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Targeting Sphingosine-1-Phosphate in Hematologic Malignancies

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Abstract

Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid mediator that regulates several processes important for hematologic cancer progression. S1P is generated by two sphingosine kinases, SphK1 and SphK2, and is exported outside the cell, where it activates specific cell surface S1P G-protein coupled receptors in autocrine/paracrine manner, coined “inside-out signaling”. In this review, we highlight the importance of SphK1 and inside-out signaling by S1P in hematologic malignancy. We also summarize the results of studies targeting the SphK1/S1P/S1P receptor axis and the effects of the S1P receptor modulator, FTY720, in hematologic malignancy.

Keywords

sphingosine-1-phosphate; sphingosine kinase; sphingosine-1-phosphate receptors; leukemia; lymphoma; hematologic malignancies

INTRODUCTION

Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid mediator that regulates numerous processes important for cancer progression including cell migration, growth, survival, and angiogenesis to name just a few [1, 2]. In contrast to S1P, which has been associated with cell growth and survival, its precursors, sphingosine and ceramide, have been shown to play a role in growth arrest and apoptosis. The balance between these counter-acting sphingolipid metabolites has been termed the “sphingolipid rheostat” and has been implicated in cell fate determination [3].

S1P is produced from sphingosine by sphingosine kinases, of which there are two isoenzymes (SphK1 and SphK2). Many growth factors, hormones, and cytokines stimulate SphK1 inducing its translocation to the plasma membrane leading to restricted formation of S1P which then can be exported by ATP-binding cassette transporters and subsequently activates its specific receptors in an autocrine/paracrine manner, defined as “inside-out signaling” [4]. S1P signals through five S1P specific G protein-coupled receptors (GPCR), known as S1P_{1–5} to exert many of its pleiotropic effects. The specific repertoire of S1P receptors that is expressed, together with their differential coupling to various heterotrimeric G-proteins that regulate numerous downstream signaling pathways, is responsible for the

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ability of S1P to regulate diverse physiological processes in a highly specific manner. A recent study demonstrated that STAT3 induces S1P₁ expression and activation of S1P₁ reciprocally regulates STAT3 activity, thus acting as a major positive feedback loop for persistent STAT3 activation in cancer cells and the tumor microenvironment for malignant progression [5].

S1P produced by SphK1 also has recently been shown to have direct intracellular actions. S1P binds to TRAF2 and is a required cofactor for its E3 ubiquitin ligase activity. This leads to lysine 63-linked polyubiquitination of RIP1, a critical event in NF- κ B activation and provides a mechanistic explanation for the numerous observations of the importance of SphK1 in inflammatory, anti-apoptotic and immune processes [6].

Elevated expression of SphK1 has been observed in multiple types of cancer, including acute leukemia [7, 8]. Upregulation of SphK1 is associated with the tumorigenic phenotype of erythroleukemia, and overexpression of SphK1 in non-tumorigenic pro-erythroblasts increased their clonogenicity as well as resistance to apoptosis. They also acquired tumorigenicity when engrafted *in vivo* [9]. These results suggest that high expression of SphK1 may be an oncogenic event required for progression of erythroleukemia.

In contrast to overexpression of SphK1, SphK2 overexpression suppresses cell proliferation [10]. Moreover, whereas expression of SphK1 reduces ceramide level, SphK2 increases it [11]. SphK1 and SphK2 have distinct and sometimes overlapping functions in cells, unique localizations depending on cell type, and the cellular location of S1P production may determine its actions. In many cancer cells SphK2 is localized to the nucleus where it is associated with the histone deacetylases, HDAC1 and HDAC2, in repressor complexes and inhibits their enzymatic activity, preventing the removal of acetyl groups from lysine residues within histone tails. This results in enhanced transcription of specific genes, such as the cyclin-dependent kinase inhibitor *p21* or the transcriptional regulator *c-fos*. This links nuclear S1P and sphingolipid metabolism in the nucleus to epigenetic regulation of gene expression [12]. Vorinostat, an HDAC inhibitor, was recently approved for the treatment of cutaneous T-cell lymphoma by US Food and Drug Administration [13]. Understanding how S1P regulates HDAC activity *in vivo* may help in the development of new therapeutics that interfere with HDAC functions in a highly specific manner.

This review summarizes research to date on the involvement and mechanisms of action of S1P and the kinases that produce it in growth and progression of the hematologic malignancies leukemia and lymphoma. We will also discuss potential new options for therapy that target S1P signaling and function in these malignancies.

ROLE OF SPHK1, S1P, AND ITS RECEPTORS IN HEMATOLOGIC MALIGNANCIES

Abundant evidence indicates that S1P stimulates growth and survival of leukemia and lymphoma cells [3, 14, 15]. The initial observation in this field was that S1P stimulates the extracellular signal-regulated kinase (ERK)/MAPK pathway and counteracts ceramide-induced activation of stress-activated protein kinase (SAPK/JNK), resulting in decreased apoptosis [3]. S1P has been shown to prevent apoptosis in several human leukemia cells lines, including U937 and HL-60 cells, counteracting activation of caspases likely by inhibiting release of cytochrome c and Smac/DIABLO from mitochondria to the cytosol [15]. Furthermore, inhibition of SphKs induced ceramide accumulation, decreased S1P, and caused apoptosis equally in chemosensitive and chemoresistant cell lines that was reversed by exogenous treatment with S1P or by overexpression of SphK1 [16]. S1P mobilized intracellular calcium in the human leukemic cell line, U937, which was required for

activation of NF- κ B, a transcription factor important for their survival [14]. In agreement, SphK1 and NF- κ B were found to be essential for the long-term survival of cytotoxic T lymphocytes in T cell large granular lymphocyte leukemia which features clonal expansion of antigen-primed competent cytotoxic T lymphocytes [17]. The *Runx* genes (*Runx1*, 2, and 3) regulate cell fate in development and can be oncogenic. Transgenic mice expressing *Runx* together with *Myc* develop lymphoma. The oncogenic potential of *Runx* was linked to key enzymes of sphingolipid metabolism (S1P phosphatase 1, UDP-glucose ceramide glycosyltransferase, and GM3 synthase) as direct targets for *Runx* transcriptional regulation in a manner consistent with survival and apoptosis resistance. The survival advantage conferred by ectopic *Runx* could be partially recapitulated by exogenous S1P and was accompanied by reduced phosphorylation of p38 MAPK [18]. In multiple myeloma cells, SphK1 is stimulated by IL-6, which influences their growth and survival. Moreover, SphK1 is involved in IL-6-mediated upregulation of myeloid cell leukaemia-1 (*Mcl-1*), leading to increased cell proliferation and survival [19], suggesting that SphK1 may contribute to this type of leukemia.

Elevation of S1P and SphK1 is an important factor that determines resistance to chemotherapy. It was reported that SphK1 expression was increased in Bcr-Abl-overexpressing leukemic cells, [20, 21]. Imatinib, a tyrosine kinase inhibitor, has been used in the treatment of certain leukemias because it blocks the formation of Bcr-Abl, which is crucial in the development of some types of leukemia. Imatinib has been shown to induce apoptosis in K562 cells, a human myelogenous leukemia cell line. Expression of SphK1 and generation of S1P were found to be increased significantly in Imatinib-resistant K562 cells [20]. Partial inhibition of SphK1 by siRNA reduced S1P levels and increased sensitivity to Imatinib-induced apoptosis in the resistant cells. Forced expression of SphK1 increased the ratio of S1P to C18-ceramide about six-fold, and prevented apoptosis significantly in response to Imatinib. This implies a role for SphK1 and S1P in the upregulation of Bcr-Abl expression at the post-transcriptional level, suggesting a mechanism for resistance to Imatinib-mediated apoptosis [20]. Similarly, it was shown that apoptosis of Imatinib-sensitive and resistant primary cells from chronic myeloid leukemia patients was induced by an inhibitor of SphK1 [22]. This study also substantiated the involvement of SphK1 in regulating Imatinib-induced apoptosis and established SphK1 as a downstream effector of the Bcr-Abl/Ras/ERK pathway inhibited by Imatinib but an upstream regulator of Bcl-2 family members [22].

Despite evidence that inside-out signaling by S1P plays an important role in cancer progression, much less is known of the importance of specific S1P receptors in hematological malignancies. T-lymphoma cell invasion is regulated by binding of S1P to its cell surface receptors that activates RhoA, phospholipase C and calcium signaling pathways leading to pseudopod formation and enhanced infiltration [23]. Yet the S1P receptors involved have not been identified. Multiple myeloma cells have been shown to express S1P₁, S1P₂, and S1P₃. S1P, which protects multiple myeloma cells from apoptosis, upregulates *Mcl-1*, an anti-apoptotic protein that is a member of the Bcl-2 family [24]. Because this effect was blocked by pertussis toxin, which inhibits Gi protein signaling, it was suggested that one of these S1P receptors was involved [24]. More work is needed to unravel the importance of the S1P receptors in leukemia.

Interestingly, S1P₅ receptor was identified as one of the differentially expressed genes in large granular lymphocyte leukemia [25]. This type of leukemia is often associated with autoimmune disease and is characterized by dysregulation of apoptosis. It is possible that this alteration in the apoptosis pathway may be due to activation of S1P₅ receptors by S1P; however, this hypothesis has yet to be explored. Microarray-based immunohistochemistry with tissue samples from mantle cell lymphoma patients demonstrated that S1P₁ was

expressed on the surface of mantle cell lymphoma cells. PCR analysis of mantle cell lymphoma lines confirmed that S1P₁ expression was upregulated [26]. Unfortunately, the role of S1P₁ in mantle cell lymphoma has not been established yet. T-cell precursor malignancies, T-lymphoblastic lymphoma (T-LBL) and acute T-lymphoblastic leukemia (T-ALL), are known to be closely related, but it was not known why T-LBL remains highly localized with accumulation of those cells in mediastinal masses in some patients while disseminating rapidly as T-ALL in others. Based on a zebrafish model, it was recently suggested that expression of S1P₁ in T-LBL cells may prevent their progression to T-ALL [27]. Feng et al reported that expression of S1P₁ and its putative target ICAM-1 (Inter-Cellular Adhesion Molecule 1), which binds integrin, is higher in human T-LBL cells than in human T-ALL cells. They suggested that this T-LBL phenotype promotes homotypic cell-cell adhesion and blocks intravasation. Inhibition of S1P₁ signaling in T-LBL cells led to decreased homotypic adhesion in vitro and increased tumor cell intravasation in vivo [27]. Thus, it seems that blockade of intravasation and hematologic dissemination in T-LBL may be due to elevated S1P₁ signaling and increased expression of ICAM1 that augments homotypic cell-cell adhesion [27].

Interestingly S1P₂ null mice develop germinal center-derived diffuse large B-cell lymphomas upon ageing, resulting in about half of all animals having this neoplasm by 1.5 to 2 years. Using histologic, immunophenotypic, and molecular analyses, a uniform tumor phenotype was noted with features of germinal center (GC)-derived diffuse large B-cell lymphoma (DLBCL). Tumor formation was preceded by increases in GC B cells, CD69 positive T cells, and increased formation of spontaneous GCs. Moreover, 26% of patients with DLBCL had multiple somatic mutations in the 5' sequences of the *s1p2* gene. This work suggests that S1P₂ signaling may play a critical role in suppression of diffuse large B-cell lymphomas derived from GCs [28].

SPHK1 TARGETED THERAPIES

Based upon increasing evidence that indicates a significant role for SphK1 and S1P in growth and survival of leukemia and lymphoma cells, it is not surprising that they have been the focus of some investigations as a target for therapy. N,N-Dimethylsphingosine (DMS), a pan-sphingosine kinase inhibitor that inhibits both SphK1 and SphK2, was shown to sensitize Jurkat, U937, and HL-60 cells to apoptosis triggered by ceramide, TNF- α , or serum deprivation [15, 29]. A caveat of this work is that DMS also inhibits protein kinase C. However, activation of protein kinase C itself leads to increased S1P levels and in some cells, DMS inhibits only SphKs and not protein kinase C [29]. Therefore, it is still possible that sensitization of cells to apoptosis by DMS is mediated by inhibition of SphKs. Because DMS is a toxic compound with very low aqueous solubility, its application as a therapeutic is severely limited. A related compound, N,N-dimethylphytosphingosine, was shown to inhibit sphingosine kinase activity in vitro [30]. N,N-Dimethylphytosphingosine decreased S1P by inhibiting SphK activity and induced caspase-dependent apoptosis of human leukemia cells which was dependent on increased reactive oxygen species [30].

F-12509a is a structurally complicated natural product inhibitor of SphK1 and SphK2 that was isolated from a culture broth of a discomycete [31]. F-12509a induced apoptosis and enhanced susceptibility to Imatinib of both sensitive and resistant chronic myeloid leukemia cells [22]. Moreover, treatment of human leukemia HL-60 cells with F-12509a, decreased S1P, increased ceramide accumulation, and caused apoptosis in both chemo-sensitive and multi-drug resistant lines [16]. As expression of multidrug resistant proteins is among the strongest prognostic factors in acute myelogenous leukemia, SphK inhibitors by overcoming apoptosis resistance and restoring sensitivity to chemotherapeutics, regardless of multidrug resistant status, may have potential for clinical development [16].

Another pan SphK inhibitor, 2-(p-hydroxyanilino)-4-(p-chlorophenyl)thiazole, which is a non-ATP competitive inhibitor and thus expected to be more specific for SphKs, was used to assess the importance of S1P formation in daunorubicin sensitive and resistant leukemia cell lines [7]. This study showed that daunorubicin decreased S1P more in the sensitive cell lines than the resistant ones with corresponding inverse effects on ceramide levels. Furthermore, this SphK inhibitor also restored daunorubicin sensitivity in the resistant leukemia cell lines suggesting that combination of the SphK inhibitor and an anti-cancer drug such as daunorubicin might also be useful for future clinical development [7]. 4-[[4-(4-chlorophenyl)-2-thiazoyl]amino]phenol, called SKI-II, which inhibits both SphK1 and SphK2, significantly induced apoptosis of T cell large granular lymphocytic leukemia cells in a dose-dependent manner but not of normal peripheral blood mononuclear cells [17]. A specific SphK2 inhibitor has been shown to inhibit growth of several types of human tumor xenografts [32–34], but its effects in leukemia have not yet been examined.

An issue that has hindered further development of SphK inhibitors is that their specificity has not been unequivocally established, and many inhibit both SphK1 and SphK2. As discussed, these two isoenzymes are distinctively different in localization and function; therefore, a pharmacological inhibitor that specifically inhibits one of the kinases was needed. (2R,3S,4E)-N-methyl-5-(4'-pentyphenyl)-2-aminopent-4-ene-1,3-diol, designated SK1-I, a potent, water soluble, isozyme-specific inhibitor of SphK1, was shown to decrease growth and survival of U937 and Jurkat cells and enhance apoptosis and cleavage of Bcl-2 [35]. SK1-I decreased S1P and concomitantly increased levels of its pro-apoptotic precursor ceramide. SK1-I also induced multiple perturbations in activation of signaling and survival-related proteins, including diminished phosphorylation of ERK1/2 and Akt. The importance of Akt survival signaling was confirmed by overexpression of constitutively active Akt which protected against SK1-I-induced apoptosis. Remarkably, SK1-I potently induced apoptosis in leukemic blasts isolated from patients with acute myelogenous leukemia but had little effects on normal peripheral blood mononuclear leukocytes. Moreover, SK1-I markedly reduced growth of AML xenograft tumors [35]. Taken together, these studies suggest that specific inhibitors of SphK1 warrant attention as potential additions to the therapeutic armamentarium in leukemia.

TARGETING S1P RECEPTORS WITH FTY720

FTY720, also known as fingolimod, is a fungal metabolite with immunosuppressive properties that has been approved by the FDA for treatment of multiple sclerosis [36]. It is a structural analogue of sphingosine and a prodrug, which is phosphorylated *in vivo* by SphK2 to phospho-FTY720, a S1P mimetic that binds to all of the S1P receptors except S1P₂. FTY720 sequesters lymphocytes in lymph nodes by inducing internalization and degradation of S1P₁, whose expression is crucial for their egress [37]. Hence, phospho-FTY720 is a “functional antagonist” of S1P₁. There is no doubt that downregulation of specific S1P receptors is one of the major actions of this pro-drug. A number of reports have surfaced indicating that FTY720 reduces growth and induces apoptosis of tumor cells in culture and also reduces xenograft tumor growth. For example, proliferation of HL-60 and U937 leukemia cells was suppressed by FTY720 in a dose-dependent manner and activation of ERK1/2 was reduced as well as its translocation to nucleus during FTY720-induced apoptosis [38]. FTY720 was also shown to induce apoptosis of T-cell large granular lymphocyte leukemia cells and sensitize them to Fas-mediated apoptotic cell death [39]. FTY 720 has also been shown to mediate cytotoxic effects in different B-cell malignancies independent of activation of caspases [40]. Moreover, in CLL B-cells, FTY720 also induced downregulation of Mcl-1 but not Bcl-2. However, overexpression of Bcl-2 failed to protect these transformed B-cells from FTY720-induced apoptosis, suggesting a Bcl-2-independent mechanism. FTY720 treatment resulted in significantly prolonged survival in a xenograft

SCID mouse model of disseminated B-cell lymphoma/leukemia [40], revealing a potential use of FTY720 as a therapeutic agent in B-cell malignancies.

FTY720 may also have intracellular actions that are independent of the S1P receptors [41–44]. Several studies have investigated the mechanism by which FTY720 induces apoptosis and cell cycle arrest. Apoptosis of Jurkat T cells was triggered by intracellular accumulation of the phosphorylated compound acting through an yet unidentified intracellular target [42]. This led to the suggestion that sphingoid base substrates for SphK2 acting intracellularly could be useful in the treatment of lymphoproliferative diseases [42]. FTY720 induced dephosphorylation of Akt in Jurkat T cells and in human B-cell leukemia cells. In these cells, okadaic acid suppressed FTY720-induced dephosphorylation of Akt, suggesting that FTY720 promotes phosphoserine/phosphothreonine protein phosphatase activity [41]. Indeed, FTY720 was found to activate purified PP2A. This suggests that FTY720 activates PP2A phosphatase which dephosphorylates Akt, thus inhibiting this survival pathway resulting in the enhancement of apoptosis via the mitochondrial apoptotic pathway [41]. It also potently inhibited blast crisis chronic myelogenous leukemia and Philadelphia chromosome–positive acute lymphocytic leukemia cells from two fatal BCR-ABL leukemias (against which Abl kinase inhibitors fail to induce a long-term response) by impairing BCR-ABL activity and expression via activation of PP2A [44]. Furthermore, pharmacologic doses of FTY720 suppressed in vivo BCR-ABL-driven leukemogenesis without exerting any toxicity [44]. This suggests that FTY720 may be useful for treating patients who are resistant to Imatinib, Dasatinib and Nilotinib, and would complement drugs that target the BCR-ABL fusion protein. Recent findings show that activation of PP2A by FTY720 also caused the dephosphorylation of the c-KIT receptor and its downstream signaling targets pAkt, pSTAT5, and pERK1/2. Additionally, administration of FTY720 to mice delayed the growth of c-KIT tumors, inhibited splenic and bone marrow infiltration, and prolonged survival [45].

CONCLUSIONS

It has been well demonstrated that S1P plays an important role in multiple hematologic malignancies. Based upon studies that utilized pharmacological inhibitors and molecular interventions, targeting the SphK1/S1P/S1P receptor axis might be beneficial in treatment of hematologic malignancies and those that develop resistance to therapies. The promising results using FTY720 raise the intriguing notion that this compound deserves consideration as a drug for therapy of hematologic malignancies.

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