Chapter 8
Sphingolipid Metabolism and Signaling as a Target for Cancer Treatment

Vinodh Rajagopalan and Yusuf A. Hannun

Abstract Sphingolipids play key roles in the regulation of several biological processes that are integral to cancer pathogenesis. Among the sphingolipid metabolites, ceramide and sphingosine-1-phosphate (S1P) have been shown to modulate cancer development and progression. The biological roles of other metabolites, such as sphingosine, and ceramide 1-phosphate, are also beginning to emerge. In general, ceramide plays a role as a tumor-suppressing lipid-inducing anti-proliferative response such as cell cycle arrest, induction of apoptosis, and senescence whereas S1P plays a role as a tumor-promoting lipid-inducing transformation, cellular proliferation, and inflammation in various cell models. Glycosphingolipids, another emerging class of bioactive sphingolipids, are believed to play anti-apoptotic roles and offer drug resistance to currently used chemotherapeutic drugs. These emerging biological roles of sphingolipids and its potential usefulness in treating cancer in the form of anticancer therapeutics are discussed in this chapter.

8.1 Sphingolipid Metabolism

Sphingolipids are a class of lipids with a sphingosine back bone that are formed from non-sphingolipid precursors in the ER and get metabolized further within different sub-cellular compartments thereby giving rise to a plethora of metabolites. Of all these metabolites, ceramide is one of the most widely studied bioactive molecules. It is formed through three distinct pathways (Fig 8.1) (1) de novo synthesis—synthesis from non-sphingolipid precursors; (2) turnover pathways—break down products from complex sphingolipids; and (3) recycling and salvage pathways—The de novo pathway starts with the condensation of serine

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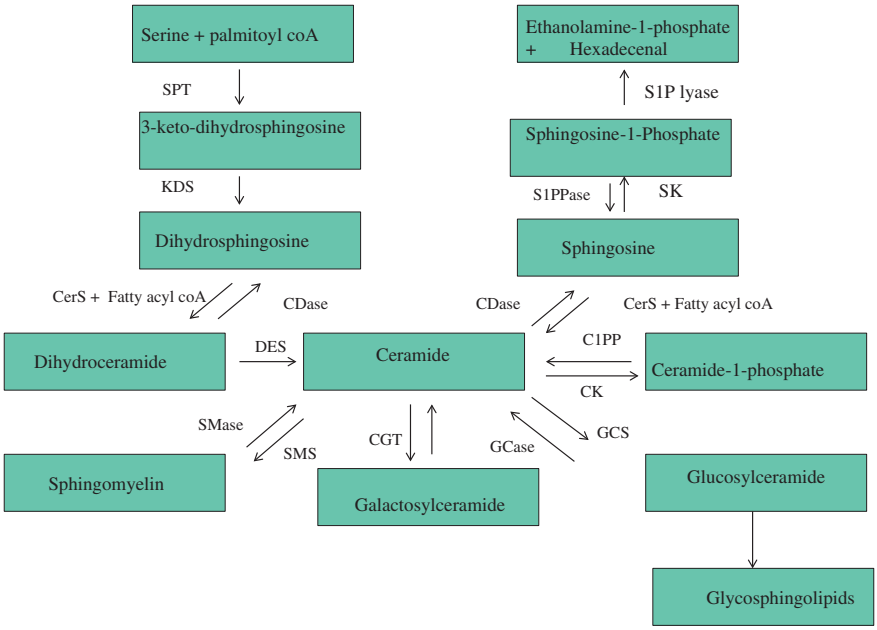

Fig. 8.1 Ceramide can be formed by de novo pathway from serine and palmitoyl-coA or from hydrolysis of sphingomyelin or cerebrosides (glucosyl or galactosyl ceramide). Ceramide, thus formed, can be phosphorylated by CK to yield ceramide-1-phosphate or serves as a substrate for the synthesis of sphingomyelin or glycosphingolipids. Ceramide can be deacylated by ceramidases to form sphingosine which can be phosphorylated by SKs to generate S1P which can be acted upon by phosphatases to generate sphingosine or by lyase to form ethanolamine-1-phosphate and hexadecanal, an aldehyde. Abbreviations: *SPT* serine palmitoyl transferase, *KDS* 3-keto-dihydrosphingosine reductase, *DES* dihydroceramide desaturase, *SPPase* Sph-1- phosphate phosphatase, *CK* cer kinase, *C1PP* C1P phosphatase, *SMS* SM synthase, *GCS* glucosylceramide synthase, *GCase* glucosyl CDase, *CGT* UDP-galactose ceramide-galactosyltransferase

and palmitoyl-CoA catalyzed by serine palmitoyl transferase (SPT) to generate 3-keto-dihydrosphingosine which is subsequently reduced to form dihydrosphingosine (sphinganine). Ceramide synthases (CerS) then act on dihydrosphingosine (or sphingosine) to form dihydroceramide (or ceramide) [1]. Dihydroceramide is subsequently desaturated by dihydroceramide desaturase (DES) which introduces a 4, 5-*trans*-double bond, thereby generating ceramide that occurs at the cytosolic face of the endoplasmic reticulum (ER) [2]. Ceramide, thus generated, can be used in biosynthetic reactions for the synthesis of sphingomyelin (SM), glucosylceramide (GluCer), galactosylceramide (GalCer) or ceramide 1-phosphate (C1P) by the attachment of head groups comprised of either phosphocholine, glucose, galactose or phosphate by sphingomyelin synthase (SMS), glucosyl ceramide synthase (GCS), UDP-galactose: ceramide-galactosyltransferase (CGT), or ceramide kinase, respectively (CK) [3, 4, 5]. In the turnover pathways of ceramide generation, sphingomyelinases act by cleaving sphingomyelin as a substrate [6] whereas

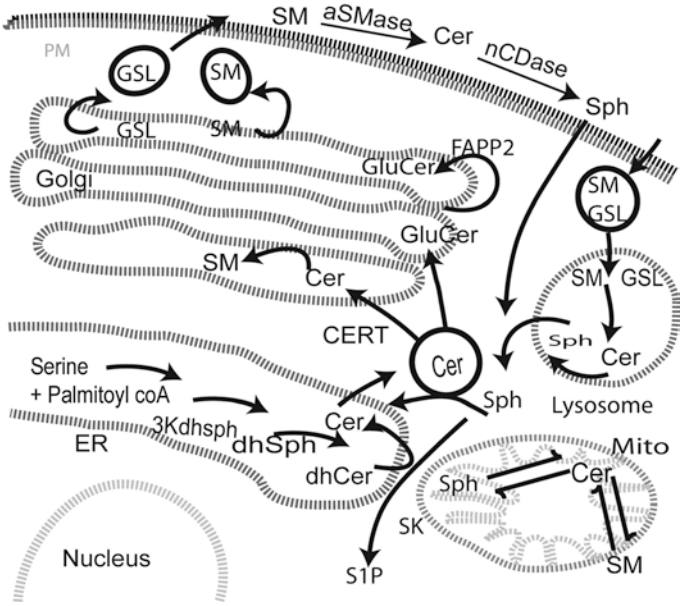

Fig. 8.2 Ceramide is generated in the ER by de novo pathway. It is transported to the Golgi membranes for SM synthesis in a CERT-dependent way or glucosylceramide (GlcCer) synthesis in FAPP2-dependent way. SM and complex GSLs are transported to the plasma membrane via vesicular trafficking where sphingomyelin gets metabolized by aSMase or neutral SMase to generate ceramide, or they are shuttled to lysosomes where aSMases and glucosidases metabolize SM and glucosylceramide, respectively, into ceramide. It is hydrolyzed by acid ceramidase to form sphingosine. Sphingosine may then traverse the lysosomal membrane to the cytosolic side where it might have two cellular fates. It can either be recycled into sphingolipid pathway in the ER or can be phosphorylated by SK1 or SK2. In mitochondria, ceramide is generated by the activation of n-SMase. Abbreviations: *3KdhSph* 3-keto-dihydrosphingosine, *dhSph* dihydrosphingosine, *CERT* ceramide transfer protein

hydrolases such as β-glucosidases and galactosidases act on glycosphingolipids such as glucosylceramide (GlcCer) and galactosylceramide as substrates to generate ceramide, respectively [7]. In the recycling and salvage pathways, ceramide generated in the lysosomes from the hydrolysis of complex sphingolipids is further broken down to sphingosine by the action of ceramidases [8] which is then re-acylated outside the lysosome by the action of ceramide synthases (CerS) to form ceramide. Since sphingosine derived from ceramide is salvaged to regenerate ceramide, it is referred to as the recycling and salvage pathway. Alternatively, sphingosine can be acted upon by sphingosine kinases (SK1 or SK2) [9] to form sphingosine-1 phosphate (S1P). S1P phosphatases can dephosphorylate S1P to regenerate sphingosine [10]. On the other hand, S1P lyase metabolizes S1P irreversibly to release ethanolamine phosphate and hexadecenal [11].

The sphingolipid enzymes discussed above are distributed in different intracellular locations. De novo ceramide synthesis takes place on the cytosolic

surface of the ER and its associated membranes. Ceramide formed in the ER is transported through ceramide transfer protein (CERT) to the trans-Golgi wherein it serves as a substrate for sphingomyelin synthase for formation of SM [12], or through vesicular transport to the Golgi wherein it serves as substrate for GCS for the formation of glucosylceramide. The transport protein, four-phosphate-adaptor protein 2 (FAPP2) delivers glucosylceramide to appropriate sites in the Golgi for synthesis of more complex glycosphingolipids (GSL) [13]. Subsequently, SM and complex GSLs are transported to the plasma membrane via vesicular trafficking. In the plasma membrane, SM can be hydrolyzed to ceramide and metabolized further by acid sphingomyelinase (aSMase) possibly acting on the outer leaflet, or by neutral sphingomelinase (nSMase) residing on the inner leaflet of the plasma membrane [14, 15].

From the plasma membrane, sphingolipids are recycled through the endosomal pathway. In lysosomes, aSMases and glucosidases metabolize complex sphingolipids (SM and glucosylceramide, respectively) into ceramide which is hydrolyzed by acid ceramidase to form sphingosine. Sphingosine may then traverse the lysosomal membrane to the cytosolic side where it has two cellular fates. Cytosolic sphingosine is either recycled into the sphingolipid pathway in the ER or phosphorylated by SK1 or SK2 [9] (refer to Fig 8.2 [16]).

8.2 Biological Targets of Ceramide

Ceramide regulates many biological processes such as cancer cell growth, differentiation, apoptosis, and senescence [17, 18]. Many signals such as cytokines, anticancer drugs, and stress-inducers upregulate ceramide through the de novo or salvage pathways [19, 20]. Ceramide triggers signaling cascades by regulating phosphatases, cathepsin D, or kinase suppressor of RAS (KSR) as described below.

Phosphatases such as protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) are activated by ceramide in vitro. Inhibition of these phosphatases inhibits the ability of ceramide to dephosphorylate (inactivate) several pro-proliferative proteins such as PKCα, Akt/PKB, c-Jun, Bcl-2, Rb, and SR [21, 22].

Many studies have documented the translocation of cathepsin D from lysosomes in response to oxidative stress followed by activation of caspase 3 and cell death [23]. Interestingly, cathepsin D was found to be a ceramide-binding and ceramide-activated protein [24]. Besides, acid sphingomyelinase-derived ceramide has been shown to favor autocatalytic proteolysis of inactive cathepsin D to enzymatically active cathepsin D isoform [25].

Similarly, KSR has been found to be ceramide responsive [26–30]. Mammalian KSR activates Raf, and activation of this pathway results in apoptosis [31, 32]. Also, ceramide has been shown to activate the zeta isoform of protein kinase C (PKCz) by phosphorylation [33]. Ceramide-activation of PKC-zeta has been

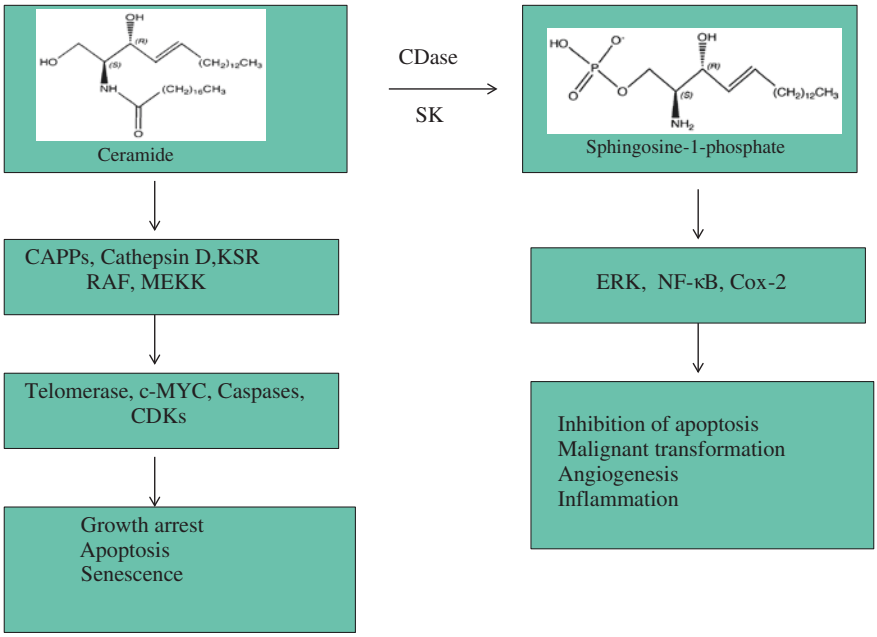

Fig. 8.3 Ceramide regulates many protein signaling molecules such as cathepsin D and ceramide-activated protein phosphatases (*CAPPs*), KSR, RAF, MEKK. Proteins that are modulated by these pathways include RB,SR, AKT, PKC-α, c-JUN, Bcl-2,telomerase, c-MYC, caspases, and cyclin-dependent kinases (*CDKs*) which in turn brings about cellular responses such as growth arrest, apoptosis, and/or senescence. Ceramide that gets metabolised to S1P by ceramidase and SK, regulates proteins such as ERK, NF-κB, Cox-2 which in turn brings about cellular responses such as inhibition of apoptosis, malignant transformation, angiogenesis, and inflammation

shown to be necessary for inactivation of Akt-dependent mitogenesis in vascular smooth muscle cells [34] (refer to Fig. 8.3).

In contrast to ceramide, S1P has emerged as a potential regulator of biological processes such as proliferation, inflammation, vasculogenesis, and tumor promotion [35]. S1P is a soluble molecule, secreted outside the cell which acts in autocrine and paracrine manner to bring about receptor-mediated cellular functions like cell motility and proliferation [35]. S1P acts directly on members of the S1P1-5 receptor family, which are G protein-coupled receptors. Non-receptor-mediated functions of S1P such as activation of TNF receptor–associated factor 2 (TRAF2) in the TNF pathway leading to NF-kB activation have been reported [36]. It has also been shown that interaction of TRAF2 with SK and activation of SK is critical for prevention of TNFα-mediated apoptosis [36] (refer to Fig. 8.3). Thus, these findings underscore the significance of uncovering bioactive sphingolipid-mediated cellular pathways that would help to conceive novel therapeutic strategies for many pathological conditions, importantly cancer.

8.3 Ceramide as a Tumor Suppressor

The involvement of sphingolipids in cancer pathogenesis is brought to light from the observation that the levels of sphingolipid metabolizing enzymes are altered in many tumors. For example, in a study, SK expression has been found to be upregulated in many human tumors originating from tissues such as breast, colon, lung, stomach, uterus, etc., [37]. In another study done by Riboni et al., an inverse correlation between the levels of ceramide and tumor malignancy in glial tumors (astrocytomas) was observed [38]. In general, exogenous ceramide-induced antiproliferative responses like apoptosis, differentiation and growth inhibition, senescence, and autophagy. Therefore, it is considered as a tumor suppressor lipid. These anti-proliferative roles of ceramide are discussed in detail in the following section.

8.3.1 Ceramide and Apoptosis

Ceramide has been implicated in many cell death paradigms. Birbes et al. [39] showed that selective targeting of bacterial sphingomyelinase (bSMase) to mitochondria and not to any other compartments such as plasma membrane, ER, or Golgi resulted in apoptosis that was associated with generation of ceramide and release of cytochrome c in MCF-7 cells. Overexpression of Bcl-2 prevented the mitochondria-targeted bSMase effects on apoptosis. Dai et al. [40] showed that UV-induced apoptosis was marked by increase in SM in all sub-cellular locations, particularly mitochondria, in HeLa cells. Ceramide levels were found to be elevated in mitochondria at 2–6 h, consistent with the cell death time course. D609, an inhibitor of sphingomyelin synthase to a marked extent and fumonisin B1 (FB1), a ceramide synthase inhibitor to a lesser extent, rescued the cells from increases in SM and ceramide, and consequently cell death. On the other hand, the SPT inhibitor myriocin did not rescue the UV effects on cell death, suggesting the involvement of the turnover pathway-generated ceramide in bringing about UV-triggered cell death.

In another study in *Caenorhabditis elegans*, upon inactivation of ceramide synthase, somatic apoptosis was unaffected, but ionizing radiation-induced apoptosis of germ cells was obliterated, and this phenotype was reversed by microinjection of long-chain natural ceramide. Radiation-induced ceramide accumulation in mitochondria consequently activated CED-3 caspase and apoptosis [41].

In Ramos B cells, surface B-cell receptor (BcR)-triggered cell death was marked by an early increase in C16 ceramide. Pulse labeling with sphinoglipid precursor, palmitate, in the presence of ceramide synthase inhibitor, FB1, demonstrated that the de novo ceramide-generating pathway was activated following BcR activation. The apoptotic cell death induced by cross-linking of BcR was mediated through mitochondrial cell death pathways followed by caspase activation [42]. In LNCaP prostate cancer cells, androgen ablation, which is considered as one of the therapeutic

modalities, was found to increase C16 ceramide level followed by G0/G1 cell cycle arrest and apoptosis. 5alpha-dihydrotestosterone (DHT) or fumonisin B1 treatment rescued LNCaP cells from apoptosis [43].

In another study, ceramide acting via PP1, dephosphorylated SR proteins that regulated the alternate splicing of Bcl-x(L) and caspase 9. In A549 lung adenocarcinoma cell lines, cell-permeable D-e-C(6) ceramide downregulated the mRNA levels of anti-apoptotic Bcl-x(L) and caspase 9b with concomitant increase in the mRNA of pro-apoptotic Bcl-x(s) and caspase 9. The chemotherapeutic agent, gemcitabine, induced de novo generation of ceramide and brought about aforementioned alternate splicing of Bcl-x(L) and caspase 9b and consequent loss of cell viability as measured by MTT assay [44].

In several studies, aSMase was shown to be necessary for radiation-induced apoptosis in endothelial cells and mice lacking aSMase were protected from gastrointestinal and CNS apoptosis [45–47]. In another study, the endolysosomal aspartate protease cathepsin D (CTSD) was identified as a target of ceramide generated by acid sphingomyelinase in response to TNFα. CTSD cleaved pro-apoptotic Bid and activated it in vitro. The lack of Bid activation in cathepsin-deficient fibroblasts suggested Bid is downstream of cathepsin D in bringing about apoptosis as a result of TNFα treatment [48].

In addition to aSMase, neutral sphingomyelinase has been implicated in stress response pathways initiated by TNFα in MCF-7 cells [49]; amyloid-β peptide in neuronal cells [50]; ethanol in HepG2 hepatoma cells [51]; and staurosporine in several neuronal cell lines [52].

In addition to the sphingomyelinases, ceramidases (CDases) were also found to regulate apoptosis. In one study, nitric oxide induced the degradation of nCDase, thereby, enabling ceramide accumulation and cell death [53]. In another study, the degeneration of photoreceptor cells was marked by an increase in ceramide which was rescued by overexpression of CDase that cleared the ceramide and prevented its apoptotic effect [54]. These studies clearly implicate ceramide in apoptosis and in mediating the cellular response to various stress causing stimuli.

8.3.2 Ceramide in Senescence

Ceramide has been implicated in cellular senescence. Their relationship stems from the observation that in WI-38 human diploid fibroblasts (HDF), there was increased neutral sphingomyelinase activity with generation of ceramide when cells entered senescence. These changes were not seen when cells entered quiescence achieved with serum withdrawal or contact inhibition [55]. Exogenous administration of ceramide (15uM) onto young WI-38 cells induced retinoblastoma protein dephosphorylation and inhibited serum-induced AP-1 activation, DNA synthesis, and mitosis, thereby inducing a senescence phenotype [55]. Involvement of ceramide in replicative senescence has been shown in human umbilical vein endothelial cells (HUVEC) as well [56]. In another study,

gemcitabine induced senescence in pancreatic cancer cells, and sphingomyelin treatment enhanced chemosensitivity to the drug by reducing the induction of senescence and redirected the cells to enter apoptosis. The authors concluded that ceramide inhibited cell cycle progression at low levels, induced senescence at moderate levels, and apoptosis at high levels [57]. Besides these studies, the yeast aging genes lac 1 and lag1 were subsequently identified as ceramide synthases, thereby providing a genetic link between ceramide to senescence and aging [58].

Ceramide also regulates senescence by inhibiting telomerase which is the enzyme that prevents the shortening of the telomeres, the long tandem repeats of G-rich sequences (5′-TTAGGG-3′) found at the ends of chromosomes. Telomerase is found to be frequently activated in many immortal cells in culture representing different tissues and malignant tumors, suggesting its role in cellular immortalization and tumorigenesis [59, 60]. In the A549 lung carcinoma cell line, daunorubicin treatment or sphingomyelinase overexpression increased ceramide generation followed by inhibition of telomerase activity. Clearance of ceramide by overexpression of GCS prevented the telomerase inhibition [61]. These studies suggest ceramide is an upstream regulator of senescence and aging.

8.3.3 Ceramide in Cell Differentiation and Growth Inhibition

Historically, the role of ceramide in cellular differentiation was discovered with the observation that vitamin D3-induced monocytic, but not neutrophilic-type cell differentiation in HL-60, and U037 leukemia cells was accompanied by increase in nSMase activity and a concomitant spike in ceramide levels [62]. In turn, exogenous ceramide was found to induce monocytic differentiation of these cells. In neuronal cell lines, ceramide-induced differentiation in T9 glioma cells, purkinje and hippocampal neurons [63].

In another study, incubation of exponentially growing *Saccharomyces cerevisiae* with short-chain ceramide inhibited cell growth with the involvement of an okadaic acid-sensitive protein phosphatase [64]. Another study uncovered the mechanism of ceramide-induced growth suppression in that serum withdrawal in MOLT-4 cells resulted in significant dephosphorylation of Rb, correlating with the induction of G0/G1 cell cycle arrest [65]. Taken together, these studies implicate a role for ceramide in cell differentiation and cell cycle progression.

8.3.4 Ceramide and Autophagy

In mammalian cells, ceramide and/or dihydroceramide have been shown to induce autophagy. For example, in glioma cells, ceramide has been shown to activate the transcription of death-inducing mitochondrial protein, BNIP3, and subsequent autophagy [66]. In the human colon cancer HT-29 cells, C2 ceramide

inhibited activation of protein kinase B, which is a negative regulator of interleukin 13-dependent macroautophic inhibition. In MCF-7 breast cancer cells, ceramide stimulated the expression of Beclin-1 which is an autophagy gene product. This study also showed that tamoxifen-induced autophagy was blocked using the SPT inhibitor myriocin (ISP1) [67]. In another study, ceramide was shown to induce autophagy by regulating calpain in MEFs [68]. In DU145 cells, fenretinide (4HPR) treatment favored autophagic induction possibly due to the increase in endogenous dihydroceramide [69].

In a study by Signorelli et al. [70], resveratrol induced autophagy in HGC-27 cells with an increase in dihydroceramides possibly by inhibition of dihydroceramide desaturase. Inhibitors of dihydroceramide desaturase mimicked the autophagic induction induced by resveratrol.

Mechanistically, Beclin-1 has been shown to be physiologically associated with the mammalian class III phosphatidylinositol 3-kinase (PI 3-kinase) Vps34, and the knockdown of Beclin-1 blunted the autophagic response of the cells to nutrient deprivation or C_2-ceramide treatment [71]. Class I PI3K and AKT pathway are known to suppress autophagy and ceramide has been shown to inhibit AKT by activation of PP2A, thereby establishing a possible mechanistic link between ceramide and autophagy induction [67, 72, 73].

Based on the above studies showing ceramide effects on mammalian autophagic regulation, combined with the observation that yeast subjected to heat stress exhibits growth suppression accompanied by upregulation of ceramide synthesis and downregulation of nutrient transporters on their cell surface [74, 75], Edinger and colleagues hypothesized that ceramide-induced mammalian autophagy might be mediated through a yeast-like response to heat stress by downregulation of nutrient transporters. They showed that C2 ceramide produced a profound downregulation of nutrient transporter proteins in mammalian cells. Inhibition of autophagy or acute limitation of extracellular nutrients increased the sensitivity of cells to ceramide. Supplementation of cells with the cell-permeable nutrient methyl pyruvate protected the cells from ceramide-induced cell death and delayed autophagic induction. So the authors concluded that ceramide killed cells (apoptosis) by provoking nutrient limitation via downregulation of nutrient transporters and subsequent autophagy [76]. Taken together, these studies implicate ceramide in the regulation of autophagic response.

8.4 S1P/S1PR as Tumor Promoters

S1P is considered a pro-survival lipid. For example, S1P stimulated the invasiveness of glioblastoma tumor cells [77], promoted estrogen-dependent tumorigenesis of breast cancer cells [78] and conferred resistance to the cytotoxic actions of TNF-α and daunorubicin [10]. A number of studies documented the role of S1P/S1PR in proliferation, inhibition of apoptosis, vasculogenesis/angiogenesis, and inflammation. These topics will be discussed in the following sections.

8.4.1 S1P in Proliferation and Inhibition of Apoptosis

Sphingosine kinase (SK) phosphorylates sphingosine to form S1P. Overexpression of the SK1 isoform induced oncogeneic transformation in NOD/SCID mice. Using inhibitors of SK, investigators implicated SK in the involvement of oncogenic H-Ras-mediated transformation [79]. In another study, addition of exogenous S1P reversed the cell death induced by ceramide [80]. Mechanistically, S1P counteracted ceramide-induced activation of stress-activated protein kinase (SAPK/JNK) and activated the extracellular signal-regulated kinase (ERK) pathway in governing the fate of the cell [80].

Neutralizing antibody to S1P substantially reduced tumor progression in murine xenograft and allograft models. The antibody arrested tumor-associated angiogenesis, neutralized S1P-induced proliferation, attenuated release of pro-angiogenic cytokines, and blocked the ability of S1P to protect tumor cells from apoptosis [81].

In yet another study, S1P-mediated inhibition of apoptosis in C3H10T 1/2 fibroblasts depended on ERK activation and MKP-1, which downregulated SAPK/JNK to bring about inhibition of apoptosis [82]. In male germ cells, S1P inhibited stress-induced cell death, possibly by inhibiting nuclear factor kappa B (NF-kappa B) and AKT phosphorylation [83].

8.4.2 S1P and Vasculogenesis/Angiogenesis

S1P promotes vasculogenesis and angiogenesis. S1P, the natural ligand for S1P3 receptor or KRX-725, a synthetic peptide that mimics S1P action on this receptor, favored angiogenesis, as demonstrated by assessment of vascular sprouting using aortic rings as an ex vivo model of angiogenesis. When S1P or KRX-725 were combined with other growth factors such as basic fibroblast growth factor (b-FGF), stem cell factor, or vascular endothelial growth factor (VEGF), the investigators observed synergistic induction of angiogenesis [84]. In a cultured mouse allantois explant model of blood vessel formation, Argraves et al. [85] showed that S1P, synthesized via the action of SK2, promoted vasculogenesis by promoting migratory activities of angioblasts and early endothelial cells to expand the vascular network.

VEGF has been shown to stimulate SK1 activity with an increase in the production of S1P and activation of H and N Ras oncogenes in T24 bladder tumor cell lines [86]. Endothelial cells undergo morphogenesis into capillary networks in response to S1P involving G protein receptors [87]. S1P has been shown to induce endothelial cell invasion and morphogenesis in physiologically relevant collagen and fibrin matrices [88]. Based on studies employing inhibitors and functional antagonists of S1P receptors, it has been hypothesized that the angiogenic function of S1P is mediated by S1P1 and S1P3 signaling [87, 89, 90].

Knockout of the S1P1 receptor resulted in vascular deficiencies in mice [91]. In a recent study, SphK1–SphK2 double-knockout mice manifested defective neural and vascular systems and exhibited embryonic lethality. The authors inferred that S1P was required for "functionally intertwined pathways of angiogenesis and neurogenesis" [92].

8.4.3 S1P in Inflammation

The SK1/S1P pathway has been implicated in inflammation. For instance, TNF-alpha resulted in activation of SK1/S1P pathway specifically leading to extracellular signal-regulated kinases and NF-kappa B activation [36] and consequently expression of vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) [93]. S1P also induced cyclooxygenase 2 (COX2) and prostaglandin E2 (PGE_2) production in L929 fibrosarcoma and A549 lung adenocarcinoma cells and genetic knockdown using siRNA blocked their production. Additionally, preventing S1P clearance using siRNAs against S1P lyase/phosphatase resulted in increased production of COX2 and PGE_2, implicating a key role of S1P in this pathway [94]. Microglial activation has been implicated in neuroinflammation. LPS treatment increased SK1 mRNA and protein levels and consequently upregulated expression of proinflammatory cytokines such as TNF-alpha, IL-1beta, and iNOS in microglia. Chronic production of inflammatory cytokines by microglia has been implicated in neuroinflammation [95, 96]. Further, the SK/S1P pathway has been implicated in many other inflammatory disease paradigms such as asthma, rheumatoid arthritis, and inflammatory bowel diseases [97].

In summary, these data collectively demonstrate that S1P regulates cancer cell viability, angiogenesis, and inflammation which favor cancer pathogenesis.

8.5 Role of Other Metabolites of Sphingolipid Metabolism in Tumorigenesis

In addition to ceramide and S1P, ceramide -1 phosphate has been implicated in cell survival pathways. Chemotherapeutic agents changed the alternative splicing of caspase 9 and Bcl-x pre-mRNA into pro-apoptotic forms which was mediated by ceramide-dependent activation of PP1 [98]. C1P has been found to inhibit PP1, and therefore, it might antagonize ceramide-mediated apoptosis, functioning as a pro-survival lipid. In another study, C1P blocked apoptosis in part by activating PI3-K/PKB/NF-κB pathways and production of anti-apoptotic Bcl-X_L [99]. CERK might play an important role in regulating the balance between ceramide and C1P and therefore cell death and cell survival similar to S1P.

Sphingosine which is the breakdown product of ceramide is also implicated in apoptotic responses. In one study, gamma irradiation along with TNF-α induced sphingosine and S1P levels. The elevation of sphingosine by exogenous administration of sphingosine or by treatment with SK inhibitor induced apoptosis in LNCaP prostate cancer cell lines [100]. In another study, phorbol myristate acetate (PMA) and tumor necrosis factor (TNF) separately induced apoptosis marked by elevation of sphingosine in HL-60 cells and neutrophils, respectively. Exogenous administration of sphingosine or its methylated derivative N, N,-dimethylsphingosine (DMS) also induced apoptosis in cells of both hematopoietic and carcinoma origin [101]. In an attempt to find the mechanism of sphingosine-induced apoptosis, Domae and colleagues found that sphingosine induced c-Jun expression and apoptosis in HL-60 cells and inhibition of protein kinase A (PKA) potentiated this effect [102]. In another study by Houghton and co-workers, rhabdomyosarcoma cell lines were found to be more sensitive to the induction of apoptosis with an increase in the cellular levels of sphingosine. Mechanistically, sphingosine-mediated cell death involved mitochondrial events such as Bax activation and translocation to the mitochondria, release of cytochrome c and Smac/Diablo, but not apoptosis-inducing factor (AIF), endonuclease G, and HtrA2/Omi, from mitochondria and finally activation of caspase-3 and caspase-9 [103].

Gangliosphingolipids have been implicated in the epithelial-to-mesenchymal cell transition (EMT) which is believed to play a role in cancer progression. Pharmacological inhibition of GlcCer synthase has been shown to result in downregulation of E-cadherin, a major epithelial marker, and upregulation of vimentin and N-cadherin, major mesenchymal cell markers, with marked changes in gangliosphingolipids (Gg4 or GM2) and increased motility, implying these specific glycosphingoplipids (Gg4 or GM2) might play a role in inhibition of EMT [104]. Some glycosphingolipids have been recognized as tumor antigens and thus could participate in tumor cell regulation and as immune targets. In many studies, it was shown that GM3 modulated receptor tyrosine kinase activity in cells [105–107]. In a recent study, ganglioside GM3 inhibited autophosphorylation of the EGFR kinase domain, thereby inactivating it in response to ligand binding, and removal of neuraminic acid of the GM3 headgroup or expression of the K642G mutant released this inhibitory effect [108].

8.6 Sphingolipids in Cancer Therapy

Among the bioactive sphingolipids, ceramide and S1P act as pro-apoptotic and anti-apoptotic lipids, respectively, and therefore, modulation of these lipids may be effective as a treatment strategy for cancer (refer to Fig. 8.4 [109]). Such strategies to increase the accumulation of ceramide and attenuation of S1P are discussed in detail in the following section.

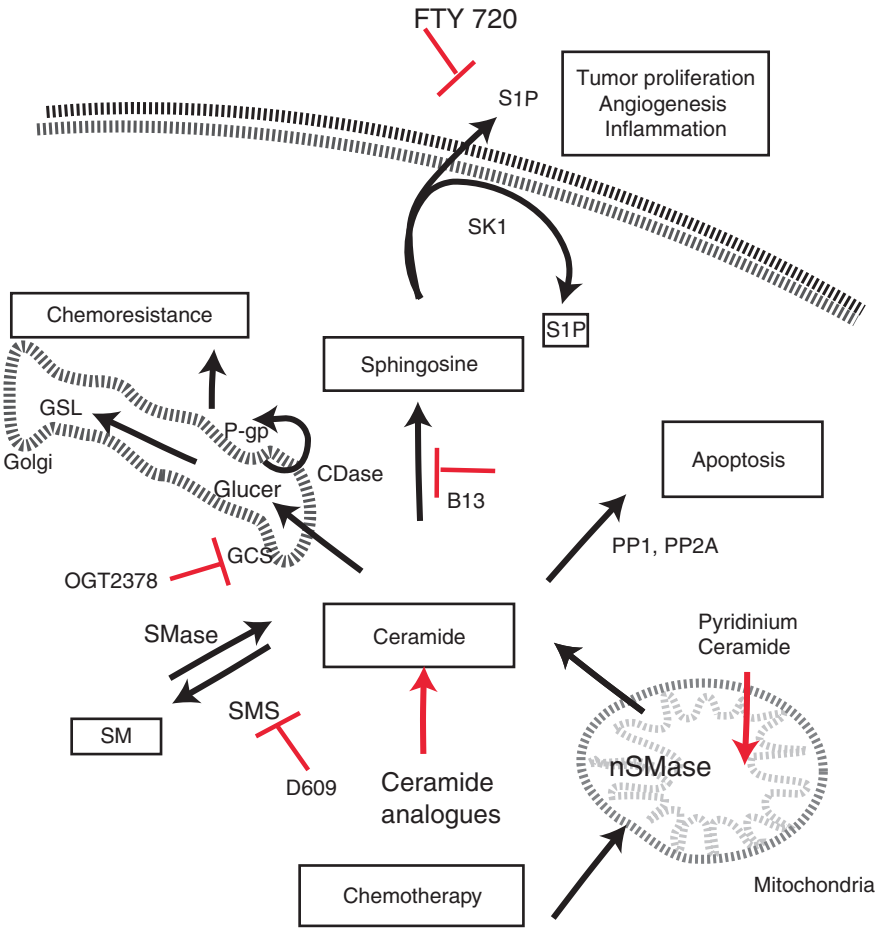

Fig. 8.4 Chemotherapeutic agents increases ceramide levels and induces apoptosis through the de novo pathway or through the neutral sphingomyelinase (*N-SMase*) pathway. Induction of SK1 in colon cancer leads to accumulation of S1P, possibly leading to tumor proliferation, angiogenesis, and inflammation. Clearance of ceramide to Glucosylceramide by GCS in breast cancer cells leads to the development of drug resistance. P-glycoprotein (*P-gp*) expression might potentiate the chemo-resistant phenotype. Marked in red arrows are modulators of SPL metabolism. B13 is an inhibitor of acid CDase, OGT2378 is GCS inhibitor, D609 is an inhibitor of SMS, FTY720 is a sphingosine analogue, and ceramide analogues mimic endogenous ceramides, and Pyridinium ceramide targets mitochondria and promotes mitochondria mediated apoptosis. Abbreviations: *CDase* ceramidase, *ER* endoplasmic reticulum, *PP1* protein phosphatase 1, *PP2A* protein phosphatase 2A, *GSL* glycosphingolipid, *GCS* glucosylceramide synthase

8.6.1 Targeting Ceramide Generation

Cytotoxic chemotherapeutic agents such as daunorubicin, etoposide, camptothecin, fludarabine, and gemcitabine were shown to induce de novo ceramide generation, and inhibition of this pathway reduced the cytotoxic responses to these drugs meaning

Table 8.1 Sphingolipid analogues and inhibitors of ceramide metabolism

Compounds	Mode of action	Cancer types
B13	Acid ceramidase inhibitor	Prostate and colon
D609	Sphingomyelin synthase inhibitor	Monocytic leukemia
C16 Serinol, 4,6-diene-ceramide, 5R-OH-3E-C_8-ceramide, adamantyl-ceramide, and benzene-C_4-ceramide	Ceramide analogue	Neuroblastoma and breast
Pyridinium ceramide	Ceramide analogue	Head and neck squamous cell carcinoma, breast and colon
Pegylated liposomes with ceramide	Improved delivery	Breast
Vincristine in SM-liposomes	Improved delivery	Acute lymphoid leukemia
FTY720	Sphingosine analogue	Lymphoma, bladder, glioma, prostate
OGT2378	GCS inhibitor	Melanoma

that these drugs manifest their cytotoxic effects partly through ceramide production [110–112]. Besides the de novo pathway, certain drugs, including daunorubicin, also induce ceramide generation through the activation of nSMase which hydrolyzes sphingomyelin to generate ceramide. For instance, in leukemia cells, cytosine arabinoside (Ara-C) induced activation of nSMase [113]. Mechanistically, this induction of nSMase was brought about by generation of reactive oxygen species followed by Jun N-terminal kinase phosphorylation and apoptosis [114]. In another study, actinomycin D and etoposide induced nSMase activity in a p53- and ROS-dependent manner [115].

Alternatively, studies on reagents that inhibit the enzymes that favor the ceramide clearance pathway, leading to accumulation of ceramide and, thereby, potentiating the cytotoxic effects were tested. For instance, compounds such as B13 that inhibited acid CDase or tricyclodecan-9-yl-xanthogenate (D609) that inhibited SMS, induced apoptosis in colon cancer and in U937 human monocytic leukemia cells, respectively [116, 117] (refer to Fig. 8.4 and Table 8.1).

Interestingly, SM was found to potentiate the chemotherapeutic response of gemicitabine in prostate cancer cell lines [118]. In a study involving combination of SM with chemotherapeutic agents, doxorubicin, epirubicin, or topotecan, it was found that the combination therapy increased the cytotoxic effect of the drugs by increasing their bioavailability, possibly by modulation of plasma membrane lipophilicity, facilitating entry of these agents into the various cancer cell lines studied [119].

8.6.2 Mimicking Ceramide Action (Analogues of Ceramide)

Ceramide analogues (such as the soluble short-chain C_2- and C_6-ceramides) have been shown to bring about cell death in many types of cancer cell lines tested [120]. C_{16}-serinol, 4, 6-diene-ceramide, 5R-OH-3E-C_8-ceramide, adamantyl-ceramide, and benzene-C_4-ceramide (Table 8.1) are some of the ceramide analogues that

induced cell death in cell lines such as neuroblastoma and breast cancer [121–124]. A novel, cationic, water soluble, pyridinium ceramide (Table 8.1) accumulated predominantly in cellular compartments that are negatively charged such as mitochondria and the nucleus and caused changes in mitochondrial structure and function and inhibited growth in various human head and neck cancer cell lines [125], while inducing apoptosis in squamous cell carcinoma (HNSCC) cell lines [126–128].

Experiments to uncover the most efficient means of delivery of these ceramide analogues have been tried extensively. Pegylated liposomes were very effective in bringing about ceramide-mediated cell death in breast cancer cell lines (Table 8.1). Liposomal delivery of ceramide decreased phosphorylated AKT and activation of caspase-3/7 more effectively than non-liposomal ceramide [129]. Vincristine incorporated in SM-liposomes called sphingosomes (Table 8.1) was found to be effective in animal models for treatment of acute lymphocytic leukemia (ALL) to the extent that it is currently in Phase II clinical trials [130]. These studies clearly demonstrate that targeting ceramide generation might be an effective method to bring about cancer cell death.

8.6.3 Attenuation of the S1P Pathway

Since S1P is found to be involved in angiogenesis and proliferation, it is intuitive to think that modulation of this pathway offers hope for the treatment of cancer. In fact, inhibition of SK1 resulted in increased cell death in many forms of cancer [131, 132] and increased the sensitivity to cell death stimuli such as TNFα and FAS ligand [133]. Dihydroxyaurone, an SK1 inhibitor, exhibits anti-tumor activity in mammary tumors [37]. Interestingly, in normal tissues of ovary and testis, exogenous S1P treatment seems to protect cells from chemotherapy-induced apoptosis [134, 135].

FTY720 (Table 8.1), an analogue of sphingosine, has been found to be phosphorylated in vivo, and the resulting FTY720 phosphate functioned as a ligand for sphingosine-1-phosphate receptors. This signaling mechanism enabled sequestration of lymphocytes in lymphoid tissues thereby causing immunosuppression [136–138]. This compound also induced apoptosis in various cancer cell lines such as lymphocyte and bladder cancer (T24, UMUC3 and HT1197), glioma (T98G), and prostate (DU145) cancer [37, 139–142]. Therefore, it is tempting to hypothesize that S1PR1 and S1PR3 antagonists might have similar anticancer effects. In another study, anti-S1P mAb greatly reduced tumor progression in murine xenograft and allograft models by inhibiting capillary formation and angiogenesis [81]. These data strongly support the candidacy of S1P as a potential therapeutic target for the treatment of cancer.

8.6.4 Dietary SM as a Cancer Therapeutic

Brasitus and co-workers demonstrated that there was a significant increase in SM levels and activity of SMS in rat colonic mucosa in response to 1, 2-dimethylhydrazine

(DMH), a chemical colonic carcinogen [143]. Merrill and co-workers found that milk sphingomyelin dietary supplementation reduced the incidence of DMH-induced pre-malignant lesions of colon tumors in CF1 mice [144]. In addition, mice fed with SM developed fewer adenocarcinomas. These findings suggest that milk SM might suppress advanced malignant tumors in colon [145]. Administration of synthetic SM and ceramide analogues also suppressed colonic crypt foci formation [146, 147]. In another study, azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced colon carcinogenesis was modulated by dietary SM in the early stages by activation of peroxisome proliferator-activated receptor γ (PPAR-γ), but its anti-carcinogenic effect was independent of PPAR-γ [148]. Therefore, dietary SM might modulate the proteins expressed during early stages of colon carcinogenesis and therefore may be a potential therapeutic candidate in the context of colon carcinogenesis.

8.7 Sphingolipids in Drug Resistance

One of the reasons for the failure of chemotherapy in cancer is the development of tumor cell resistance. Part of the basis for chemoresistance might be attributed to a re-wiring of sphingolipid metabolism. For instance, in many cases of leukemia, breast cancer, and melanoma, chemotherapeutic agents increase the activity of GCS which thereby attenuates ceramide levels, resulting in a drug resistance phenotype [20, 149]. Overexpression of GCS offered increased resistance to doxorubicin whereas siRNA knockdown promoted increased sensitivity to doxorubicin, paclitaxel, and etoposide in breast cancer cells [150–152]. Mechanistically, GCS upregulated P-glycoprotein (P-gp) which is an ABC transporter implicated in drug resistance. Knockdown of GCS inhibited MDR1, a gene that encodes P-gp, reversing drug resistance [153, 154].

Based on the above studies, GCS inhibition has been predicted to improve the effectiveness of chemotherapeutic drugs. Some studies suggest that this hypothesis is in fact true. For instance, OGT2378 (Table 8.1), an inhibitor of GCS, inhibited melanoma growth in a syngeneic orthotopic murine model [155]. In separate studies, combination of fenretinide, a compound that induces accumulation of dihydroceramide [156] through direct inhibition of dihydroceramide desaturase [157], with GCS inhibitors resulted in synergistic suppression of the growth of various tumors. Additionally, fenretinide combined with SK inhibitors such as PPMP or safingol caused growth inhibition [158, 159].

Sphingosine kinase and S1P have also been implicated in drug resistance phenotypes. For instance, it has been brought to light that certain drug-resistant melanoma cell lines such as Mel-2a and M221 are resistant to Fas-induced cell death due to a decrease in ceramide and an increase in S1P compared with Fas-sensitive counterparts such as A-375 and M186. Downregulation of SK1 with siRNA decreased the resistance of Mel-2a cells to apoptosis [160]. Similar inference was made in camptothecin resistant prostate cancer cell lines [161]. In a recent study, SK1 was found to be upregulated in imatinib-resistant chronic myeloid leukemia

cell line concomitant with increased BCR-ABL mRNA and protein levels. The PI3K/AKT/mTOR pathway was also found to be upregulated. Knocking down SK1 expression using siRNA reversed the imatinib resistance to apoptosis and returned BCR-ABL to normal levels [162], suggesting a role for SK1 in conferring drug resistance.

8.8 Conclusions

There is compelling evidence to suggest that sphingolipid metabolism plays an integral part of cancer pathogenesis and therapeutic response. Ceramide is a bioactive lipid that activates signaling pathways to induce apoptosis of various cancer cell lines. S1P, on the other hand, is emerging as a pro-proliferative lipid that is frequently upregulated in tumors. Therapeutic regimens targeting the balance of ceramide and S1P may prove useful in the treatment of many carcinomas.

In spite of our understanding of sphingolipid metabolism, and its relevance in cancer models, we still have a long way to go in understanding the intricacies of sphingolipid metabolism, how the metabolism proceeds in different cancer subtypes, how compartmentalization of metabolism may offer unique regulatory roles, and how different species of individual lipid molecules provide unique signaling functions. With the advent of sophisticated lipidomics and bioinformatic approaches, more and more sphingolipid functions/signaling mechanisms are being uncovered, and this area of research holds and will continue to hold promise as a potential avenue of therapy.

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