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Review Article

Tumor-suppressive sphingosine-1-phosphate receptor-2 counteracting tumor-promoting sphingosine-1-phosphate receptor-1 and sphingosine kinase 1

———Jekyll Hidden behind Hyde

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Abstract: Sphingosine-1-phosphate (S1P) is a plasma lipid mediator with multiple roles in mammalian development, physiology and pathophysiology. It is constitutively produced mostly by erythrocytes by the action of sphingosine kinase 1 (SphK1), resulting in high (~0.5 micromolar) steady-state plasma S1P content and steep S1P concentration gradient imposed between plasma/lymph/tissue interstitial fluid. S1P is also locally produced by activated platelets and tumor cells, in the latter case SphK1 is a downstream target of activated Ras mutant and hypoxia, and is frequently upregulated especially in advanced stages of tumors. Most if not all of the S1P actions in vertebrates are mediated through evolutionarily conserved G protein-coupled S1P receptor family. Ubiquitously expressed mammalian subtypes S1PR1, S1PR2 and S1PR3 mediate pleiotropic actions of S1P in diverse cell types, through coupling to distinctive repertoire of heterotrimeric G proteins. S1PR1 and S1PR3 mediate directed cell migration toward S1P through coupling to G_i and activating Rac, a Rho family small G protein essential for cell migration. Indeed, S1PR1 expressed in lymphocytes directs their egress from lymph nodes into lymph and recirculation, serving as the target for downregulation by the immunosuppressant FTY720 (fingolimod). S1PR1 in endothelial cells plays an essential role in vascular maturation in embryonic stage, and mediates angiogenic and vascular protective roles of S1P which include eNOS activation and maintenance of barrier integrity. It is likely that S1PR1 and SphK1 expressed in host endothelial cells and tumor cells act in concert in a paracrine loop to contribute to tumor angiogenesis, tumor invasion and progression. In sharp contrast, S1PR2 mediates S1P inhibition of Rac at the site downstream of $G_{12/13}$-mediated Rho activation, thus identified as the first G protein-coupled receptor that negatively regulates Rac and cell migration. S1PR2 could also mediate inhibition of Akt and cell proliferation/survival signaling via Rho-ROCK-PTEN pathway. S1PR2 expressed in tumor cells mediates inhibition of cell migration and invasion *in vitro* and metastasis *in vivo*. Moreover, S1PR2 expressed in host endothelial cells and tumor-infiltrating myeloid cells in concert mediates potent inhibition of tumor angiogenesis and tumor growth *in vivo*, with inhibition of VEGF expression and MMP9 activity. These recent findings provide further basis for S1P receptor subtype-specific, novel therapeutic tactics for individualized treatment of patients with cancer.

Keywords: Sphingosine-1-phosphate, S1P receptors, tumor angiogenesis, metastasis, sphingosine kinase 1

Introduction

More than two decades ago S1P was first suggested to be an intracellular second messenger for Ca^{2+} mobilization in the field of signal transduction [1]. Around the same time Spiegel and colleagues [2] found that S1P induced a transient Ca^{2+} release from the intracellular Ca^{2+} store and stimulated DNA synthesis in Swiss 3T3 fibroblasts. They previously observed that sphingosine, which had been suggested to be an endogenous inhibitor of protein kinase C [3], unexpectedly stimulated DNA synthesis, depending on the concentration employed [4].

They showed that sphingosine was phosphorylated by sphingosine kinase (SphK) to become S1P before exerting mitogenic effect. Indeed, platelet-derived growth factor (PDGF) and serum mitogens induced a transient activation of SphK in cultured fibroblasts, leading to a proposal that S1P generated intracellularly acted as a second messenger for mitogenesis [5].

Igarashi and colleagues reported, on the other hand, that S1P potently inhibited B16 melanoma cell migration and invasion of Matrigel [6]. They suggested that S1P acted via a putative cell surface receptor rather than as a second messenger, based upon their observations including effectiveness of immobilized S1P and detection of saturable high affinity cell surface binding sites for S1P [7].

These pioneering works disclosed two distinct, major aspects of S1P actions, i.e. stimulation of cell proliferation and inhibition of cell migration, which are now proven to exert stimulatory and inhibitory effects, respectively, in tumor progression via different subtypes of the G protein-coupled S1P receptors.

It is now well established that the S1P signaling system plays crucial roles in mammalian development and physiology, maintaining homeostasis of such diverse systems as cardiovascular, immune, respiratory, endocrine, reproductive and nervous systems, and liver, kidney, bone and so on; it is also implicated in human diseases including cancer and atherosclerosis, the two leading causes of death in developed countries, among many others [8-19]. The S1P signaling system consists of S1P synthesizing and degrading enzymes, transmembrane S1P transporters and S1P carrier proteins in the plasma, in addition to five members of the G protein-coupled S1P receptor subtypes and downstream intracellular signaling molecules. S1P could also act on intracellular targets, which at present are yet to be fully elucidated [12, 14 and references therein].

In this review, we will briefly overview basic architectures of the S1P signaling through three ubiquitously expressed S1P receptor (S1PR) subtypes, and then focus on S1PRs in tumor cells, S1PRs in host cells in the context of tumor angiogenesis. We will also overview molecular mechanisms for overexpression of sphingosine kinase 1 (SphK1) in tumor cells and its roles in tumor progression, which include sustained activation of Akt, stabilization of HIF1α and S1P production.

Identification of the G protein-coupled receptor (GPCR) family for S1P and LPA

The lysosphingolipid S1P shows structural similarity to lysophosphatidic acid (LPA), which is a classical plasma lipid mediator with pleiotropic activities [20-26]. Moolenaar and colleagues first demonstrated that LPA stimulated DNA synthesis and phosphoinositide hydrolysis with cellular Ca^{2+} mobilization in pertussis toxin-sensitive and -insensitive manners, respectively, in fibroblasts [23]. Their observations and other studies also indicated that a transient intracellular Ca^{2+} mobilization at the G0/G1 interface was neither required nor sufficient as a mitogenic signal in fibroblasts and other cell types [23, 27-31]. In N1E-115 neuronal cells LPA induced neurite retraction in a C3 toxin-sensitive, Rho-dependent manner [32]. Exogenously added S1P, but not its microinjection, was also shown to induce neurite retraction and Ca^{2+} mobilization, with the latter showing homologous desensitization but not cross-desensitization to LPA [33]. In guinea pig atrial myocytes S1P but not LPA activated $I_{K(Ach)}$ in a pertussis toxin-sensitive manner [25]. These observations strongly suggested the existence of distinct, yet closely related, GPCRs for S1P and LPA.

In an attempt to explore a novel signaling system in the vasculature our group cloned a putative GPCR from rat aortic cDNA library [34]. This clone, designated as AGR16(=EDG5/H218/S1PR2), was abundantly expressed in vascular smooth muscle cells but showed no similarity to any known GPCR except for EDG1(=S1PR1), which was reported by Hla and Maciag [35] as an mRNA upregulated in differentiating human umbilical vein endothelial cells (HUVECs) in response to a phorbol ester. Chun and colleagues identified *vzg-1*(=EDG2/LPA1), which has a substantial homology with EDG1 and EDG5, as a GPCR specific for LPA [36]. After this discovery, EDG1, EDG5, and another closely related one (S1PR3/EDG3), were identified as receptors specific for S1P by several laboratories including our laboratory [37-44],

Distinct signaling mechanisms of S1PR1, S1PR2 and S1PR3

S1PR1, S1PR2 and S1PR3 are ubiquitously expressed S1P receptor subtypes that are re-

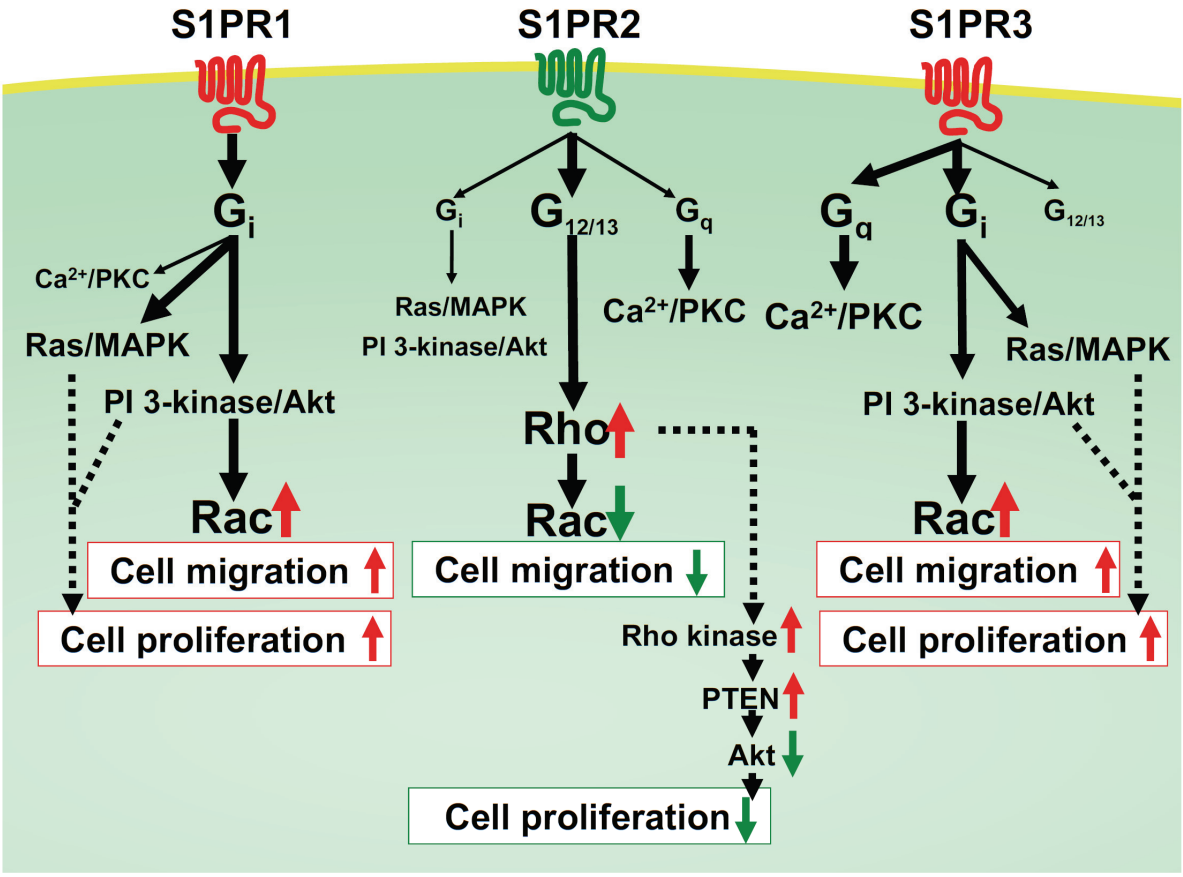

Figure 1. S1P receptor subtype-specific heterotrimeric G protein coupling and intracellular signaling mechanisms. S1PR1 couples exclusively to G_i to activate Ras-ERK and PI 3-kinase-Akt/Rac pathways, leading to stimulation of chemotaxis and cell proliferation. S1PR2 couples to multiple G proteins, especially to $G_{12/13}$ to induce potent Rho activation, leading to inhibition of Rac and cell migration, and also inhibition of cell proliferation via inhibition of Akt. S1PR3 activates G_q-PLC-Ca^{2+} pathway, and G_i-Ras-ERK and -PI 3-kinase-Akt/Rac pathways. S1PR3- $G_{12/13}$ –Rho pathway becomes evident only when G_i is inhibited by pertussis toxin.

sponsible for mediating diverse actions of S1P in a variety of cell types, through overlapping yet distinctive intracellular signaling mechanisms (**Figure 1**) [36-47, 48-55 for review]. The expression of the other two S1P receptors S1PR4 and S1PR5 are relatively restricted to the immune and the nervous system, respectively [8].

S1PR1 couples exclusively to G_i to activate Ras/ERK and PI 3-kinase/Akt pathways, leading to mitogenic and prosurvival signaling, and also to activate Rho family small GTPase Rac, which is essential for cell migration and cellular cortical actin assembly known as lamellipodia or membrane ruffling. S1PR1 thus mediates directed cell migration or chemotaxis toward S1P. S1PR1 could also activate phospholipase C (PLC) and consequent Ca^{2+} mobilization via G_i [38-40, 44, 45, 48-55].

Differently from S1PR1, S1PR2 couples to multiple heterotrimeric G proteins, among which $G_{12/13}$ coupling to RhoA activation is most prominent [41, 44, 45, 47-59]. S1PR2 exerts, at the site downstream of $G_{12/13}$-RhoA, a potent inhibitory effect on Rac via stimulation of Rac GAP activity, with consequent inhibition of cell migration toward chemotactic growth factors and chemokines [45, 48-59]. S1PR2-mediated, $G_{12/13}$-coupled RhoA activation also exerts potent inhibition of Akt [60, 62], but not ERK, leading to inhibition, rather than stimulation, of cell

proliferation [62, 63]. This inhibition of Akt and cell proliferation via S1PR2 is likely achieved by Rho kinase-mediated phosphorylation and activation of PTEN [60-62]. However, S1PR2-mediated inhibition of cell migration is independent of Rho kinase in CHO cells and B16 melanoma cells [45, 56, 59], and is observed in PTEN-deficient glioma cells [13, 64]. S1PR2 also mediates S1P stimulation of PLC and Ca^{2+} mobilization via G_q, and activation of Ras/ERK and PI 3-kinase pathways via G_i [41, 45, 47-56].

S1PR3, which also couples to multiple G proteins, potently activates PLC/Ca^{2+} signaling pathway via G_q, in addition to Ras/ERK, PI 3-kinase and Rac via G_i, mediating mitogenic/prosurvival and chemotactic effects of S1P [42-56, 59]. S1PR3 also couples to $G_{12/13}$-Rho, although to a lesser extent as compared to S1PR2 [45, 59].

Since S1PR1, S1PR2 and S1PR3 are widely expressed in various types of cells, an integrated outcome of S1P signaling in a given cell type largely depends upon relative expression levels of the S1P receptor subtypes (see below). In addition, ever growing numbers of examples of cross talks between S1P receptor signaling and growth factor or cytokine receptor signaling have been reported. For example, under certain conditions S1PR3 activation leads to activation of TGFβ signaling pathway and fibrosis [18, 65]. Update information regarding detailed cross talk mechanisms is available in recently published excellent reviews [8, 12, 13, 66].

Sphingolipid metabolizing enzymes and S1P transporters

S1P is generated through phosphorylation of sphingosine by sphingosine kinases SphK1 [67] and SphK2 [68], which share a conserved catalytic domain, but are distinct in other aspects including their structures of non-catalytic domains and expression patterns. S1P is either dephosphorylated by S1P phosphatases (SPPs) [69, 70] to regenerate sphingosine, or degraded by S1P lyase (SPL) to ethanolamine phosphate and hexadecenal [71, 72], the latter reaction serving as the exit from sphingolipid metabolic pathway (**Figure 2**).

SphKs, SPPs and SPL play important roles not only for production and degradation of S1P but also for controlling cellular levels of sphingolipids including sphingosine and its metabolic precursor ceramide. These sphingolipid species exert growth inhibitory and proapoptotic effects upon elevation of their cellular levels, through multiple mechanisms including activation of protein phosphatases and inhibition of Akt, which leads to activation of apoptotic signaling pathways [73-75].

In the mammalian body, there is a steep S1P concentration gradient across the capillary wall [76, 77]: the plasma S1P concentration is around 500 nM, which is considered to be markedly higher than that in tissue interstitial fluid. The vast majority of plasma S1P derives from red blood cells [78, 79], which have a moderate level of SphK1 but lack S1P degrading enzymes [80], thus function as a huge S1P reservoir and supplier of S1P in blood. Indeed, anemia causes a reduction in the plasma S1P level. Release of S1P from erythrocytes strictly requires acceptor plasma proteins, mostly HDL and albumin [79, 81]. A high concentration of plasma S1P, derived from erythrocytes, is crucial in maintaining vascular integrity, which is achieved by endothelial S1PR1-mediated enforcement of adherence junctions [82, 83]. Endothelial cells and activated platelets also contribute to plasma S1P [84, 85], although to lesser extents. Nevertheless, S1P generated by activated platelets contributes to serum S1P, which is approximately twice as much as that in plasma.

The major part of plasma S1P is bound to HDL (~60%), albumin (~30%) and other plasma proteins, with only a few percentages of total S1P circulating in a free form [15, 79, 81, 86]. It is known that the major determinants of total plasma S1P concentration are the plasma HDL and albumin levels [15]. Many of beneficial effects of HDL, including activation of eNOS, maintenance of endothelial integrity and NO-dependent functions, and protection of cardiomyocytes after ischemia/reperfusion injury, are attributed to HDL-bound S1P [15].

S1P is secreted out of erythrocytes via a transmembrane S1P transporter, whose molecular identity is yet to be established [87]. In a zebrafish mutant *miles apart* (but not in mammals), deletion of *S1PR2* results in an anomaly termed *cardia bifida* (two primordial heart tissues remaining separated) [88]. Deletion of Spns2, the first physiologically relevant S1P transporter

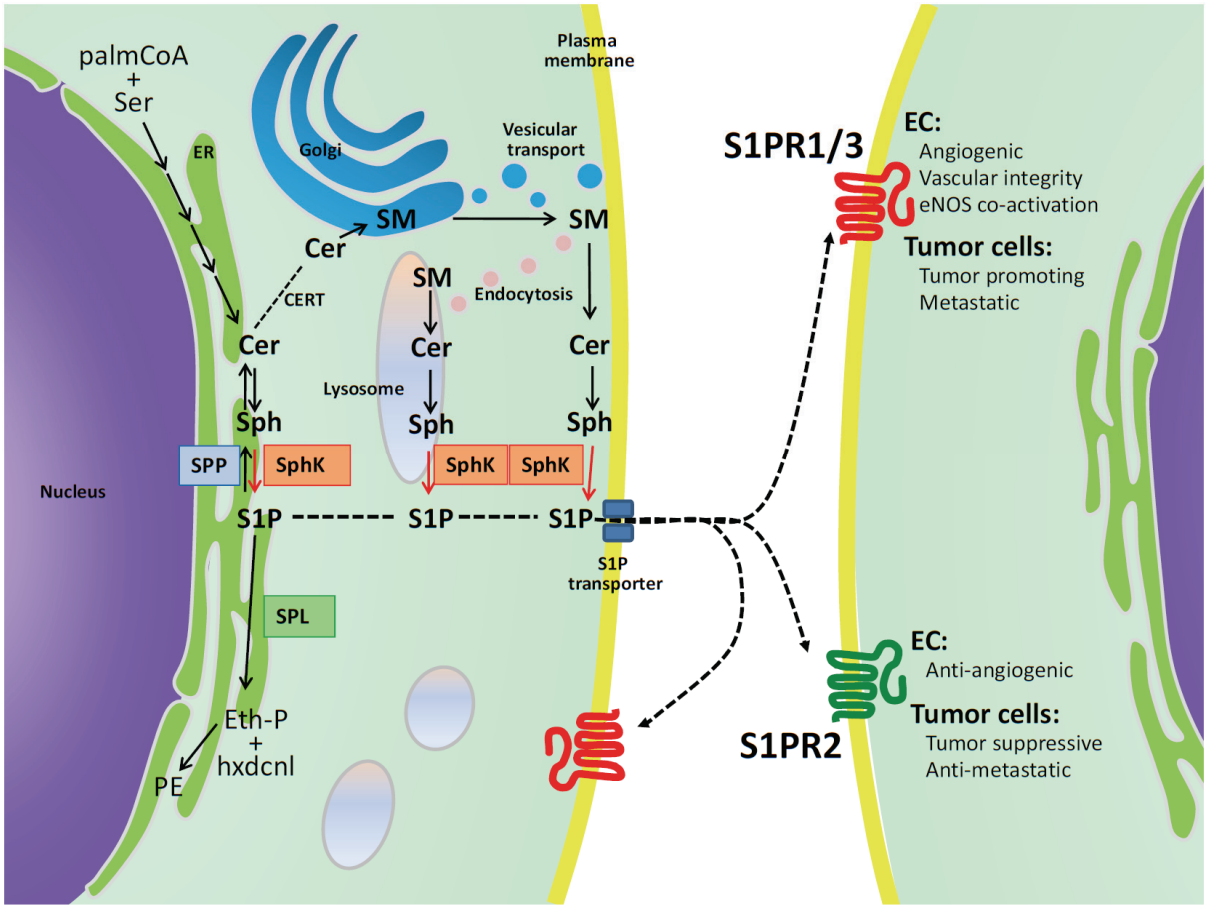

Figure 2. Sphingolipid metabolism in various subcellular compartments. Ceramide (Cer) is produced either by de novo synthesis from palmitoyl CoA (palmCoA) and L-serine with sequential enzymatic reactions in endoplasmic reticulum (ER) or through degradation of sphingomyelin (SM) by the action of sphingomyelinases in the plasma membrane and intracellular membranes including lysosomes. Cer is deacylated by ceramidase to yield sphingosine (Sph), which is then phosphorylated by SphK1/2 to generate S1P. S1P is exported through a plasma membrane S1P transporter, leading to activation of the G protein-coupled S1P receptor subtypes (S1PR1~S1PR5). S1P could be either dephosphorylated by S1P phosphatase1/2 (SPP) back to Sph or degraded to ethanolamine-phosphate (Eth-P) and hexadecenal (hxdcnl) by S1P lyase (SPL) to leave sphingolipid metabolic pathway. SphK1 is present in both cytosolic and membrane-bound fractions, both being enzymatically active. SPPs and SPL are located in ER. Intracellular transfer of Cer from ER to Golgi is facilitated by transfer proteins such as CERT, and both Cer and SM traffic between membrane compartments via vesicular transport [172, 173]. PE, phosphatidylethanolamine.

identified in vertebrates, which belongs to the major facilitator superfamily type transporter, gives rise to the same phenotype [89, 90]. It is not known at present whether erythrocyte S1P transporter is a homologue of Spns2.

S1P in the lymph is produced exclusively by lymphatic endothelial cells [91]. There is an increasing concentration gradient of S1P as follows: lymphoid organ tissue interstitial fluid<lymph<plasma. S1PR1 expressed in lymphocytes mediates their egress from thymus, bone marrow and secondary lymphoid organs into lymph and peripheral blood, thus allowing their recirculation that is necessary for immunosurveillance and adaptive immunity [92-94]. S1PR1 is also implicated in innate immunity [16, 95].

SphK1 knockout (KO) mice were phenotypically normal except for 60% reductions in plasma and serum S1P concentrations as compared to

WT mice, without significant decrease in tissue S1P levels [96]. *SphK2*KO mice were also phenotypically normal, except that they failed to show lymphopenia after administration of FTY720, which is a potent immunosuppressant that acts after being phosphorylated *in vivo* to cause retention of lymphocytes in secondary lymphoid organs via downregulation of S1PR1 expressed in lymphocytes (see below) [97]. These results indicate that SphK1 is the major isozyme responsible for production of plasma and serum S1P, and that SphK2 could compensate for SphK1 in its absence, and that SphK2 but not SphK1 is responsible for phosphorylating FTY720.

SphK1$^{-/-}$*SphK2*$^{-/-}$ double knockout mice, which have no detectable level of S1P, are embryonic lethal around embryonic day E11.5 with defects in vascular and neural development [98], indicating that S1P is produced exclusively by SphK1 and SphK2 *in vivo*. It is remarkable that severely SphK-deficient *Sphk1*$^{-/-}$*Sphk2*$^{+/-}$ mice are viable with normal phenotype, except for female infertility due to marked accumulation of sphingosine and dihydrosphingosine in deciduum [99]. S1P levels in uterus and deciduum in *Sphk1*$^{-/-}$*Sphk2*$^{+/-}$ mice were comparable to WT mice.

SPL-null mice show markedly high levels of S1P in tissue and serum with accumulation of ceramide and long chain bases, and also generalized derangements in lipid metabolism, resulting in multi-organ abnormalities with vacuolar changes and inclusion bodies, severe lymphopenia, granulocytosis and monocytosis, leading to early lethality after weaning [100, 101].

S1PR2 is a chemorepellant receptor whereas S1PR1 and S1PR3 are chemotactic receptors

Cell migration is a process essential for morphogenesis, angiogenesis, immune surveillance, inflammation, tumor cell invasion and metastasis. It is regulated through receptor-mediated processes in response to a variety of ligands, which are either soluble, bound to extracellular matrix or expressed on cell surface.

One of outstanding biological activities of S1P is the ability to regulate cell migration either negatively or positively, which was first recognized to be apparently cell type-dependent. S1P potently inhibits cell migration in a variety of tumor cells including B16 melanoma, breast cancer, and glioblastoma cells [6, 7, 56, 58, 64, 102, 103], as well as vascular smooth muscle cells [104, 105] and neutrophils [106]. By contrast, S1P induces chemotaxis in vascular endothelial cells [107-109], murine embryonic fibroblasts [110], and T and B lymphocytes [111, 112].

Chinese hamster ovary (CHO) cells are an excellent model for studying mechanism of cell migration [45, 48-50, 55, 59]. They vigorously exhibit stimulation or inhibition of cell migration, depending on stimuli. CHO cells overexpressing either S1PR1 or S1PR3 showed pertussis toxin-sensitive, G_i- and PI 3-kinase-dependent stimulated cell migration toward S1P, with characteristic bell-shaped dose-response curves in a Boyden chamber assay. In contrast, S1PR2-expressing cells or vector control cells did not show a change in cell migration response to S1P. All of the four cell types showed comparable extents of stimulated cell migration in response to their chemoattractant insulin-like growth factor-1 (IGF-1), which was dependent upon PI 3-kinase but not G_i. Importantly, CHO cells overexpressing S1PR2, but not S1PR1 or S1PR3, showed S1P dose-dependent inhibition of cell migration toward IGF-1 [45]. Rho family GTPases Rac, Rho and Cdc42 stimulate actin polymerization to induce lamellipodia, stress fibers and filopodia, respectively [113]. The chemoattractant receptors S1PR1 and S1PR3 mediated Rac activation, whereas chemorepellant receptor S1PR2 did not. S1PR2 and S1PR3 but not S1PR1 mediated activation of RhoA. S1PR1 ~ S1PR3 did not alter Cdc42 activity. The chemoattractant IGF1 induced activation of Rac but not Rho or Cdc42. S1PR2 but not S1PR1 or S1PR3 inhibited IGF-1-stimulated Rac activation. The expression of either a dominant negative (DN) Rac mutant N^{17}Rac or DN Cdc42 mutant N^{17}Cdc42, but not DN Rho mutant N^{19}RhoA inhibited chemotaxis toward IGF-1. N^{17}Rac and N^{17}Cdc42 but not N^{19}RhoA also inhibited S1PR1- and S1PR3-mediated chemotaxis. Thus, stimulated Rac activity and probably basal Cdc42 activity are required for chemotaxis toward IGF-1 and S1P [45]. We also found that S1PR2-mediated inhibition of Rac activity and cell migration in response to IGF-1 were abolished either by expression of DN N^{19}Rho, inactivation of Rho by pretreatment with C3 toxin, or interfering of S1PR2-$G_{12/13}$ coupling by expression of the peptide with a sequence of $G_{12}\alpha$ C-

terminus or $G_{13}\alpha$ C-terminus [45, 59]. Rho kinase inhibitors, which completely abolished formation of stress fibers and focal adhesions in response to S1P, failed to prevent S1P inhibition of Rac and cell migration [45, 59]. These results indicate that S1P engagement of S1PR2 results in inhibition of Rac and cell migration at the site downstream of $G_{12/13}$ and Rho activation but not Rho kinase. Rho-dependent, Rho kinase-independent inhibition of Rac seems to involve stimulation of Rac GTPase activating protein (GAP) [45], but not PTEN (unpublished observation) or inhibition of PI 3-kinase [45]. Interestingly, S1PR3 chemotactic receptor, which couples to both G_i and $G_{12/13}$, functions as a migration inhibitory receptor just like S1PR2 when G_i is inhibited by pertussis toxin [59].

Consistent with the findings obtained in the CHO cell expression system, S1PR1 was predominantly expressed in HUVECs, in which S1P induced Rac activation and chemotaxis in a pertussis toxin-sensitive manner [105, 107, 114-116]. Enforced expression of S1PR2 in endothelial cells dampened or inhibited the stimulatory effect of S1P on cell migration and tube formation on Matrigel [57, 62]. On the other hand, S1PR2 was abundantly expressed in rat aortic smooth muscle (RASM) cells [34, 105], in which S1P potently stimulated RhoA with concomitant inhibition of Rac and cell migration in response to PDGF [105]. Enforced expression of S1PR1 in RASM cells attenuated S1P inhibition of cell migration [105].

S1PR2 in tumor cells mediates potent inhibition of cell migration, invasion and metastasis, whereas S1PR1 and S1PR3 mediate their stimulation

By adopting B16 melanoma cells which abundantly expressed endogenous S1PR2 as a model system, we provided the first evidence that endogenous S1PR2 was responsible for S1P inhibition of cell migration and invasion of Matrigel [56]. B16 cells showed a high basal Rac activity under a serum-deprived condition as compared to CHO cells. S1P dose-dependently and markedly suppressed Rac activity, cell migration and invasion. The inhibitory effects of S1P on Rac and cell migration were completely abolished by an S1PR2-selective antagonist JTE-013. In addition, pretreatment with C3 toxin or the expression of a DN N^{19}Rho, but not Rho kinase inhibitors, abolished S1P inhibition of B16 cell migration, indicating that endogenous S1PR2 mediated inhibition of Rac and cell migration via a Rho-dependent, but Rho kinase-independent manner, just like S1PR2 overexpressing CHO cells [45]. Overexpression of S1PR2 sensitized B16 melanoma cells to S1P-induced inhibition of Rac activity and cell migration [56].

Differently from S1PR2, overexpression of either S1PR1 or S1PR3 in B16 melanoma cells blunted the inhibitory effects of S1P on Rac activity and cell migration with rightward shifts of the dose response curves. In these cell types blockade of endogenous S1PR2 by JTE-013 resulted in S1P dose-dependent stimulation of chemotaxis toward S1P [56].

Importantly, S1PR2 mediates S1P inhibition of hematogenous metastasis of B16 nelanoma cells in a S1P dose-dependent manner [58]. Tail vein injection of B16 cells resulted in the formation of metastatic nodules in the lung three weeks later. Pretreatment of B16 cells with S1P for just 5 min prior to intravenous injection potently reduced the number of nodules in a dose-dependent manner, with the maximal 40 % inhibition obtained at 10^{-7} M S1P, suggesting that the inhibitory mechanism involved an early phase of metastasis process [58]. Indeed, studies with fluorescent dye-labeled B16 cells demonstrated that tumor microembolus formation in pulmonary capillaries after intravenous injection was significantly inhibited when tumor cells were pretreated with S1P (Yamaguchi et al., unpublished observation). The expression of N^{17}Rac in B16 cells, which persisted for at least several days, also potently inhibited metastasis by 80% with a reduction in tumor microembolus formation. The expression of N^{17}Rac also inhibited B16 cell proliferation *in vitro*, although to a much lesser extent (18% inhibition) as compared to its effect on metastasis, whereas S1P did not show any detectable inhibition on B16 cell proliferation *in vitro*. The inhibitory effect of S1P on metastasis was also observed (40% inhibition) when it was administered intraperitoneally to mice 30 min before injection of B16 cells and afterward once every day (10 micrograms/day), indicating a possible involvement of host cell S1PR2 and also an inhibitory S1P target late in the metastatic process as well [58]. The combination of S1P pretreatment of B16 cells and systemic S1P administration into

mice resulted in a greater than 60% inhibition.

S1PR2 overexpression markedly sensitized B16 cells to S1P-induced inhibition of hematogenous metastasis, with a maximal 80% inhibition [58]. Conversely, overexpression of S1PR1 in B16 cells resulted in marked enhancement of metastasis by *in vitro* pretreatment of B16 cells with S1P at a relatively low concentration range [58]. Aggravation of metastasis with S1P pretreatment was also observed for B16 cells overexpressing S1PR3 (Yamaguchi et al., unpublished observation). These composite results provided the first molecular basis for S1PR2-selective agonists and S1PR1- and S1PR3-selective antagonists as promising anti-cancer therapeutics in the future [58].

S1PR2-mediated inhibition of tumor cell migration has also been demonstrated in glioblastoma cells in the presence and absence of PTEN [13, 60, 64]. In gastric cancer cells, which express S1PR2 and S1PR3, S1P inhibition of cell migration is strictly related to a higher endogenous expression level of S1PR2 over S1PR3; when S1PR2 expression level is higher than that of S1PR3, then S1P inhibits cell migration, and vice versa [117]. The stimulatory action on cell migration and invasion of S1PR1 and S1PR3 is also evident for thyroid cancer, ovarian cancer and Wilms tumor cells, when their actions overcome inhibitory effects of S1PR2 [118-120].

Endothelial S1PR1 mediates angiogenic activity

In cultured endothelial cells (ECs) S1P induced proangiogenic cellular responses including stimulation of cell proliferation, migration and morphogenic capillary-like tube formation in Matrigel, in pertussis toxin-sensitive manners. S1P enhanced angiogenesis in vivo in corneal micropocket assay and Matrigel plug assay [82, 107-109, 114-116]. In accordance with the original observation [35] that endothelial morphogenic activity was associated with upregulation of S1PR1/EDG1 expression, angiogenic effects of S1P on endothelial cells were mediated via S1PR1. S1PR1-selective antagonist inhibited endothelial cell tube formation in vitro and angiogenesis in vivo [121]. S1PR3 also shows proangiogenic activities [114, 116]. In a mouse model of subcutaneous tumor implantation, host S1PR1 was upregulated in tumor microvessels. Intratumoral injections of S1PR1-specific siRNA every three days into LLC tumors, which itself did not express S1PR1, inhibited tumor angiogenesis and tumor growth in vivo [122]. Tumor angiogenesis and tumor growth were inhibited by FTY720, the functional antagonist for S1PR1 (see below) [123-125].

*S1PR1*KO mice were embryonic lethal between embryonic days E13.5 and E14.5, not because of defective angiogenesis, but because of defective maturation of blood vessels with impaired mural cell coverage and consequent massive hemorrhage [110]. This phenotype of defective vascular maturation was reproduced in mice with vascular endothelial cell-specific conditional deletion of S1PR1 [126]. The investigation into the interaction between vascular endothelial and mural cells revealed that endothelial S1PR1 was required for Rac-dependent, N-cadherin-mediated adhesion between the two cell types [127]. *S1PR1/S1PR2/S1PR3* triple KO mice showed more severe phenotype in vascular formation as compared to *S1PR1*KO mice [128].

Endothelial and myeloid S1PR2 mediates anti-angiogenic and tumor suppressive activities

The observation that S1PR2-selective antagonist JTE-013 [56] markedly stimulated in vivo angiogenesis in Matrigel plug assay [57] suggested that S1PR2 might play an inhibitory role in angiogenesis. We created *S1PR2*KO ($S1P2^{-/-}$) and *S1PR2*KO/*LacZ* knock-in ($S1P2^{LacZ/LacZ}$) mice to understand in depth the role of host *S1PR2* in tumor angiogenesis and tumor growth *in vivo* [62].

Subcutaneously inoculated Lewis lung carcinoma (LLC) and B16BL6 melanoma grew much more rapidly in *S1PR2*KO mice as compared to those inoculated in wild type (WT) littermates. LLC tumors in littermate mice that were heterozygous for *S1PR2* allele grew at a rate between those of WT and KO mice (unpublished observation). Tumors grown in *S1PR2*KO mice showed twice as much tumor microvessels as those grown in WT littermates, with enhanced maturity, namely increased mural cell coverage and perfusion *in vivo*. The numbers of proliferating tumor cells, which were abundant in areas close to tumor microvessels, were also greater in *S1PR2*KO mice. Analysis on tumor-bearing $S1PR2^{+/LacZ}$ mice demonstrated increased expression of S1PR2 in tumor microvessels, both

in endothelial and smooth muscle cells, as well as tumor-infiltrating cells outside of vessels. Lung microvasculature endothelial cells obtained from *S1PR2*KO and WT littermates showed that S1PR2 in WT endothelial cells mediated inhibition of cell proliferation, migration and morphogenic tube formation. When inoculated into mice together with LLC and B16BL6 cells, *S1PR2*KO endothelial cells greatly stimulated tumor growth with enhanced angiogenesis as compared to WT endothelial cells. These results indicate that S1PR2 expressed on host endothelial cells inhibits tumor angiogenesis in a cell-autonomous manner.

Accumulating evidence indicates that bone marrow-derived, tumor-infiltrating myeloid cells play crucial roles in tumor development. These cells, including tumor-associated/educated macrophages and $CD11b^+$ population, are responsible for production of proangiogenic growth factors including VEGF-A and activation of matrix metalloproteases (MMPs), which allow liberation of matrix-bound proangiogenic factors and growth factors for tumor cells, and also provides a suitable environment for tumor cell invasion, thus leading to stimulation of angiogenesis, invasion and metastasis [129]. We characterized tumor-infiltrating, S1PR2 expressing cells by bone marrow transplantation experiments with GFP transgenic and *S1PR2*$^{+/LacZ}$ mice and found that the majority of these cells were bone marrow-derived myeloid lineage cells, which included $CD11b^+$ cell population that expressed S1PR2, by using double staining for β-galactosidase activity and CD11b immunoreactivity. Bone marrow-derived S1PR2 expressing cells were not detected in the tumor microvessel wall, neither in endothelial nor smooth muscle layer. As compared to tumors grown in WT mice that had received bone marrow transplantation from *S1PR2*WT mice as a donor, tumors grown in WT mice that had received bone marrow transplantation from *S1PR2*KO mice showed greatly increased numbers of tumor infiltrating myeloid cells, which suggested their enhanced recruitment from the bone marrow to tumors. Notably, WT mice that had been reconstituted with *S1PR2*KO bone marrow showed significantly stimulated tumor angiogenesis and rapid tumor growth. Studies on bone marrow-derived macrophages revealed that S1PR2 was expressed on these cells and mediated inhibition by S1P`of cell migration, expression of VEGF-A and other proangiogenic factors. Indeed, tumors grown in *S1PR2*KO mice showed increased expression of VEGF-A, bFGF and TGFβ, and MMP9 activity as compared to WT littermates.

These results indicate that host S1PR2 has a unique tumor-suppressive activity: S1PR2 expressed in bone marrow-derived, tumor-infiltrating myeloid cells and vascular endothelial cells in concert mediates inhibition of tumor angiogenesis and tumor growth [62].

SphK1 could be involved in tumor progression, rather than tumor initiation

Spiegel and colleagues first demonstrated that overexpression of SphK1 in NIH3T3 fibroblasts and HEK293 cells resulted in reduced serum-dependence and increased rate of cell proliferation, with increased survival under serum-deprived condition or against ceramide challenge, implicating the involvement of SphK1 in cancer development [130].

It was then reported that overexpression of SphK1 in NIH3T3 fibroblasts resulted in full transformation with the ability to form tumors in NOD/SCID mice, which led to a proposal that *SphK1* was an oncogene capable of inducing tumor initiation [131]. They also showed that phosphorylation of SphK1 at Ser225 by ERK1/2 was required for translocation to the plasma membrane and also for transformation [132, 133]. A mutant SphK1, $SphK1^{S225A}$, which was enzymatically active but defective in ERK1/2-dependent phosphorylation, failed to show either plasma membrane localization or transformation, whereas the addition of a myristoylation/palmitylation motif to $SphK1^{S225A}$ conferred constitutive plasma membrane location and recovery of transforming activity in soft agar colony formation [133].

These observations obtained in NIH3T3 cells may not be generalized, however, as in 3T3-L1 cells the overexpression of a myristoylated SphK1 construct, which showed plasma membrane localization, resulted in a potent inhibition, rather than stimulation, of cell proliferation, whereas overexpression of WT SphK1, which distributed in both cytosol and perinuclear membrane structures, did stimulate cell proliferation [134]. Differently from widely accepted concept of oncogene (active mutant) vs. proto-oncogene (WT) paradigm, SphK1 is constitutively active in its WT form, and mutation in

SphK1 gene or *SphK1* gene amplification in cancer has not been reported to date, raising the question whether SphK1 represents an oncogene [13, 135].

Accumulating evidence indicates that SphK1 could be involved in the process of tumor progression, rather than tumor initiation, through multiple mechanisms as discussed below. The mechanisms include amplification of HIF1-dependent responses, paracrine stimulation of endothelial S1PR1-dependent angiogenesis, paracrine/autocrine stimulation of tumor cell invasion, and intracellular roles of SphK1 as a sphingolipid metabolizing enzyme and as a signaling molecule that interacts with an intracellular target.

Multistep carcinogenesis and hypoxia leads to overexpression and activation of SphK1 in tumor cells

The expression levels of *SphK1* mRNA and protein are elevated in a variety of tumor cells and tumor tissues of mouse and human origin, with positive correlations observed between the SphK1 expression levels and either the extents of resistance to chemotherapeutic agents or radiation, advanced stages or poor prognosis [13, 136-144]. Immunohistochemical analysis shows cytosolic localization of SphK1 protein in SphK1 overexpressing tumor tissues [143, 145]. *SphK1* gene amplification in tumors has not been reported thus far. The underlying mechanism for SphK1 overexpression in tumor tissue as compared to normal tissue has not been fully addressed until recently.

Several lines of evidence indicate that either an activating mutation of a proto-oncogene or inactivation of a tumor suppressor gene could lead to SphK1 overexpression. First, the expression of a constitutively active mutant of Ras induces transcriptional upregulation of SphK1 [131]. Similarly, an activating mutation of RET, which is responsible for papillary thyroid carcinoma and multiple endocrine neoplasia (MEN) type 2, also induces upregulation of *SphK1* mRNA and protein levels [146]. Second, the expression of v-Src stabilizes *SphK1* mRNA, leading to *SphK1* mRNA upregulation and protein overexpression [147]. Third, degradation of SphK1 is dependent upon p53 function, and loss of function of p53, which is frequently observed in human cancer, results in reduced rate of SphK1 degradation and upregulation of SphK1 protein level [148]. It is therefore reasonable to postulate that increased expression of SphK1 in tumor cells may be at least in part a consequence of multistep carcinogenesis.

SphK1 overexpression could also result from activation of GPCRs or growth factor receptor/tyrosine kinases. For example, engagement of LPA receptor-1 strongly induces overexpression of SphK1 through a mechanism involving transactivation of EGF receptor signaling and S1PR3 upregulation [149]. In addition, it is known that engagement of GPCRs and growth factor receptors induce a rapid and transient activation of SphK1 activity [5, 12-14].

Another crucial event that leads to upregulation of SphK1 in tumor cells is hypoxia, which induces *SphK1* transcriptional upregulation in a hypoxia inducible factor (HIF)-dependent manner [150, 151]. *SphK1* gene has two HIF-responsive elements (HREs) in its promoter region, through which both HIF1α and HIF2α contribute to stimulate *SphK1* gene transcription. Indeed, conditioned media from hypoxia-treated tumor cells induced angiogenic responses in human umbilical vein endothelial cells in a S1PR1/S1PR3-dependent manner [150]. In addition to hypoxia-induced transcriptional upregulation of *SphK1* gene expression, hypoxia induces stimulation of SphK1 activity upon reoxygenation through a reactive oxygen species (ROS)-dependent mechanism, which is reported to involve Akt-mediated phosphorylation and functional regulation of GSK3β with downstream signaling [152]. Consistent with this, cardiac hypoxia/reperfusion injury is alleviated in *SphK1* transgenic mice [18] and aggravated in *SphK1*KO mice, as compared to littermate WT mice [10], and the protective effect of SphK1 is mimicked by S1P and HDL.

Overexpression of SphK1 in tumor cells could enhance tumor angiogenesis and tumor cell invasion through multiple mechanisms involving HIF1 and S1PR1

It is of note that SphK1, which is upregulated in a HIF1α-dependent mechanism, in turn stabilizes HIF1α [152, 153]. The SphK1-dependent stabilization of HIF1α levels is mediated by the Akt/GSK3β signaling pathway that prevents von Hippel-Lindau protein-mediated proteasomal degradation of SphK1. The pharmacological and

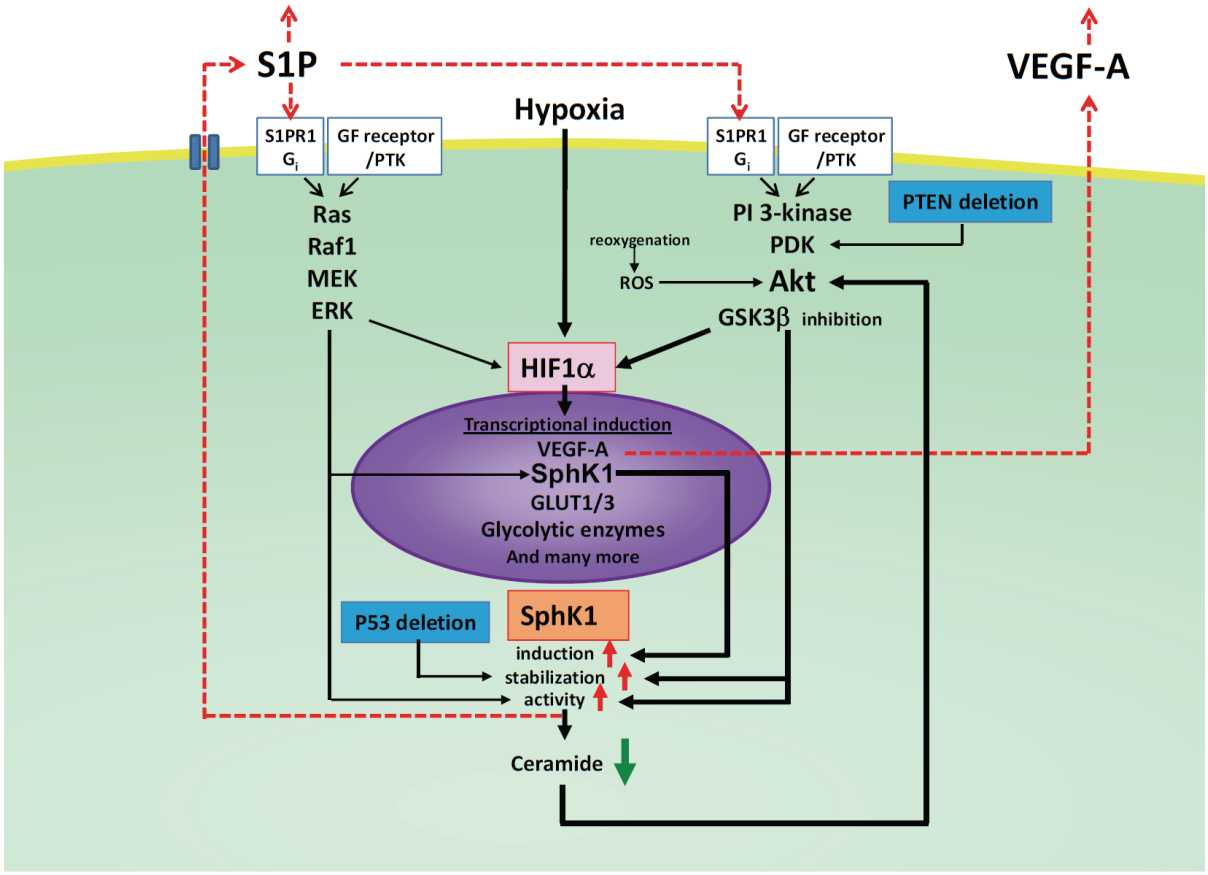

Figure 3. Hypoxia, activating *Ras* mutation and *p53* deletion in concert upregulate SphK1 expression level and activity, leading to Akt-dependent growth promoting/anti-apoptotic signaling and autocrine/paracrine activation of S1PR1 signaling. SphK1 in turn stabilizes HIF1α. Thus, HIF1α and SphK1 likely constitute a feed-forward amplification loop that could lead to sustained tumor progression.

RNA silencing inhibition of SphK1 activity prevents the accumulation of HIF1α and its transcriptional activity [152, 153].

These composite findings strongly suggest that SphK1 and HIF1α constitute a feed-forward amplification loop under hypoxia, resulting in further enhancement of HIF1α-dependent responses, which include increased expression of glucose transporter and glycolytic enzymes for metabolic adaptation to hypoxia, and induction of angiogenic growth factors such as VEGF-A and many others that are involved in tumor progression and metastasis (**Figure 3**). This amplification loop likely functions in normoxic condition as well, since both SphK1 and HIF1α are upregulated by growth factor stimulation via a Ras-dependent mechanism under normoxia [154]. At present it is not known whether S1P receptors are involved in SphK1-induced, Akt-dependent stabilization of HIF1α. Since Akt is a dominant downstream signaling molecule of Gi-coupled receptors S1PR1 and S1PR3, it is likely that tumor-expressing S1PR1/3 participate in the HIF1α-mediated signaling of tumor progression, through autocrine/paracrine stimulation of the receptors.

In addition to HIF1α-dependent production of angiogenic growth factors in SphK1-overexpressing tumor cells, SphK1-catalyzed production of S1P in tumor cells leads to S1P release into cell exterior through a transmembrane S1P transporter, which enables

paracrine/autocrine activation of Gi-coupled S1P receptors, S1PR1 and S1PR3, in both host endothelial cells and tumor cells. Indeed, a recent study by Obeid and colleagues [155] demonstrated for the first time that S1P secreted from SphK1-overexpressing HEK cells and tumor cells induced vascular and lymphatic endothelial cell migration and tube formation in a coculture system. It is also reported in U-118MG glioblastoma cells that overexpression of S1PR1 and S1PR3, but not S1PR2, resulted in potent activation of urokinase type plasminogen activator (uPA), which is involved in tumor cell invasion [156]. Inhibition of SphK1 by either a pharmacological SphK inhibitor or RNA silencing potently inhibited uPA activation and invasion [156].

Recent studies [123-125, 157] demonstrate that anti-S1P monoclonal antibody and FTY720, both being functional inhibitors of S1PR1 signaling, effectively inhibit angiogenesis and tumor cell proliferation *in vivo*. These observations strongly suggest that autocrine/paracrine S1P signaling through S1PR1 plays a significant role in these processes.

Overexpression of SphK1 in tumor cells could confer resistance to chemotherapeutics through reduction in ceramide level and potentiation of Akt activity

Overexpression of SphK1 confers not only acceleration of cell proliferation but also resistance to proapoptotic stimuli, which include serum deprivation, ceramide toxicity and chemotherapeutic agents [130]. It is widely recognized that ceramide, which is located upstream of SphK1-catalyzed reaction in the sphingolipid metabolic pathway, accumulates in cells in response to apoptosis-inducing stimuli, such as chemotherapeutic agents and radiation, and mediates cell growth arrest and apoptosis [73, 74, 136, 158]. Underlying mechanisms for ceramide-mediated induction of apoptosis may involve inhibition of Akt activity through enhanced dephosphorylation of Ser473 [159]. It is reported recently by Banno and colleagues [160] that resistance to oxaliplatin was well correlated with high SphK1/2 expression levels and low ceramide level. Oxaliplatin stimulated neutral sphingomyelinase in sensitive cells, resulting in production of ceramide, inhibition of Akt and induction of apoptotic signaling. By contrast, resistant cells showed a high basal Akt activity, which was inhibited by oxaliplatin only when either SphK1 or SphK2 was pharmacologically inhibited or knocked down by RNA interference. Inhibition of Akt was associated with an increase in ceramide synthesis via salvage pathway, followed by apoptosis. These results suggest that SphK1 overexpression confers resistance to oxaliplatin through mechanisms involving suppression of the ceramide level and potentiation of Akt activation [160]. They further provided evidence that oxaliplatin induces upregulation of p53 when SphK1/2 expression and Akt activity were suppressed [160]. Consistent with this, it was previously demonstrated that Akt phosphorylates and activates MDM2, which leads to ubiquitination and degradation of p53 [161], whereas p53 and caspases are involved in degradation of Akt [162].

In addition to ceramide reduction and Akt activation, SphK1 could directly interact with an intracellular target protein to exert anti-apoptotic effect. Upon TNFα stimulation, SphK1 directly interacts with TNF receptor associated factor 2 (TRAF2), leading to SphK1 activation and production of S1P, which are required for NF-kB activation and inhibition of apoptosis [163, 164].

SphK1 deletion could inhibit tumor formation through accumulation of sphingosine rather than reduced S1P production

The inhibition of *SphK1* by either RNA silencing or SphK inhibitors leads to inhibition of cell proliferation and induction of apoptosis. The effect of *SphK1* deletion on tumor formation was examined in two different mouse tumor models using *SphK1*KO mice [143, 145]. In both models development of adenoma and adenocarcinoma was inhibited in *SphK1*KO mice, although underlying mechanism may not be identical.

In azoxymethane- and dextran sulfate-induced colon carcinogenesis model, in which chronic inflammation is involved as a mechanism underlying cancer development, adenocarcinoma lesion in WT mice showed a several fold increase in the *SphK1* mRNA expression and twofold elevation in the S1P level [143]. Adenoma and adenocarcinoma did develop in *SphK1*KO mice, however, their numbers were both 50% reduced as compared to WT littermates, with reduced numbers of cells in S phase (60%) and increased numbers of apoptotic cells (200%) as

compared to WT tumors [143]. In addition, mRNA levels of inflammation related genes, TNFα and COX2, were elevated in WT cancer tissues by 5 to 10-fold, respectively, whereas in cancer tissues in *SphK1*KO mice *COX2* mRNA level was elevated by only 3-fold and TNFα mRNA was not elevated. The tissue levels of S1P or other sphingolipids in tumor and normal mucosa of *SphK1*KO mice were not addressed.

In $Apc^{Min/+}$ mouse model of intestinal adenomatous polyposis, polyp lesions showed increases in the levels of S1P and sphingosine, with no change in that of ceramide [145]. Differently from azoxymethane model, $Apc^{Min/+}Sphk1^{-/-}$ mice showed a 50% reduction in polyp size, but not polyp numbers, as compared to $Apc^{Min/+}Sphk1^{+/+}$ mice. Interestingly, the S1P levels in polyp lesion and normal mucosa in $Apc^{Min/+}Sphk1^{-/-}$ mice were comparable to those in $Apc^{Min/+}Sphk1^{+/+}$ mice, respectively, whereas the sphingosine level in tumors of $Apc^{Min/+}Sphk1^{-/-}$ mice was 150% of that in $Apc^{Min/+}Sphk1^{+/+}$ mice. It was suggested that a marked reduction in proliferating cells in polyps of $Apc^{Min/+}Sphk1^{-/-}$ mice were due to increased level of sphingosine, which was associated with reduced expression of CDK4 and *myc*; genetic studies indicated that S1PR1 ~ S1PR3 were not likely involved in adenoma development. An increase in the tissue sphingosine level, but not a reduction in the S1P level, was also observed in deciduum of severely *SphK*-deficient $Sphk1^{-/-}Sphk2^{+/-}$ female mice, which led to lamellar body formation reminiscent of sphingolipidosis, decreased cell proliferation, increased apoptosis and infertility [99].

Further studies are required to fully elucidate molecular mechanisms for SphK1-dependent tumor progression. In addition, generalized overexpression of SphK1 *in vivo* does not necessarily stimulate tumor formation (our unpublished observations).

Targeting S1P receptors and SphK1 as anticancer treatments

Targeting the angiogenic and tumor promoting S1P signaling would be promising as an anticancer treatment. Anti-S1P monoclonal antibody (Sphingomab) sequesters S1P from binding to cell surface receptors. It is reported to effectively inhibit tumor growth in xenograft and allograft mouse models, through inhibition of tumor angiogenesis, and also through direct inhibitory effects on tumor cells, in which the antibody competed against S1P effects including stimulation of cell proliferation, prevention of apoptosis and production of proangiogenic cytokines [157; 165 for review]. Because the antibody, which specifically binds S1P with a high affinity, is reported to trigger S1P release from erythrocytes [79], which constitute a large reservoir of plasma S1P, the antibody dose that is required to effectively reduce plasma S1P concentration would be high in clinical settings. Delineating suitable antibody delivery system that bypass bloodstream to target primary and metastatic tumor tissue would improve cost performance status. Since anti-S1P antibody should reduce availability of S1P for anti-angiogenic and tumor suppressive S1P receptor S1PR2 as well, addition of selective inhibitors of S1PR1 and S1PR3 is expected to potentiate the effectiveness of the antibody in inhibiting angiogenesis and tumor cell migration and invasion.

FTY720, an analogue of sphingosine, acts after SphK2-mediated phosphorylation as an S1P analogue *in vivo*, with two opposing actions: it could act as an agonist for S1PR1, S1PR3, S1PR4 and S1PR5 but not S1PR2, and also as a functional antagonist for S1PR1. The latter action is due to internalization, ubiquitination and proteasomal degradation of S1PR1 [166]. Thus, FTY720 acts as a potent immunosuppressant through downregulation of S1PR1 in lymphocytes to inhibit lymphocyte egress from lymphoid organs and recirculation [91-94, 167]. FTY720 also suppresses angiogenic activity of S1P through downregulation of S1PR1 in endothelial cells [166]. Pretreatment of HUVECs with nanomolar concentrations of FTY720 or FTY720 -phosphate downregulated S1PR1 and inhibited endothelial cell migration and Ca^{2+} mobilization in response to S1P. FTY720 potently inhibited angiogenesis in corneal micropocket assay, subcutaneously implanted chamber assay, and tumor angiogenesis, tumor growth and metastasis in vivo [123-125]. These results are consistent with the notion that S1PR1 expressed in endothelial cells is involved in stimulation of tumor angiogenesis and metastasis. In addition to its inhibitory effect on endothelial cells via downregulation of S1PR1 at nanomolar concentrations, it is reported that micromolar concentrations of FTY720 directly induced apoptosis of a breast cancer cell line [123]. It is also reported in ovarian cancer cells that FTY720 induced

autophagy and necrosis [168]. These observations may be related to recently reported novel actions of FTY720, which include inhibition of SphK1 [169] and autotaxin, the latter being phospholipase D in the plasma responsible for lysophosphatidic acid production [170]. Since FTY720 is a potent immunosuppressant, it is a concern that this effect might reduce immunological anti-tumor activity. Infectious complications should also be carefully watched. Another concern is endothelial dysfunction after prolonged usage of the anti-S1P antibody or FTY720 because S1P maintains vascular barrier integrity and supports eNOS activity via S1PR1 and S1PR3.

Inhibitors of SphK1 for clinical use are currently under intensive investigation [13]. Upon entering bloodstream, SphK1 inhibitors would get into erythrocytes to inhibit SphK1 therein, before being delivered to tumor tissues, which could reduce plasma S1P level to alter vascular barrier integrity and anti-atherogenic effects of HDL. Tumor-directed drug delivery system for SphK1 inhibitors would reduce such adverse effects. Very recently, the first phase I trial of SphK1 inhibitor L-threo-dihydrosphingosine (Safingol) has been conducted [171].

Another promising strategy of anti-cancer treatment that inhibits tumor angiogenesis, tumor growth and metastasis is to potentiate S1PR2 tumor suppressive signaling. A combination of a S1PR2-selective agonist with a S1PR1-selective antagonist [121] is expected to be more potent than either alone.

Conclusion

Recent studies unequivocally demonstrate the existence of the tumor suppressive S1P signaling that is mediated through $G_{12/13}$-coupled S1PR2 in tumor cells, endothelial cells and bone marrow-derived myeloid cells. S1PR2 negatively regulates tumor angiogenesis, tumor growth and metastasis, counteracting Gi-coupled S1PR1/S1PR3 signaling, which mediates tumor angiogenesis and tumor promotion. Selective activation of S1PR2 in combination with selective blockade of S1PR1/S1PR3 could be an effective anti-cancer therapeutic strategy targeting S1P signaling system, with possibly a reduced side effect.

In tumor cells, hypoxia induces HIF1α-dependent transcription of SphK1 with consequent increases in its mRNA and protein levels, and also its post-translational activation. Oncogenic mutations, including activating Ras mutation and p53 deletion, also contribute to upregulation of SphK1 protein levels and activity. These mechanisms in concert lead to SphK1 overexpression that is occasionally observed in tumor cells. SphK1 thus upregulated in tumor cells in turn stabilizes HIF1α through Akt–dependent regulation of GSK3β, providing tumor cells a feed-forward amplification loop for HIF1α–dependent processes, which include upregulation of angiogenic growth factors, glucose transporters, glycolysis enzymes and many others. SphK1 overexpressed in tumor cells also acts to protect Akt from ceramide-induced deactivation, thus conferring resistance to proapoptotic stresses such as chemotherapeutics and irradiation. These multiple mechanisms, which brings about elevated production and release of S1P and consequent activation of S1PR1 on tumor cells and endothelial cells through autocrine/paracrine mechanisms, in concert lead to tumor promotion, rather than tumor initiation. Significance and precise molecular mechanisms for SphK1 overexpression in tumor biology, as well as S1PR subtype-targeted, selective therapeutic tactics deserve further investigation.

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References

[1] Ghosh TK, Bian J and Gill DL. Intracellular calcium release mediated by sphingosine derivatives generated in cells. Science 1990; 248: 1653-6.

[2] Zhang H, Desai NN, Olivera A, Seki T, Brooker G and Spiegel S. Sphingosine-1-phosphate, a

novel lipid, involved in cellular proliferation. J Cell Biol. 1991; 114: 155-67.
[3] Hannun YA, Bell RM. Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. Science 1989; 243: 500-7.
[4] Zhang H, Buckley NE, Gibson K, Spiegel S. Sphingosine stimulates cellular proliferation via a protein kinase C-independent pathway. J Biol Chem 1990; 265: 76-81.
[5] Olivera A and Spiegel S. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature 1993; 365: 557-60.
[6] Sadahira Y, Ruan F, Hakomori S, Igarashi Y. Sphingosine 1-phosphate, a specific endogenous signaling molecule controlling cell motility and tumor cell invasiveness. Proc Natl Acad Sci U S A. 1992; 89: 9686-90.
[7] Yamamura S, Yatomi Y, Ruan F, Sweeney EA, Hakomori S, Igarashi Y. Sphingosine 1-phosphate regulates melanoma cell motility through a receptor-coupled extracellular action and in a pertussis toxin-insensitive manner. Biochemistry. 1997; 36: 10751-9.
[8] Chun J, Hla T, Lynch KR, Spiegel S, Moolenaar WH. International Union of Basic and Clinical Pharmacology. LXXVIII. Lysophospholipid receptor nomenclature. Pharmacol Rev. 2010; 62: 579-87.
[9] Skoura A, Hla T. Regulation of vascular physiology and pathology by the S1P2 receptor subtype. Cardiovasc Res. 2009; 82: 221-8.
[10] Karliner JS. J Sphingosine kinase and sphingosine 1-phosphate in cardioprotection. Cardiovasc Pharmacol. 2009; 53: 189-97.
[11] Sattler K, Levkau B. Sphingosine-1-phosphate as a mediator of high-density lipoprotein effects in cardiovascular protection. Cardiovasc Res. 2009; 82: 201-11.
[12] Strub GM, Maceyka M, Hait NC, Milstien S, Spiegel S. Extracellular and intracellular actions of sphingosine-1-phosphate. Adv Exp Med Biol. 2010; 688: 141-55.
[13] Pyne NJ, Pyne S.Sphingosine 1-phosphate and cancer. Nat Rev Cancer. 2010; 10: 489-503.
[14] Fyrst H, Saba JD. An update on sphingosine-1-phosphate and other sphingolipid mediators. Nat Chem Biol. 2010; 6: 489-97.
[15] Lucke S, Levkau B. Endothelial functions of sphingosine-1-phosphate. Cell Physiol Biochem. 2010; 26: 87-96.
[16] Rivera J, Proia RL, Olivera A. The alliance of sphingosine-1-phosphate and its receptors in immunity. Nat Rev Immunol. 2008; 8: 753-63.
[17] Wang F, Okamoto Y, Inoki I, Yoshioka K, Du W, Qi X, Takuwa N, Gonda K, Yamamoto Y, Ohkawa R, Nishiuchi T, Sugimoto N, Yatomi Y, Mitsumori K, Asano M, Kinoshita M, Takuwa Y. Sphingosine-1-phosphate receptor-2 deficiency leads to inhibition of macrophage proinflammatory activities and atherosclerosis in apoE-deficient mice. J Clin Invest. 2010; 120: 3979-95.
[18] Takuwa N, Ohkura S, Takashima S, Ohtani K, Okamoto Y, Tanaka T, Hirano K, Usui S, Wang F, Du W, Yoshioka K, Banno Y, Sasaki M, Ichi I, Okamura M, Sugimoto N, Mizugishi K, Nakanuma Y, Ishii I, Takamura M, Kaneko S, Kojo S, Satouchi K, Mitumori K, Chun J, Takuwa Y. S1P3-mediated cardiac fibrosis in sphingosine kinase 1 transgenic mice involves reactive oxygen species. Cardiovasc Res. 2010; 85: 484-93.
[19] Oyama O, Sugimoto N, Qi X, Takuwa N, Mizugishi K, Koizumi J, Takuwa Y. The lysophospholipid mediator sphingosine-1-phosphate promotes angiogenesis in vivo in ischaemic hindlimbs of mice. Cardiovasc Res. 2008; 78: 301-7.
[20] Tokumura A, Fukuzawa K, Akamatsu Y, Yamada S, Suzuki T, Tsukatani H. Identification of vasopressor phospholipid in crude soybean lecithin. Lipids. 1978; 13: 468-72.
[21] Gerrard JM, Kindom SE, Peterson DA, Peller J, Krantz KE, White JG. Lysophosphatidic acids. Influence on platelet aggregation and intracellular calcium flux. Am J Pathol. 1979; 96: 423-38.
[22] Tokumura A, Fukuzawa K, Yamada S, Tsukatani H. Stimulatory effect of lysophosphatidic acids on uterine smooth muscles of non-pregant rats. Arch Int Pharmacodyn Ther. 1980; 245: 74-83.
[23] van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH. Lysophosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell. 1989; 59: 45-54.
[24] Fernhout BJ, Dijcks FA, Moolenaar WH, Ruigt GS. Lysophosphatidic acid induces inward currents in Xenopus laevis oocytes; evidence for an extracellular site of action. Eur J Pharmacol. 1992; 213: 313-5.
[25] Bünemann M, Liliom K, Brandts BK, Pott L, Tseng JL, Desiderio DM, Sun G, Miller D, Tigyi G. A novel membrane receptor with high affinity for lysosphingomyelin and sphingosine 1-phosphate in atrial myocytes. EMBO J. 1996; 15: 5527-34.
[26] Tokumura A. Physiological and pathophysiological roles of lysophosphatidic acids produced by secretory lysophospholipase D in body fluids. Biochim Biophys Acta. 2002; 1582: 18-25.
[27] Takuwa N, Takuwa Y, Rasmussen H. Stimulation of mitogenesis and glucose transport by 1-monooleoylglycerol in Swiss 3T3 fibroblasts. J Biol Chem. 1988; 263: 9738-45.
[28] Takuwa N, Takuwa Y, Ohue Y, Mukai H, Endoh K, Yamashita K, Kumada M, Munekata E. Stimulation of calcium mobilization but not proliferation by bombesin and tachykinin neuropeptides in human small cell lung cancer cells. Cancer Res. 1990; 50: 240-4.
[29] Takuwa N, Iwamoto A, Kumada M, Yamashita K, Takuwa Y. Role of Ca2+ influx in bombesin-

induced mitogenesis in Swiss 3T3 fibroblasts. J Biol Chem. 1991; 266: 1403-9.
[30] Takuwa N, Zhou W, Takuwa Y. Calcium, calmodulin and cell cycle progression. Cell Signal. 1995; 7: 93-104.
[31] Takuwa N, Zhou W, Kumada M, Takuwa Y. Involvement of intact inositol-1,4,5-trisphosphate -sensitive Ca2+ stores in cell cycle progression at the G1/S boundary in serum-stimulated human fibroblasts. FEBS Lett. 1995; 360: 173-6.
[32] Jalink K, van Corven EJ, Hengeveld T, Morii N, Narumiya S, Moolenaar WH. Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. J Cell Biol. 1994; 126: 801-10.
[33] Postma FR, Jalink K, Hengeveld T, Moolenaar WH. Sphingosine-1-phosphate rapidly induces Rho-dependent neurite retraction: action through a specific cell surface receptor. EMBO J. 1996; 15: 2388-92.
[34] Okazaki H, Ishizaka N, Sakurai T, Kurokawa K, Goto K, Kumada M, Takuwa Y. Molecular cloning of a novel putative G protein-coupled receptor expressed in the cardiovascular system. Biochem Biophys Res Commun. 1993; 190: 1104-9.
[35] Hla T, Maciag T. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors. J Biol Chem. 1990; 265: 9308-13.
[36] Hecht JH, Weiner JA, Post SR, Chun J.Ventricular zone gene-1 (vzg-1) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J Cell Biol. 1996; 135: 1071-83.
[37] An S, Bleu T, Huang W, Hallmark OG, Coughlin SR, Goetzl EJ. Identification of cDNAs encoding two G protein-coupled receptors for lysosphingolipids. FEBS Lett. 1997; 417: 279-82.
[38] Zondag GC, Postma FR, Etten IV, Verlaan I, Moolenaar WH. Sphingosine 1-phosphate signalling through the G-protein-coupled receptor Edg-1. Biochem J. 1998; 330: 605-9.
[39] Lee MJ, Van Brocklyn JR, Thangada S, Liu CH, Hand AR, Menzeleev R, Spiegel S, Hla T. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science. 1998; 279: 1552-5.
[40] Okamoto H, Takuwa N, Gonda K, Okazaki H, Chang K, Yatomi Y, Shigematsu H, Takuwa Y. EDG1 is a functional sphingosine-1-phosphate receptor that is linked via a Gi/o to multiple signaling pathways, including phospholipase C activation, Ca2+ mobilization, Ras-mitogen-activated protein kinase activation, and adenylate cyclase inhibition. J Biol Chem. 1998; 273: 27104-10.
[41] Gonda K, Okamoto H, Takuwa N, Yatomi Y, Okazaki H, Sakurai T, Kimura S, Sillard R, Harii K, Takuwa Y. The novel sphingosine 1-phosphate receptor AGR16 is coupled via pertussis toxin-sensitive and -insensitive G-proteins to multiple signalling pathways. Biochem J. 1999; 337: 67-75.
[42] Sato K, Kon J, Tomura H, Osada M, Murata N, Kuwabara A, Watanabe T, Ohta H, Ui M, Okajima F. Activation of phospholipase C-Ca2+ system by sphingosine 1-phosphate in CHO cells transfected with Edg-3, a putative lipid receptor. FEBS Lett. 1999; 443: 25-30.
[43] Okamoto H, Takuwa N, Yatomi Y, Gonda K, Shigematsu H, Takuwa Y. EDG3 is a functional receptor specific for sphingosine 1-phosphate and sphingosylphosphorylcholine with signaling characteristics distinct from EDG1 and AGR16. Biochem Biophys Res Commun. 1999; 260: 203-8.
[44] Windh RT, Lee MJ, Hla T, An S, Barr AJ, Manning DR. 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. 1999; 274: 27351-8.
[45] Okamoto H, Takuwa N, Yokomizo T, Sugimoto N, Sakurada S, Shigematsu H, Takuwa Y. Inhibitory regulation of Rac activation, membrane ruffling, and cell migration by the G protein-coupled sphingosine-1-phosphate receptor EDG5 but not EDG1 or EDG3. Mol Cell Biol. 2000; 20: 9247-61.
[46] Ishii I, Friedman B, Ye X, Kawamura S, McGiffert C, Contos JJ, Kingsbury MA, Zhang G, Brown JH, Chun J. 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. 2001; 276: 33697-704.
[47] Ishii I, Ye X, Friedman B, Kawamura S, Contos JJ, Kingsbury MA, Yang AH, Zhang G, Brown JH, Chun J. 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. 2002; 277: 25152-9.
[48] Takuwa Y, Okamoto H, Takuwa N, Gonda K, Sugimoto N, Sakurada S. Subtype-specific, differential activities of the EDG family receptors for sphingosine-1-phosphate, a novel lysophospholipid mediator. Mol Cell Endocrinol. 2001; 177: 3-11.
[49] Takuwa Y, Takuwa N, Sugimoto N. The Edg family G protein-coupled receptors for lysophospholipids: their signaling properties and biological activities. J Biochem. 2002; 131: 767-71.
[50] Takuwa Y. Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors. Biochim Biophys Acta. 2002; 1582: 112-20.
[51] Rosen H, Goetzl EJ. Sphingosine 1-phosphate and its receptors: an autocrine and paracrine

network. Nat Rev Immunol. 2005; 5: 560-70.
[52] Sanchez T, Hla T. Structural and functional characteristics of S1P receptors. J Cell Biochem. 2004; 92: 913-22.
[53] Anliker B, Chun J. Cell surface receptors in lysophospholipid signaling. Semin Cell Dev Biol. 2004; 15: 457-65.
[54] Takuwa Y, Okamoto Y, Yoshioka K, Takuwa N. Sphingosine-1-phosphate signaling and biological activities in the cardiovascular system. Biochim Biophys Acta. 2008; 1781: 483-8.
[55] Takuwa Y, Du W, Qi X, Okamoto Y, Takuwa N and Yoshioka K. Roles of sphingosine-1-phosphate signaling in angiogenesis. World J Biol Chem 2010; 1: 298 -306.
[56] Arikawa K, Takuwa N, Yamaguchi H, Sugimoto N, Kitayama J, Nagawa H, Takehara K, Takuwa Y. 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. 2003; 278: 32841-51.
[57] Inoki I, Takuwa N, Sugimoto N, Yoshioka K, Takata S, Kaneko S, Takuwa Y. Negative regulation of endothelial morphogenesis and angiogenesis by S1P2 receptor. Biochem Biophys Res Commun. 2006; 346: 293-300.
[58] Yamaguchi H, Kitayama J, Takuwa N, Arikawa K, Inoki I, Takehara K, Nagawa H, Takuwa Y. Sphingosine-1-phosphate receptor subtype-specific positive and negative regulation of Rac and haematogenous metastasis of melanoma cells. Biochem J. 2003; 374: 715-22.
[59] Sugimoto N, Takuwa N, Okamoto H, Sakurada S, Takuwa Y. 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. 2003; 23: 1534-45.
[60] Sanchez T, Thangada S, Wu MT, Kontos CD, Wu D, Wu H, Hla T. PTEN as an effector in the signaling of antimigratory G protein-coupled receptor. Proc Natl Acad Sci U S A. 2005; 102: 4312-7.
[61] Li Z, Dong X, Wang Z, Liu W, Deng N, Ding Y, Tang L, Hla T, Zeng R, Li L, Wu D. Regulation of PTEN by Rho small GTPases. Nat Cell Biol. 2005; 7: 399-404.
[62] Du W, Takuwa N, Yoshioka K, Okamoto Y, Gonda K, Sugihara K, Fukamizu A, Asano M, Takuwa Y. S1P(2), the G protein-coupled receptor for sphingosine-1-phosphate, negatively regulates tumor angiogenesis and tumor growth in vivo in mice. Cancer Res. 2010; 70: 772-81.
[63] Schüppel M, Kürschner U, Kleuser U, Schäfer-Korting M, Kleuser B. Sphingosine 1-phosphate restrains insulin-mediated keratinocyte proliferation via inhibition of Akt through the S1P2 receptor subtype. J Invest Dermatol. 2008; 128: 1747-56.
[64] Malchinkhuu E, Sato K, Maehama T, Mogi C, Tomura H, Ishiuchi S, Yoshimoto Y, Kurose H, Okajima F. S1P(2) receptors mediate inhibition of glioma cell migration through Rho signaling pathways independent of PTEN. Biochem Biophys Res Commun. 2008; 366: 963-8.
[65] Keller CD, Rivera Gil P, Tölle M, van der Giet M, Chun J, Radeke HH, Schäfer-Korting M, Kleuser B. Immunomodulator FTY720 induces myofibroblast differentiation via the lysophospholipid receptor S1P3 and Smad3 signaling. Am J Pathol. 2007; 170: 281-92.
[66] Lebman DA, Spiegel S. Cross-talk at the crossroads of sphingosine-1-phosphate, growth factors, and cytokine signaling. J Lipid Res. 2008; 49: 1388-94.
[67] Kohama T, Olivera A, Edsall L, Nagiec MM, Dickson R, Spiegel S. Molecular cloning and functional characterization of murine sphingosine kinase. J Biol Chem. 1998; 273: 23722-8.
[68] Liu H, Sugiura M, Nava VE, Edsall LC, Kono K, Poulton S, Milstien S, Kohama T, Spiegel S. Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. J Biol Chem. 2000; 275: 19513 -20.
[69] Mandala SM, Thornton R, Galve-Roperh I, Poulton S, Peterson C, Olivera A, Bergstrom J, Kurtz MB, Spiegel S. Molecular cloning and characterization of a lipid phosphohydrolase that degrades sphingosine-1- phosphate and induces cell death. Proc Natl Acad Sci U S A. 2000; 97: 7859-64.
[70] Ogawa C, Kihara A, Gokoh M, Igarashi Y. Identification and characterization of a novel human sphingosine-1-phosphate phosphohydrolase, hSPP2. J Biol Chem. 2003; 278: 1268-72.
[71] Zhou J, Saba JD. Identification of the first mammalian sphingosine phosphate lyase gene and its functional expression in yeast. Biochem Biophys Res Commun. 1998; 242: 502-7.
[72] Serra M, Saba JD. Sphingosine 1-phosphate lyase, a key regulator of sphingosine 1-phosphate signaling and function. Adv Enzyme Regul. 2010; 50: 349-62.
[73] Hannun YA, Obeid LM. Ceramide: an intracellular signal for apoptosis. Trends Biochem Sci. 1995; 20: 73-7.
[74] Taha TA, Mullen TD, Obeid LM. A house divided: ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. Biochim Biophys Acta. 2006; 1758: 2027-36.
[75] Schubert KM, Scheid MP, Duronio V. Ceramide inhibits protein kinase B/Akt by promoting dephosphorylation of serine 473. J Biol Chem. 2000; 275: 13330-5.
[76] Schwab SR, Pereira JP, Matloubian M, Xu Y, Huang Y, Cyster JG. Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. Science. 2005; 309: 1735-9.
[77] Hla T, Venkataraman K, Michaud J. The vascular S1P gradient-cellular sources and biological

significance. Biochim Biophys Acta. 2008; 1781: 477-82.
[78] Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, Camerer E, Zheng YW, Huang Y, Cyster JG, Coughlin SR. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. Science. 2007; 316: 295-8.
[79] Bode C, Sensken SC, Peest U, Beutel G, Thol F, Levkau B, Li Z, Bittman R, Huang T, Tölle M, van der Giet M, Gräler MH. Erythrocytes serve as a reservoir for cellular and extracellular sphingosine 1-phosphate. J Cell Biochem. 2010; 109: 1232-43.
[80] Ito K, Anada Y, Tani M, Ikeda M, Sano T, Kihara A, Igarashi Y. Lack of sphingosine 1-phosphate-degrading enzymes in erythrocytes. Biochem Biophys Res Commun. 2007; 357: 212-7.
[81] Okajima F. Plasma lipoproteins behave as carriers of extracellular sphingosine 1-phosphate: is this an atherogenic mediator or an anti-atherogenic mediator? Biochim Biophys Acta. 2002; 1582: 132-7.
[82] Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M, Volpi M, Sha'afi RI, Hla T. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. Cell. 1999; 99: 301-12.
[83] Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D, Pham TH, Wong JS, Pappu R, Coughlin SR. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. J Clin Invest. 2009; 119: 1871-9.
[84] Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y, Bonkovsky HL, Parikh NS, Habrukowich C, Hla T. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. Circ Res. 2008; 102: 669-76.
[85] Yatomi Y, Ruan F, Hakomori S, Igarashi Y. Sphingosine-1-phosphate: a platelet-activating sphingolipid released from agonist-stimulated human platelets. Blood. 1995; 86: 193-202.
[86] Murata N, Sato K, Kon J, Tomura H, Yanagita M, Kuwabara A, Ui M, Okajima F. Interaction of sphingosine 1-phosphate with plasma components, including lipoproteins, regulates the lipid receptor-mediated actions. Biochem J. 2000; 352: 809-15.
[87] Kobayashi N, 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.
[88] Kupperman E, An S, Osborne N, Waldron S, Stainier DY. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. Nature. 2000; 406: 192-5.
[89] Osborne N, Brand-Arzamendi K, Ober EA, Jin SW, Verkade H, Holtzman NG, Yelon D, Stainier DY. The spinster homolog, two of hearts, is required for sphingosine 1-phosphate signaling in zebrafish. Curr Biol. 2008; 18: 1882-8.
[90] Kawahara A, Nishi T, Hisano Y, Fukui H, Yamaguchi A, Mochizuki N.The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. Science. 2009; 323: 524-7.
[91] Pham TH, Baluk P, Xu Y, Grigorova I, Bankovich AJ, Pappu R, Coughlin SR, McDonald DM, Schwab SR, Cyster JG. Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. J Exp Med. 2010; 207: 17-27.
[92] Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature. 2004; 427: 355-60.
[93] Cyster JG.Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu Rev Immunol. 2005; 23: 127-59.
[94] Allende ML, Tuymetova G, Lee BG, Bonifacino E, Wu YP, Proia RL. S1P1 receptor directs the release of immature B cells from bone marrow into blood. J Exp Med. 2010; 207: 1113-24.
[95] Lee H, Liao JJ, Graeler M, Huang MC, Goetzl EJ. Lysophospholipid regulation of mononuclear phagocytes. Biochim Biophys Acta. 2002; 1582: 175-7.
[96] Allende ML, Sasaki T, Kawai H, Olivera A, Mi Y, van Echten-Deckert G, Hajdu R, Rosenbach M, Keohane CA, Mandala S, Spiegel S, Proia RL. Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720. J Biol Chem. 2004; 279: 52487-92.
[97] Kharel Y, Lee S, Snyder AH, Sheasley-O'neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KS, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR. Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem. 2005; 280: 36865-72.
[98] Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL. Essential role for sphingosine kinases in neural and vascular development. Mol Cell Biol. 2005; 25: 11113-21.
[99] Mizugishi K, Li C, Olivera A, Bielawski J, Bielawska A, Deng CX, Proia RL. Maternal disturbance in activated sphingolipid metabolism causes pregnancy loss in mice. J Clin Invest. 2007; 117: 2993-3006.
[100] Vogel P, Donoviel MS, Read R, Hansen GM, Hazlewood J, Anderson SJ, Sun W, Swaffield J, Oravecz T. Incomplete inhibition of sphingosine 1-phosphate lyase modulates immune system function yet prevents early lethality and non-lymphoid lesions. PLoS One. 2009; 4: e4112.
[101] Bektas M, Allende ML, Lee BG, Chen W, Amar MJ, Remaley AT, Saba JD, Proia RL. Sphingosine 1-phosphate lyase deficiency disrupts lipid homeostasis in liver. J Biol Chem. 2010; 285: 10880-9.
[102] Wang F, Nohara K, Olivera A, Thompson EW,

Spiegel S. Involvement of focal adhesion kinase in inhibition of motility of human breast cancer cells by sphingosine 1-phosphate. Exp Cell Res. 1999; 247: 17-28.
[103] Lepley D, Paik JH, Hla T, Ferrer F. The G protein-coupled receptor S1P2 regulates Rho/Rho kinase pathway to inhibit tumor cell migration. Cancer Res. 2005; 65: 3788-95.
[104] Bornfeldt KE, Graves LM, Raines EW, Igarashi Y, Wayman G, Yamamura S, Yatomi Y, Sidhu JS, Krebs EG, Hakomori S, et al. 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. 1995; 130: 193-206.
[105] Ryu Y, Takuwa N, Sugimoto N, Sakurada S, Usui S, Okamoto H, Matsui O, Takuwa Y. Sphingosine-1-phosphate, a platelet-derived lysophospholipid mediator, negatively regulates cellular Rac activity and cell migration in vascular smooth muscle cells. Circ Res. 2002; 90: 325-32.
[106] Kawa S, Kimura S, Hakomori S, Igarashi Y. Inhibition of chemotactic motility and transendothelial migration of human neutrophils by sphingosine 1-phosphate. FEBS Lett. 1997; 420: 196-200.
[107] Wang F, Van Brocklyn JR, Hobson JP, Movafagh S, Zukowska-Grojec Z, Milstien S, Spiegel S. Sphingosine 1-phosphate stimulates cell migration through a G(i)-coupled cell surface receptor. Potential involvement in angiogenesis. J Biol Chem. 1999; 274: 35343-50.
[108] English D, Welch Z, Kovala AT, Harvey K, Volpert OV, Brindley DN, Garcia JG. Sphingosine 1-phosphate released from platelets during clotting accounts for the potent endothelial cell chemotactic activity of blood serum and provides a novel link between hemostasis and angiogenesis. FASEB J. 2000; 14: 2255-65.
[109] Lee OH, Kim YM, Lee YM, Moon EJ, Lee DJ, Kim JH, Kim KW, Kwon YG. Sphingosine 1-phosphate induces angiogenesis: its angiogenic action and signaling mechanism in human umbilical vein endothelial cells. Biochem Biophys Res Commun. 1999; 264: 743-50.
[110] 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. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. J Clin Invest. 2000; 106: 951-61.
[111] Graeler M, Goetzl EJ. Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic T cells. FASEB J. 2002; 16: 1874-8.
[112] Durand CA, Westendorf J, Tse KW, Gold MR. The Rap GTPases mediate CXCL13- and sphingosine1-phosphate-induced chemotaxis, adhesion, and Pyk2 tyrosine phosphorylation in B lymphocytes. Eur J Immunol. 2006; 36: 2235-49.
[113] Raftopoulou M, Hall A. Cell migration: Rho GTPases lead the way. Dev Biol. 2004; 265: 23-32.
[114] Kimura T, Watanabe T, Sato K, Kon J, Tomura H, Tamama K, Kuwabara A, Kanda T, Kobayashi I, Ohta H, Ui M, Okajima F. 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.
[115] Okamoto H, Yatomi Y, Ohmori T, Satoh K, Matsumoto Y, Ozaki Y. Sphingosine 1-phosphate stimulates G(i)- and Rho-mediated vascular endothelial cell spreading and migration. Thromb Res. 2000; 99: 259-65.
[116] Paik JH, Chae Ss, Lee MJ, Thangada S, Hla T. 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. 2001; 276: 11830-7.
[117] Yamashita H, Kitayama J, Shida D, Yamaguchi H, Mori K, Osada M, Aoki S, Yatomi Y, Takuwa Y, Nagawa H. Sphingosine 1-phosphate receptor expression profile in human gastric cancer cells: differential regulation on the migration and proliferation. J Surg Res. 2006; 130: 80-7.
[118] Balthasar S, Samulin J, Ahlgren H, Bergelin N, Lundqvist M, Toescu EC, Eggo MC, Törnquist K. Sphingosine 1-phosphate receptor expression profile and regulation of migration in human thyroid cancer cells. Biochem J. 2006; 398: 547-56.
[119] Park KS, Kim MK, Lee HY, Kim SD, Lee SY, Kim JM, Ryu SH, Bae YS. S1P stimulates chemotactic migration and invasion in OVCAR3 ovarian cancer cells. Biochem Biophys Res Commun. 2007; 356: 239-44.
[120] Li MH, Sanchez T, Yamase H, Hla T, Oo ML, Pappalardo A, Lynch KR, Lin CY, Ferrer F. S1P/S1P1 signaling stimulates cell migration and invasion in Wilms tumor. Cancer Lett. 2009; 276: 171-9.
[121] Yonesu K, Kawase Y, Inoue T, Takagi N, Tsuchida J, Takuwa Y, Kumakura S, Nara F. Involvement of sphingosine-1-phosphate and S1P1 in angiogenesis: analyses using a new S1P1 antagonist of non-sphingosine-1-phosphate analog. Biochem Pharmacol. 2009; 77: 1011-20.
[122] Chae SS, Paik JH, Furneaux H, Hla T. Requirement for sphingosine 1-phosphate receptor-1 in tumor angiogenesis demonstrated by in vivo RNA interference. J Clin Invest. 2004; 114: 1082-9.
[123] Azuma H, Takahara S, Ichimaru N, Wang JD, Itoh Y, Otsuki Y, Morimoto J, Fukui R, Hoshiga M, Ishihara T, Nonomura N, Suzuki S, Okuyama A, Katsuoka Y. Marked prevention of tumor growth and metastasis by a novel immunosuppressive agent, FTY720, in mouse breast can-

cer models. Cancer Res. 2002; 62: 1410-9.
[124] Schmid G, Guba M, Papyan A, Ischenko I, Brückel M, Bruns CJ, Jauch KW, Graeb. FTY720 inhibits tumor growth and angiogenesis. Transplant Proc. 2005; 37: 110-1.
[125] LaMontagne K, Littlewood-Evans A, Schnell C, O'Reilly T, Wyder L, Sanchez T, Probst B, Butler J, Wood A, Liau G, Billy E, Theuer A, Hla T, Wood J. Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. Cancer Res. 2006; 66: 221-31.
[126] Allende ML, Yamashita T, Proia RL. G-protein-coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation. Blood. 2003; 102: 3665-7.
[127] Paik JH, Skoura A, Chae SS, Cowan AE, Han DK, Proia RL, Hla T. Sphingosine 1-phosphate receptor regulation of N-cadherin mediates vascular stabilization. Genes Dev. 2004; 18: 2392-403.
[128] Kono M, Mi Y, Liu Y, Sasaki T, Allende ML, Wu YP, Yamashita T, Proia RL. The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis. J Biol Chem. 2004; 279: 29367-73.
[129] Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer. 2008; 8: 618-31.
[130] Olivera A, Kohama T, Edsall L, Nava V, Cuvillier O, Poulton S, Spiegel S. Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival. J Cell Biol. 1999; 147: 545-58.
[131] Xia P, Gamble JR, Wang L, Pitson SM, Moretti PA, Wattenberg BW, D'Andrea RJ, Vadas MA. An oncogenic role of sphingosine kinase. Curr Biol. 2000; 10: 1527-30.
[132] Pitson SM, Moretti PA, Zebol JR, Lynn HE, Xia P, Vadas MA, Wattenberg BW.Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. EMBO J. 2003; 22: 5491-500.
[133] Pitson SM, Xia P, Leclercq TM, Moretti PA, Zebol JR, Lynn HE, Wattenberg BW, Vadas MA. Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling. J Exp Med. 2005; 201: 49-54.
[134] Safadi-Chamberlain F, Wang LP, Payne SG, Lim CU, Stratford S, Chavez JA, Fox MH, Spiegel S, Summers SA. Effect of a membrane-targeted sphingosine kinase 1 on cell proliferation and survival. Biochem J. 2005; 388: 827-34.
[135] Vadas M, Xia P, McCaughan G, Gamble J. The role of sphingosine kinase 1 in cancer: oncogene or non-oncogene addiction? Biochim Biophys Acta. 2008; 1781: 442-7.
[136] Nava VE, Cuvillier O, Edsall LC, Kimura K, Milstien S, Gelmann EP, Spiegel S. Sphingosine enhances apoptosis of radiation-resistant prostate cancer cells. Cancer Res. 2000; 60: 4468-74.
[137] French KJ, Schrecengost RS, Lee BD, Zhuang Y, Smith SN, Eberly JL, Yun JK, Smith CD. Discovery and evaluation of inhibitors of human sphingosine kinase. Cancer Res. 2003; 63: 5962-9.
[138] Pchejetski D, Golzio M, Bonhoure E, Calvet C, Doumerc N, Garcia V, Mazerolles C, Rischmann P, Teissié J, Malavaud B, Cuvillier O. Sphingosine kinase-1 as a chemotherapy sensor in prostate adenocarcinoma cell and mouse models. Cancer Res. 2005; 65: 11667-75.
[139] Kawamori T, Osta W, Johnson KR, Pettus BJ, Bielawski J, Tanaka T, Wargovich MJ, Reddy BS, Hannun YA, Obeid LM, Zhou D. Sphingosine kinase 1 is up-regulated in colon carcinogenesis. FASEB J. 2006; 20: 386-8.
[140] Akao Y, Banno Y, Nakagawa Y, Hasegawa N, Kim TJ, Murate T, Igarashi Y, Nozawa Y. High expression of sphingosine kinase 1 and S1P receptors in chemotherapy-resistant prostate cancer PC3 cells and their camptothecin-induced up-regulation. Biochem Biophys Res Commun. 2006; 342: 1284-90.
[141] Li J, Guan HY, Gong LY, Song LB, Zhang N, Wu J, Yuan J, Zheng YJ, Huang ZS, Li M. Clinical significance of sphingosine kinase-1 expression in human astrocytomas progression and overall patient survival. Clin Cancer Res. 2008; 14: 6996-7003.
[142] Bonhoure E, Lauret A, Barnes DJ, Martin C, Malavaud B, Kohama T, Melo JV, Cuvillier O. Sphingosine kinase-1 is a downstream regulator of imatinib-induced apoptosis in chronic myeloid leukemia cells. Leukemia. 2008; 22: 971-9.
[143] Kawamori T, Kaneshiro T, Okumura M, Maalouf S, Uflacker A, Bielawski J, Hannun YA, Obeid LM. Role for sphingosine kinase 1 in colon carcinogenesis. FASEB J. 2009; 23: 405-14.
[144] Malavaud B, Pchejetski D, Mazerolles C, de Paiva GR, Calvet C, Doumerc N, Pitson S, Rischmann P, Cuvillier O. Sphingosine kinase-1 activity and expression in human prostate cancer resection specimens. Eur J Cancer. 2010; 46: 3417-24.
[145] Kohno M, Momoi M, Oo ML, Paik JH, Lee YM, Venkataraman K, Ai Y, Ristimaki AP, Fyrst H, Sano H, Rosenberg D, Saba JD, Proia RL, Hla T. Intracellular role for sphingosine kinase 1 in intestinal adenoma cell proliferation. Mol Cell Biol. 2006; 26: 7211-23.
[146] Murakami M, Ichihara M, Sobue S, Kikuchi R, Ito H, Kimura A, Iwasaki T, Takagi A, Kojima T, Takahashi M, Suzuki M, Banno Y, Nozawa Y, Murate T. RET signaling-induced SPHK1 gene expression plays a role in both GDNF-induced differentiation and MEN2-type oncogenesis. J Neurochem. 2007; 102: 1585-94.
[147] Sobue S, Murakami M, Banno Y, Ito H, Kimura A, Gao S, Furuhata A, Takagi A, Kojima T, Suzuki M, Nozawa Y, Murate T. v-Src oncogene product increases sphingosine kinase 1 expres-

sion through mRNA stabilization: alteration of AU-rich element-binding proteins. Oncogene. 2008; 27: 6023-33.
[148] Taha TA, Osta W, Kozhaya L, Bielawski J, Johnson KR, Gillanders WE, Dbaibo GS, Hannun YA, Obeid LM. Down-regulation of sphingosine kinase-1 by DNA damage: dependence on proteases and p53. J Biol Chem. 2004; 279: 20546-54.
[149] Shida D, Fang X, Kordula T, Takabe K, Lépine S, Alvarez SE, Milstien S, Spiegel S. Cross-talk between LPA1 and epidermal growth factor receptors mediates up-regulation of sphingosine kinase 1 to promote gastric cancer cell motility and invasion. Cancer Res. 2008; 68: 6569-77.
[150] Anelli V, Gault CR, Cheng AB, Obeid LM. Sphingosine kinase 1 is up-regulated during hypoxia in U87MG glioma cells. Role of hypoxia-inducible factors 1 and 2. J Biol Chem. 2008; 283: 3365-75.
[151] Schwalm S, Döll F, Römer I, Bubnova S, Pfeilschifter J, Huwiler A. Sphingosine kinase-1 is a hypoxia-regulated gene that stimulates migration of human endothelial cells. Biochem Biophys Res Commun. 2008; 368: 1020-5.
[152] Ader I, Brizuela L, Bouquerel P, Malavaud B, Cuvillier O. Sphingosine kinase 1: a new modulator of hypoxia inducible factor 1alpha during hypoxia in human cancer cells. Cancer Res. 2008; 68: 8635-42.
[153] Ader I, Malavaud B, Cuvillier O. When the sphingosine kinase 1/sphingosine 1-phosphate pathway meets hypoxia signaling: new targets for cancer therapy. Cancer Res. 2009; 69: 3723-6.
[154] Semenza GL. HIF-1: upstream and downstream of cancer metabolism. Curr Opin Genet Dev. 2010; 20: 51-6.
[155] 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.
[156] Young N, Pearl DK, Van Brocklyn JR. Sphingosine-1-phosphate regulates glioblastoma cell invasiveness through the urokinase plasminogen activator system and CCN1/Cyr61. Mol Cancer Res. 2009; 7: 23-32.
[157] Visentin B, Vekich JA, Sibbald BJ, Cavalli AL, Moreno KM, Matteo RG, Garland WA, Lu Y, Yu S, Hall HS, Kundra V, Mills GB, Sabbadini RA. Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages. Cancer Cell. 2006; 9: 225-38.
[158] Selzner M, Bielawska A, Morse MA, Rüdiger HA, Sindram D, Hannun YA, Clavien PA. Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. Cancer Res. 2001; 61: 1233-40.
[159] Schubert KM, Scheid MP, Duronio V. Ceramide inhibits protein kinase B/Akt by promoting dephosphorylation of serine 473. J Biol Chem. 2000; 275: 13330-5.
[160] Nemoto S, Nakamura M, Osawa Y, Kono S, Itoh Y, Okano Y, Murate T, Hara A, Ueda H, Nozawa Y, Banno Y. Sphingosine kinase isoforms regulate oxaliplatin sensitivity of human colon cancer cells through ceramide accumulation and Akt activation. J Biol Chem. 2009; 284: 10422-32.
[161] Feng J, Tamaskovic R, Yang Z, Brazil DP, Merlo A, Hess D, Hemmings BA. Stabilization of Mdm2 via decreased ubiquitination is mediated by protein kinase B/Akt-dependent phosphorylation. J Biol Chem. 2004; 279: 35510-7.
[162] Feng Z. p53 regulation of the IGF-1/AKT/mTOR pathways and the endosomal compartment. Cold Spring Harb Perspect Biol. 2010; 2: a001057.
[163] Xia P, Wang L, Moretti PA, Albanese N, Chai F, Pitson SM, D'Andrea RJ, Gamble JR, Vadas MA. Sphingosine kinase interacts with TRAF2 and dissects tumor necrosis factor-alpha signaling. J Biol Chem. 2002; 277: 7996-8003.
[164] Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, Maceyka M, Jiang H, Luo C, Kordula T, Milstien S, Spiegel S. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. Nature. 2010; 465: 1084-8.
[165] Sabbadini RA. Sphingosine-1-phosphate antibodies as potential agents in the treatment of cancer and age-related macular degeneration. Br J Pharmacol. 2010 in press.
[166] Oo ML, Thangada S, Wu MT, Liu CH, Macdonald TL, Lynch KR, Lin CY, Hla T. 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.
[167] Thangada S, Khanna KM, Blaho VA, Oo ML, Im DS, Guo C, Lefrancois L, Hla T. Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines lymphocyte egress kinetics. J Exp Med. 2010; 207: 1475-83.
[168] Zhang N, Qi Y, Wadham C, Wang L, Warren A, Di W, Xia P. FTY720 induces necrotic cell death and autophagy in ovarian cancer cells: a protective role of autophagy. Autophagy. 2010; 6: 1157-67.
[169] Pchejetski D, Bohler T, Brizuela L, Sauer L, Doumerc N, Golzio M, Salunkhe V, Teissié J, Malavaud B, Waxman J, Cuvillier O. FTY720 (fingolimod) sensitizes prostate cancer cells to radiotherapy by inhibition of sphingosine kinase -1. Cancer Res. 2010; 70: 8651-61.
[170] van Meeteren LA, Brinkmann V, Saulnier-Blache JS, Lynch KR, Moolenaar WH. Anticancer activity of FTY720: phosphorylated FTY720 inhibits autotaxin, a metastasis-enhancing and angiogenic lysophospholipase D. Cancer Lett. 2008; 266: 203-8.

[171] Dickson MA, Carvajal RD, Merrill AH, Gonen M, Cane LM, Schwartz GK. A Phase I Clinical Trial of Safingol in Combination with Cisplatin in Advanced Solid Tumors. Clin Cancer Res. 2011. In press.
[172] Kono M, Allende ML and Proia RL. Sphingosine-1-Phosphate Regulation of Mammalian Development. Biochim Biophys Acta. 2008; 1781: 435–441.
[173] Bartke N and Hannun YA. Bioactive sphingolipids: metabolism and function. J Lipid Res. 2009 50(Supplement): S91–S96.