Review

# The structure and function of the S1P1 receptor

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**Sphingosine 1-phosphate (S1P) receptors (S1PRs) belong to the class A family of G protein-coupled receptors (GPCRs). S1PRs are widely expressed on many cell types, including those of the immune, cardiovascular, and central nervous systems. The S1PR family is rapidly gaining attention as an important mediator of many cellular processes, including cell differentiation, migration, survival, angiogenesis, calcium homeostasis, inflammation and immunity. Importantly, S1PRs are known drug targets for multiple sclerosis (MS), for which the newly developed oral therapy fingolimod, an S1PR modulator, has recently been approved for clinical use. Much progress has also recently been made in the field of structural biology and in the modeling of heterotrimeric GPCRs allowing the crystal structure of the S1PR1 subtype to be elucidated and key interactions defined. Here, we outline the structure and function of S1PR1, highlighting the key residues involved in receptor activation, signaling, transmembrane interactions, ligand binding, post-translational modification, and protein–protein interactions.**

## S1P and its receptors

The role of S1PRs in autoimmune illnesses has received a great deal of attention since recent clinical approval of the drug fingolimod. This compound targets S1PRs and has been approved as the first oral therapy for MS. The functional roles of modulating S1PRs by its natural ligand, S1P, as well as newly designed pharmacological compounds, are also the subject of intense research activity in the academic, clinical, and pharmaceutical sectors. Here, we summarize the pharmacological tools generally used to investigate S1PR function. We also highlight new insights gained from the recently elucidated S1PR1 crystal structure and combine this information with functional studies.

S1P is a zwitterionic lysophospholipid that has been implicated as a crucial regulator in many physiological and pathophysiological processes (Table 1). S1P is derived from the related sphingolipid ceramide, which is itself synthesized either via the actions of sphingomyelinases in the membrane or via *de novo* pathways initiated in the endoplasmic reticulum (Figure 1). Deacylation of ceramide by ceramidase results in release of sphingosine ((2*S*,3*R*,4*E*)-2-amino-4-octadecen-1,3-diol), which is subsequently phosphorylated in an ATP-dependent manner by sphingosine kinase (SphK) 1 or 2 to form S1P [1]. Thus, sphingolipid turnover yields various potent signaling molecules including ceramide and sphingosine, both of which promote cell-cycle arrest and apoptosis. The phosphorylated form, ceramide 1-phosphate, antagonizes the proapoptotic effects of ceramide, is mitogenic, and promotes inflammation. By contrast, S1P enhances cell proliferation and has prosurvival, proinflammatory, and promotility characteristics. Together, these sphingolipids perform a vital balancing act that regulates the survival, trafficking, and function of many cells [2]. Not surprisingly, therefore, tight control is kept over S1P production by the enzymes that catalyze the formation of its substrate sphingosine, by the SphKs and by S1P-degrading enzymes including two S1P-phosphatases, three lipid phosphate phosphatases, and S1P lyase (Figure 2) [3].

As indicated above, the newly developed oral therapy fingolimod targets S1PRs and has been approved for clinical use in MS [4]. This formulation contains fingolimod hydrochloride, which is converted *in vivo* to the active metabolite fingolimod phosphate (pFTY720). After enantiospecific monophosphorylation by SphK2, pFTY720 binds with high affinity to S1PRs and results in sequestration of lymphocytes into secondary lymphoid tissues. This sequestration subsequently modulates the recirculation of lymphocytes between blood and lymphoid tissues [5]. pFTY720 was first synthesized through structural simplification of ISP-I (a natural immunosuppressive product), leading to the discovery of a nonchiral symmetric 2-substituted 2-aminopropane-1,3-diol framework and, subsequently, discovery of pFTY720 [6] (Table 2). pFTY720 contains a prochiral quarternary carbon atom bearing two hydroxymethyl groups ($CH_2$–OH). Substitution of one $CH_2$–OH group with an alkyl group (such as methyl) generates a racemate mixture of the pFTY720 analog. Pharmacological evaluation of each enantiomer revealed a biologically critical role for the pro-(*S*)-$CH_2$–OH group, in which only the (*S*)-enantiomer of pFTY720 binds all S1PRs (except S1PR2) [7,8]. By contrast, the pro-(*R*)-$CH_2$–OH group generating the (*R*)-enantiomer appears inactive on S1PRs, but may have chemically and physicochemically important roles in improving the solubility of the molecule [5,9].

Since the development of pFTY720, numerous S1PR agonists and, more recently, antagonists have been

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**Table 1. Association of S1P/S1PR signaling with disease**

| System | Disease | Notes | Refs |
|---|---|---|---|
| **Central nervous system** | Alzheimer's disease | S1P increases BACE proteolytic activity | [53] |
| | Ischemia | S1P levels reduced in brain samples from Alzheimer's disease | [54] |
| | Stress | S1P levels increased in ischemic brain of a mouse model of middle cerebral artery occlusion | [55] |
| | Pain | S1P levels increased in rat model of stress | [56] |
| | Multiple sclerosis | S1P levels decreased in the CSF of rat models of pain | |
| | | S1P levels elevated in CSF (but not blood) from MS patients | [57] |
| | | | [58] |
| **Peripheral nervous system** | Peripheral neuropathy and pain | S1P induces hyperalgesia via S1PR1 | [59] |
| **Eye** | Retinal inflammation | Retinal neovascularization retarded by S1PR2 KO | [60] |
| | Diabetic neuropathy | Role of S1P in VEGF-induced retinal EC migration and proliferation and reduced retinal vascular leakage | [61] |
| | AMD | Role of S1P in AMD-associated neovascularization, inflammation, and fibrosis | [62] |
| **Ear** | Deafness | Loss of S1PR2 and S1PR3 display cochlear and vestibular defects resulting in deafness | [63] |
| **Lung** | Asthma | S1P levels elevated in BAL fluid from patients with asthma | [64] |
| | COPD | S1P levels decreased in lungs of patients with cystic fibrosis and COPD; reduced S1PR5 levels in COPD | [65] |
| | Anaphylaxis | Anaphylactic responses attenuated S1PR2 antagonist JTE-013 and in S1PR2 KO mice | [66,67] |
| **Vasculature** | Sepsis | TLR4 signaling activates SphK1, causes S1P production and sepsis-induced inflammation | [68] |
| | Atherosclerosis | pFTY720 limits atherosclerosis in apolipoprotein E KO mice on high-cholesterol diet (suppression of monocytes/macrophages) | [69] |
| | Coronary artery disease | S1P levels elevated in coronary artery disease and considered predictor of occurrence and severity of coronary stenosis | [70,71] |
| **Heart** | Myocardial infarction and ischemia | S1P protects heart against ischemia–reperfusion injury; treatment with pFTY720 improves recovery during reperfusion | [72] |
| | Heart rate | S1PR3 implicated in sinus bradycardia | [73,74] |
| **Liver** | Hepatitis | S1P levels reduced in chronic hepatitis C with liver fibrosis | [75] |
| | Portal hypertension | S1PR2 activation of Rho kinase plays a role in portal hypertension, S1PR2 antagonists may aid portal hypertension | [76] |
| **Pancreas** | Diabetes | S1P (via S1PR1) prevents interaction of monocytes with type 1 diabetic endothelium | [77] |
| | Insulin resistance | S1PR2 KO mice have increased plasma insulin levels and polymorphisms in S1PR2, show association with onset of diabetes | [78] |
| | | SphK activity and S1P levels are suggested to be critical for glucose-stimulated insulin secretion | [79] |
| | | pFTY720 treatment of diet-induced obese mice prevented weight gain and improved insulin sensitivity | [80] |
| **Skin** | Atopic dermatitis | Linked to increased release of S1P from mast cells and type 2 helper T cell immune response, where pFTY720 may prove useful | [81,82] |
| | Acne vulgaris | S1P downregulates keratinocyte proliferation and has been considered for use in psoriasis vulgaris and acne vulgaris | [83] |
| | Lupus erythematosus | Increased concentration of serum S1P in JSLE; pFTY720 treatment was effective in SLE | [84] |
| **Skeleton** | Rheumatoid arthritis | Altered S1P levels in arthritis | [85] |
| | Osteoporosis | S1PR1–3 regulate synoviocyte survival, migration, and cytokine production; antagonists of SphK and S1PR may be useful | [86,87] |
| | | pFTY720 reduces osteoclast number and osteoporosis in an ovariectomized mouse model of postmenopausal osteoporosis | [88] |
| **Cancer** | Cancer | Cancer cells promote production of S1P via upregulation of SphK1; this promotes infiltration of mast cells, ECs, platelets, fibroblasts, and neutrophils, resulting in an inflammatory response and tumor angiogenesis | [3,89–93] |

The table summarizes studies reporting links between aberrant S1P/S1PR signaling and disease. Abbreviations: BACE1, beta-site amyloid precursor protein-cleaving enzyme 1; CSF, cerebrospinal fluid; KO, knockout; VEGF, vascular endothelial growth factor; EC, endothelial cell; AMD, age-related macular degeneration; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; TLR4, toll-like receptor 4; JSLE, juvenile-onset systemic lupus erythematosus; SLE, systemic lupus erythematosus.

synthesized. Early suggestions, although over simplistic, have proposed that the clinical benefits of pFTY720 are attributable to the regulation of S1PR1, whereas the side effects of this drug are linked to S1PR3 activation. Thus, as a group, these pharmacological agents have become more selective, with the development of S1PR1 drugs being favored over S1PR3. The lack of commercially available selective S1PR3 compounds has limited our understanding of how S1PR3 plays a role in the efficacy and side effects of pFTY720. Noteworthy compounds among these structures are those preferably used as tool compounds and those currently in clinical trials. These include the pharmacological tool compounds AUY954, a selective S1PR1 agonist, and the newly developed S1PR1