Functions of the Multifaceted Family of Sphingosine Kinases and Some Close Relatives*

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Sphingosine kinases (SphK) are prototypical members of a highly conserved family of signaling enzymes. They are present in organisms as diverse as mammals, flies, worms, yeast, and plants and catalyze the phosphorylation of sphingosine to form the bioactive sphingolipid metabolite sphingosine 1-phosphate (SIP). Two distinct mammalian isoforms have been identified, SphK1 and SphK2. Other close relatives, ceramide kinase (CerK) and the more promiscuous acylglycerol kinase (AGK), are now emerging as lipid-signaling kinases with important functions. This brief review will focus on the biochemical properties of this novel family of lipid kinases with emphasis on recent studies that have begun to uncover the biological functions of their phosphorylated products.

SIP in Immunity and Cancer

As a specific ligand for a family of five G protein-coupled receptors, termed SIP1–5, SIP regulates diverse physiological processes important for cancer as well as lymphocyte trafficking, immunity, and allergy. SIP also has receptor-independent intracellular functions in mammalian cells important for calcium homeostasis, cell growth, and suppression of apoptosis (reviewed in Ref. 1), consistent with the observations that lower intracellular functions in mammalian cells important for calcification, immunity, and allergy. SIP also has receptor-independent intracellular functions in mammalian cells important for calcium homeostasis, cell growth, and suppression of apoptosis (reviewed in Ref. 1), consistent with the observations that lower.

Many reports have shown that SIP is an important player in the regulation of cancer cell survival and tumor progression. Growth factors, hormones important for progression of cancer, and cytokines stimulate SphK1 and production of SIP (1). SphK1 is a critical regulator of the balance between the pro-growth and anti-apoptotic SIP and its pro-apoptotic precursors ceramide and sphingosine. Numerous previous studies have shown that overexpression of SphK1 promotes tumorigenesis (12). In accordance, down-regulating its expression in cancer cells reduces growth, increases apoptosis, and enhances chemosensitivity (13). Recently it was suggested that SphK1 regulates autophagy, a normal physiologic self-digestion mechanism for the turnover of cellular proteins and excess or damaged organelles, to protect cancer cells from apoptosis during nutrient starvation (14). Moreover, SphK1 is up-regulated in a variety of solid tumors, including breast, colon, lung, ovary, stomach, uterus, kidney, and rectum (15), and several lipid and non-lipid SphK inhibitors have anti-tumor activity in xenograft models (16). An elegant study recently demonstrated that intravenous administration of a monoclonal antibody that neutralizes SIP drastically reduced tumor progression and associated angiogenesis in several animal models of human cancer (17). These results suggest that SIP not only has effects on tumor cells themselves but also is permissive or required for the actions of angiogenic factors and provide proof of concept that targeting of this important sphingolipid signaling molecule is a novel strategy for the development of new types of cancer treatments.

Transactivation of growth factor receptor tyrosine kinases by G protein-coupled receptor ligands, such as SIP, is important for amplification of signaling and regulation of cell growth. A reciprocal mechanism of receptor cross-talk has been shown to regulate movement of cells whereby activation of receptor tyrosine kinases stimulates and translocates SphK1 to the plasma
membrane, resulting in spatially restricted formation of S1P that in turn activates S1P₁ (or other S1P receptors present on the cell surface) and downstream signaling events critical for directed cell movement (Fig. 1) (18). Thus, S1P might be the central controller of several amplification loops, in line with the emerging view of the intricacy and nonlinearity of signaling via S1P receptors and receptor tyrosine kinases and the importance of membrane compartmentalization of a signaling complex (signalplex). This web has become even more entangled by the recent demonstration that estrogen acting on its own receptors stimulates SphK1 and the release of S1P, which in turn activates S1P₃ leading to EGFR transactivation in a matrix metalloprotease-dependent manner (19).

**How Is S1P Transported?**

It is still a mystery how S1P produced inside cells by two SphKs can reach its receptors on the cell surface. Although it has been suggested that extracellular production of S1P by exported SphK1 may contribute to the establishment of the vascular S1P gradient (20), data from many studies indicate that intracellularly produced S1P itself is secreted. Studies originally related to multidrug resistance in cancer cells identified several ATP-binding cassette (ABC) transporters, including ABCB1 (previ-
SphK1 and SphK2 have different kinetic properties and but diverges in its central region and has a longer amino terminus. SphK1 and SphK2 have different physiological functions. Little is known of the functions of SphK2, although its endogenous overexpression suppresses cell growth and enhances apoptosis in mast cell-mediated immune responses. It will be important to determine whether S1P release from other cells, particularly cancer cells, is mediated by ABC transporters.

**SphK1 Versus SphK2: Castor and Pollux**

The ubiquitously expressed SphK2 also has the same five evolutionarily conserved domains found in all SphKs (Fig. 2) but diverges in its central region and has a longer amino terminus. SphK1 and SphK2 have different kinetic properties and also have different developmental and tissue expression patterns, suggesting that they have distinct physiological functions. Little is known of the functions of SphK2, although its overexpression suppresses cell growth and enhances apoptosis (24). These effects of SphK2 might be related to its localization to the nucleus (25) or endoplasmic reticulum (26) or to its putative BH3 domain (24). However, the role of endogenous SphK2 is still controversial as down-regulating its expression suppresses cell growth and enhanced apoptosis of glioma cells (27) yet protected HEK 293 cells from serum withdrawal-induced apoptosis (25).

Importantly, expression of SphK2 increased production of ceramide, whereas SphK1 decreased it (26). In accordance, down-regulating SphK2 reduced conversion of sphingosine to ceramide in the recycling pathway, and conversely, down-regulating SphK1 increased it (13, 26). How do SphK1 and SphK2 exert opposite effects on ceramide levels? Sphingosine is not produced by de novo biosynthesis; rather, it is derived from cleavage of ceramide by ceramidas and can then be re-utilized for ceramide and complex sphingolipid synthesis or phosphorylated by SphKs to form S1P. Thus, SphK2 might play a role in a sphingosine salvage pathway of mammalian cells, acting in concert with S1P phosphatase to convert S1P back to sphingosine and then to ceramide. In contrast, S1P formed by SphK1 may inhibit ceramide biosynthesis as a cellular sensing mechanism to regulate levels of ceramide (26).

The two SphK isoenzymes might also have some overlapping and/or complementary functions. For example, both SphK1 and SphK2 were required for EGF-induced migration of MDA-MB-453 breast cancer cells but not for motility of HEK 293 cells (28). Furthermore, although the single knock-out mice have no phenotype, the SphK1/SphK2 double knock-out is lethal with severely disturbed neurogenesis, including neural tube defects and aberrant angiogenesis, accompanied by a dramatic increase in apoptosis and a decrease in mitosis in the developing nervous system (29).

Much progress has been made in the last several years in understanding how external stimuli regulate SphK1 activity. Although activation of protein kinase C by phorbol ester and VEGF induced phosphorylation of SphK1 and translocation to the plasma membrane, protein kinase C does not phosphorylate SphK1 directly. Rather, activated ERK2 phosphorylates SphK1 on serine 225, which both increases its activity and is necessary for its translocation from the cytosol to the plasma membrane and also for its oncogenic function (30). Recently, it was suggested that this phosphorylation may induce a conformational change or electrostatic switch of SphK1 that allows it to specifically interact with phosphatidylserine in the plasma membrane (31). It has also been suggested that release of calcium and association of Ca$^{2+}$/CaM with hSphK1 through its CaM binding site is also involved in translocation of SphK1 to the plasma membrane (32). Whether SphK2 is also regulated similarly is still unknown.

**Ceramide Kinase and Ceramide 1-Phosphate: Little Brother Grows Up**

Based on sequence homology to SphK1 and SphK2, a related lipid kinase was cloned that catalyzed the phosphorylation of ceramide to form ceramide 1-phosphate (C1P) (33). Ceramides with acyl chains of 8 or 16 carbons were the best substrates, whereas short-chain ceramides were poor substrates. CerK contains several regions conserved in SphKs (Fig. 1), an N-terminal pleckstrin homology domain that binds phosphatidylinositol 4,5-bisphosphate and a calcium/CaM binding motif (33). Recent studies demonstrated that the pleckstrin homology domain of CerK regulates its membrane targeting and activity and that calmodulin is involved in calcium-dependent activation (34, 35). Expression of a CerK homolog (CerKL) has been detected in several human tissues, and a truncated form of CerKL in the nucleus has been associated with retinitis pigmentosa (36). Surprisingly, however, CerKL has no detectable ceramide phosphorylating activity, and its biological function is thus not clear.

Down-regulation of CerK expression led to the discovery of a novel role for its product C1P as a proximal mediator of arachidonic acid release (37), a critical event in the production of all eicosanoids. Interleukin-1β activated CerK in A549 human lung cancer cells leading to increased C1P and concomitant increased arachidonic acid release, and down-regulation of CerK suppressed arachidonic acid release (37). Moreover, treatment with exogenous C1P induced translocation of cPLA$_2$ from the cytosol to the Golgi apparatus, a known site of trans-
location in response to agonists. Indeed, CerK is also localized to the Golgi (34), and in vitro binding assays showed that C1P interacts directly with cPLA2 (38) to not only increase its membrane affinity but also to allosterically activate it (Fig. 1) (39). Notably, the binding site for C1P is within the C2 domain of cPLA2α, in a region distinct from the pleckstrin homology domain.

There is also evidence that SphK1 and S1P mediate the effects of cytokines on induction of cyclooxygenase 2 (COX-2), the enzymes of CerK and formation of C1P can trigger the eicosanoid cascade. This mechanism would ensure coordinated activation/translocation of cPLA2 and induction of COX-2, the enzymes that generate and metabolize arachidonic acid, respectively, leading to formation of prostaglandins, suggesting that these two phosphorylated sphingolipid metabolites and the kinases responsible for their formation, SphK1 and CerK, may act in concert to regulate inflammatory responses. A recent study suggests that CerK and C1P also regulate phagocytosis (41) as CerK-transfected cells displayed a significant increase in phagocytic index in association with increased activity and translocation to lipid rafts and increased membrane liquid crystalline order after activation with opsonized erythrocytes leading to promotion of phagosome formation (41).

Exogenous C1P was originally shown to have mitogenic properties, and more recently, it has been described as a potent inhibitor of apoptosis and inducer of cell survival (42). Interestingly, CerK was identified as the accelerated cell death 5 (ACD5) gene product in Arabidopsis thaliana (43). Plants harboring an ACD5 mutation undergo programmed cell death accompanied by increased accumulation of ceramide prior to death. It is also worth mentioning in this regard that down-regulation of CerK reduced proliferation of A549 cells, progression into S phase, and enhanced apoptosis induced by serum starvation. Thus, CerK may determine the balance between pro-apoptotic ceramide and anti-apoptotic C1P to regulate mammalian cell fate.

AGK: the Profligate Sibling

While searching for additional isoforms of SphK, a related gene was identified that when expressed encoded an AGK that phosphorylated monoacylglycerols and diacylglycerols to form LPA and PA, respectively (44). Both of these phospholipids regulate pivotal processes related to pathogenesis of cancer, and LPA has long been implicated as an autocrine and paracrine growth stimulatory factor for many cancers (45). AGK, also known as MULK (multisubstrate lipid kinase), might be more promiscuous in vitro (46) than in vivo (44). It is still not clear why AGK is localized to the mitochondria or what the functions of LPA and PA are there. In addition to actions of LPA through its conventional G protein signaling pathways, activation of LPA receptors can indirectly regulate cell functions by transactivating the EGF tyrosine kinase receptor (47). AGK, which is highly expressed in prostate cancers, could play a role in prostate cancer progression as its overexpression in prostate cancer cells increased formation and secretion of LPA, resulting in transactivation of EGFR and activation of ERK1/2 leading to increased cell growth (44). Conversely, its down-regulation blocked EGF-induced ERK1/2 activation and cell proliferation and also decreased EGFR-mediated cell motility, which plays an important role in androgen–refractory prostate cancer. Because AGK can also phosphorylate 2-arachidonoyl glycerol, an endogenous cannabinoid and pro-apoptotic lipid, converting it to LPA may regulate the dynamic levels of these counterregulatory lipids that have been shown to play opposing roles in growth and survival.

Of note, an LPA-specific phosphatase localized to the mitochondria has been implicated in the progression of prostate cancer (48). A recent study suggests that expression of AGK in esophageal cancer tissue was significantly lower than in corresponding normal esophageal mucosa and correlated with poor prognosis (49). Interestingly, LPA fatty acyl transferase, endophilin B1, is required for the maintenance of mitochondrial morphology and is translocated to the mitochondria during the synchronous remodeling of the mitochondrial network that occurs during apoptosis (50).

AGK could play an important role in cellular responses induced by EGF as it regulates production and secretion of LPA, which in turn stimulates the release of mature EGF and thus activates the EGF receptor, amplifying mitogenic and survival signals (Fig. 1). Remarkably, expression of AGK is stimulated by EGF and even by LPA itself (44), thereby providing a positive feed-forward stimulus that could further enhance EGFR-dependent and –independent processes important for cancer progression. Therefore, targeting AGK, which is upstream of EGFR, might offer additional therapeutic benefits in treatment of many types of cancers in which EGF and LPA play a synergistic role.

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