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Lyase to live by: Sphingosine phosphate lyase as a therapeutic target

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Abstract

Background—Sphingosine 1-phosphate (S1P) is a bioactive lipid that regulates cell proliferation, survival and migration and plays an essential role in angiogenesis and lymphocyte trafficking. S1P levels in the circulation and tissues are tightly regulated for proper cell functioning, and dysregulation of this system may contribute to the pathophysiology of certain human diseases. Sphingosine phosphate lyase (SPL) irreversibly degrades S1P and thereby acts as a gatekeeper that regulates S1P signaling by modulating intracellular S1P levels and the chemical S1P gradient that exists between lymphoid organs and circulating blood and lymph. However, SPL also generates biochemical products that may be relevant in human disease. SPL has been directly implicated in various physiological and pathological processes, including cell stress responses, cancer, immunity, hematopoietic function, muscle homeostasis, inflammation and development.

Objective—This review will summarize the current knowledge of SPL structure, function, regulation, its involvement in various disease states, and currently available small molecules known to modulate SPL activity.

Results/Conclusion—This review provides the evidence that SPL presents itself as a potential target for pharmacological manipulation for the treatment of malignant, autoimmune, inflammatory and other diseases.

Keywords

FTY720; Sphingosine 1-Phosphate Lyase; S1P Lyase; Sphingolipid; *Sgpl1*

1.0 Introduction

Sphingosine 1-phosphate (S1P) is a bioactive metabolite of sphingolipid metabolism. S1P has been shown to promote cell survival, proliferation and migration, ischemia preconditioning and plays an essential role in angiogenesis and lymphocyte trafficking^{1–3}. S1P exerts its functions either by acting as an intracellular second messenger⁴ or by activating its cognate G protein-coupled receptors (GPCR). Five GPCRs that respond specifically to S1P ligand have been identified (designated S1P₁ to S1P₅) and they show differential expression patterns depending on tissue and cell type^{5, 6}. In mammals, S1P₁, S1P₂, and S1P₃ are expressed ubiquitously, whereas S1P₄ is restricted to lymphoid tissues and lung and S1P₅, to brain and skin. Activation of S1P receptors stimulates downstream signaling pathways, including those involving mitogen activated protein kinases (MAPK), phosphoinositide-3 kinase, cyclic AMP and other downstream mediators^{6, 7}. The effects of S1P signaling through these receptors include cytoskeletal rearrangements, cell proliferation and migration, invasion, vascular

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development, platelet aggregation, and lymphocyte trafficking^{1,8}. In contrast to the well characterized receptor-mediated effects of S1P, much less is known about the intracellular actions of S1P. It has been suggested that intracellular actions of S1P can regulate calcium mobilization, cell growth and suppression of apoptosis in response to a variety of extracellular stimuli in a receptor-independent manner^{1, 4}. Because of the importance of S1P, considerable interest has developed in understanding the function and regulation of the enzymes involved in its metabolism.

S1P is formed from sphingosine in a reaction catalyzed by sphingosine kinases (Sphk)^{9, 10}. Once formed, S1P phosphatases and nonspecific lipid hydrolases can dephosphorylate S1P¹¹. Alternatively, S1P can be irreversibly degraded by sphingosine phosphate lyase (SPL), which catalyzes the cleavage of the carbon chain at positions 2–3, leading to the formation of a long-chain aldehyde (hexadecenal) and phosphoethanolamine^{12–14}. SPL plays an important role in development, reproduction, cell survival, stress responses, the maintenance of tissue integrity, lymphocyte trafficking and tumorigenesis^{15–22}. Here, we summarize the current understanding of SPL function, regulation and its role in physiology and disease. We further hypothesize how SPL might serve as a pharmacological target for cancer therapy, immune modulation and therapeutic intervention for other diseases.

2.0 Biochemical actions of SPL

SPL catalyzes the final step of sphingolipid catabolism. The substrates of SPL are phosphorylated long chain bases that include phosphorylated sphingosine, dihydrosphingosine, sphingadiene, and phytosphingosine as well as dihydrosphingosine-1-phosphonate. SPL shows specificity towards the D(+)-*erythro* isomer of its substrates²³. SPL is a pyridoxal 5'-phosphate-dependent enzyme and its lysine residue (Lys-353) is an active site that forms an internal Schiff base with pyridoxal 5'-phosphate, facilitating the cleavage of S1P to hexadecenal and phosphoethanolamine²⁴. Dysregulation of paracrine and autocrine S1P signaling through its receptors, as well as potentially receptor-independent effects of intracellular S1P and abnormal accumulation of other sphingolipid intermediates appear to be responsible for many of the effects associated with SPL inhibition, as described at length below (Figure 1). However, product depletion may also be a consideration. For example, a lack of phosphoethanolamine production was accountable for the lack of viability and differentiation of *Leishmania major* spl-mutants, as the supplementation of ethanolamine completely reversed these defects²². The products of the SPL reaction can also serve as precursors to phospholipid metabolism²⁵, and products of the SPL reaction were found to promote F9 embryonal carcinoma cell proliferation through an S1P-independent mechanism²⁶. Therefore, SPL exerts effects on pathophysiological processes through mechanisms involving both the substrate and products of the reaction.

3.0 Structure, expression and intracellular localization of SPL

The first SPL gene to be cloned was *DPL1* (dihydrosphingosine phosphate lyase). Its identification in yeast facilitated the identification of functional SPL homologs within the genomes of *Caenorhabditis elegans*, *Dictyostelium discoideum*, *Leishmania major*, *Drosophila melanogaster*, *Mus musculus*, *Homo sapiens* and recently in the plants^{12, 13, 16, 17, 22, 27, 28}.

The human SPL gene, *sphingosine phosphate lyase 1* (*Sgpl1*), encodes a protein of 568 amino acids with a predicted molecular mass of 63.5 kDa¹⁴. Human SPL displays 84% amino acid identity and 91% similarity to the mouse ortholog. Immunofluorescence and subcellular fractionation studies revealed that SPL is predominantly localized within the endoplasmic reticulum (ER)^{18, 23, 24}. Based on structural modeling and biochemical characterization, SPL is predicted to be a single-pass type III membrane protein. SPL contains one transmembrane

segment close to the N-terminus. Its N-terminal domain resides in the ER lumen, whereas its large C-terminal domain which contains the catalytic site is exposed to the cytosol¹⁴. Several conserved amino acid residues have been mapped to the C-terminal domain of human SPL, including a 20 amino-acid stretch spanning positions 344–364, which contains predicted cofactor binding lysine residues at position K353 and K359. Site-directed mutagenesis of human SPL revealed the importance of two conserved cysteine residues at positions C218 and C317, which are required for proper enzymatic activity¹⁴. Interestingly, recombinant SPL lacking the transmembrane domain and the ER luminal sequence was fully active *in vitro* when expressed in bacteria¹⁴. Conversely, a mutagenesis study conducted on the yeast ortholog Dpl1p revealed the important role of the N-terminal domain²⁹. The luminal domain of Dpl1p appears to be required to maintain protein stability, and a Dpl1p NΔ57 mutant lacking the entire luminal domain was completely inactive when evaluated using an *in vivo* complementation assay in yeast²⁹. Dpl1p has been shown to form higher order complexes that are required for its *in vivo* function. Polar amino acid residues in the transmembrane domain of Dpl1p play an important role in protein oligomerization²⁹.

4.0 Tissue distribution of SPL

SPL is expressed in many mammalian tissues to variable degrees. In mice and rats, SPL activity/ expression is highest in the small intestine, colon, thymus, spleen, and harderian gland, whereas moderate expression is observed in liver, kidney, lung, stomach and testis. SPL expression is lowest in the heart, skeletal muscle and brain, with the exception of the olfactory mucosal epithelium, where the enzyme is highly enriched^{14, 30} (Our unpublished observations). SPL expression is low in lymphocytes and absent in erythrocytes and platelets, which are major sources of plasma S1P^{31, 32}. SPL is expressed in some inflammatory cells, but its activity in macrophages, monocytes, dendritic cells and neutrophils has not been characterized to date.

SPL expression seems to be high in tissues characterized by rapid cell turnover. This is exemplified by the pronounced expression and activity of SPL in intestinal epithelial cells, which are renewed every 12 hours. High SPL expression in intestinal epithelial cells suggests that it may play an important role in catabolizing dietary sphingolipids³³. SPL may also be required to maintain low S1P levels in the cells at the villus tips, facilitating cell death and tissue turnover in response oxidative stress and as a mechanism of gut immunity. SPL expression is high in the olfactory mucosa, a unique neuronal tissue that is subject to high rates of apoptosis due to inhaled toxic-induced cell damage. Interestingly, olfactory mucosa is unique among adult neuronal tissues for its ability to sustain continuous neurogenesis³⁰. The high expression of SPL in the thymus might be required to maintain low tissue S1P levels compared to the surrounding plasma. The S1P concentration gradient between thymus and the plasma enables T cell egress from thymus into the circulation²⁰.

5.0 Regulation of SPL

There is evidence that SPL activity is regulated at multiple levels, including epigenetic, transcriptional and post-translational. GATA family transcription factors were shown to regulate SPL expression in nematode and human cells at the transcriptional level³⁴. SPL was also identified as an immediate early gene and transcriptional target of platelet-derived growth factor (PDGF)³⁵. PDGF signaling plays a role in embryonic development, cell proliferation, cell migration and angiogenesis and has been linked to several diseases including atherosclerosis, fibrosis and tumorigenesis³⁶. Furthermore, an SPL knockout mouse demonstrated phenotypes consistent with PDGF signaling abnormalities, including vascular developmental abnormalities, skeletal defects, and defects of cell migration³⁷. Analysis of the 5' flanking region of the SPL gene suggests that other mechanisms of transcriptional control may be involved in the regulation of SPL expression. In addition, *Sgpl1* has also been identified

as a microRNA (mir-125b) target in prostate cancer cells³⁸. A comparative genomics approach to the analysis of immune system genes identified common *cis*-elements, including early growth response factor, zinc-binding protein factor, and GC-box factor motifs in the promoters of *Sgpl1* and other thymic-enriched genes³⁹. *Sgpl1* was also shown along with other pro-apoptotic genes to be a downstream target of mSin3A, a core component of a co-repressor complex involved in embryonic development and in regulating cellular functions such as cell cycle progression, DNA replication, DNA repair, apoptosis and mitochondrial metabolism⁴⁰.

In addition to transcriptional regulation, post-translational regulation of human SPL has also been suggested by the finding of SPL in a screen for nitrosylated proteins. SPL is predicted to be nitrosylated on tyrosine residues Y356 and Y366⁴¹. By altering the charge of the tyrosine residues, nitrosylation may interfere with enzyme-substrate binding. Multiple phosphorylation sites are also predicted by protein sequence analysis, but these findings have not been verified experimentally⁴². A recent study has demonstrated that Dpl1p forms higher order complexes, and oligomerization of Dpl1p is required for its *in vivo* function²⁹. However, to date formation of high order complexes by human SPL has not been tested.

6.0 Potential roles for SPL in human disease

The involvement of S1P in diverse essential cellular processes necessitates precise control and regulation of its intracellular and extracellular levels. The importance of SPL in modulating the levels of S1P, other sphingolipid intermediates and cell fate is likely to contribute to tissue homeostasis. Alternatively, its dysregulation could contribute to the pathophysiology of disease (Figure 2). In support of this notion, genetically modified mice lacking SPL expression in all tissues demonstrate stunted growth and early mortality with defects reported in the kidney, bone and vasculature³⁷, as well as myeloid cell hyperplasia, and significant lesions in the lung, heart, urinary tract⁴³. The potential role of SPL in specific disease states is described below.

6.1 SPL in cell growth and cancer

Sphingolipids regulate various aspects of cell growth, proliferation, and cell death⁴⁴. Ceramide, the backbone of all higher order sphingolipids from which S1P derives, generally mediates anti-proliferative responses such as inhibition of cell growth, induction of apoptosis, and/or modulation of senescence. Ceramide generation is considered to be a key mechanism by which chemotherapeutic agents induce apoptosis in cancer cells. On the other hand, S1P promotes cell growth, migration, tumor angiogenesis, invasion and metastasis. Consistent with their key roles in the regulation of cancer growth and therapy, genetic changes acquired by malignant cells that result in either a reduction in ceramide generation and/or an accentuation of S1P generation are implicated in the development of resistance to drug-induced apoptosis and escape from cell death⁴⁵. A dynamic balance between ceramide and S1P is, thus, maintained within the cells, contributing to the determination of cellular outcome in response to stress. SPL has the ability to shift the balance towards cell death by attenuating the proliferative S1P signal.

A potential role of SPL in regulating cell fate and stress responses has been investigated in several model systems. For example, an insertional mutagenesis study revealed that mutations in the *Dictyostelium sglA* gene, which encodes SPL, confer resistance to the anticancer drug cisplatin⁴⁶. Conversely, overexpression of SPL enhanced the sensitivity of *Dictyostelium* cells to the drug⁴⁷. SPL has also been shown to sensitize mammalian cells to various stressful stimuli including serum starvation and DNA damage. Enforced expression of SPL in malignant and non-transformed human cells sensitizes these cells to platinum-based chemotherapy drugs (cisplatin and carboplatin), daunorubicin and etoposide^{18, 21, 48}. The ability of SPL to potentiate cell death requires the actions of p53 and p38 MAPK signaling pathways.

Conversely, knockdown of endogenous SPL expression in HEK293 cells by siRNA results in diminished apoptosis in etoposide-treated cells²¹. Furthermore, elevation in *Sgpl1* mRNA levels has been associated with testicular degeneration caused by increased apoptosis due to leptin deficiency in mice⁴⁹. Conversely, SPL deficiency can also lead to cell death, as evidenced by increased apoptosis observed in the reproductive organs of *Drosophila Sply* null mutant⁵⁰. The sphingolipid metabolite responsible for this effect is unknown, although these tissues show a profound accumulation of long-chain bases including $\Delta 4,6$ -sphingadienes, which are found to promote apoptosis in *Drosophila* cell lines^{19,51}. Interestingly, it has been proposed that accumulation of intracellular S1P above a certain threshold can also induce apoptosis⁵². Cerebellar granule neurons isolated from SPL-deficient mice showed a similar elevation in the intracellular S1P levels upon treatment with either sphingosine or exogenous S1P. However, only S1P addition to the cells induces apoptosis⁵².

Consistent with a role for S1P in various processes associated with tumorigenesis, the genes regulating S1P metabolism are altered in variety of human cancers^{44, 53}. *Sphingosine kinase 1 (SK1)*, which encodes the main enzyme responsible for S1P synthesis, functions as an oncogene⁵⁴ and is regulated in a variety of solid tumors, such as colorectal, breast, stomach, lung, ovary, uterus and kidney^{55, 56}. SPL expression and activity are downregulated during intestinal tumorigenesis in the *Apc^{Min/+}* mouse model of intestinal tumorigenesis, as well as in human colon cancer specimen compared with normal adjacent tissues²¹. Alterations of *Sgpl1* expression in several types of cancers have been demonstrated by cDNA microarrays. *Sgpl1* is repressed during myc-mediated β -cell tumorigenesis in mice and is re-expressed upon Myc deactivation⁵⁷. Moreover, Myc binds to the *Sgpl1* promoter in Burkitt's lymphoma cells and negatively regulates its expression⁵⁸. Fatty acid synthase (FAS) is upregulated in a wide variety of cancers, and is considered a potential metabolic oncogene by virtue of its ability to enhance tumor cell survival in certain cancers, especially those with the lipogenic phenotype⁵⁹. Knockdown of FAS in mammary carcinoma cells upregulates the expression of *Sgpl1*⁶⁰. Furthermore, *Sgpl1* is among a set of genes downregulated in metastatic tumor tissues compared to primary tumors from the same patients⁶¹. These findings suggest that SPL may act as tumor suppressor and may be involved in cancer surveillance pathways. Presumably SPL would act by preventing intracellular S1P accumulation, whereas loss of SPL expression or activity might promote tumorigenesis through activation of S1P-mediated signaling. Although, the role of SPL in tumor suppression has not been established *in vivo*, such a notion is consistent with a study wherein administration of a monoclonal S1P antibody substantially reduced tumor progression and angiogenesis in murine xenograft and allograft models⁶². However, upregulation of SPL expression has been observed in ovarian cancer⁶³. *Sgpl1* was also identified in a group of genes whose expression is upregulated in ovarian tumors that were resistant to chemotherapy⁶⁴. Lysophospholipids, including S1P and lysophosphatidic acid, are elevated in malignant ovarian tissues and malignant ascites and stimulate tumor cell proliferation⁶⁵. Whether induction of SPL is a causative factor or, conversely, a manifestation of tissue responses to high levels of lysophospholipids remain to be determined. Interestingly, S1P signaling is radioprotective to oocytes and ovarian function and has been proposed as a therapeutic strategy for preventing reproductive failure in cancer patients^{66, 67}.

Future studies are warranted to address whether SPL expression is causally related to intestinal tumorigenesis and other forms of neoplasia. If SPL downregulation in intestinal adenomas is a reversible phenomenon, it might be exploited as an adjuvant approach to enhance the therapeutic response of tumor cells to DNA-damaging agents. Conversely, SPL might also be a target for pharmacological intervention to transiently raise S1P levels as a way of protecting reproductive organs and potentially other organ functions in patients receiving radio- and chemotherapy⁶⁶.

6.2 SPL as a target for immunological disorders

S1P signaling is essential for immune cell trafficking and plays a role in immune surveillance, cytokine secretion, immune cell differentiation and allergic responses^{68,7}. Importantly, a recent study demonstrated that the immune system is exquisitely sensitive to partial loss of SPL activity, suggesting that activation of the S1P pathway is more stringently controlled in the immune system than the non-lymphoid environment⁴³. Interest in the physiological role of S1P signaling in the immune system developed in last decade after it was recognized that the potent immunosuppressive drug FTY720 functions as a sphingosine analog⁶⁹. Currently, FTY720 is under clinical trials for the treatment of allograft rejection in renal transplant patients and for multiple sclerosis. FTY720 is rapidly phosphorylated *in vivo* by Sphk2^{70,71}, and the phosphorylated compound acts as a ligand for four of the five known S1P receptors⁶⁹. Although FTY720-phosphate is an S1P₁ receptor agonist, chronic exposure to the drug induces internalization and degradation of the S1P₁ receptor, resulting in functional antagonism⁸. FTY720 treatment deprives thymocytes and lymphocytes of an S1P signal that stimulates their egress from thymus and secondary lymphoid tissues⁸. The majority of circulating lymphocytes are, thus, sequestered in lymph nodes, causing lymphopenia. FTY720 has been shown to inhibit SPL activity, which raises the possibility that SPL inhibition might contribute to the immunological effects of the drug⁷².

S1P levels in the blood and lymph are high (0.1–1.0 μ M) compared with those in most tissues, which maintain baseline levels in the range of 0.5 to 75 pmol/mg². This difference establishes an S1P concentration gradient between circulatory fluids (lymph and blood) and lymphoid organs, which is tightly maintained by SPL activity in the tissues. This S1P gradient is essential for lymphocyte egress from lymphoid organs²⁰. Inhibition of SPL activity by a food colorant tetrahydroxybutylimidazole (THI) or by reducing hematopoietic cell SPL expression through RNA interference-mediated knockdown prevents lymphocyte egress from thymus and secondary lymphoid organs²⁰. Inhibition of SPL by THI raised the bioavailable S1P levels ~100–1000-fold in thymus and secondary lymphoid organs without altering plasma S1P levels, thereby causing a disruption of the S1P gradient. The critical role of SPL in lymphocyte trafficking was recently confirmed in a study showing that genetically modified mice lacking *Sgpl1* expression exhibit lymphopenia, with sequestration of mature T cells in the thymus and lymph nodes⁴³. Furthermore, humanized knock-in mice lacking murine *Sgpl1* expression but harboring one or two alleles of human *Sgpl1* also exhibited immune defects. Replacement of the human SPL gene in the *Sgpl1* null background resulted in SPL expression at 10–20% of normal mouse SPL levels, yet failed to restore normal T-cell development and trafficking. These data suggest that immune functions including lymphocyte trafficking are exquisitely sensitive to alterations in SPL activity⁴³.

Apart from its role lymphocyte trafficking, S1P signaling plays a role in cell proliferation, survival and differentiation of lymphocytes⁷. S1P treatment enhances the survival of B and T cells⁷³, inhibits both homeostatic proliferation and T-cell receptor-induced proliferation of T cells⁷⁴, and inhibits cytokine production⁷⁵. Importantly, it has recently been demonstrated that genetic ablation of *Sgpl1* in mice hampers B and T cell development in addition to lymphocyte egress⁴³. *Sgpl1* knockout (*Sgpl1*^{−/−}) and humanized knock-in mice harboring one human allele (*Sgpl1*^{H/−}) mice showed severe hypocellularity and increased apoptosis of lymphocytes within the thymic cortex, as well as a paucity of T-cells in the splenic periarteriolar lymphoid sheaths and paracortical areas of lymph nodes. It was suggested that excess tissue S1P levels in *Sgpl1*^{−/−} mice may interfere with lymphocyte-stromal interactions. SPL deficiency also caused the vacuolization of thymic epithelial and stromal cells required for T-cell selection and maturation⁴³.

Multiple sclerosis (MS) is an autoimmune condition in which autoreactive T-cells migrate across the blood-brain barrier and attack myelin sheaths, leading to demyelination. Clinical

manifestations include visual loss, extra-ocular movement disorders, paresthesias, loss of sensation, weakness, dysarthria, spasticity, ataxia, and bladder dysfunction. The usual pattern is one of recurrent attacks followed by partial recovery but acute fulminating and chronic progressive forms also occur⁷⁶. Based on the observations in the SPL knockout mouse models, targeting SPL to reduce T-cell circulation might be an effective treatment for MS and other autoimmune diseases. Many studies have shown that FTY720 is highly effective in animal models of MS^{77, 78}, and phase II clinical trials in patients with relapsing MS are encouraging⁷⁹. Patients with relapsed MS who were treated FTY720 showed reduced number of gadolinium-enhanced lesions detected on magnetic resonance imaging scan. Moreover, 70% of patients with MS, treated with FTY720 were relapse-free even after three years, showing the advantage over current treatments for MS that reduce the relapse rate by only 30%. In addition, only transient and relatively mild side effects such as nasopharyngitis, dyspnea, headache, diarrhea, and nausea were observed⁷⁹. Thus, strategies targeting SPL may provide alternative or adjuvant therapy for MS and other autoimmune diseases.

Type 1 diabetes is a Th1-mediated autoimmune disease that results in destruction of pancreatic β cells. S1P and its analogs show protective effects on islet β -cells against cytokine-induced cell death⁸⁰. Treatment of a nonobese diabetic (NOD) mouse model of autoimmune type 1 diabetes with FTY720 has been shown to either prevent the onset of diabetes or cure spontaneous diabetes⁸¹. In addition, CD4⁺ T-cells from diabetic NOD mice are highly activated and secrete twofold more interferon- γ and interleukin-17 than nondiabetic lymphocytes. S1P or a S1P₁ receptor agonist inhibit CD4⁺ T-cell activation through regulation of hypoxia-inducible factor 1 α short isoform, resulting in decreased cytokine production⁸². The role of SPL in type I diabetes has not been investigated but could potentially be a therapeutic target in this widespread disease.

There is evidence that S1P signaling plays a role in inflammation, particularly in relation to secretion and function of pro-inflammatory cytokines TNF- α and interleukin-1. SphK1 is activated by TNF- α stimulation in monocytes and contributes to subsequent intracellular signaling, degranulation, cytokine production and activation of nuclear factor- κ B in these cells⁸³. Furthermore, knockdown studies revealed that both S1P and SphK1 are required for TNF- α -mediated induction of cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2) and activation of endothelial nitric oxide synthase⁸⁴. Concomitantly, knockdown of SPL or S1P phosphatase augmented COX-2 induction, leading to enhanced generation of PGE2. The S1P₁ receptor was found to be expressed in rheumatoid arthritis (RA) synovium, and S1P signaling enhanced synoviocyte proliferation⁸⁵. Inhibition of SphK1 by N, N-dimethylsphingosine reduced the levels of inflammatory cytokines in peripheral blood mononuclear cells from RA patients and inhibited disease severity, articular inflammation and joint destruction in a murine model of RA⁸⁵. An anti-inflammatory function of S1P has also been reported⁸⁶, wherein pretreatment of mouse macrophages inhibited LPS-induced cytokine production. Expression of SPL in lymphocytes is low, but other cells in inflammatory infiltrates express SPL²¹. This raises the possibility that SPL might regulate S1P levels and inflammatory cell functions.

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease often associated with autoimmune diseases. Increased *Sgpl1* expression has been reported in the skin of patients affected by AD⁸⁷. This finding might be related to inflammatory changes in the skin associated with the disease. Alternatively, changes in sphingolipid metabolism, including SPL upregulation, might be responsible for a breakdown in the skin barrier function, because the stratum corneum is enriched in ceramides that provide much of the barrier function of the epidermis⁸⁸. Interestingly, we have found significant expression *Sgpl1* in the sebaceous glands of normal murine skin (our unpublished results). Migration of Langerhans cells from the skin to the lymph node is an essential step in the pathogenesis of allergic contact dermatitis.

Therefore, inhibition of Langerhans cells-migration could be a promising strategy to improve AD. Interestingly, in a murine model of the disease, topical application of S1P and FTY720 inhibited the inflammatory reaction in the elicitation phase allergic contact dermatitis through inhibition of dendritic cell migration⁸⁹. These studies suggest that dysregulation of the S1P/SPL axis could be important in mediating multiple aspects of this disease and, conversely, may serve as an avenue for therapeutic intervention.

6.3 Role of SPL in vascular disease

S1P signaling through S1P₁ receptor is essential for vascular maturation during embryogenesis and contributes to pathophysiology of vascular diseases such as atherosclerosis, vascular permeability and tumor angiogenesis⁹⁰. Platelets were widely considered as the major source of plasma S1P levels³¹. However, recent findings show that erythrocytes and vascular endothelium are also major contributors of plasma S1P^{32,91}. Moreover, laminar shear stress downregulates the expression of SPL and S1P phosphatase in vascular endothelial cells. Furthermore, knockdown or overexpression of *Sgpl1* expression in endothelial cells directly affects intracellular S1P levels and subsequent release into the extracellular compartment, implicating a role for SPL in maintenance of vascular homeostasis⁹¹.

Disruption of vascular barrier integrity increases permeability to fluid and solutes and is the central pathophysiologic mechanism of many inflammatory disease processes, including inflammation, atherogenesis, sepsis and acute lung injury⁹². S1P regulates endothelial barrier function by activating S1P₁ receptor which triggers intracellular signaling that promotes adherens junction integrity and cytoskeleton organization⁹³. Treatment of mice with an S1P₁ receptor antagonist compromises vascular integrity in the skin and lungs⁹⁴. In addition, administration of intravenous S1P reduces acute lung injury caused by combined intrabronchial endotoxin administration or high tidal volume mechanical ventilation⁹⁵. Furthermore, Sphk1 plays a crucial role in regulating endothelial barrier function in lungs⁹⁶. In comparison to wild-type mice, Sphk1-deficient mice show markedly enhanced pulmonary edema formation in response to LPS and protease-activating receptor-1 activation, and endothelial barrier function is restored upon exogenous S1P treatment⁹⁶. Conversely, alveolar epithelial cells express S1P₃ receptors, and airway administration of S1P disrupts epithelial cell tight junctions, leading to pulmonary edema⁹⁷. Interestingly, SPL-deficient mice showed lesions in lungs and alveoli that contained variable sized irregularly shaped polygonal flakes and clumps of smooth homogenous material accompanied by increased numbers of alveolar macrophages⁴³. It was postulated that respiratory distress caused by pulmonary lesions was the cause of the reduced life span of SPL knockout mice. It might be possible that in the absence of SPL, high levels of extracellular S1P may play a direct role in the development of pulmonary lesions by compromising the integrity of the pulmonary epithelial barrier^{43, 97}.

Angiogenesis and vascular maturation are pivotal for wound healing. When tissues are wounded, damaged blood vessels recruit and activate platelets that release soluble mediators of vascular dilatation, permeability and cell proliferation. Endothelial cells also participate in wound healing through enhancement of cell proliferation, blood coagulation and angiogenesis⁹⁸. Diabetes impairs wound healing by impairing neo-vascularization, migration and proliferation of endothelial, keratinocytes and fibroblasts⁹⁹. Treatment of diabetic mice with S1P accelerated cutaneous wound healing¹⁰⁰. In addition, S1P₂ receptor is essential for wound healing response to acute liver injury¹⁰¹. Furthermore, embryonic fibroblast cells from SPL null mice demonstrate defects in migration in vitro^{37, 102}. These findings suggest the possibility that SPL could be a useful therapeutic target for diabetic wound healing.

6.4 Role of SPL in Muscle Biology

There is growing evidence that S1P regulates skeletal muscle differentiation and regeneration, myoblastic cell contraction and survival of cardiac myocyte^{103, 104}. Striated muscle possesses the full enzymatic machinery to generate S1P and expresses the transcripts of S1P receptors¹⁰⁵. Further, S1P acts as a trophic factor of skeletal muscle¹⁰⁶. *Drosophila Sply* mutants lacking SPL accumulate sphingolipid intermediates and develop a progressive myopathy in the thoracic muscles needed to power flight^{17, 19}. Although *Sgpl1* expression in the skeletal muscle is low at baseline, its expression is increased 3-fold in the gastrocnemius dystrophin-deficient mice¹⁰⁷. These studies suggest that SPL expression may be dynamically regulated in muscle and predict a role for SPL in muscle regeneration.

Cardiac myocytes predominantly express S1P₁ receptors, and activation of this receptor inhibits cAMP formation and antagonizes adrenergic receptor-mediated contractility¹⁰⁵. Expression of S1P₃ receptors is low on cardiac myocytes. These receptors were shown to be responsible for mediating the bradycardic effect of S1P agonists^{108, 109}. Cardiac smooth muscle cells express S1P₂ receptors, activation of which can induce coronary vasoconstriction¹¹⁰. Intriguingly, levels of circulatory S1P are abnormally high in patients with coronary artery disease¹¹¹. Conversely, activation of S1P signaling plays a cardioprotective role in response to acute ischemia/perfusion injury^{3, 112}. Sphk1 knockout mice demonstrate increased ischemia/reperfusion injury and respond poorly to ischemic pre- and post-conditioning^{3, 113}. However, the role of SPL in the cardiovascular system is largely unknown. SPL null mice developed cardiac lesions characterized by patchy to diffuse expansion of the interstitium by numerous variably sized vacuoles and vacuolated mesenchymal cells separated by cardiomyocytes⁴³. The physiological consequence of these changes has not been determined, but these findings suggest that SPL expression is important in cardiac tissue, even though baseline expression is very low. As in skeletal muscle, SPL may be induced in response to injury, a concept that will require further investigation.

6.5 SPL in neurodegenerative disease

Inappropriate induction of apoptosis has been proposed to underlie the progressive neuronal attrition associated with various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and other neurological disorders characterized by the gradual loss of specific populations of neurons. Alterations in sphingolipid metabolism have been reported in Alzheimer's disease patients^{114, 115}. Accumulation of ceramide and reduction in S1P levels in the brains of Alzheimer's patients shift sphingolipid metabolism in the direction of cell death. Consistent with this is the finding of upregulation in the mRNA expression levels of S1P degrading enzymes, SPL and phosphatidic acid phosphatase type 2B and ceramide synthase in the different regions of brain of Alzheimer's disease patients¹¹⁴. Additionally, upregulation of SPL expression was correlated with the progression of clinical dementia¹¹⁴. Furthermore, two single nucleotide polymorphisms were detected in the *Sgpl1* gene in late-onset Alzheimer's disease patients, which suggests that variation in *Sgpl1* expression and/or function may confer susceptibility to late-onset Alzheimer's disease¹¹⁶.

7.0 SPL inhibition by small molecules

Modulation of SPL activity through the use of small molecule inhibitors appears to hold promise for therapeutic purposes, particularly for immunomodulation in the treatment of autoimmune disorders and for transplantation, as well as for enhancement of wound healing and protection of normal tissues from various insults such as radiation, hypoxia and ischemia. Different pharmacological categories of SPL inhibitors have been described. First are the substrate analogs, including 1-desoxydihydrosphingosine-1-phosphonate¹¹⁷ and the 2D,3L-

isomer of DHS1P and 2-vinyldihydrosphingosine-1-phosphate^{117, 118}. The second group of SPL inhibitors includes pyridoxal 5'-phosphate analogs or compounds that inhibit the binding of the cofactor. Deoxypyridoxine acts as a competitive inhibitor of the enzyme and semicarbazide, cyanide, and bisulfite also inhibit the enzyme activity. The ceramide analog and dihydroceramide desaturase inhibitor N-[(1R,2S)-2-hydroxy-1-hydroxymethyl-2-(2-tridecyl-1-cyclopropenyl)ethyl]octanamide (GT11) also inhibits SPL activity, but it appears to do so only *in vivo*, suggesting that a metabolite of the compound may be the active inhibitor¹¹⁹. FTY720 is a modest inhibitor of SPL activity both *in vivo* and *in vitro*⁷². New and more specific agonists and antagonists of S1P receptor subtypes are also being developed, but their effects on SPL activity have not been reported. THI is a caramel food colorant that has been shown to inhibit SPL activity *in vivo*, and inhibition of the enzyme by THI causes potent immunomodulatory effects²⁰. Finally, SPL activity is also inhibited by divalent metal ions such as Ca²⁺ and Zn²⁺²³.

FTY720 has shown promise in preventing rejection with both standard and reduced cyclosporine exposure. However, the results of large multicenter clinical trials with FTY720 in renal transplant patients have been disappointing^{120–122}. Phase III clinical trial studies not only failed to show an advantage of FTY720 over standard immunosuppressive drugs but also demonstrate lower creatine clearance and increased risk of macular edema. FTY720 was associated with higher incidence of bradycardia and respiratory disorders¹²². Thus, there remains a need for additional approaches to modulating S1P-mediated effects on the immune system for therapeutic purposes. Despite the number of small molecules reported to inhibit SPL, none is specific and non-cytotoxic. However, Lexicon Pharmaceutical, Inc., The Woodlands, Texas, has developed an orally-delivered small molecule inhibitor of SPL, LX2931, which has completed Phase I clinical trial for the treatment of rheumatoid arthritis (Lexicon Pharmaceuticals, Inc. <http://www.lexicon-genetics.com/pipeline/lx2931-lx2932.html>). Preclinical studies with LX2931 showed a consistent reduction in circulating lymphocyte counts in multiple species. In addition, LX2931 reduced joint inflammation and prevented arthritic destruction of joints in mouse and rat models of arthritis^{123, 124}. These findings strongly suggest that SPL inhibitors could be a powerful addition to the arsenal of immunomodulatory therapy for autoimmune diseases.

8.0 Expert opinion

Alterations of S1P signaling and metabolism induce marked effects on various organ systems. S1P exerts its effects either by activating its cognate receptors or by initiating intracellular signaling pathways. There is growing evidence that S1P plays an important role in angiogenesis, immune function, inflammatory responses, wound healing, muscle regeneration, tumor invasion and metastasis. S1P levels in tissues and in the circulation are tightly maintained, as would be expected of a potent signaling molecule with such diverse functions. SPL appears to be an important gatekeeper for regulating sphingolipid homeostasis and S1P signaling.

SPL can exert its effects on physiological processes by depleting its substrate (S1P) or by forming the products (phosphoethanolamine and hexadecenal). The fate and importance of the trans-2-hexadecenal and phosphoethanolamine produced in SPL reaction are not well understood. Phosphoethanolamine produced in the SPL reaction regulates viability and differentiation of *Leishmania major*. Thus, SPL may be a target for anti-leishmanial antibiotic therapy. In addition, products of SPL reaction have shown to promote mammalian cell proliferation. However, the product responsible for these effects is not known. Due to the reactivity of the trans-2-hexadecenal, it is possible that it may react with other cellular components, including DNA and proteins and influence cellular function and may contribute

to the some of the effects of SPL upregulation. Studies addressing this will be important next steps in understanding the full impact of SPL on physiology and disease.

Future studies should also address whether SPL downregulation is causally related to intestinal tumorigenesis and other forms of neoplasia, and whether mutations of SPL are present in cancer specimens. Tumorigenesis studies with SPL null mice have been limited due to their reduced life span. The development of tissue-specific knockout mice and cell lines with inducible SPL expression or knockdown would provide an invaluable set of models with which to directly address whether SPL is an anti-oncogene. If SPL downregulation in cancer is a reversible phenomenon, it might be exploited as an adjuvant approach to enhance the therapeutic response of tumor cells to DNA-damaging agents. Conversely, SPL blockade might be used to transiently raise SIP levels in tissues for a variety of therapeutic purposes, such as for protecting reproductive function in patients that receive cytotoxic therapy and radiation, to enhance wound healing or stimulate heart muscle survival after ischemic insult.

SPL holds great promise as a therapeutic target for designing novel immunosuppressant drug for the prevention of allograft rejection, multiple sclerosis and treatment of T-lymphocyte-driven inflammatory skin diseases, such as lupus erythematosus, psoriasis, and atopic dermatitis. Clinical trials with LX2931 for rheumatoid arthritis appear promising. Identifying additional regulatory mechanisms that control SPL expression might reveal novel ways to pharmacologically reactivate this enzyme. Mechanisms responsible for transcriptional and postranslational control of SPL are beginning to emerge. It is noteworthy that *Sgpl1* has been identified as a target for a microRNA mir-125b in prostate cancer cells. While this remains to be validated, microRNAs present a unique small molecule approach to regulating gene expression for therapeutic purposes, especially in cancer. Similarly, postranslational modifications such as phosphorylation, nitrosylation and glycosylation of SPL are intriguing possibilities for pharmacological modulation. The topology and the catalytic site of the enzyme have been determined, but its tertiary structure has not been fully elucidated. Studies of SPL structure including its crystallization should expand our understanding of the molecular requirements for enzyme activity, facilitating the design of more specific and potent inhibitors. Our current knowledge of SPL's expression patterns and the phenotypes associated with its pharmacological modulation or genetic deletion in rodents, human pathogens and model organisms together illustrate the important role of SPL in physiology and its potential impact upon human disease. Advances in the molecular and biochemical understanding of SPL regulation and function should lead to the development of novel therapeutic strategies against human cancers, immunological disorders, vascular and neurodegenerative diseases.

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Figure 1.

Autocrine and paracrine actions of S1P and its degradation by SPL. S1P is cleaved by SPL to yield phosphoethanolamine and hexadecenal. S1P can exert its effects through autocrine (intracellular), paracrine (receptor-dependent) or through formation of its products. Hexadecenal is unstable and rapidly converted to fatty acid and fatty alcohol. Physiological importance of phosphoethanolamine and hexadecenal produced in the SPL reaction in mammalian system is not known.

Figure 2.

Potential role of SPL in human diseases. Sphingosine (So) is phosphorylated by sphingosine kinase (SK), yielding S1P. S1P can be recycled back to sphingosine by S1P phosphatase (SPP). Alternatively, S1P can be irreversibly degraded by SPL yielding phosphoethanolamine (EP) and hexadecenal (Hex). SPL has been implicated in various human diseases such as cancer, autoimmune, neurodegenerative, cardiovascular and infectious diseases. Inhibition of SPL activity by THI or novel inhibitor LX2931 prevents lymphocyte egress from thymus and secondary lymphoid organs, indicating SPL can serve as a potential drug target for immunomodulatory drug.