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Sphingosine-1-phosphate metabolism: A structural perspective

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Abstract

Sphingolipids represent an important class of bioactive signaling lipids which have key roles in numerous cellular processes. Over the last few decades, the levels of bioactive sphingolipids and/or their metabolizing enzymes have been realized to be important factors involved in disease development and progression, most notably in cancer. Targeting sphingolipid-metabolizing enzymes in disease states has been the focus of many studies and has resulted in a number of pharmacological inhibitors, with some making it into the clinic as therapeutics. In order to better understand the regulation of sphingolipid-metabolizing enzymes as well as to develop much more potent and specific inhibitors, the field of sphingolipids has recently taken a turn toward structural biology. The last decade has seen the structural determination of a number of sphingolipid enzymes and effector proteins. In these terms, one of the most complete arms of the sphingolipid pathway is the sphingosine-1-phosphate (S1P) arm. The structures of proteins involved in the function and regulation of S1P are being used to investigate further the regulation of said proteins as well as in the design and development of inhibitors as potential therapeutics.

Keywords

Sphingolipid metabolism; sphingosine kinases; sphingosine-1-phosphate receptors; sphingosine-1-phosphate; structural biology

Introduction

Sphingolipids were once thought to only serve in roles to provide integrity to the cell membrane or in energy metabolism (Hannun & Obeid, 2008). However, over the past three decades it has become clear that certain sphingolipids, termed bioactive sphingolipids, play important roles in both normal and pathological physiology. Sphingolipids can be produced

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via two separate mechanisms: (1) *de novo* synthesis, which starts with a condensation reaction between serine and palmitate or (2) the salvage pathway in which complex sphingolipids are metabolized to ceramide, the central hub of sphingolipids, via sphingolipid specific enzymes (Hannun & Obeid 2008). There are three well-studied bioactive sphingolipids: ceramide, sphingosine and sphingosine-1-phosphate (S1P). These three bioactive sphingolipids can all be inter-converted by specific enzymes (Figure 1). Changes in the levels of these bioactive sphingolipids have a diverse array of cellular effects which can affect disease progression and development.

In cancer, it has become clear that changes in sphingolipid levels as well as the levels of sphingolipid-metabolizing enzymes or their activities are critical in disease development and progression (Ogretmen & Hannun, 2004). In the context of cancer, ceramide is a tumor-suppressing lipid due to its ability to induce apoptosis (Obeid *et al.*, 1993), quiescence (Dbaibo *et al.*, 1995) and senescence (Venable *et al.*, 1995). On the other hand, S1P is understood to function as a tumor-promoting lipid through the stimulation of angiogenesis (Kimura *et al.*, 2000) and anti-apoptotic pathways (Cuvillier *et al.*, 1996). There have been numerous studies which implicate the importance of these lipids and the enzymes that produce and metabolize them in disease states such as cancer. As such, there have been a number of inhibitors and lipid-analogs studied for their potential therapeutic uses (Delgado *et al.*, 2006).

One area of sphingolipid research that could provide a critical perspective into sphingolipid-metabolizing enzymes is structural biology. Within the last decade, the number of available sphingolipid enzyme structures has been on the rise. There are atomic structures of bacterial homologues of serine palmitoyltransferase (Yard *et al.*, 2007), neutral ceramidase (Inoue *et al.*, 2009), sphingomyelinase (Ago *et al.*, 2006), the human ceramide transfer protein (Kudo *et al.*, 2008) and ceramide 1-phosphate transfer protein (Simanshu *et al.*, 2013). While the sphingolipid community has made much progress to date, there are still a number of key enzymes for which there is structural clarity missing, including: ceramidases, glucosylceramide synthase, ceramide synthases, ceramide kinase (CERK), sphingomyelin synthases, sphingosine kinase 2 (SK2), and ceramide- and S1P phosphatases. However, the sphingosine/S1P arm of the sphingolipid metabolism pathway has made significant structural advances and is the most complete so far with structures of sphingosine kinase 1 (SK1), the S1P transporter Apolipoprotein M, sphingosine-1-phosphate receptor 1 (S1PRs) and S1P lyase (SPL).

In this review, we will take a look at the progress made on understanding the metabolism and function of S1P from a structural viewpoint. We will cover the current understanding of the structures of the proteins responsible for S1P metabolism and function, insights into the mechanisms of these proteins, and how we can target these proteins pharmacologically for therapeutics purposes.

Sphingosine kinase: the sole source of S1P

There have been two isoforms of sphingosine kinase discovered to date in humans: SK1 and SK2 (Kohama *et al.*, 1998; Liu *et al.*, 2000a; Pitson *et al.*, 2000a). Over the past several

years, it has become apparent that these enzymes have many important roles including cell growth, survival (Olivera *et al.*, 1999) and angiogenesis (Anelli *et al.*, 2010). The SK1/SK2 double knockout mouse is embryonic lethal showing severe defects in development, including angiogenesis and neurogenesis (Mizugishi *et al.*, 2005). On the other hand, the SK1 knockout and the SK2 knockout mice do not show any obvious phenotype, suggesting there is at least a partial redundancy among these enzymes. Aside from the roles that SKs play in normal physiology, we are beginning to understand their complex role in other diseases such as autoimmune diseases and cancer. SK1 is over-expressed in a number of different cancers such as breast (Ruckhaberle *et al.*, 2008), colon (Kawamori *et al.*, 2006) and lung (Johnson *et al.*, 2005). In addition, there is a strong link between SK1 and inflammation (Snider *et al.*, 2010), which is now being recognized as a precursor to cancerous lesions. In this section, we will review the structure of SK1, SK1 biochemistry and discuss new and exciting opportunities for the development of SK1-targeted therapeutics.

The sphingolipid kinases (including SK1, SK2 and ceramide kinase) comprise a unique family of lipid kinases bearing little or no resemblance to protein kinases; it has been suggested that this family of kinases is distantly related to the phosphofructokinase-like (PFK-like) superfamily (Cheek *et al.*, 2005; Labesse *et al.*, 2002) and the diacylglycerol kinases (Pitson, 2011; Wattenberg *et al.*, 2006). Wang and colleagues have published the long-awaited structure of SK1 and indeed show that there is overall structural homology between diacylglycerol kinases and nicotinamide adenine dinucleotide kinases. Among the sphingolipid kinases, there are five conserved motifs denoted C1–C5 (Kohama *et al.*, 1998; Sugiura *et al.*, 2002; Taha *et al.*, 2006a). Among these motifs, the C4 domain varies the most between SK1, SK2 and CERK, suggesting C4 is the motif responsible for substrate specificity.

Overall architecture

The overall architecture of SK1 is bi-lobal, with N- and C-lobes (Figure 2A) consisting of 15 β -sheets, 10 α -helices and a 3_{10} helix. The N-lobe harbors the ATP-binding site (Figure 2A and B), whereas the sphingosine-binding pocket is buried within the C-lobe (Figure 2A–D). The overall fold of the N-lobe is similar to the Rossmann fold of a three-layer $\alpha/\beta/\alpha$ sandwich. The C-lobe is comprised an 11-strand anti-parallel β -sheet flanked by helices $\alpha 7$ – $\alpha 9$ and one 3_{10} helix. The catalytic cleft sits between the N- and C-lobes and is where the catalytic motif (S/G)GDG is positioned. This catalytic motif is conserved among the PFK-like superfamily and partially conserved with the glycine-rich loops of protein kinases (Pitson *et al.*, 2002).

Nucleotide binding site and catalysis

Among the five linear motifs conserved between SKs, motifs C1–C3 are located within the N-lobe of SK1, whereas motifs C4 and C5 are in the C-lobe. A number of studies have identified residues important for ATP and GTP (Pitson *et al.*, 2002) binding. Structural studies reveal that all of the motifs, C1–C5, contribute to ADP binding (Figure 2B). R24, T54, E86 and E343 stabilize the adenine and ribose groups through direct hydrogen-bonding with additional stability from π -stacking interactions with the side chain of N22 and the

backbones of E55 and R56 (Figure 2B). A Mg^{2+} ion, coordinated by D341 and E343, in combination with seven residues (S79, G80, G82, S112, G113, R185 and R191) helps to orient the α - and β -phosphate groups stereochemically (Figure 2B).

In SK1, mutation of G80 or G82 of the conserved (S/G)GDG motif to aspartate results in catalytically inactive mutants (Pitson *et al.*, 2000b, 2002). This is likely due to the electrostatic repulsion of negatively charged phosphate groups, as both G80 and G82 make hydrogen-bonds with ADP (Figure 2B). Wang *et al.* (2013) suggest that the conserved sequence of (S/G)GDG contains the catalytic residue D81 of SK1, by modeling ATP into the SK1/sphingosine co-crystal structure and demonstrate by site-directed mutagenesis that the D81A mutation obliterates SK1 activity. D81 is thought to act as a general base catalyst by deprotonating the primary hydroxyl thereby priming sphingosine for the transfer of the γ -phosphate from ATP. Recently, however, molecular modeling of inhibitors bound to SK1 has suggested D178 is the catalytic residue (Baek *et al.*, 2013a). Baek *et al.* propose that D178 is the deprotonating base catalyst since the inhibitors were predicted to interact with D81 but were not substrates. A structure of SK1 in complex with both sphingosine and a non-hydrolysable analog of ATP would provide invaluable information on the exact mechanism of S1P generation.

The current structure of SK1 can now explain some of the previously published biochemistry of SK1 mutations. Stahelin *et al.* (2005) report that mutations of T54 and N89 to alanine resulted in mutants with reduced activity in cells upon stimulation with 12-phorbol 13-myristate acetate. These mutations were originally identified as being important for SK1 activation after addition of a stimulus. The structure depicts Thr54 as a key residue for ATP binding through two hydrogen bonds with the adenine ring of ATP. N89 does not make any apparent interactions with either ATP or sphingosine, although it does hydrogen bond to one of the β -strands in the C-lobe. Mutation of N89 to alanine might disrupt the stability of the two lobes of SK1 making the transfer of the γ -phosphate more difficult.

Other inactivation mutations include the mutation of G111 and G113 to aspartate (Pitson *et al.*, 2001, 2002). G113 along with S112, the residue between G111 and G113, is shown to hydrogen bond with the β -phosphate of the bound ADP in the structure, in what appears to be aiding in the proper positioning of the γ -phosphate. Therefore, mutation of this segment to a negatively charged amino acid (such as aspartate or glutamate) will result in electrostatic repulsion of the phosphate groups interfering with ATP binding. Interestingly, mutation of G113 to alanine resulted in an increase in SK1 activity, which suggests that the hydrogen bonding to G113 can accommodate a small side chain without interfering with said hydrogen bond. To define an exact mechanism for this activation will require additional research.

Another catalytically inactive mutation, K103A, has been reported to be in the ATP binding site of SK1 and was identified by affinity labeling with the ATP analog 5-*p*-fluorosulfonyl-benzoyl-adenosine (FSBA) (Pitson *et al.*, 2002). The positioning K103 directly opposite of the ATP-binding site indicates that it is not involved in ATP binding. K103 is surface exposed and would be capable of binding to FBSA which might explain why it was labeled with FSBA. While we can speculate that K103 might be indirectly involved through some

kind of bonding network, exactly how the K103A mutation is causing catalytic inactivity warrants further investigation.

Sphingosine binding site

Another feature revealed by the structural elucidation of SK1 is a deep, well-defined, “J-shaped” hydrophobic pocket that binds sphingosine (Figure 2C). The distinct contour of this pocket accommodates the substrate in a bent conformation. As evidenced by previous studies the substrate-binding pocket of SK1 is comprised, in part, by the less-conserved C4 domain as evidenced in previous studies (Taha *et al.*, 2006a; Yokota *et al.*, 2004). Since there was no substrate added to any of the crystallization experiments, it was surprising to find a sphingosine-like substrate bound within the sphingosine-binding pocket. Unfortunately, the authors were unable to identify whether the bound lipid was sphingosine or the lipid tail of detergent used during the purification (Wang *et al.*, 2013). There have been a couple of key residues identified that are responsible for the recognition of sphingosine. Yokota *et al.* (2004) show that D178 (D177 in mouse) is important for the recognition of sphingosine by SK1 as suggested by the decrease in Michaelis–Menten kinetics upon mutation. D178 has been substantiated by the structure to be involved in binding sphingosine which is discussed in the following section (Wang *et al.*, 2013).

The defining mark of sphingolipids, the 2' amine group, is recognized by S168 through hydrogen bonding. Surprisingly, upon the introduction of the S168A mutation there is only an overall 4% decrease in SK1 activity in the presence of lipid vesicles (Stahelin *et al.*, 2005). This phenomenon might be explained by the overwhelming number of favorable van der Waals interactions between the carbon backbone of sphingosine and the hydrophobic residues lining the pocket. The sphingosine binding pocket, beyond the binding site for the polar headgroup, is lined with a number of hydrophobic/non-polar residues including: 7 leucines, 4 phenylalanines, 4 alanines, 2 valines, 2 methionines and 1 isoleucine (Figure 2D).

SK1 inhibitors

SK1 has emerged as a potential therapeutic target in cancer as there is an increase in protein level as well as enzymatic activity in a number of different cancers. Prior to elucidation of the SK1 structure, many of the available inhibitors were sphingosine analogs along with some non-lipid inhibitors. The major problems associated with these early inhibitors were a lack of specificity and potency. Some of the first SK inhibitors were sphingosine analogs including D,L-threo-dihydrosphingosine (safingol) (Buehrer & Bell, 1992), dimethylsphingosine (DMS) (Igarashi & Hakomori, 1989), FTY720 (Fingolimod) and its daughter compound (*S*)-FTY720-vinylphosphonate (Tonelli *et al.*, 2010), and SK1-I (Paugh *et al.*, 2008).

Along with inhibition of SK1 activity (from whole platelets) with an IC₅₀ of ~13 μM (Buehrer & Bell, 1992), safingol has been shown to inhibit a host of protein kinase C isoforms as well as inhibit the phosphorylation of several growth associated pathways (Coward *et al.*, 2009). With that being said, safingol has been used in a phase I clinical trial for the treatment of solid tumors (Dickson *et al.*, 2011). In addition to inhibiting SK1, DMS

has been shown to inhibit both SK2 (Liu *et al.*, 2000a) and CERK (Sugiura *et al.*, 2002) making it difficult to decipher the role of SK1. On the other hand, SK1-I has been shown to be an isozyme specific SK1 inhibitor that does not inhibit SK2 (Paugh *et al.*, 2008). FTY720, after it becomes phosphorylated by SK2 to become FTY720-P, was ultimately identified as a functional antagonist of sphingosine-1-phosphate receptors (S1PRs) (Graler & Goetzl, 2004; Oo *et al.*, 2007) (further discussed below) and is used in the clinic for the treatment of multiple sclerosis (MS; Willis & Cohen, 2013). However, it has been shown that SK1 can be competitively inhibited by FTY720 (Lim *et al.*, 2011). In addition, the FTY720 family of SK1 inhibitors is able to cause the proteosomal degradation of SK1 (Tonelli *et al.*, 2010). These inhibitors have been extensively reviewed elsewhere (Orr Gandy & Obeid, 2013).

Additional non-lipid SK inhibitors have been identified, including SKI-II (French *et al.*, 2003), PF-543 (Schnute *et al.*, 2012), SK1-178 (Hengst *et al.*, 2010) and RB-005 (Baek *et al.*, 2013b). Following the elucidation of the SK1 structure, there has been an explosion of new molecules identified using structure-guided design and computational modeling (Baek *et al.*, 2013a; Gustin *et al.*, 2013). To date, three non-lipid small molecules, all sphingosine-competitive inhibitors, have been co-crystallized with SK1 (Figure 2E): SKI-II (magenta sticks), PF-543 (cyan sticks) and compound 23 (yellow sticks).

SKI-II was identified from a screen of library compounds and was found to inhibit SK1 in a non-ATP competitive manner with an approximate IC_{50} of 500 nM (French *et al.*, 2003). This compound was shown to have anti-tumor activity as it was able to reduce tumor volume in mice (French *et al.*, 2006). SKI-II has recently been crystallized with SK1 and shown to be bound at the bottom of the sphingosine-binding pocket making van der Waals interactions with several hydrophobic residues (Wang *et al.*, 2013). It also makes a hydrogen bond with D178 and interacts with the hydroxyl group of T196 (Wang *et al.*, 2013).

The other two co-crystallized compounds, PF-543 (Figure 2E, cyan) and compound 23 (Figure 2E, yellow) take full advantage of the entire sphingosine binding pocket. PF-543 takes advantage of numerous van der Waals interactions but unlike SKI-II has additional interactions in the head-group binding region that confer its potency. In addition, it makes hydrogen bonds with D264 (equivalent to D178 in human SK1 variant 1). These characteristics make PF-543 the most potent and specific of any of the SK1 inhibitors identified to date. PF-543 has an IC_{50} in cells of about 1 nM and is more than 300 times more selective for SK1 than SK2 (Lynch, 2012; Schnute *et al.*, 2012). Use of this inhibitor has produced some controversial results in the field due to recent papers, which suggest SK1 is not required to maintain tumor cell viability (Rex *et al.*, 2013; Schnute *et al.*, 2012). Treatment of cells with PF-543 does not increase endogenous ceramides (Schnute *et al.*, 2012) which is intriguing because it would be expected that inhibition of SK1 would result in a concomitant increase in ceramide levels. It is possible that inhibition of SK1 by PF-543 does not affect any biological processes because it does not possess desirable pharmacokinetic properties which are required for the ablation of said biological activity. Another possibility is that SK1 might play a role in the cell that is independent of its activity, since there is a survival and growth phenotype for the SK1 knockdown in cell lines (Taha *et*

et al., 2006b). The exact reason for the discrepancy between the inhibition and knock-down of SK1 remains unclear and warrants further investigation.

Compound 23 was identified from a structure-based series of SK inhibitors by Gustin *et al.* (2013). In a similar fashion to the other two co-crystallized inhibitors, compound 23 assumes a bent conformation (in a similar fashion binding configuration as sphingosine) to fit within the shape of the sphingosine-binding pocket. Analogous to the PF-543 binding mechanism, compound 23 interacts with the hydrophobic residues and D178 (Gustin *et al.*, 2013). In contrast, however, compound 23 hydrogen bonds with D81 and participates in the water-mediated hydrogen-bond network (Figure 2D) (Gustin *et al.*, 2013). A number of compounds from this series are specific for SK1 and have favorable pharmacokinetic and pharmacodynamic properties that show promise from therapeutic purposes (Gustin *et al.*, 2013).

There are plenty of opportunities for structural studies of SK1. Based on previous structural studies it was suggested that upon substrate binding there is movement within SK1 (Wang *et al.*, 2013). It would be easy to hypothesize that there are different conformations that SK1 can take and that these conformations might be related to its activation. Nuclear magnetic resonance studies are necessary to understand the mechanisms underlying SK1 activation and substrate binding. Further work to understand membrane binding and development of novel mechanisms of inhibition (such as bi-substrate competitive inhibitors) will be imperative in understanding how SK1 works and how to abrogate its activity in disease.

S1P transportation

S1P uses two mechanisms to elicit its physiological responses: (1) it is exported out of the cell and transported to elicit a paracrine (or autocrine) effect or (2) it can bind to intracellular targets and elicit a response. Since the latter is not a well understood mechanism, this section will focus on the former (export and transportation). S1P is a charged lipid and therefore cannot freely diffuse across the membrane as the phosphate will not cross the hydrophobic interior. In order for S1P to be exported out of the cell, it requires the assistance of a number of transporters. The first hint of transporter mediated S1P export was reported in rat platelets. Although the authors were unable to definitively identify the transporter, they suggested that it was most likely an ATP-binding cassette (ABC) transporter (Kobayashi *et al.*, 2006).

S1P transporters

Several members of the ABC transporter superfamily have been reported to be involved in the export of S1P. S1P release from both human and rat mast cells has been shown to be independent of degranulation and dependent on ABCC1 protein and activity (Mitra *et al.*, 2006). In the central nervous system, specifically in astrocytes, S1P release is coupled to both the formation of lipoprotein and ABCA1 (Sato *et al.*, 2007). In addition, in rat erythrocytes it has been suggested that ABCA1 is involved in S1P export as the release of S1P can be reduced by the ABCA1 transporter inhibitor glyburide. However, S1P transport was not affected by MK571, a multidrug resistance-associated protein inhibitor, or cyclosporine A, the multidrug resistance protein inhibitor (Kobayashi *et al.*, 2009). In breast

cancer cells, estradiol stimulates SK1 activity and S1P export via both ABCC1 and ABCG2 (Takabe *et al.*, 2010). In a recent paper, it was shown that S1P transporters including ABCA1, ABCB1, ABCC1 and Spns2 are upregulated and can lead to chemoresistance in SPL-deficient mouse embryonic fibroblasts (Ihlefeld *et al.*, 2015). Interestingly, while these ABC transport proteins can transport S1P out of cells, their downregulation does not decrease the S1P levels in plasma (Lee *et al.*, 2007). Another ABC transporter, the cystic fibrosis transmembrane regulator (CFTR), has been reported to be involved in the uptake of S1P, dhS1P and lysophosphatidic acid (Boujaoude *et al.*, 2001). A common mutation found in CFTR in cystic fibrosis patients, the deletion of phenylalanine 508, was shown to reduce the amount of S1P taken into the cell (Boujaoude *et al.*, 2001).

Spns2 has also recently been reported as a major transporter for S1P in cells. Spns2 was originally identified in zebrafish as a S1P transporter (Kawahara *et al.*, 2009; Osborne *et al.*, 2008). Spns2 plays an important role in the immune system as it creates the necessary S1P gradient for the proper egression of T and B cells (Fukuhara *et al.*, 2012). In Spns2 mutant mice, which express a prematurely terminated mRNA, it was found that the S1P levels in lymph were decreased significantly, whereas in the blood S1P levels were only slightly lower (Mendoza *et al.*, 2012). In contrast, Spns2 knockout mice show reduced levels of S1P, and dihydrosphingosine-1-phosphate (dhS1P), in blood and plasma (Nagahashi *et al.*, 2013). In non-small cell lung cancer, the knockdown of Spns2 by siRNA reduced the mRNA expression of S1PRs and SPL as well as increased migration of the A549 cell line (Bradley *et al.*, 2014).

Pharmacologically targeting the ABC transporters and/or Spns2 might prove to be a viable method of inhibiting the binding of S1P to its receptors or intracellular effector proteins if uptake is inhibited. In cancer, these inhibitors could serve as anti-angiogenic treatments as S1P has been shown to be an inducer of angiogenesis and lymphangiogenesis (Anelli *et al.*, 2010). In addition, as S1P has many downstream effects including cell growth and migration inhibition of the exporters might be an effective way of inhibiting the “inside-out signaling” of the SK1/S1P axis. Currently, there are no structures of any of the transporters that export S1P from the interior of cells (although there are structures of the nucleotide binding domains of a few of the ABC transporters). The elucidation of these structures will surely guide rational drug design efforts as well as provide insight into the underlying mechanisms of their action.

S1P transport in the blood stream

Once S1P has been exported from cells it must bind apolipoproteins and albumin in order to be transported effectively in the blood stream. The fraction of plasma which contains the largest portion of S1P is the high density lipoprotein (HDL) fraction with more than 50% of plasma S1P (Murata *et al.*, 2000). One of the lipoproteins associated with HDL is apolipoprotein M (ApoM) (Christoffersen *et al.*, 2008; Xu & Dahlback, 1999). ApoM has been shown to bind to multiple lipids including myristate and glycerol-1-myristate (Sevvana *et al.*, 2009). However, using the intrinsic fluorescent properties of ApoM, Sevvana *et al.* (2009) were able to show that S1P was the best lipid for quenching the fluorescence. This result suggested that S1P has a better binding affinity compared to other lipids.

Christoffersen *et al.* (2011) solved the crystal structure of ApoM in complex with S1P (Figure 3A and B). The structure revealed how the headgroup of S1P is recognized via direct hydrogen binding with R98, W100 and R116. Additional interactions are provided by a complex water-mediated hydrogen-bond network (Figure 3C). Recently, it was found that the Q22A mutation in ApoM resulted in increased secretion of S1P and dhS1P from hepatocytes (Liu *et al.*, 2015). The Q22A mutation introduces a cleavable signal peptide which, once cleaved, increases the secretion rate of ApoM^{Q22A}. The authors conclude that the uncleavable ApoM is the rate-limiting step in the formation of large S1P-enriched HDL particles.

Since S1P is a potent chemoattractant for endothelial cells it is possible that targeting S1P-containing ApoM might provide yet another avenue for anti-angiogenic therapeutics by inhibiting the delivery of S1P within the blood stream. However, there are reports that suggest S1P has atheroprotective effects (reviewed in Poti *et al.*, 2014). Therefore, there is a need for further research into the effects of ApoM inhibition in cancer models as well as in cardiovascular disease models to dissect the roles played by the ApoM/S1P axis in both types of diseases.

S1P signaling: the S1P receptor

There are five mammalian S1P receptors, S1P₁₋₅, of which the structure of S1P₁ has been determined and will be discussed in detail in this section. The S1P receptors are cell-surface class A G-protein coupled receptors (GPCRs), which bind and are activated by the endogenous ligand S1P. The five S1P receptors each couple to different intracellular G-proteins, leading to regulation of different responses, including cell proliferation, apoptosis, cell adhesion, cell motility, angiogenesis, inflammation and immunity (Adada *et al.*, 2013).

S1P₁

S1P₁ is the only receptor with evident non-redundant functions; S1P₁ deletion proves to be embryonic lethal, whereas deletion of other S1P receptors is not (Liu *et al.*, 2000b). Strong evidence has shown S1P₁ to be involved in a number of important physiological functions, making it a pertinent therapeutic target. In particular, S1P₁-mediated signaling is essential for the egress of T and B cells from the thymus and secondary lymphoid tissues (Matloubian *et al.*, 2004). Activation of S1P₁ by the phosphorylated form of FTY720 (FTY720-P) has been shown to cause receptor internalization and degradation, ultimately leaving the receptor unresponsive to S1P signaling (Brinkmann *et al.*, 2004; Lamontagne *et al.*, 2006). Hence, treatment with FTY720 results in lymphocyte sequestration in lymphoid bodies, causing immunosuppression (Galicía-Rosas *et al.*, 2012). FTY720, also known as Fingolimod or Gilenya^{TM}, is currently used for treatment of relapsing-remitting MS (Yeh & Weinstock-Guttman, 2011). It acts as a pro-drug modulator and is rapidly phosphorylated by SK2 to produce a functional antagonist for S1P₁ (Zemann *et al.*, 2006). However, the same drug is an agonist for receptors S1P₃, S1P₄ and S1P₅ causing off-target side effects, creating a strong demand for an improved S1P₁ antagonist treatment.

S1P₁ crystallization and structural determination

The structure of S1P₁ was determined in 2012 as part of the widespread effort to increase representation of GPCRs in the protein data bank (PDB), in order to elucidate common mechanisms of ligand recognition and receptor activation (Hanson *et al.*, 2012). The problematic hydrophobic nature of S1P₁ in crystallization was overcome through the addition of a T4-lysozyme fusion, which introduces suitable crystal contacts and vastly facilitates the crystallization process (Rosenbaum *et al.*, 2007). In addition, a C-terminal truncation was introduced to the S1P₁ construct to prevent oligomerization and maintain the receptor in its monomeric form throughout purification and crystallization (Rosen *et al.*, 2013). The receptor was co-crystallized in complex with the sphingolipid mimic antagonist ML056. The apoprotein form of the S1P₁ receptor was found to be unstable, and ML056 was found to have the greatest stabilizing effect of a number of ligands tested (Rosen *et al.*, 2013).

The data set reached a resolution of 3.35 Å using traditional data processing (PDB 3V2W), and 2.8 Å using pioneering microdiffraction data assembly (MDA) methods (PDB 3V2Y). Crystallization of GPCRs commonly generates microcrystals, which are susceptible to rapid radiative decay in the microbeam upon data collection. In the traditional data processing pipeline, data sets are merged from multiple microcrystals and data are included in processing following indexing and integration based on isotropic cut-offs of descriptive statistics. This results in the exclusion of large amounts of data solely due to poor merging statistics. The novel MDA methods allowed for inclusion or exclusion of individual reflections based on their correlation to established peak profiles, enabling higher-resolution structural determination. In the case of S1P₁, the use of these MDA methods made it possible to reveal the most interesting structural aspects of the receptor.

S1P₁ structural features

The overall topology of the S1P₁ receptor is consistent with the known structures of typical GPCRs. S1P₁ is composed of a seven transmembrane (7TM) helical bundle, in which the transmembrane helices are annotated I–VII (Figure 4A). Three extracellular loops ECL1, 2 and 3 are formed between helices II and III, IV and V, and VI and VII, respectively (Figure 4A). There are, however, some striking unique features of S1P₁, which reflect its lipid-binding function.

The positioning of the extracellular N-terminal tail of S1P₁ is distinct in that it forms an ordered α -helix that packs tightly onto ECL1 and ECL2 to form a cap over the top of the receptor. The helical cap is precisely oriented such that the *N*-acetyl glucosamine of N16 protrudes perpendicularly from the receptor (Figure 4A) surface into the extracellular environment. In addition, ECL1, 2 and 3 display particular secondary structure elements; ECL2 and 3 are held in tight hairpin bends by disulfide bonds, while ECL1 forms a small α -helix (Figure 4A). The positioning of the N-terminal cap and the features of ECL1, 2 and 3 all serve to limit extracellular access to the ligand-binding pocket. Furthermore, helices I and II are observed to be relatively shifted toward helix III, leaving an opening between helices I and VII (Figure 4B). These structural features provide insight into the mechanism of S1P receptor binding and suggest that S1P is first deposited into the membrane bilayer from a

circulating pool in which it is in complex with HDL cholesterol/ApoM and albumin. S1P will likely need to be embedded into the outer leaflet of the membrane, once it is there it may move laterally to gain direct access to its receptor binding pocket (Hanson *et al.*, 2012; Mandala *et al.*, 2002; Murata *et al.*, 2000).

S1P₁ ligand-binding pocket

The endogenous receptor ligand S1P is highly hydrophobic-zwitterionic, owing to its extended acyl tail and its headgroup composed of an amine and phosphate group. This is reflected in the amphipathic nature of the receptor's ligand binding pocket. The primary amine and phosphonate groups of the S1P antagonist ML056 mimic the zwitterionic headgroup of S1P, producing equivalent contacts in the S1P₁ receptor ligand-binding pocket (Hanson *et al.*, 2012). Receptor residues N101 and E121 are seen to form ionic interactions with the primary amine of ML056, while R120 interacts with the agonist phosphonate group (Figure 4C). In addition, positive and polar residues from helices III and VII, ECL2, and the N-terminal helical cap of the receptor surround the ML056 phosphonate group (Figure 4C). Together, these interactions amass a high-affinity binding site for ML056 or S1P (Hanson *et al.*, 2012). Prior to publication of the structure by Hanson *et al.*, S1P₁ was previously modeled using a 9-Å structure of bovine rhodopsin as a template, into which S1P was subsequently docked to elucidate the ligand binding site on S1P₁ (Parrill *et al.*, 2000). This homology modeling was able to correctly identify E121 and R120 as critical ligand-binding residues, confirmed by the ML056 co-crystal structure (Figure 4C). It also predicted R292 would form a key interaction with the phosphate headgroup of S1P. These three previously predicted interacting residues have been shown to be critical for receptor response to S1P (Jo *et al.*, 2005). In the S1P₁ complex with ML056, however, R292 does not form any evident ligand interaction (Hanson *et al.*, 2012). It has been hypothesized that R292 plays a role in extracting S1P from the membrane bilayer, which is consistent with its position at the proposed entry point of S1P from the membrane bilayer (Figure 4B) (Parrill *et al.*, 2012; Rosen *et al.*, 2013).

Hanson *et al.* suggest that residues N101, Y29 and K34 are involved in important ligand-binding interactions in S1P₁. This suggestion is based on their proximity (<4 Å) to the ML056 headgroup in the 2.8-Å structure of the ligand-binding pocket (Figure 4C). Hanson and colleagues then pursued this prediction by determining EC₅₀ values for S1P-induced Erk phosphorylation using cells transfected with mutant forms of S1P₁. The N101A mutation was shown to cause an increase in the EC₅₀, which is consistent with previous EC₅₀ analyses for mutations to N101 in S1P₁ (Parrill *et al.*, 2000). Mutations Y29A and K34A were only able to affect EC₅₀ significantly when introduced together as a double mutation (Hanson *et al.*, 2012). Although these mutations are seen to increase the EC₅₀, it is worth noting that they still maintain EC₅₀ values in the nM range (compared to the low nM EC₅₀ for wild-type S1P₁). Considering that mutation of R120 or E121 causes almost a complete loss of ligand binding (resulting in EC₅₀>1 mM) (Parrill *et al.*, 2000), it is likely that the newly identified residues merely contribute to ligand binding, but are not critical.

Prior to 2012, there had been little insight into the portion of the ligand-binding pocket responsible for binding the acyl tail of S1P. The phenyl-acyl tail of ML056, which mimics

the acyl chain of S1P, is precisely held in an extended conformation within a hydrophobic tunnel of the ligand-binding pocket. Helices III, V, VI and VII, and ECL2 all contribute residues toward lining this selective tail binding site. The structure shows short aliphatic residues defining the shape of the pocket, while four aromatic residues (F125, F210, W269 and F273) provide the specific interactions required for ligand tail binding (Figure 4D). In particular, W269 of helix VI located at the base of the binding pocket provides crucial ligand-binding interactions, as well as a hypothesized rotamer toggle mechanism for S1P₁. Such a mechanism has been proposed based on molecular dynamics simulations (Yuan *et al.*, 2013). Accordingly, S1P binding causes W269 to flip between rotameric states, altering the conformation of the neighboring residue F265. These residues make up the center of a transmission switch, which redirects the flow of water molecules inside the receptor (Foss *et al.*, 2007). Ultimately, this results in an opening of the cytoplasmic portion of S1P₁, leaving the receptor available for G-protein binding.

Antagonism versus agonism

Docking studies carried out by Hanson *et al.* demonstrate that ligands that bind to S1P₁ function either as an agonist or antagonist based on minor differences in their structures, which translates to activating/deactivating shifts in critical receptor residues. Overall, the activating effect of a ligand on S1P₁ is dependent on the length and position of its acyl chain in the ligand-binding pocket with respect to its amine and phosphate/phosphonate headgroup. Interestingly, the S1P₁ structure shows that the antagonist ML056 is entirely in its meta conformation. Hanson and colleagues observed that switching the conformation pattern from meta to para switches the ligand effect from antagonism to agonism. Hanson *et al.* performed induced-fit docking of ligands into the S1P₁ hydrophobic binding pocket based on this conformational switch. Their method allows for shifts in the side chains of residues lining the tail-binding portion of the pocket in response to the meta-to-para switch, while keeping the ligand headgroup in a fixed position.

In addition to the conformation of ligand, the volume of the ligand-binding pocket that is occupied also plays a critical role in receptor activation. An increase in length of the acyl chain of a ligand effectively switches its effect from antagonism to agonism. ML056 variants extending the acyl chain up to three additional methylenes can be accommodated in the binding pocket with no significant side chain shifts. However, once the total number of methylenes in the acyl chain reaches 10 (i.e. ML056 extended by four methylenes), the ligand reaches the point at which significant positional shifts are needed in the binding pocket residues to accommodate the ligand. The largest positional shift was observed in F273, with lesser shifts also observed in W269 and F125; together these shifts expanded the ligand-binding pocket to fit the docked ligand. The expanded pocket is also able to accommodate S1P and other antagonists FTY720-P and SEW2871. Hence, Hanson *et al.* predict that these residues are important in receptor activation. Although significant insight has been gained through the S1P₁-ML056 structure and ligand docking studies, structural determination of S1P₁ in complex with an agonist is needed to confirm these predictions and fully understand the mechanism of receptor activation.

S1P₁ ligands

Hanson *et al.* propose that S1P₁ ligands can be segregated into two classes. Class I ligands can be either lipid-like S1P mimics (e.g. FTY720-P), or non-lipid-like molecules possessing a polar headgroup in place of the zwitterionic one (e.g. SEW2871) (Jo *et al.*, 2005). Class II ligands (e.g. CYM-5442) are bitopic molecules which lack any interactions with the headgroup-binding region of the ligand-binding pocket. They were identified from a group of library screening hits that do not exhibit interactions with R120 or E121, yet still signal through S1P₁ (Gonzalez-Cabrera *et al.*, 2008). Mutagenesis identified F210, F265 and W269 as the critical residues involved in class II ligand binding (Hanson *et al.*, 2012). F210L, F265L and W269L mutations were seen to abolish CYM-5442 binding to S1P₁, whereas they had only a minimal effect on S1P and FTY720-P binding. Interestingly, substitution of phenylalanine or tyrosine at W269 restored some CYM-5442 binding, indicating that class II ligands depend on the presence of aromatic residues in the binding site (compared with the requisite hydrophobicity for S1P and class I ligands). This dependence on aromatic residues makes class II ligands more dependent on a precise shape complementarity with the ligand-binding pocket than class I ligands.

All five S1P receptors show a complete conservation of all predicted headgroup-binding residues, which is reflected in the lack of selectivity of class I ligands which rely primarily on these interactions for their affinity. This is a real problem in the clinical use of FTY720, since there are significant adverse side effects attributed to the off-target activation of other S1P receptors, such as bronchoconstriction and cardiovascular dysfunction (Behjati *et al.*, 2014; Chiba *et al.*, 2010). Conversely, S1P receptors display significant distinctions in the acyl tail region of the hydrophobic ligand-binding pocket, making class II ligands a promising source of S1P₁-selective molecules. For example, S1P₃ has F263 in the equivalent position to L276 of S1P₁, and S1P₂ has F86 in place of Y98; these bulky residues introduce steric clashes with the aromatic rings in CYM-5442, preventing ligand binding. S1P₄ has L125 in place of M124 of S1P₁, which constricts the ligand-binding pocket at a critical point, preventing binding of the indole ring of CYM-5442. These structural details are reflected in the selectivity and efficacy of CYM-5442 as a S1P₁ inhibitor and immunosuppressant *in vivo* (Gonzalez-Cabrera *et al.*, 2008). This has led to a surge in second-generation S1P receptor ligands that are currently being developed based on class II ligands. CYM-5520 is a class II ligand selective toward S1P₂, and CYM-5541 is selective for S1P₃ (Jo *et al.*, 2012; Satsu *et al.*, 2013). Interestingly, the entire ligand-binding pocket of S1P₅ is very similar to that of S1P₁, which is reflected in the lack of ligands that are able to distinguish between the two receptors.

Degradation: S1P lyase

Levels of S1P are tightly controlled in part by its synthesis by SK, and in part by its degradation. The latter can occur via two pathways: it can be dephosphorylated back to sphingosine through the action of S1P phosphatases, or it can be irreversibly degraded by SPL to phosphoethanolamine (PE) and hexadecenal (Figure 1). While the structure of SPL has been well-characterized, that of S1P phosphatase has yet to be determined, and in fact

there is very little biochemical data to identify structural features in this enzyme. Further work is needed on S1P phosphatases to elucidate this step in S1P regulation.

The SPL structure has been determined for the human (PDB ID 46QR), yeast (Dpl1p: PDB ID 3MC6) and bacterial (StSPL: PDB IDs 3MAD, 3MAF, 3MAU, 3MBB) homologues of the enzyme. The bacterial homologue from *Symbiobacterium thermophilum* was identified by Bourquin *et al.* based on a BLAST search for Dpl1p-related prokaryotic proteins. The structures of StSPL and Dpl1p were originally solved in 2010 and were used to identify critical residues involved in ligand and co-factor binding, as well as proposing a mechanism for S1P cleavage (Bourquin *et al.*, 2010). The structure of human SPL has been recently solved, and shows very good conservation with the previous structures – demonstrated by an RMSD of 0.8 Å over 400 Ca atoms in an overlay of the human SPL and Dpl1p (Weiler *et al.*, 2014) (Figure 5A). This is also reflected in the high sequence similarity between homologues (Bourquin *et al.*, 2010). For the purposes of consistency and reference, residues are referred to in this section by their numbering in the bacterial StSPL sequence, unless otherwise indicated.

Structural features and mechanism of SPL

Human SPL and yeast Dpl1p possess a single trans-membrane domain that inserts into the membrane of the endoplasmic reticulum, positioning the C-terminal catalytic domain of the enzyme on the cytosolic face (Ikeda *et al.*, 2004). The bacterial StSPL lacks this trans-membrane domain, which has been shown to be expendable for catalytic activity in the human and yeast homologues (Mandon *et al.*, 1992; Van Veldhoven *et al.*, 2000). For the purposes of purification and crystallization, N-terminal deletions of SPL and Dpl1p were used, encompassing only the soluble catalytic domain. While there is one PDB entry for each human SPL and yeast Dpl1p, there are four entries for bacterial StSPL in different states of complex.

SLP is a pyridoxal phosphate (PLP)-dependent enzyme, and displays the typical structure and overall folds common to type I pyridoxal-dependent decarboxylases. SLP is active as a dimer, with two active sites to which each protomer contributes residues simultaneously (Bourquin *et al.*, 2010; Weiler *et al.*, 2014). The phosphate group of the PLP cofactor is held in the three-dimensional “phosphate cup” common to all PLP-dependent enzymes (Denesyuk *et al.*, 2003). In structures of StSPL, the PLP phosphate group is hydrogen-bonded to residues H310, T169, G168 and S353 (from the opposite protomer) (Figure 5C). The PLP pyridium ring sits facing D274, between H201 and C276, although these residues are too distant to be involved in any direct interactions (Figure 5C). In the symmetric StSPL structure 3MAD, PLP is also observed forming a covalent bond with K311, reflecting the crucial role of residue K311 in PLP binding and the catalytic mechanism of S1P cleavage, as explained below.

Although the solved SPL structures are lacking bound S1P, almost all show a phosphate ion that mimics binding of the S1P headgroup (Bourquin *et al.*, 2010; Weiler *et al.*, 2014). In StSPL, this phosphate interacts with the phenolic moiety of Y105, the amide group of A103 and the imidazolium ring of H129 from the opposite protomer (Figure 5C). K317 is also predicted to have a role in substrate binding (Bourquin *et al.*, 2010). Although it appears to

be too distant in solved structures to make a direct interaction with the phosphate, it is very possible that the actual positioning of bound S1P is such that K317 is able to form a hydrogen bond (Figure 5C). The structure of StSPL in complex with PE confirms these substrate-binding residues, since this is the product of S1P cleavage that ends up with the polar headgroup from the original substrate (PDB 3MAU). In addition, PE is observed to form a Schiff base with PLP, which lends to the mechanism of S1P cleavage.

These structural insights, together with knowledge of the mechanism of action of PLP-dependent decarboxylases, enabled Bourquin *et al.* to propose a scheme for the mechanism of S1P cleavage by SPLs. In the enzyme's unbound state, the active site contains an internal aldimine formed by the bond between PLP and K311. When S1P binds the active site, it replaces K311 as Schiff base partner of PLP, forming an external aldimine. Retro-aldol cleavage occurs by nucleophilic attack on S1P, releasing hexadecenal. The remaining quinonoid intermediate is re-protonated (most likely by K311 or Y105) to form a second external aldimine with PE. The final PE product is then released, allowing the active site to revert to its original internal aldimine. C276 was originally thought to act as the attacking nucleophile in S1P cleavage (Van Veldhoven *et al.*, 2000). Although the nucleophile in this mechanism is not yet identified, structures indicate that it is not C276, and that this residue serves a purely structural role maintaining the position of PLP for catalysis. Predicted critical residues A103, H129, Y105, K311 and K317 have all been demonstrated to be important for SPL function, since they produce either totally or partially inactive mutants (Bourquin *et al.*, 2010). Furthermore, all critical residues are completely conserved in all three species (apart from A103, which is threonine in human).

SPL inhibition

SPL is responsible for maintaining the S1P gradient that controls egress of T cells from primary lymphoid organs to return to circulation (discussed in the previous section on S1P₁). Hence, SPL inhibition causes T cell sequestration and immunosuppression, as demonstrated by pharmacological inhibition *in vivo* (Schwab & Cyster, 2007; Schwab *et al.*, 2005) and SPL knock-out mice (Billich *et al.*, 2013). Such findings put SPL in a similar position to S1P₁: they are both important therapeutic targets in the treatment of MS (Bagdanoff *et al.*, 2009). In addition, SPL inhibition has also been indicated in Phase II clinical trials to benefit patients with rheumatoid arthritis, due to its inhibitory effect on lymphocyte migration (Billich *et al.*, 2013; Fleischmann, 2012). Such findings have generated an impetus for finding a high-affinity, selective SPL inhibitor.

Weiler *et al.* performed a high-throughput screen to identify novel inhibitors of human SPL. Prior to this search, previously identified "SPL inhibitors" had only been shown to function *in vivo* to increase S1P levels; none had been shown to bind SPL directly or to inhibit its activity *in vitro* (Bagdanoff *et al.*, 2009; Loetscher *et al.*, 2013). The inhibitor described by Weiler *et al.* was slightly modified from an original hit from their library, resulting with a final IC₅₀ in the mid-nM range. This inhibitor protects from autoimmune encephalomyelitis, which is a model of MS (Weiler *et al.*, 2014), following oral administration making it a promising starting point for a novel MS therapeutics.

The structure of human SPL displays a narrow hydrophobic tunnel linking the exterior of the enzyme to its active site. The inhibitor identified by Weiler *et al.* sits at the entrance of this tunnel (Figure 5B), with the cyano pyridylmethyl piperazine side chain pointing toward the exterior. The affinity between inhibitor and SPL is predominantly mediated by hydrophobic interactions. This hydrophobic tunnel is likely to form the binding site for the acyl tail portion of S1P. However, it is not clear whether the S1P binding site directly overlays with the inhibitor-binding site. A structure of SPL in complex with S1P is needed to confirm the exact substrate-binding site in order to improve the design and development of small-molecule inhibitors. Given the emergence of SPL as a viable therapeutic target, these insights into its structure, active site and potential antagonist binding sites offer good foundations for further study to find a clinically useful SPL inhibitor.

Conclusion

The sphingolipid field is beginning to assemble a complete map, at atomic resolution, of how S1P is generated, how it binds to its effector proteins, and how it is degraded and exits the sphingolipid metabolism cycle (Figure 6). We can identify residues which are common to proteins which bind sphingosine and/or S1P. For example, the phosphate headgroup is stabilized by positively charged residues including both lysine and arginine residues in S1P₁, SPL and ApoM. In addition, all of the sphingosine/S1P binding pockets are lined with hydrophobic/non-polar residues for interaction with the acyl tail of the sphingolipid. Curiously, residues that interact with the 2' amine, the hallmark of sphingolipids, are not very conserved among the proteins reviewed in this paper. In SK1 there is a hydrogen bond between the 2' amine and a serine residue. However, in ApoM the residue that interacts with the 2' amine is a glutamate. It is possible that with continued work on the elucidation of sphingolipid binding proteins in complex with substrates or substrate analogs we will be able to identify binding motifs that are suited for specifically binding sphingolipids.

Concurrently with the elucidation of more structures, it is now possible that some of these structures can be computationally modeled. S1P₁ can now be used as a template to predict the structures of the other four S1P receptors by homology modeling. However, use of SK1 to predict the full structure of SK2 will be hindered by the large insertions in both the N-terminus and within the catalytic domain. Therefore, SK2 and the S1P transporters are the main puzzle pieces of sphingosine/S1P arm which remain unresolved.

On the other hand, we are only beginning to elucidate the structures of other sphingolipid metabolizing enzymes and to build a more complete structural view of sphingolipid metabolism as a whole. There is still much more work to be done to understand the structures of sphingolipid-metabolizing enzymes, the molecular mechanisms which regulate their activity and the potential use of this knowledge for therapeutic advances. Furthermore, as the ever-increasing biology and pathological physiology associated with bioactive sphingo-lipids is discovered and characterized, the importance of such an understanding will only grow with time.

References

- Adada M, Canals D, Hannun YA, Obeid LM. Sphingosine-1-phosphate receptor 2. FEBS J. 2013; 280:6354–66. [PubMed: 23879641]
- Ago H, Oda M, Takahashi M, et al. Structural basis of the sphingomyelin phosphodiesterase activity in neutral sphingomyelinase from *Bacillus cereus*. J Biol Chem. 2006; 281:16157–67. [PubMed: 16595670]
- Anelli V, Gault CR, Snider AJ, Obeid LM. Role of sphingosine kinase-1 in paracrine/transcellular angiogenesis and lymphangiogenesis *in vitro*. FASEB J. 2010; 24:2727–38. [PubMed: 20335228]
- Baek DJ, Macritchie N, Anthony NG, et al. Structure–activity relationships and molecular modeling of sphingosine kinase inhibitors. J Med Chem. 2013a; 56:9310–27. [PubMed: 24164513]
- Baek DJ, Macritchie N, Pyne NJ, et al. Synthesis of selective inhibitors of sphingosine kinase 1. Chem Commun (Camb). 2013b; 49:2136–8. [PubMed: 23388656]
- Bagdanoff JT, Donoviel MS, Nouraldean A, et al. Inhibition of sphingosine-1-phosphate lyase for the treatment of autoimmune disorders. J Med Chem. 2009; 52:3941–53. [PubMed: 19489538]
- Behjati M, Etemadifar M, Abdar Esfahani M. Cardiovascular effects of fingolimod: a review article. Iran J Neurol. 2014; 13:119–26. [PubMed: 25422729]
- Billich A, Baumrucker T, Beerli C, et al. Partial deficiency of sphingosine-1-phosphate lyase confers protection in experimental autoimmune encephalomyelitis. PLoS One. 2013; 8:e59630. [PubMed: 23544080]
- Boujaoude LC, Bradshaw-Wilder C, Mao C, et al. Cystic fibrosis transmembrane regulator regulates uptake of sphingoid base phosphates and lysophosphatidic acid: modulation of cellular activity of sphingosine 1-phosphate. J Biol Chem. 2001; 276:35258–64. [PubMed: 11443135]
- Bourquin F, Riezman H, Capitani G, Grutter MG. Structure and function of sphingosine-1-phosphate lyase, a key enzyme of sphingo-lipid metabolism. Structure. 2010; 18:1054–65. [PubMed: 20696404]
- Bradley E, Dasgupta S, Jiang X, et al. Critical role of Spns2, a sphingosine-1-phosphate transporter, in lung cancer cell survival and migration. PLoS One. 2014; 9:e110119. [PubMed: 25330231]
- Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am J Transplant. 2004; 4:1019–25. [PubMed: 15196057]
- Buehrer BM, Bell RM. Inhibition of sphingosine kinase *in vitro* and in platelets. Implications for signal transduction pathways. J Biol Chem. 1992; 267:3154–9. [PubMed: 1310683]
- Cheek S, Ginalski K, Zhang H, Grishin NV. A comprehensive update of the sequence and structure classification of kinases. BMC Struct Biol. 2005; 5:6. [PubMed: 15771780]
- Chiba Y, Suzuki K, Uechi M, et al. Downregulation of sphingosine-1-phosphate receptors in bronchial smooth muscle of mouse experimental asthma. Pharmacol Res. 2010; 62:357–63. [PubMed: 20554039]
- Christoffersen C, Jauhiainen M, Moser M, et al. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice. J Biol Chem. 2008; 283:1839–47. [PubMed: 18006500]
- Christoffersen C, Obinata H, Kumaraswamy SB, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. Proc Natl Acad Sci USA. 2011; 108:9613–8. [PubMed: 21606363]
- Coward J, Ambrosini G, Musi E, et al. Safingol (L-threo-sphinganine) induces autophagy in solid tumor cells through inhibition of PKC and the PI3-kinase pathway. Autophagy. 2009; 5:184–93. [PubMed: 19098447]
- Cuvillier O, Pirianov G, Kleuser B, et al. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature. 1996; 381:800–3. [PubMed: 8657285]
- Dbaibo GS, Pushkareva MY, Jayadev S, et al. Retinoblastoma gene product as a downstream target for a ceramide-dependent pathway of growth arrest. Proc Natl Acad Sci USA. 1995; 92:1347–51. [PubMed: 7877980]

- Delgado A, Casas J, Llebaria A, et al. Inhibitors of sphingolipid metabolism enzymes. *Biochim Biophys Acta*. 2006; 1758:1957–77. [PubMed: 17049336]
- Denesyuk AI, Denessiouk KA, Korpela T, Johnson MS. Phosphate group binding “cup” of PLP-dependent and non-PLP-dependent enzymes: leitmotif and variations. *Biochim Biophys Acta*. 2003; 1647:234–8. [PubMed: 12686139]
- Dickson MA, Carvajal RD, Merrill AH Jr, et al. A phase I clinical trial of safinol in combination with cisplatin in advanced solid tumors. *Clin Cancer Res*. 2011; 17:2484–92. [PubMed: 21257722]
- Fleischmann R. Novel small-molecular therapeutics for rheumatoid arthritis. *Curr Opin Rheumatol*. 2012; 24:335–41. [PubMed: 22357358]
- Foss FW Jr, Snyder AH, Davis MD, et al. Synthesis and biological evaluation of gamma-aminophosphonates as potent, subtype-selective sphingosine 1-phosphate receptor agonists and antagonists. *Bioorg Med Chem*. 2007; 15:663–77. [PubMed: 17113298]
- French KJ, Schrecengost RS, Lee BD, et al. Discovery and evaluation of inhibitors of human sphingosine kinase. *Cancer Res*. 2003; 63:5962–9. [PubMed: 14522923]
- French KJ, Upson JJ, Keller SN, et al. Antitumor activity of sphingosine kinase inhibitors. *J Pharmacol Exp Ther*. 2006; 318:596–603. [PubMed: 16632640]
- Fukuhara S, Simmons S, Kawamura S, et al. The sphingosine-1-phosphate transporter Spns2 expressed on endothelial cells regulates lymphocyte trafficking in mice. *J Clin Invest*. 2012; 122:1416–26. [PubMed: 22406534]
- Galicia-Rosas G, Pikor N, Schwartz JA, et al. A sphingosine-1-phosphate receptor 1-directed agonist reduces central nervous system inflammation in a plasmacytoid dendritic cell-dependent manner. *J Immunol*. 2012; 189:3700–6. [PubMed: 22933630]
- Gonzalez-Cabrera PJ, Jo E, Sanna MG, et al. Full pharmacological efficacy of a novel S1P1 agonist that does not require S1P-like headgroup interactions. *Mol Pharmacol*. 2008; 74:1308–18. [PubMed: 18708635]
- Graler MH, Goetzl EJ. The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. *FASEB J*. 2004; 18:551–3. [PubMed: 14715694]
- Gustin DJ, Li Y, Brown ML, et al. Structure guided design of a series of sphingosine kinase (SphK) inhibitors. *Bioorg Med Chem Lett*. 2013; 23:4608–16. [PubMed: 23845219]
- Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol*. 2008; 9:139–50. [PubMed: 18216770]
- Hanson MA, Roth CB, Jo E, et al. Crystal structure of a lipid G protein-coupled receptor. *Science*. 2012; 335:851–5. [PubMed: 22344443]
- Hengst JA, Wang X, Sk UH, et al. Development of a sphingosine kinase 1 specific small-molecule inhibitor. *Bioorg Med Chem Lett*. 2010; 20:7498–502. [PubMed: 21050755]
- Igarashi Y, Hakomori S. Enzymatic synthesis of *N,N*-dimethyl-sphingosine: demonstration of the sphingosine: *N*-methyltransferase in mouse brain. *Biochem Biophys Res Commun*. 1989; 164:1411–16. [PubMed: 2686639]
- Ihlefeld K, Vienken H, Claas RF, et al. Upregulation of ABC transporters contributes to chemoresistance of sphingosine 1-phosphate lyase-deficient fibroblasts. *J Lipid Res*. 2015; 56:60–9. [PubMed: 25385827]
- Ikeda M, Kihara A, Igarashi Y. Sphingosine-1-phosphate lyase SPL is an endoplasmic reticulum-resident, integral membrane protein with the pyridoxal 5'-phosphate binding domain exposed to the cytosol. *Biochem Biophys Res Commun*. 2004; 325:338–43. [PubMed: 15522238]
- Inoue T, Okino N, Kakuta Y, et al. Mechanistic insights into the hydrolysis and synthesis of ceramide by neutral ceramidase. *J Biol Chem*. 2009; 284:9566–77. [PubMed: 19088069]
- Jo E, Bhattacharai B, Repetto E, et al. Novel selective allosteric and bitopic ligands for the S1P(3) receptor. *ACS Chem Biol*. 2012; 7:1975–83. [PubMed: 22971058]
- Jo E, Sanna MG, Gonzalez-Cabrera PJ, et al. S1P1-selective *in vivo*-active agonists from high-throughput screening: off-the-shelf chemical probes of receptor interactions, signaling, and fate. *Chem Biol*. 2005; 12:703–15. [PubMed: 15975516]
- Johnson KR, Johnson KY, Crellin HG, et al. Immunohistochemical distribution of sphingosine kinase 1 in normal and tumor lung tissue. *J Histochem Cytochem*. 2005; 53:1159–66. [PubMed: 15923363]

- Kawahara A, Nishi T, Hisano Y, et al. The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. *Science*. 2009; 323:524–7. [PubMed: 19074308]
- Kawamori T, Osta W, Johnson KR, et al. Sphingosine kinase 1 is up-regulated in colon carcinogenesis. *FASEB J*. 2006; 20:386–8. [PubMed: 16319132]
- Kimura T, Watanabe T, Sato K, et al. Sphingosine 1-phosphate stimulates proliferation and migration of human endothelial cells possibly through the lipid receptors, Edg-1 and Edg-3. *Biochem J*. 2000; 348:71–6. [PubMed: 10794715]
- Kobayashi N, Nishi T, Hirata T, et al. Sphingosine 1-phosphate is released from the cytosol of rat platelets in a carrier-mediated manner. *J Lipid Res*. 2006; 47:614–21. [PubMed: 16371645]
- Kobayashi N, Yamaguchi A, Nishi T. Characterization of the ATP-dependent sphingosine 1-phosphate transporter in rat erythrocytes. *J Biol Chem*. 2009; 284:21192–200. [PubMed: 19531471]
- Kohama T, Olivera A, Edsall L, et al. Molecular cloning and functional characterization of murine sphingosine kinase. *J Biol Chem*. 1998; 273:28722–8.
- Kudo N, Kumagai K, Tomishige N, et al. Structural basis for specific lipid recognition by CERT responsible for nonvesicular trafficking of ceramide. *Proc Natl Acad Sci USA*. 2008; 105:488–93. [PubMed: 18184806]
- Labesse G, Douguet D, Assairi L, Gilles AM. Diacylglyceride kinases, sphingosine kinases and NAD kinases: distant relatives of 6-phosphofructokinases. *Trends Biochem Sci*. 2002; 27:273–5. [PubMed: 12069781]
- Lamontagne K, Littlewood-Evans A, Schnell C, et al. Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. *Cancer Res*. 2006; 66:221–31. [PubMed: 16397235]
- Lee YM, Venkataraman K, Hwang SI, et al. A novel method to quantify sphingosine 1-phosphate by immobilized metal affinity chromatography (IMAC). *Prostaglandins Other Lipid Mediat*. 2007; 84:154–62. [PubMed: 17991617]
- Lim KG, Tonelli F, Li Z, et al. FTY720 analogues as sphingosine kinase 1 inhibitors: enzyme inhibition kinetics, allosterism, proteasomal degradation, and actin rearrangement in MCF-7 breast cancer cells. *J Biol Chem*. 2011; 286:18633–40. [PubMed: 21464128]
- Liu M, Allegood J, Zhu X, et al. Uncleaved apoM signal peptide is required for formation of large apoM/S1P-enriched HDL particles. *J Biol Chem*. 2015; 290:7861–70. [PubMed: 25627684]
- Liu H, Sugiura M, Nava VE, et al. Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. *J Biol Chem*. 2000a; 275:19513–20. [PubMed: 10751414]
- Liu Y, Wada R, Yamashita T, et al. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J Clin Invest*. 2000b; 106:951–61. [PubMed: 11032855]
- Loetscher E, Schneider K, Beerli C, Billich A. Assay to measure the secretion of sphingosine-1-phosphate from cells induced by S1P lyase inhibitors. *Biochem Biophys Res Commun*. 2013; 433:345–8. [PubMed: 23499842]
- Lynch KR. Building a better sphingosine kinase-1 inhibitor. *Biochem J*. 2012; 444:e1–2. [PubMed: 22533672]
- Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science*. 2002; 296:346–9. [PubMed: 11923495]
- Mandon EC, Ehse I, Rother J, et al. Subcellular localization and membrane topology of serine palmitoyltransferase, 3-dehydrosphinganine reductase, and sphinganine *N*-acyltransferase in mouse liver. *J Biol Chem*. 1992; 267:11144–8. [PubMed: 1317856]
- Matloubian MCGL, Cinamon G, Lesneski MJ, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*. 2004; 427:355–60. [PubMed: 14737169]
- Mendoza A, Breart B, Ramos-Perez WD, et al. The transporter Spns2 is required for secretion of lymph but not plasma sphingosine-1-phosphate. *Cell Rep*. 2012; 2:1104–10. [PubMed: 23103166]
- Mitra P, Oskeritzian CA, Payne SG, et al. Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. *Proc Natl Acad Sci USA*. 2006; 103:16394–9. [PubMed: 17050692]
- Mizugishi K, Yamashita T, Olivera A, et al. Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol*. 2005; 25:11113–21. [PubMed: 16314531]

- Murata N, Sato K, Kon J, et al. Interaction of sphingosine 1-phosphate with plasma components, including lipoproteins, regulates the lipid receptor-mediated actions. *Biochem J.* 2000; 352:809–15. [PubMed: 11104690]
- Nagahashi M, Kim EY, Yamada A, et al. Spns2, a transporter of phosphorylated sphingoid bases, regulates their blood and lymph levels, and the lymphatic network. *FASEB J.* 2013; 27:1001–11. [PubMed: 23180825]
- Obeid LM, Linardic CM, Karolak LA, Hannun YA. Programmed cell death induced by ceramide. *Science.* 1993; 259:1769–71. [PubMed: 8456305]
- Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer.* 2004; 4:604–16. [PubMed: 15286740]
- Olivera A, Kohama T, Edsall LC, et al. Sphingosine kinase expression increases intracellular sphingosine 1 phosphate and promotes cell growth and survival. *J Biol Chem.* 1999; 274:545–57.
- Oo ML, Thangada S, Wu MT, et al. Immunosuppressive and anti-angiogenic sphingosine 1-phosphate receptor-1 agonists induce ubiquitinylation and proteasomal degradation of the receptor. *J Biol Chem.* 2007; 282:9082–9. [PubMed: 17237497]
- Orr Gandy KA, Obeid LM. Targeting the sphingosine kinase/ sphingosine 1-phosphate pathway in disease: review of sphingosine kinase inhibitors. *Biochim Biophys Acta.* 2013; 1831:157–66. [PubMed: 22801037]
- Osborne N, Brand-Arzamendi K, Ober EA, et al. The spinster homolog, two of hearts, is required for sphingosine 1-phosphate signaling in zebrafish. *Curr Biol.* 2008; 18:1882–8. [PubMed: 19062281]
- Parrill AL, Lima S, Spiegel S. Structure of the first sphingosine 1-phosphate receptor. *Sci Signal.* 2012; 5:pe23. [PubMed: 22623751]
- Parrill AL, Wang D, Bautista DL, et al. Identification of Edg1 receptor residues that recognize sphingosine 1-phosphate. *J Biol Chem.* 2000; 275:39379–84. [PubMed: 10982820]
- Paugh SW, Paugh BS, Rahmani M, et al. A selective sphingosine kinase 1 inhibitor integrates multiple molecular therapeutic targets in human leukemia. *Blood.* 2008; 112:1382–91. [PubMed: 18511810]
- Pitson SM. Regulation of sphingosine kinase and sphingolipid signaling. *Trends Biochem Sci.* 2011; 36:97–107. [PubMed: 20870412]
- Pitson SM, D'Andrea RJ, Vandeleur L, et al. Human sphingosine kinase: purification, molecular cloning and characterization of the native and recombinant enzymes. *Biochem J.* 2000a; 350:429–41. [PubMed: 10947957]
- Pitson SM, Moretti PA, Zebol JR, et al. A point mutant of human sphingosine kinase 1 with increased catalytic activity. *FEBS Lett.* 2001; 509:169–73. [PubMed: 11741582]
- Pitson SM, Moretti PA, Zebol JR, et al. Expression of a catalytically inactive sphingosine kinase mutant blocks agonist-induced sphingosine kinase activation. A dominant-negative sphingosine kinase. *J Biol Chem.* 2000b; 275:33945–50. [PubMed: 10944534]
- Pitson SM, Moretti PA, Zebol JR, et al. The nucleotide-binding site of human sphingosine kinase 1. *J Biol Chem.* 2002; 277:49545–53. [PubMed: 12393916]
- Poti F, Simoni M, Nofer JR. Atheroprotective role of high-density lipoprotein (HDL)-associated sphingosine-1-phosphate (S1P). *Cardiovasc Res.* 2014; 103:395–404. [PubMed: 24891400]
- Rex K, Jeffries S, Brown ML, et al. Sphingosine kinase activity is not required for tumor cell viability. *PLoS One.* 2013; 8:e68328. [PubMed: 23861887]
- Rosen H, Stevens RC, Hanson M, et al. Sphingosine-1-phosphate and its receptors: structure, signaling, and influence. *Annu Rev Biochem.* 2013; 82:637–62. [PubMed: 23527695]
- Rosenbaum DM, Cherezov V, Hanson MA, et al. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science.* 2007; 318:1266–73. [PubMed: 17962519]
- Ruckhaberle E, Rody A, Engels K, et al. Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer. *Breast Cancer Res Treat.* 2008; 112:41–52. [PubMed: 18058224]
- Sato K, Malchinkhuu E, Horiuchi Y, et al. Critical role of ABCA1 transporter in sphingosine 1-phosphate release from astrocytes. *J Neurochem.* 2007; 103:2610–19. [PubMed: 17931360]

- Satsu H, Schaeffer MT, Guerrero M, et al. A sphingosine 1-phosphate receptor 2 selective allosteric agonist. *Bioorg Med Chem*. 2013; 21:5373–82. [PubMed: 23849205]
- Schnute ME, McReynolds MD, Kasten T, et al. Modulation of cellular S1P levels with a novel, potent and specific inhibitor of sphingosine kinase-1. *Biochem J*. 2012; 444:79–88. [PubMed: 22397330]
- Schwab SR, Cyster JG. Finding a way out: lymphocyte egress from lymphoid organs. *Nat Immunol*. 2007; 8:1295–301. [PubMed: 18026082]
- Schwab SR, Pereira JP, Matloubian M, et al. Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. *Science*. 2005; 309:1735–9. [PubMed: 16151014]
- Sevvana M, Ahnstrom J, Egerer-Sieber C, et al. Serendipitous fatty acid binding reveals the structural determinants for ligand recognition in apolipoprotein M. *J Mol Biol*. 2009; 393:920–36. [PubMed: 19733574]
- Simanshu DK, Kamlekar RK, Wijesinghe DS, et al. Non-vesicular trafficking by a ceramide-1-phosphate transfer protein regulates eicosanoids. *Nature*. 2013; 500:463–7. [PubMed: 23863933]
- Snider AJ, Orr Gandy KA, Obeid LM. Sphingosine kinase: role in regulation of bioactive sphingolipid mediators in inflammation. *Biochimie*. 2010; 92:707–15. [PubMed: 20156522]
- Stahelin RV, Hwang JH, Kim JH, et al. The mechanism of membrane targeting of human sphingosine kinase 1. *J Biol Chem*. 2005; 280:43030–8. [PubMed: 16243846]
- Sugiura M, Kono K, Liu H, et al. Ceramide kinase, a novel lipid kinase – molecular cloning and functional characterization. *J Biol Chem*. 2002; 277:23294–300. [PubMed: 11956206]
- Taha TA, Hannun YA, Obeid LM. Sphingosine kinase: biochemical and cellular regulation and role in disease. *J Biochem Mol Biol*. 2006a; 39:113–31. [PubMed: 16584625]
- Taha TA, Kitatani K, El-Alwani M, et al. Loss of sphingosine kinase-1 activates the intrinsic pathway of programmed cell death: modulation of sphingolipid levels and the induction of apoptosis. *FASEB J*. 2006b; 20:482–4. [PubMed: 16507765]
- Takabe K, Kim RH, Allegood JC, et al. Estradiol induces export of sphingosine 1-phosphate from breast cancer cells via ABCC1 and ABCG2. *J Biol Chem*. 2010; 285:10477–86. [PubMed: 20110355]
- Tonelli F, Lim KG, Loveridge C, et al. FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells. *Cell Signal*. 2010; 22:1536–42. [PubMed: 20570726]
- Van Veldhoven PP, Gijssbers S, Mannaerts GP, et al. Human sphingosine-1-phosphate lyase: cDNA cloning, functional expression studies and mapping to chromosome 10q22(1). *Biochim Biophys Acta*. 2000; 1487:128–34. [PubMed: 11018465]
- Venable ME, Lee JY, Smyth MJ, et al. Role of ceramide in cellular senescence. *J Biol Chem*. 1995; 270:30701–8. [PubMed: 8530509]
- Wang Z, Min X, Xiao SH, et al. Molecular basis of sphingosine kinase 1 substrate recognition and catalysis. *Structure*. 2013; 21:798–809. [PubMed: 23602659]
- Wattenberg BW, Pitson SM, Raben DM. The sphingosine and diacylglycerol kinase superfamily of signaling kinases: localization as a key to signaling function. *J Lipid Res*. 2006; 47:1128–39. [PubMed: 16520486]
- Weiler S, Braendlin N, Beerli C, et al. Orally active 7-substituted (4-benzylphthalazin-1-yl)-2-methylpiperazin-1-yl]nicotinonitriles as active-site inhibitors of sphingosine 1-phosphate lyase for the treatment of multiple sclerosis. *J Med Chem*. 2014; 57:5074–84. [PubMed: 24809814]
- Willis MA, Cohen JA. Fingolimod therapy for multiple sclerosis. *Semin Neurol*. 2013; 33:37–44. [PubMed: 23709211]
- Xu N, Dahlback B. A novel human apolipoprotein (apoM). *J Biol Chem*. 1999; 274:31286–90. [PubMed: 10531326]
- Yard BA, Carter LG, Johnson KA, et al. The structure of serine palmitoyltransferase; gateway to sphingolipid biosynthesis. *J Mol Biol*. 2007; 370:870–86. [PubMed: 17559874]
- Yeh EA, Weinstock-Guttman B. Fingolimod: an oral disease-modifying therapy for relapsing multiple sclerosis. *Adv Ther*. 2011; 28:270–8. [PubMed: 21394595]

- Yokota S, Taniguchi Y, Kihara A, et al. Asp177 in C4 domain of mouse sphingosine kinase 1a is important for the sphingosine recognition. FEBS Lett. 2004; 578:106–10. [PubMed: 15581625]
- Yuan S, Wu R, Latek D, et al. Lipid receptor S1P(1) activation scheme concluded from microsecond all-atom molecular dynamics simulations. PLoS Comput Biol. 2013; 9:e1003261. [PubMed: 24098103]
- Zemann B, Kinzel B, Muller M, et al. Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood. 2006; 107:1454–8. [PubMed: 16223773]

Figure 1.

Overview of the interconversion of bioactive sphingolipids. The scheme shows the structure of each of the bioactive sphingolipids as well as the products of the breakdown of S1P which is the exit point of sphingolipid metabolism. Ceramide can be broken down to sphingosine and fatty acid by ceramidases and can be made by ceramide synthases which use acyl-CoA and sphingosine as substrates. Sphingosine can be phosphorylated to S1P by sphingosine kinases and dephosphorylated by S1P phosphatases. The final step in sphingolipid metabolism is catalyzed by sphingosine phosphate lyase which breaks down S1P to PE and hexadecanal. (see colour version of this figure at www.informahealth-care.com/bmg).

Figure 2.

Structural view of SK1. (A) An overview of the SK1 structure. The conserved domains, C1–C5, are highlighted in firebrick red, tv blue, light teal, light orange and lime green, respectively. The structure takes an overall two-lobe fold with the N-lobe and C-lobe containing binding sites for ADP and sphingosine (circled), both of which are shown as magenta sticks. (B) ATP binding interactions. ADP (magenta sticks) is shown with the interacting residues of SK1. The interacting residues are colored in coordination with the domain that they belong to as in panel A. Dashed lines represent hydrogen bonds. All dashed lines are within hydrogen bonding distance. (C) Sphingosine binding interactions. Residues within in 5 Å of sphingosine (magenta) are shown as sticks. Dashed lines represent hydrogen bonds. Dashed lines are within hydrogen bonding distance unless otherwise noted. There are many hydrophobic/non-polar residues that line the pocket where the hydrocarbon chain binds whereas near the headgroup there are polar/ charged residues. (D) Surface cutaway of SK1. A surface representation of SK1 has been clipped near the sphingosine binding pocket. This reveals the overall depth and “J-shaped” nature of the sphingosine (magenta sticks) binding pocket of SK1. (E) Overview of inhibitor binding. The inhibitors

(SKI-II, magenta sticks; PF-543, cyan sticks; compound 23, yellow sticks) are shown as sticks. The inhibitors shown bind nicely within the sphingosine binding pocket of SK1. It is notable that the inhibitors take on a similar bent conformation as sphingosine. (see colour version of this figure at www.informahealthcare.com/bmg).

Figure 3.

Transport of S1P by ApoM. (A) An overview of ApoM bound to S1P. A cartoon representation (grey cartoon) bound to S1P (magenta sticks). S1P binds to the central cavity of ApoM which is within the center of the β -barrel. (B) Surface cutaway of ApoM bound to S1P. A surface representation of ApoM (grey surface) to show S1P (magenta sticks) is bound. S1P takes a bent conformation upon binding to ApoM, similar to SK1. (C) S1P recognition by ApoM using a complex, water-mediated hydrogen-bond network. S1P (magenta sticks) is recognized by several residues (grey sticks) and water molecules (red spheres). The black dashed lines represent hydrogen bonds between residue side chains, water molecules and S1P. (see color version of this figure at www.informahealthcare.com/bmg).

Figure 4.

S1P₁ structure. (A) Overview of S1P₁. S1P₁ is shown as a rainbow-colored cartoon with dark blue representing the N-terminus and red representing the C-terminus. The T4-lysozyme fusion has been removed for clarity. The extracellular loops are labeled and disulfide bonds are shown as sticks of the corresponding color. The *N*-acetyl glucosamine is shown as blue sticks. The S1P₁ antagonist ML056 is shown bound to S1P₁ as spheres. (B) Side entry point for S1P. A semi-transparent surface representation of S1P₁ overlaid on top of the cartoon. R292, proposed to be responsible for guarding entry, is shown as sticks underneath the semi-transparent surface. The darkened area represents the proposed side entry point for S1P from within the membrane. (C) Binding of the ML056 headgroup. ML056 is shown as magenta sticks and interacting residues are shown as sticks colored according to the helix to which they are located. Dashed lines represent hydrogen bonds with the distances shown. (D) Binding of the tail of ML056. ML056 is shown as magenta sticks and important residues are shown as sticks colored according to the helix to which they are located. van der Waal interactions dominate in the binding of ML056 to S1P₁. (see color version of this figure at www.informahealthcare.com/bmg).

Figure 5.

S1P degradation by SPLs. (A) Overview of SPLs. The human and *S. thermophilum* S1P lyases are shown as magenta and cyan cartoons, respectively. In the inset image, the presumed substrate binding site has a phosphate bound (orange sticks) and the co-factor (5-hydroxy-4,6-dimethylpyridin-3-yl)methyl dihydrogen phosphate bound (cyan sticks). The overlay shows that there is significant structural homology between the two species. (B) Cutaway of a surface representation of human SPL complexed with an inhibitor. The human SPL in complex with 6-[2(*R*)-4-(4-benzyl-7-chlorophthalazin-1-yl)-2-methylpiperazin-1-yl]pyridine-3-carbonitrile (an SPL inhibitor, yellow sticks) and overlaid substrate mimic and co-factor (cyan sticks) from *S. thermophilum*. It shows that the inhibitor is most likely a competitive inhibitor as it sits within the entry tunnel to the active site, blocking access of S1P to the catalytic site. (C) Overview of residues which are involved in binding the substrate and co-factor. The substrate mimic (bound phosphate) is shown as orange sticks and the co-factor is shown as cyan sticks. In the structure, residue 311 is mutated to an alanine; here we have mutated said alanine to the natural lysine which is shown as grey sticks. The black dashed lines represent hydrogen bonds based on either the structure or on

published biochemistry. (see color version of this figure at www.informahealthcare.com/bmg).

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Figure 6.

Structural view of S1P metabolism. Starting with sphingosine (magenta sticks) generated either *de novo* or from the salvage pathway, SK1 (shown as cartoon from Figure 2A; PDB ID: 3VZB) can phosphorylate sphingosine to generate S1P. S1P can then be exported by certain members of the ABC transporter family or by Spns2 (grey box). Once S1P is outside the cell ApoM (grey cartoon from Figure 3; PDB ID: 2YG2) is one of the proteins which can mediate its transport throughout the blood stream. Upon its arrival to cells, S1P can diffuse into the membrane, move laterally and bind to S1PRs (represented by the rainbow cartoon; PDB ID: 3V2Y) from within the membrane. After the effect of S1P has been elicited by the receptors, S1P diffuses out of the receptor binding site into the membrane where it encounters SPL (magenta cartoon; PDB ID: 4Q6R). S1P is then broken down into PE and hexadecanal which are no longer able to be used in the sphingolipid metabolic cycle. (see color version of this figure at www.informahealthcare.com/bmg).