Journal of Cellular Biochemistry 92:913–922 (2004)

Structural and Functional Characteristics of S1P Receptors

Teresa Sanchez and Timothy Hla*

Department of Cell Biology, Center for Vascular Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmngton, Connecticut 06030-3501

Abstract The sphingosine-1-phosphate (S1P) family of G protein-coupled receptors (GPCR) regulates essential cellular processes such as proliferation, migration, cytoskeletal organization, adherens junction assembly, and morphogenesis. S1P, a product from the breakdown of sphingomyelin, binds to the five members of this receptor family, $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$, previously referred to as endothelial differentiation gene (*EDG*)-1, -5, -3, -6, and -8. S1P receptors are widely expressed in different tissues, so it is not surprising that the S1P receptor family regulates many physiological processes, such as vascular maturation, cardiac development, lymphocyte trafficking, and vascular permeability. FTY720, a new S1P receptor agonist, is undergoing clinical trials as an immunosuppressor. Understanding the physiological role of these receptors and the basics of the ligand-receptor interaction will potentially provide new therapies to control a variety of diseases. J. Cell. Biochem. 92: 913–922, 2004. © 2004 Wiley-Liss, Inc.

Key words: Rho GTPases; migration; vascular maturation; vascular permeability; sphingosine kinase

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid that mediates a wide variety of cellular responses in different cell types through the interaction with the members of the endothelial differentiation gene (*EDG*) family of plasma membrane-localized G protein-coupled receptors (GPCR). Before the discovery of the S1P receptors, it was believed that S1P acted as an intracellular mediator [Zhang et al., 1991]. This idea is supported by the fact that increases in intracellular S1P levels take place upon cell stimulation by growth factors [Olivera and Spiegel, 1993], cytokines [Xia et al., 1999], and hormones [Sukocheva et al., 2003], which activate sphingosine kinase activity. However, the intracellular molecular targets of S1P remain to be identified.

Early in 1992, Igarashi's group suggested that S1P could act as an extracellular mediator in the control of cell motility through a putative transmembrane receptor [Sadahira et al., 1992]. To date, five S1P receptors have been identified, and recently renamed as $S1P_1$(EDG-1), $S1P_2$ (EDG-5), $S1P_3$ (EDG-3), $S1P_4$ (EDG-6), and $S1P_5$ (EDG-8). They have overlapping as well as distinct patterns of expression in different tissues. In addition, the coupling of these receptors to different G proteins explains their differential signal transduction properties, and also the varied cellular effects of S1P. Herein, we will review the interaction of S1P with its receptors, signaling properties and the function of the different S1P receptors, as well as their potential significance in human health and disease.

CLONING, DISCOVERY, AND CHARACTERIZATION OF S1P RECEPTORS

The first member of the S1P receptor family to be cloned was $S1P_1$. It was originally discovered

Abbreviations used: $CB_{1,2}$, cannabinoid receptor 1 and 2; CHO, Chinese hamster ovary; EDG, endothelial differentiation gene; ERK, extracellular signal regulated kinase; FTY720-P, FTY720-phosphate; GPCR, G protein coupled receptor; HEK, human embryonic kidney cells; HEL, human erythroleukemia cells; HUVEC, human umbilical vein endothelial cells; JNK, c-jun N-terminal kinase; LPA_{1-3}, lysophosphatidic acid receptor 1–3; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; PI3K, phosphoinositide-3-kinase; S1P, sphingosine-1-phosphate; $S1P_{1-5}$, sphingosine-1-phosphate receptors 1–5; VEGF, vascular endothelial cell growth factor.

*Correspondence to: Timothy Hla, Department of Cell Biology, Center for Vascular Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3501. E-mail: hla@nso2.uchc.edu

Received 14 November 2003; Accepted 13 March 2004

DOI 10.1002/jcb.20127

© 2004 Wiley-Liss, Inc.

as a transcript induced during endothelial cell differentiation in vitro [Hla and Maciag, 1990]. Later, two other S1P receptors were cloned, $S1P_2$, from rat brain and rat vascular smooth muscle cell [Okazaki et al., 1993; MacLennan et al., 1994] and $S1P_3$, from a human genomic library [Yamaguchi et al., 1996]. The identity of the ligand was still unknown at that time but later studies revealed that S1P was a high affinity ligand for $S1P_1$ [Lee et al., 1998b]. Subsequently, $S1P_4$ was cloned from in vitro differentiated human and murine dendritic cells [Graler et al., 1998]. Finally, the gene *nrg-1*, which was cloned from a rat PC12 cell cDNA library [Glickman et al., 1999], was characterized and shown to be a S1P receptor, $S1P_5$ [Im et al., 2000].

The sequence analysis of these receptors indicates that they are members of the superfamily of GPCR, and therefore, they have related structural elements. S1P receptors exhibit ~20% amino acid sequence identity with cannabinoid receptors (CB_1 and CB_2) and ~30% with lysophosphatidic acid receptors (LPA_{1-3}). The relationship of S1P receptors with LPA and CB receptors is shown in Figure 1. S1P receptors consist of the extracellular NH_2 terminus, which contain potential N-linked glycosylation sites, seven transmembrane domains, and the respective hydrophilic extracellular and intracellular loops. Interestingly, the Asp residue near the end of the third transmembrane domain is conserved in all GPCR activated by cationic ligands and is thought to be essential for the binding of charged amines. This Asp residue is replaced with Glu in the S1P receptor family. The Cys residue at the C terminus is also conserved among most of GPCR and in the S1P familiy, and it is important as a palmitoylation site. The intracellular hydrophilic loop regions and the C-terminus contain several potential recognition sites for phosphorylation by serine/threonine protein kinases. In the case of $S1P_1$, our laboratory has shown the phosphorylation of the Thr^{236} residue, at the third intracellular loop, by the protein kinase Akt [Lee et al., 2001].

All five receptors from this family bind to S1P with high affinity except for the $S1P_4$ receptor. Phytosphingosine-1-phosphate was actually shown to be a much better agonist for this receptor [Candelore et al., 2002]. Non-phosphorylated sphingosine derivatives (sphingosine, sphinganine, ceramide) have been shown not to compete with the binding of S1P. Phospholipids lacking a basic amine (sphingomyelin, lysophosphatidic acid, phosphatidyl inositol) also failed to compete with S1P at physiologically relevant concentrations. Of the many compounds evaluated for $S1P_1$ interaction, only dihydro-S1P [Van Brocklyn et al., 1998], sphingosylphosphorylcholine [Okamoto

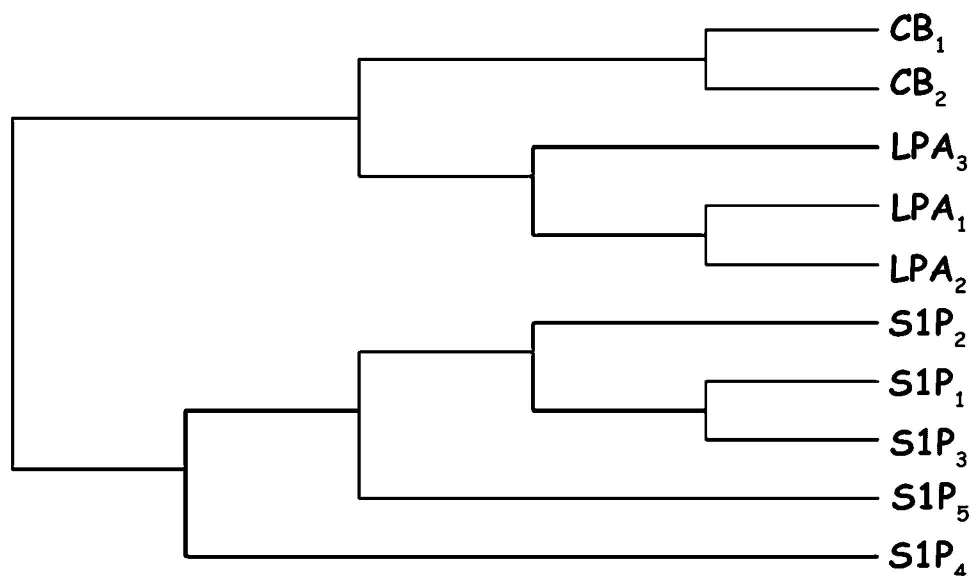

Fig. 1. Phylogenetic tree representation of CB, LPA, and S1P receptors. The amino acid sequences of CB, LPA, and S1P receptors were aligned and analyzed by the CLUSTAL program. Phylogenetic reconstruction was done with Mac Vector 7.1.1 program by using neighbor joining method. The best tree, midpoint rooted is shown.

et al., 1998], and S1P-homophosphonate [Van Brocklyn et al., 1999], which maintains the appropriate chain length between the cationic and the anionic moieties, have been shown to displace S1P from $S1P_1$. Lastly, competition studies with S1P steroisomers showed that the presence and configuration of the C3 hydroxyl group of S1P (Fig. 2) is important for the binding to S1P receptors [Lim et al., 2003].

In additional studies, a computational model of the $S1P_1$ receptor was developed to predict the interactions with S1P [Parrill et al., 2000]. The model established three ion-pairing interactions critical to the recognition of S1P by its receptor: Arg^{120} and Arg^{292} with the phosphate group of S1P, and Glu^{121} with the protonated amino group of S1P. These predictions were confirmed by site-directed mutagenesis followed by binding and receptor activation assays as well as by internalization studies. All S1P receptors share an anionic residue that corresponds to the Glu^{121} in $S1P_1$, and two basic residues that are predicted to interact with the phosphate group. However, the confirmation of this theoretical model for $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ is lacking.

Recently, a new pharmacological modulator of S1P receptors has been described, namely, the immunomodulatory agent FTY720 [Brinkmann et al., 2002; Mandala et al., 2002] (Fig. 2). FTY720 is phosphorylated in vivo and the phosphorylated form is a potent agonist of $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$. FTY720-phosphate (FTY720-P) shares structural similarities with S1P: it contains a lipophilic tail, a 2-amino group, and a phosphate head group. It has a phenyl ring inserted between the polar head and the lipophilic tail, which has been recently shown to confer an increase in agonism at $S1P_5$, loss of activity at $S1P_2$, and loss of the stereospecificity of position 2 at $S1P_1$ and $S1P_3$ receptors [Clemens et al., 2003].

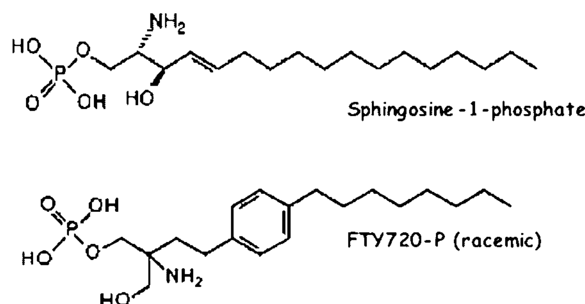

Fig. 2. Chemical structures of S1P and FTY720-P.

In conclusion, further studies need to be done in order to elucidate the structure-activity relationship of S1P for each of its receptors. In addition, identification of S1P receptor specific agonists/antagonists will provide further information about the physiological/pathophysiological role of each S1P receptor and may lead to the development of new therapeutic agents.

SIGNALING OF S1P VIA ITS RECEPTORS: CELLULAR EFFECTS OF S1P

Binding of S1P to its receptors activates different signaling pathways via the heterotrimeric G proteins. The coupling of $S1P_1$, $S1P_2$, and $S1P_3$ to G proteins has been studied by using chimeric G proteins in the oocyte system [Ancellin and Hla, 1999] and also by determining the binding of ^{35}S-GTPγS to G_i, G_q, G_s, and G_{13} in cell membranes, using a heterologous expression system of insect Sf9 cells [Windh et al., 1999]. In both studies, $S1P_1$ coupled exclusively to Gi, as it had been previously reported by Lee et al. [1996] when $S1P_1$ was still an orphan receptor. On the other hand, $S1P_2$ and $S1P_3$ coupled to G_i, G_q, and G_{13}. Subsequently, $S1P_5$ was shown to couple to $G_{i/o}$ and G_{12} in the binding assays using $S1P_5$-overexpressing CHO cells [Malek et al., 2001].

The downstream signaling activated by S1P binding to its receptors has been extensively studied in different types of mammalian cells, such as human embryonic kidney (HEK) cells, Chinese hamster ovary (CHO) cells, human erythroleukemia (HEL) cells, Jurkat T cells, and HTC4 rat hepatoma cells (reviewed in [Pyne and Pyne, 2000; Kluk and Hla, 2002]) (Fig. 3). In $S1P_1$ overexpressing cells, S1P induced the stimulation of extracellular signal-regulated kinase (ERK), phospholipase C (PLC), and phosphoinositide 3-kinase (PI3K) β, as well as inhibition of cyclic AMP accumulation. All these responses were *Pertussis* toxin sensitive, indicating that they were mediated via G_i. $S1P_3$ and $S1P_2$ also mediate activation of ERK in a G_i dependent way. However, these two receptors also couple to PLC in a *Pertussis* toxin insensitive way, suggesting that this response is mediated by G_q. $S1P_3$ and $S1P_2$ can also mediate Rho activation through $G_{12/13}$.

Lastly, the signaling through $S1P_4$ and $S1P_5$ has yet to be studied thoroughly. However, it is believed that $S1P_4$ couples to G_i, based on the *Pertussis* toxin sensitivity of ERK [Van

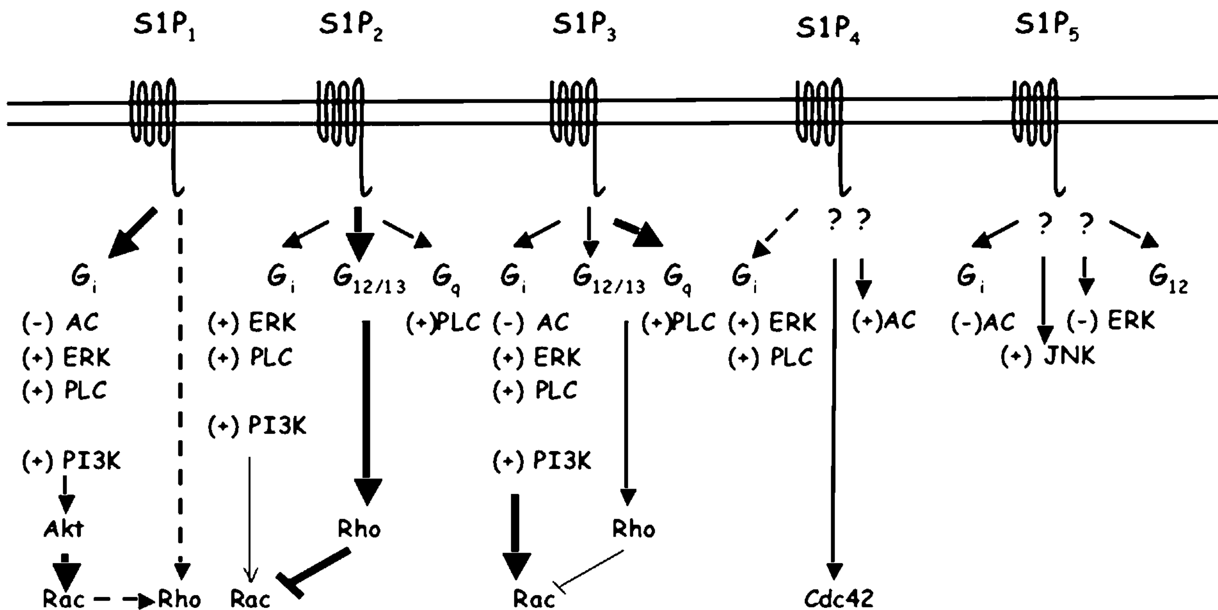

Fig. 3. Dowstream signaling pathways activated by S1P receptors. $S1P_1$ stimulation leads to activation of ERK, PLC, PI3K β, and inhibition of adenylate cyclase (AC) via G_i. Akt phosphorylation of $S1P_1$ is necessary for Rac activation. In endothelial cells Rac is upstream of Rho, at least in the induction of stress fibers. $S1P_2$ couples via G_i to ERK and PLC activation, via $G_{12/13}$ to Rho and via G_q to PLC. Similarly, $S1P_3$ activates ERK and PLC and inhibits AC via Gi, it activates PLC via G_q and Rho via $G_{12/13}$. Studies with $S1P_2$ and $S1P_3$ null cells indicate that $S1P_2$ couples more strongly to $G_{12/13}$, and $S1P_3$ to G_q. $S1P_1$, $S1P_2$, and $S1P_3$ can activate Rac, via PI3K. $S1P_4$ activates ERK and PLC in a *Pertussis* toxin sensitive way, and activates AC. It has recently been reported that it brings about Cdc42 activation. $S1P_5$ couples to Gi and $G_{12/13}$. It inhibits AC via Gi and it also inhibits ERK activation and activates JNK.

Brocklyn et al., 2000] and PLC activation [Yamazaki et al., 2000]. It has also recently been reported that $S1P_4$ brings about activation of Cdc42 [Kohno et al., 2003], a member of the Rho family of GTPases. With respect to $S1P_5$, it has been shown to mediate inhibition of cyclic AMP accumulation via G_i [Im et al., 2000; Malek et al., 2001]. In addition, $S1P_5$ activated c-jun N-terminal kinase (JNK) and inhibited serum-induced ERK activation in a *Pertussis* toxin insensitive way, although the mechanisms are still unclear [Malek et al., 2001].

Through the activation of different signaling pathways, S1P can regulate many different cellular functions, among them, adherens junction assembly, cytoskeletal changes, migration, proliferation, and apoptosis. The different activities triggered by S1P depend on the pattern of expression of S1P receptors in each cell type. $S1P_1$ was discovered as a transcript induced in endothelial cells after phorbol 12-myristate 13-acetate (PMA) treatment [Hla and Maciag, 1990]. Since PMA promotes differentiation of endothelial cells into tubular structures, it was hypothesized that $S1P_1$ played a role in this phenomenon. Later on, S1P was identified as the ligand for $S1P_1$ and was shown to induce cell aggregation and the expression of P- and E-cadherins. These effects were dependent on the small GTPase Rho. S1P also induced the formation of well-developed adherens junctions in $S1P_1$ overexpressing HEK293 cells [Lee et al., 1998b]. In human umbilical vein endothelial cells (HUVEC), which express $S1P_1$ and $S1P_3$, it was later shown that S1P induced translocation of VE-cadherin and β-catenin to the adherens junctions. This phenomenon was mediated by $S1P_1$ and $S1P_3$ and required the activity of the small GTPases Rho and Rac [Lee et al., 1999]. S1P also induced Rho-dependent stress fiber formation, Rac-dependent cortical actin assembly, and morphogenesis into capillary-like structures.

The Rho familiy of GTPases are important regulators of the actin cytoskeleton and cell motility [Hall, 1998]. Indeed, S1P, which activates the small GTPases Rac and Rho [Paik et al., 2001] is also a potent chemoattractant for endothelial cells. S1P induced Rho-dependent integrin clustering into focal contact sites, which was essential for cell adhesion, spreading, and migration. Another important player in S1P-induced migration is the protein kinase Akt. S1P triggered phosphorylation of Akt in

endothelial cells [Igarashi et al., 2001; Morales-Ruiz et al., 2001], which was essential for S1P-induced migration [Lee et al., 2001]. In addition, Akt binds to $S1P_1$ and phosphorylates the third intracellular loop at the Thr^{236} residue. This phosphorylation is necessary for Rac activation, cortical actin assembly, and chemotaxis. A $S1P_1$ mutant, T236A, failed to activate Rac and induce migration, thereby, acting as a dominant negative receptor.

Interestingly, in other cell types, like vascular smooth muscle cells [Bornfeldt et al., 1995] and melanoma cells [Sadahira et al., 1992], S1P is an inhibitor of migration. This effect has been attributed to the expression of $S1P_2$ in these cells [Ryu et al., 2002; Arikawa et al., 2003]. In B16 melanoma cells, expression of $S1P_2$ but not $S1P_1$ or $S1P_3$ was detected. In these cells, S1P inhibited migration and invasion [Arikawa et al., 2003]. This effect was abrogated by JTE013, a specific $S1P_2$ antagonist, indicating again the involvement of this receptor in inhibiting the migratory response to S1P. The mechanism whereby $S1P_2$ mediates inhibition of migration is an interesting topic to address. Both $S1P_2$ and $S1P_3$ couple to G_i, G_q, and G_{13}. However, while $S1P_3$ promotes migration, $S1P_2$ acts as a repellant receptor. This question has been addressed by Takuwa's group [Sugimoto et al., 2003]. They showed a counteraction between G_i and $G_{12/13}$ with regards to Rac activation and regulation of migration. S1P receptor coupling to G_i lead to activation of Rac and migration, while coupling to $G_{12/13}$ brought about a Rho-dependent inhibition of Rac and migration (Fig. 3). Thus, in the case of $S1P_2$, a robust coupling to $G_{12/13}$ and a more modest coupling to G_i could explain the effects of this receptor. This is in agreement with the fact that S1P stimulation of Rho is considerably impaired in $S1P_2$ null mouse embryonic fibroblasts [Ishii et al., 2002].

S1P has also been shown to modulate the migratory response of naïve lymphocytes, which express predominantly $S1P_1$ and $S1P_4$. [Graeler and Goetzl, 2002; Graeler et al., 2002]. S1P, at lower concentrations, was a chemoattractant for naïve CD4 and CD8 T cells and also enhanced chemotaxis to CCL-21 and CCL-5. However, higher physiological concentrations of S1P inhibited this response. In addition, activation of T cell receptors resulted in decreased expression of $S1P_1$ and $S1P_4$, thereby suppressing the effects of S1P on chemotaxis. Interestingly, S1P inhibits proliferation of lymphocytes induced by T cell receptor activation [Dorsam et al., 2003]. Although the mechanisms are not completely understood, this effect is apparently mediated by $S1P_1$ and requires Ca^{2+} signaling and low levels of cyclic AMP. Since S1P is present in serum [Yatomi et al., 1997] and it can be released by inflammatory cells [Prieschl et al., 1999; MacKinnon et al., 2002], it may be an important regulator of the homeostasis of the immune system during an inflammatory response. Moreover, the levels of S1P in a specific physiological compartment can differentially regulate the response of lymphocytes.

Another cellular effect mediated by S1P is mitogenesis and protection from apoptosis. These effects have been shown in fibroblasts, endothelial cells, and vascular smooth muscle cells. In fibroblasts, mitogenesis was attributed to intracellular actions of S1P [Lee et al., 1998a], although the intracellular targets of S1P are yet to be identified. In other cellular systems the proliferative effect of S1P has been shown to be *Pertussis* toxin sensitive. In vascular smooth muscle cells, $S1P_1$ and G_i signaling were required for S1P-induced DNA synthesis and cellular proliferation. This effect was mediated by activation of p70 S6 kinase and increased levels of cyclin D1 [Kluk and Hla, 2001].

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLE OF S1P RECEPTORS

Among the S1P receptors, $S1P_1$, $S1P_2$, and $S1P_3$ are widely expressed in various tissues, whereas the expression of $S1P_4$ is confined to lymphoid and hematopoietic tissue and $S1P_5$ to the central nervous system. From this pattern of expression, it is likely to expect an important role for these receptors in the regulation of many different physiological functions.

The role of S1P receptors in embryonic development is underscored by gene targeting studies. Indeed, the phenotype of the $S1P_1$ knock out mice shows the crucial role of this receptor in vascular maturation [Liu et al., 2000]. The null $S1P_1$ embryos died between E12.5 and E14.5 due to massive hemorrhage. The aortae were incompletely covered with vascular smooth muscle cells. This defect in mural cell coverage was also found in the vessels at the brain and limbs, which were undeveloped

and rounded. The similar phenotype of the conditional $S1P_1$ knock out in endothelial cells indicates that $S1P_1$ signaling in the endothelium is essential to regulate the coverage of vessels by vascular smooth muscle cells [Allende et al., 2003].

A zebrafish mutation in the *mil* gene called "miles apart" indicates a role of S1P receptors in the development of the cardiovascular system [Kupperman et al., 2000]. Mutants showed a defective migration of the cardiac muscle progenitor cells from lateral positions to the midline resulting in the development of two hearts. Interestingly, the product of the *mil* gene seems to function in the midline cells and not in the migrating cardiomyocytes. They also had a defect in epithelial integrity. Mil encodes a product, most similar to $S1P_2$, which shares $\sim$50% identity with S1P receptors, and it is activated by S1P, indicating the role of this lipid mediator in embryogenesis.

Disruption of $S1P_2$ in mice did not cause any abnormality in embryonic development [MacLennan et al., 2001; Ishii et al., 2002], similar to $S1P_3$ null mice [Ishii et al., 2001]. $S1P_2$ null mice and $S1P_3$ null mice were fertile but the litter sizes were slightly smaller. This suggests a role of $S1P_2$ and $S1P_3$ in reproduction, in agreement with the expression of these receptors in the gonads [Ishii et al., 2001] and prevention of oocyte apoptosis by S1P [Morita et al., 2000]. MacLennan reported episodes of seizures in $S1P_2$ knock out mice, accompanied by electroencephalographic abnormalities and hyperexcitability in neocortical pyramidal neurons, that often resulted in death [MacLennan et al., 2001]. However this was not observed in other knock-out studies of $S1P_2$ [Ishii et al., 2002]. Considering the role of $S1P_2$ in decreasing the length of neurites [MacLennan et al., 2000], it can be hypothesized that $S1P_2$ could regulate dendritic and/or axonal growth in different types of neurons and in the absence of this receptor, these neurons may form inappropriate connections leading to seizures. That could be the cause for the marked perinatal lethality observed in $S1P_2$/$S1P_3$ double-null mice. Double null mice that survived were apparently normal in overall health, general behavior, and longevity. Since $S1P_2$ and $S1P_3$ null mice survive, it will possible to study the physiological and pathophysiological role of these receptors in the adult, when they are challenged by injury or other type of stress.

In the case of $S1P_1$, future studies of conditional knock out or overexpression in different organs/systems will provide a better understanding of the pathophysiological role of this receptor. An alternative would be the use of specific antagonists and agonists.

The immunosuppresor FTY720 is a novel pharmacological modulator of S1P receptors [Brinkmann et al., 2002; Mandala et al., 2002]. After in vivo administration, FTY720 is metabolized to its phosphorylated derivative, FTY720-P, which is an agonist of $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors. So far, sphingosine kinase-2 is the best candidate for the activation of FTY720 into a S1P receptor agonist [Billich et al., 2003; Sanchez et al., 2003]. FTY720-P elicits lymphopenia in blood and thoracic duct by sequestering lymphocytes from circulation into the secondary lymphoid organs, away from peripheral tissues and graft sites. The non-hydrolyzable phosphonate analog of FTY720-P was also able to cause lymphopenia [Mandala et al., 2002], indicating that the active principle of FTY720 is its S1P receptor agonist phosphorylated form.

The lymphopenic effect of this immunosuppressor may be the result of the combination of a modulation of both the chemotactic responses of lymphocytes [Chen et al., 2001] and the endothelial cell barrier at the sinus-lining endothelium. Indeed, FTY720-P has similar effects as S1P on endothelial cells, such as adherens junction assembly and protection from apoptosis [Sanchez et al., 2003]. This protective effect of FTY720-P on endothelial cells may have a particular relevance in the prevention of chronic rejection after graft transplantation. Chronic allograft vasculopathy remains a leading cause of graft failure after organ transplantation [Uretsky et al., 1992; Aranda and Hill, 2000; Tovbin et al., 2000]. Many different risk factors determine the accelerated arteriosclerosis after transplantation, both immunological and non-immunological, such as genetic factors, injury of endothelial cells after ischemia-reperfusion, viral infections, or the immunosuppressor therapy itself. Indeed, many of the current immunosuppressive drugs are associated with an increase of one or more risk factors for the development of atherosclerosis [Miller, 2002]. Both immunological and non-immunological factors converge in the endothelial cell injury, which predisposes to inflammation, thrombosis, vasoconstriction,

and vascular smooth muscle cell proliferation [Miller, 2002]. Thus, the protective role of FTY720-P in endothelial cells may be important to prevent chronic rejection.

Another potential clinical application of FTY720 is the treatment of vascular permeability disorders. We have shown that FTY720 is actually a potent regulator of vascular permeability, both in vitro and in vivo [Sanchez et al., 2003]. FTY720-P blocked vascular endothelial cell growth factor (VEGF)-induced paracellular permeability in vitro and microvascular permeability induced by intradermal injection of VEGF in the mouse ear. Since VEGF is involved in many different pathological conditions, like tumor-induced angiogenesis, intraocular neovascular syndromes, or inflammatory disorders (reviewed in [Ferrara et al., 2003]), among others, S1P receptor ligands may antagonize the effects of VEGF in these situations, by promoting the formation of well-developed adherens junctions. Furthermore, S1P receptor agonists can regulate vascular tone since endothelial nitric oxide synthase is phosphorylated and activated by Akt upon S1P stimulation [Igarashi et al., 2001; Morales-Ruiz et al., 2001].

In smooth muscle cells, the balance between $S1P_{1/3}$ and $S1P_2$ seems to define the migratory response of these cells towards S1P [Kluk and Hla, 2001; Ryu et al., 2002]. This may be relevant in the pathogenesis of atherosclerosis and restenosis after angioplasty, which is characterized by the migration of vascular smooth muscle cells from the media to the intima and proliferation. The role of PDGF, a potent chemoattractant and mitogen for smooth muscle cells, on regulation of this phenomenon has been well established [Newby and Zaltsman, 2000]. S1P may also be involved. $S1P_1$, $S1P_2$, and $S1P_3$ receptors are expressed in pup-intimal cells, which have similar characteristics to cultured rat neointimal cells isolated from arteries after injury [Schwartz et al., 1995]. Adult-medial vascular smooth muscle cells also express $S1P_2$ and $S1P_3$, but much lower levels of $S1P_1$ in comparison with pup-intimal cells. We showed that high levels of $S1P_1$ expression result in an increased proliferative and migratory responses to S1P, suggesting a role of $S1P_1$ in the pathogenesis of atherosclerosis and restenosis after angioplasty. This is supported by the fact that $S1P_1$ is one of the genes induced in neointimal lesions of human in-stent restenosis, as determined by cDNA array analysis [Zohlnhofer et al., 2001].

Since S1P regulates proliferation and migration, two important functions for tumor progression and metastasis, it is possible that S1P receptor expression pattern in tumor cells may determine the prognosis of a tumor. Indeed, S1P has been shown to stimulate motility in several glioma cell lines, in particular those lines which had the highest expression of $S1P_1$ and $S1P_3$ [Van Brocklyn et al., 2003]. Additionally, an interesting in vivo report indicates that S1P treatment of B16 melanoma cells inhibited lung metastasis after injection of the cells in the mouse tail vein [Yamaguchi et al., 2003]. Daily intraperitoneal administration of S1P also reduced the number of metastasis. Interestingly, in $S1P_1$ overexpressing B16 melanoma cells, S1P treatment increased the number of pulmonary metastatic nodules. In conclusion, the study of the expression pattern of S1P receptors in different tumors as well as the synthesis of selective agonist/antagonists for these receptors may contribute to the development of new therapies to inhibit tumor growth and metastasis.

FUTURE PERSPECTIVES

We are just beginning to understand the pathophysiological role of S1P receptors. There are still many questions to be answered, among them, how are S1P levels modulated, what regulates the expression and activity of S1P receptors or which genes are regulated upon receptor activation. Transgenic models, gene targeting approaches, and in vivo use of small interference RNA will help to understand the role of S1P receptor on human health and disease, so that in the future, specific agonist or antagonists can be used as therapeutic agents.

ACKNOWLEDGMENTS

We thank Dr. Michael J. Kluk for critical comments during the preparation of the manuscript.

REFERENCES

Allende ML, Yamashita T, Proia RL. 2003. G-protein coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation. Blood 102:3665–3667.

Ancellin N, Hla T. 1999. Differential pharmacological properties and signal transduction of the sphingosine

1-phosphate receptors EDG-1, EDG-3, and EDG-5. J Biol Chem 274:18997–19002.
Aranda JM, Jr., Hill J. 2000. Cardiac transplant vasculopathy. Chest 118:1792–1800.
Arikawa K, Takuwa N, Yamaguchi H, Sugimoto N, Kitayama J, Nagawa H, Takehara K, Takuwa Y. 2003. Ligand-dependent inhibition of B16 melanoma cell migration and invasion via endogenous S1P2 G protein-coupled receptor. Requirement of inhibition of cellular RAC activity. J Biol Chem 278:32841–32851.
Billich A, Bornancin F, Devay P, Mechtcheriakova D, Urtz N, Baumruker T. 2003. Phosphorylation of the imunomodulatory drug FTY720 by sphingosine kinases. J Biol Chem 278:47408–47415.
Bornfeldt KE, Graves LM, Raines EW, Igarashi Y, Wayman G, Yamamura S, Yatomi Y, Sidhu JS, Krebs EG, Hakomori S, et al. 1995. Sphingosine-1-phosphate inhibits PDGF-induced chemotaxis of human arterial smooth muscle cells: Spatial and temporal modulation of PDGF chemotactic signal transduction. J Cell Biol 130:193–206.
Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR. 2002. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457.
Candelore MR, Wright MJ, Tota LM, Milligan J, Shei GJ, Bergstrom JD, Mandala SM. 2002. Phytosphingosine 1-phosphate: A high affinity ligand for the S1P(4)/EDG-6 receptor. Biochem Biophys Res Commun 297:600–606.
Chen S, Bacon KB, Garcia G, Liao R, Pan ZK, Sullivan SK, Nakano H, Matsuzawa A, Brinkmann V, Feng L. 2001. FTY720, a novel transplantation drug, modulates lymphocyte migratory responses to chemokines. Transplant Proc 33:3057–3063.
Clemens JJ, Davis MD, Lynch KR, Macdonald TL. 2003. Synthesis of para-alkyl aryl amide analogues of sphingosine-1-phosphate: Discovery of potent S1P receptor agonists. Bioorg Med Chem Lett 13:3401–3404.
Dorsam G, Graeler MH, Seroogy C, Kong Y, Voice JK, Goetzl EJ. 2003. Transduction of multiple effects of sphingosine 1-phosphate (S_1P) on T cell functions by the S_1P_1 G protein-coupled receptor. J Immunol 171:3500–3507.
Ferrara N, Gerber HP, LeCouter J. 2003. The biology of VEGF and its receptors. Nat Med 9:669–676.
Glickman M, Malek RL, Kwitek-Black AE, Jacob HJ, Lee NH. 1999. Molecular cloning, tissue-specific expression, and chromosomal localization of a novel nerve growth factor-regulated G-protein-coupled receptor, nrg-1. Mol Cell Neurosci 14:141–152.
Graeler M, Goetzl EJ. 2002. Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic T cells. Faseb J 16: 1874–1878.
Graeler M, Shankar G, Goetzl EJ. 2002. Cutting edge: Suppression of T cell chemotaxis by sphingosine 1-phosphate. J Immunol 169:4084–4087.
Graler MH, Bernhardt G, Lipp M. 1998. EDG6, a novel G-protein-coupled receptor related to receptors for bioactive lysophospholipids, is specifically expressed in lymphoid tissue. Genomics 53:164–169.
Hall A. 1998. Rho GTPases and the actin cytoskeleton. Science 279:509–514.
Hla T, Maciag T. 1990. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors. J Biol Chem 265:9308–9313.
Igarashi J, Bernier SG, Michel T. 2001. Sphingosine 1-phosphate and activation of endothelial nitric-oxide synthase. differential regulation of Akt and MAP kinase pathways by EDG and bradykinin receptors in vascular endothelial cells. J Biol Chem 276:12420–12426.
Im DS, Heise CE, Ancellin N, O'Dowd BF, Shei GJ, Heavens RP, Rigby MR, Hla T, Mandala S, McAllister G, George SR, Lynch KR. 2000. Characterization of a novel sphingosine 1-phosphate receptor, EDG-8. J Biol Chem 275:14281–14286.
Ishii I, Friedman B, Ye X, Kawamura S, McGiffert C, Contos JJ, Kingsbury MA, Zhang G, Brown JH, Chun J. 2001. Selective loss of sphingosine 1-phosphate signaling with no obvious phenotypic abnormality in mice lacking its G protein-coupled receptor, LP(B3)/EDG-3. J Biol Chem 276:33697–33704.
Ishii I, Ye X, Friedman B, Kawamura S, Contos JJ, Kingsbury MA, Yang AH, Zhang G, Brown JH, Chun J. 2002. Marked perinatal lethality and cellular signaling deficits in mice null for the two sphingosine 1-phosphate (S1P) receptors, S1P(2)/LP(B2)/EDG-5 and S1P(3)/LP(B3)/EDG-3. J Biol Chem 277:25152–25159.
Kluk MJ, Hla T. 2001. Role of the sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration. Circ Res 89:496–502.
Kluk MJ, Hla T. 2002. Signaling of sphingosine-1-phosphate via the S1P/EDG-family of G-protein-coupled receptors. Biochim Biophys Acta 1582:72–80.
Kohno T, Matsuyuki H, Inagaki Y, Igarashi Y. 2003. Sphingosine 1-phosphate promotes cell migration through the activation of Cdc42 in EDG-6/S1P4-expressing cells. Genes Cells 8:685–697.
Kupperman E, An S, Osborne N, Waldron S, Stainier DY. 2000. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. Nature 406:192–195.
Lee MJ, Evans M, Hla T. 1996. The inducible G protein-coupled receptor edg-1 signals via the G(i)/mitogen-activated protein kinase pathway. J Biol Chem 271: 11272–11279.
Lee MJ, Thangada S, Liu CH, Thompson BD, Hla T. 1998a. Lysophosphatidic acid stimulates the G-protein-coupled receptor EDG-1 as a low affinity agonist. J Biol Chem 273:22105–22112.
Lee MJ, Van Brocklyn JR, Thangada S, Liu CH, Hand AR, Menzeleev R, Spiegel S, Hla T. 1998b. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science 279:1552–1555.
Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M, Volpi M, Sha'afi RI, Hla T. 1999. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. Cell 99:301–312.
Lee MJ, Thangada S, Paik JH, Sapkota GP, Ancellin N, Chae SS, Wu M, Morales-Ruiz M, Sessa WC, Alessi DR, Hla T. 2001. Akt-mediated phosphorylation of the G protein-coupled receptor EDG-1 is required for endothelial cell chemotaxis. Mol Cell 8:693–704.
Lim HS, Oh YS, Suh PG, Chung SK. 2003. Syntheses of sphingosine-1-phosphate stereoisomers and analogues

and their interaction with EDG receptors. Bioorg Med Chem Lett 13:237–240.

Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, Hobson JP, Rosenfeldt HM, Nava VE, Chae SS, Lee MJ, Liu CH, Hla T, Spiegel S, Proia RL. 2000. EDG-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. J Clin Invest 106:951–961.

MacKinnon AC, Buckley A, Chilvers ER, Rossi AG, Haslett C, Sethi T. 2002. Sphingosine kinase: A point of convergence in the action of diverse neutrophil priming agents. J Immunol 169:6394–6400.

MacLennan AJ, Browe CS, Gaskin AA, Lado DC, Shaw G. 1994. Cloning and characterization of a putative G-protein coupled receptor potentially involved in development. Mol Cell Neurosci 5:201–209.

MacLennan AJ, Devlin BK, Marks L, Gaskin AA, Neitzel KL, Lee N. 2000. Antisense studies in PC12 cells suggest a role for H218, a sphingosine 1-phosphate receptor, in growth-factor-induced cell-cell interaction and neurite outgrowth. Dev Neurosci 22:283–295.

MacLennan AJ, Carney PR, Zhu WJ, Chaves AH, Garcia J, Grimes JR, Anderson KJ, Roper SN, Lee N. 2001. An essential role for the H218/AGR16/EDG-5/LP(B2) sphingosine 1-phosphate receptor in neuronal excitability. Eur J Neurosci 14:203–209.

Malek RL, Toman RE, Edsall LC, Wong S, Chiu J, Letterle CA, Van Brocklyn JR, Milstien S, Spiegel S, Lee NH. 2001. Nrg-1 belongs to the endothelial differentiation gene family of G protein-coupled sphingosine-1-phosphate receptors. J Biol Chem 276:5692–5699.

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H. 2002. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 296:346–349.

Miller LW. 2002. Cardiovascular toxicities of immunosuppressive agents. Am J Transplant 2:807–818.

Morales-Ruiz M, Lee MJ, Zollner S, Gratton JP, Scotland R, Shiojima I, Walsh K, Hla T, Sessa WC. 2001. Sphingosine 1-phosphate activates Akt, nitric oxide production, and chemotaxis through a Gi protein/phosphoinositide 3-kinase pathway in endothelial cells. J Biol Chem 276:19672–19677.

Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, Fuks Z, Xie Z, Reed JC, Schuchman EH, Kolesnick RN, Tilly JL. 2000. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. Nat Med 6:1109–1114.

Newby AC, Zaltsman AB. 2000. Molecular mechanisms in intimal hyperplasia. J Pathol 190:300–309.

Okamoto H, Takuwa N, Gonda K, Okazaki H, Chang K, Yatomi Y, Shigematsu H, Takuwa Y. 1998. EDG1 is a functional sphingosine-1-phosphate receptor that is linked via a Gi/o to multiple signaling pathways, including phospholipase C activation, Ca^{2+} mobilization, Ras-mitogen-activated protein kinase activation, and adenylate cyclase inhibition. J Biol Chem 273:27104–27110.

Okazaki H, Ishizaka N, Sakurai T, Kurokawa K, Goto K, Kumada M, Takuwa Y. 1993. Molecular cloning of a novel putative G protein-coupled receptor expressed in the cardiovascular system. Biochem Biophys Res Commun 190:1104–1109.

Olivera A, Spiegel S. 1993. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature 365:557–560.

Paik JH, Chae S, Lee MJ, Thangada S, Hla T. 2001. Sphingosine 1-phosphate-induced endothelial cell migration requires the expression of EDG-1 and EDG-3 receptors and Rho-dependent activation of alpha vbeta3- and beta1-containing integrins. J Biol Chem 276:11830–11837.

Parrill AL, Wang D, Bautista DL, Van Brocklyn JR, Lorincz Z, Fischer DJ, Baker DL, Liliom K, Spiegel S, Tigyi G. 2000. Identification of EDG1 receptor residues that recognize sphingosine 1-phosphate. J Biol Chem 275:39379–39384.

Prieschl EE, Csonga R, Novotny V, Kikuchi GE, Baumruker T. 1999. The balance between sphingosine and sphingosine-1-phosphate is decisive for mast cell activation after Fc epsilon receptor I triggering. J Exp Med 190:1–8.

Pyne S, Pyne N. 2000. Sphingosine 1-phosphate signalling via the endothelial differentiation gene family of G-protein-coupled receptors. Pharmacol Ther 88:115–131.

Ryu Y, Takuwa N, Sugimoto N, Sakurada S, Usui S, Okamoto H, Matsui O, Takuwa Y. 2002. Sphingosine-1-phosphate, a platelet-derived lysophospholipid mediator, negatively regulates cellular Rac activity and cell migration in vascular smooth muscle cells. Circ Res 90:325–332.

Sadahira Y, Ruan F, Hakomori S, Igarashi Y. 1992. Sphingosine 1-phosphate, a specific endogenous signaling molecule controlling cell motility and tumor cell invasiveness. Proc Natl Acad Sci USA 89:9686–9690.

Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V, Claffey K, Hla T. 2003. Phosphorylation and action of the immunomodulator FTY720 inhibits VEGF-induced vascular permeability. J Biol Chem 278:47281–47290.

Schwartz SM, deBlois D, O'Brien ER. 1995. The intima: Soil for atherosclerosis and restenosis. Circ Res 77:445–465.

Sugimoto N, Takuwa N, Okamoto H, Sakurada S, Takuwa Y. 2003. Inhibitory and stimulatory regulation of Rac and cell motility by the G12/13-Rho and Gi pathways integrated downstream of a single G protein-coupled sphingosine-1-phosphate receptor isoform. Mol Cell Biol 23:1534–1545.

Sukocheva OA, Wang L, Albanese N, Pitson SM, Vadas MA, Xia P. 2003. Sphingosine kinase transmits estrogen signaling in human breast cancer cells. Mol Endocrinol 17:2002–2012.

Tovbin D, Feldman L, Basok A, Shnaider A, Hertzanu Y, Lantsberg S, Mostoslavsky M, Zlotnik M. 2000. Renal transplant dysfunction due to severe aorto-iliac atherosclerosis in the presence of patent renal transplant artery. Am J Nephrol 20:487–490.

Uretsky BF, Kormos RL, Zerbe TR, Lee A, Tokarczyk TR, Murali S, Reddy PS, Denys BG, Griffith BP, Hardesty RL, et al. 1992. Cardiac events after heart transplantation: Incidence and predictive value of coronary arteriography. J Heart Lung Transplant 11:S45–S51.

Van Brocklyn JR, Lee MJ, Menzeleev R, Olivera A, Edsall L, Cuvillier O, Thomas DM, Coopman PJ, Thangada

S, Liu CH, Hla T, Spiegel S. 1998. Dual actions of sphingosine-1-phosphate: Extracellular through the Gi-coupled receptor EDG-1 and intracellular to regulate proliferation and survival. J Cell Biol 142:229–240.

Van Brocklyn JR, Tu Z, Edsall LC, Schmidt RR, Spiegel S. 1999. Sphingosine 1-phosphate-induced cell rounding and neurite retraction are mediated by the G protein-coupled receptor H218. J Biol Chem 274:4626–4632.

Van Brocklyn JR, Graler MH, Bernhardt G, Hobson JP, Lipp M, Spiegel S. 2000. Sphingosine-1-phosphate is a ligand for the G protein-coupled receptor EDG-6. Blood 95:2624–2629.

Van Brocklyn JR, Young N, Roof R. 2003. Sphingosine-1-phosphate stimulates motility and invasiveness of human glioblastoma multiforme cells. Cancer Lett 199: 53–60.

Windh RT, Lee MJ, Hla T, An S, Barr AJ, Manning DR. 1999. Differential coupling of the sphingosine 1-phosphate receptors EDG-1, EDG-3, and H218/EDG-5 to the G(i), G(q), and G(12) families of heterotrimeric G proteins. J Biol Chem 274:27351–27358.

Xia P, Wang L, Gamble JR, Vadas MA. 1999. Activation of sphingosine kinase by tumor necrosis factor-alpha inhibits apoptosis in human endothelial cells. J Biol Chem 274:34499–35505.

Yamaguchi F, Tokuda M, Hatase O, Brenner S. 1996. Molecular cloning of the novel human G protein-coupled receptor (*GPCR*) gene mapped on chromosome 9. Biochem Biophys Res Commun 227:608–614.

Yamaguchi H, Kitayama J, Takuwa N, Arikawa K, Inoki I, Takehara K, Nagawa H, Takuwa Y. 2003. Sphingosine-1-phosphate receptor subtype-specific positive and negative regulation of Rac and haematogenous metastasis of melanoma cells. Biochem J 374: 715–722.

Yamazaki Y, Kon J, Sato K, Tomura H, Sato M, Yoneya T, Okazaki H, Okajima F, Ohta H. 2000. EDG-6 as a putative sphingosine 1-phosphate receptor coupling to $Ca^{(2+)}$ signaling pathway. Biochem Biophys Res Commun 268:583–589.

Yatomi Y, Igarashi Y, Yang L, Hisano N, Qi R, Asazuma N, Satoh K, Ozaki Y, Kume S. 1997. Sphingosine 1-phosphate, a bioactive sphingolipid abundantly stored in platelets, is a normal constituent of human plasma and serum. J Biochem (Tokyo) 121:969–973.

Zhang H, Desai NN, Olivera A, Seki T, Brooker G, Spiegel S. 1991. Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. J Cell Biol 114:155–167.

Zohlnhofer D, Richter T, Neumann F, Nuhrenberg T, Wessely R, Brandl R, Murr A, Klein CA, Baeuerle PA. 2001. Transcriptome analysis reveals a role of interferon-gamma in human neointima formation. Mol Cell 7:1059–1069.