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Regulation and functions of sphingosine kinases in the brain

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Abstract

It has long been known that sphingolipids, especially sphingomyelin, a principal component of myelin, are highly enriched in the central nervous system and are structural components of all eukaryotic cell membranes. In the last few years, substantial evidence has accumulated from studies of many types of cells demonstrating that in addition to their structural roles, their breakdown products form a new class of signaling molecules with potent and myriad regulatory effects on essentially every cell in the body. While the sphingolipid metabolites sphingosine and its precursor ceramide have been associated with cell growth arrest and apoptosis, sphingosine-1-phosphate (S1P) enhances proliferation, differentiation, and cell survival as well as regulates many physiological and pathological processes. The relative levels of these three interconvertible sphingolipid metabolites, and thus cell fate, are strongly influenced by the activity of sphingosine kinases, of which there are two isoforms, designated SphK1 and SphK2, the enzymes that phosphorylate sphingosine to produce S1P. Not much is yet known of the importance of S1P in the central nervous system. Therefore, this review is focused on current knowledge of regulation of SphK1 and SphK2 on both transcriptional and post-translational levels and the functions of these isozymes and their product S1P and its receptors in the central nervous system.

Keywords

Sphingosine kinase; Sphingsosine-1-phosphate; Phosphorylation; Transcription; Post-translational modifications; Central nervous system

1. Introduction

The interconvertible sphingolipid metabolites, ceramide, sphingosine, and sphingosine-1-phosphate (S1P), are now recognized as important bioactive mediators that regulate many cellular and physiological processes [1]. To highlight just a few of these, ceramide and sphingosine have been shown to be involved in cell cycle arrest and apoptosis [2], while S1P has been implicated in cell proliferation, survival, migration, angiogenesis, and differentiation [1]. The relative levels of ceramide and sphingosine compared to S1P, also known as the sphingolipid rheostat, is critical in determining cell fate [1]. Thus, increased levels of S1P can protect against apoptosis mediated by increases in cellular ceramide, a major response to stress [3]. The synthesis and metabolism of sphingolipids was recently discussed in an excellent

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review [4] and will not be discussed here. However these pathways are shown in Fig. 1 and the reader is referred to this comprehensive review for detailed information.

S1P can act intracellularly to increase DNA synthesis and suppress apoptosis and regulate calcium mobilization [5], although the intracellular targets of S1P are still elusive. The most well-characterized functions of S1P are mediated by binding to five ubiquitously expressed G protein-coupled receptors (named $S1P_{1–5}$) [1,6]. The S1P receptors couple to a variety of G proteins with varying affinities for different effector molecules that activate numerous downstream signaling pathways [7]. Thus, depending on the spectrum of S1P receptors expressed in a given cell type, S1P can activate various pathways regulating numerous important cellular and physiological functions [8].

Sphingosine kinases (SphKs), the enzymes that produce S1P by phosphorylating sphingosine, are essential elements in the regulation of S1P levels, and hence the levels of its precursors, sphingosine and ceramide. There are two isoforms of SphK, SphK1 and SphK2, that have different properties and subcellular locations [9], suggesting that they have distinct biological functions, although they may be able to complement each other for some vital functions since production of S1P is critical for brain and cardiovascular system development [10]. Many studies have established that expression of SphK1 is associated with cell survival and proliferation. Presumptive evidence suggests that *sphk1* may be an oncogene: overexpression of SphK1 in NIH 3T3 cells enhances foci formation, colony growth in soft agar, and tumor formation in SCID mice [11]; MCF7 human breast cancer cells overexpressing SphK1 produce larger and more abundant tumors in xenografts [12]; and SphK1 is expressed at high levels in many types of cancers [13]. The biological functions of SphK2 are not yet clearly defined and appear to vary depending on the cell type. However, when overexpressed, SphK2 generally acts as a “bad” kinase and induces cell cycle arrest and apoptosis [14,15]. Because there is such a paucity of information on the role of SphKs and S1P at the molecular level in the central nervous system, this review will first focus on current knowledge of transcriptional and post-transcriptional regulation of SphKs gleaned from studies in various types of cells.

2. Structure and localization of sphingosine kinases

In humans, the *sphk1* gene is located on chromosome 17 (17q25.2) while the *sphk2* gene is on chromosome 19 (19q13.2). SphK1 and SphK2 are highly homologous and contain five conserved domains, one of which includes the conserved diacylglycerol kinase ATP binding domain [16]. Although SphK1 and SphK2 display 80% amino acid sequence similarity [17], they differ in their central regions and N termini. SphK1 lacks transmembrane domains or identifiable signal sequences and is mainly cytosolic [18]. SphK1 is abundantly expressed in adult mouse heart, spleen, lung, and brain, whereas SphK2 expression is highest in brain, kidney, and liver [17]. SphK2 is about 240 amino acids longer than SphK1 at its N terminus and contains several transmembrane domains [17]. In addition, SphK2 possesses a nuclear localization signal within its N terminal region, which when mutated, prevents it from entering the nucleus and inhibiting DNA synthesis [14]. Unlike SphK1, which is mainly localized to the cytosol in all cells, SphK2 localization is cell type-specific. For example, in HEK 293 cells, SphK2 can be detected in the plasma membrane, mitochondria, ER, Golgi, and in the cytosol [9], whereas, in COS7, HeLa, MCF7, and NIH 3T3 cells, it is predominantly localized to the nucleus [19,20].

2.1. Activation of sphingosine kinases

A broad range of external stimuli has been reported to activate SphK1, among which are various growth factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), basic fibroblast growth factor (bFGF), transforming growth factor beta (TGFβ), and insulin-like growth

factor-1 (IGF-1), cytokines such as TNF-α and interleukins, and hormones (estradiol and prolactin) (reviewed in [21]). Many of these stimuli activate SphK1 in a biphasic manner. That is to say, the first phase of activation is rapid (minutes) and transient, most likely via post-translational modifications that increase enzymatic activity and its translocation to the plasma membrane where its substrate sphingosine resides, and a second phase of activation over the next 24 h that entails upregulation of transcription. Much less is known about regulation of SphK2 activity.

2.2. Post-translational activation of SphK1 and SphK2

Several SphK1 interacting proteins have been identified by the yeast two-hybrid approach [22]. Although some have been shown to interact with SphK1 in mammalian cells, none have yet been implicated in the regulation of SphK1 activity or S1P production. Crosslinking of the high affinity IgE receptor (FcεRI) on mast cells activates SphK1, increasing production of S1P, which is secreted and regulates mast cell functions in an autocrine or paracrine manner by binding to S1P receptors. Recently, activation of SphK1 was shown to be due to direct interaction with Lyn tyrosine kinase [23]. This interaction explicitly enhanced the enzymatic activities of both SphK1 and Lyn, although SphK1 was not phosphorylated by Lyn. More recently, SphK2 was also reported to be activated upon FcεRI crosslinking [24]. In addition, Fyn, another Src protein tyrosine kinase, is also essential for SphK1 and SphK2 activation, since mast cells from Fyn deficient mice exhibit impaired SphK1 and SphK2 enzyme activity and S1P production [24]. However, neither SphK1 nor SphK2 are substrates for Fyn. Rather, activation of SphK1 by Fyn involves the adapter Grb2-associated binder 2 and phosphatidylinositol 3-kinase, but activation of SphK2 is independent of this pathway [24].

In contrast, some activators require Ca^{2+} to stimulate SphK. For instance, activation of SphK1 by platelet-derived growth factor BB (PDGF-BB) is blocked by chelation of intracellular Ca^{2+} with BAPTAAM [25]. Specifically, PDGF activation of SphK1 requires phosphorylation of the PDGF receptor on tyrosine-1021, which is necessary for the subsequent association with PLCγ, thus leading to production of $InsP_3$ and downstream mobilization of calcium [25]. Moreover, it has been proposed that SphK1 contains several putative Ca^{2+}/calmodulin binding sites [18]. In agreement, translocation of SphK1 to the plasma membrane induced by the PKC activator phorbol ester is inhibited by the loss of the functional calmodulin binding site [26]. In spite of these findings, direct activation of SphK1 by Ca^{2+} or calmodulin has not been detected.

EGF is one of the most potent, and most well studied, activators of SphK1. In MCF7 cells, EGF stimulates SphK1 activity and induces its translocation from the cytosol to the plasma membrane [27]. A novel EGF pathway involving sequential activation of c-Src, PKCδ, and subsequent activation and translocation of SphK1 to the plasma membrane was recently described in glioblastoma cells [28]. Several lines of evidence suggest that PKCδ and SphK1 may be downstream targets of EGF-activated c-Src in these glioma cells: (i) EGF induced rapid phosphorylation and translocation of PKCδ to the plasma membrane, which was blocked by the Src inhibitor PP2; (ii) EGF also induced translocation of SphK1 to the plasma membrane which was blocked by inhibition of c-Src and by downregulation of PKCδ; and (iii) down-regulation of PKCδ and SphK1 abolished EGF-induced plasminogen activator inhibitor-1 (PAI-1) expression. It is important to note that although, the high expression of EGFR alone is not a prognostic marker in gliomas, patients expressing high levels of both EGFR and PAI-1 have a shorter overall survival prognosis [29]. Thus, our recent results suggest that SphK1 is a downstream target of PKCδ that is indispensable for PAI-1 upregulation by EGF and might have important implications for glioma invasiveness. Interestingly, in primary cytotrophoblasts, the PI3K inhibitor LY294002 blocked EGF-stimulated SphK1 activity, indicating the possible involvement of the PI3K/Akt pathway [30]. Altogether, these studies

suggest that EGF might utilize diverse signaling pathways to rapidly enhance SphK1 activity. Additionally, EGF induced sustained activation of SphK1 that lasted for 24 h. Similar sustained activation of SphK1 was also described in both primary human fibroblasts and in WI-38 fibroblasts in response to TGFβ, suggestive of an increase in transcription [31,32]. NGF and bFGF also incite persistent activation of SphK1 in pheochromocytoma PC12 cells; however, EGF and IGF-1 only transiently stimulate SphK1 in these cells [33]. Moreover, NGF instigates translocation of SphK1 to the plasma membrane and local production of S1P, which differentially activates the $S1P_1$ and $S1P_2$ receptors [34]. While the precise mechanism of SphK1 activation by NGF is still not known, it is mediated by the TrkA tyrosine kinase receptor for NGF [35], which activates the Ras/ERK, PI3K/Akt, and PLCγ pathways [36]. In T24 bladder tumor cells, activation of the tyrosine kinase VEGF receptor by VEGF, a potent proangiogenic growth factor, induces phosphorylation and activation of PKC via Flk-1, which in turn transiently activates SphK1 by direct phosphorylation [37].

The protein kinase C activator, phorbol 12-myristate 13-acetate (PMA), also has been shown to transiently stimulate SphK1 by inducing PKC-dependent phosphorylation and translocation to the plasma membrane [38]. Furthermore, stimulation of SphK1 by PMA, as well as the pro-inflammatory cytokine TNF-α, requires its phosphorylation on Ser225 [39]. SphK1 possesses a docking site for ERK1/2 [40] and both ERK1 and ERK2 phosphorylate SphK1 at Ser225 [39], which is crucial for its translocation to the plasma membrane [39]. This translocation is also important for its oncogenic signaling [41]. TNFα and interleukin-1β (IL-1β) have also been shown to transiently increase SphK1 enzyme activity in A549 epithelial lung carcinoma cells [42]. This activation does not stem from increases in its mRNA or protein levels; therefore, it is most likely due to a phosphorylation event similar to that described above.

As SphK1 has been identified as an important pro-survival factor, it seems logical that its activity or expression might be regulated by other pro-survival components. One of these is Bcl-2 which was initially described as an oncogene in follicular lymphomas [43], and later found to be an anti-apoptotic protein that promotes cell survival [44]. Interestingly, Bcl-2 expression protects SphK1 from proteolysis induced in response to DNA damaging drugs [45]. Additionally, overexpression of Bcl-2 in A-375 human melanoma cells increased SphK1 enzyme activity as well as its expression [46]. Of note, SphK1 mediates BCR/ABL-induced upregulation of another anti-apoptotic protein Mcl-1 in chronic myeloid leukemia cells. In these cells, SphK1 expression and activity are upregulated by BCR/ABL via the ERK1/2, PI3K, and JAK2 pathways [47].

A novel “criss-cross” transactivation mechanism important for proliferation that involves activation of SphK1 by 17β-estradiol (E_2) has also been reported [48]. In MCF7 human breast cancer cells, E_2 binding to the estrogen receptor activates SphK1 and increases S1P production. Subsequently, S1P is secreted and signals through the $S1P_3$ receptor to increase metalloproteinase activity and production of EGF which then activates the EGF receptor [49]. More recently, the hormone prolactin as well as E_2 were also reported to biphasically activate SphK1 in MCF-7 cells [50]. The initial activation of SphK1 occurred rapidly after stimulation, followed by a much later increase in mRNA and protein expression and both phases of activation were ablated by MEK and PKC inhibitors [50]. Moreover, the increase in the proliferation and migration in response to either E_2 or prolactin was significantly reduced by downregulating SphK1 expression, suggesting that activation of SphK1 is critical for these responses.

Very few studies have examined the mechanism of activation of SphK2. In addition to activation of SphK2 by crosslinking of FcεRI [51], EGF-induced activation of SphK2 was observed in HEK 293 and MDA-MB-453 breast cancer cells [52]. EGF-induced migration of MDA-MB-453 cells was decreased by knock-down of either SphK1 or SphK2, suggesting that

activation of both isozymes by EGF contributes independently to the motility responses in these cells. In a follow-up study, it was shown that activation of SphK2 induced by EGF resulted from EGFR-mediated ERK1/2-depenent phosphorylation of SphK2 [53]. Site-directed mutagenesis indicated that hSphK2 is phosphorylated on Ser351 and Thr578 by ERK1 and that phosphorylation of these residues is important for EGF-stimulated migration of MDA-MB-453 cells [53].

Lastly, it was recently shown that PMA-induced phosphorylation of SphK2 on a putative nuclear export sequence (NES) located in the central region of the protein stimulated CRM1-mediated export from the nucleus to the cytosol [54]. Furthermore, mutation of two serine residues (Ser-419 and Ser-421) phosphorylated within this NES-like motif abolished SphK2 export. Thus, post-translational modification via phosphorylation of SphK2 may be an important regulator of its activity and localization.

2.3. Transcriptional regulation of sphk1

To date, a great deal of our knowledge of the regulation of the *sphk1* gene stems from studies that were first performed in rodent cells. The rat *sphk1* gene contains a 3.7 kb CpG island, within which there are six transcription start sites, potentially yielding six alternative first exons; thus indicating that multiple splice variants of the rat *sphk1* gene exist (*sphk1a-f*) [55]. In the brain, the hypomethylated region of *sphk1a* is in the 5′ end of the CpG island, and a tissue-dependent, differentially methylated region (T-DMR) is situated approximately 800 bp upstream of the *sphk1a* first exon. The T-DMR is hypomethylated in the brain, where *sphk1a* is the sole isoform. In contrast, the T-DMR is hypermethylated in the heart where *sphk1a*, as well as the other isoforms, is not expressed. These results suggest that hypermethylation of the T-DMR is associated with *sphk1a* suppression. Moreover, several endogenous antisense transcripts to *sphk1*, termed *Khps1*, have also been described [56]. Of these, *Khps1a* spans the T-DMR within the CpG island, and its expression induces the demethylation of CG sites within the T-DMR. Interestingly, the expression of *sphk1* or *Khps1* is mutually exclusive, suggesting that the CpG island regulates the intrinsic expression of *sphk1*.

Little is known of the regulation of Sphk1 expression, but several transcription factors that regulate both the human and rat *sphk1* genes have been identified. The 55-bp fragment localized in front of exon 1d mediates NGF induction of rat *sphk1* gene transcription [57]. This fragment contains one activator protein-2 (AP-2), and two specificity protein 1 (Sp1) binding sites. Interestingly, the proximal Sp1 site, and not the distal Sp1 site, is required for the intrinsic expression of the rat *sphk1* gene. However, analysis of the 5′ flanking region of the human *sphk1* gene revealed that a 300 bp fragment containing one Sp1 and two AP-2 binding motifs, effectively mediates *sphk1* transcription upon PMA treatment in human megakaryoblastic leukemia MEG-01 cells (Fig. 2) [58]. Moreover, an unknown protein, other than AP-2, binds to the AP-2 binding motifs of the promoter. Furthermore, MEK and PKC inhibitors also prevented the *sphk1* promoter activity induced by PMA. These results imply that PMA-induced *sphk1* gene expression is mediated by transcription factors that are activated by the PKC and ERK signaling pathways.

Histamine also upregulates SphK1 expression in endothelial cells via the PKC and ERK pathways and requires the AP-2 and Sp1 binding motifs of the *sphk1* gene [59]. The importance of these binding motifs in the transcriptional regulation of *sphk1* was solidified by the observations that increased promoter activity induced by prolactin and GDNF also utilized AP-2 and Sp1 binding motifs in MCF-7 cells [50] and TGW human neuroblastoma cells [60]. Moreover, upregulation of SphK1 expression by prolactin also required STAT5 activation, while GDNF induced transcription through the PI3K pathway in addition to the ERK1/2 pathway.

Subjecting glioma cells to hypoxic stress activates SphK1 and increases its expression, effects that may be important in more hypoxic areas in the center of a brain tumor [61]. The increase in SphK1 activity resulted in increased intracellular S1P production and secretion. Hypoxia is known to enhance stability hypoxia-inducible factors (HIFs), transcription factors that bind to hypoxia response elements (HREs) and regulate hypoxia-inducible genes (Fig. 2). In this regard, the *sphk1* 5′ flanking region possesses multiple putative HRE sites and a reporter construct containing 3124 bp upstream of the transcription start site conferred response to hypoxic stress [61]. Furthermore, HIF-1α and HIF-2α both bind exclusively to a region within the 5′ flanking region that contains an evolutionarily conserved HRE site. Notably, HIF-2α, and not HIF-1α, activates transcription of the *sphk1* gene under hypoxic conditions [61]. In contrast to *sphk1*, *sphk2* transcriptional regulation remains unexplored.

3. Functions of sphingosine kinases and S1P in the brain

The brain is the organ that contains the highest concentration of S1P [62]. During pathological conditions, such as brain injury or stroke, local concentrations of S1P may be further increased as S1P could be released from platelets in blood clots [63]. Moreover, high expression levels of SphK1 have been correlated with decreased rates of survival for patients diagnosed with glioblastoma multiforme, the most invasive primary brain tumor [64]. SphK activity and S1P also protect cultured mesencephalic neurons against glutamate-induced neurotoxicity [65].

There have been contradictory reports as to which SphK isoform is predominantly responsible for S1P production in normal brain tissue [66,67]. One study has reported that SphK1 is the primary isoform in mouse brain [66], and is highly abundant in the cerebellum where it is located within the dendrites and dendritic spines of Purkinje cells [68]. As discussed above, it seems most likely that both SphK1 and SphK2 are expressed in the brain and probably even within the same cells as neither of the single SphK knockout mice display a remarkable CNS phenotype, while the double SphK1–SphK2 knockout mice have a severe brain defect [10].

A substantial presence of SphK2 in the brain is more consistent with reports on the effects of the potent immunosuppressant FTY720 in the CNS. It is well established that FTY720 is phosphorylated *in vivo* by SphK2, converting it to a S1P mimetic and potent S1P receptor agonist that accumulates in CNS white matter [69]. In experimental allergic encephalitis models, FTY720-phosphate affects the blood-brain barrier and glial repair mechanisms, which restores nerve function [70]. Moreover, FTY720 seems to be a highly promising drug for treatment of relapsing multiple sclerosis and is currently in Phase III trials [71].

Thus, clearly both SphK1 and SphK2 must be expressed in the brain, but far more is known about the functions of SphK1 there. bFGF, another growth factor present in the brain, induced secretion of S1P from cerebellar astrocytes, which was prevented by inhibiting SphK1 [72]. S1P in turn increased DNA synthesis and activated ERK1/2 via one of its G_i/G_o coupled receptors, of which $S1P_1$, $S1P_2$, and $S1P_3$ are expressed in cerebellar astrocytes. S1P also signals in autocrine and/or paracrine manners in hippocampal neurons to facilitate glutamate secretion induced by secretagogues and SphK1 may be involved in the underlying regulation of synaptic transmission [73]. SphK1 is not only capable of regulating functions in the brain via its product S1P, but also through corresponding effects on intracellular levels of its substrate sphingosine. Thus, conversion of sphingosine to S1P can also regulate calcium levels in neurons as sphingosine inhibits voltage-operated calcium channels (VOCC), which prevents calcium entry in response to depolarization [74,75]. In agreement, expression of SphK1 in GH_4C_1 rat pituitary cells reduced inhibition of the VOCC by sphingosine [76].

An interesting observation was made that SphK1 interacts with and is activated by neural plakophilin-related armadillo repeat protein (δ-catenin/NPRAP) in hippocampal neurons [77]. Moreover, a SphK inhibitor decreased δ-catenin/NPRAP-dependent neuronal cell

migration [77]. S1P also plays an important role in motility of glioblastoma cells [78], and as previously mentioned, is critical for the process of invasion. Furthermore, S1P signals through $S1P_1$, $S1P_2$, and $S1P_3$ to stimulate glioma cell proliferation, and activation of $S1P_1$ and $S1P_3$ also amplifies glioma migration and invasion [79].

4. S1P receptors in the CNS

Although receptors for S1P are abundant in the central nervous system, only a few studies have addressed cell-specific functions of S1P via its receptors and most of these have been focused on isolated cells in culture (Table 1). It has previously been demonstrated that in PC12 cells and dorsal root ganglion (DRG) neurons, NGF induces translocation of SphK1 to the plasma membrane [34]. S1P thus causes activation of $S1P_1$ leading to Rac activation and neurite extension. In addition, NGF downregulates $S1P_2$ and $S1P_3$, which leads to neurite retraction by activation of Rho [34]. Evidence for the role of S1P in CNS neuroexcitability emerged from some observations of $S1P_2$ knockout mice. It was noticed that homozygous knockout mice occasionally exhibited seizure activity characterized behaviorally by a 2–10 s wild running episode followed by a 15–60 s period of freezing [80]. These events occurred only in 3–7 week old mice and were accompanied by changes in electrical activity of the brain [80]. However, recent studies have demonstrated that $S1P_2$ knockout mice are profoundly deaf from postnatal day 22 and display a progressive loss of vestibular function [81–83].

S1P enhances the excitability of cultured rat DRG neurons that is likely mediated via S1P receptors, as blockade of G-protein signaling abrogated these effects of S1P [84]. Moreover, S1P generated intracellularly from ceramide can also increase neuronal excitability by mechanisms that are poorly understood [85]. S1P was also recently shown to have two actions on glutamate secretion in primary hippocampal neurons: (i) it can act as a secretagogue to trigger glutamate secretion and (ii) to potentiate depolarization-evoked glutamate secretion [73]. Depolarization-induced glutamate release was dependent on SphK1, S1P formation, and subsequent $S1P_1$ activation. These findings indicate that S1P, through its autocrine action, facilitates glutamate secretion in hippocampal neurons and may be involved in mechanisms underlying regulation of synaptic transmission [73]. Furthermore, neural stem/progenitor cells migrate toward a damaged area of the CNS to reduce the damage. It was recently reported that S1P concentration in the spinal cord was increased after a contusion injury, due to accumulation of microglia and reactive astrocytes in the injured area. Moreover, this locally increased S1P induced migration of transplanted neural stem/progenitor cells through its receptor $S1P_1$ [86].

S1P is also involved in growth and survival of oligodendrocytes [87], the myelinating cells of the CNS. The survival promoting effect of neuotrophin-3 (NT-3) on oligodendrocyte precursors was shown to be dependent on SphK1 and S1P itself was able to stimulate CREB phosphorylation, an important NT-3 survival signaling pathway [88]. $S1P_5$, which is exclusively expressed on oligodendrocytes and throughout their development, has different functions depending on the developmental stage. It mediates process retraction of oligodendrocyte precursors and promotes survival of mature oligodendrocytes [89]. Moreover, activation of $S1P_5$ inhibits oligodendrocyte progenitor migration [90]. S1P receptors are differentially modulated in oligodendrocyte progenitors by PDGF resulting in downregulation of $S1P_5$ and upregulation of $S1P_1$ [91]. Moreover, $S1P_1$ is involved in PDGF-induced proliferation of oligodendrocyte progenitors. Thus, $S1P_1$ and $S1P_5$ may have different functions during oligodendroglial development, and possibly during remyelination [91].

Destruction of oligodendrocytes is a key pathological process in multiple sclerosis (MS). The current therapies available for MS utilize an immunomodulatory approach to prevent T-cell- and macrophage-mediated destruction of brain-resident oligodendrocytes and axonal loss. Recently, the sphingosine analogue, FTY720 (Fingolimod), was shown to significantly reduce

relapse rates in MS patients and is currently in Phase III clinical trials [92]. FTY720 is a pro-drug that is phosphorylated *in vivo* by SphK2 but not SphK1 [93,94] to biologically active FTY720-phosphate (FTY720-P), a mimetic of S1P. FTY720-P binds to four of the five known S1P receptors, but not to $S1P_2$. Although it is a $S1P_1$ receptor agonist, it has been shown to cause prolonged receptor downregulation [95]. As $S1P_1$ is required for lymphocytes to sense and move towards the S1P gradient between tissues and blood, its loss prevents egress of thymocytes and lymphocytes from secondary lymphoid tissues into the circulation[95–97]. It has been assumed that the beneficial effects of FTY720 result from retardation of lymphocyte mobilization to sites of inflammation without an induction of a generalized state of immunosuppression. However, the mechanisms underlying the action of FTY720 in MS have not yet been definitively identified. For example, FTY720 has been shown to also have direct effects on oligodendrocyte progenitors [98]. Treatment of these cells with FTY720 causes activation of ERK1/2 and Akt, accompanied by protection from apoptosis [91,98]. FTY720P also regulates oligodendrocyte progenitor differentiation into mature oligodendrocytes [91]. However, FTY720 also arrested oligodendrocyte differentiation, an effect that was counteracted by NT-3, which not only enhanced the survival of oligodendrocyte progenitors induced by FTY720, but also stimulated their maturation [98]. FTY720 also was recently shown to induce time-dependent modulation of S1P receptors on human oligodendrocyte progenitors with consequent functional responses that were directly relevant for the remyelination process [91]. Altogether, these observations suggest that in addition to its immunosuppressive functions, FTY720 could also have a beneficial effect in MS by these direct actions on oligodendrocyte progenitors. However, the finding that FTY720 impedes the differentiation of these cells raises the question of whether FTY720 therapy for MS should include the use of differentiation-enhancing factors, such as NT-3 [98]. This approach could ensure both protection of existing oligodendrocyte progenitor pools against immune-mediated insults as well as stimulation of remyelination by enhancing the maturation of these cells.

Much less is known of the roles of S1P in other types of glial cells. Astrocytes, the major type of glial cell in the brain, are another target of FTY720 in the CNS as they express $S1P_1$, $S1P_2$, and $S1P_3$ [99]. FTY720-Pas well as another agonist of $S1P_1$, SEW2871, stimulate astrocyte migration [100]. Astrocytes also express SphKs and can synthesize S1P and release it for autocrine/paracrine actions [101]. An interesting finding was that fibroblast growth factor (FGF),whose synthesis was earlier shown to be increased by S1P [102], can also stimulate S1P release from astroglial cells [72], suggesting a signaling amplification loop that further adds to the complexity of the relationship between growth factors and S1P. Moreover, S1P is present at high levels in blood and can enter the brain during CNS injury. Reactive astrogliosis, a prominent component of CNS injury with potentially harmful consequences, could also involve S1P-mediated proliferation of astrocytes [103].

In injured spinal cords, reactive astrocytes and microglia around the injury sites colocalize to regions with high levels of S1P [86], suggesting that the accumulation of reactive astrocytes and microglia after spinal cord injury may be responsible for the increased level of S1P. Only one study so far has examined functions of S1P and the expression of S1P receptors in microglia, resident brain macrophages [104]. While $S1P_1$, $S1P_2$, $S1P_3$, and $S1P_5$ were all expressed by acutely isolated microglia, $S1P_3$ expression was lost after 2 weeks in culture [104]. In addition, stimulation of microglia with S1P induced the release of TNF-α, suggesting the potential involvement of S1P in CNS inflammation [104].

5. Perspectives

Knowledge of SphKs and the functions of S1P has grown immensely over the past several years, and this trend will surely continue. It is truly amazing that so much has been written about the functions of S1P, yet so little is still known of the regulation of the enzymes that

determine its levels. It is a growing field, with important implications for future therapeutics for neurodegenerative disorders, particularly MS. Progress in developing specific agonists and antagonists of S1P receptors as well as specific inhibitors of SphK1 and SphK2 will provide the necessary tools to understand their functions and development of new approaches to target the S1P axis.

Abbreviations

CNS, central nervous system
DRG, dorsal root ganglion
EGF, epidermal growth factor
EGFR, EGF receptor
ERK, extracellular regulated kinase
E_2, 17β-estradiol
FGF, fibroblast growth factor
GDNF, glial-derived nerve growth factor
HIF, hypoxia-inducible factor
HRE, hypoxia-responsive element
IGF-1, insulin-like growth factor-1
MEK, mitogen-activated protein kinase kinase
MS, multiple sclerosis
NES, nuclear export signal
NGF, nerve growth factor
NPRAP, plakophilin-related armadillo repeat protein
NT-3, neurotrophin-3
PDGF, platelet-derived growth factor
PI3K, phosphatidylinositol 3-kinase
PKC, protein kinase C
PLC, phospholipase C
PMA, phorbol 12-myristate 13-acetate
S1P, sphingosine-1-phosphate
SphK, sphingosine kinase
SPL, S1P lyase
VEGF, vascular endothelial growth factor
VOCC, voltage-operated calcium channels

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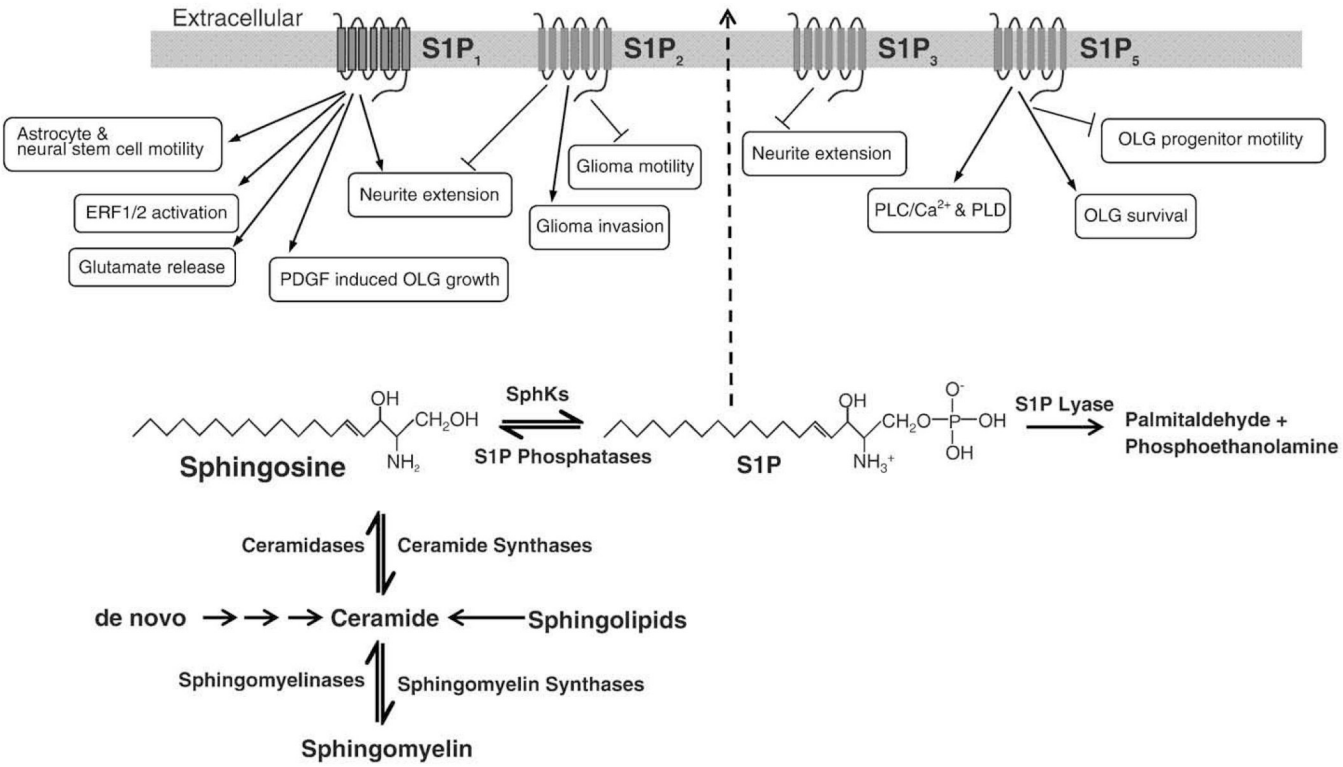

Fig. 1.

Abridged scheme showing pathways for synthesis and degradation of S1P and its actions through S1P receptors expressed in the brain.

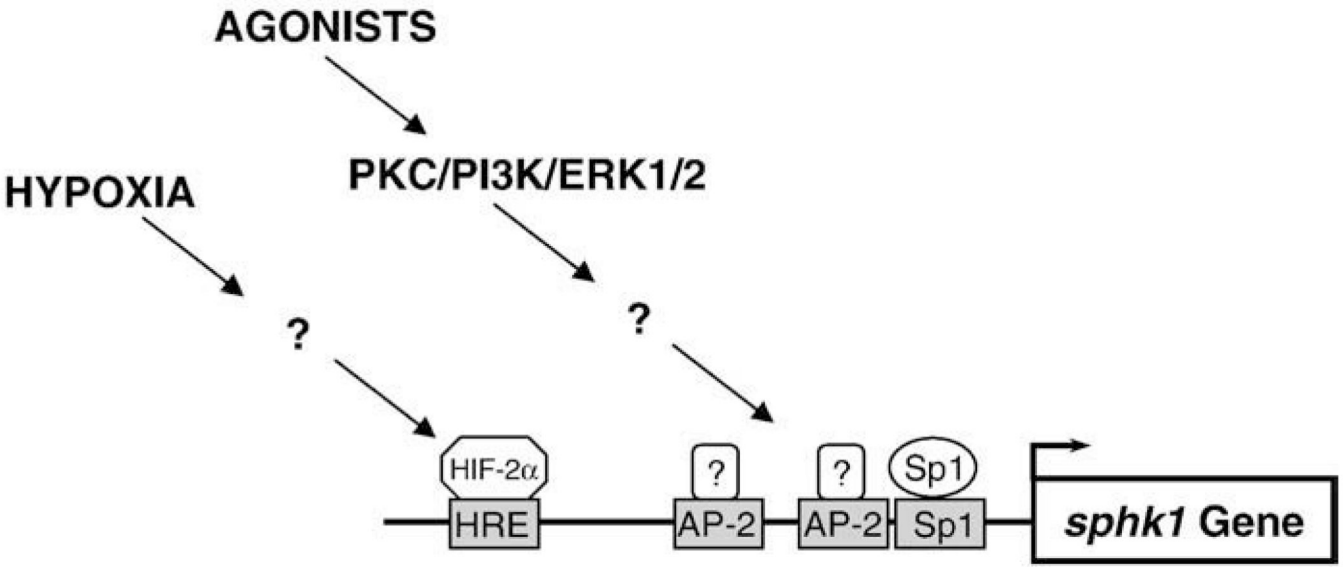

Fig. 2.
Regulatory elements within the 5′ flanking region of the *sphk1* gene. See text for more details.

Table 1

S1P receptors and functions in neurons and glial cells

Receptor	Functions	Reference
$S1P_1$	Regulates neurite outgrowth/extension in neurons	[34]
	Depolarization-induced glutamate release is dependent on	[73]
	SphK1, S1P formation, and subsequent $S1P_1$ activation	
	Migration of neural stem cells toward a site of spinal cord injury	[86]
	Mediates stimulation of ERK/Egr-1/FGF-2 in C6 glioma	[105]
	Mediates ERK activation in astrocytes	[106]
	Promotes astrocyte motility	[100]
	Mediates PDGF-induced mitogenesis of oligodendrocyte precursors	[91]
$S1P_2$	Inhibits neurite extension in neurons	[34]
	Maintenance of vestibular and cochlear hair cells	[82]
	Inhibits glioblastoma motility	[107,108]
	Increases glioma invasion	[79]
	Induces morphology changes in C6 glioma cells	[109]
	Induced PLC/Ca^{2+} in C6 glioma cells	
	Essential for neuronal development/excitability in zebrafish	[80]
	Spontaneous seizures in $S1P_2$ null mice	[80]
	Loss of vestibular function in $S1P_2$ null mice	[81–83]
$S1P_3$	May inhibit neurite extension in PC12 cells	[34]
$S1P_4$	Only expressed in lymphatic and hematopoietic tissues	
$S1P_5$	Stimulation of PLC-Ca^{2+} and PLD in C6 glioma	[105]
	Inhibits oligodendrocyte progenitor migration	[90]
	Induces process retraction and cell survival in oligodendroglial cells	[89]