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S1P metabolism in cancer and other pathological conditions

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Abstract

Nearly two decades ago, the sphingolipid metabolite sphingosine 1-phosphate was discovered to function as a lipid mediator and regulator of cell proliferation. Since that time, sphingosine 1-phosphate has been shown to mediate a diverse array of fundamental biological processes including cell proliferation, migration, invasion, angiogenesis, vascular maturation and lymphocyte trafficking. Sphingosine 1-phosphate acts primarily via signaling through five ubiquitously expressed G protein-coupled receptors. Intracellular sphingosine 1-phosphate molecules are transported extracellularly and gain access to its cognate receptors for autocrine and paracrine fashion and for signaling at distant sites reached through blood and lymphatic circulation systems. Intracellular pools of sphingosine 1-phosphate available for signaling are tightly regulated by three enzymes that include sphingosine kinase, S1P lyase and S1P phosphatase. Alterations in S1P levels as well as the enzymes involved in its synthesis and catabolism have been observed in many types of malignancy. These enzymes are being evaluated for their role in mediating cancer formation and progression, as well as their potential to serve as targets of anti-cancer therapeutics. In this review, the impact of sphingosine 1-phosphate, its cognate receptors, and the enzymes of sphingosine 1-phosphate metabolism on cell survival, apoptosis, autophagy, cellular transformation, invasion, angiogenesis and hypoxia in relation to cancer biology and treatment are discussed.

Keywords

sphingolipid; sphingosine 1-phosphate; tumorigenesis; sphingosine kinase; S1P lyase; S1P phosphatase; lysophospholipid; cancer

1. Introduction

Sphingolipids are structural membrane components and have also become widely recognized as the reservoir for a family of bioactive lipid mediators including ceramide, sphingosine, sphingosine 1-phosphate (S1P) and ceramide 1-phosphate derived from sphingolipid metabolism. S1P is the final common product of the sphingolipid degradative pathway and a bioactive molecule that mediates a diverse range of cellular processes. S1P acts extracellularly in paracrine and autocrine manner through five specific G protein-coupled receptors S1P₁–S1P₅ [1]. S1P circulates in blood and lymphatic systems and, thus, potentially gains access to receptors distant from its site of synthesis. There is some evidence that S1P may have direct intracellular functions, although this mechanism of action is poorly understood and remains controversial [2–4]. Since the first discovery of S1P's involvement in cellular proliferation in

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1991 [5], numerous studies have revealed roles for S1P signaling in regulating conserved cell death pathways, cytoskeletal rearrangements, cell motility and vascular development [6–8]. Increased generation of S1P triggers signaling pathways that mediate cell survival, malignant transformation, and regulate apoptosis, invasion, angiogenesis, and hypoxia [9–11]. These fundamental biological processes are integral to cancer pathogenesis, thereby implicating S1P in cancer biology.

S1P homeostasis is tightly regulated by the balance between its synthesis and degradation via three enzymes (Fig. 1). These include sphingosine kinase (SphK), which generates S1P through phosphorylation of its precursor, sphingosine, S1P phosphatases (SPP1 and SPP2), which reversibly convert S1P back to sphingosine, and S1P lyase (SPL), which irreversibly degrades S1P to generate ethanolamine phosphate and hexadecenal, representing the last step in the sphingolipid degradation pathway [12]. Modulation of these enzymes affects S1P-mediated biology, and alterations of these enzymes have been observed in various pathological conditions including cancer. This review focuses on our current understanding of how S1P signaling and the enzymes involved in S1P homeostasis impact cancer biology.

2. Alterations in S1P levels and related genes in cancer

Sphingolipid metabolism is often found to be dysregulated in cancer. Numerous studies have shown that SphK1 acts as an oncogene [13] and is upregulated in a variety of human tumors, including lung, colon, breast, ovary, brain, stomach, uterus and kidney, compared with their healthy tissue counterparts [14–16]. Increased tumor Sphk1 expression correlated with a shorter survival for patients with glioblastoma multiforme [17]. Moreover, microarray analyses revealed a worse outcome for breast cancer patients with elevated Sphk1 expression, further supporting the notion that increased S1P generation confers a worse prognosis for cancer patients [18]. In a transgenic mouse model of erythroleukemia, increased SphK1 expression and activity correlated with an increase in tumorigenic potential [19].

Consistent with the growth promoting effects of S1P in many tumors [20], S1P was found to be elevated in the plasma and malignant ascites of ovarian cancer patients [21,22]. Increased S1P levels and decreased SPL expression and enzyme activity were also observed in the polyps of the *Apc^{Min/+}* mouse model of intestinal tumorigenesis compared to surrounding tissues [23]. The two enzymes responsible for S1P catabolism, SPP and SPL, were also downregulated in human colon cancer tissues compared to uninvolved colonic tissue [23]. These findings indicate that S1P metabolism is altered in neoplastic tissue and that SPL and SPP could potentially function as anti-oncogenes [24]. In addition, SPL expression is downregulated in association with myc-mediated β -cell tumorigenesis in mice [25]. Significant downregulation of SPL was also observed in metastatic tumor tissues compared with primary tumors from the same patient, which suggests a role for SPL in tumor progression [26]. Conversely, SPL was found to be upregulated in ovarian cancer [27] and in ovarian tumors that were resistant to chemotherapy [28]. Whether SPL upregulation is a manifestation of tissue responses to elevated levels of S1P or is a causative factor remains to be determined.

3. S1P metabolism in relation to cell proliferation, transformation and conserved cell death pathways

A recent study demonstrated marked attenuation of tumor progression in murine xenograft and allograft models with the administration of a specific S1P-targeted monoclonal antibody that acts by interrupting S1P signaling [29]. This hallmark study illustrates the contribution of S1P signaling to tumorigenesis and the potential of targeting the S1P pathway for cancer treatment.

S1P acts as a mitogen and suppressor of apoptosis in most cell types [30,31]. S1P promoted cell growth and survival via extracellular signal-regulated kinase activation in human glioblastoma cells [32]. S1P upregulated myeloid cell leukemia-1 Mcl-1, an antiapoptotic member of the bcl-2 family, and protected the multiple myeloma cells and chronic myeloid leukemia cells from dexamethasone-induced apoptosis [33]. Although the mitogen effect of S1P in many tumors is well recognized [20], S1P has also been shown to exert an apoptotic effect in prostate cancer cell [34]. It has been speculated that the conversion of S1P to sphingosine by SPP or the accumulation of ceramide when the cells were stimulated with S1P may account for this finding. However, it is also possible that S1P may have anti-proliferative effects in certain cell populations. Intriguingly, S1P induced the expression of connective tissue growth factor CTGF, which possessed an anti-proliferative effect in Wilms tumor cells. The induction of CTGF is reported to be mediated by the S1P₂ receptor [35].

Some of the mitogenic and anti-apoptotic actions of S1P may be mediated independently of S1P receptors [4]. In the *Apc^{Min/+}* mouse model, deletion of Sphk1 attenuated tumor formation, whereas the deletion of S1P receptors did not [36]. Redundancy of receptor signaling could be responsible for this observation. Alternatively, these findings could represent a role for sphingosine-mediated growth inhibition or a receptor-independent effect of S1P on the regulation of adenoma growth. S1P has been shown to mobilize calcium from internal stores [5,37] and activate phospholipase D [38] independent of S1P₁ receptor expression [4]. In addition, S1P-induced focal adhesion kinase phosphorylation was not affected by suramin, an inhibitor of S1P receptor-ligand interactions [39]. S1P inhibited changes in mitochondrial membrane potential and prevented cytochrome c release from mitochondria [40]. S1P signaling has also been shown to downregulate Bax, prevent its translocation to mitochondria and inactivate Bad in response to Fas-induced apoptosis [41]. To date, the precise intracellular targets of S1P remain elusive, and the role, if any, of receptor-independent S1P actions in tumorigenesis is unclear.

Besides regulating cell proliferation, S1P is able to trigger autophagy in cancer cells, which may experience nutrient starvation and has to rely on autophagy for early development. In human prostate cancer PC 3 cells, S1P inhibits rapamycin signaling and induces autophagy through S1P₅ receptor [42]. Nutrient deprivation stimulates both autophagy and Sphk activity. Overexpression of Sphk1 in MCF-7 cells stimulates autophagy by increasing the formation of LC3-positive autophagosomes and the rate of proteolysis sensitive to the autophagy inhibitor 3-methyladenine. Conversely, knocking down Sphk1 diminish nutrient deprived autophagy, indicating Sphk/S1P induced autophagy and prevent cell death from apoptosis [43].

Two isotypes of Spk (Sphk1 and Sphk2) have been identified. There is strong evidence supporting the importance of Sphk1 in tumorigenesis, whereas the role of Sphk2 in this process remains to be determined [44]. Sphk1 is activated to produce S1P by a variety of growth factors (epidermal growth factor, EGF and insulin-like growth factor-1, IGF-1), hormones (estradiol) and angiogenic factors (vascular endothelial growth factor, VEGF) implicated in mediating cancer progression [2]. Overexpression of Sphk1 in mouse fibroblasts resulted in H-Ras-mediated cellular transformation and tumor formation in nude mice, thereby revealing the oncogenic role of Sphk1 [13]. Promotion of tumor growth was also observed in mice injected with prostate cancer cells overexpressing Sphk1, whereas, downregulating Sphk1 resulted in apoptosis and reduced cell growth [45,46]. Overexpression of Sphk1 in MCF-7 breast cancer cells elevated S1P levels, stimulated estrogen-dependent cell proliferation, promoted breast cancer growth in soft agar and enhanced tumorigenesis in mice [47], whereas silencing of Sphk1 resulted in growth arrest and apoptosis [48]. In *Apc^{Min/+}* mice, knockdown of Sphk1 resulted in reduction of adenoma size and attenuation of epithelial cell proliferation in the polyps, suggesting that Sphk1 plays a role in tumor progression [36]. Similarly, knockdown of Sphk1 in mice treated with azoxymethane led to reduction of crypt foci formation, indicating

the importance of Sphk1/S1P pathway in colon carcinogenesis [16]. Importantly, Sphk inhibitors have been shown to reduce gastric, lung, mammary adenocarcinoma tumor growth in mice [14,19,49].

Sphk1 appears to be crucial for human breast cancer cell growth in vitro, as inhibition of Sphk1 suppressed EGF stimulated growth and enhanced sensitivity to chemotherapeutic agents [50]. Estrogen signaling was shown to be mediated through Sphk activation in human breast cancer cells [51]. In addition, elevated S1P levels resulting from S1PP1 inhibition or Sphk1 overexpression endowed MCF-7 cells with protection against cell death induced by sphingosine and TNF α [47,52]. Similarly, overexpression of Sphk1 in HL-60 acute myeloid leukemia cells led to reduction of etoposide-induced apoptosis [53], and stimulation of Sphk1 by vitamin D3 resulted in increased HL-60 cell survival [54]. Prosaposin treatment, which activates Sphk1 via ERK1/2, increased cell proliferation and prevented apoptosis in PC12 pheochromocytoma cells [55]. Moreover, overexpressing Sphk1 in these cells resulted in increased resistance to ceramide-mediated apoptosis [56]. Similar observation was found in human melanoma cells [57]. Both expression and activity of Sphk1 were activated by the antiapoptotic protein Bcl-2 in the melanoma cells [57], linking Sphk1 with anti-apoptosis. Consistent with its oncogenic role, activation of Sphk by VEGF in T24 bladder-tumor cells increased DNA synthesis [58]. Inhibition of Sphk1 suppressed cell growth, enhanced apoptosis and accompanied with decreased S1P and increased ceramide levels in PC3 prostate cancer cells [45,46]. These observations indicate that Sphk1's role in mediating survival and blocking cell death pathways is common to numerous malignant cell types. Although Sphk1 appears to be the primary enzyme responsible for mediating the oncogenic effect of S1P in cancer, a supportive role of Sphk2 in tumor progression was recently reported. A substantial decrease in the growth of Sphk2-deficient MCF-7 breast tumor xenografts was observed when compared with control cells [59].

SPL, which degrades S1P irreversibly, plays an important role in modulating intracellular S1P levels. In HEK293 cells, an apoptotic effect of SPL was demonstrated via the action of p53 and p38 tumor-suppressor signaling, whereas cellular depletion of SPL led to diminished apoptosis [24]. A study employing insertional mutagenesis in *Dictyostelium* demonstrated that mutation in the SPL-encoding gene *sglA* confer resistance to cisplatin [60]. In contrast, overexpression of the SPL gene resulted in enhanced sensitivity to chemotherapy drugs [61]. Consistent with these findings, SPL has been shown to sensitize mammalian cells to chemotherapeutic agents and DNA damage [62]. Despite these finding with SPL and the observed alteration of SPL in tumors, a direct role for SPL in mediating tumor suppression remains to be determined.

4. SphK/S1P on migration, invasion and metastasis

Cell migration and invasion are the initial critical steps in tumor metastasis. The effects of S1P on cell movement appear to be receptor-dependent events that are influenced by the subtypes of receptors expressed on the cell surface. In many cell types, activation of S1P₁ receptor by S1P exerts a pro-migratory effect, whereas activation of S1P₂ receptor endows the cells with an inhibitory effect on cell migration.

Urokinase plasminogen activator uPA and its receptor uPAR stimulate invasiveness of glioblastoma multiforme cells. The basal expression of uPA and uPAR was suppressed by inhibition of Sphk. S1P and overexpression of S1P₁ led to the dramatic induction of the uPA system, suggesting that S1P/S1P₁ signaling enhances invasion of glioblastoma multiforme [63]. Other studies in human glioblastoma cells support the notion of a pro-migratory effect of S1P [64,65]. In addition, S1P was reported to activate plasminogen activator inhibitor-1 PAI-1, which contributes to the invasiveness of glioblastoma cells [66]. However, profound and

differential S1P effects on glioblastoma cell migration were observed. S1P exerts anti-migratory effect on glioblastoma cells which predominately expressed S1P₂ receptor. Activation of RhoA/Rho-kinase was implicated in the anti-migratory effect of S1P/S1P₂ receptor signaling [65].

These observations were consistent with studies employing B16 mouse melanoma cells, which expressed only the S1P₂ receptor [67,68]. In this cell model, S1P negatively regulated cell migration and invasion through inhibition of Rac, which mediates signaling events contributing to the protrusion of lamellipodia and forward movement, and activation of Rho, which mediates stress fiber formation and focal adhesion. When S1P₁ receptor was overexpressed in these cells, cell migration was stimulated. These findings demonstrate that S1P positively and negatively regulates cell migration in a receptor subtype-dependent manner. The implications of this finding for malignant cell invasion and metastasis was demonstrated when B16 cells treated with S1P were injected into mouse of hematogenous metastasis model, wherein S1P inhibited lung metastasis three weeks after injection. Importantly, overexpression of S1P₂ receptor potentiated the inhibition, whereas overexpression of S1P₁ receptor potentiated metastasis [68].

S1P₃ receptor was also shown to play a role in cancer cells migration. S1P₃ receptor demonstrated a positive role in the ML-1 thyroid carcinoma cell migration. In this model system, overexpression of Sphk1 increased S1P generation and exerted a pro-migratory effect through both S1P₁ and S1P₃ receptors [69]. Migration of gastric cancer cells lines that overexpressed the S1P₃ receptor was stimulated by S1P. Conversely, S1P inhibited migration of cells that exclusively expressed the S1P₂ receptor. Thus, the balance between S1P receptor subtypes appears to be an important determinant in the metastatic response to S1P [70]. Study in Wilms tumor cells also suggested that the relative ratio of S1P receptor subtypes determines the ability of the tumor cells to migrate toward S1P [71]. In these cells, the S1P₁ receptor mediates cell migration and invasion via downstream PI3K and Rac1 activation.

S1P was also found to stimulate ovarian cancer cell invasion in a receptor-dependent fashion involving activation of ERK, AKT and p38 [72].

Sphk may also play a role in cancer cells migration. In pancreatic carcinoma cells, overexpression of the tumor suppressor KAI led to reduction of SphK1 activity and enhanced migration [73]. Sphk1 activation by EGF was shown to be required for EGF-directed cell migration and metastasis in breast cancer cells [50]. EGF also activated Sphk2, and inhibition of Sphk2 diminished cell migration toward EGF [74,75].

5. Sphk/S1P in angiogenesis

Angiogenesis is pivotal for solid tumor progression. Cancer cells undergo adaptive changes that stimulate angiogenesis to facilitate oxygenation, enabling cancer cells to proliferate and survive in the anoxic tumor environment. The S1P/S1P₁ receptor pathway has been clearly implicated in the promotion of vascular development in vivo [1,29,76–78]. The essential function of S1P₁ receptor is further demonstrated by impaired vascular maturation observed in S1P₁ receptor knockout mice. These mice exert embryonic hemorrhage and subsequently intrauterine death due to incomplete coverage of blood vessels by smooth muscle cells [79]. S1P signaling plays a key role in blood vessel formation by stimulating the proliferation and migration of endothelial cells. S1P prevents apoptosis of endothelial cells in response to serum deprivation and TNF α [80]. VEGF signaling is crucial for angiogenesis, as this pathway controls proliferation, migration, adhesion and assembly of both vascular endothelial cells and vascular smooth muscle cells. S1P/S1P₁ receptor signaling and VEGF signaling collaborate in promoting angiogenesis. Inhibition of Sphk decreased VEGF-induced expression of adhesion molecules [81]. In addition, S1P₁ receptor expression was induced by VEGF in

endothelial cells [82]. S1P together with VEGF or other pro-angiogenic factors produced synergistic enhancement of vascular sprouting and neo-vascularization in an ex vivo model of angiogenesis [83]. The mechanism of S1P-mediated neo-vascularization involved activation of S1P receptors and downstream regulation of the Rho family of small GTPases, which regulate cell motility [84,85]. The membrane type-1 matrix metalloproteinase MT1-MMP has been shown to promote blood vessels sprouting in the rat aortic ring assay [86]. Importantly, MT1-MMP is activated by S1P to induce endothelial cell migration and morphogenic differentiation [87].

The pathophysiological role of S1P signaling in tumor angiogenesis is supported by several studies. S1P₁ receptor expression is strongly induced in tumor vessels, and injection of S1P₁ receptor siRNA into murine tumors dramatically decreased vascular stabilization, angiogenesis and tumor growth in vivo [77]. The neutralization of S1P with anti-S1P monoclonal antibodies inhibited VEGF- and basic fibroblast growth factor, bFGF-induced blood vessel formation, resulting in the arrest of tumor-associated angiogenesis in murine xenograft and allograft models. S1P antibodies also blocked endothelial cell migration [29]. These findings demonstrate that S1P signaling contributes to the pathological angiogenesis needed to support tumor progression, and further that some of the anti-tumor effects of S1P signaling blockade are likely mediated by inhibiting tumor angiogenesis.

6. S1P in hypoxia

It has been estimated that hypoxic conditions exist in up to 50–60% of solid tumors as a result of imbalance between oxygen supply and demand from continuously growing tumors [88]. This common characteristic of solid tumors triggers signaling pathways that promote angiogenesis, tumor growth, metastasis as well as resistance to cytotoxic therapy and DNA damage. Consequently, these adaptive changes enable cancer cells to survive and proliferate. HIF-1 is the recognized master regulator of adaptation to hypoxia [89]. Under well-oxygenated conditions, HIF-1 is hydroxylated, resulting in recognition and binding by the von Hippel-Lindau tumor suppressor gene product (pVHL). Following binding, polyubiquitylation and degradation of HIF-1 occur. Under hypoxic condition, unhydroxylated HIF-1 accumulates, translocates to the nucleus and bind to hypoxia response elements (HREs) located in the promoter region of its numerous target genes, such as VEGF, thereby regulating angiogenesis. Recently Sphk1 was found to modulate HIF-1 α accumulation under low oxygen tension in human cancer cells [90]. During hypoxia, Sphk1 activity is stimulated in a reactive oxygen species (ROS)-dependent manner. The generation of S1P then activates the Akt/glycogen synthase kinase-3 β signaling (AKT/GSK3 β), which favors the nuclear translocation of HIF-1 α . In U87 glioma cells, Sphk1 was found to be upregulated in response to cobalt chloride-induced hypoxia in a HIF-2 α -dependent manner. HIF-2 α was shown to bind to the Sphk1 promoter. In contrast, when HIF-1 α was inhibited, both HIF-2 α and Sphk1 were increased [91].

The S1P₂ receptor was shown to be induced in hypoxia-triggered pathological angiogenesis of the mouse retina. Interestingly, pathologic neo-vascularization was suppressed in the S1P₂^{-/-} mice subjected to ischemia-driven retinopathy, suggesting that the S1P₂ receptor is essential for endothelial cell responses to hypoxia [92]. In A549 lung cancer cells, hypoxia was found to activate Sphk2, promoting S1P generation. The binding of S1P to S1P₁/S1P₃ receptors protected the cancer cells from etoposide-induced cell death through activation of p42/44 mitogen-activated protein kinase [93]. These findings implicate S1P signaling in mediating hypoxia-related molecular changes that allow cancer cells to adapt to hypoxic tumor environments.

7. Targeting S1P signaling and metabolism in cancer therapy

S1P mediates a diverse array of signaling pathways, impacting fundamental biological processes that are integral to cancer pathogenesis (Fig 2). Understandably, the S1P signaling pathway has been the subject of interest in potential anti-cancer therapeutics. Currently, a number of pharmaceutical companies have established major programs investigating the potential of targeting S1P signaling for cancer treatment.

Some strategies for inhibiting S1P signaling in cancer focus on modulating S1P receptor signaling. The S1P agonist FTY720 (fingolimod) bears structural similarity to S1P and is an immunomodulatory drug that binds to four of five S1P receptors (S1P₁, S1P₃, S1P₄, S1P₅). However, its binding induces internalization and degradation of the S1P₁ receptor, resulting in prolonged downregulation of the receptor. Administration of FTY720 to mice harboring human hepatocellular and bladder cancer xenografts prevented their outgrowth [94,95]. FTY720 also inhibited tumor development in a carcinogen-induced model of lung cancer and murine breast cancer models [96–98]. In addition, FTY720 inhibits angiogenesis and tumor vascularization and mediates tumor cell death [99]. A nonselective competitive antagonist at both S1P₁ and S1P₃ receptors, VPC23019, had been shown to suppress S1P-induced migration of thyroid cancer cells, ovarian cancer cells and neural cells [72,100,101].

Novel strategies neutralizing S1P with the anti S1P monoclonal antibody may also prove successful in cancer therapeutic. LT1002 and LT1009, antibodies against mouse and human S1P were generated and were shown to block S1P-mediated release of the pro-angiogenic and pro-metastatic cytokine from human ovarian cancer cells in vitro. Further, anti-angiogenic activity of the antibodies was demonstrated in vivo. LT1009 has been formulated for Phase 1 clinical trials, which will be important to demonstrate the potential therapeutic use in humans [102].

Sphk inhibitors represent another strategy for inhibiting S1P signaling in cancer. Dimethylsphingosine (DMS), an N-methylated metabolite of sphingosine, inhibits both Sphk1 and Sphk2. DMS has been used extensively in vitro to examine the effects of blocking S1P production in tumor cells and has also been employed in several preclinical trials using animal models. For example DMS was shown to inhibit the growth of lung and gastric cancer cells in athymic mice [103] and to decrease lung metastasis of melanoma cells in rodent models [104]. Another Sphk inhibitor, SKI-I (BML-248), which is a specific inhibitor of Sphk1 was shown to reduced growth and survival of human leukemia and Jurkat cells and decreased growth of xenograft tumors [105]. SKI-II, which is orally bioavailable, also exhibited in vivo antitumor activity [106]. These sphingosine kinase inhibitors demonstrating antitumor activity showed potential as therapeutic treatments in cancer therapy.

8. Potential roles of SPL in other pathological diseases

Considering S1P's role in autophagy, apoptosis, cell migration and stress responses, it is not surprising that SPL has been found to play a role in other disease states such as neurodegenerative disease, vascular disease and wound healing. In addition, S1P's recently discovered role as a regulator of immune cell trafficking has now uncovered a role for SPL as a target of immunomodulation.

The immunomodulatory drug, FTY720, which deprives thymocytes and lymphocytes of an S1P signal that stimulates their egress from thymus and secondary lymphoid tissues [107], had been shown to inhibit SPL activity, suggesting that SPL inhibition may contribute to the immunological effects of FTY720 [108]. Inhibition of SPL activity by a food colorant tetrahydroxybutylimidazole (THI), increases S1P levels in the thymus and secondary lymphoid organs without changing plasma S1P levels, therefore, disrupts the S1P gradient, and

subsequently prevents lymphocyte egress from thymus and secondary lymphoid organs [109]. The important role of SPL in lymphocyte trafficking was further confirmed by the lymphopenia observed in the genetically modified mice lacking SPL [110]. In addition to lymphocyte egress, genetic ablation of SPL in mice hampers B and T cell development. SPL knockout mice showed severe hypocellularity and increased apoptosis of lymphocytes within the cortex. Thus, the immune system is exquisitely sensitive to alterations in SPL activity [110].

Altered sphingolipid metabolism has been reported in Alzheimer's disease patients and reduction in brain S1P levels shifts sphingolipid signaling in the direction of cell death [111, 112]. SPL expression was upregulated in the Alzheimer's disease patients and increased SPL expression was correlated with clinical dementia progression [111]. In addition, two single nucleotide polymorphisms in the SPL gene confer susceptibility to late-onset Alzheimer's disease were described [113]. These findings suggest that SPL might hold a potential role in the progression of neurodegenerative diseases such as Alzheimer's disease.

S1P signaling is critical for vascular maturation. Both inhibition and overexpression of SPL in endothelial cells directly affects intracellular S1P levels, resulting in S1P release into the extracellular compartments. Moreover, laminar shear stress downregulated the expression of SPL and S1P phosphatase in vascular endothelial cells. Together, these findings suggest a role of SPL in maintenance of vascular homeostasis [114].

Delay wound healing is often seen with diabetes due to impair neo-vascularization, migration and proliferation of endothelial cells, keratinocytes and fibroblasts [115], resulting in malformation of the extra cellular matrix. Treatment of S1P enhances wound healing in the diabetic mice [116]. Moreover, S1P₂ receptor has been shown to trigger wound healing in response to acute liver injury [117]. Embryonic fibroblasts from SPL knockout mice demonstrated defects of migration *in vitro*, suggesting that SPL might have a potential role in S1P mediated wound healing.

SPL knockout mice exert a reduced life span and developed lesions in the lung, heart, urinary tract and bone. The reduced life span is believed to be due to respiratory failure. Lesions in lungs and alveoli contain smooth homogeneous material accompanied by increased numbers of alveolar macrophages. And cardiac lesions were characterized by expansion of the interstitium by vacuoles and vacuolated mesenchymal cells that separated the cardiomyocytes. Although the precise role of SPL in lung and cardiac function remain unclear, yet these findings suggest that SPL expression is important for normal physiology in various tissues [110].

9. Conclusion

An increasingly more comprehensive picture of S1P signaling and its role in cancer biology have developed over the past decade. Substantial evidence supports the role of S1P, enzymes maintaining its homeostasis, and S1P receptors in cancer biology. Nevertheless, more studies are needed to fully elucidate the many ways in which S1P signaling impacts tumor initiation, progression, metastatic spread and the development of patterns of drug resistance. Still undetermined are potential intracellular targets of S1P, alterations in the regulation and function of S1P receptors subtypes in cancer cells, potential alterations in S1P transport in cancer, and a defined role for SPL and SPP as tumor suppressors. A more detailed picture of the molecular events contributing to altered S1P signaling in the cancer cell combined with improvements in selective modulation of S1P receptors and enzymes of S1P metabolism are likely to contribute to the development of more efficient novel therapeutic strategies targeting this pathway for therapeutic benefit in the clinical management of cancer.

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Figure 1.

S1P homeostasis is tightly regulated by the balance between its synthesis and degradation via three enzymes. (1) sphingosine kinase (Sphk), which generates S1P through phosphorylation of its precursor, (2) S1P phosphatases (SPP1 and SPP2), which reversibly convert S1P back to sphingosine, and (3) S1P lyase (SPL), which irreversibly degrades S1P to generate ethanolamine phosphate and hexadecenal, representing the last step in the sphingolipid degradation pathway.

Figure 2.

A range of growth factors, hormones, angiogenic factor and other stimuli activate Sphk by phosphorylation, resulting in translocation of Sphk from cytosol to the plasma membrane. S1P is then generated and act intracellularly or exported out of the cells to engage with its receptors (S1P₁₋₅) to mediate mediates a diverse array of signaling pathways, impacting fundamental biological processes that are integral to cancer pathogenesis.