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REVIEW

Sphingosine 1-phosphate as a therapeutic agent

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The bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P), formed by activation of sphingosine kinase in response to diverse stimuli, is an important lipid mediator that has novel dual actions – both inside and outside of cells. S1P is the ligand for a family of five G protein-coupled receptors. Activation of these GPCRs by S1P or dihydro-S1P regulates diverse processes, including cell migration, angiogenesis, vascular maturation, heart development, and neurite retraction. There is also abundant evidence that S1P can function as a second messenger important for regulation of calcium homeostasis, cell growth, and suppression of apoptosis. In many cases, the intracellular level of S1P and ceramide, another important sphingolipid metabolite associated with cell death and cell growth arrest, coordinately determine cell fate. Changes in S1P and ceramide have been implicated in a number of pathological conditions in which apoptosis plays an important role. Importantly, radiation-induced oocyte loss in adult female mice, the event that drives premature ovarian failure and infertility in female cancer patients, was completely prevented by *in vivo* therapy with S1P. Understanding the biosynthesis, metabolism and functions of S1P can uncover new targets for the pharmaceutical and therapeutic applications of S1P.
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Introduction

Sphingolipids are a family of membrane lipids whose structure is made up of a long-chain sphingoid base backbone (such as sphingosine), an amide-linked fatty acid of varying chain length (usually, long chain or very long chain), and one of various polar head groups (hydroxyl for ceramide, phosphorylcholine for sphingomyelin, and carbohydrate residues for glycosphingolipids) (Figure 1). Although sphingolipids were originally thought to play a predominantly structural role as components of the lipid bilayer,[1] sphingolipid metabolism is now known to be a dynamic process and sphingolipid metabolites – including ceramide, sphingosine, and sphingosine 1-phosphate (S1P) – are active mediators that play essential roles in cell growth, survival and death.[2–6] Figure 1 illustrates pathways for the formation and metabolism of these sphingolipids in eukaryotic cells. This review focuses on the biosynthesis and degradation of S1P and on the biological roles of this important metabolite. It also highlights the extracellular and intracellular actions of S1P as well as the potential for the development of therapeutic interventions in various pathophysiological conditions associated with aberrant S1P signaling.

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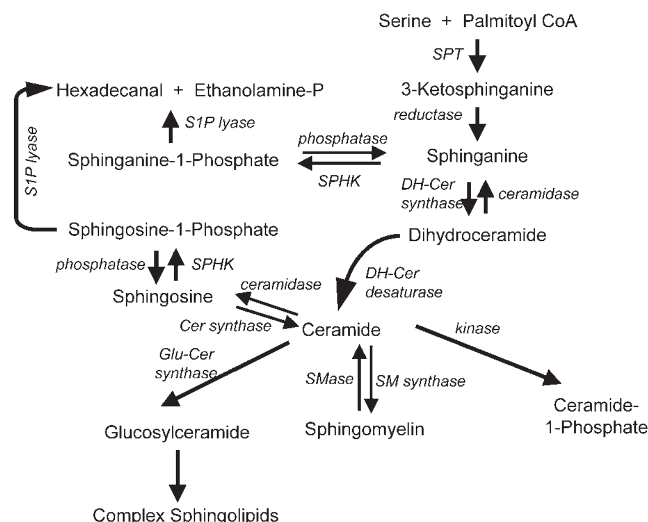

Figure 1 Sphingolipid metabolism. Cer, ceramide; DH-Cer, dihydroceramide; DH-SPH, dihydrosphingosine or sphinganine; Ethanolamine-P, phosphoethanolamine; SMase, sphingomyelinase; SM, sphingomyelin; S1P, sphingosine-1-phosphate; SPHK, sphingosine kinase, SPT, serine palmitoyltransferase.

S1P metabolism

Various stressful stimuli, including radiation, as well as proinflammatory cytokines, anticancer drugs, and growth factor withdrawal, activate sphingomyelinase, which catalyzes the hydrolysis of sphingomyelin to produce ceramide (*N*-acyl sphingosine) (Figure 1).[5,7] Ceramide, in turn, regulates various events that lead to arrest of cell growth, stress responses or apoptosis.[5,7] Evidence also suggests that apoptosis, or programmed cell death, is sometimes mediated by ceramide synthesized *de novo*.[5] Ceramide is further metabolized by ceramidase to sphingosine, which also has been found to have biological actions, including inhibition of protein kinase C and induction of apoptosis.[8] Several recent reviews have focused on sphingolipid metabolism and the enzymes that regulate ceramide and sphingosine concentrations,[5,7,9] and hence these topics will not be discussed further here. In contrast to the growth-inhibitory and pro-apoptotic effects of ceramide and sphingosine, S1P, which is produced by phosphorylation of sphingosine in a reaction catalyzed by sphingosine kinase (SPHK), has been implicated in cell growth[10] and inhibition of ceramide-mediated apoptosis.[11–14]

The intracellular concentration of S1P is low and regulated by the balance between its synthesis, catalyzed by SPHK, and its metabolism and degradation catalyzed by endoplasmic reticulum S1P lyase, and still not well-characterized phosphohydrolase activities. To date, seven SPHK isozymes with confirmed SPHK activity have been molecularly cloned. These

include SPHK type 1 from mouse and human,[15–17] type 2 from mouse and human,[18] Lcb4 and Lcb5 from the yeast *Saccharomyces cerevisiae*,[19] and a SPHK from the plant *Arabidopsis thaliana*.[20] Homology searches of protein databases with the amino acid sequences of known SPHKs have yielded at least an additional four putative members of the SPHK family. Although mammalian SPHK1 and SPHK2 share sequence homology, both in a conserved region known as the C1–C4 domains, and at their carboxyl termini, these isoforms exhibit distinct tissue distributions and characteristics.[18] SPHK1 is a 42.4 kDa protein that is most abundant in the lung, spleen and liver, whereas the 65.6 kDa SPHK2 is expressed predominantly in the liver and heart.[15,18] These two isoforms also differ in the temporal patterns of their appearance during development and they possess distinct kinetic properties,[15,18] implying that they perform distinct cellular functions and may be regulated by different mechanisms.

SPHK is activated by numerous external stimuli, among which growth and survival factors are prominent. These stimuli include platelet-derived growth factor (PDGF),[10,21,22] nerve growth factor,[23] muscarinic acetylcholine agonists,[24] cytokines such as TNF-alpha[13] and IL-1-beta,[25] and crosslinking of the immunoglobulin receptors FcepsilonRI[26] and FcgammaRI.[27] Intracellular S1P mobilizes Ca^{2+} from internal stores independently of inositol trisphosphate,[24,28] as well as triggering diverse signaling pathways that lead to cell proliferation[29,30]and suppression of apoptosis.[11,12,23,30,31] Forced expression of SPHK1 in NIH 3T3 fibroblasts increased the proportion of cells in S phase of the cell cycle by promoting the G_1–S transition; it also reduced the doubling time of these cells, an effect that was especially marked under low-serum conditions, indicating that intracellular S1P may be an important regulator of cell growth.[32] Furthermore, overexpression of human SPHK1 in NIH 3T3 fibroblasts resulted in their acquisition of a transformed phenotype, as determined by assays of focus formation, colony growth in soft agar, and the ability to form tumors in NOD/SCID mice,[33] suggesting that the corresponding wild-type gene may act as an oncogene. With the use of a SPHK inhibitor and a dominant negative mutant of this enzyme, Xia *et al*[33] also showed that SPHK contributes to cell transformation mediated by oncogenic H-Ras. Overexpression of SPHK1 also protected against apoptosis induced by serum deprivation or by ceramide in NIH 3T3 fibroblasts,[32] and PC12 cells. In the latter cells, the anti-apoptotic effect correlated with inhibition of caspases-2, -3 and -7 and of the stress-activated protein kinase known as JNK.[34] Collectively, these various observations implicate SPHK1 and S1P formation in cell growth, transformation and cancer. Hence, SPHK inhibitors might be useful for treatment of cancers (see below).

Several mammalian lipid phosphate phosphohydrolases (LPPs) have been identified. Type 2 LPPs consist of a family of Mg^{2+}-independent, membrane-associated phosphatases that share sequence homology and *N*-ethylmaleimide sensitivity. Two genes, designated *LBP1* (also known as *YSR2* or *LCB3*) and *LBP2* (also known as *YSR3*), encoding specific sphingoid base phosphate phosphatases that regulate the concentrations of phosphorylated sphingoid bases and ceramide have been identified in *S. cerevisiae*. These S1P phosphatases share the conserved sequence motif that is present in all lipid phosphatases and thus belong to the family of LPPs. On the basis of sequence homology with *LBP1*, a cDNA encoding mammalian S1P phosphatase, SPP-1, was recently cloned from mouse. This 400 amino acid hydrophobic enzyme contains eight to 10 putative membrane-spanning regions and degrades S1P, but not lysophosphatidic acid, phosphatidic acid, or ceramide-1-phosphate. It may therefore contribute to regulation of the dynamic balance between the concentrations of sphingolipid metabolites in mammalian cells and, consequently, influence cell fate.[35]

S1P lyase is a pyridoxal-dependent enzyme that cleaves S1P at the C2–C3 bond to yield ethanolamine phosphate and hexedecanal. The first S1P lyase gene to be characterized was cloned from *S. cerevisiae*.[36] The corresponding deletion mutant accumulates endogenous phosphorylated sphingoid bases and exhibits an increased rate of proliferation during respiratory growth; this latter effect is associated with failure of normal recruitment of cells into the G_1 phase of the cell cycle.[37] On the basis of their sequence similarity to the yeast lyase gene, cDNAs encoding murine and human S1P lyases were subsequently cloned.[38,39] Human S1P lyase is a 568 amino acid protein and is abundant in the liver.[39] Site-directed mutagenesis revealed the importance of cysteine residues 218 and 317 of the human enzyme for the cleavage reaction.[39] S1P lyase, like S1P phosphatase, appears to be localized to the endoplasmic reticulum of cells.

SPOTLIGHT

Functions of S1P

S1P is a sphingolipid metabolite that plays important roles in the regulation of a variety of biological processes, including Ca^{2+} mobilization, reorganization of the cytoskeleton, as well as cell growth, differentiation, survival and motility.[6,40] Similar to other important phospholipid mediators, S1P exerts dual actions in cells: it acts intracellularly as a second messenger and extracellularly as a ligand for G protein-coupled receptors (GPCRs). S1P is thus the ligand for specific GPCRs that were originally known as the endothelial differentiation gene-1 (EDG-1) family of proteins but were recently renamed S1P receptors (S1PRs).[6,40] To date, five members of the S1PR family have been cloned – including $S1P_1$ (EDG-1), $S1P_2$ (EDG-5), $S1P_3$ (EDG-3), $S1P_4$ (EDG-6), and $S1P_5$ (EDG-8) – all of which bind and are activated specifically by S1P and dihydro-S1P (also known as sphinganine 1-phosphate, whose structure is identical to that of S1P with the exception that it lacks the 4,5-*trans* double bond). Members of this family of receptors are differentially expressed and coupled to a variety of G proteins. S1P is thus able to activate and regulate a diverse array of signal transduction pathways in different cell types, depending on the relative abundance of S1PRs and associated G proteins and resulting in a wide range of responses.[6,40] Despite their diversity, all of the S1PRs have been implicated in regulation (positive or negative) of cell motility.[6] For example, activation of $S1PR_1$ or $S1PR_3$, either by S1P or dihydro-S1P, in various cell types induces chemotaxis and membrane ruffling, whereas activation of $S1PR_2$ in the same cell types inhibits these same processes.[41] Consistent with these observations, stimulation of these receptors results in either activation or inhibition of members of the Rho family of small GTPases, most prominently Rho and Rac.[42] Members of the Rho family act downstream of the heterotrimeric G proteins and play important roles in reorganization of the cytoskeleton. Activated Rho thus induces the formation of stress fibers, and activated Rac induces formation of the cortical actin network.[43,44] Activation of $S1PR_1$ promotes this latter function of Rac,[42] whereas activation of $S1PR_2$ inhibits it and thereby prevents Rac-induced chemotaxis and membrane ruffling.[41] However, the binding of S1P to $S1PR_2$ or $S1PR_3$ triggers Rho-mediated stress fiber assembly.[41] The differential expression

SPOTLIGHT

of S1PRs is thus able to determine the chemotactic responses of cells to extracellular gradients of S1P.

Cell motility is important in many physiological and pathological processes, including inflammation, wound healing, angiogenesis, as well as tumor growth and metastasis, and it plays a crucial role during development. Several recent studies suggest that S1PRs contribute to cell motility during development. Disruption of the zebrafish gene *miles apart*, which encodes a homologue of mammalian $S1PR_2$, thus results in defective migration of myocardial cells during heart development.[45] Furthermore, disruption of the murine gene for $S1PR_1$ revealed that this receptor is essential for vascular maturation; although the $S1PR_1$ knockout embryos appeared to possess normal blood vessel networks, they died *in utero* as a result of a marked deficiency of smooth muscle cells and pericytes in the vessel walls and consequent massive hemorrhage.[46] Notably, fibroblasts from these embryos exhibited an attenuated Rac-mediated chemotactic response when positioned in an S1P gradient, emphasizing that $S1PR_1$ is essential for the recruitment of cells during microvasculature maturation.

$S1PR_1$ is also essential for cell migration toward a source of PDGF.[47] PDGF activates SPHK and thereby increases the intracellular concentration of S1P in a variety of cell types.[6] PDGF has also been shown to activate $S1PR_1$, as revealed by phosphorylation of the latter and its interaction with β-arrestin, thus providing a new example of cross-communication between a tyrosine kinase receptor (the PDGF receptor) and a GPCR ($S1PR_1$).[47] It is thus thought that the accumulation of S1P triggered by the PDGF-induced stimulation of SPHK results in the activation of $S1PR_1$ and of downstream signaling events important for cell locomotion.[47] Mice lacking the B chain of PDGF or the receptor for PDGF-BB manifest a phenotype similar to that of the $S1PR_1$ knockout mice; namely, embryonic death as a result of incomplete vasculogenesis.[48,49] The observation that $S1PR_1$ plays a critical role in directed cell motility thus unveils the underlying mechanism by which S1P acts as a regulator of angiogenesis.

S1P as an intracellular second messenger

In addition to its extracellular actions, S1P functions as a second messenger in the regulation of Ca^{2+} homeostasis,[24,28,50,51] and suppression of apoptosis.[11,12,14,23,31,52] Although the intracellular targets of S1P remain to be identified, several lines of evidence support the second messenger role of S1P: (1) dihydro-S1P binds to and activates all of the identified S1PRs, but it does not mimic all of the effects of S1P, especially those related to cell survival;[30] (2) yeast cells do not possess GPCRs, yet the abundance of phosphorylated long-chain sphingoid bases regulates environmental stress responses, cell proliferation, and cell survival in a manner reminiscent of the function of S1P in mammalian cells; (3) in plants, which do not express S1PRs, S1P regulates Ca^{2+} homeostasis and ion channels;[53] and (4) microinjection of S1P into, or photolysis of caged S1P within, mammalian cells induces both Ca^{2+} mobilization[54] and cell proliferation.[30]

S1P has been implicated as a second messenger in cellular proliferation and survival,[10] as well as in the protection against ceramide-mediated apoptosis.[11,12,14,23,31,52] The dynamic balance between the intracellular concentration of S1P and those of sphingosine and ceramide (the 'sphingolipid rheostat'), and the consequent activities of the respective opposing signaling pathways, has therefore been suggested to be an important factor in determination of survival or death in mammalian cells.[11,12,14,23] Thus, whereas stressful stimuli increase the concentrations of ceramide and sphingosine, leading to apoptosis, survival factors activate SPHK, resulting in accumulation of S1P and consequent suppression of apoptosis. The induction of ceramide-mediated apoptosis by the anticancer drug doxorubicin in unfertilized mouse oocytes is blocked by S1P, but not by the ceramide synthase inhibitor fumonisin B1.[12] This effect of S1P was not mimicked by lysophosphatidic acid[12] or by an equimolar concentration of dihydro-S1P. Furthermore, the protective effect of S1P in female germ cells appeared entirely independent of S1PRs coupled to G_i, as it was not influenced by treatment of the cells with pertussis toxin.[14] Collectively, these results underscore the specificity of S1P protection against ceramide-mediated apoptotic death as well as the likely S1PRs-independent nature of the anti-apoptotic effect of S1P in oocytes.

The sphingolipid rheostat is evolutionarily conserved, given that it also regulates the growth and survival of yeast cells during stressful situations.[55–57] It has also been implicated in several other biological processes, including Ca^{2+} homeostasis and allergic responses. Cross-linking of FcεRI induces activation of SPHK and conversion of sphingosine to S1P, which mediates Ca^{2+} mobilization from intracellular stores by an inositol trisphosphate-independent pathway,[26,28,54] whereas the Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) is a direct target of sphingosine.[58] The balance between sphingosine and S1P has been suggested to determine the allergic responsiveness of mast cells.[59] A high intracellular concentration of sphingosine thus markedly inhibits leukotriene synthesis and cytokine production in response to the combination of immunoglobulin E and antigen by preventing both activation of the ERK (extracellular signal-regulated kinase) signaling pathway and gene expression mediated by the transcription factor AP-1. In contrast, an increase in the intracellular concentration of S1P counteracts the inhibitory effects of sphingosine. SPHK thus likely plays a critical role in the activation of signaling cascades by FcεRI through its effect on the balance between S1P and sphingosine. SPHK also plays an important regulatory role in the cardiovascular system. Activation of this enzyme and the consequent generation of S1P contribute both to the stimulation of endothelial cells by TNF-α,[60] and to the triggering of a signaling pathway by this cytokine that protects the cells against apoptosis.[13] The ability of high density lipoprotein (HDL) to inhibit the cytokine-induced expression of adhesion molecules in endothelial cells has also been correlated with resetting of the sphingolipid rheostat.[61] This effect of HDL might be responsible, at least in part, for the protective function of this lipoprotein against the development of atherosclerosis and associated coronary heart disease.

S1P in hematopoietic malignancies: potential use of sphingosine kinase inhibitors

Disregulation of the sphingolipid biostat may be important in the acquisition of malignant phenotypes in which transformed cells circumvent existing apoptotic mechanisms that normally target destruction of mutated cells. The regulation of the sphingolipid biostat may also have implications for treatment of cancer, because many therapeutic approaches cause accumulation of ceramide, including the chemotherapeutic drugs cytosine arabinoside, vincristine, daunorubicin, and ionizing radiation. Further, inhibition of sphingosine kinase strongly affects induction of apoptosis of cancer cells and enhances sensitivity to gamma irradiation (reviewed in Refs 2

and 5). Indeed, some therapies that induce ceramide-mediated apoptotic pathways have been successfully manipulated to improve treatment of leukemias and lymphomas.

Modulation of the sphingolipid biostat to manipulate apoptosis susceptibility has been examined in numerous cancer cell lines. In fact, N,N-dimethylsphingosine (DMS), an inhibitor of sphingosine kinase, appears to be an ubiquitous inducer of apoptosis of cancer cells, including hematopoietic malignancies.[62–65] DMS also potentiates TNF- and FasL-induced apoptosis in the human acute leukemia Jurkat, U937 and HL-60 cell lines,[11,66] which suggests that combining DMS with cytotoxic drugs might be a useful chemotherapeutic approach. In addition, DMS inhibits *in vivo* growth of human gastric carcinoma cells in nude mice.[67] Although in these animal experiments, DMS also caused some hemolysis, liposomal delivery enhanced its potency and reduced toxicity.[68]

An analog of sphingosine, L-threo-dihydrosphingosine (known as safingol), which also acts as a sphingosine kinase inhibitor, enhanced doxorubicin accumulation and sensitivity of MCF-7 drug-resistant cells.[69] Because DMS and L-threo-dihydrosphingosine induce apoptosis regardless of P-glycoprotein expression, they may provide a new strategy for the treatment of anticancer drug-resistant cancers. Indeed, in pilot clinical phase I trials with safingol, which also potentiated the tumor-inhibiting effect of doxorubicin in tumor-bearing animals, it was found that safingol can be given safely with doxorubicin.[70]

A recent study showed that DMS was equipotent for primary leukemic and drug-resistant leukemia cells,[71] while a P-glycoprotein-positive HL-60 line was even more sensitive than parental HL-60 cells.[71] Because P-glycoprotein expression is among the strongest prognostic factors in acute myologenous leukemia, agents such as DMS, might be useful therapeutically.[72] These results with leukemia specimens that have not been subjected to *in vitro* selection validate the sphingolipid rheostat regulation of the apoptotic pathway in fresh human tumors, and support initiation of pilot studies and phase I clinical trials that include DMS and other sphingosine kinase inhibitors as part of the regimen.

In vivo studies with S1P

Gametogenesis during fetal ovarian development is associated with a high rate of germ cell apoptosis.[73] Evidence that hydrolysis of sphingomyelin to ceramide is a key event in the apoptosis of these cells was provided by a histological evaluation of oocyte endowment in neonatal (postpartum day 4) female *ASMase*$^{-/-}$ mice, which lack the gene for sphingomyelinase. Compared with their wild-type female littermates, *ASMase*$^{-/-}$ females possessed an excess of more than 1100 oocyte-containing primordial follicles per ovary as well as exhibited marked hyperplasia of the early-growing (primary and small preantral) follicle populations. The oocyte reserve was increased in *ASMase*$^{-/-}$ females throughout young adulthood, well before the onset of Niemann–Pick disease-like symptoms previously demonstrated to occur in these knockout animals during postnatal life.[74] The basis of the extensive oocyte hyperplasia in *ASMase*$^{-/-}$ neonates was investigated by *ex vivo* culture of ovaries harvested from *ASMase*$^{+/+}$ and *ASMase*$^{-/-}$ mice at embryonic day 13.5.[75] Whereas culture of the wild-type ovaries without hormonal support for up to 72 h resulted in a time-dependent activation of programmed cell death in germ cells, germ cell apoptosis was substanitally attenuated in the *ASMase*$^{-/-}$ fetal ovaries. These observations are indicative of a cell death defect intrinsic to the ovaries of *ASMase*$^{-/-}$ mice, and they point to enhanced survival of the developing germ line during oogenesis as the mechanism responsible for the marked enlargement of the oocyte pool at birth.

Consistent with the notion of a ceramide-S1P rheostat, treatment with S1P was shown to mimic the effect of ASMase deficiency in the *ex vivo* fetal ovarian culture model. Whereas culture of wild-type fetal ovaries in the presence of the selective ceramide synthase inhibitor fumonisin B1 did not affect the rate of oocyte survival,[76,77] the reduced incidence of germ cell apoptosis associated with ASMase deficiency was recapitulated by culture of wild-type ovaries with S1P. Indeed, approximately equivalent levels of germ cell survival were achieved by either disruption of *ASMase* or treatment with S1P at a concentration of 10 μM. The cell autonomous (germ line-intrinsic) nature of these effects was confirmed by culture of individual oocytes from adult wild-type and *ASMase*$^{-/-}$ female mice in the presence of the anticancer drug doxorubicin to induce apoptosis.[78] Whereas a marked apoptotic response was apparent in wild-type oocytes after 24 h, *ASMase*$^{-/-}$ oocytes were almost completely resistant to doxorubicin. Moreover, S1P at a concentration of 10 μM mimicked the inhibitory effect of the peptide caspase inhibitor zVAD-fmk on doxorubicin-induced oocyte apoptosis.

These studies were extended to an *in vivo* model of oocyte apoptosis and ovarian failure. Exposure of C57/BL6 mice to a single dose of 0.1 Gy of ionizing radiation results in almost complete destruction of the oocyte-containing primordial follicle reserve within 2 weeks. However, *in vivo* administration of S1P 2 h before irradiation induced a significant and dose-dependent protective effect, with complete protection of the quiescent (primordial) and growing (primary, preantral) follicle populations achieved by the highest dose of S1P administered (200 μM). Protection of the oocyte pool was also reflected in ovary size, which was markedly reduced in vehicle-pretreated mice compared with that in S1P-pretreated mice.[14]

In addition to its effect on oocyte number, treatment with S1P before irradiation also resulted in higher quality oocytes, as determined by subsequent *in vitro* fertilization. For these studies, oocytes were harvested from vehicle- or S1P-pretreated mice 2 weeks after exposure to 0.1 Gy of ionizing radiation and were then fertilized *in vitro* to examine their competency for preimplantation embryonic development. Whereas the rates of fertilization and the numbers of two-cell embryos produced did not differ between the two groups of oocytes, progression of embryonic development to the blastocyst stage was markedly impaired for oocytes harvested from the vehicle-pretreated mice compared with oocytes from the S1P-pretreated animals. The anomalies characteristic of poor quality blastocysts that were apparent at an increased frequency with the oocytes from vehicle-pretreated mice included arrested development, failure to differentiate an inner (embryonic) cell mass, the occurrence of apoptosis, and the inability to hatch from the zona pellucida. Collectively, these data,[12,14] support the idea that pretreatment with S1P *in vivo* effectively preserves ovarian follicular dynamics and oocyte competency in animals exposed to radiation. Further, preliminary studies show that S1P preserves fertility in irradiated female mice without propagating genomic damage in offspring (Kolesnick and Tilly, unpublished).

The results of these studies thus provide substantive evidence for a germ cell-autonomous death defect caused by ASMase deficiency, and they indicate that this phenotype can

SPOTLIGHT

be completely reproduced in oocytes by the ceramide antagonist S1P. They also suggest that pharmacological agents that increase the concentration of S1P, perhaps through the activation of SPHK, or treatment with S1P itself might prove beneficial for the prevention of oocyte destruction induced by cancer therapy.

Future challenges

The results of the many studies described in this review provide strong support for the notion that S1P functions as both a first messenger and a second messenger. It thus acts extracellularly by binding to members of the S1PR family of GPCRs, thereby regulating cell motility, and it acts intracellularly to regulate both cell growth, survival and Ca^{2+} homeostasis. Future challenges include further characterization of the specific roles of the various S1PRs as well as identification both of the intracellular targets of S1P and of the source of extracellular S1P. The development of antagonists or agonists of S1PRs or of inhibitors or activators of enzymes that affect the intracellular concentration of S1P may make it possible to manipulate S1P function for therapeutic purposes, as suggested above for the protection of oocytes in women undergoing radiation treatment or chemotherapy for cancer. These intriguing studies,[14] suggest that S1P may be useful as a radioprotective or chemoprotective agent for the ovaries of young girls and women. Furthermore, S!P might be a useful agent to improve the quality of the eggs used for *in vitro* fertilization, a major reason for the common failure of this procedure.

Gastrointestinal tract damage by chemotherapy or radiation limits their efficacy in cancer treatment. In ground-breaking studies, it was recently reported that radiation-induced crypt damage, organ failure, and death from the gastrointestinal syndrome could be prevented when endothelial apoptosis was inhibited pharmacologically by intravenous basic fibroblast growth factor or genetically by deletion of the acid sphingomyelinase gene.[79] Exogenous S1P mimics the phenotype of ASMase knockout in multiple assays of ovarian apoptosis *ex vivo* or *in vivo*. If similar relationships exist in other organ systems, it is tempting to speculate that S1P, like deletion of the *ASMase* gene, might be able to protect endothelium and secondarily, the gastrointestinal tract from radiation and endotoxin damage.[79] Studies designed to address this issue are currently in progress (Kolesnick, unpublished).

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