and J. Rivera. 2006. IgE-dependent activation of sphingosine kinases 1 and 2 and secretion of sphingosine 1-phosphate requires Fyn kinase and contributes to mast cell responses. *J. Biol. Chem.* **281**: 2515–2525.
21. Olivera, A., K. Mizugishi, A. Tikhonova, L. Ciaccia, S. Odom, R. L. Proia, and J. Rivera. 2007. The sphingosine kinase-sphingosine-1-phosphate axis is a determinant of mast cell function and anaphylaxis. *Immunity.* **26**: 287–297.
22. Oskeritzian, C. A., S. E. Alvarez, N. C. Hait, M. M. Price, S. Milstien, and S. Spiegel. 2008. Distinct roles of sphingosine kinases 1 and 2 in human mast-cell functions. *Blood.* **111**: 4193–4200.
23. Schwab, S. R., and J. G. Cyster. 2007. Finding a way out: lymphocyte egress from lymphoid organs. *Nat. Immunol.* **8**: 1295–1301.
24. Massberg, S., P. Schaerli, I. Knezevic-Maramica, M. Kollnberger, N. Tubo, E. A. Moseman, I. V. Huff, T. Junt, A. J. Wagers, I. B. Mazo, et al. 2007. Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell.* **131**: 994–1008.
25. Herr, D. R., N. Grillet, M. Schwander, R. Rivera, U. Muller, and J. Chun. 2007. Sphingosine 1-phosphate (S1P) signaling is required for maintenance of hair cells mainly via activation of S1P2. *J. Neurosci.* **27**: 1474–1478.
26. Hobson, J. P., H. M. Rosenfeldt, L. S. Barak, A. Olivera, S. Poulton, M. G. Caron, S. Milstien, and S. Spiegel. 2001. Role of the sphingosine-1-phosphate receptor EDG-1 in PDGF-induced cell motility. *Science.* **291**: 1800–1803.
27. Shida, D., X. Fang, T. Kordula, K. Takabe, S. Lepine, S. E. Alvarez, S. Milstien, and S. Spiegel. 2008. Cross-talk between LPA1 and epidermal growth factor receptors mediates up-regulation of sphingosine kinase 1 to promote gastric cancer cell motility and invasion. *Cancer Res.* **68**: 6569–6577.
28. Maceyka, M., S. E. Alvarez, S. Milstien, and S. Spiegel. 2008. Filamin A links sphingosine kinase 1 and sphingosine-1-phosphate receptor 1 at lamellipodia to orchestrate cell migration. *Mol. Cell. Biol.* **28**: 5687–5697.
29. De Palma, C., S. Falcone, C. Panzeri, S. Radice, M. T. Bassi, and E. Clementi. 2008. Endothelial nitric oxide synthase overexpression by neuronal cells in neurodegeneration: a link between inflammation and neuroprotection. *J. Neurochem.* **106**: 193–204.
30. Tauseef, M., V. Kini, N. Knezevic, M. Brannan, R. Ramchandaran, H. Fyrst, J. Saba, S. M. Vogel, A. B. Malik, and D. Mehta. 2008. Activation of sphingosine kinase-1 reverses the increase in lung vascular permeability through sphingosine-1-phosphate receptor signaling in endothelial cells. *Circ. Res.* **103**: 1164–1172.
31. Niessen, F., F. Schaffner, C. Furlan-Freguia, R. Pawlinski, G. Bhattacharjee, J. Chun, C. K. Derian, P. Andrade-Gordon, H. Rosen, and W. Ruf. 2008. Dendritic cell PAR1–S1P3 signalling couples coagulation and inflammation. *Nature.* **452**: 654–658.
32. Mitra, P., C. A. Oskeritzian, S. G. Payne, M. A. Beaven, S. Milstien, and S. Spiegel. 2006. Role of ABCCL1 in export of sphingosine-1-phosphate from mast cells. *Proc. Natl. Acad. Sci. USA.* **103**: 16394–16399.
33. Kobayashi, N., T. Nishi, T. Hirata, A. Kihara, T. Sano, Y. Igarashi, and A. Yamaguchi. 2006. Sphingosine 1-phosphate is released from the cytosol of rat platelets in a carrier-mediated manner. *J. Lipid Res.* **47**: 614–621.
34. Anada, Y., Y. Igarashi, and A. Kihara. 2007. The immunomodulator FTY720 is phosphorylated and released from platelets. *Eur. J. Pharmacol.* **568**: 106–111.
35. Sato, K., E. Malchinkhuu, Y. Horiuchi, C. Mogi, H. Tomura, M. Tosaka, Y. Yoshimoto, A. Kuwabara, and F. Okajima. 2007. Critical role of ABCA1 transporter in sphingosine 1-phosphate release from astrocytes. *J. Neurochem.* **103**: 2610–2619.
36. Pappu, R., S. R. Schwab, I. Cornelissen, J. P. Pereira, J. B. Regard, Y. Xu, E. Camerer, Y. W. Zheng, Y. Huang, J. G. Cyster, et al. 2007. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science.* **316**: 295–298.
37. Venkataraman, K., Y. M. Lee, J. Michaud, S. Thangada, Y. Ai, H. L. Bonkovsky, N. S. Parikh, C. Habrukowich, and T. Hla. 2008. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ. Res.* **102**: 669–676.
38. Hanel, P., P. Andreani, and M. H. Graler. 2007. Erythrocytes store and release sphingosine 1-phosphate in blood. *FASEB J.* **21**: 1202–1209.
39. Lee, Y. M., K. Venkataraman, S. I. Hwang, D. K. Han, and T. Hla. 2007. A novel method to quantify sphingosine 1-phosphate by immobilized metal affinity chromatography (IMAC). *Prostaglandins Other Lipid Mediat.* **84**: 154–162.
40. Ohkawa, R., K. Nakamura, S. Okubo, S. Hosogaya, Y. Ozaki, M. Tozuka, N. Osima, H. Yokota, H. Ikeda, and Y. Yatomi. 2008. Plasma sphingosine-1-phosphate measurement in healthy subjects: close correlation with red blood cell parameters. *Ann. Clin. Biochem.* **45**: 356–363.
41. Jang, S., S. H. Suh, H. S. Yoo, Y. M. Lee, and S. Oh. 2008. Changes in iNOS, GFAP and NR1 expression in various brain regions and elevation of sphingosine-1-phosphate in serum after immobilized stress. *Neurochem. Res.* **33**: 842–851.
42. Kawamori, T., T. Kaneshiro, M. Okumura, S. Maalouf, A. Uflacker, J. Bielawski, Y. A. Hannun, and L. M. Obeid. 2009. Role for sphingosine kinase 1 in colon carcinogenesis. *FASEB J.* **23**: 405–414.
43. Serrano-Sanchez, M., Z. Tanfin, and D. Leiber. 2008. Signaling pathways involved in sphingosine kinase activation and sphingosine-1-phosphate release in rat myometrium in late pregnancy: role in the induction of cyclooxygenase 2. *Endocrinology.* **149**: 4669–4679.
44. Pettus, B. J., J. Bielawski, A. M. Porcelli, D. L. Reames, K. R. Johnson, J. Morrow, C. E. Chalfant, L. M. Obeid, and Y. A. Hannun. 2003. The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-alpha. *FASEB J.* **17**: 1411–1421.
45. Snider, A. J., T. Kawamori, S. G. Bradshaw, K. A. Orr, G. S. Gilkeson, Y. A. Hannun, and L. M. Obeid. 2009. A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. *FASEB J.* **23**: 143–152.
46. McVerry, B. J., and J. G. Garcia. 2005. In vitro and in vivo modulation of vascular barrier integrity by sphingosine 1-phosphate: mechanistic insights. *Cell. Signal.* **17**: 131–139.
47. Machesky, N. J., G. Zhang, B. Raghavan, P. Zimmerman, S. L. Kelly, A. H. Merrill, Jr., W. J. Waldman, J. R. Van Brocklyn, and J. Trgovcich. 2008. Human cytomegalovirus regulates bioactive sphingolipids. *J. Biol. Chem.* **283**: 26148–26160.