Sphingosine 1-phosphate (S1P), generated by the action of sphingosine kinase, is a potent bioactive phospholipid that regulates a remarkably broad spectrum of biological processes, including cell proliferation, apoptosis, calcium homeostasis, cell migration, angiogenesis and vascular maturation (1). The reasons for these diverse actions of S1P have begun to be elucidated in the last few years with the discovery that this phospholipid has novel dual actions, both inside and outside of cells (2). Extracellularly, S1P acts as a ligand for a family of five G protein-coupled receptors which bind only S1P and dihydro-S1P with high affinity. The various S1P receptors are differentially expressed, and couple to different G proteins that regulate numerous downstream signalling pathways. This allows extracellular S1P the ability to regulate a diverse range of physiological processes, including cell migration, angiogenesis and differentiation, in a highly cell-specific manner depending on the relative expression of S1P receptors and G proteins (3). In addition to this extracellular role, S1P also acts as an intracellular second messenger in the regulation of other physiological processes, including enhancing cell proliferation, suppressing apoptosis and calcium homeostasis (2). Although the direct intracellular targets of S1P have not yet been identified, considerable evidence, reviewed in more detail elsewhere (2), supports this intracellular role, including studies employing microinjection of S1P into, or photolysis of caged S1P within, mammalian cells, the use of yeast and plant cells that naturally lack cell-surface S1P receptors, and mammalian cells genetically manipulated to be devoid of these receptors.

The cellular levels of S1P are largely controlled through its formation from sphingosine by the activity of sphingosine kinase, and to a lesser extent by its degradation by S1P lyase and S1P phosphatases (Fig. 1). In most cells this balance between S1P generation and degradation results in low basal levels of S1P in the cell. However, when cells are exposed to specific growth factors and other agonists, S1P levels can increase rapidly and transiently as a direct consequence of rapid changes in sphingosine kinase activity in the cell (4). As described above, the S1P generated by sphingosine kinase activation can act either intracellularly as a second messenger or be released from cells, via a secretory mechanism not yet understood, to activate cell-surface S1P receptors to initiate paracrine or autocrine signalling cascades (Fig. 2). This agonist-induced increase in S1P, and its downstream consequences can be prevented by the addition of sphingosine kinase inhibitors or expression of a dominant-negative sphingosine kinase (4). This places sphingosine kinase, and its activation, in a central and obligatory role in controlling the observed effects attributed to S1P signalling. This review describes the current understanding of the regulatory mechanisms controlling the activity of this signalling enzyme, and outlines the implications of this work in the development of therapeutics to combat cancer and other conditions where the disregulation of sphingosine kinase has been implicated in disease progression.

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**Fig. 1. Formation and degradation of sphingosine 1-phosphate (S1P).**
Sphingosine kinases catalyse the formation of S1P from sphingosine. Degradation of S1P can occur via two pathways; S1P phosphatases convert S1P back to sphingosine, while S1P lyases cleave S1P into hexadecenal and phosphoethanolamine which are reused for biosynthesis of phosphatidylethanolamine.
SK1 dysregulation in naturally occurring tumours, including the findings that SK1 mRNA levels are increased in a variety of human tumours (8); brain tumours with high SK1 expression correlated with poor survival of patients (9); high expression of SK1 appears to be an oncogenic event required for erythroblastic leukaemia progression (10); SK1 is integral in estrogen-dependent regulation of breast tumour cell growth and survival (11); and perhaps most compellingly, the use of sphingosine kinase inhibitors significantly reduced tumour growth in vivo in mice (8). Furthermore, sphingosine kinase inhibitors, expression of a dominant-negative SK1 mutant, or siRNA-mediated suppression of SK1 expression have also been recently shown to sensitize many tumour cells to chemotherapeutics (12).

In addition to this apparent role in tumourigenesis, sphingosine kinase has also been implicated in a number of other (patho)physiological processes. These include inflammation where sphingosine kinase appears to act at several levels, including regulating adhesion molecule expression by vascular endothelial cells, as well as being integral in neutrophil priming and macrophage responses (13). Sphingosine kinase has also been implicated in asthma, being involved in both determining the allergic responsiveness of mast cells and inducing constriction of airway smooth muscle cells (14), and hypertension, through regulation of vascular smooth muscle cell constriction (15).

These findings have provided considerable impetus for the targeting of sphingosine kinase in the development of therapies, primarily for cancer and inflammation. Thus, in addition to the continuing search for effective sphingosine kinase inhibitors, much of the current work in this area is directed towards understanding both the mechanisms regulating the catalytic and functional activity of sphingosine kinase (the focus of the remainder of this review), and the downstream signalling pathways controlled by this enzyme.

Sequence and Structural Features of Sphingosine Kinases

Sphingosine kinases have been cloned from a variety of eukaryotic organisms, ranging from human to yeast and plants. The sphingosine kinases are evolutionarily well conserved and, together with the ceramide kinases, comprise a novel family of proteins distinguished by five conserved regions (C1 to C5) in their polypeptide sequences (16). Apart from sharing some similarity with the ‘catalytic domain’ of the diacylglycerol kinases, the sphingosine kinase sequences are surprisingly devoid of other known protein domain motifs that could allow predictions of its regulation, localisation or interaction partners. Notably, the SK sequences show no clear homology to the well established ATP-binding motifs of other kinases. However, we identified Gly82 and Lys103 to be essential in nucleotide-binding in human SK1, and have suggested a motif of SGDGx17-21K to represent the nucleotide-binding region of sphingosine kinases (16).
This nucleotide-binding region is unique to this family of proteins, and only shows weak sequence similarity to the well characterised and highly conserved glycine-rich loop motifs of protein kinases and phosphatidylinositol phosphate kinases. This divergence in sequence, and likely structure (the structure of sphingosine kinase has not yet been determined), of the ATP-binding site of the sphingosine kinases from that of other known kinases raises the possibility of generating specific ATP-binding site-directed sphingosine kinase inhibitors.

Human Sphingosine Kinases

Two human sphingosine kinase isoforms have been identified. While these two enzymes, designated SK1 and SK2, originate from different genes and differ in size (43 kDa and 65 kDa, respectively), they possess a high degree of sequence similarity. Almost all of the SK1 polypeptide sequence aligns with regions of the larger SK2, with 80% similarity (17). SK2, however, also possesses two additional polypeptide regions, one at the N-terminus and the other within the central region that are quite distinct from SK1 and any other protein. Both SK1 and SK2 generate S1P from sphingosine, although SK2 has approximately 10-fold lower specific activity, appears somewhat more promiscuous than SK1 in the substrates it can utilise, and has different developmental expression (17, 18).

Despite their sequence and catalytic similarity, SK1 and SK2 appear to have contrasting roles in the cell. As noted earlier, SK1, on which most work has focussed, has a pro-survival, pro-proliferative effect on cells. Recent studies have suggested, however, that SK2 may have an opposing role to SK1 and enhance apoptosis (19). As detailed later, the reasons for these conflicting roles for SK1 and SK2 are now beginning to be elucidated with the cellular localisation of the isoforms appearing pivotal in determining their function.

Sphingosine Kinase Activation

Sphingosine kinase activity is always present in cells due to the intrinsic catalytic activity of SK1 that is not dependent on post-translational modifications (20). This basal level of cellular sphingosine kinase activity is thought to function in a ‘house-keeping’ role in maintaining low sphingosine levels in the cell. As noted earlier, however, exposure of cells to various growth factors, cytokines and other agonists results in sphingosine kinase activation, and the subsequent signalling functions of this pathway. Recently, the mechanism of activation of human SK1 has been identified and shown to be brought about by phosphorylation at Ser225 (21). This phosphorylation, which is mediated by ERK1/2, results in a direct 14-fold increase in the $k_{cat}$ of the enzyme, while having no effect on the $K_m$ values for either substrate. Notably, this single phosphorylation not only controls the catalytic activity of SK1, but is also necessary for agonist-induced translocation of the enzyme from the cytosol to the plasma membrane. The mechanism of agonist-induced translocation of SK1 remains to be elucidated, although recent studies provide evidence that a phosphorylation-induced conformational change in SK1 enhances its binding to phosphatidylserine and, thus, prolongs its retention at the plasma membrane (22).

Comparatively little is known about the regulation of SK2 activity, with only a few very recent studies reporting activation of this enzyme following cell exposure to some cytokines and growth factors that are also known to induce SK1 activation. Interestingly, however, the phosphorylation site involved in SK1 activation is not conserved in SK2.
Importance of Sphingosine Kinase Localisation for its Cellular Function

As inferred previously, there is now growing evidence that the roles of the sphingosine kinases may be largely determined by their cellular localisation. SK1 is predominately a cytosolic enzyme. However, agonist-induced activation of SK1 results in the rapid translocation of the enzyme to the plasma membrane (21). This phosphorylation-dependent localisation of SK1 to the plasma membrane is integral for its signalling role in enhancing cell proliferation and survival since, in contrast to wild-type SK1, expression of a non-phosphorylatable SK1 provided no survival or proliferative effects on cells (7). Artificial localisation of this non-phosphorylatable mutant to the plasma membrane, however, restored its pro-survival, pro-proliferative signalling (7).

The localisation of SK2 appears more complex than that of SK1. While the most convincing data suggests SK2 resides predominantly in internal membranes, various other studies report SK2 localisation to the cytosol, nucleus and plasma membrane and appears dependent on cell type and also cell density (19). The levels of SK2 at the endoplasmic reticulum (ER) are enhanced during serum starvation and this localisation appears critical for its pro-apoptotic function. In keeping with this, artificial targeting of SK1 to the ER converts the normally anti-apoptotic enzyme into one with a pro-apoptotic function (19).

Thus, current understanding is that localisation of sphingosine kinase activity to the plasma membrane provides pro-survival, pro-proliferative signalling to the cell, while ER localisation imparts apoptotic signalling. But, how does this differential localisation result in such different signalling outcomes? Plasma membrane localisation of SK1 enhances secretion of S1P from the cell (7), allowing for engagement of the cell-surface S1P receptors. However, as noted earlier, these receptors do not appear involved in the pro-survival, pro-proliferative functions of SK1. Thus, the effectors of S1P generated at the plasma membrane that control these functions remain to be resolved, with one recent study even suggesting that the break-down products of S1P lyase action on S1P, phosphoethanolamine and hexadecenal (Fig. 1), may be the true intracellular effectors, and not S1P itself (23).

The mechanisms behind the pro-apoptotic effects of S1P generated at the ER are also unclear. One possible explanation proposed by Sarah Spiegel (19) draws upon the dynamic nature of the sphingolipid biosynthetic/metabolic pathway, and suggests that the subsequent metabolism of S1P at the ER by S1P phosphatase to eventually generate ceramide, a known pro-apoptotic molecule, may drive its apoptotic signalling in this location (Fig. 2). Many questions, however, remain unanswered in this model, such as: (i) how ER-generated S1P is preferentially directed to the S1P lyase and away from S1P phosphatase, and; (iii) why the phosphorylation and subsequent dephosphorylation of sphingosine appears necessary for channelling of this lipid into ceramide biosynthesis.

Although the studies described above provide strong evidence for opposing cellular roles for SK1 and SK2 through their differential localisation, in vivo studies using SK1 or SK2 knockout mice suggest that these enzymes may have compensatory roles under these conditions, at least in mouse development (24, 25). Notably, SK1/SK2 double-knockout mice die in vivo due to severe defects in neurogenesis and angiogenesis (25).

Perspectives

The sphingosine kinases are a novel family of proteins that have effects on a diverse array of signalling pathways. SK1, in particular, has been the subject of considerable interest in the development of potential anti-cancer and anti-inflammatory therapeutics. Recent studies describing the differential signalling of SK1 and SK2, combined with the known ‘house-keeping’ functions of these enzymes, suggest that direct inhibitors of total cellular sphingosine kinase activity may not be the best therapeutics. Instead, targeting the mechanisms of activation and differential localisation of these enzymes that appear to control their signalling functions may provide more specific therapeutic options. Clearly, further work is required to understand the precise mechanisms regulating the phosphorylation status and localisation of SK1 before such therapeutics can be developed.

References