Review Article

Sphingosine-1-phosphate (S1P) in cancer immunity and development

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Abstract: Sphingosine-1-phosphate (S1P) is a cellular and extracellular signaling molecule that acts locally in different organs and is also systemically present in blood and lymph. S1P is a well characterized immune modulator influencing lymphocyte circulation and immune cell differentiation, survival, function, migration, and locality. Many of these functions are also relevant for the pathology of tumor cells, and there is a body of evidence supporting the fact that S1P fulfils similar functions in cancer. This review aims to summarize the current knowledge about the roles of S1P in tumor growth and survival, cancer cell invasion and metastasis, and cancer development and neoplasia. Different mechanistic concepts are discussed, including S1P receptor and inside-out signaling, adaptation, immune escape mechanisms, and the sphingolipid rheostat.

Keywords: Adaptation; immune escape; inside-out signaling; receptor; sphingolipid rheostat

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Introduction

Sphingosine-1-phosphate (S1P) is a lipid metabolite and signaling molecule that influences many different cellular functions. It is produced by two different sphingosine kinases, SphK1 and SphK2 (*Figure 1*) (1-3). Interestingly cells devoid of both SphKs are still able to grow and to proliferate, which demonstrates that S1P is not required for normal cell survival (4). Numerous studies however show that S1P can rescue cells from apoptosis and cell death induced by other stimuli, suggesting its potential role as a survival factor under certain pathologic conditions including cancer (5-15). Mice deficient of both SphK1 and SphK2 die around embryonic day 11.5 mainly due to vascular and neurologic defects, which emphasizes its role in developmental processes (4). While various different phosphatases like lipid phosphate phosphatases (LPPs) (16), sphingosine phosphate phosphatases (SPPs) (17,18) and also alkaline phosphatases are able to dephosphorylate S1P (19), which obviously is a rather unspecific event that can be initiated by many different enzymes, only the retro-aldolase S1P-lyase is able to cleave and irreversibly degrade S1P into hexadecenal and phosphoethanolamine (*Figure 1*) (20-23). The activity of anabolic SphKs on one side and catabolic phosphatases and the S1P-lyase on the other side together with the availability of the substrate sphingosine basically determine the cellular amount of S1P. The balance of intracellular S1P and ceramides determines cell fate (*Figure 1*) (24). More cellular S1P induces cell survival, while an increase in ceramides shifts cells into apoptosis. A potential reason for the survival effect of S1P could be the induction of autophagy (25). Accumulation of S1P in thymocytes from S1P-lyase deficient mice however cannot prevent apoptosis induced by increased ceramide levels (22). Therfore the interconversion of S1P and ceramides may be more relevant for cell fate decision than the total amounts of these metabolites present in cells, and this may well be adapted by cancer cells to increase survival. Current data

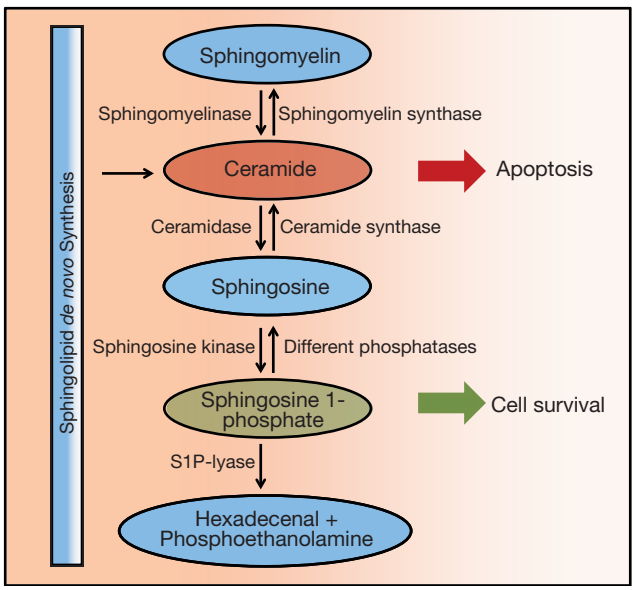

Figure 1 The sphingolipid metabolism. The degradation of sphingosine-1-phosphate (S1P) by S1P-lyase is the only irreversible step in this pathway. Ceramide and S1P have antagonizing functions, which makes their balance essential for cell fate.

indicate that SphK2 predominantly feeds into the catabolic metabolism determined by the activity of the S1P-lyase (26), while SphK1 is inducibly transported to the cell membrane to produce S1P that is subsequently secreted by cells (27). Spinster homolog 2 (Spns2) is a transporter integrated into the cell membrane that is involved in export of S1P from the cytosol into the extracellular matrix (28,29). S1P is an extracellular signaling molecule and ligand of five G protein-coupled S1P receptors designated $S1P_{1-5}$ (30-33). S1P secretion can therefore lead to autocrine and paracrine signaling in the local tissue environment and also to endocrine signaling at distant locations via release into circulation. In fact S1P concentrations are highest in plasma and lymph, and very low in tissues due to the activity of the S1P-lyase which is predominantly expressed by tissue cells (21,22,34,35). These differences produce concentration gradients between the circulatory system and peripheral tissues which are important to induce lymphocyte egress from lymphoid organs and to maintain lymphocyte circulation (36-40). Neutralizing these S1P gradients by increasing the S1P concentration in lymphoid tissues prevents lymphocyte egress from thymus and lymph nodes and results in lymphopenia, although S1P as the primary exit signal is still present in blood at high micromolar levels (21,22,35,41). The reason for the unresponsiveness of lymphocytes to the exit signal S1P in blood and lymph is the premature downregulation of the $S1P_1$ receptor (36,38). S1P is also a very potent inducer of angiogenesis and vascular barrier stability (42-45). The latter requires some kind of constitutive signaling activity that is also important for cancer cells, although under different circumstances. Constitutive signaling particularly of the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) is frequently observed in cancer cells and under normal conditions prevented by receptor desensitization and internalization (46). How constitutive signaling of high blood S1P concentrations is possible is currently not clear (42). Recent data suggest a role of the S1P-lyase, at least for the endothelial barrier-stabilizing activity of S1P (47). Thus, many cancer-related functions of S1P are known like cell survival, autophagy, constitutive signaling, migration, cell differentiation, and development.

The sphingolipid rheostat

A key feature of tumor cells is unrestricted cell growth and enhanced survival, although isolated tumor cells are frequently more sensitive and die earlier than related normal primary cells when taken into cell culture, pointing to exogenous factors that promote tumor growth and survival *in vivo* (48). While early reports focused on sphingosine and its blocking activity on protein kinase C (PKC) (49,50), which can be attributed to its competition with the PKC activator phosphatidylserine (PS) for PKC binding, further studies revealed significant non-PKC mediated side effects that pointed to other relevant signaling events (51,52). Subsequently S1P and ceramides as the closest sphingosine metabolites came into the focus as potential mediators of sphingosine-related effects (53,54), although sphingosine itself still has unique physiological functions apart from ceramide and S1P signaling (55). Ceramide evolved as a pro-apoptotic molecule (56,57), while S1P was regarded as a pro-survival factor (8,9). These opposing physiological functions of the two closest metabolites of sphingosine shaped the concept of the sphingolipid rheostat (24). It postulates that the intracellular balance of ceramide and S1P generation determines cell fate (*Figure 2*). Increased ceramide production would lead to apoptosis while increased S1P production would promote cell survival (24). S1P-induced autophagy was later on identified as a possible mechanism to promote cell survival and to avoid ceramide-induced apoptosis (25,58). Cellular production of S1P by SphKs appears to be a key event of this hypothesis, and indeed many publications report increased expression

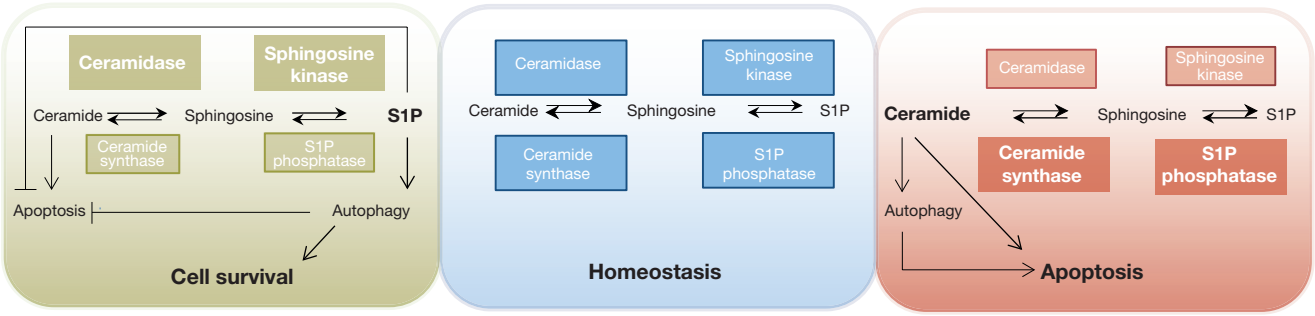

Figure 2 The concept of the sphingolipid rheostat. The intracellular balance of the two closest metabolites of sphingosine, sphingosine-1-phosphate (S1P) and ceramide, determine cell fate. Homeostasis is maintained through interconversion of ceramide and S1P via sphingosine as an intermediate (blue box). Increased S1P production promotes cell survival by suppression of ceramide induced apoptosis and induction of autophagy (green box). Apoptosis is associated with increased ceramide levels within the cell (red box).

particularly of SphK1 in different tumors (59). On the other hand, accumulation of S1P in thymocytes of S1P-lyase deficient mice did not prevent ceramide-induced apoptosis and thymus atrophy, indicating that the sole presence of high amounts of intracellular S1P was not sufficient to rescue them from cell death (22). In addition specific SphK inhibitors failed to inhibit tumor cell growth and viability, also questioning the relevance of the sphingolipid rheostat concept in cancer (60). Since intracellular targets of both S1P and ceramide are still not worked out very well, defining relevant intracellular signaling processes for both metabolites will be required to understand this system more thoroughly and to potentially use it for medical interventions.

S1P receptor signaling

In contrast to ceramide, S1P is also an extracelluar signaling molecule and a ligand for five G protein-coupled cell surface receptors. Type 1 and type 2 S1P-receptors are particularly often described in the literature in the context of cancer. $S1P_1$ signaling is frequently linked to promotion of tumor cell survival, providing an alternative pathway for S1P-mediated vitality (13,61). A major downstream signaling molecule of $S1P_1$ is the serine/threonine kinase Akt (62-65). $S1P_1$ is also known to induce cell migration, and several studies link $S1P_1$ expression and function with tumor cell migration, invasion, and metastasis (66-68). Notably $S1P_2$ appears to induce opposing effects at least on germinal center B cells by dampening Akt activation, growth, and $S1P_1$-mediated migration (69,70). Loss of function mutations in $S1P_2$ are thus linked with the occurrence of germinal center-like (GCB) diffuse large B cell lymphomas (DLBCL) (71,72). In contrast to the concept of the sphingolipid rheostat where SphKs play a major role for the intracellular generation of S1P, signaling of S1P receptors requires the presence of extracellular S1P, which can be produced locally by tumor cells via SphKs, but it can also be provided via the circulatory system from distant sources. Tumor angiogenesis is an important step in the development of solid cancers, and S1P is a strong angiogenic factor that mainly acts via activation of $S1P_1$ on endothelial cells (45,73-77). Evidence exists that $S1P_1$-mediated angiogenesis can also be opposed by $S1P_2$ signaling (78).

Inside-out signaling

The concomitant presence of S1P receptors and SphKs in one cell shaped the concept of inside-out signaling (79,80). SphKs are intracellular enzymes, and in order to stimulate S1P receptors on the cell surface, S1P needs to be released by cells (*Figure 3*). This release is supported by ATP binding cassette (ABC) transporters, a large family of ubiquitously expressed integral membrane proteins that actively transport ligands across biological membranes (81). Although several reports indicate the involvement of ABC transporters in S1P exportation (82-86), other studies did not find a specific role of ABC transporters for S1P exportation from erythrocytes and endothelial cells (87,88). A clear function as a S1P-transporter however could be assigned to the membrane protein Spns2 (28,29). Recent reports indicate the involvement of Spns2 in tumor angiogenesis, cancer cell survival and migration (89,90). As mentioned before, many tumors upregulate expression of SphKs, particularly SphK1 (59), and it is an appealing hypothesis to suggest that tumor cells produce their own

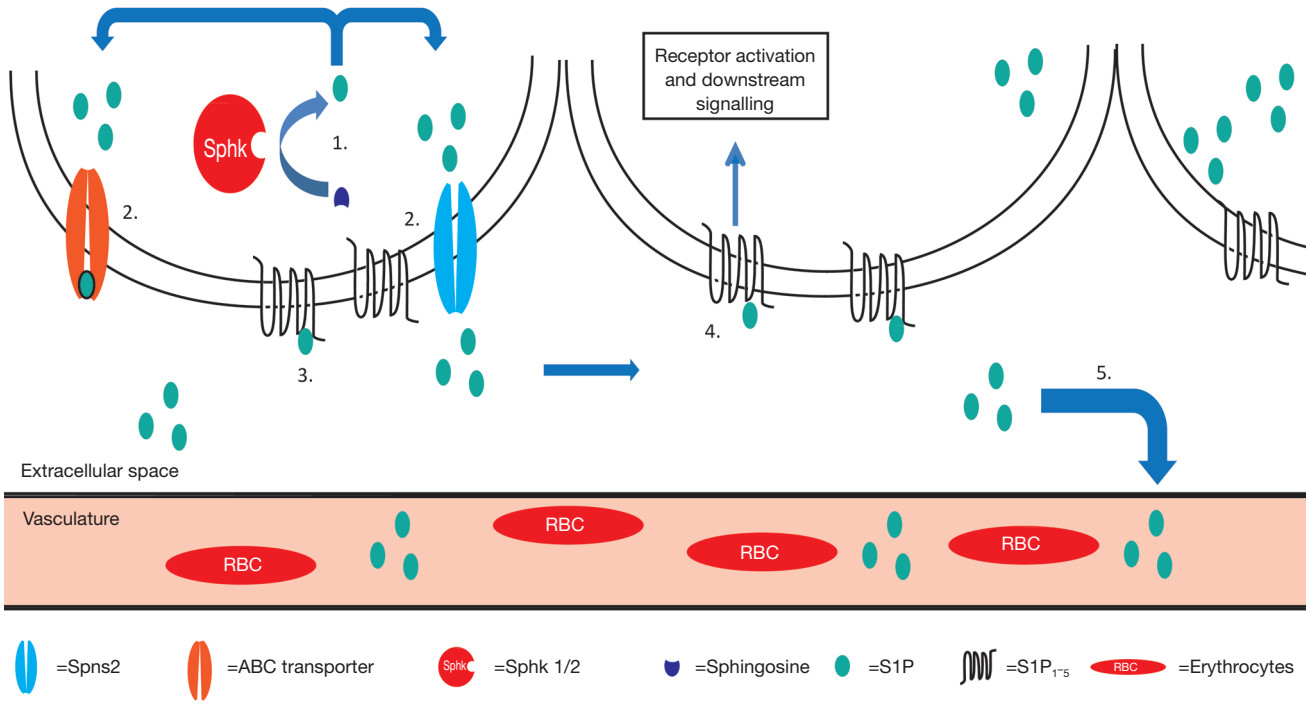

Figure 3 Inside-out signaling. Within tumor cells, endogenous sphingosine is phosphorylated by SphK1/2 to sphingosine-1-phosphate (S1P) [1]. Subsequently, S1P is exported into the extracellular space via transmembrane S1P transporters [2] namely Spns2 and/or ABC transporters. Once there, free S1P can activate surface S1P receptors in an autocrine [3] or paracrine [4] fashion. Further, it is possible that S1P produced and exported from tumor cells can be taken up into the vasculature [5] inducing receptor activation on distant target cells (endocrine signaling).

survival factor S1P that is secreted by ABC transporters and/or Spns2 into the tumor matrix to induce autocrine and paracrine survival signals via S1P-receptors, particularly $S1P_1$ (*Figure 3*). But so far there are no reports available demonstrating increased S1P concentrations in tumors compared to normal tissues, and also the contribution of systemic S1P supply via the circulatory system is not clear. Blocking strategies for systemic S1P by either using an anti-S1P antibody (91) or an S1P-neutralizing L-aptamer (92) hold promise that systemic eradication of S1P could dampen tumor progression (45,93-99).

Adaptation

Adaptation is a process that allows cells to survive and proliferate under difficult conditions. A classical case of adaptation is the occurrence of drug resistance, and there is strong evidence that S1P and particularly the expression of SphK1 confers resistance of cancer cells to different therapeutic drugs and treatments (77,82,95,100-111). A common mechanistic pattern is S1P-mediated increase in vitality and prevention of ceramide-induced apoptosis (101,109) together with S1P receptor-mediated survival signaling (108,111). Another variant of adaptation is the establishment of constitutive NF-κB signaling via the $S1P_1$-STAT3 axis (46,112). The signal transducer and activator of transcription-3 (STAT3) is a transcription factor for $S1P_1$, so that $S1P_1$ expression is elevated in STAT3-positive tumors. As a positive feedback-loop, upregulation of $S1P_1$ activates STAT3 and results in increased interleukin-6 (IL6) production, which accelerates tumor growth and metastasis in a STAT3-dependent manner (46,112).

Immune escape mechanisms

Degenerated cells are usually detected and killed by immune cells. Obviously this endogenous defense system is not working properly anymore when tumors are formed. One reason for a failed immune response against tumors are certain immune escape strategies of the tumors that prevent either their recognition or their efficient attack by the immune system. Accumulation of regulatory T cells (Tregs)

for example blocks the onset of an immune response, and $S1P_1$ expression in T cells impairs their generation and their immune suppressive function (65,113). Under physiological conditions, increased expression of $S1P_1$ therefore reduces the amount and activity of Tregs. Tumors however reverse the activity of $S1P_1$ signaling in T cells by promoting the migration of Tregs into the tumor in a STAT3 dependent manner (114). Concomitantly activity and accumulation of CD8 T cells in tumors are reduced (114). There is also evidence that S1P released by apoptotic cells within tumors induce the generation of regulatory macrophages (14). These tumor-associated macrophages infiltrate tumors and promote tumor growth by, for example, activating hypoxia inducible factor 1α and releasing vascular endothelial growth factor (14).

Concluding remarks

Main functions of S1P include inhibition of apoptosis and induction of cell survival, cell migration, and angiogenesis. Tumor cells make use of this system to enhance their growth and proliferation, to invade tissues, to adapt to different environments, and to hide from the immune system. Although S1P signaling is used by tumor cells for their benefit, none of the S1P-related receptors and enzymes are proto-oncogenes except for $S1P_2$, which is involved in GCB-DLBCL lymphoma development when loss-of-function-mutations occur. Because of the tumor-promoting role of S1P signaling, agonists and antagonists for S1P receptors, inhibitors of SphKs, and S1P-blocking substances are promising candidates for cancer treatment.

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Footnote

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