Introduction

Extensive studies have cemented sphingolipids as reservoirs of critical bioactive molecules and undeniably propelled the study of these lipids in biology and disease. Previously considered purely structural lipids, sphingolipids, particularly ceramide and sphingosine-1-phosphate (S1P), are now established critical regulators of myriad biological processes. Sphingosine, which serves as the backbone structure of many sphingolipid species, was originally named by the German chemist, J. L. W. Thudichum in the 1880s, in reference to the many mysteries the study of this lipid presented. In Greek mythology, the sphinx guarded the entrance to the city of Thebes and only granted entry to travelers who answered its riddle. Today, the field of sphingolipid research is one that is equally complex and promising. Currently, sphingolipids are being studied as potential targets in cancer research due to their role in regulating cellular and pathological processes such as cell growth and survival, apoptosis, inflammation, vascular integrity and angiogenesis. The complexity is accentuated by the metabolic interconnected nature of these bioactive lipids. The levels of sphingolipids are regulated by multiple enzymes either through synthesis or breakdown reactions. This review will describe the fundamental interconnectivity of metabolic sphingolipids, will review and highlight recent data pertaining to the subcellular compartmentalization of sphingolipids, and will provide evidence of the opposite roles that ceramide and S1P play in various cellular processes relevant to cancer biology.
Sphingolipid biosynthetic pathways

Regulation of ceramide synthesis and breakdown

The central hub of the sphingolipid pathway is the bioactive lipid ceramide, which can be generated through different pathways (Figure 1) (1,2). The de novo generation of sphingolipids starts with the condensation of serine and palmitoyl CoA to generate 3-ketodihydrosphingosine in a reaction catalyzed by a family of enzymes, the serine palmitoyl transferases (SPTs) (3). SPTs also have the ability to utilize alanine and glycine instead of serine in a pathway that eventually forms deoxysphingolipids, a terminal class of lipids that is unable to exit the sphingolipid pathway due to the absence of enzymes that metabolize it (4,5). Following formation of 3-ketodihydrosphingosine, dihydrosphingosine is generated via a reduction reaction. The latter is subsequently N-acylated to dihydroceramide by ceramide synthases (CerS) (1,2). There are six mammalian CerS characterized to date. These differ by their fatty acyl chain length specificity and tissue distribution. As such, this generates ceramide species differing in the chain length of their fatty acid (6,7) which are then acted on by dihydroceramide reductase to insert a double bond at the 4,5 position of the sphingosine backbone to form ceramide (8).

Subsequently, ceramide can have multiple fates. Addition of a phosphocholine headgroup by sphingomyelin synthases (SMS) results in the formation of sphingomyelin (SM), a major component of cell membranes (9). Ceramide can also be glycosylated by glycosyl or galactosyl CerS to form more complex glycosphingolipids (2), or phosphorylated by ceramide kinase to form ceramide-1-phosphate (C1P) (10). Conversely, ceramide can be generated back from these complex sphingolipids via lipid hydrolases. Thus, an intricate balance of ceramide synthesis and breakdown is present in the cell and is regulated by multiple mechanisms as will be discussed in subsequent sections.

Ceramide and sphingosine-1-phosphate (S1P) axis

Another fate of ceramide metabolism is the generation of sphingosine via a deacetylation reaction catalyzed by ceramidases. Five of these enzymes have been cloned and are classified based on their pH optimas of activity into acid, neutral and alkaline (3 alkaline ceramidases exist) (11-13). It should be noted that some ceramidases have reverse activities and can convert sphingosine back to ceramide (14,15). Moreover sphingosine may be reacylated by one of the 6 CerS and this pathway is termed the “salvage pathway” as it rescues ceramide back and prevents its exit from the sphingolipid pathway (1,2). Additionally, sphingosine can be targeted by sphingosine kinase isoenzymes (SK1 or SK2) for phosphorylation on its hydroxyl head group to yield S1P (16). S1P can be dephosphorylated by the function of S1P phosphatases to generate sphingosine (17,18). Finally, a nonreversible step is the breakdown of S1P by a lyase to generate hexadecenal and ethanolamine phosphate, constituting the point of exit of the sphingolipid metabolic pathway (19).

Metabolic interconnectivity regulated by subcellular compartmentalization

Endoplasmic reticulum (ER)

The de novo production of ceramide constitutes the only point of entry into the sphingolipid pathway. The main reactions occur in the ER and once ceramide is produced, it is transferred to the Golgi apparatus where it can form SM by the addition of a phosphocholine head group, glycosphingolipids through the addition of sugar head groups, or C1P through phosphorylation (1,2). SM synthesis in the Golgi is thought to occur through sphingomyelin synthase 1 (SMS1) and sphingomyelin synthase 2 (SMS2) (20,21). It is believed that the ER to Golgi transport occurs in a non-vesicular fashion through the ceramide transfer protein (CERT) for SM and likely for C1P production and via vesicular transport for the generation of glucosylceramide, the precursor of complex glycosphingolipids (Figure 2) (22,23). After synthesis in the Golgi, complex sphingolipids are distributed in a vesicular fashion to different cellular compartments (24).

Plasma membrane

In the plasma membrane, SM is localized primarily on the outer leaflet where it is hydrolyzed by acid sphingomyelinase (aSMase) (25). However, a small pool of sphingomyelin is localized in the inner leaflet of the plasma membrane and is used as a substrate for the action of neutral sphingomyelinase-2 (nSMase2) to form ceramide (26). The reverse reaction of conversion of ceramide to sphingomyelin can occur at the plasma membrane and is catalyzed by SMS2 (27). Furthermore, neutral ceramidase (nCDase) is also present in the plasma membrane, albeit on the outer leaflet (28). It is unclear whether the substrate of nCDase comes from ceramide targeted to the plasma membrane in
vesicular fashion or from the flipping of nSMase2-generated ceramide from the inner leaflet of the plasma membrane. Sphingosine kinase 1 (SK1) is also present in the PM and localizes there in response to different stimuli to produce S1P (29-32).

**Lysosome**

The bulk of sphingomyelin degradation occurs in the lysosome through the action of the lysosomal form of aSMase. Defective activity of this enzyme is the genetic basis for Niemann-Pick disease that is characterized by failure of growth and psychomotor regression in children as well as progressive worsening of respiratory functions (33). Ceramide generated in the lysosome can serve as a substrate for the generation of sphingosine through acid ceramidase (aCDase). The deficiency in the activity of this enzyme results in the lipid storage disorder Farber lipogranulomatosis (Farber disease). Affected patients suffer from progressive joint deformation and neurological complications, which typically result in early death (15,34). To date, there is no characterized presence of any of the two SK in the lysosome. As such, it is assumed that lysosomal sphingolipids must be transported into other organelles to exit the sphingolipid pathway.

**Nucleus**

SM presence in the nucleus has been reported and mapped to different subnuclear localizations (35). The intricacy of how SM gets to the nucleus is still unclear. Two distinct possibilities arise: the first is a vesicular transport from the Golgi and the second is its nuclear generation from ceramide that is transported from the ER. The second possibility is more probable as SMS activity was detected in nuclear enriched fractions (36). SM can be degraded in the nucleus by neutral sphingomyelinase-1 (nSMase1), which has been shown to localize to the nucleus (37). Furthermore, sphingosine can be generated through the action of nuclear ceramidases as well as S1P through sphingosine kinase two (SK2) (38-40).

**Mitochondria**

Mitochondrial sphingolipids have also been detected.
Figure 2 Subcellular compartmentalization of the interconnected sphingolipid metabolic pathways. The de novo synthesis of sphingolipids originates in the endoplasmic reticulum (ER), where through a series of condensation and reduction reactions ceramide (Cer) is produced. Cer may also be formed in the mitochondria-associated membranes. De novo-generated Cer is transported in a non-vesicular fashion by the ceramide transfer protein (CERT) and via vesicular transport by four-phosphate adaptor protein 2 (FAPP2) to the Golgi. In the Golgi, Cer is modified to form sphingomyelin (SM), ceramide-1-phosphate (C1P) and glucosylceramide (GluCer), the latter serving as the precursor for complex glycosphingolipids (GSL). After synthesis in the Golgi, SM and complex GSL are delivered to the plasma membrane by vesicular transport. Various sphingolipid metabolic enzymes are present at the plasma membrane to regulate the levels of SM, Cer, sphingosine (Sph) and sphingosine-1-phosphate (S1P). S1P is produced by sphingosine kinase (SK) isoenzymes 1 and 2 and can be transported across the bilayer to signal via the S1P receptor (not shown). During endocytosis, membrane sphingolipids are internalized and transported to the lysosome via endocytic vesicles, where hydrolysis is catalyzed by acid sphingomyelinase (aSMase), acid ceramidase (aCDase), and glycosidase. Sph can be phosphorylated by SK to form S1P and follow the metabolic axis of glycerolipid production regulated by S1P lyase. Alternately, S1P can go through the salvage pathway for generation of Cer. In the nucleus, SM can be targeted by neutral sphingomyelinase 1 (nSMase1), and subsequently, sphingosine generated via this axis can be phosphorylated by SK2. dhSph, dihydrosphingosine; 3KdhSph, 3 ketosphinganine; dhCer, dihydroceramide; nSMase2, neutral sphingomyelinase 2; nCDase, neutral ceramidase; SMS2, sphingomyelinase synthase 2; S1PP, S1P phosphatase; Ma-nSMase, mitochondria-associated neutral sphingomyelinase.
Ceramide synthase activity has been detected in partially purified mitochondrial-enriched fractions suggesting the potential for either de novo synthesis of ceramide or salvage generation to occur in the mitochondria (41). Furthermore, the generation of ceramide from sphingosine and palmitoyl-CoA regulated by nCDase has been identified in liver mitochondria (42). Lastly, a recently cloned mitochondria-associated neutral sphingomyelinase was found to localize to the outer mitochondrial membrane (43,44).

Taken together, these results point to a very specific compartmentalization of sphingolipid metabolism. Functionally, as we continue to gain the molecular tools to study the enzymes, it would be of utmost importance to assign specific functions to organelle-specific sphingolipids and to be able to manipulate those organelle-specific pools. This could have major implications for understanding signaling processes, and using sphingolipids as potential targets for disease treatments.

**Opposing roles of ceramide and S1P in cellular biology**

**Apoptosis vs. cell survival**

Cellular amounts of ceramide and S1P are important regulators of cell survival. Extensive literature has described the effect of ceramide on cell death. The first reports described an effect of exogenous C2-ceramide treatments on induction of DNA fragmentation and programed cell death in leukemia cells (45). This was very selective to ceramide as dihydroceramide was unable to induce the same biology (46). Furthermore, exogenous addition of SMase to fibrosarcoma cells was demonstrated to induce DNA damage and apoptosis (47). At the time, that was the first report of an endogenously generated ceramide mediating an apoptotic function. Subsequently many stimuli were shown to induce ceramide generation and apoptosis and these included TNF-α, Fas ligand and ionizing radiation (1). Most of these effects were thought to occur through the hydrolysis of SM either by neutral or acid sphingomyelinase activities. However, the first reports of the involvement of de novo ceramide synthesis in mediating apoptosis came from studies on daunorubicin. These studies demonstrated a role for daunorubicin-generated ceramide in mediating cell death that was inhibited by the ceramide synthase inhibitor, Fumonisn B1 (48). Following these initial studies, many of the subsequent literature elaborated on different stimuli that induce ceramide generation and cell death in multiple cell lines. Interestingly, ceramide levels can also be regulated by inhibition of ceramide breakdown including inhibition of sphingosine kinase or S1P lyase (49,50).

The mechanism by which ceramide induces cell death involves activation of both the intrinsic and the extrinsic pathways of apoptosis. The intrinsic pathway is characterized by mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release. Interestingly, MOMP directly correlates with the level of ceramide in the outer mitochondrial membrane (51). However, some studies suggest that ceramide is not sufficient to induce MOMP but rather its synergism and activation of BAX is required for apoptosis (52-54). Ceramide can also induce MOMP through caspase-2 and caspase-8 activation. This can occur following glycogen synthase kinase-3β (GSK3β) activation in response to induction of cathepsins, downregulation of AKT or activation of protein phosphatase 2A (PP2A) (55-58). Finally, ceramide has also been shown to induce the mitochondrial translocation of PKC-δ which induces cytochrome c release and caspase-9 activation (59).

The extrinsic pathway of apoptosis is mediated mainly through receptor activation of endogenous caspases and cell death. Many receptors engage the extrinsic pathway and these include TNF receptors and the TNF-related apoptotic ligand (TRAIL) receptors. It is widely believed that these receptors generate ceramide at the plasma membrane and localize to ceramide-enriched membrane platforms (60,61). Much of the understanding of ceramide function comes from cancer cell resistance to the extrinsic pathway of apoptosis and the modulation of that resistance by sphingolipids and ceramide generation. For instance, resistance to TRAIL-induced cell death is overcome by expression of ceramide synthase 6 (CerS6) (62). Furthermore, TNF receptor 1 promotes the formation of ceramide at the plasma membrane through nSMase2 activation. Failure of that activation is associated with resistance to TNF-induced apoptosis of breast tumors (62). A last possible way of ceramide activating the extrinsic pathway is through downregulation of the FLICE protein, an endogenous inhibitor of caspases-8. This has been shown to occur in glioblastoma and prostate cancer (63,64).

The role of S1P as an anti-apoptotic regulator was first described in 1996, when it was demonstrated that S1P negatively regulates ceramide-mediated apoptosis (65). Early studies implicated SK1-derived S1P in cell survival, since overexpression of SK1 in NIH 3T3 fibroblasts and HEK293 cells was demonstrated to prevent apoptosis
induced by ceramide and serum deprivation (66). Numerous studies have since investigated the regulatory role of S1P in cell survival, establishing this bioactive lipid as a regulator of cancer cell proliferation, with elevated levels of SK1 and S1P present in various tumor tissues and cancers (67,68). S1P is a pleiotropic and mitogenic bioactive sphingolipid that is present in both tissues and circulation. S1P can regulate pro-survival cellular responses in an autocrine or paracrine manner (inside-out signaling), by activating a class of G-protein coupled receptors, S1P receptor 1-5 (S1PR1-5). Additionally, S1P can regulate cellular responses by S1PR-independent mechanisms (69), as it has been shown to promote growth and survival in S1PR-deficient mouse embryonic fibroblast (70).

S1P inhibits the intrinsic apoptotic machinery by regulating the release of cytochrome c and Smac/DIABLO from mitochondria, blocking the activation of executioner caspases and regulating Bax oligomerization (49,71,72). SK1 can also regulate the extrinsic apoptotic pathway by activating phosphatidylinositol-3 kinase (PI3K)/AKT and nuclear factor (NF)-κB (NFκB) signaling downstream of TNF receptor activation (73,74). In fact, inhibition of SK1 in apoptotic resistant cells enhances sensitivity to TNF-mediated cell death (75).

SK1/S1P play an important role in regulating chemotherapeutic responses, as inhibition of SK1 enhances sensitivity to apoptotic chemotherapeutic agents (76). Negative regulation of S1P, either through SK1 or S1P lyase, prevents drug resistance in tumor cells treated with the chemotherapeutic agent cisplatin (77). In triple-negative breast cancer cell lines, SK1 is overexpressed and this phenotype correlates with poor prognosis and resistance to doxorubicin therapy. Attenuation of SK1 expression sensitizes cells to chemotherapeutic drugs by regulating oncogenic signaling via ERK1/2 and AKT, thus representing a potential target for combinatorial therapy (78-80). In in vivo prostate cancer models, the efficacy of docetaxel and camptothecin treatment is mediated by inhibiting SK1, which results in decreased tumor cell growth (65,81). Furthermore, the generation of SK1 inhibitors has spurred the focus on SK1 as a potential drug target (82,83). Recent studies demonstrate that SKI-II inhibits growth of acute myeloid leukemia cells via a caspase-dependent mechanism and suppresses leukemic xenograft tumor growth in severe combined immunodeficient (SCID) mice (84). The S1P receptor antagonist FTY720 (Fingolimod), synergizes with cisplatin to reduce survival of melanoma cell lines by inhibiting the PI3K/AKT/mTOR pathway (85). The role of S1P in regulating oncogenic signaling via the PI3K pathway has also been shown in non-small cell lung cancer (NSCLC) cells (86).

The particular contribution of SK2 in cell survival is not as well established. Inhibition of SK2 using small-molecule inhibitors reduces the growth of prostate cancer cells by downregulating Myc and the androgen receptor (AR) (87). Using RNAi or the SK2-specific inhibitor ABC294640, another group demonstrated that SK2 regulates apoptosis in multiple myeloma (88). Other studies indicate only partial overlapping roles for the two SK isoenzymes in regulating cell survival, and suggest an optimal role for SK2 as a cancer therapeutic target (89).

Cell cycle regulation

The initial characterization of ceramide in cell growth arrest came from studies in the Hannun laboratory on serum-starved Molt-4 leukemia cells. It was noticed that, following serum withdrawal, cells arrested in G0/G1 phase with accumulation of ceramide from sphingomyelin hydrolysis. Conversely, exogenous addition of C6 ceramide recapitulated the same phenotype (90). Subsequent studies suggested that this effect was possibly mediated through the retinoblastoma (Rb) gene product (91). Studies on the involvement of ceramide in G0/G1 arrest demonstrated also the involvement of nSMase2 in mediating confluence induced growth arrest (92). This was attributed to the dephosphorylation of β-catenin in a protein phosphatase 1-γ (PP1C-γ) dependent manner, suggesting a signaling mechanism mediating this effect (93). Recent studies have identified dihydroceramide-mediated regulation of G0/G1 arrest induced by cell confluence in neuroblastoma cells (94).

More importantly, some studies on ceramide function in growth arrest concentrated on a potential role of ceramide in regulating cell cycle checkpoints. This is of particular interest as cell cycle regulators represent one of the major areas where focus is intense to develop novel chemotherapeutics to treat malignant tumors. The most developed of these studies suggest the major function of ceramide to occur at the G1/S transition through two signaling avenues. The first involved the activation of p21 and the dephosphorylation of Rb (95) and this can occur both in a p53-dependent and independent manner (96). The second involves the inhibition of the G1 cyclin-dependent kinase CDK2 (97). Interestingly, recent studies suggested a third mechanism by which ceramide can control the G1/S transition. In response to all trans retinoic acid...
(ATRA), nSMase2 is activated and appears to mediate a G1 arrest that is dependent on the dephosphorylation of S6 kinase with no effect on either Rb or p21 (98). Ceramide has also been implicated in the G2/M cell cycle checkpoint. Increase of ceramide levels in NIH 3T3 cells mediated by threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), a glucosylceramide synthase inhibitor, demonstrated decreased activity of cyclin dependent kinase CDK1 and cell cycle arrest at the G2/M checkpoint (99). Likewise, inhibition of ceramide generation via the knockdown of β-glucosidase, also demonstrated regulation of G2/M arrest induced by the chemotherapeutic agent paclitaxel (100). Increase in ceramide levels has also been reported to occur right after the G2/M progression to regulate Rb dephosphorylation (101). More recent studies suggest that in rhabdomyosarcoma cells, ceramide induces a G2/M arrest that is concomitant with increased p21 and downregulation of cyclin D (102). The other suggested that ceramide suppresses survivin which controls the G2/M transition (103). As such, the distinct mechanisms of how ceramide controls the G2/M checkpoint, as well as its application to endogenous situations of ceramide upregulation, require further investigation. To note, there has not been to date studies that pointed to roles of ceramide in either the S phase checkpoint or in the mitotic spindle checkpoint.

Cell cycle progression is also regulated by S1P signaling as a mechanism to promote cell proliferation (104,105). Early reports identified the involvement of SK1 in cell cycle regulation activated in response to ganglioside GM1 mitogenic signaling (106). These authors found that inhibition of SK1 mediated by D,L-threo-dihydrosphingosine, results in a concomitant decrease in CDK2, and important regulator of G1 cell cycle progression. Following, SK nuclear activity was detected and found to correlate with cell cycle transition from G1 to the S phase (107). While both SK isoforms have been associated with cell cycle progression in glioblastoma cell lines (108), other conflicting studies have characterized SK2 as the only nuclear SK isoform, and correlated SK2 activity with G1/S cell cycle arrest (39). SK1-mediated regulation of G1 to S phase cell cycle progression has been investigated by loss of function studies utilizing RNAi in MCF7 cells (49) and in genetic studies of intestinal tumor epithelial cells (109). Mechanistically, these studies show that SK1 regulates the G1/S transition by increasing the expression of CDK4 and c-myc. Additional studies in breast cancer cells show that SK1 downregulates CDK1 and CHK1, which modulate spindle checkpoint function and cytokinesis (110). Pharmacological inhibition of SK1 using SKI-II and SKI-178 have also demonstrated regulation of CDK1 in natural killer-lymphocyte leukemia cells and associate this role with a G2/M cell cycle arrest (111). Conversely, inhibition of S1P receptor signaling with FTY720 in MDA-MB-361 breast cancer cells induces cell cycle arrest at the G0/G1 phase and a decrease in cells at S and G2/M phase. FTY720 treatment in these cells increases the anti-proliferative effect induced by single dose ionizing radiation (112).

Moreover, studies from the Obeid lab identified SK1 as a target of p53, a tumor suppressor and cell cycle regulator (113). While SK1 is elevated in p53 deficient mice, a double knock out model showed increase of cell cycle inhibitors p21 and p16 and decreased tumor formation. Though these studies have characterized S1P as an important regulator of cell cycle progression, the targets and biochemical pathways involved may be cell specific.

Senescence

Cellular senescence is a process by which cells lose the ability to proliferate. It is different from quiescence in that it is thought to be irreversible and associated with aging. The first observation implicating ceramide in senescence regulation came from the Obeid laboratory; ceramide was found to increase in senescent human diploid fibroblast due to neutral sphingomyelinase activity (114,115). This was also associated with dephosphorylation of Rb and inhibition of AP-1 activation (114,116). Subsequent studies showed induction of β-galactosidase by exogenous ceramide addition in these fibroblasts (117), and in human umbilical vain endothelial cells (118). Furthermore, exogenous treatment of ceramide in these cell lines was also found to dephosphorylate Rb, commonly seen with ceramide-induced cellular senescence. Recent evidence suggests that metformin, a drug used in the treatment of diabetes, can reverse ceramide-induced senescence in C2C12 myoblasts (119). However, it is unclear if the action of metformin is in the same pathway or in a parallel pathway to ceramide. While most of these studies implicated a neutral-sphingomyelinase generated ceramide in the induction of senescence, a recent study suggest that the lack of CERT, and thereby the lack of transfer of ceramide from the ER to the Golgi resulted in premature senescence in mouse embryonic fibroblasts (120).

Despite limited studies focusing on the role of S1P in regulating senescence, is not surprising that the metabolic
interconnectivity of these lipids supports a role for SK/SIP in this response. Deletion of SK1 in p53 null mice results in induction of senescence and decreased thymus tumor burden (113). Recent studies have shown that SK2-generated SIP inhibits senescence by promoting telomerase stability. S1P regulates senescence and cell proliferation by binding the catalytic subunit of the telomerase reverse transcriptase (TERT) and thus stabilizing the enzyme during DNA replication (121).

**Autophagy**

Autophagy is a cellular protective mechanism aimed at the degradation of unused cellular metabolites and organelles to preserve cellular energy. However, cancer cells use this mechanism to promote their survival and as such, understanding the processes that regulate this phenomenon can lead to its efficacious targeting. Ceramide has long been implicated in autophagy (122-125). Many mechanisms have been proposed to elucidate the role of ceramide in regulating autophagy (126,127), the most convincing of which show regulation of the mTOR pathway and nutrient uptake (128). Some studies have implicated ceramide in the generation of autophagic vacuoles by upregulating Beclin-1 and inhibiting protein kinase B (122); others implicate ceramide in regulating the dissociation of Beclin-1 and Bcl-2 to promote autophagy (129). Interestingly enough, there seems to be a consensus that regulation of autophagy seems to occur through the long-chain dihydroceramides (122,130-132). The drug fenretinide has been used extensively in these studies due to its dual action as an activator of SPTs as well as an inhibitor of dihydroceramide desaturase, which results in the accumulation of dihydroceramide. Briefly, fenretinide was shown to induce autophagy in breast, pancreatic and cervical cancer cell lines (133-135).

S1P has also been implicated as a regulator of prosurvival autophagy (136,137). In PC-3 prostate cancer cells, exogenous S1P and dihydro-S1P treatment induced autophagy upon serum starvation (138). Similarly, increased expression of SK1 and depletion of the S1P phosphatase phosphohydrolase-1 promotes autophagy as determined by the formation of LC3-positive autophagosomes (139). In these studies, autophagy was specifically induced by SK1, since both dimethylsphingosine and overexpression of a catalytically inactive SK1 mutant abrogated autophagy induction. Interestingly, S1P-induced prosurvival autophagy is characterized by inhibition of mTOR but independent of AKT signaling (140). The role of dihydro-S1P in promoting autophagy is controversial (139). Loss of function studies demonstrated the need for S1PR5 in S1P-induced ER stress and autophagy (141), although the receptor specificity may be cell specific (125). Other studies showed that in a subpopulation of T-cell acute lymphoblastic leukemia (T-ALL), the use of SK1 and SK2 inhibitors induced the unfolded protein response (UPR) leading to ER stress, and autophagic cell death (142).

**Cell motility and invasion**

The cellular balance of sphingolipids metabolites, ceramide and SIP, plays an important role in regulating adhesion, migration and invasion, which are important precedents of cancer metastasis (143). The opposing roles of ceramide and SIP have been studied in vivo and in vitro cancer models. Increased expression of CerS2 and ceramide levels correlate with poorly invasive phenotypes of breast cancer cell lines. Mechanistically, CerS2 overexpression correlates with activation of metalloproteinases (MMP) and degradation of extracellular matrix, resulting in decreased invasion (144). CerS2 expression also correlates with less metastasis in human bladder cancer cell lines (145). The overexpression and increased metabolic function of aCDase in prostate cancer cells, results in decreased levels of long chain ceramide species and increased adhesion and migration on the ECM. Furthermore, acute treatment with C6 ceramide nanoliposomes suppresses tumor migration in a PKCζ-dependent manner to promote stress fiber depolymerization and focal adhesion disassembly (146). In addition, inhibition of aSMase— and thus decreased generation of ceramide—in Hela and MDA-MB-231 cells, reduced invasion in vitro (147). At the same time, other studies have identified a role for aSMase-derived ceramide in adhesion and metastasis. aSMase-deficient mice show reduced hematogenous melanoma metastasis. Melanoma cells triggered secretion of aSMase from platelet cells, resulting in increased ceramide and clustering of α5β1 integrin in melanoma cells to promote adhesion (148,149).

The role of SK1 in cell migration and invasion is an area of intense research focus (150). Migration regulated by extracellular SIP is dependent on the expression of specific SIPR, with SIPR1 and SIPR3 generally promoting migration and SIPR2 inhibiting it with some exceptions. The migratory role associated with different SIPRs is regulated by downstream coupling to different Gi proteins and consequently, activation of distinct downstream
signaling pathways (136). For instance, coupling of S1PR2 to G12/13 results in Rho activation and inhibition of Rac and cell migration (151). Moreover, the role of S1PR in migration is also regulated by tissue distribution; for example, in Jurkat and U937 immune cells, S1PR3 but not S1PR1, is required for S1P-mediated migration and adhesion to the endothelial monolayer (152,153). While in melanoma and glioblastoma cells, expression of S1PR2 is predominant, thus S1P signaling results in reduced migration (136,154,155). The role of SIP in migration is further regulated by the extensive crosstalk with growth factor signaling (136). In MCF7 and glioblastoma cells, EGFR signaling promotes the translocation and activation of SK1 to the membrane (79,156). And is possible that this signal is amplified in a loop, since in other cells S1P has been shown to stimulate the expression of EGFR (157). Furthermore, through different S1PRs, S1P also mediates EGFR and VEGFR2 transactivation to regulate migration (158,159).

In addition to its pro-migratory role, S1P also regulates tumor cell invasion to promote metastasis (160,161), making this potent bioactive lipid, a “swiss army knife” for cancer cells. While S1P can also promote the secretion and activation of proteases and metalloproteinases to degrade the ECM, it can also transcriptionally regulate PAR and PAI-1, which are key modulators of invasion (162-164). More recently, our group described a mechanism by which EGF and S1P induce cell adhesion and invasion (165). By regulating ERM proteins, sphingolipids regulate cellular cytoskeleton dynamics and invasion (166,167). Furthermore, it is probable that different pools of S1P target different processes in cancer cells, as some studies have implicated that inhibition of SK2 upregulates SK1 protein, activity levels, and increases intracellular S1P (89). On the other hand, SK1 inhibition does not regulate SK2, but decreases intracellular S1P and increases ceramide. In this study, inhibition of SK2 resulted in a more dramatic regulation of invasion, supporting a non-overlapping role for SK1 and SK2 in cancer cells (89). Clearly, the multifaceted role of S1P in migration and invasion requires additional focus and investigation in order to understand the complex regulation of these processes as they represent important targets for cancer therapeutics.

Conclusions

Our current model of sphingolipid signaling has been defined by a vast number of studies that have provided deeper understanding of the mechanism of enzyme/lipid regulation as well as associated cellular targets. What is becoming clear is that the various levels of complexity associated with bioactive sphingolipid metabolites continue to riddle and challenge us to consider novel mechanistic avenues. Recent advances in molecular and analytical tools have been instrumental in uncovering intricacies and new paradigms. While ceramide and S1P have been traditionally considered to play pro-death and pro-growth roles, respectively, newer roles are continuing to surface and perpetuating the enigmatic facet of these lipids. For instance, SK2-derived S1P has been implicated in both pro- and anti-apoptotic functions (68). In addition, is becoming clear that not all ceramide species “are created equal” and that different chain length ceramides may play different roles in promoting or suppressing tumors (68). We expect that advances in the field will allow us to clearly characterize these new models.

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Footnote

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