Sphingosine kinase-1 – a potential therapeutic target in cancer

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Sphingolipid metabolites play critical functions in the regulation of a number of fundamental biological processes including cancer. Whereas ceramide and sphingosine mediate and trigger apoptosis or cell growth arrest, sphingosine 1-phosphate promotes proliferation and cell survival. The delicate equilibrium between the intracellular levels of each of these sphingolipids is controlled by the enzymes that either produce or degrade these metabolites. Sphingosine kinase-1 is a crucial regulator of this two-pan balance, because it produces the prosurvival sphingosine 1-phosphate, and reduces the content of both ceramide and sphingosine, the proapoptotic sphingolipids. Sphingosine kinase-1 controls the levels of sphingolipids having opposite effects on cell survival/death, its gene was found to be of oncogenic nature, its mRNA is overexpressed in many solid tumors, its overexpression protects cells from apoptosis and its activity is decreased during anticancer treatments. Therefore, sphingosine kinase-1 appears to be a target of interest for therapeutic manipulation via its pharmacological inhibition. Strategies to kill tumor cells by increasing their ceramide and/or sphingosine content while blocking sphingosine 1-phosphate generation should have a favorable therapeutic index. *Anti-Cancer Drugs* 18:105–110 © 2007 Lippincott Williams & Wilkins.

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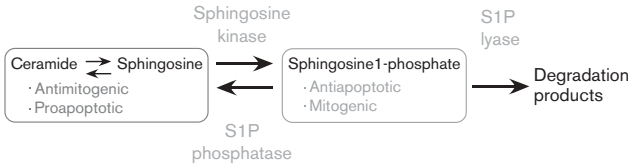
Introduction

Sphingolipids are a family of compounds that, in addition to being structural constituents of cell membranes, play key roles as signaling molecules (Fig. 1). In particular, two of these sphingolipid metabolites, ceramide and sphingosine 1-phosphate (S1P), have garnered considerable attention over the last decade as critical mediators of cell death or survival (reviewed in [1–3]). Ceramide, the central molecule in sphingolipid metabolism, mediates apoptosis in response to a wide array of anticancer treatments through de-novo synthesis and/or the hydrolysis of sphingomyelin (reviewed in [2,4]). Chemotherapy and radiotherapy elicit an increase in the endogenous ceramide level occurring before the first biochemical signs of apoptosis, i.e. mitochondrial release of apoptogenic proteins such as cytochrome *c* and activation of effector caspases (reviewed in [2]). Moreover, addition of exogenous short-chain ceramides causes apoptosis in numerous cancer cell lines (reviewed in [2]). In contrast to ceramide, S1P promotes cell survival in response to apoptotic stresses that induce ceramide generation *in vitro*, *ex vivo* and *in vivo* [5–7]. The opposing directions of ceramide-mediated and S1P-mediated signaling gave birth to the concept of a ceramide/S1P biostat, and the postulate that the ratio between these two lipids could determine the cell fate [5].

Fig. 1



The sphingolipid biostat: a conversed stress regulator. Ceramide, sphingosine and sphingosine 1-phosphate (S1P) are interconvertible sphingolipids. Both ceramide and sphingosine have been associated with growth arrest and apoptosis in response to multiple stress signals (e.g. chemotherapeutics, irradiation). On the contrary, many prosurvival stimuli can trigger increased sphingosine kinase-1 activity leading to S1P content augmentation. The levels of S1P can also be regulated by the S1P phosphatase and lyase activities.

A crucial regulator of this ceramide/S1P balance is the sphingosine kinase (SphK1 and SphK2) that phosphorylate sphingosine (the catabolite of ceramide) to form S1P [8,9]. SphK1 has been shown to be a key enzyme in

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sphingolipid metabolism, because it serves the dual function of modulating ceramide and S1P levels; not only does it produce the progrowth, antiapoptotic messenger S1P but it also decreases the intracellular levels of the proapoptotic ceramide, counterbalancing its effect. SphK1 can be stimulated by a variety of growth factors, cytokines and mitogens (reviewed in [3]), and its localization seems to be crucial to its signaling capability as it would involve a translocation to the plasma membrane [10,11]. Intriguingly, in contrast to SphK1, the SphK2 isoform has been found to induce apoptosis [12,13]. We, however, are not discussing the case of the SphK2 in this review inasmuch as its role in cancer pathogenesis or treatment has not been studied in detail so far.

Sphingosine kinase-1 is an oncogene and is upregulated in cancer

Strong evidence supporting SphK1 as a potential target in cancer is based on the data from Vadas *et al.* who found that SphK1 was an oncogene [14]. In line with the classical model of cell transformation, it was found that fibroblasts overexpressing SphK1 acquire the transformed phenotype in tissue culture and capability to form tumors in nude mice [14]. Furthermore, the content of SphK1 mRNA was found to be significantly higher in various tumor tissues (brain, breast, colon, lung, ovary, stomach, uterus, kidney, rectum, small intestine) than in its healthy counterparts [15–17]. The elevated SphK1 mRNA expression was confirmed by a strong immunopositive staining for SphK1 in the cancerous lesion in a variety of non-small-cell carcinomas of the lung as compared with normal adjacent tissue [16]. The expression of SphK1 was also found upregulated in colon cancer tissues induced by azoxymethane, a well-characterized carcinogen that induces colon cancer in rodents. Of interest, the expression of SphK1 was dependent on the tumor stage. A low percentage of colon adenomas showed weak staining for SphK1, whereas colon adenocarcinomas showed intense SphK1 immunostaining. The upregulation of SphK1 expression by azoxymethane was at the level of transcription as real-time polymerase chain reaction demonstrated that colon carcinoma expressed a higher level of SphK1 mRNA than that of adjacent normal mucosa. These results suggest that SphK1 may play a role in colon carcinogenesis, and has the potential to be used as a novel biomarker for colon malignancy and target for the development of mechanism-based chemoprevention and therapeutic strategies against colon cancer development [18]. Finally, expression of SphK1 in human astrocytoma grade 4 (glioblastoma multiforme) tissue has been shown to correlate with a short patient survival. Patients whose tumors had low SphK1 expression survived a median of 357 days, whereas those with high levels of SphK1 survived a median of 102 days [17].

Sphingosine kinase-1 acts as a sensor during anticancer therapies

Consistent with the general idea that ceramide is a mediator of apoptosis, lack of generation of ceramide has been linked to tumor cell resistance (reviewed in [4,19]). Radiation-resistant and chemo-resistant cell lines do not generate ceramide after irradiation or chemotherapy [20–24], a defect that could be bypassed by the addition of cell-permeable ceramides. Interestingly, a correlation was originally established between SphK1 activity and resistance to irradiation in prostate cancer cells. We have indeed demonstrated that SphK1 activity was not affected by ionizing radiation in radiation-resistant LNCaP cells, whereas SphK1 was markedly inhibited in radiation-sensitive TSU cells [24]. More recently, the downregulation of SphK1 protein in Molt-4 and HL-60 leukemia cells responsive to DNA-damaging agents was reported [25,26], but not in MDR1-positive or MRP1-positive cells refractory to doxorubicin and etoposide [25]. To further substantiate a role for SphK1 as an indicator of tumor cell sensitivity or resistance, we took advantage of the differential effect of the topoisomerase inhibitor camptothecin and the antimicrotubule agent docetaxel (taxotere) on human PC-3 and LNCaP prostate cancer cells to determine their effect on SphK1 and subsequent ceramide/S1P balance in relation to cell survival [27]. A strong inhibition of SphK1 and elevation of the ceramide/S1P ratio was seen only in cell lines sensitive to these drugs. The differential effect of both chemotherapeutics was confirmed in an orthotopic PC-3/green fluorescent protein model established in nude mice. Docetaxel induced a stronger SphK1 inhibition and ceramide/S1P ratio elevation than camptothecin, and this was accompanied by a smaller tumor volume and a reduced occurrence and number of metastases [27]. These results were the first in-vivo demonstration of SphK1 as a sensor of chemotherapy [27].

The mechanism underlying SphK1 inhibition during apoptosis in cancer cells is not known. It has been proposed that SphK1 downregulation after treatment with DNA-damaging agents was a p53-dependent mechanism in Molt-4 cells [26]. We, however, found a downregulation of SphK1 after doxorubicin and etoposide treatments in HL-60 cells, which are p53-null, hence indicating that SphK1 inhibition could be independent of tumor suppressor p53 expression in these cells [25]. Moreover, our recent data in LNCaP and PC-3 prostate cancer cells showed SphK1 inhibition in response to chemotherapeutics regardless of the p53 status of the cells [27], thus implying that multiple pathways are involved in SphK1 downregulation. Nonetheless, one can speculate that ceramide generation is needed for the downregulation of SphK1 activity, given that – in leukemia cells [25] – SphK1 inhibition is seen after treatment with C_2-ceramide, a short-chain homolog of

natural ceramide which is known to be metabolized to form natural long-chain ceramides when added to the cells [28]. With respect to the meaning of SphK1 inhibition during apoptosis, one can instinctively envision it as a way of ensuring that the ceramide produced in response to stress agents, will not give rise to increased antiapoptotic S1P. The metabolic conversion of ceramide into S1P may switch cancer cells from an apoptotic state to a cell growth/survival state. As mentioned previously, sphingolipid metabolism can be viewed as a dynamic flow in which an increase in ceramide level can lead to an augmented S1P level as well, unless SphK1 is inhibited. Ceramide generation alone might not be enough to successfully mediate apoptosis without inhibition of the prosurvival SphK1.

Sphingosine kinase-1 overexpression protects against anticancer treatments

A strategic role for SphK1 in regulating chemotherapeutics-induced apoptosis in cancer cells is supported by its overexpression that can markedly inhibit cell death induced by: (i) anthracyclines in MCF7 breast cancer cells [29], (ii) doxorubicin and etoposide in HL-60 acute myeloid leukemia cells [25], and (iii) camptothecin and docetaxel in PC-3 and LNCaP prostate cancer cells [27]. These results corroborate other findings showing that SphK1 overexpression protects from various proapoptotic stimuli that provoke generation of endogenous ceramide such as serum withdrawal in NIH 3T3 cells and Jurkat T cells [30], and PC12 cells [31]; cell-permeable ceramides in HEK293 cells and Jurkat T cells [30], PC12 cells [31] and A-375 melanoma cells [32]; and tumor necrosis factor in MCF7 breast cancer cells [29]. Multiple evidence exists that SphK1 overexpression can abrogate apoptosis, notably by shifting the ceramide/S1P balance towards the cytoprotective S1P [27,29–32]. Enhanced SphK1 activity reduces the ceramide level by driving ceramide metabolism toward the synthesis of S1P, which is known to thwart the apoptotic machinery at the premitochondrial level via the inhibition of cytochrome *c* release [33], possibly in a MEK/extracellular kinase-dependent mechanism [34]. Moreover, it has been demonstrated that SphK1 overexpression inhibits cytochrome *c* release induced by chemotherapeutics [25].

Importantly, using an orthotopic model for prostate cancer, Pchejetski *et al.* [27] recently demonstrated that animals injected with SphK1-overexpressing PC-3 prostate cells developed larger tumors and resistance to docetaxel treatment. Accordingly, the ceramide elevation normally triggered by docetaxel treatment was attenuated in animals implanted with PC-3/SphK1. As a result of this, as observed *in vitro*, the increase in the ceramide/S1P ratio was strongly diminished in PC-3/SphK1 tumors compared with the PC-3/neo tumors [27].

Sphingosine kinase-1 inhibition as a cancer therapy ?

On the basis of the aforementioned information indicating that (i) SphK1 activity is downregulated in cancer cells that are responsive to apoptosis-induced anticancer treatments and (ii) SphK1 enforced expression – by pushing the sphingolipid metabolism toward S1P – can offer protection to various chemotherapies and irradiation, one might hence ask whether SphK1 inhibition could be of therapeutic interest.

It was recently shown that the specific knockdown of SphK1 by a small interference RNA approach could trigger apoptosis of tumor models including leukemic [25,26], breast [35], glioblastoma [17] and prostate [27] cancer cells. The proapoptotic effect induced by SphK1 small interference RNA, i.e. activation of effector caspases and cytochrome *c* release [25,35], was associated with a significant increase in ceramide and sphingosine levels [35], both known to act upstream of the mitochondrial pathway [33,36,37].

During the last two decades, several pharmacological compounds have been either synthesized or isolated from natural sources (mostly from fungi) that inhibit SphK1 activity. The most commonly utilized compounds are the sphingosine derivatives, DL-*threo*-Dihydrosphingosine and dimethylsphingosine that are potent competitive inhibitors of purified SphK1, with K_i values of around 10 and 5 μmol/l, respectively [38]. DL-*threo*-Dihydrosphingosine and dimethylsphingosine can elicit growth inhibition and cause apoptosis in various tumor cells: acute myeloid leukemia [33,39–50], chronic myeloid leukemia [43], acute lymphoid leukemia [36,33], cervix carcinoma [51], pheochromocytoma [31,52], prostate adenocarcinoma [24], gastric cancer [50,53], lung cancer [53], colon cancer [50,53], melanoma [53,54], epidermoid carcinoma [50,53], hepatoma [55], neuroblastoma [56] and breast adenocarcinoma [29,57]. In almost, if not, all cancer cell lines studied, the toxicity as well as the pathways initiated by dimethylsphingosine or DL-*threo*-dihydrosphingosine were quite similar (for further details, see [58]). More interesting is the finding that these sphingoid bases can either potentiate anticancer regimen-induced cell death or overcome radiation resistance or chemo resistance in acute myeloid leukemia [33,43,45] or solid tumors such as breast adenocarcinoma [57,59], prostate adenocarcinoma [24,59], cervix carcinoma [51], gastric adenocarcinoma [60], neuroblastoma [59], melanoma [59], lung [59], colon [59] or pancreatic [59] tumors. Low doses of DL-*threo*-dihydrosphingosine could also inhibit carcinogenesis of mouse embryo fibroblasts exposed to γ-rays or phorbol ester [61].

DL-*threo*-Dihydrosphingosine or dimethylsphingosine, however, cannot be considered as specific inhibitors of SphK1 because they are known to potently (like

sphingosine) inhibit protein kinase C (nPKC/cPKC) activity *in vitro* [62–64]. Although dimethylsphingosine has been shown to induce apoptosis through the inhibition of sphingosine kinase without affecting PKC in PC12 cells [65], it is not currently established whether dimethylsphingosine induces cell death mainly through sphingosine kinase inhibition rather than inhibiting nPKC/cPKC in other systems. A negative regulatory role for dimethylsphingosine on sphingosine kinase and/or PKC is of interest because the existence of a sphingosine methylation pathway that produces dimethylsphingosine was demonstrated in cancer cells [66–68] and brain tissue [69,70]. The intracellular amounts of dimethylsphingosine detected are, however, so minute that the physiological relevance of cellular dimethylsphingosine is still unclear and remains to be studied.

DL-*threo*-Dihydrosphingosine or dimethylsphingosine have already been successfully employed in cancer therapy preclinical trials on animal models. For instance, administration of dimethylsphingosine was reported to dose dependently inhibit the in-vivo growth of lung and gastric carcinoma tumors in athymic mice [53], and to severely decrease lung metastasis of melanoma cells in syngeneic mice [71]. Interestingly, the in-vivo tumor growth inhibitory effect of dimethylsphingosine also applies to drug-resistant KB-2 cervix carcinoma [51]. L-*threo*-Dihydrosphingosine (Safingol), another sphingoid base derivative with SphK-inhibiting properties [72], has even reached the phase I clinical evaluation level. Administration of Safingol either alone or in combination with doxorubicin has proven to be nontoxic and does not alter the pharmacokinetics of the anticancer drug [73]. Although the initial phase I trial with Safingol ended before achieving a maximally tolerated dose and had entered too few patients to assess activity, a modest activity was seen [73].

Even though sphingosine derivatives were found equipotent for many cancer cell in-vitro models, and capable of inhibiting tumor growth *in vivo*, they did cause strong hemolysis. Clearly, potent inhibitors of SphK1 that can be easily synthesized (or purified from natural sources) and are not toxic to animals are needed to evaluate the clinical potential of SphK1 inhibition in animal models.

By screening a library of synthetic compounds, French *et al.* [15] recently identified a panel of inhibitors. These compounds are selective towards SphK in comparison with other lipid (phosphatidylinositol 3-phosphate kinase) and protein kinases (PKCa and extracellular kinase 2), and are not competitive inhibitors of the ATP-binding site of SphK. Among them, 2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl)thiazole was the most selective compound isolated (IC_{50} = 0.5 μmol/l for SphK activity), and demonstrated a strong cytotoxicity toward T24 bladder carcinoma cells (IC_{50} = 4.6 μmol/l), MCF-7 breast cancer (IC_{50} = 1.2 μmol/l), MCF-7/VP that is resistant to several anticancer drugs because of the overexpression of the transport protein MRP1 (IC_{50} = 1.3 μmol/l) and NCI/ADR (IC_{50} = 0.9 μmol/l), a cell line resistant to many anticancer drugs because of the overexpression of the drug transporter P-glycoprotein [15]. More recently, 2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl)thiazole was found to be able to kill both androgen-sensitive (LNCaP) and hormone-resistant human prostate cancer cells (PC-3), regardless of their p53 status, notably by tilting the ceramide/S1P biostat towards ceramide [27]. A synthesized variant of one of the inhibitors, compound V (IC_{50}s for inhibition of SphK and cell viability around 2 μmol/l), was tested for its effects on tumors growing in mice (mouse JC mammary adenocarcinoma cells subcutaneously implanted in immunocompetent BALB/c mice). Interestingly, tumors in mice treated with compound V had between 50 and 85% less growth than tumors in untreated animals. Importantly, there were no significant differences in the body weights of those animals, suggesting that it was not toxic to the animals [15].

A few natural product inhibitors have also been isolated from marine bacterium or fungi [74–77]. The S-12183a and b compounds were isolated from the *Zopfiella inermis* fungus [77]. They exhibit an IC_{50} of 2.5 and 1.6 μmol/l towards SphK activity in cell-free systems, respectively [77]. Unfortunately, no information is currently available regarding their effect on cell cultures. B-5354c and F-12509A were isolated from a novel marine bacterium and the discomycete *Trichopezizella barbata*, respectively [74–76]. The K_i values of B-5354c and F-12509A for recombinant SphK1 isoforms are 3.7 and 4 μmol/l, respectively, compared with 3.2 μmol/l for dimethylsphingosine [78]. Lineweaver–Burk plot analysis indicated that F-12509A inhibited SphK1 competitively (like dimethylsphingosine) with respect to sphingosine, suggesting that the sesquiterpene moiety of F-12509A may mimic the conformation of sphingosine in binding to the active site of SphK1 [78]. On the other hand, B-5354c showed noncompetitive-type inhibition with respect to sphingosine, thus indicating that B-5354c may interact with domains distinct from the sphingosine-binding sites to regulate SphK1 activity [78]. Importantly, these two compounds did not exhibit the unfavorable activities on PKC as observed with the sphingosine analogs DL-*threo*-dihydrosphingosine and dimethylsphingosine. In addition, B-5354c and F-12509A did not inhibit other lipid activities such as sphingomyelinase or phosphatidylinositol 3-phosphate kinase even at doses above 500 μmol/l. So far, only F-12509A has been used for cell culture studies in acute myeloid leukemia models. The F-12509a compound induced SphK1 inhibition, resulting in simultaneous S1P biosynthesis blocking and ceramide accumulation in chemo-sensitive HL-60 as well as in chemo-resistant HL-60/Doxo and HL-60/VP16 cells overexpressing MRP1 and MDR1, respectively [25]. At

low micromolar amounts, F-12509a clearly triggered apoptosis in HL-60 cells regardless of their MDR status through a mitochondria-dependent pathway as cytochrome *c* and SMAC/Diablo proteins were released from mitochondria to the cytosol followed by the inactivation of X-linked mammalian inhibitor of apoptosis protein characterized by its cleavage [25].

Conclusion

Collectively, the results of the many studies described in this review provide strong support that strategies to kill tumor cells via SphK1 inhibition are valid and could have a favorable therapeutic index. Indeed, not only is the oncogenic SphK1 activity upregulated in many tumors, making it a putative target for therapy, but also its inhibition – by increasing their ceramide content and by preventing the shunting of ceramide into S1P – could overcome chemoresistance or resensitize tumor cells to chemotherapy and radiation therapy.

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