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SPHINGOSINE-1-PHOSPHATE SIGNALING AND ITS ROLE IN DISEASE

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Abstract

The bioactive sphingolipid metabolite, sphingosine-1-phosphate (S1P), is now recognized as a critical regulator of many physiological and pathophysiological processes, including cancer, atherosclerosis, diabetes and osteoporosis. S1P is produced in cells by two sphingosine kinase isoenzymes, SphK1 and SphK2. Many cells secrete S1P, which can then act in an autocrine or paracrine manner. Most of the known actions of S1P are mediated by a family of five specific G protein-coupled receptors. More recently, it was shown that S1P also has important intracellular targets involved in inflammation, cancer and Alzheimer's disease. This suggests that S1P actions are much more complex than previously thought, with important ramifications for development of therapeutics. This review highlights recent advances in our understanding of mechanisms of action of S1P and its roles in disease.

S1P: a signaling molecule

Like glycerophospholipids, sphingolipids are ubiquitous components of mammalian membranes that are metabolized to form signaling molecules. It is now recognized that sphingolipid metabolites play important roles in regulation of many cellular processes important for health and disease. One of the most important of these metabolites is sphingosine-1-phosphate (S1P, Figure 1). It has been 20 years since the discovery that S1P is a signaling molecule that regulates cell growth¹ and suppresses apoptosis², suggesting that it may play a role in cancer. Later, it was shown that S1P acts through a family of cell surface receptors, subsequently shown to be critical for migration of immune cells throughout the body. Indeed, one of the hallmarks of S1P involvement in disease is its control of cell trafficking³. New tools, such as specific agonists and antagonists and the generation of targeted knockouts has led to a surge of interest in the role of S1P in numerous diseases. Remarkable progress has been made in understanding its mechanism of action, though many questions are still unanswered. In this review, we will discuss how S1P is generated, how it signals, and briefly summarize its involvement in several diseases.

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Localized production of S1P

S1P is formed by the phosphorylation of sphingosine – the backbone of sphingolipids – by two kinases, sphingosine kinase 1 and 2 (SphK1 and SphK2). S1P levels are tightly controlled both by the enzymes that produce its substrate sphingosine, by the SphKs themselves, and by the enzymes that degrade S1P, which include S1P lyase (SPL), two S1P-specific phosphatases, and three lipid phosphate phosphatases.

Numerous agonists activate SphK1, including growth factors, hormones, pro-inflammatory cytokines, lipopolysaccharide, ligation of the IgE and IgG receptors, and many GPCR ligands, and this activation is critical for their full actions. Many of these agonists induce extracellular signal regulated kinase (ERK)-dependent phosphorylation at serine 225 of SphK1, leading to its translocation from the cytosol to the plasma membrane where its substrate sphingosine resides⁴. The SphK1-S225A mutant acts like a dominant negative, even though it retains full kinase activity. Targeting this mutant to the plasma membrane reverses the inhibitory phenotype, however, and even transforms quiescent fibroblasts⁴. Translocation of SphK1 to the plasma membrane may also be mediated by interaction with other proteins. For example, it was recently shown that calcium and integrin-binding protein 1 bind SphK1 in a calcium-dependent manner resulting in its translocation to the plasma membrane through a calcium-myristoyl switch⁵. Several other proteins that interact with SphK1 also directly increase its activity⁴. One interesting recent example is elongation factor 1A (eEF1a). Although well known for its role in translation, eEF1a is a G protein that activates SphK1 only in its GDP-bound state⁶.

In contrast to SphK1, which is mainly localized in the cytosol, SphK2 is present in several intracellular compartments, depending on cell type. Its functions in these compartments have only recently begun to be elucidated. Consistent with its nuclear localization signal, SphK2 in the nucleus regulates gene transcription, at least in part by producing S1P, which acts as an endogenous inhibitor of histone deacetylases⁷. Although lacking an identifiable mitochondrial targeting signal, SphK2 is also present in mitochondria where it is required for correct assembly of the cytochrome oxidase complex⁸. SphK2 also is postulated to bind to phosphatidylinositol monophosphates through an N-terminal domain, targeting it to intracellular membranes⁹.

Compared to SphK1, much less is known about activation of SphK2. Both EGF and phorbol ester activate SphK2 through ERK1-dependent phosphorylation^{7, 10}, and phosphorylation by protein kinase D promotes its nuclear export¹¹. Hypoxia activates SphK2 in A549 lung cancer cells¹² and brain microvasculature¹³, producing S1P that protected against apoptosis and promoted ischemic tolerance, respectively.

In sum, the diverse compartment-specific localizations of the SphKs indicate that the specific microenvironment in which S1P is produced dictates its functions.

S1P acts extracellularly through cell surface receptors

There are five specific cell surface G protein-coupled receptors for S1P, termed S1PR1–5, all with low nM Kd's. Differential signaling induced by binding of S1P to these receptors is due to distinct, though sometimes overlapping, coupling to diverse heterotrimeric G proteins (Figure 1). These receptors have been implicated in a variety of developmental and disease-related processes. For example, S1PR1 activation plays a critical role in the trafficking of immune cells¹⁴. The pro-drug FTY720, approved by the FDA for treatment of the autoimmune disease multiple sclerosis¹⁵, is phosphorylated *in vivo* by SphK2 and induces downregulation and degradation of S1PR1, but not other S1PRs, on lymphocytes. Loss of S1PR1 prevents lymphocytes from sensing the S1P gradient from the lymphoid organs into

the blood, blocking egress and inducing lymphopenia¹⁶. The role of this S1P/S1PR axis in the trafficking of immune cells is becoming paradigmatic³, as it is also important for B cell migration through the lymph node follicle¹⁷. Furthermore, T-bet-dependent S1PR5 expression is required for natural killer T cell egress from lymph organs¹⁸, and S1PR4 plays a role in neutrophil trafficking¹⁹. S1P also may be important for stem cell recruitment, differentiation, and maintenance in a pluripotent state²⁰.

S1P/S1PR axes also are important in controlling vascular tone and permeability. S1P in the circulation regulates basal and inflammation-induced vascular leak via S1PR1²¹. TNF- α induces vasoconstriction of the spiral modiolar artery, the sole provider of blood to the cochlea, by activating SphK1 and producing S1P that stimulates S1PR2, whose downstream signaling leads to vasoconstriction²². This finding provides a link between inflammation- and vasculopathy-induced hearing loss and may explain why S1PR2 receptor null mice are deaf^{23, 24}.

These, together with many other studies, raise a broader issue with agonist-induced activation of SphK1 leading to S1PR signaling: SphK1 is a cytosolic enzyme, and yet the S1PRs bind S1P on the exoplasmic leaflet. How does the S1P get outside the cell? Several transporters are now implicated in exporting S1P, where it can locally activate S1PRs. These include the ATP binding cassette transporters ABCA1²⁵, ABCC1, and ABCG2^{26,27}, as well as the putative transporter, Spnster 2²⁸. This process – whereby agonists activate SphK1 and induce its recruitment to the plasma membrane, producing S1P that is then locally secreted to activate S1PRs – is called “inside-out signaling” and explains the astounding variety of autocrine and paracrine actions of S1P (Figure 1).

Intriguingly, the S1P-S1PR signaling axis can also be controlled by the activity of S1P lyase (SPL), an ER-localized enzyme. Inhibition or genetic ablation of SPL increases circulating S1P and induces lymphopenia, reminiscent of the effects of FTY720^{19, 29}. Others have suggested that extracellular S1P-mediated motility of lung endothelial cells is dependent on intracellular S1P levels, which are regulated by both SphK1 and SPL³⁰. Moreover, it has been suggested that intracellular localization of SphK1 determines access to sphingosine substrate pools but does not affect the degradative fate of S1P³¹. These studies not only reinforce the importance of “inside-out” signaling but also highlight SPL as a potential therapeutic target³².

S1P is an intracellular messenger

Many lines of evidence point to an intracellular role for S1P. S1P counteracts apoptosis mediated by its pro-apoptotic precursor ceramide and SphK1 is suggested to play a critical role in this “sphingolipid rheostat” (reviewed in³³, Figure 1). Moreover, early studies demonstrated that S1P could induce calcium release from the ER,^{34–36} though no target has yet been conclusively identified. For nearly two decades, no *bona fide* intracellular targets of S1P had been identified. Recently, this changed with the demonstration that S1P binds and alters the function of several disparate intracellular proteins.

HDAC

Although SphK2 is present in the nucleus of many cells, its function there was unknown. Recently, it was shown for the first time that nuclei contain significant amounts of both S1P and sphingosine. Moreover, SphK2 is in a repressor complex with histone H3 and histone deacetylases (HDACs), producing S1P that regulates histone acetylation at specific lysine residues and gene transcription⁷. Although S1P has no effect on histone acetyl transferases, it binds to and inhibits both HDAC1 and its close homolog, HDAC2. Inhibition of HDAC1/2 by S1P is physiologically relevant, as SphK2 was detected at the promoters of the

cyclin-dependent kinase inhibitor *p21*, and the transcriptional regulator *c-fos*, where it regulates histone acetylation and enhances their transcription⁷. These results indicate that S1P produced in the nucleus by SphK2 influences the dynamic balance of histone acetylation and thus the epigenetic regulation of specific target genes.

TRAF2

TNF receptor-associated factor 2 (TRAF2) is an adaptor protein containing a RING domain that is implicated in the regulatory ubiquitination of RIP1, a critical event in activation of NF- κ B in response to TNF- α . All attempts to demonstrate that TRAF2 was the E3 ligase for RIP1 failed, however. TRAF2 is known to bind SphK1 and stimulate its activity³⁷, though the role of S1P in the canonical NF- κ B pathway was unclear. Recently, it was discovered that S1P is a missing cofactor required for the E3 ligase activity of TRAF2 and, consequently, Lys-63-linked polyubiquitination of RIP1 and NF- κ B activation³⁸. Interestingly, only S1P, and not dihydro-S1P, which lacks the double bond in S1P, binds to and activates TRAF2. This explains why, although S1P and dihydro-S1P are equally potent ligands for the five S1P receptors, only S1P suppresses apoptosis³⁸. These data provide the first mechanistic explanation for the numerous observations of the importance of SphK1 and S1P in cytoprotective, inflammatory, and immune responses.

PKC δ

SphK1 plays a key role in a number of immune responses, including sepsis³⁹. Investigation of the molecular mechanism revealed a role for protein kinase C delta (PKC δ) in mediating endotoxin-induced activation of NF- κ B. Moreover, S1P, or recombinant SphK1 incubated with sphingosine and ATP to produce S1P, activates recombinant human PKC δ *in vitro*³⁹. Although direct binding was not determined, these data suggest that S1P binds and activates PKC δ *in vivo*, and implicate SphK1 as a critical component and therapeutic target for septic shock.

PHB2

Prohibitin 2 (PHB2), a highly conserved protein that regulates mitochondrial assembly and function, was recently shown to bind S1P *in vitro* and *in vivo*⁸. PHB2 predominantly localizes to the inner mitochondrial membrane where it is thought to form a large, macromolecular complex with PHB1 that is involved in mitochondrial biogenesis and metabolism⁴⁰. This study showed for the first time that SphK2 also localizes to the mitochondria, where it produces the majority of mitochondrial S1P. Mitochondria respiration was reduced in SphK2 knockout mice due to aberrant assembly and reduced activity of complex IV (cytochrome oxidase) of the electron transport chain, suggesting that interaction of S1P with PHB2 is important for cytochrome-c oxidase assembly and mitochondrial respiration. Further work is needed to elucidate the role of mitochondria S1P in mitochondrial function during oxidative stress and aging.

BACE1

A recent study showed that the activity of β -site amyloid precursor protein (APP) cleaving enzyme-1 (BACE1), the rate-limiting enzyme for amyloid- β peptide (A β) production, is modulated by S1P in neurons⁴¹. Inhibition or downregulation of SphK1, or overexpression of S1P-degrading enzymes, all decreased BACE1 activity and A β production⁴¹. S1P also specifically bound to BACE1 *in vitro* and increased its proteolytic activity, suggesting that cellular S1P directly modulates BACE1 activity. Importantly, SphK2 was upregulated in the brains of patients with Alzheimer's disease (AD). These results suggest a unique link between S1P, SphK2, and AD.

S1P in disease

As summarized above, the SphKs/S1P/S1PRs axis is implicated in regulation of many physiological processes, drawing attention to their potential functions in pathophysiology and diseases (Figure 2). The important and rapidly emerging area of targeting S1PRs in inflammation and multiple sclerosis, has been extensively reviewed recently ^{3, 15, 42}. Therefore, we will focus on selected recent advances in understanding the role of S1P in cancer, atherosclerosis, diabetes, and osteoporosis.

Cancer

Numerous studies have shown that SphK1 and production of S1P promotes tumor growth, resistance to apoptosis, tumor angiogenesis and metastasis (reviewed in ⁴³). SphK1 message and protein levels are often upregulated in cancerous tissue and expression is correlated with chemo- and radio-resistance and poor prognosis. Consistent with these observations, overexpression of SphK1 promotes, while its inhibition reduces, tumor growth, angiogenesis and chemoresistance in numerous xenograft models ^{43, 44}.

S1P secreted from tumor cells can act through S1PRs either in an autocrine manner to promote growth, survival, motility, and metastasis ^{45, 46} or in a paracrine manner to induce endothelial adhesion molecules, angiogenesis and regulate tumor-stromal interactions as well as immune cells ⁴⁷ (Figure 3). As discussed above, S1P may also promote cancer progression via intracellular targets, such as HDAC1/2 and NF- κ B. Finally, S1P can promote resistance of cancer cells to therapy by counteracting the pro-apoptotic effects of ceramide ² (Figure 1). An intriguing study recently identified S1PR1 as a key element involved in persistent activation of signal transducer and activator of transcription-3 (STAT3) in tumor cells and the tumor microenvironment ⁴⁸. STAT3 is a transcription factor for *S1pr1* and, conversely, enhanced *S1pr1* expression activates STAT3 and upregulates IL-6 expression, a pro-inflammatory cytokine crucial for STAT3 activation and inflammatory cell-mediated transformation and tumor progression. Thus, this is a new feed-forward mechanism that explains persistent STAT3 activation in cancer cells and the tumor microenvironment that is important for malignant progression and metastasis ⁴⁸.

Because of the potential importance of SphK1 in cancer and other diseases, much emphasis is now on development of isotype-specific inhibitors of SphK1 (Table 1). First-generation SphK inhibitors lacked isoform specificity, although one of these, safingol, is in phase I clinical trials ⁴⁹. One SphK1-specific inhibitor, known as SKI-I, inhibits the growth of acute myelogenous leukemia ⁵⁰ and glioblastoma tumors ⁵¹ in xenografts by affecting multiple survival signaling pathways. Another inhibitor, SKI-II, enhances the sensitivity of non-small cell lung cancer cells to chemotherapeutics both *in vitro* and *in vivo* ⁵². Surprisingly, SKI-II also induces proteosomal degradation of SphK1 ⁵³ and binds directly to the antagonist ligand-binding domain of the estrogen receptor, inhibiting estrogen receptor-stimulated transcriptional activity in human breast cancer cells ⁵⁴. Recently, progress was reported in the design of specific SphK1 inhibitors based on a homology model of SphK1 trained with a library of amidine-based compounds. Inhibitors with nM K_i 's for SphK1 were developed and found to significantly reduce endogenous S1P levels in leukemia U937 cells ⁵⁵. It will be interesting to see the results of *in vivo* studies with these compounds.

In contrast, the connection between SphK2 and cancer is still not well defined. SphK2 downregulation was more effective than SphK1 downregulation in inhibiting growth of glioblastoma cells ⁵⁶. Downregulation of SphK2 in MCF7 cells also decreased G₂-M arrest and markedly enhanced apoptosis induced by doxorubicin, probably due to effects on p21 expression ⁵⁷. SphK2-deficient breast cancer cells have impaired growth in a mouse tumor model ⁵⁸. A proposed SphK2-specific inhibitor, ABC294640, inhibited the proliferation of a

variety of cancer cells in culture and reduced the S1P content and growth of mammary tumors in nude mice ⁵⁹. Interpretation of these results is complicated by the recent demonstration that this inhibitor also has anti-estrogenic effects and binds to the estrogen receptor ⁶⁰. The demonstration that S1P produced by SphK2 is an endogenous HDAC inhibitor ⁷ also suggests that the role of SphK2 in cancer progression may be dependent on tissue-specific and perhaps tumor-specific properties.

Atherosclerosis

Atherosclerosis is a chronic inflammatory condition, a hallmark of which is the extensive accumulation of lipids and immune cells, called plaques, in subendothelial regions, causing the arterial lumen to narrow and thrombose after rupture of the plaque. Interest in S1P in the pathogenesis of atherosclerosis stems from observations that S1P is in high concentrations in the circulation, where it is largely bound to high density lipoprotein (HDL) and albumin. S1P has diverse effects on a variety of cell types central to the development of atherosclerosis, including monocyte attachment and migration, proliferation of smooth muscle cells, vascular tone, and stimulation of the NF- κ B pathway leading to production of pro-inflammatory cytokines ⁶¹. S1P actions are complex and it is still not clear whether S1P is pro- or anti-atherogenic. The stimulatory pathway is mediated mainly by NF- κ B activation and adhesion molecule expression, probably via S1PR3, and the inhibitory pathway is mediated mainly by activation of endothelial nitric oxide synthase and nitric oxide-dependent vasorelaxation by activation of S1PR1 ⁶² (Figure 4). Although there are no major differences in atherosclerotic lesions and lipid volume in the aorta of apolipoprotein E (ApoE)^{-/-} S1PR3^{-/-} double knockout mice, there is significant reduction in macrophage and smooth muscle content of the lesions ⁶³. These results suggest that S1PR3 promotes inflammatory monocyte/macrophage recruitment and alters smooth muscle cell behavior. Because proliferation of smooth muscle cells *in vitro* was unaffected by S1PR3 deletion, further studies with targeted deletions of this receptor are needed to confirm its role. Both pharmacologic and genetic approaches to silence S1PR2 in ApoE^{-/-} mice attenuated atherosclerotic lesion formation as shown by reduced plaque area, inhibition of macrophage accumulation in the aorta and increased cholesterol efflux in macrophages ^{64, 65}. These effects were attributed to lower S1PR2-dependent macrophage retention and/or transmigration ⁶⁵ and decreased ROCK and NF- κ B activities, leading to decreased expression of pro-inflammatory cytokines, adhesion molecules and the chemokine MCP-1 ⁶⁴ (Figure 4). Nevertheless, the anti-atherogenic actions of HDL independent of cholesterol metabolism are suggested to be mediated by HDL-associated S1P ⁶². Indeed, a recent study showed that HDL-associated S1P is bound solely to apolipoprotein M (ApoM), which is known to have anti-atherogenic effects ⁶⁶. Hence, in addition to increasing cholesterol efflux from macrophage foam cells, and pre- β -HDL formation and its antioxidative effects, ApoM is vasculoprotective by delivering S1P to S1PR1 on endothelial cells ⁶⁶, and S1P-ApoM may well be anti-atherogenic. It is possible that binding of S1P to ApoM in HDL is one factor that determines the specificity of the physiological effects of S1P.

Interestingly, FTY720 markedly reduced atherosclerotic lesions in both low density lipoprotein receptor-null and ApoE-null mice ^{67, 68}. This is likely due to FTY720-mediated downregulation of S1PR1 on lymphocytes and resulting lymphopenia, as well as to shifting classical inflammatory M1 macrophages to anti-inflammatory M2 types ⁶⁷. FTY720 conferred atheroprotective effects in human primary macrophages by reducing transport of scavenged lipoprotein cholesterol to the ER and facilitating its release ⁶⁹. Surprisingly, however, this effect was independent of S1PRs. Rather, FTY720 stimulated production of 27-hydroxycholesterol from cholesterol, providing an additional explanation for its atheroprotective effects and identifying a novel mechanism for the actions of FTY720 ⁶⁹.

Diabetes and obesity

Diabetes is a chronic disease affecting hundreds of millions of people worldwide. Sphingolipid metabolism is altered in diabetic conditions, however, focus to date in this area has been on ceramide^{70, 71} and only a few studies have examined the involvement of S1P. Fatty acid (palmitate)-induced stimulation of *de novo* ceramide synthesis is required, but not sufficient, for the onset of insulin resistance and ceramide is linked to insulin resistance, in part due to inhibition of Akt^{70, 71}. Moreover, inhibition of sphingolipid synthesis increased insulin sensitivity, resolved hepatic steatosis, and prevented the onset of diabetes in obese rodents.

Inflammatory responses in obesity exacerbate insulin resistance and development of diabetes. Activation of toll-like receptor 4 (TLR4) by saturated fatty acids is one pathway of innate immunity contributing to increased inflammation associated with obesity. An intriguing study recently demonstrated that TLR4 stimulates IKK β and NF- κ B, which increases the transcription of genes involved in ceramide synthesis⁷². These genes drive ceramide elevation, inhibiting Akt, which is required for insulin signaling (Figure 5). Interestingly, increased ceramide production was dispensable for TLR4-dependent induction of inflammatory cytokines, but required for TLR4-dependent insulin resistance⁷². Altogether, this work suggests that ceramide is a key mediator that links lipid-induced inflammatory pathways to insulin resistance and diabetes.

Another intriguing link was recently uncovered between adipocyte-derived adiponectin – a hormone with insulin sensitizing, anti-inflammatory and anti-apoptotic functions – and ceramide and its metabolites with insulin sensitivity⁷³. Binding of adiponectin to its two receptors, AdipoR1 and AdipoR2, stimulates ceramidase activity (deacylation to sphingosine) and formation of its anti-apoptotic metabolite S1P. S1P may then bind to its receptors, leading to activation of AMPK. These actions of adiponectin via S1P might promote survival of pancreatic beta cells, nutrient uptake, nutrient utilization and mitochondrial proliferation⁷³ (Figure 5).

Plasma S1P levels are elevated in two animal models of type 1 diabetes (streptozotocin-induced diabetic rats and Ins2 Akita diabetic mice), yet no changes in levels were detected in the livers of these animals⁷⁴, suggesting other sources of S1P. An S1PR2 antagonist prevented the onset of diabetes in a streptozotocin diabetes mouse model and S1PR2^{-/-} mice displayed lower blood glucose levels and reduced beta cell apoptosis together with higher insulin/glucose ratios (an index of relative insulin deficiency)⁷⁵. Glucose induces a rapid and sustained increase in islet sphingosine kinase activity that is dependent on glucose phosphorylation, but independent of ATP generation and protein biosynthesis. Moreover, glucose-supported beta cell growth appears to be, in part, mediated by this increased activity. Although several studies suggest that S1P might be pro-survival for pancreatic beta cells, it is still not clear whether this is mediated by intracellular or extracellular actions of S1P, nor do we know the mechanism or which S1PRs are involved.

S1P in osteoporosis

Osteoporosis is a systemic skeletal disorder and one of the most common types of metabolic bone diseases⁷⁶. In the past few years there has been increasing interest in the role of S1P in normal bone homeostasis as well as in the pathogenesis of bone destructive disorders. S1P has multiple functions in osteoclasts, including stimulating their motility, providing dynamic control over the migration of osteoclast precursors, and maintaining bone mineral homeostasis and increased osteoclastogenesis^{77–80}. S1P stimulates osteoblast migration and promotes their survival, and thus secretion of S1P at sites of bone resorption may be important for osteogenesis⁷⁷. Osteoclasts might recruit osteoprogenitors to the site of bone

remodeling through the S1P/S1PR1 axis and BMP6, and stimulate bone formation through increased activation of Wnt/BMP pathways ⁷⁸.

Low concentrations of S1P are chemotactic for osteoclast precursors, while high concentrations inhibit their movement ⁷⁹. This suggests that these precursors, which express both S1PR1 and S1PR2, are kept in the vicinity of the bone by the S1P gradient: S1PR2 prevents S1PR1-induced migration of these bone-resorbing cells away from bone. S1PR2 null mice develop osteopetrosis due to a decrease in bone resorption ⁸⁰. Likewise, in ovariectomized mice, the S1PR2 antagonist JTE013 prevented bone loss by decreasing osteoclast attachment to bone ⁸⁰. Conversely, osteoclast-specific ablation of S1PR1 induced osteoporosis by increasing their attachment to bone ⁷⁹. Thus, the balance between the S1PRs and their sensing of local S1P concentrations plays a critical physiological role in bone homeostasis and likely in osteoporosis, although this remains to be examined. Similarly, in a TNF transgenic mouse model for inflammatory arthritis, knockout of SphK1 significantly reduced arthritis symptoms as well as bone erosive disease ⁸¹. Moreover, the synovial joints had fewer osteoclasts, suggesting that SphK1 plays a key role in TNF-induced inflammatory arthritis by regulating synovial inflammation and osteoclast number.

Concluding remarks

The sum of a plethora of *in vitro* and *in vivo* studies in the decades since S1P was first discovered to be a second messenger has taught us much about its mechanisms of action. We now understand why S1P is so important for regulation of many normal and pathophysiological processes. The successful development of the sphingosine analogue FTY720, a pro-S1P mimetic, as a useful drug for treatment of multiple sclerosis has proven that it is possible and beneficial to specifically target S1P signaling in humans without serious side effects. Inhibitors of S1P metabolism and its receptors are effective in many animal models of human disease. There is also now tantalizing evidence, summarized briefly here, that dysregulation of S1P correlates with progression of diseases such as cancer and autoimmune disorders. Thus, the time is ripe for translating this knowledge into a new class of sphingolipid-centric therapeutics.

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Figure 1. Intracellular and extracellular actions of S1P

S1P produced intracellularly can inhibit functions of its pro-apoptotic precursor ceramide. Ceramide is implicated in growth arrest, apoptosis and autophagy (red quadrant). S1P also has intracellular targets (green quadrant) or can be exported out of cells to act in autocrine and/or paracrine manners through the S1P receptors (blue quadrant).

Figure 2. The pathobiology of S1P

S1P is implicated in numerous pathophysiological conditions and diseases that affect almost every organ in the human body. Because S1P is also linked to almost every type of cancer, only a few are shown.

Figure 3. Involvement of S1P in cancer

As described in the text, S1P regulates many processes in cancer cells and the tumor microenvironment that are important for malignant progression, including growth, survival and metastasis of the tumor, migration of stromal cells, and angiogenesis. S1P is also involved in recruitment of inflammatory cells and secretion of cytokines and chemokines that are important for inflammation and tumorigenesis.

Figure 4. Role of S1P and its receptors in atherogenesis

Binding of S1P to distinct S1P receptors regulates vascular tone and barrier function, monocyte attachment and migration, macrophage infiltration and retention, proliferation of smooth muscle cells, and activation of NF- κ B leading to production of pro-inflammatory cytokines and adhesion molecules. S1PR1, red; S1PR2, green; S1PR3, blue.

Figure 5. Role of S1P in diabetes and obesity

Saturated fatty acid-induced insulin resistance is mediated by the proinflammatory receptor TLR4, which stimulates IKK β and leads to NF- κ B activation. This results in the upregulation of biosynthetic genes involved in *de novo* ceramide formation. Ceramide, in turn, inhibits Akt to induce apoptosis and suppress insulin function. Binding of adiponectin to its receptors enhances deacylation of ceramide to sphingosine, which can then be phosphorylated to form S1P. Inside-out signaling via S1PRs can activate AMPK, important for mitochondria biogenesis. S1P also stimulates Akt and prevents apoptosis.

Table 1

Small molecules targeting SIP.

Name	Mechanism	Diseases	References
FTY720 (Fingolimod, Gilenya) 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol	<ul style="list-style-type: none"> • Prodrug; phosphorylated by SphK2 • SIPR1, SIPR3–5 agonist • Downregulates SIP1 	<ul style="list-style-type: none"> • FDA approved for multiple sclerosis * • Dermatitis • Arthritis • Allergy 	<ul style="list-style-type: none"> [15] [82] [83] [84]
SK1-I (BML-258) (2R,3S,4E)-N-methyl-5-(4'-pentyphenyl)-2-aminopent-4-ene-1,3-diol	<ul style="list-style-type: none"> • SphK1 inhibitor 	<ul style="list-style-type: none"> • Glioblastoma • Leukemia 	<ul style="list-style-type: none"> [51] [50]
Safingol L-threo-dihydrospingosine	<ul style="list-style-type: none"> • pan SphK inhibitor 	<ul style="list-style-type: none"> • Solid tumors* (Phase I) 	[49]
SK1 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole	<ul style="list-style-type: none"> • SphK1 inhibitor • Induces proteasomal and lysosomal degradation of SphK1 	<ul style="list-style-type: none"> • Pancreatic cancer • Leukemia • Asthma 	<ul style="list-style-type: none"> [85] [86] [87]
ABC294640 3-(4-chlorophenyl)-adamantane-1-carboxylic acid (pyridinyl-4-methyl) amide	<ul style="list-style-type: none"> • SphK2 inhibitor • Estrogen receptor agonist 	<ul style="list-style-type: none"> • Cancer • Rheumatoid arthritis • Inflammatory bowel disease 	<ul style="list-style-type: none"> [59] [88] [89]
ABC294735 3-(4-chlorophenyl)adamantane-1-carboxylic acid [2-(3,4-dihydroxyphenyl)ethyl]amide	<ul style="list-style-type: none"> • pan SphK inhibitor 	<ul style="list-style-type: none"> • Cancer 	[90]
SK1-II (SK1-2, SPHK I ₂) 4-[4-(4-chlorophenyl)-thiazolyl-2-amino]-phenol	<ul style="list-style-type: none"> • SphK1 inhibitor 	<ul style="list-style-type: none"> • Asthma 	[91]
THI 2-acetyl-4(5)-[1(R),2(S),3(R),4-tetrahydroxybutyl]-imidazole	<ul style="list-style-type: none"> • SIP Lyase inhibitor 	<ul style="list-style-type: none"> • Ischemia/reperfusion injury • Lung injury 	<ul style="list-style-type: none"> [92] [93]
LX2931 (E)-1-(4-((1R,2S,3R)-1,2,3,4-tetrahydroxybutyl)-1H-imidazol-2-yl)ethanone oxime	<ul style="list-style-type: none"> • SIP Lyase inhibitor 	<ul style="list-style-type: none"> • Rheumatoid arthritis* (Phase II) 	[32]
5c 2,2-dimethyl-4S-(1-oxo-2-hexadecyn-1-yl)-1,1-dimethylethyl ester-3-oxazolidinecarboxylic acid	<ul style="list-style-type: none"> • SphK1 inhibitor 	<ul style="list-style-type: none"> • Sepsis 	[39]
SEW2871 5-[4-phenyl]-5-(trifluoromethyl)-2-thienyl]-3-[3-(trifluoromethyl) phenyl]-1,2,4-oxadiazole	<ul style="list-style-type: none"> • SIPR1 agonist 	<ul style="list-style-type: none"> • Diabetic nephropathy • Renal protection • Emphysema 	<ul style="list-style-type: none"> [94] [95] [96]

Name	Mechanism	Diseases	References
AAL(R) 2-amino-4-(4-heptyloxyphenyl)-2-methylbutanol	• Prodrug; phosphorylated by SphK2 • SIPR1/SIPR3 agonist	• Neuroprotection	[97]
		• Spontaneous autoimmune polyneuropathy	[98]
		• Influenza	[99]
AUY954	• SIPR1 agonist	• Sepsis	[100]
		• Transplantation	[101]
		• Experimental autoimmune neuritis	[102]
JTE013	• SIPR2 antagonist	• Anaphylaxis	[103]
		• Atherosclerosis	[65]
		• Cancer	[45]

* Effects in humans. All other compounds have only been tested in animal models.