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

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Abstract The sphingolipid metabolite sphingosine-1-phosphate (SIP) 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 SIP, which in turn can be secreted out of the cell and bind to and signal through SIP receptors (SIPRs) in an autocrine and/or paracrine manner. Recent studies suggest that this “inside-out” signaling by SIP may play a role in many human diseases. As the roles of the SIPRs 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 SIP reaches its receptors, the role of SphKs and SIP in regulating sphingolipid homeostasis, and the potential importance of the SphK/SIP 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 (SIP), 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 SIP. There are two pathways of SIP degradation: reversible dephosphorylation to sphingosine by nonspecific phosphatases, including lysosomal and lipid-specific phosphatases, and by two SIP-specific phosphatases, SPP1 and SPP2; and irreversible cleavage by SIP hydrolase (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 SIP 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, SIP inhibits apoptosis and promotes proliferation (2). Thus, the dynamic balance between SIP 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 SIP result from its action as a ligand for a family of five G protein-coupled receptors, denoted SIP1-5, there is some evidence indicating that SIP can also act through still not yet well-characterized intracellular targets (2).

COULD SIP 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-SIP, suggesting that SphK1-generated SIP may negatively regulate CerS(s) activity, leading to a buildup of...
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 anticancer 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 phosphatidyserine (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 FceRI, the high-affinity receptor for IgE, stimulates SphK1 and SphK2, increasing production and secretion of S1P.
of S1P from cells? A few studies have begun to provide evidence for the involvement of ATP-binding cassette (ABC) family of transporters. Release of S1P from mast cells activated by FceRI crosslinking was mediated by ABCG1 and not by ABCB1 (32). Similarly, it was suggested that thrombin-stimulated S1P secretion from platelets is also mediated by an ABC transporter (33, 34), and in astrocytes, the ABCA1 transporter is critical for release of S1P (35). Taken together, these studies suggest that members of the large family of ABC transporters might be important for export of S1P out of cells.

THE S1P GRADIENT

Mammals maintain an S1P gradient between blood, lymph, and tissues: S1P levels in plasma are near μM 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 S1P, though SphK1 plays a larger role (21). Platelets were originally suggested to be the major source of S1P in plasma, as these cells release large amounts of S1P 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 S1P in either plasma or lymph. Lethal irradiation followed by wild-type bone-marrow reconstitution restored normal S1P levels in plasma, but not in lymph. Conversely, plasma S1P 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 S1P 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 S1P. Thus, the authors concluded that plasma and lymph S1P levels are maintained by different cell types, with circulating S1P being mainly contributed by erythrocytes (36). Indeed, another study demonstrated that S1P is released from isolated erythrocytes, but not platelets or lymphocytes, in a plasma-dependent manner (38). It is still not clear whether erythrocytes produce S1P or just store and release it. Moreover, studies with ABC receptor knockout mice showed that neither ABCG1, ABCA1, nor ABCA7 contribute to plasma S1P levels (39). Nevertheless, these studies are consistent with recent findings in humans that plasma S1P 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 S1P (37) and that ABCA1
and ABCC1 might be involved in S1P release from endothelial cells (39). Thus, it is likely that multiple sources contribute to maintaining the high levels of S1P in blood. Moreover, these levels of S1P can change in pathological conditions and diseases, such as anaphylaxis (21), stress (41), and inflammation-induced cancer (42).

### ROLES OF S1P IN HEALTH AND DISEASE

Many of the initial studies on the roles of S1P in disease focused on the growth promoting and angiogenic properties of S1P 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 FYT720, a potent immunosuppressive prodrug that is phosphorylated in vivo to a S1P mimic, there has been an explosion of research on S1P 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 S1P 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 S1P 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 S1P is known to play an important role in the lung in stabilizing interendothelial junctions to prevent microvessel leakiness (46), whether this S1P 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 S1P1 receptor was required for normal endothelial barrier function and indicate a crucial role for S1P inside-out signaling in the regulation of endothelial barrier homeostasis (30).

An emerging new area of interest is the role of SphK1 and S1P 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-S1P 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).

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