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Targeting Sphingosine-1-Phosphate Receptors in Cancer

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Abstract

Sphingosine 1-phosphate (S1P) is a bioactive lipid with diverse biological functions, including cell proliferation, differentiation, angiogenesis, chemotaxis, and migration. Many of the activities of S1P are mediated through five closely related G-protein-coupled receptors of the sphingosine-1-phosphate receptor family (S1PR) which play a crucial role in sphingolipid metabolism. Each of these receptors appears to be tissue specific and to have demonstrated roles in the regulation of cell proliferation and survival in various cancer types. Further analysis of the function that S1PRs serve in hematological malignancies offers a great potential for the discovery of novel and selective therapeutic agents targeting these receptors. This review focuses on the characterization of S1PRs and their roles in cancer development in various signaling pathways mediated through specific G coupled protein. In particular, pharmacological agents targeting these S1PRs will be discussed and their potential will be examined.

Keywords

Sphingosine-1-phosphate receptors (S1PR); Sphingosine-1-phosphate (S1P); leukemia; G-protein coupled receptors (GPCR)

G-PROTEIN COUPLED RECEPTORS (GPCR)

G-protein coupled receptors (GPCR) comprise a family of more than 900 members.¹ While they are regulated by different agonists², all GPCRs, by virtue of their serpentine motif, utilize heterotrimeric GTP-binding regulatory proteins (G proteins) as a primary means of regulating enzymes for initiation of intracellular signaling.³ G proteins are comprised of three subunits, α , β , and γ . Because the signal transducing properties of the various $\beta\gamma$ combinations do not appear to radically differ from one another, G proteins are classified into four distinct groups based on the isoform of their α -subunit (G_s , $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$).⁴ The first class of G proteins, the stimulatory G protein or G_s (G_s and G_{olf}), activates the target adenylyl cyclase by catalyzing cAMP formation and inducing activation of protein kinase A (PKA), resulting in the downstream activation of many signaling transduction pathways.³ The second class consists of inhibitory G protein or G_i (G_{i1} , G_{i2} , G_{i3} , G_{o1} , G_{o2} , G_z , G_t , and G_{gu}), which is structurally similar to G_s , but works by inhibiting adenylyl cyclase activity and lowering cAMP production. The third class G_q (G_q , G_{11} , G_{14} , $G_{15/16}$)⁴ stimulates membrane bound phospholipase C (PLC), which cleaves the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate to form inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG).³ Lastly, G_{12} (G_{12} , G_{13}),⁴ is part of the Rho family GTPases and works by targeting Rho-specific guanine nucleotide exchange factors, thereby playing

an integral role in cell cytoskeleton remodeling and cell migration.³ GPCRs can be activated by different ligands and extracellular signals to initiate a myriad of molecular signaling pathways, which make them interesting drug targets.⁵ In addition, there are ~120 orphan GPCRs for which no ligands have been identified yet.^{1, 6} Importantly, GPCRs are involved in many diseases, and are also the targets of approximately 30% of all modern medicinal drugs.⁷ GPCRs have been extensively characterized in cancer since 1986 when a link between cellular transformation and the *MAS* oncogene which encodes for a GPCR was established.⁸

GPCRs are activated after binding of a ligand which stimulates the G protein to exchange its bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP). The GTP-bound G protein dissociates from the β/γ subunits, and targets a nearby enzyme, such as adenyl cyclase and therefore activates various downstream pathways including those involved in cell migration, growth, differentiation, and apoptosis. Hydrolysis of the bound GTP leads to G protein α subunit's re-association with the β/γ subunits.⁴

GPCRs have been implicated in the biological process of cell migration. In particular, actin cytoskeleton remodeling is responsible for the cell's ability to change shape and therefore migrate.⁹ GPCRs can activate ERK, which enables ERK to phosphorylate the proteins involved in the cytoskeletal organization such as myosin light chain kinase, calpain, and focal adhesion kinase.⁹ In addition, GPCRs such as the chemokine receptor CXCR2 is pivotal in the process of wound healing, chemotaxis, and inflammation to induce enhanced cellular motility of leukocytes.^{9, 10} In non small cell lung cancer, CXCR4 has been shown to be responsible for the increased cell migration.¹¹ GPCRs have also been shown to transactivate EGFR, enabling cell migration by activation of the MAPK, PI3K, and the JAK/STAT pathways.⁹ $G_{12/13}$ /Rho activation stimulates additional signals necessary for cell proliferation.¹² In particular, RhoA interacts with small G protein Ras by increasing its activity which leads to the MAPK signaling where it translocates to the nucleus and initiates transcription to enable cell growth and survival.¹² GPCRs have also been implicated in the differentiation of many cell lineages through the involvement in the development of stem cells and progenitor cells.¹³ Examples include neuropeptides in neuronal differentiation and axonal cell growth; differentiation of smooth muscle cells, adipocytes, and osteoblasts; and chemokines involved in lymphocyte differentiation.¹³ For example, receptors for part of the complement system, C5a and C3a, have been shown to stimulate T cell proliferation and differentiation. However, inhibiting these receptors results in defective MHC class II expression and reduced T cell responses.¹³ Interestingly, GPCRs play a role in apoptosis through stimulation of the JNK-dependent pathway ENREF 10 via the $G\alpha$ and $G\beta\gamma$ subunits' regulation of the signaling transduction molecules Rho, PLC and PI3K. Upon activation, the JNK proteins translocate to the nucleus and phosphorylate c-Jun, SMAD, and p53 transcription factors that are involved in the regulation of apoptosis.¹ Additionally, GPCRs have been shown to interact with NF- κ B, a molecule that promotes both pro- and anti-apoptotic responses.¹ GPCRs have also been linked to the transcription factor p53, which serves as a substrate for GPCR kinases (GRKs). In particular, degradation of p53 by GRK-5 prevented apoptosis in osteosarcoma cells.^{1, 14}

SPHINGOSINE-1 PHOSPHATE RECEPTORS (*S1PR*)

Sphingosine 1-phosphate receptors (*S1PR*), a family of plasma membrane G-protein coupled receptors, play important roles in the sphingolipid pathway due to their high affinity and receptor specificity for sphingosine-1-phosphate (*S1P*).¹⁵ *S1P*, the ligand for *S1PR* has been implicated in cell proliferation, suppression of apoptosis, and enhancement of cell survival.^{16, 17} ENREF 10 *S1PRs* were originally termed endothelial differentiation gene (*EDG*) receptors *EDG* 1, 3, 5, 6, and 8 which correspond to *S1PR*1, *S1PR*3, *S1PR*2, *S1PR*4,

and S1PR5, respectively¹⁵ (Fig. 1). S1PRs differ in their tissue expression and cell lineage: S1PR1-3 are expressed throughout the immune, central nervous system, and cardiovascular system;¹⁸ S1PR4 is primarily expressed in lymphoid tissue;^{18, 19} and S1PR5 is a predominant receptor in CNS white matter, spleen and natural killer (NK) cells.^{18, 20} ENREF 5

The coupling of S1PRs to the G proteins has been investigated in great details. It is known that S1PR1 is coupled to G_i; S1PR2 and S1PR3 are coupled to G_i and G_q, G₁₂, G₁₃; and S1PR4 and S1PR5 are coupled to G_i, G₁₂, and/or G₁₃ but not G_s and G_q.²¹⁻²⁴ ENREF 15 ENREF 15 Downstream targets of G_i include Ras/ERK (extracellular-signal-regulated kinase) and Rac/PAK (p21-activated kinase) signaling pathways.²⁴ PAKs have recently been discovered as important regulators in cancer-cell signaling networks.²⁵ Rho/ROCK (Rho-associated protein kinase) reside downstream of G₁₂²⁴ and have been shown to have the altered expression or activity in tumors,²⁶⁻²⁸ regulating several important processes in cancer including cellular transformation, survival, invasion, and metastasis.²⁶

SPHINGOSINE-1-PHOSPHATE

Sphingosine-1-phosphate (S1P) plays an important part in migration, survival, and growth of mammalian cells because of its involvement in multiple signaling cascades. It has been known that S1P exists in the ceramide-sphingosine-S1P rheostat, where S1P promotes cell growth and survival while ceramide and sphingosine exhibit pro-apoptotic properties.²⁹ This rheostat has been implicated in cancer cell survival as well as resistance to various therapies.¹⁹ S1P levels are kept constant through its irreversible degradation with S1P lyase and its synthesis by phosphorylation of sphingosine by two sphingosine kinase isoforms, SPHK1 and SPHK2.^{30, 31} SPHK1 is primarily located in the cytosol while SPHK2 is mainly located in the nucleus or endoplasmic reticulum.¹⁹ SPHK1 translocates to the plasma membrane to be in close proximity to sphingosine after phosphorylation of Ser225 by ERK1 and ERK2.^{19, 32} SPHK2 is phosphorylated by protein kinase D which enables its translocation from the nucleus to the cytoplasm.³³ S1P can also be dephosphorylated by S1P phosphatases³⁰ into hexadecenal and phosphoethanolamine.¹⁹ S1P has also been reportedly stored in erythrocytes which act as a buffer system to protect S1P from degradation.³⁴ S1P acts as a tumorigenic growth factor in the tumor microenvironment to promote cancer progression.³⁵ For example, inhibition with siRNA targeting SPHK1 in K562 chronic myeloid leukemia cells decreased S1P levels and increased apoptosis.³⁶ In addition, activation of SPHK results in increased intracellular levels of S1P that can function intracellularly as a second messenger or extracellularly by being secreted outside of the cell and binding to S1PRs which could result in their activation in many types of human diseases.³⁷ ENREF 23 This binding can occur in an autocrine or paracrine manner and is described as “inside-out” signaling.³⁷ S1P is also a proven chemotactic mediator for the trafficking of CD34+ hematopoietic progenitor cells.³⁸ S1PRs belong to the same group of GPCRs as chemokine receptors and evidence shows a dose-dependent chemotactic effect of S1P on human CD34+ hematopoietic progenitor cells (HPCs).³⁸ It has also been observed *in vitro* that withdrawal of S1P results in the inhibition of progenitor migration beneath the stromal cell layer, which is mediated by chemokine receptor 4.^{38, 39}

Currently, new therapies are in development to target S1P signaling in cancer. LT1009 (ASONEP), an S1P-specific antibody, depletes extracellular S1P by acting as a “molecular sponge”.^{19, 40} This drug is currently in Phase I trials for cancer and age related macular degeneration. It has been shown to deplete S1P from the blood and reduce levels of circulating lymphocytes markedly after intravenous administration.⁴⁰ The mechanism of action is designed to work by outcompeting S1P in binding to S1PRs.⁴⁰

SPHINGOSINE-1-PHOSPHATE RECEPTOR 1

Sphingosine-1-phosphate receptor 1 (S1PR1 or EDG-1), a G_i family protein, appears to be the predominant receptor expressed on T and B lymphocytes.¹⁸ S1P binds to S1PR with a K_d of ~ 8 nM.⁴¹ S1PR1 has shown to increase chemotaxis of macrophages and dendritic cells as well as T cells and B cells at high concentrations of S1P ~ 0.3 -3 μ M.⁴² However, at low concentrations of ~ 0.1 -100 nM, S1P decreases chemotaxis for T and B cells.⁴² Overexpression of S1PR1 has been implicated in the progression of T-lymphoblastic lymphoma (T-LBL) to acute T-lymphoblastic leukemia (T-ALL) and inhibition of S1PR1 signaling resulted in decreased homotypic adhesion and increased tumor cell intravasation.⁴³ This led to the conclusion that dissemination of T-LBL is related to high levels of S1PR1 expression.⁴³ S1PR1 also interacts with platelet derived growth factor (PDGF) receptor activating p42/p44 MAPK and enhancing cell growth and migration.⁴⁴ In addition, S1PR1 is not only responsible for T-cell egression from lymphoid tissue, but also implicated in T cell proliferation and cytokine production.⁴⁵ Increased expression levels of S1PR1 have also been shown to positively correlate with the survival in glioblastoma patients.⁴⁶ In particular, downregulation of S1PR1 increases the proliferative activity of glioblastomas, which results in poor survival.⁴⁶

Transgenic S1PR1-null mice typically result in lethal embryonic hemorrhage and do not survive past E12.5 to E14.5.⁴⁷ These mice have deficient ensheathment of new blood vessels by pericytes and smooth muscle cells⁴⁷, indicating S1PR1 plays an essential role in vascular development.⁴⁸

SPHINGOSINE-1-PHOSPHATE RECEPTOR 2

Sphingosine-1-phosphate receptor 2 (S1PR2 or EDG-5), couples to G_i , G_q , and G_{13} and is mainly expressed in the immune, cardiovascular and central nervous systems resulting in essential effects on cellular proliferation, survival, and migration.⁴⁹ S1PR2 displays a 50-60% homology to S1PR1 and has a high binding affinity for S1P ($K_d=16$ -27 nM).⁵⁰ Targeted disruption or inhibition of S1PR2 has led to B-cell lymphoma formation. For example, transgenic S1PR2^{-/-} mice developed clonal B-cell lymphomas with age.⁴⁹ S1PR2 has also been shown to negatively regulate tumor angiogenesis and growth in mice by its G_{12}/G_{13} regulatory effects on Akt and Rac (member of small Rho GTPases).⁵¹ In this study, S1PR2 knockout mice demonstrated an increase in tumor growth and angiogenesis.⁵¹ In particular, mouse embryonic fibroblasts (MEFs) that were S1PR2^{-/-} showed decreased Rho activation,⁵² implicating its role as a negative regulator of PDGFR to mediate proliferation, migration, and SPHK1 activation.⁵³ Also, inhibition of S1PR2 by a selective antagonist showed its role in mediating suppression of migration and invasion.⁵⁴ Astrocytomas of a variety of histological grades expressed S1PR2, however no important correlation between patient survival and grade were found.⁵⁵ S1PR2 has been found to play a significant role by its interaction with COX-2 which is ubiquitously expressed in all Wilms tumor, one of the most common malignant renal tumors in children.⁵⁶ The mechanism of regulation for COX-2 was unknown until overexpression or downregulation of S1PR2 resulted in significant increase or decrease of COX-2 expression levels, respectively.⁵⁶

SPHINGOSINE-1-PHOSPHATE RECEPTOR 3

Sphingosine-1-phosphate receptor 3 (S1PR3 or EDG-3), couples to G_i , G_q , and G_{13} and is mainly expressed in tissues such as the heart, brain, lung, liver, kidney, pancreas, thymus, and spleen.⁵⁰ S1PR3 has about 50% homology to S1PR1 and S1PR2 with a high binding affinity for S1P ($K_d=23$ -26 nM).⁵⁰ No obvious phenotypic abnormalities were observed upon knockout of S1PR3, which suggests that other receptors, S1PR1 and S1PR2 might compensate for the loss of S1PR3 due to their structural and functional similarities.⁵⁰

However, S1PR3 has been shown to be co-amplified with SHC3 (Src homology 2 domain containing) transforming protein 3, in ~60% of ependymomas, which are the third most common brain tumor type in children and the most common primitive tumor of the spinal cord in adults.⁵⁷ Human breast cancers have also shown to predominantly express S1PR3 and treatment with all-trans retinoic acid (ATRA) decreased migration.⁵⁸

S1PR3-null mice are still viable and fertile but suffer from significant decrease in litter sizes, indicating an important role in normal development.⁵² The cells derived from these particular transgenic mice reveal defects in S1P signaling.⁴⁸

SPHINGOSINE-1-PHOSPHATE RECEPTOR 4

Sphingosine-1-phosphate receptor 4 (S1PR4 or EDG-6), couples to G_i and $G_{12/13}$ with a high affinity for S1P ($K_d=12-63$ nM). S1PR4 showed more limited expression and appeared to be confined to tissues and cells of hematopoietic system and lung.⁵⁰ S1PR4 has ~44% identity to S1PR1 and ~46% identity to S1PR3. In CHO-K1 cells, S1PR4 was shown to activate small Rho GTPase and result in cytoskeletal rearrangements after stimulation with S1P.⁵⁹ Its predominance in the lymphoid system suggests it may play an important role in immune surveillance and maintenance. For instance, S1PR4 has been implicated in the development of megakaryocytes; S1PR4 deficient mice which displayed atypical and reduced formation of proplatelets.⁶⁰ In addition, overexpression of S1PR4 in human erythroleukemia cells resulted in release of platelet sized particles and formation of cytoplasmic extensions.⁶⁰ S1PR4 has also been shown to interact with human epidermal growth factor receptor 2 (HER2) that results in stimulation of ERK1 and ERK2 in MDA-MB-453 breast cancer cells.⁶¹ This link between S1PR4 and oncogene HER2 suggests an important role of S1PR4 in breast cancer progression.⁶¹

SPHINGOSINE-1-PHOSPHATE RECEPTOR 5

Sphingosine-1-phosphate receptor 5 (S1PR5 or EDG-8), couples to G_i and $G_{12/13}$. S1PR5 has the highest affinity for S1P ($K_d=2-6$ nM).⁵⁰ S1PR5 is present in brain and spleen; and it was initially discovered in peripheral blood mononuclear cells (PBMC) from T cell large granular lymphocyte (LGL) leukemia as an upregulated EST.¹⁶ Indeed, in a microarray analysis S1P5 was found to be upregulated in ~80% of T-LGL leukemia patient samples compared to normal PBMCs.⁶² ENREF 10 Human S1PR5 shows two alternatively spliced forms; a 5.4-kb transcript that is predominantly expressed in peripheral tissues, and a 2.4-kb transcript preferentially expressed in oligodendrocytes and leukemic LGL cells.^{16, 63, 64} S1PR5 appears to mediate S1P-induced survival of mature oligodendrocytes through the G_i -sensitive pathway.⁶⁵ In addition, S1PR5 mediated activation of S1P has been implicated in a cytoprotective role for prostate cancer cell line PC3, through induction of autophagy.⁶⁶ In addition, S1PR5 is the predominant type of S1P receptor expressed on normal NK cells. NK cell homing was aberrant in S1PR5^{-/-} mice during steady-state conditions⁶⁷, indicating that S1PR5 plays a role in NK cell trafficking. As previously mentioned, S1PR5 is highly expressed in central nervous system, in particular oligodendrocytes.²³ Although S1PR5-null mice did not display the myelination defects⁶⁵, they seemed to suffer from the migration defects.⁶⁸

PHARMACOLOGICAL AGENTS TARGETING S1PRS

FTY720

FTY720, also known as Fingolimod (GilenyaTM) is a pharmacological agonist of S1P receptors inhibiting the egress of T cells and B cells from lymphoid organs.^{67, 69} At low nanomolar concentrations, FTY720 becomes phosphorylated *in vivo* and acts as a potent

agonist for most of sphingosine-1 phosphate-receptors.¹⁸ In this capacity as an immunomodulatory drug and a lipophilic oral agent, FTY720 was recently approved by the FDA for treatment of multiple sclerosis, based on Phase III efficacy data.^{70, 71} ENREF_62 ENREF_62 Upon phosphorylation by sphingosine kinase, FTY-720 is converted *in vivo* to FTY720-phosphate, a close structural analog of S1P⁷², which then acts as an agonist targeting S1PR1, S1PR3, S1PR4, and S1PR5 in a wide variety of tissues¹⁸ (Table 1). However, in lymphocytes it works as an antagonist to stimulate the internalization and degradation of the receptors, leading to receptor downregulation.¹⁸ FTY720 has various immunologic effects on lymphocytes as an immunomodulatory drug. Downregulation of S1P receptors from FTY720 prevents lymphocyte egression from lymph nodes, resulting in depletion of circulating lymphocytes by sequestration of lymphocytes in the lymphoid tissue.¹⁸ However, FTY720 does not affect circulation of CCR7-effector memory T cells (T_{EM}) while it retains expression of CCR7⁺ naïve and central memory T cells in the lymph nodes, thus maintaining many normal immune responses.^{18, 73} Treatment in animals and humans has resulted in overall reductions in peripheral blood mononuclear cells (PBMC).^{18, 74} Discontinuation of FTY720 results in lymphocytes returning to normal counts within 4-8 weeks since it works through an immunomodulating function by redirecting lymphocytes rather than inducing a cytotoxic response.¹⁸ This is favorable because this compound does not alter lymphocyte function or proliferation.¹⁸

In contrast, at high micromolar concentrations, FTY720 has shown anti-tumor activity, inducing apoptosis of solid tumor cells or leukemic cells through S1PR-dependent or S1PR-independent mechanisms.^{18, 75} Efficacy of FTY720 in sensitizing cells to treatment has been reported in a wide variety of solid tumors. In hepatocellular carcinoma, it has been shown to induce reactive oxygen species (ROS) production, which leads to protein kinase C delta activation and subsequent caspase-3-dependent apoptosis.⁷⁶ FTY720 was able to induce a strong time- and dose-dependent cytotoxic response in ovarian cancer cells that were resistant to cisplatin⁷⁵; cell death was shown to be caspase-3 independent occurring through induction of necrosis and autophagy.⁷⁵ Prostate cancer cells were also shown to become sensitized to radiotherapy and S1PR-independent apoptosis after FTY720 treatment through inhibition of sphingosine kinase 1 (SPHK1). In chronic lymphocytic leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia, FTY720 demonstrated preclinical anti-tumor activity through induction of PP2A-dependent apoptosis.^{47, 77} Notably, the high micromolar doses of FTY720 have the ability to inhibit SPHK1 activity leading to the accumulation of sphingosine.⁷⁸ As a result, sphingosine functions as a second messenger by activation of JNK and p38 to mediate apoptosis.⁷⁹ FTY720 was also found to induce apoptosis in leukemic LGL cells and sensitize LGL cells to Fas-mediated cell death.⁶² Potential side effects of administration of FTY720 include first-dose bradycardia and macular edema, necessitating patient observation for the first 6 hours after treatment.¹⁸ ENREF_4 ENREF_4 ENREF_4 ENREF_4 ENREF_4 ENREF_4 ENREF_4 ENREF_4

KRP-203

KRP-203, 2-amino-2-[2-[4-(3-benzoyloxyphenylthio)-2-chlorophenyl]-1,3-propanediol hydrochloride, is an immunosuppressant that has been identified as an agonist for S1PR1 and appears to behave similarly to FTY720, by sequestering lymphocytes in lymphoid tissues³¹ (Table 1). In particular, KRP-203 enabled skin and heart engraftment in animal models of organ transplantation.⁸⁰ Immunohistochemical analysis was performed 100 days after transplant and revealed that typical infiltration of macrophages and T cells were significantly reduced by KRP-203 treatment.⁸⁰

SEW2871

SEW2871, 5-(4-phenyl-5-trifluoromethylthiophen-2-yl)-3-(3-trifluoromethylphenyl)-(1, 2, 4)-oxadiazole is a highly selective S1PR1 agonist which also induces lymphocytopenia similar to FTY720.⁸¹ Similar to S1P, SEW2871 induces the internalization and recycling of S1PR1;⁸¹ however, unlike FTY720, SEW2871 is able to induce the receptors to recycle back to the plasma membrane⁸², while FTY720 results in degradation of the receptors.⁸¹

JTE-013

JTE-013, 1-[1,3-Dimethyl-4-(2-methylethyl)-1H-pyrazolo[3,4-b]pyridine-6-yl]-4-(3,5-dichloro-4-pyridinyl)-semicarbazide is an antagonist for S1PR2 (Table 1) which was shown to inhibit the ability of S1P to increase outflow resistance and therefore increase intraocular pressure in the eye.⁸³ Treatment with JTE-013 led to enhanced migration in endothelial cells and smooth muscle cells⁸⁴, as well as glioma cells⁸⁵ and it was found that S1PR2 has a negative regulatory role.⁸¹ For instance, combination injection of JTE-013 with S1P into cutaneous wounds resulted in accelerated healing due to the stimulation of cell proliferation, differentiation, and angiogenesis.⁸⁶

Suramin

Suramin, a symmetrical polysulfonated naphthylamine derivative of urea⁸⁷, is a selective S1PR3 and S1PR5 antagonist (Table 1). S1P/S1PR3 plays a role in liver fibrogenesis through migration of bone marrow cells to damaged liver tissue⁸⁸. In a mouse model of cholestasis-induced liver fibrosis, suramin was able to drastically reduce bone marrow derived cells during cholestasis and alleviate bile duct ligation induced hepatic fibrosis.⁸⁸ Suramin is currently in clinical trials for bladder, prostate, and breast cancers (<http://www.clinicaltrials.gov/ct2/results?term=sur-amin>).

VPC23019 (Aryl-amide Analogs)

Aryl-amide containing compounds act as competitive antagonists for S1PR1 and S1PR3 and were developed as a tool to better understand S1P biology.⁸⁹ VPC-23019, (R)-phosphoric acid mono-[2-amino-2-(3-octyl-phenylcarbamoyl)-ethyl] ester) is the lead compound in the series. VPC-23019 interferes with the ability of S1P to prevent activation of these receptors³¹ (Table 1) ENREF 27 ENREF 23. This drug reverses the effects of SEW2871 and as a result, inhibits mitogen-activated protein kinase activation, cell migration, and T-cell receptor internalization.^{81, 90} These analogs block the agonist-mediate migration and calcium mobilization in T24 cells, a form of human urinary bladder carcinoma.⁸⁹ Analogs of VPC23019 have also been tested on the S1PRs. In particular, shortening the alkyl chain by one carbon or two carbon atoms resulted in VPC25239 and VPC23031, respectively.⁸⁹ These analogs also worked similarly as antagonists for both S1PR1 and S1PR3.⁸⁹

W123, W146

W123, 3-{[(3-hexyl-phenylcarbamoyl)-methyl]-amino} -propionic acid), is a competitive antagonist of S1PR1 that reverses T cell motility and egression^{81, 90} (Table 1). In addition, W123 is also a competitive antagonist of S1PR1 by SEW2871.⁹⁰ W146, (R)-3-Amino-4-(3-hexylphenylamino)-4-oxobutylphosphonic acid, is also a selective S1PR1 antagonist which through its inhibition results in loss of capillary integrity in mouse skin and lung, but ENREF 82 does not affect the number of blood lymphocytes⁹¹ (Table 1) ENREF 79. ENREF 77

Concluding Remarks

S1PRs play critical roles in cell survival, proliferation, migration, and differentiation during cancer development and progression of cancers, including hematological malignancies,

which make them critical and important targets for potential therapeutics in cancers. As GPCRs, these receptors make ideal targets for drug development due to the specificity of compounds that can be generated. S1PRs seem to have discreet functions such as pro- or anti-survival signaling depending on the cell type and cancer involved. Therefore it will be necessary to develop specific antagonists or agonists for each tumor type for optimum treatment because a particular S1PR expressed in one tissue may play a different role in another tissue. For example, activating S1PR1 in glioblastoma to decrease proliferation could yield completely different results in another cancer such as T-lymphoblastic lymphoma where overexpression is linked to its progression. Many new pharmacological compounds are currently being investigated, although FTY720 appears the most advanced. Further research needs to be conducted in *in vivo* models to determine efficacy of the rest of these compounds on S1PRs. In addition, the improved understanding of the molecular signaling of S1PRs may lead to the discovery of a new generation of S1PR-based therapeutics.

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Fig. (1).

Sphingosine-1-Phosphate Receptors. S1PR1-5 regulate many biological processes in various cell types. S1PR1 is the predominant receptor expressed on T and B lymphocytes and couples to the G_i protein. S1PR2 couples to G_i , G_q , and G_{12} and is mainly expressed in the immune, cardiovascular, and central nervous systems. S1PR3 also couples to G_i , G_q , and G_{12} with expression found in the heart, brain, lung, liver, kidney, pancreas, thymus, and spleen. S1PR4, coupled to G_i and G_{12} , has more limited expression and is confined to tissues of the hematopoietic system. S1PR5 also coupled to G_i and G_{12} is present in the brain, spleen, and natural killer cells.

Key: AC=adenylate cyclase, ERK=extracellular regulated kinase, cAMP=cyclic adenosine monophosphate, PLC=Phospholipase C, Rho=rhodopsin, ROCK=Rho associated protein kinase, Ras=Rat sarcoma

Table 1

Pharmacological Inhibitors Targeting Sphingosine-1-Phosphate Receptors

Pharmacological Agent	Structure	Receptor Specificity	Receptor Type	Experimental or Clinical Data
<i>FTY720</i>		S1PR1 S1PR3 S1PR4 S1PR5	Agonist	Approved by FDA for treatment of multiple sclerosis ^{70,71}
<i>SEW2871</i>		S1PR1	Agonist	Induces lymphocytopenia ⁵¹
<i>KSP-203</i>		S1PR1	Agonist	Applied in animal models of organ transplantation ⁸⁰ Currently in Phase II trials for subacute cutaneous lupus erythematosus [NCT01294774]
<i>Suramin</i>		S1PR3 S1PR5	Antagonist	Reduced ability of bone marrow derived cells to induce fibrogenesis in a mouse model of cholestasis induced liver fibrosis ⁸⁸ Currently in phase II trials for Multiforme Glioblastoma [NCT0000294774] and adrenocortical carcinoma [NCT00002921]
<i>W146</i>		S1PR1	Antagonist	Results in loss of capillary integrity in mouse skin and lung ⁹¹
<i>W123</i>		S1PR1	Antagonist	Reverses T cell motility and egressions ^{81,90}
<i>VPC23019</i>		S1PR1 S1PR3	Antagonist	Blocked agonist activity and calcium mobilization in urinary bladder carcinoma cells ⁸⁹
<i>JTE-013</i>		S1PR2	Antagonist	Enhanced migration in endothelial, smooth muscle, ⁸⁴ and glioma cells ⁸⁵