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“Evolving concepts in cancer therapy through targeting sphingolipid metabolism”

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Abstract

Traditional methods of cancer treatment are limited in their efficacy due to both inherent and acquired factors. Many different studies have shown that the generation of ceramide in response to cytotoxic therapy is generally an important step leading to cell death. Cancer cells employ different methods to both limit ceramide generation and to remove ceramide in order to become resistant to treatment. Furthermore, sphingosine kinase activity, which phosphorylates sphingosine the product of ceramide hydrolysis, has been linked to multidrug resistance, and can act as a strong survival factor. This review will examine several of the most frequently used cancer therapies and their effect on both ceramide generation and the mechanisms employed to remove it. The development and use of inhibitors of sphingosine kinase will be focused upon as an example of how targeting sphingolipid metabolism may provide an effective means to improve treatment response rates and reduce associated treatment toxicity.

Keywords

Cancer; ceramide; chemoresistance; sphingosine kinase; sphingosine-1-phosphate; inhibitors

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Introduction

The first generally accepted case of a chemotherapeutic agent being successfully used to treat cancer was in 1865 by Lissauer using potassium arsenite on a patient with chronic myelogenous leukemia [1]. There is reference from texts over 1000 years old of the use of wild chervil roots (containing deoxypodophyllotoxin) for the treatment of cancer [2]. However, for the first half of the twentieth century, radiation therapy (along with surgery) was generally the most effective cancer therapy. The discovery that nitrogen mustard (from mustard gas) could be used in the treatment of lymphoma in 1943 began a new era in the development and use of more effective cancer therapies [3]. Unfortunately, follow ups of patients treated with nitrogen mustard showed that remission was both incomplete and short [4]. This has been a recurring problem faced during cancer therapy throughout medical history.

The loss of normal growth control in cancer cells provides one common characteristic that can be generally targeted by cancer therapy [5]. Radiation therapy produces DNA damage in the cancer cell leading to its death by apoptosis or replicative shutdown due to irreparable and widespread DNA damage [6]. The majority of traditional chemotherapeutic drugs used today target various cellular components vital for cellular division [7].

Many connections have been developing between cancer therapies and sphingolipid metabolism. Several cancer treatments often result in the generation of ceramide which has been implicated in mediating or at least regulating the cell death response. As such, abrogation of ceramide generation is one of the mechanisms exploited by cancer cells in order to evade treatment as first demonstrated with induction of glucosylceramide synthesis [8]. Another common survival strategy employed by cancer cells is the synthesis of sphingosine 1-phosphate (S1P), formed by phosphorylating the product of ceramide hydrolysis [9]. In this review the role of sphingolipids in the induction of apoptosis or resistance to some of the most commonly used cancer therapies will be examined. In addition, strategies inhibiting sphingosine kinase (SK) activity will be reviewed, along with their effects on chemotherapy and their effect on chemoresistance. It is hoped that this will highlight the potential of manipulating the sphingolipid pathway as a means to both increase the efficiency of treatment and potentially reduce its more serious side effects.

Part 1. Sphingolipids and the initiation of apoptosis

Sphingolipids comprise a broad group of both structural and bioactive lipids [10, 11], and they are generated *de novo* in the endoplasmic reticulum from non-sphingolipid precursors. Ceramide can be considered the central hub of the sphingolipid pathway, and its generation has been observed following diverse treatments that can induce many different cellular effects including apoptosis, growth arrest, senescence and differentiation [12]. Induction of ceramide can be achieved either through hydrolysis of sphingomyelin by sphingomyelinases, hydrolysis of cerebroside, or via the *de novo* pathway by ceramide synthases [13, 14]. The sphingomyelinase and *de novo* pathways are the best studied so far.

1.1. Generation of ceramide

1.1.1. Sphingomyelinases—Sphingomyelinases exist as three major groups depending on the pH required for optimal activity, neutral, acid and alkaline, and can hydrolyze sphingomyelin to form ceramide [15]. The potential role of sphingomyelinases in cancer therapy remains to be properly elucidated. Studies have shown levels of alkaline SMase activity are reduced in human colorectal carcinomas, suggesting a role in the development of malignancy [16]. Treatment of several diverse cell lines (including multidrug resistant prostate cancer cell line DU-1. 45) with either Sunitinib or SU11652, both multitargeting-tyrosine kinase inhibitors, inhibited acid sphingomyelinase (ASMase) activity leading to lysosomal destabilization and cell death [17]. Another somewhat contradictory report showed that treatment of implanted hepatocellular carcinoma cells with both sorafenib (a multi-serine/threonine kinase inhibitor) and recombinant ASMase increased cell death relative to sorafenib alone [18]. This is backed up by a study showing that liver ASMase activity can inhibit the growth of metastatic colon cancer [19]. It therefore appears that the activity of ASMase in promoting cancer death may be tied to both the cell type and the protein kinases that are present.

At present three different neutral SMase (nSMase) isoforms, encoded in separate genes, have been identified in mammals [20]. In the mid 1990's a role for nSMase activity in chemotherapy was reported in 1- β -D-Arabinofuranosylcytosine (Ara-C) treatment of HL-60 (human promyelocytic leukemia cells) [21]. A role for nSMase in cell growth was suggested when cells overexpressing nSMase 2 exhibited slower proliferation, while growth arrested MCF-7 breast cancer cells had increased levels of nSMase 2 [22, 23]. Conversely, treatment of human mammary epithelial cells 184B5/HER with either exogenous nSMase or C₂ or C₆ ceramide could increase both cyclooxygenase 2 gene and protein expression and increase proliferation [24]. Analysis of nSMase genes showed that 5% of human acute myeloid leukemias and 6% of acute lymphoid leukemias tested had inactivating mutations [25]. Furthermore, nSMase 2 has been reported to promote angiogenesis and regulate metastasis through regulation of exosomal microRNA secretion [26]. Different isoforms of nSMase have been found within the nuclear envelope, nuclear matrix and associated with chromatin [27]. SMase activity is associated with chromatin unwinding and the initiation of replication, although nuclear SMase activity can also induce an apoptotic response [27, 28]. Interestingly, SMase-treatment of RNase-resistant RNA can render it more sensitive to degradation, suggesting a role for sphingomyelin in RNA stability [29].

1.1.2. Ceramide synthases—Ceramide synthases are integral membrane proteins localized in the endoplasmic reticulum, and 6 different enzymes have been identified and have been named CerS1-6 [30, 31]. Each CerS shows specificity towards a fatty acyl CoA of different chain length, resulting in the synthesis of ceramides of different chain length [31]. Ceramide generated by CerS can be transported to the Golgi by either vesicular trafficking or through ceramide transfer protein, CERT [32]. Studies in knockout (KO) mice have shown that while activity of other expressed CerS seems to increase to compensate for total cellular levels of ceramide, severe pathologies based on the particular CerS KO have been reported [33, 34]. The CerS family has yet to be fully characterized, although the formation of CerS homo- and hetero- dimers appears to be a factor in modulating their activity [35].

CerS expression may be altered in several different human cancers such as head and neck, and is often associated with enhanced cancer survival [31].

1.2. Mechanisms of ceramide-induced apoptosis

Ceramide formation (mostly through the activity of sphingomyelinases) can enhance the signaling of proapoptotic membrane molecules such as CD95, part of the extrinsic pathway [36, 37]. Ceramide can also initiate apoptosis through intrinsic pathways involving intracellular mechanisms. Through hydrophobic interactions, ceramide can form transmembrane channels in the mitochondria that may allow the release of apoptosis-inducing proteins [38] and reviewed in [39]. The formation of these channels can be regulated by members of the Bcl-2 family such as Bax and Bcl-xL [39].

Ceramide may initiate apoptotic signaling by down-regulating the activity of the serine/threonine protein kinase Akt, a kinase that can mediate many anti-apoptotic functions [40]. Inactivation or alteration of Akt signaling in cancer is associated with chemoresistance and generally indicates a poor prognosis [41]. Ceramide can activate apoptosis signal-regulating kinase 1 (ASK1 or MAP3K5), which in turn activates p38 and JNK, resulting in apoptosis [42]. Ceramide accumulation can also activate p53 which may lead to the upregulation of the proapoptotic Bax and the subsequent initiation of apoptosis [42] although other studies place ceramide as downstream of p53 [43]. This could be related to differences in studying the function of exogenous ceramides from that of endogenous ceramides, respectively.

Ceramide activated protein phosphatases (CAPP) represent another mechanism that ceramide can exert its effects, the best studied being CAPP1 and CAPP2A [44, 45]. Pretreatment of human squamous carcinoma cells A431 with okadaic acid, an inhibitor of protein phosphatase 2A (including CAPP2A), rendered the cells resistant to C₆ ceramide or tumor necrosis factor α (TNF α)-induced apoptosis [46]. Another potential CAPP substrate is Akt, suggesting a link between CAPP activation and cell survival [40]. While CAPP activation is often associated with anti-apoptotic signaling, CAPP2A activation has been shown to dephosphorylate Bcl-2 and render it inactive [47].

The role of ceramide in senescence was first determined when high levels of ceramide were measured in high-passage fibroblasts [48]. Addition of exogenous C₆ ceramide was able to recapitulate this finding [48]. Treatment of human pancreatic cancer cells with gemcitabine could produce ceramide at low levels that was associated with cell cycle arrest [49]. Intermediate levels of cellular ceramide through addition of C₆ ceramide caused senescence, and at high levels (by adding exogenous sphingomyelin concomitantly with gemcitabine) caused apoptosis [49]. Senescent fibroblasts are more resistant to TNF α -induced apoptosis due at least in part to interrupted ceramide signaling, suggesting that senescence may be another strategy to escape apoptosis [50, 51].

Ceramide has also been associated with telomerase activity and telomere length [52]. Telomeres, long tandem repeats of guanine-rich sequences, protect the ends of chromosomes and are progressively shortened after each cell division [53]. Once the telomere decreases beyond a critical length, the cell undergoes replicative senescence or apoptosis. Many cancer cells upregulate telomerase activity in order to lengthen telomeres [54]. Increased cellular

ceramide levels have been shown to both decrease telomerase activity and accelerate telomere shortening [52, 55, 56].

Thus, increased ceramide commonly (although not exclusively) leads to cell death. Levels of ceramide can be regulated through various different mechanisms to produce new sphingolipids that may also have different cellular effects. Reduction in the amount of ceramide present is therefore one mechanism that can mediate cell survival. Strategies that can inhibit ceramide metabolism may provide new avenues to both enhance specificity and enhance sensitivity.

1.3. Removal of ceramide

1.3.1. Ceramidases—There are three major groups of ceramidases which, like the sphingomyelinases, are characterized by their optimal pH, acid, neutral and alkaline [57]. Sphingosine, the product of ceramide hydrolysis by ceramidases, has been shown to induce apoptosis and also act as an opposing regulator of ERK and SAPK signaling pathways [58, 59]. Interestingly, sphingosine has also been reported to form small, short-lived channels in the outer mitochondrial membrane [60]. Both neutral and acid ceramidase (ACDase) activity have been detected within liver nuclear membranes, although their role is at present undetermined [61]. Elevated acid ceramidase expression has been reported in cancers such as prostate, breast, and head and neck and has been linked to increased malignancy and worse clinical outcomes to treatment [62–64]. Gemcitabine-treatment of polyoma middle T transformed mouse endothelial cells downregulated neutral CDase expression leading to cell cycle arrest [65]. Knock down of neutral CDase by siRNA confirmed its role in cell cycle progression [65]. Inhibition of ceramidase may be of therapeutic effect as treatment of colon cancer cells with ceramidase inhibitor B13 completely suppressed tumor growth in nude mice [66]. Additionally, overexpression of ACDase in prostate cancer cells was associated with increased proliferation and migration, while knock down increased sensitivity to several chemotherapeutic drugs including gemcitabine [67]. While sphingosine can cause cell death, its conversion by SK into sphingosine-1 phosphate (S1P) turns it into a potent survival-associated sphingolipid [68].

1.3.2. Glucosylceramide synthase—Glycosylation of ceramide can occur via glucosyl ceramide synthase (GCS). Glucosylceramide has been reported to play a role in cell proliferation, differentiation and metastasis [69, 70]. In addition, glucosylceramide synthesis has been reported in the development of multi-drug resistant cancers and in upregulating the expression of Multidrug Resistance protein 1 (MDR1) [8, 71–74]. Levels of GCS mRNA may also be a marker of disease progression as levels were most elevated in late stage breast cancer [75]. Use of DL-*threo*-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP), a GCS inhibitor, along with C₆ ceramide-carrying liposomes has been shown to synergistically induce apoptosis in natural killer cell leukemias [76]. Suppression of GCS may also restore p53-dependent apoptosis of p53 mutant ovarian cancer cells [77]. However, while studies using PPMP have demonstrated chemosensitization effects in cancer cells, the non-specific inhibition of other cellular processes hide the true contribution of GCS to the development of multidrug resistance (MDR) [78]. To address this problem, GCS levels were knocked down using siRNA, which conferred sensitivity to both TNF α and doxorubicin

[79]. Conversely, over-expression of GCS conferred resistance to both TNF α and doxorubicin-induced apoptosis through suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and subsequent reactive oxygen species generation [79]. Drugs designed to inhibit GCS in the context of Gaucher disease type 1 (lysosomal storage disorder) have been developed and show good specificity and are well tolerated [80]. However, caution in using GCS inhibitors is warranted as it has been reported that PPMP (along with RNAi) can cause cytokinesis failure and multinucleation in human cervical adenocarcinoma cells HeLa, which can lead to chemoresistance [81, 82].

1.3.3. Ceramide kinase—Ceramide kinase can phosphorylate ceramide to produce ceramide-1 phosphate (C1P) [83]. The synthesis of C1P is associated with inflammatory processes and with the development of metabolic syndrome [83]. Exogenous C1P can promote bone marrow-derived macrophage proliferation, while addition of C1P to growth factor deprived macrophages could protect them from apoptosis through inhibition of ASMase [84, 85]. The primary sequence of ceramide kinase has been shown to possess both nuclear import and export sequences suggesting an as yet unknown nuclear role for C1P [86].

1.4. Sphingosine kinase

Sphingosine kinase has two major isoforms (SK1 and SK2) that are encoded by separate genes and which share 80% similarity of their amino acid sequences [87]. Along with GCS, SK1 and possibly SK2 have been majorly implicated in cellular survival pathways. The sphingosine kinases phosphorylate sphingosine to S1P which can exert its effects either through multiple G protein-coupled receptors (named S1PR1-5) or through direct intracellular effects [88]. The different S1P receptors may play diverse roles, such as S1PR1 being implicated in the trafficking of immune cells and its inhibition (through FTY-720, trade name Fingolimod) is now used to decrease the relapse rate of patients with relapsing-remitting multiple sclerosis [89, 90]. Cancer cells frequently generate S1P that can act through either an autocrine or paracrine manner to promote growth, survival, metastasis, angiogenesis or regulate immune function [91–93]. Exogenous S1P has been shown to upregulate matrix metalloproteinase 2 gene expression and protein, providing a mechanism on how S1P may promote both metastasis and angiogenesis [94, 95]. Nuclear S1P has been associated with progression through the S phase of the cell cycle [96]. It has been shown that S1PR1 signaling can act in a positive feedback loop in increasing signal transducer and activator of transcription-3 activity and driving cancer initiation, development and progression [97]. Another important role of S1P is in mediating the Hippo-YAP pathway that can both regulate adult organ size and also modulate proliferation, apoptosis and differentiation [98]. Extracellular S1P has also potent angiogenic and inflammatory effects that can also affect tumor growth [99–101]. Elevated levels of S1PR1 promote the development of localized T lymphoblastic lymphoma into disseminated T lymphoblastic leukemia [102]. Of potential major importance to cancer research are the effects of S1P to suppress ceramide-induced apoptosis [103].

Once S1P is generated, it can be irreversibly cleaved through the action of S1P lyase or converted back to sphingosine by S1P phosphatase [104, 105]. Through the action of S1P

Lyase, S1P provides a link between sphingolipids and glycerophospholipids [106]. Interestingly, over expression of S1P lyase in human embryonic kidney, A549 lung cancer, MCF-7 and DLD-1 colon cancer cells has been shown to sensitize cells to apoptotic stress, probably through reduction of intracellular S1P levels [107–109]. Conversely, S1P lyase has been reported to be significantly downregulated in human colon cancer and in prostate cancer when compared to normal tissues [110, 111]. In addition, the chromosomal region containing the gene for S1P lyase is often deleted in human cancers [112–114].

1.4.1. Sphingosine kinase 1—Of particular interest to potential cancer chemotherapy is SK1. Located primarily in the cytoplasm, SK1 can undergo translocation to the plasma membrane in response to activation of protein kinase C (PKC) and/or phospholipase D and after phosphorylation by ERK [115–117]. Through alternative splicing, SK1 had been identified as having three different forms in humans, named SK1a, SK1b, and SK1c, of which the isoform SK1a may be extracellularly exported [118]. There may be a role for SK in regulating cellular ceramide levels by phosphorylating dihydrosphingosine, thus removing it from the ceramide biosynthetic pathway [119, 120]. By mediating oncogenic H-ras transformation, SK1 has been shown to act in some cases as an oncogene [121]. Furthermore, SK1 has been described in ‘oncogene addiction’ of human epidermal growth factor-2 expressing breast cancer cells [122]. The concept of ‘oncogene addiction’ is evoked when a cancer type requires expression of an oncogene to maintain its disease phenotype. Since SK1 does not usually display any mutations that change its function, it can also be referred to as ‘non-oncogene addiction’ [123]. Proteolytic cleavage of SK1 (and its inactivation) mediated by p53 activation in response to DNA damage appears to be an important step in initiating the apoptotic process [124]. Overexpression of SK1 has been described in many different cancer types including lung, kidney, breast, ovarian, stomach and glioblastoma [125–128]. The overexpression of SK1 has been linked to advanced disease progression, resistance to chemotherapeutic drugs like doxorubicin and also to a poor prognosis [100, 127, 129–132]. Colon cancer is often preceded by chronic inflammation, and levels of SK are upregulated in mouse models of ulcerative colitis [133]. Mice with SK1 KO appear resistant for the development of colorectal cancer [101]. It was later shown that and an increase in SK1 expression in both inflammation and mutagen-treated mice drove the development of colon cancer [134].

1.4.2. Sphingosine kinase 2—While less studied than SK1, overexpression of SK2 *in vitro* appears to have opposite effects to SK1 expression [135]. The subcellular localization of SK2 may alter its effects on growth. While in the nucleus, SK2 can halt DNA replication through inhibition of histone deacetylase 2 [136, 137]. Interestingly, when COS-7 fibroblasts become confluent, the amount of nuclear SK2 increases [136]. This raises the possibility that SK2 may contribute to epigenetic changes associated with cancer. However, localization of SK2 to the endoplasmic reticulum under stress conditions promotes apoptosis [135]. Underlining the importance of subcellular localization to function, artificial targeting of the normally anti-apoptotic SK1 to the endoplasmic reticulum or nucleus could also promote apoptosis [135, 136]. Interestingly, despite the apparent differences in SK1 and 2 function, mice with either SK1 or SK2 knocked out are both viable and fertile suggesting a level of functional redundancy between the two SK forms [138]. On the other hand, mice

lacking both SK1 and SK2 were embryonically lethal, displaying widespread vascular and neural defects [138]. Studies using siRNA against SK2 in A498 kidney adenocarcinoma cells could inhibit their growth more effectively than siRNA against SK1 [139]. Interestingly, ablation of SK2 led to increased intracellular S1P and SK1 expression, while ablation of SK1 did not lead to any corresponding increase in SK2 or S1P [139].

1.4.3. The S1P/ceramide ‘rheostat’ model—Because of the mainly pro-apoptotic effects of ceramide versus the mainly pro-survival effects of S1P, the rheostat model has been conceived [140]. According to the model, if S1P levels were to go down relative to ceramide levels, the cell would undergo apoptosis, and vice versa. While this model may account for gross cellular changes in either S1P or ceramide, the subcellular compartmentalization of either sphingolipid and the diversity of ceramide structure and function are over-looked [12, 141].

Part 2. Mechanisms behind resistance to cancer therapy

Chemotherapeutic drug resistance can be either innate and/or acquired, and result in recurrent cancers often being more resistant to treatment [142]. Innate mechanisms are inherent features of the particular cancer that render it drug resistant, and share many of the same processes that that acquired resistance of relapsed cancer can utilize. The mechanisms that can make cancer resistant to chemotherapy can be mediated through several different pathways.

2.1. Principles of resistance: Innate and acquired resistance

Entry of chemotherapeutic drugs into a cancer cell can be reduced through either a loss of or a mutation of drug transporters [143]. Chemotherapeutic drugs can be removed from the cell, thus lowering the intracellular drug concentration through enhanced efflux by over-expressed ATP-binding cassette proteins (such ABCB1 or MDR1) [144]. Incomplete drug penetration into solid tumors presents another innate mechanism reducing chemotherapeutic drug efficiency. The tumor microenvironment can be either hypoxic or have defective vasculature preventing optimal chemotherapeutic drug delivery [145, 146]. Furthermore, some tumors of the central nervous system or the brain are protected by the blood/brain barrier which can severely limit the penetration of chemotherapeutic drugs [147]. Enhanced or acquired detoxification mechanisms or drug metabolism such as through superoxide dismutase, glutathione S-transferase, or cytochrome P450 can mitigate drug efficacy [148–150]. Mutation of cell cycle checkpoints or deregulation of apoptotic pathways can suppress drug-induced cell death reviewed in [151, 152]. For example, an important study based on an unbiased screen of RNAi data showed that resistance to several chemotherapeutic drugs was mediated by regulators of mitotic arrest and chromosomal instability [153, 154]. These identified CERT and GCS as potentially important regulators of responses to paclitaxel. Conversely, chemotherapy-induced DNA damage can be reduced through activation of DNA repair mechanisms [143]. Therefore, in order to increase the efficacy of chemotherapeutic drug-treatment, one or more of these mechanisms must be overcome.

Part 3. The effect of sphingolipids on cancer treatment

3.1. Radiation Therapy

For many years DNA damage was considered the primary focus behind radiation-induced cell death. However, in recent years it has been reported that cell membranes may also be a target [155]. Later research showed that elements within the cytoplasm, when targeted with microbeam radiation, could also induce radiosensitivity [156]. Agents that could disrupt the formation of lipid rafts (filipin) could inhibit the effect, showing again that membranes can be a target of ionizing radiation. Ionizing radiation has been shown to induce cell death via at least two separate mechanisms, one involving ASMase and one involving CerS. A major problem involved in radiotherapy is the radiation-induced damage of nearby non-cancerous tissue. Furthermore, radiation damage to neighboring tissues can increase the probability of future malignancies forming [157]. While new technologies such as stereotactic radiosurgery can lessen the radiation dosage delivered to normal tissues while increasing the delivered radiation dose, the inherent resistance of some tumors to radiation treatment remains a significant limiting factor [158].

3.1.1. Role of ASMase on radiation-induced apoptosis—The importance of membranes and ceramide in the cellular response to ionizing radiation was first shown in 1994 when ceramide generation was observed in irradiated nuclei-free plasma membranes isolated from endothelial cells [155]. Concomitantly with the ceramide increase, sphingomyelin levels decreased, suggested the activation of a SMase. Remarkably, the radiation-induced ceramide generation occurred within a span of around 2 minutes. Lymphoblasts lacking in ASMase are insensitive to ionizing radiation-induced apoptosis and can be sensitized by genetic reintroduction of ASMase [159]. However, ionizing radiation-induced ASMase activation has been mostly described in endothelial cells. Whether the rapid activation of ASMase after irradiation is due to its relatively high abundance in endothelial cells compared to other cell types is still unknown [160]. The importance of the endothelial response to radiation treatment was underlined when implanted tumors, responsive to radiation in a mouse expressing ASMase, were rendered radioresistant in an ASMase KO mouse, thus suggesting a role for ASMase in host tissue and not tumor [161]. Immunohistochemistry showed that blood vessels infiltrating the tumor were undamaged in the irradiated ASMase KO mouse. Mice with either B16-F1 melanomas or MCA/129 fibrosarcomas had their tumors radiosensitized after intravenous injection of an adenoviral vector containing ASMase under control of an endothelial cell-specific promoter [162]. Interestingly, while the total numbers of transduced tumor endothelial cells was relatively low, *in vitro* transduction of endothelial cells showed most ASMase activity to be in the conditioned medium, suggesting a role for secretory ASMase in initiating radiation-induced cell death [162]. Therefore, through modification of radiation-induced ASMase activation, it may be possible to both increase damage to tumor vasculature and through inhibition to lessen damage to normal blood vessels.

3.1.2. Role of *de novo* ceramide generation in radiation-induced apoptosis—The second ceramide-mediated pathway activated by ionizing radiation is via ceramide synthase. Studies on endothelial cells showed that CerS activation occurred 12 hours

following radiation [163]. Inhibition of CerS activity with fumonisin B1 inhibited the radiation-induced increase in ceramide and the subsequent apoptosis. Interestingly, irradiation of phorbol ester (potent activator of conventional and novel PKC families)-pretreated prostate cancer cell line LNCaP did not produce any measurable increase in ASMase activation, but did lead to an increase in CerS activity and apoptosis. While the mechanism is unknown, the inhibition of radiation-induced CerS activity by ataxia-telangiectasia mutated protein has been reported [163, 164]. Interestingly, some reports suggest that the ceramide chain length generated in response to ionizing radiation may also play a role in determining radiation sensitivity [165]. While ionizing radiation activated CerS 2, 5 and 6, overexpression of CerS2, responsible for the generation of C_{24:0} ceramide, partially protected HeLa cells from radiation-induced apoptosis [165]. Conversely, overexpression of CerS5 led to increased C_{16:0} ceramide and greater radiation-induced apoptosis [165]. Thus, selectively suppressing or enhancing CerS activation in response to ionizing radiation may increase both selectivity and efficiency of the treatment.

3.1.3. Role of ACDase and SK1 in radiation-resistance—Some cancer cells such as prostate or head and neck are able to escape ceramide-induced cell death by over-expressing ceramidases [62, 166]. Recent studies have shown that radiation treatment of prostate cancer cells can upregulate ACDase expression [167]. Surviving prostate cancer clonogens had increased ACDase expression which mediated both their increased radiation resistance and increased proliferation [167]. Inhibition of ACDase activity following irradiation of U87-MG glioma cells or of the PPC-1 prostate cancer cell line could confer sensitivity to radiation-induced apoptosis [168, 169]. The origin of the generated ceramide (either through the activity of ASMase or CerS) is unknown. These results suggest that inhibition of ceramidase activity may confer increased radiation sensitivity, allowing for more efficient tumor control and for the lowering of the effective dose.

Analogues of S1P have been shown to reduce murine radiation-induced lung injury, a serious potential side-effect to thoracic radiation treatment [170]. Pre-treatment of female macaque monkeys with S1P one week before ionizing radiation treatment could maintain fertility after irradiation of the ovaries [171]. Furthermore, xenografts of human ovarian cortical tissue also showed a suppression of radiation-induced oocyte depletion after pretreatment with S1P [171]. Protection of male mouse germ-cells from radiation treatment by S1P has also been reported [172]. Through activation of the Akt pathway, S1P can also protect the small intestine from radiation-induced endothelial apoptosis [173].

3.2. Doxorubicin

Doxorubicin was first isolated from a strain of *Streptomyces peucetius* treated with the mutagen N-nitroso-N-methyl urethane. It has been used to treat a wide spectrum of cancers including Hodgkin's lymphoma, breast, bladder and stomach [174]. Doxorubicin inhibits topoisomerase II activity through intercalation of DNA, inhibiting synthesis of DNA and RNA and causing DNA damage [175, 176]. The most serious adverse effect associated with doxorubicin chemotherapy is cardiotoxicity, where the mortality rate for induced congestive heart failure leading from cardiomyopathy can reach 50% [177, 178]. In addition, doxorubicin treatment has also been associated with male infertility [179, 180].

Treatment of follicular thyroid carcinoma cells with doxorubicin increased ceramide via the *de novo* pathway [181]. Interestingly, it has been shown that in resistant HL-60 cells, doxorubicin can increase GCS expression via activation of the transcription factor sp-1 [182]. Recapitulating this result, doses of doxorubicin (0.1 μ M – 0.7 μ M) in MCF-7 cells could also increase GCS protein levels through increased transcription mediated by the transcription factor sp1 [183, 184]. Furthermore, the addition of C₆ ceramide to MCF-7 could upregulate transcription of GCS, showing a link between ceramide and GCS expression. Increased GCS resulting from sub-optimal treatment with chemotherapeutic drugs may represent one mechanism through which multi-drug resistant (MDR) cells could be selected for. Conversely, treatment of Burkitt lymphoma cells or mouse *ex-vivo* T cells with sub-optimal doses of doxorubicin could produce ASMase-generated ceramide which sensitized the cells to subsequent TRAIL-induced apoptosis [185]. This further underlines the importance of ceramide for the initiation of the apoptotic pathway. Doxorubicin treatment (along with paclitaxel, tamoxifen and methotrexate) has been shown to disrupt mitochondrial function and stimulate NADPH oxidase activity which was blocked with increased GCS function [79]. Treatment of neonatal rat cardiomyocytes with doxorubicin caused mitochondrial fragmentation in a manner similar to C₂ ceramide, suggesting a role for ceramide in doxorubicin-induced cardiomyopathy [186]. Furthermore, adult rat cardiomyocytes treated with doxorubicin slowly accumulated ceramide, which by 7 days could initiate an apoptotic response [187]. In rats treated with 7.5mg/kg doxorubicin, an increase in C₁₆ ceramide was observed in the testis 2 days following treatment [188]. This suggests that the level of ceramide generated in response to treatment determines, at least in part, whether the cell will undergo apoptosis. Addition of exogenous C₆ ceramide can sensitize several different cancer cell lines to doxorubicin by promoting AMP-activated kinase activation and inhibiting mammalian target of rapamycin complex 1 [189]. High levels of SK1 in non small cell lung cancer significantly contributed to doxorubicin resistance [132]. Down-regulation of SK1 expression in gastric cancer cell lines and MCF-7 cells could sensitize them to doxorubicin-treatment [190, 191]. Interestingly, binding of high-density lipoprotein to S1PR2 (but not S1PR1 or S1PR3) could protect cardiomyocytes from doxorubicin-induced cell death [192].

3.3. Paclitaxel

Paclitaxel (often also referred to as Taxol) is a chemotherapeutic used in the treatment for breast, ovarian and lung cancer. Paclitaxel binds to β -tubulin, and as a result stabilizes microtubules and prevents their normal depolymerization/polymerization [193, 194]. Microtubule assembly and disassembly are essential during mitosis, thus paclitaxel treatment causes cell cycle arrest at G2/M and apoptosis [195]. At higher doses, paclitaxel has also been observed to promote the polymerization of stable microtubules [196]. In addition to its effects on microtubule stability, paclitaxel has also been shown to cause mitochondrial depolarization and calcium release [197, 198]. Seriously limiting paclitaxel's usage is that up to 30% of patients treated with higher doses of paclitaxel experience neuropathy of sensory axons [199, 200].

Jurkat cells treated concomitantly with ceramide and paclitaxel displayed a higher level of apoptosis [201]. Nanoparticles containing paclitaxel and C₆ ceramide were more effective at

inhibiting the proliferation of aortic smooth muscle cells than with paclitaxel alone [202]. Direct addition of ceramide along with paclitaxel could also increase the apoptotic response of pancreatic cells (L3.6) [203]. Replicative senescence, a loss of cellular ability to proliferate, was induced in human lung cancer cells when C₂ ceramide was added together with paclitaxel [204]. Resistance to paclitaxel treatment of ovarian carcinoma cells was associated with a lack of ceramide generation and not to its hydrolysis or glycosylation [205]. One mechanism perhaps underlying this is the finding that in several cancer types CERT is downregulated [153]. On the other hand, glioblastoma cell lines express high levels of GCS that renders these cell lines intrinsically chemoresistant to paclitaxel treatment [206]. Furthermore, paclitaxel-treated MCF-7 cells activated ASMase and the generated ceramide led to cell rounding and a reduction in cell motility, suggesting that ceramide may play an important role in metastasis [207].

3.4. Etoposide

Etoposide was developed in 1966 from analogues of modified podophyllum toxin [208]. It kills cancer cells through inducing single and double strand breaks in DNA by increasing the steady-state concentration of topoisomerase II-containing DNA cleavage complexes [208]. As topoisomerase II is mostly found in actively dividing cells, it has found use as a potent chemotherapeutic drug [209]. Etoposide is commonly used for the treatment of various cancers including testicular, small cell lung, lymphomas and ovarian cancer [2, 208]. One of the major side-effects of etoposide use is the later development of acute myeloid leukemia in some patients, possibly through oxidative DNA damage [210].

Ceramide generated through the *de novo* pathway, and through increases in serine palmitoyl transferase activity, was reported following etoposide treatment of acute lymphoblastic leukemia cells MOLT-4 [211, 212]. Interestingly, ceramide generation after treatment was biphasic, reaching a peak 4 hours and another at 24–48h post treatment, somewhat reminiscent of radiation-induced ceramide increase [213]. Underlining the importance of ceramide in inducing etoposide (and doxorubicin) cell death, pretreatment of MDR breast cancer cell line MCF-7TN-R cells with a ceramide analogue significantly increased sensitivity to treatment [214]. Additionally, the suppression of dihydroceramide desaturase activity, an enzyme that introduces a 4,5-trans-double bond into the sphinganine backbone, has been shown to confer resistance to etoposide-induced apoptosis in mouse embryonic fibroblast cultures [215]. Pretreatment of mouse T-cell hybridomas with lithium could inhibit etoposide and ceramide-induced protein phosphatase 2A activation and protect the cells from apoptosis [216]. Removal of ceramide by increased expression of GCS has also been linked to etoposide resistance [217]. Additionally, mice lacking S1P lyase are resistant to etoposide treatment, suggesting a role for S1P in mediating resistance [218].

3.5. Cisplatin

First approved by the FDA in 1978, cisplatin is either used as a first line treatment or in combination for bladder cancer, cervical cancer, ovarian cancer, non small cell lung cancer, squamous cell carcinoma of the head and neck and testicular cancer [219]. Cisplatin forms DNA intrastrand crosslinks causing DNA damage, and can also cause mitochondrial damage leading to the generation of reactive oxygen species [220]. While not life threatening,

cisplatin can cause severe hearing loss, especially in children [221]. In addition, and more seriously, cisplatin can also cause nephrotoxicity [219].

Ceramide generation in cisplatin-induced apoptosis of glioma cells has been reported [222]. Inhibition of sphingomyelinases suppressed cisplatin-induced apoptosis, while inhibition of ceramidase activity with N-oleoylethanolamine (OE) potentiated the apoptotic effect [222]. Furthermore, treatment of resistant cell cultures with OE increased their sensitivity to cisplatin, suggesting that ceramidase activity is responsible for cisplatin resistance. Knock down of CerS2 led to a reduction of C₂₄ ceramide and an increase in C₁₆ ceramide which increased sensitivity of HeLa cells to cisplatin treatment, suggesting that C₁₆ ceramide induces apoptosis while C₂₄ promotes survival [223]. Other studies showed that cisplatin treatment of baby mouse kidney cells could activate CerS through a Bak-mediated pathway [224]. Interestingly, over-expression of CerS1 could sensitize human embryonic kidney HEK293 cells to cisplatin, while over-expression of SK1 (but not SK2) in those same cells abrogated sensitivity [225]. Plasma membrane redistribution of CD95 in HT-29 colon cancer cells following cisplatin treatment suggest a role for the extrinsic pathway of ceramide-induced apoptosis [226].

3.6. Fluoropyrimidines

The first fluoropyrimidine, 5-fluorouracil (5-FU), was synthesized in 1957, and represents one of the first 'rationally designed' chemotherapeutic agents [227]. For many years 5-FU was the only drug approved for use in the US for the treatment of colorectal cancer, although response rates of 10–15% and a median survival of 8–12 months limit its usefulness [227]. Other cancers treated with 5-FU include gastric cancer and breast cancer. Treatment with 5-FU can kill cells via two mechanisms. The first is inhibition of thymidylate synthase, which synthesizes de novo deoxythymidine monophosphate, which after double phosphorylation, is incorporated into DNA [228]. The second method is by competing with uridine triphosphate inhibiting RNA and protein synthesis. Common side effects include hand/foot syndrome, diarrhea and nausea, although severe cardiotoxicity has also been reported [229].

Inhibition of thymidylate synthase with folate analog GW1843 can increase activity of both ASMase and nSMase leading to apoptosis in MOLT-4 cells [230]. Interestingly, pretreatment with a low dose of the rotenoid deguelin, a potentially chemo-preventative drug against several different cancers, increased ceramide which in turn sensitized the cells to further 5-FU treatment [231]. Another study showed that adding exogenous sphingomyelin could enhance the sensitivity of human colonic xenografts to 5-FU treatment [232]. It is possible that increasing SMase substrate could potentiate the amount of ceramide generated in response to treatment, thereby increasing apoptosis. The role of SMase activity on the extrinsic pathway of apoptotic signaling is suggested by the finding that 5-FU treatment could kill mouse thymocytes via the CD95 pathway [233]. Treatment with carmofur (1-hexylcarbamoyl-5-fluorouracil), a drug that can intracellularly release 5-FU, can significantly inhibit ACDase activity in human colon adenocarcinoma SW403 while increasing ceramide levels [234]. This in turn could sensitize the cells to 5-FU treatment, while pretreatment of ACDase overexpressing cells with carmofur showed no potentiating effect [234].

3.7. Sphingomyelinases vs *de novo* synthesis

Although several studies have found either activation of ASMase or the *de novo* pathway in response to specific chemotherapeutic agents, these two events may not be ‘contradictory’. First, these two pathways could be turned on independently of each other in the same cell. This is supported by the different kinetics observed usually with activation of ASMase (usually early) vs the *de novo* pathway, which is usually later. This was well illustrated in one study with a thymidylate synthase inhibitor, where ASMase activity first peaked 50 mins following treatment then slowly increased to a maximum after 48hrs [230]. On the other hand, nSMase activity first peaked at 20min then peaked again around 18–24hrs after treatment, before dropping [230]. However, total cellular ceramide levels rose 4-fold after 60mins treatment, then started increasing again at 24hrs to reach levels nearly 8-fold higher after 48hrs [230]. Second, our group has demonstrated regulation of the salvage pathway of sphingolipid metabolism whereby hydrolysis of sphingomyelin or cerebroside in the lysosomes leads to the formation of ceramide which is then acted upon by ACDase. The liberated sphingosine can then be reincorporated into ceramide through the action of CerS. Thus, ASMase and CerS can be part of one coordinated pathway, the salvage pathway. Importantly, the accumulated ceramide in this pathway can be inhibited by Fumonisin B1 which therefore does not distinguish the *de novo* pathway from the salvage one. In contrast, myriocin (ISP1) would selectively inhibit *de novo* synthesis. Therefore, the conclusion from some studies that implicate activation of the *de novo* pathway based on using Fumonisin must be considered as inconclusive as to whether it is the *de novo* pathway or the salvage pathway.

3.8. Takeaway lessons from sphingolipid studies

It is therefore clear that for many of the different ‘traditional’ chemotherapeutic drugs, ceramide accumulation is important for the initiation of the apoptotic pathway. While chemotherapeutic drugs can raise ceramide levels in a specific way, cancer cells can also upregulate specific ceramide metabolizing enzymes to counteract the treatment (summarized in Figure 1). Therefore, a tailored approach specific for the cancer type (and maybe even to the individual) might provide a way to both sensitize cancer cells and also limit the dosage to mitigate serious side effects in clinical applications. This kind of approach is still in the early stages of testing, and with the advent of better, more selective inhibitors of sphingolipid metabolism, a potentially promising avenue to explore.

Part 4. Inhibitors of SK1 and their application

4.1.1. Early inhibitors

Of all the sphingolipid-modifying enzymes known, probably research on SK is the most developed at this stage. The role of S1P as a potent survival and resistance factor has further driven research into its role in cancer chemoresistance. With the recent publication of the structure of SK1, the design of specific inhibitors should in the future be facilitated [235]. Representative structures of SK1, SK2, and dual SK1 and 2 inhibitors are shown in Figure 2. Early inhibitors of SK were sphingosine analogues that competitively inhibited both SK1 and SK2 such as *L-threo*-dihydrosphingosine (DHS), *D-erythro-N,N*-dimethylsphingosine (DMS), and *N,N,N*-trimethylsphingosine. The first described SK inhibitor, DHS (also known

as safinol), has a good affinity for SK1 but can also potentially inhibit protein kinase C α [236–238]. A phase 1 clinical trial used safinol, in combination with cisplatin for the treatment of various different solid tumors either refractory for treatment or for which there was no treatment available [239]. Blood levels of S1P decreased in a dose-dependent manner, and disease was stabilized in 6 patients (16%) [239]. Interestingly, the best response was observed in patients with adrenal cortical cancer [239]. DMS has been shown to induce apoptosis in both leukemias and colon carcinoma cells and sensitize TNF- α treated LNCaP cells to ionizing radiation-induced apoptosis [240, 241]. However, these compounds are all non-specific for SK and have other diverse cellular targets [125].

4.1.2. FTY720 (Fingolimod)

FTY720 has emerged as a novel sphingoid analog that suppresses the S1P pathway. It was first described in 1992 after modification of myriocin, a potent inhibitor of serinepalmitoyl transferase [242]. When administered, FTY720 is phosphorylated by SK2. The FTY720-P is a potent functional inhibitor of S1PR1 (and of S1PR3, 4 and 5). It can also induce the proteolysis of SK1 [242]. Interestingly, treatment of ovarian cancer cells with FTY720 could cause both autophagic and necrotic cell death [243]. Treatment of several different prostate cancer cells with Fingolimod caused S1PR-independent apoptosis and increased radiosensitivity [244]. In addition, Fingolimod treatment of mice carrying orthotopic prostate cancer xenografts potentiated the radiation response [244]. (R)-FTY720 methyl ether has been developed to specifically target SK2, and *in vitro* was able to inhibit DNA synthesis of MCF-7 cells [245]. A role for FTY720 in cancer prevention was demonstrated when daily treatment reduced colitis in both SK2 KO and wild type mice [134].

It has been recently shown that FTY720 can also disrupt lysosomes through proteolysis of ASMase protein in a manner similar to that observed after treatment with the tricyclic antidepressant desipramine. Since desipramine-treatment can induce apoptosis in various cell lines possibly through endoplasmic stress, induction of apoptosis via FTY720-treatment may use a similar, non-S1PR/SK-specific pathway [246, 247]

4.1.3. SKI-II

More selective compounds with IC₅₀ in the submicromolar range were described in 2003, the best was the non-isoform selective 2-(p-hydroxyanilino)-4-(p-chlorophenyl)-thiazole, designated SKI or SKI-II [125]. While designed to be a specific SK1 inhibitor, it has been subsequently shown to also inhibit SK2 activity through competitive inhibition [125]. The initial evaluation of the drug showed that it could inhibit cell proliferation in a number of different cell lines (including MCF-7), and could induce apoptosis in the T24 human bladder cancer cells [125]. Later studies showed that SKI-II could induce apoptosis of androgen-responsive prostate cancer cells [248]. In addition, SKI-II was orally bioavailable and could inhibit murine mammary adenocarcinoma tumor growth and suppress ulcerative colitis in dextran-sulfate sodium (DSS)-treated mice [249, 250]. Inhibition of the Ras/ERK pathway in HepG2 human hepatoma cells using U0126 could sensitize them to SKI-II induced cell death [251]. In combination with doxorubicin or etoposide, SKI-II can increase the percentage of dead MCF-7TN-R cells [252]. SKI-II treatment of MDA-MB-231, HCT-116 colon cancer and NCI-H358 lung cancer cells increased sensitivity to doxorubicin treatment

and formation of reactive oxygen species [253]. Head and neck squamous cell carcinoma cell lines can also be radiosensitized with SKI-II [254].

4.1.4. Amidine-based inhibitors

Selective, amidine-based SK inhibitors 100 times more potent towards SK1 than SK2 have been used in U937 (human monocytic leukemia) and Jurkat (human T-cell leukemia) cells, of which Compound 1a demonstrated the highest potency [255]. When using Compound 1a, cellular S1P levels decreased within 10mins, indicating that S1P generated by SK1 has a very rapid turnover [255]. Interestingly, Compound 1a could induce apoptosis in Jurkat cells after 16hrs of treatment and at a dose of 10 μ M, far higher than the dose required to maximally inhibit SK1 activity [255].

4.1.5. PF-543

A more potent newly described competitive inhibitor PF-543 is 100 times more potent towards SK1 than SK2, and did not inhibit the activity of protein kinases from a panel of almost 50 tested [256]. It has an IC₅₀ of approximately 2nM and was not significantly inactivated in blood due to metabolism or the presence of binding proteins, so can theoretically be injectable without losing potency. Another important characteristic is that sphingosine levels increased during treatment with PF-543, underlining the fact that virtually all SK activity was abolished [256].

4.2. Inhibitors of SK2

4.2.1. ABC294640—ABC294640 is a selective inhibitor of SK2 activity with good oral bioavailability [257]. Treatment of melanoma cells along with several other cancer cell lines including prostate, colon, and breast showed decreased proliferation. In several different cell lines the compound could induce autophagy [258]. Furthermore, oral treatment of mice carrying subcutaneous JC mouse mammary adenocarcinoma tumors demonstrated a significant reduction in palpable tumor growth [257]. Further studies showed that ABC294640 could inhibit NF- κ B-mediated chemoresistance of MCF-7TN-R cells implanted in mice [259]. The potential utility of this drug as an adjuvant to established chemotherapy was demonstrated using *in vivo* mouse models [260, 261]. Adding ABC294640 to sorafenib treatment, a multi-kinase inhibitor, showed a modest but significant suppression of hepatocellular carcinoma, pancreatic adenocarcinoma and kidney carcinoma [260, 261]. In addition, inhibition of SK2 activity in Caov-3 ovarian cancer cells could potentiate the apoptotic effects of paclitaxel treatment [262].

4.2.2. K145—A Thiazolidine-2,3-dione analogue, K145 has an IC₅₀ of 4.3 μ M, and at concentrations of 10 μ M did not demonstrate any inhibitory effect on SK1 [263]. Treatment of HEK293 cells with K145 resulted in significantly reduced intracellular S1P levels and could induce apoptotic cell death in both U937 and JC cells *in vitro* [263]. Furthermore, oral treatment of mice carrying U937 tumors could significantly suppress growth [263].

4.3. Selective inhibitors of both SK1 and SK2

Potent inhibitors of both SK1 and SK2 have also been recently described [264]. Named Compound A and Compound B, their use in several different cell lines led to a significant reduction in intracellular S1P levels to undetectable levels [264]. While Compound A was orally bioavailable, it could not significantly suppress growth of MDA-MB-231 xenografts when used alone [264].

4.4. Differences of effects of SK1 inhibitors versus SK1 knockdown

Treatment of MCF-7 cells with siRNA against SK1 and treatment of human bladder cancer cells with SK1-II can induce apoptosis [125, 265]. Other experiments have shown that knockdown of SK1 or SK2 expression suppresses cell cycle progression but do not induce apoptosis [139]. However, experiments using the newer generation of potent and specific SK1 inhibitors have failed to induce apoptosis in a number of different cancer cell lines [255, 256, 264]. In contrast, both ABC294640 and K145, specific SK2 inhibitors, have been shown to suppress proliferation and cause cell death in some cell lines [257, 263]. It is easy to speculate that the apoptotic effects of the older SK1 inhibitors are simply due to off-target effects, and are thus non-specific. However, the fact that knock down of SK1 expression has been reported to induce either cell cycle arrest or apoptosis raises a couple of interesting possibilities. Either the S1P/ceramide rheostat theory is wrong (or overly simplistic), or that SK1 protein has other effects in addition to its kinase activity. Methods inhibiting SK1 activity have been to date mostly focused on analyzing their effects on cancer cells as monotherapy. Cancer models where SK1 is involved in oncogene/non-oncogene addiction may turn out to be suitable targets for therapeutic drugs directed against SK1. More research needs to be done using SK1 inhibitors in combination with chemotherapeutics to fully assess their potential efficacy in the treatment of cancer.

4.5. Hurdles to overcome before clinical applications targeting sphingolipid metabolism

Sphingolipid metabolism holds may hold promise over established methods of chemotherapy as a more mechanistically-driven approach to cancer therapeutics; however at this point there exist a few hurdles to accomplish such goals. One problem to be overcome is the redundancy of function of different sphingolipid metabolizing enzymes, such as CerS [32]. Another problem to face is that simply raising total cellular ceramide levels may not be sufficient to induce apoptosis without some corresponding driving apoptogenic stimulus [266]. A third problem is that until recently many of the compounds used to inhibit sphingolipid metabolism exhibit non-specific effects or were not effective at clinically tolerable doses [125, 267, 268]. While there is accumulating evidence that targeting sphingolipid metabolism may be of use in cancer therapy, a better understanding of the role of sphingolipid metabolism in cancer cell survival and other key cancer cell functions is required [162]. This, coupled with better, more specific inhibitors will be required before they can be reliably used in a clinical setting.

Summary

While there are other pathways initiated to induce radiation or chemoresistance, sphingolipids play a major role. Commonly, C₁₆ ceramide induction will lead to apoptosis,

while strategies to remove ceramide lead to enhanced chemoresistance. Therefore, maintaining C₁₆ abundance may increase the sensitivity of cells to chemotherapeutic drugs. The disappointment of initial results using SK1 inhibitors alone is tempered by the encouraging results obtained inhibiting either SK1 or SK2 alone or together in combination with chemotherapeutic drug treatment.

Abbreviations

ACDase	Acid ceramidase
ASMase	Acid sphingomyelinase
C1P	Ceramide 1-phosphate
CAPP	Ceramide activated protein phosphatase
CerS	Ceramide synthase
CERT	Ceramide transfer protein
DHS	<i>L-threo</i> -dihydrosphingosine
DMS	<i>D-erythro-N,N</i> -dimethylsphingosine
GCS	Glucosyl ceramide synthase
KO	Knockout
MDR	Multi drug resistance
NADPH	Nicotinamide adenine dinucleotide phosphate
nSMase	Neutral sphingomyelinase
OE	N-oleoylethanolamine
PKC	Protein kinase C
PPMP	<i>DL-threo</i> -1-phenyl-2-palmitoylamino-3-morpholino-1-propanol
RNAi	Interfering RNA
S1P	Sphingosine 1-phosphate
S1PR	Sphingosine 1-phosphate receptor
siRNA	Small interfering RNA
SMase	Sphingomyelinase
SK1/2	Sphingosine kinase 1/2
SKI-II	2-(p-hydroxyanilino)-4-(p-chlorophenyl)-thiazole
TNFα	Tumor necrosis factor α

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Highlights

- We review how common chemotherapeutic agents can affect sphingolipid metabolism.
- We also review resistance mechanisms cancer cells employ to escape treatment.
- Sphingosine kinase inhibitors are examined as potential modifiers to chemotherapy.
- Targeting sphingolipid metabolism may improve treatment outcomes.

Figure 1. Summary of the pathways cancer cells use to escape chemotherapy

Chemotherapeutics can induce ceramide production through ceramide synthase or acid sphingomyelinase activity. While the ceramide generated may cause cell death, some cancer cells will glycosylate or hydrolyze ceramide in order to develop resistance. Activation of sphingosine kinase, phosphorylating the sphingosine by-product of acid ceramidase activity, is another strategy employed by many different cancer types to increase survival. The subcellular locations of the above mentioned sphingolipid metabolizing enzymes, along with the subcellular targets of chemotherapeutics described in this review, are noted.

PF-543 **ABC254640** **SKI-II** **Compound A** **Figure 2. Representative molecular structures of SK inhibitors**

The structures of SK1-specific inhibitor PF-543, SK2-specific inhibitor ABC254640, and dual SK1/2 inhibitors SKI-II and Compound A are shown.