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Sphingosine-1-Phosphate Regulation of Mammalian Development

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Summary

Sphingosine-1-phosphate (S1P) was first identified as a lysophospholipid metabolite whose formation is required for the irreversible degradation of sphingolipids. Years later, it was elucidated that S1P is a bioactive lipid that provokes varied cell responses by acting through cell-surface receptors to drive cell signaling. More recent findings in model organisms have now established that S1P metabolism and signaling are integrated into many physiological systems. We describe here the surprising breadth of function of S1P in mammalian development and the underlying biologic processes that S1P regulates.

Keywords

Lysophospholipid; S1P; development; vascular; neural; maternal:fetal interface; decidualization

1. Introduction

Sphingosine-1-phosphate (S1P) is a lysophospholipid that is generated by the action of sphingosine kinases on sphingosine [1]. Because the formation of S1P is required for the irreversible degradation of sphingolipids and entry into the pathway of membrane phospholipid synthesis (Fig. 1), it has a key role in cellular lipid metabolism. However, S1P also serves another important function as a cellular signaling molecule. Like lysophosphatidic acid (LPA), another well-studied lysophospholipid, S1P regulates basic cell behaviors through a family of cell-surface receptors. Originally known as endothelial differentiation gene (Edg) receptors, those specific for S1P are now called S1P receptors (Fig. 1) [2]. The functions of S1P synthesis and signaling have now been explored in a number of model organisms. The reverse genetics approach and analysis of embryonic development, particularly in mouse models, have revealed a great deal about the *in vivo* role of S1P by highlighting the physiologic systems dependent on S1P synthesis and receptor signaling. Due to functional redundancy of the genes encoding sphingosine kinase and S1P receptors, a clearer picture of the importance and function of the pathway began to emerge when the consequences of simultaneous gene disruptions could be evaluated. In this review, we will focus on the role of S1P synthesis and S1P receptors on

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mammalian development and how studies have illuminated the physiologic processes that S1P regulates.

De novo synthesis of sphingoid bases is initiated at the cytoplasmic face of the endoplasmic reticulum from condensation of palmitoyl Co-A and serine by serine palmitoyltransferase to form 3-ketosphinganine (Fig. 1). Next, reduction of 3-ketosphinganine to dihydrosphingosine occurs, followed by acylation to form dihydroceramide. After desaturation of dihydroceramide to produce ceramide, sphingosine can be liberated by ceramidase action. Sphingosine kinase (Sphk) catalyzes the ATP-dependent phosphorylation of sphingosine and dihydrosphingosine to form S1P and dihydroS1P, respectively. Mammals have two evolutionarily conserved *Sphk* genes, *Sphk1* and *Sphk2* [3,4]. However, these genes show differences in tissue distribution, cellular localization, and expression during development, as well as kinetic differences [4]. The phosphorylated sphingoid bases S1P and dihydroS1P can be degraded by S1P lyase and the products used for phospholipid synthesis (Fig. 1). Phosphoethanolamine, derived from S1P degradation, is a direct precursor for phosphatidylethanolamine synthesis.

In addition to a role as a intermediate in lipid metabolism, S1P can also act as a ligand for the S1P receptor family of G-protein-coupled receptors. Five members have been identified: S1P₁ (formerly known as Edg1), S1P₂ (Edg5), S1P₃ (Edg3), S1P₄ (Edg6), and S1P₅ (Edg8) [2]. After binding of S1P, each member of the S1P receptor family activates different and, in some cases, overlapping G-protein-mediated signaling pathways [5]. S1P receptor signaling triggers diverse cellular effects such as proliferation, migration, survival, and cytoskeletal and cell junction changes [1,6].

2. S1P and development of the vascular system

During embryonic development, the vascular system is formed by a multi-step process that requires intricate coordination between cellular proliferation, differentiation, cell matrix changes, and migration [7]. The first step in the process is *vasculogenesis*, where mesodermally derived endothelial precursors, known as angioblasts, form the primitive vasculature, consisting of tubular endothelial structures. The next step, *angiogenesis*, involves the sprouting, splitting, and remodeling of existing vessels to generate a more complex, hierarchical network of small and large vessels. Finally, *maturation* of the vessels occurs as the vascular network is invested and surrounded by mural cells--vascular smooth muscle cells in arteries and pericytes in capillaries--to reinforce and stabilize them.

2.1. Expression of sphingosine kinases and S1P receptors in the developing vascular system

Sphk2 is expressed in the prevascularized embryonic tissues of mice before day 8 postcoitum (pc), with the bulk of *Sphk1* transcripts appearing slightly later [8]. Around the developmental stage that includes the process of vasculogenesis, S1P₁, S1P₂, and S1P₃ receptors are expressed in embryonic tissues [8–10]. At E10.5, S1P₁ receptor is found abundantly expressed in endothelial cells of the dorsal aorta and intersomitic vessels, with lesser expression detected in the vascular smooth muscle cells that surround the aorta and in the common atrial chamber of the heart [11]. At E14, S1P₁, S1P₂, and S1P₃ receptors are expressed in blood vessels of the brain [10].

2.2. Vascular development in sphingosine kinase knockout mice

Sphk1-null mice are viable [12]. Although S1P levels in most tissues from the *Sphk1*-null mice are not markedly decreased, serum levels of S1P are about half that of wild-type mice, indicating that *Sphk1* is important for maintaining serum S1P levels. *Sphk2*-null mice are also viable, but reports from separate studies of different mouse *Sphk2* knockout lines have differed in their findings about changes in serum levels of S1P in these mice [13–16]. In one line there

was a reported 25% decrease in serum and plasma S1P levels [13]. However, in two other independently derived lines of *Sphk2*-null mice, significantly higher levels of S1P in serum and plasma was reported [14–16]. In contrast to the single-null mice, the simultaneous deletion of both *Sphk1* and *Sphk2* genes in embryos causes them to die at mid-gestation [14]. These double-null embryos have undetectable levels of S1P, indicating that the two sphingosine kinases are responsible for S1P production. When both *Sphk1* and *Sphk2* genes are deleted simultaneously in the adult, S1P levels fall to undetectable levels in plasma and lymph proving that both kinases contribute to S1P levels in the vascular compartments [17]. These mice do not exhibit major vascular defects. However, they have a block in lymphocyte egress from thymus and lymph nodes demonstrating a key role for plasma and lymph S1P levels in lymphocyte trafficking.

At E11.5–E12.5, *Sphk1/Sphk2* double-null embryos exhibit bleeding in the head and body. Coverage of the dorsal aorta by vascular smooth muscle cells is incomplete, indicating a defect in vascular maturation. In addition, blood vessels in the mesenchymal region of the head are poorly remodeled, enlarged and dilated, and have severely defective endothelial cells, suggesting problems related to angiogenesis [14].

2.3. Vascular development in S1P receptor knockout mice

Deletion of *SIP₁*, either globally or within endothelial cells, causes embryonic death in mice at around E12.5 as a result of excessive bleeding [11,18]. These studies led to the conclusion that the expression of *SIP₁* in endothelial cells is absolutely essential for vascular maturation, the process in which nascent vessels are covered by vascular smooth muscle cells. Mechanistically, S1P₁ signaling regulates N-cadherin interactions, which are important for interactions between endothelial and vascular smooth muscle cells [19].

Although single-null *SIP₂* or *SIP₃* mice do not show embryonic lethality, when mice are null for both *SIP₂* and *SIP₃*, about 50% of embryos die [20,21]. These double-null mice have angiogenic defects and begin to bleed around E13.5. Their vessels have endothelial cells that appear defective, with abnormally thin cell bodies, although the vessels appear covered by vascular smooth muscle cells.

Embryos lacking all three receptors (*SIP₁*, *SIP₂*, and *SIP₃*) are the most severely affected of any of the combinations of receptor mutations, bleeding and dying at E10.5–E11.5 [21]. Taken together, these results indicate that S1P₁ is the most important S1P receptor for vascular development, while the S1P₂ and S1P₃ receptors provide redundant and/or enhancing functions for S1P receptor signaling. The S1P₁ receptor couples selectively to the G_i signaling pathway, whereas the S1P₂ and S1P₃ receptors both couple to the G_i, G_q, and G_{12/13} pathways [5].

S1P receptor signaling is also essential for the regulation of lymphocyte trafficking patterns within the vascular system. This was first unveiled by discovery of the mode of action of the immunosuppressant FTY720, a sphingosine analogue [22]. When administered to mice, FTY720 after phosphorylation by Sphk2, blocks emigration of mature thymocytes out of thymus and traps lymphocytes in lymph nodes causing profound lymphopenia. The immunosuppressive action and effect on immune cell trafficking by FTY720 appear to be largely due to its activity as a ligand for the S1P₁ receptor [23].

The genetic deletion of the lymphocyte S1P₁ gene has demonstrated that S1P₁ receptor expression is required for controlling the exit of maturing CD4 and CD8 single-positive T lymphocytes from the thymus into the peripheral blood [24,25]. Similarly, NKT cells use S1P₁ receptor signaling to emigrate out of the thymus [26]. Exit of T and B lymphocytes and plasma cells from secondary lymphoid organs into lymph also depends on S1P₁ receptor

expression on the lymphocyte plasma membrane [24]. Within the spleen marginal zone B cells localize to the marginal zone in response to S1P signaling through S1P₁ receptor [27].

2.4. Conclusions

The mouse knockouts of the Sphks and S1P receptors demonstrate that the Sphk/S1P receptor axis is essential for vascular development. The dominant defect is in the later stages of angiogenesis, where remodeling of vessels and their maturation occur. However, the expression patterns of the kinases and S1P receptors, and some of the potential downstream signaling pathways [28], suggest that they could also be engaged in earlier vascular functions. An earlier role in vascular development is also supported by in vitro studies using the mouse allantois explant model, which demonstrated that S1P drives the migratory event during de novo blood vessel formation [8]. The lack of early vascular defects in the Sphk and S1P receptor null mice may be due to other sources of S1P, possibly maternal, or S1P receptors other than S1P₁, S1P₂, and S1P₃. Another possibility is that early S1P signaling functions may be masked by redundant signaling pathways, such as those mediated through LPA, whose formation is essential for early vascular development [29,30].

After the vascular system has been formed, S1P signaling through the S1P₁ receptor on lymphocytes serves an essential function in the development of a functional immune system by mediating lymphocyte egress from lymphoid tissues into the vascular compartments. Key to this process is the generation of plasma S1P levels by Sphk1 and Sphk2. Although not proven, a functional plasma S1P pool may also drive vascular development.

3. S1P and development of the nervous system

The central nervous system develops from the neural plate that folds and fuses to create a fluid-filled neural tube. The original neural tube is composed of one cell layer of germinal neuroepithelium. During embryonic development, a proliferative region adjacent to the cerebral ventricles, called the ventricular zone, gives rise to neural cells and ultimately the neurons that form the cerebral cortex.

3.1. Expression of sphingosine kinases and S1P receptors in the developing nervous system

In E10.5 mouse embryos, *Sphk1*, *Sphk2*, and *S1P1* mRNA are all expressed in the developing central nervous system, with overlapping expression in the forebrain (telencephalon) [11,14]. The *S1P1* receptor mRNA is concentrated in the proliferative ventricular zones in E14 mouse embryos [10]. *S1P2* and *S1P3* mRNA is expressed in a punctate pattern in the embryonic brain that may be associated with endothelial cells, reflecting the role of the receptors in vascular development. *S1P5* is expressed within the developing oligodendroglia [31].

3.2. Nervous system development in sphingosine kinase knockout mice

Sphk1-null and *Sphk2*-null mice live to adulthood and display no gross brain abnormalities. However, *Sphk1/Sphk2* double-null embryos show exencephaly at frequencies of 18% at E10.5, 13% at E11.5, and 20% at E12.5. The brains of *Sphk1 Sphk2* double-null mice at E11.5 have a very thin, poorly developed wall of neuroepithelium, along with ventricular dilatation [14]. Substantially increased numbers of apoptotic cells, together with decreased mitotic cells, are present in the neuroepithelium of almost all brain regions, particularly in the telencephalon, and correlate with the thinning of the neuroepithelial layer.

3.3. Nervous system development in S1P receptor knockout mice

S1P1 receptor-null embryos at E12.5 have cell loss in the forebrain. They have increased apoptotic cells in the neuroepithelium of the telencephalon and diencephalon, and decreased

mitotic cells in the telencephalon [14]. These defects, although similar to those in the *Sphk1/Sphk2* double-null embryos, are less severe.

Adult *SIP₂*-null mice show spontaneous, sporadic seizures between 3 and 7 weeks [32]. Moreover, *SIP₂*-null mice are deaf, with multiple inner ear pathologies. The simultaneous deletion of *SIP₂* and *SIP₃* genes results in additional inner ear defects [33–35]. Deletion of the *SIP₂* gene results in degeneration of sensory hair cells and the spiral ganglion neurons. An early change in *SIP₂* null mice is an abnormal capillary bed in stria vascularis, which may serve to trigger or exasperate the other inner ear defects.

3.4. Conclusions

The similarity of nervous system phenotypes in the *Sphk1/Sphk2* double-null and *SIP₁*-null embryos indicates that the effect of S1P on nervous system development is mediated, at least in part, through the *SIP₁* receptor. *Sphk1/Sphk2* double-null and *SIP₁*-null embryos exhibit increased apoptosis and decreased mitosis in the neuroepithelia, most prominently in the forebrain, where these genes are highly expressed, suggesting important functions for S1P signaling in cortical neurogenesis. However, the *SIP₁*-null mice are much less severely affected than the *Sphk1/Sphk2* double-null mice, suggesting redundant components, possibly other S1P receptors that may be involved in neurogenesis.

Lpa1 and Lpa2 receptors, which are G-protein receptors for LPA, have an embryonic nervous system expression pattern similar to the *SIP₁* receptor within the ventricular zone and also regulate cortical neurogenesis [10,36]. Thus, there appear to be dual lysophospholipid ligands--S1P and LPA--and multiple receptors all engaged in cortical neurogenesis.

4. Maternal S1P and control of embryonic development

The connection between maternal and embryonic tissue begins with embryonic implantation, which occurs after the synchronized development of the embryo to the blastocyst stage and an intricate program of uterine preparation [37]. Very soon after implantation, endometrial stromal cells surrounding implanting blastocysts undergo dramatic transformation (decidualization) during which they proliferate and differentiate into decidual cells. Decidualization begins in the stromal region immediately surrounding the embryo (antimesometrial site). Next to the implanting blastocyst, a thin, dense, avascular cell layer called the primary decidual zone is formed. Adjacent to the primary decidual zone is the broad, well-vascularized secondary decidual zone. The decidua provides a vascular network to enable nutrition and gas change for the developing embryo before the establishment of a functional placenta. Other functions include protection of the embryo from the maternal immune system and regulation of trophoblast invasion.

4.1. Expression of sphingosine kinases and S1P receptors during early pregnancy

The de novo synthesis pathway for sphingolipids, and in particular the portion leading to the production and degradation of S1P (Fig. 1A), is activated at the transcriptional level within the uterus during pregnancy [38]. Sphk enzyme activity is highly elevated in uteri of pregnant mice and rats [39,40]. The increase in Sphk enzyme activity appears to be due largely to a transcriptional increase in *Sphk1*, but not *Sphk2*, mRNA [39]. Other key genes involved in the synthesis of S1P are upregulated in uteri during pregnancy, including serine palmitoyltransferase 1 and 2 (*Sptlc1* and *Sptlc2*), S1P lyase (*Sgpl1*), S1P phosphatase 1 (*Sgpp1*), and sphingomyelinase 1 (*Smpd1*). Interestingly, the *Lass* genes, which encode ceramide synthases, are not substantially elevated during pregnancy.

In day 7.5 pc uteri, *Sphk1* mRNA expression is confined to the decidual cells surrounding the embryo, particularly in the antimesometrial portion. In uteri from pregnant rats, *Sphk1* is

localized to the glandular epithelium, vasculature, and the myometrium [38]. Early in pregnancy, S1P₁, S1P₂, and S1P₃ receptors are upregulated in the decidua, while S1P₄ and S1P₅ receptor expression remains unchanged. Expression of S1P₁ and S1P₂ receptors is colocalized with prostaglandin-endoperoxide synthase (2PTGS2) at the maternal:fetal interface [41].

4.2. Pregnancy defects in sphingosine kinase knockout mice

Strikingly, female mice of the genotype *Sphk1*^{-/-} *Sphk2*^{+/-} are infertile, in contrast to mice with all other combinations of *Sphk1* and *Sphk2* mutant alleles studied [39]. The infertile *Sphk1*^{-/-} *Sphk2*^{+/-} female mice do not show any obvious abnormalities in their ovaries or during early implantation of fertilized embryos. Instead, uterine decidualization is severely impaired in these females, leading to the death of embryos by day 7.5 pc. The decidua of pregnant *Sphk1*^{-/-} *Sphk2*^{+/-} females has increased numbers of apoptotic cells and decreased numbers of proliferating cells. The blood vessels in the mutant decidua contain damaged endothelial cells. Single-null *Sphk1*^{-/-} mice exhibit similar decidual defects during pregnancy, but to a lesser degree [39]. Single-null *Sphk2*^{-/-} mice do not show decidual defects.

Uteri from pregnant sphingosine kinase-deficient mice (*Sphk1*^{-/-} *Sphk2*^{+/-}) exhibit an enormous accumulation of dihydrosphingosine and sphingosine, along with a reduction in phosphatidylethanolamine levels. This accumulation results from limited sphingosine kinase activity in a tissue with highly elevated de novo production of sphingoid bases, both dihydrosphingosine and sphingosine. Because S1P is degraded by S1P lyase to produce the intermediate phosphoethanolamine, which then can be used for biosynthesis of phosphatidylethanolamine, the decreased levels of phosphatidylethanolamine in *Sphk1*^{-/-} *Sphk2*^{+/-} uteri may be the result of the block of substrate flux through the sphingolipid metabolic pathway.

4.3. Pregnancy defects in S1P receptor knockout mice

Double-null *S1P2*/*S1P3* female mice have decreased litter sizes, although the underlying reason is unknown [20]. The role of maternally expressed S1P₁ receptor in pregnancy has not yet been investigated.

4.4. Conclusions

The sphingolipid metabolic pathway is highly activated in the decidua during normal pregnancy. Disruption of *Sphk* genes causes sphingoid base accumulation and a reduction of phosphatidylethanolamine levels, along with defects in decidual cells and disruption of decidual blood vessels, which likely contribute to maternally derived early pregnancy loss in the *Sphk*-deficient mice. The severely defective decidualization observed in these *Sphk1*^{-/-} *Sphk2*^{+/-} females may be due to the large accumulation of sphingoid bases, which are known to cause be antiproliferative and proapoptotic [39]. Even so, the highly activated S1P/dihydroS1P pathway in the decidua suggests that the phosphorylated sphingoid bases may serve important other important functions during pregnancy:

Phosphatidylethanolamine production—Maternal synthesis of phosphatidylethanolamine is essential for optimal embryonic development. Mice deficient in ethanolamine kinase-2, and thus lacking one of the pathways for phosphatidylethanolamine synthesis, display intrauterine-growth retardation of embryos due to extensive placental thrombosis [42]. It is believed that phosphatidylethanolamine may regulate hemostasis at the maternal:fetal interface. The production of S1P may be needed as an additional source to provide ethanolamine phosphate for phosphatidylethanolamine biosynthesis.

Prostaglandin biosynthesis—The prostaglandin pathway plays crucial roles in female reproduction [43]. Prostaglandin-endoperoxide synthase 2 (Ptgs2), which converts arachidonic acid to prostaglandin H₂ (PGH₂), is a key enzyme in the biosynthesis of prostaglandins. The PGH₂ is then converted to various prostaglandins by specific enzymes. Deficiency of *ptgs2* causes multiple maternal reproductive failures, including defective attachment reaction and defective decidualization in mice [44]. The expression of S1P receptors (*S1P₁* and *S1P₂*) in the decidual microvasculature was found to overlap with *ptgs2* expression, and treatment of predecidualized stromal cells with S1P induced *ptgs2* expression, indicating a potential link between S1P and prostaglandin signaling pathways [41].

Recently, the G-protein-coupled receptor LPA₃ has been reported to be crucial for embryo implantation and spacing [45]. The *lpa₃*-deficient females show deferred implantation, which leads to inappropriate embryo spacing and reduced litter size. In *lpa₃*-null females, levels of *ptgs2* expression and prostaglandins are reduced, and treatment of mice with prostaglandins resumes on-time implantation, suggesting a linkage between LPA signaling and prostaglandin biosynthesis [45]. Although embryo spacing and in utero litter size appear normal in the *Sphk*-deficient females, a similar function in regulating prostaglandins by S1P may be masked by a redundant LPA-prostaglandin pathway.

Vascular functions—Increased vascular permeability and angiogenesis are key events for implantation and placentation. As described earlier in this review, S1P signaling has well-established roles in vascular development by acting directly on endothelial cells. During early pregnancy, S1P₁ and S1P₂ receptors are expressed on decidual endothelial cells, suggesting that they may be responsive to increased levels of S1P [41].

Immune cell trafficking—S1P has important functions in the control of immune cell trafficking [24,25] and may be involved in leukocyte homing into the decidua during pregnancy. Natural killer (NK) cells, in particular, accumulate in the decidua during pregnancy and secrete angiogenic factors important for vascular development [46]. Recently, the S1P₅ receptor was shown to mediate the trafficking of NK cells to peripheral tissues [47], raising the possibility that S1P-mediated signaling also targets NK cells to the decidua.

Maternal S1P transfer to embryonic tissues—After embryonic implantation, trophoblasts derived from the outer layers of the blastocyst invade the decidua, and ultimately differentiate into the cells of the placenta. S1P₁, S1P₂, and S1P₃ receptors are expressed on human trophoblasts, and S1P inhibits their differentiation through a G_i-mediated signaling pathway [48]. S1P produced by decidual cells may regulate differentiation of trophoblasts and subsequent placental development.

S1P stimulates key steps in vasculogenesis in vitro, yet this process is largely unaffected in embryos lacking *Sphk1* and *Sphk2* and the capability to synthesize S1P. The presence of highly elevated S1P synthetic machinery in uterine decidual cells may enable transfer of this small lysosphospholipid into the developing embryonic tissue to trigger the early events in blood vessel formation.

5. Concluding remarks

The targeted deletion of genes for *Sphk* and the S1P receptors in mice has revealed that they have remarkably diverse and important functions in mammalian development (Fig. 2, Table 1). Embryonic vascular and nervous system development and maternal support of early pregnancy are all processes that are critically dependent on the S1P system. Previous studies have also contributed to our basic understanding of S1P biology. For instance, they have shown that S1P synthesis within embryos is almost totally dependent on the two known *Sphks*. This

finding indicates that embryonic S1P synthesis is not needed to support proliferation, migration, and other cell functions until mid-gestation. In addition, earlier studies have shown that the S1P-directed vascular and neuronal development are receptor dependent. During pregnancy, S1P synthesis has a biological purpose besides receptor signaling: it is metabolically linked to the production of phosphatidylethanolamine, a glycerophospholipid with important placental functions.

Intriguingly, the LPA/LPA receptor and the S1P/S1P receptor systems appear to occupy closely related functions in vascular and nervous system development and in early pregnancy. A full understanding of the relationship of these “sister” lysophospholipid pathways remains to be determined. Finally, the elucidation of developmental road blocks induced by deletion of Sphk and S1P receptor genes has identified focal points that can be examined to begin to decipher the role of S1P and its receptors in human disease. As evidenced by the progress already made in the area of S1P and disease [6,49], this promises to be a fruitful realm for study.

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Figure 1.

S1P metabolism and receptor signaling. De novo sphingolipid metabolism begins in the endoplasmic reticulum (ER) with the condensation of palmitoyl-CoA and serine to produce 3-ketosphinganine (3-KS). Next, conversion to dihydrosphingosine (DH-Sph) occurs, followed by acylation to form dihydroceramide (DH-Cer). Ceramide (Cer) is derived by desaturation and is transported to the Golgi for conversion to sphingomyelin (SM). Ceramide, generated either from the de novo pathway or from degradation of plasma membrane sphingolipids such as sphingomyelin, is deacylated to yield sphingosine (Sph), which is phosphorylated by sphingosine kinases 1 and 2 (Sphk1, 2) to produce S1P. S1P can be secreted from cells (dashed line) and can stimulate one of the members of the S1P receptor family (S1P₁₋₅), which transmit signals through heterotrimeric G proteins. In addition, S1P and dihydroS1P (DH-S1P), from the phosphorylation of dihydrosphingosine, can be degraded to yield ethanolamine phosphate (Eth-P), which is utilized for phosphatidylethanolamine (PE) synthesis.

Figure 2.

Role of S1P and its receptors in mammalian development. S1P and S1P receptors are essential for embryonic vascular and nervous system development. During pregnancy, maternal S1P synthesis, mediated by sphingosine kinases 1 and 2 (Sphk1, 2), is also essential for embryonic development. Possible underlying biologic roles of S1P are indicated in the boxes.

Table 1Developmental phenotypes of *Sphk* and *S1P* receptor null mice

Mutation	Viability	Phenotype	Reference
<i>SIP₁</i> Null	Death between E12.5–E14.5	Vascular maturation defect, Hemorrhage starts at E12.5. Cell loss in the forebrain at E12.5.	[11,18]
<i>SIP₂</i> Null	Viable	None described.	[20,21]
<i>SIP₃</i> Null	Viable	None described.	[21,50]
<i>SIP₁/SIP₂</i> Null	Death between E10.5–E12.5	Hemorrhage starts at E11.5.	[21]
<i>SIP₁/SIP₃</i> Null	Death between E12.5–E13.5	Hemorrhage starts at E12.5.	[21]
<i>SIP₂/SIP₃</i> Null	Death after E13.5; ~50% of embryos	Subcutaneous hemorrhage after E13.5.	[20,21]
<i>SIP₁/SIP₂/SIP₃</i> Null	Death between E10.5–E11.5	Vascular remodeling defect in head. Hemorrhage starts at E10.5.	[21]
<i>SIP₄</i> Null			Not reported
<i>SIP₅</i> Null	Viable	None described.	[31]
<i>Sphk1</i> Null	Viable	In pregnant females, uterine decidualization defects and vascular endothelial damage at E7.5. Females are fertile.	[12]
<i>Sphk2</i> Null	Viable	None described.	[13–15]
<i>Sphk1</i> ^{-/-} <i>Sphk2</i> ^{+/-}	Viable	In pregnant females, uterine decidualization defects and vascular endothelial damage at E7.5. Females are infertile.	[39]
<i>Sphk1/Sphk2</i> Null	Death between E11.5–E13.5	Vascular remodeling and maturation defects, Hemorrhage starts at E10.5, Poorly developed neuroepithelium, along with ventricular dilatation at E11.5.	[14]