

Review

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Novel mechanisms of action of classical chemotherapeutic agents on sphingolipid pathways

Abstract: The prevailing mechanisms of action of traditional chemotherapeutic agents have been challenged by sphingolipid cancer research. Many studies have shown that ceramide generation in response to cytotoxic agents is central to tumor cell death. Ceramide can be generated either via hydrolysis of cell-membrane sphingomyelin by sphingomyelinases, hydrolysis of cerebrosides, or via *de novo* synthesis by ceramide synthases. Ceramide can act as a second messenger for apoptosis, senescence or autophagy. Inherent or acquired alterations in the sphingolipid pathway can account for resistance to the classic chemotherapeutic agents. In particular, it has been shown that activation of the acid ceramidase can lead to the formation of sphingosine 1-phosphate, which then antagonizes ceramide signaling by initiating a pro-survival signaling pathway. Furthermore, ceramide glycosylation catalyzed by glucosylceramide synthase converts ceramide to glucosylceramide, thus eliminating ceramide and consequently protecting cancer cells from apoptosis. In this review, we describe the effects of some of the most commonly used chemotherapeutic agents on ceramide generation, with a particular emphasis on strategies used to enhance the efficacy of these agents.

Keywords: apoptosis; autophagy; ceramide; chemotherapeutic agents; resistance; senescence.

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Introduction

The term chemotherapy was coined in the early 1900s by Paul Ehrlich to refer to the use of chemicals in the treatment of diseases (DeVita and Chu, 2008). Most of the classical chemotherapeutic agents fall into one of three classes: alkylating agents, antimetabolites, or antibiotics. The newer and most widely used agents are not part of these classes; these include the platinum compounds, procarbazine, vinca alkaloids, taxanes, topoisomerase inhibitors and ‘targeted therapy’ agents that target a specific pathway which may be elevated or vulnerable in some tumor cells. Anticancer agents have been shown to target diverse intracellular elements that are vital for cellular division. In particular, most agents are thought to work by affecting DNA synthesis or function with subsequent failure to maintain normal replication (Chabner and Roberts, 2005). This prevailing hypothesis has been challenged by many studies showing that these agents can induce cell death via activation of sphingolipid metabolism. Several chemotherapeutic agents have been shown to generate ceramide, a potent second messenger involved in apoptosis, senescence and autophagy (DeJesus et al., 2002; Klionsky, 2007). Moreover, correlations between drug resistance and alterations in the ceramide pathway have been reported (Truman et al., 2014). Manipulating the sphingolipid pathways could be central in increasing efficacy of chemotherapeutic treatment.

Generation of ceramide

Ceramide can be generated by two distinct sphingolipid pathways. The first pathway is initiated by hydrolysis of the phospholipid sphingomyelin that is preferentially concentrated in the plasma membrane of mammalian cells. This hydrolysis occurs within seconds to minutes after exposure to cytokines (i.e., TNF α , Fas), hormones,

radiation, or environmental stresses (Verheij et al., 1996). Rapid catabolism of membrane-bound sphingomyelin to ceramide (Figure 1) is mediated by the action of the neutral or the acid sphingomyelinase (ASMase), which are sphingomyelin-specific forms of phospholipase C (Fuks et al., 1995). Alternatively, the second pathway involves *de novo* synthesis of ceramide via the condensation of the sphingoid base sphinganine and fatty acyl-CoA catalyzed by the enzyme (dihydro-)ceramide synthase (CerS) to form dihydroceramide, which is then oxidized to ceramide by dihydroceramide desaturase (Figure 1). Ceramide is generated in the endoplasmic reticulum and transferred to the Golgi apparatus to be functionalized as the primary hydroxyl (Hirschberg et al., 1993; Shimeno et al., 1995; Spiegel et al., 1996). *De novo* sphingomyelin biosynthesis depends on non-vesicular ceramide trafficking by the CERamide Transfer (CERT) protein (Hanada et al., 2003).

The ceramide-producing pathways are cell-type specific. In bovine aortic endothelial cells, it was shown that ionizing radiation, like TNF α , induces rapid sphingomyelin hydrolysis to ceramide (Haimovitz-Friedman et al., 1994). Conversely, in epithelial cells, the slower CerS pathway is predominant (Kolesnick and Fuks, 2003; Mesicek et al., 2010). Moreover, a selective tissue and sub-cellular distribution of six mammalian CerS isoforms was

described combined with distinct fatty acyl chain length substrate preferences, implicating differential functions of specific ceramide species in cellular signaling (Mesicek et al., 2010). Interestingly, overexpression of CerS2 results in partial protection from irradiation-induced apoptosis, whereas overexpression of CerS5 increases apoptosis in HeLa cells (Mesicek et al., 2010). Generation of long chain ceramides C₁₆-ceramide and C₁₈-ceramide led to the inhibition of cell proliferation and induction of apoptosis, whereas very long chain ceramide, such as C_{24:0}-ceramide and C_{24:1}-ceramide, increased cell proliferation in MCF-7 (breast cancer) and HCT-116 (colon cancer) cells (Hartmann et al., 2012). Thus, in addition to the balance of ceramide-producing and metabolizing pathways, the chain length of the resulting ceramide species generated seems to be important for inducing cell death or survival.

Mechanisms of ceramide-induced apoptosis

Ceramide acts as a second messenger in initiating apoptosis via the mitochondrial system. Apoptotic cell death refers to an inducible preprogrammed pathway that

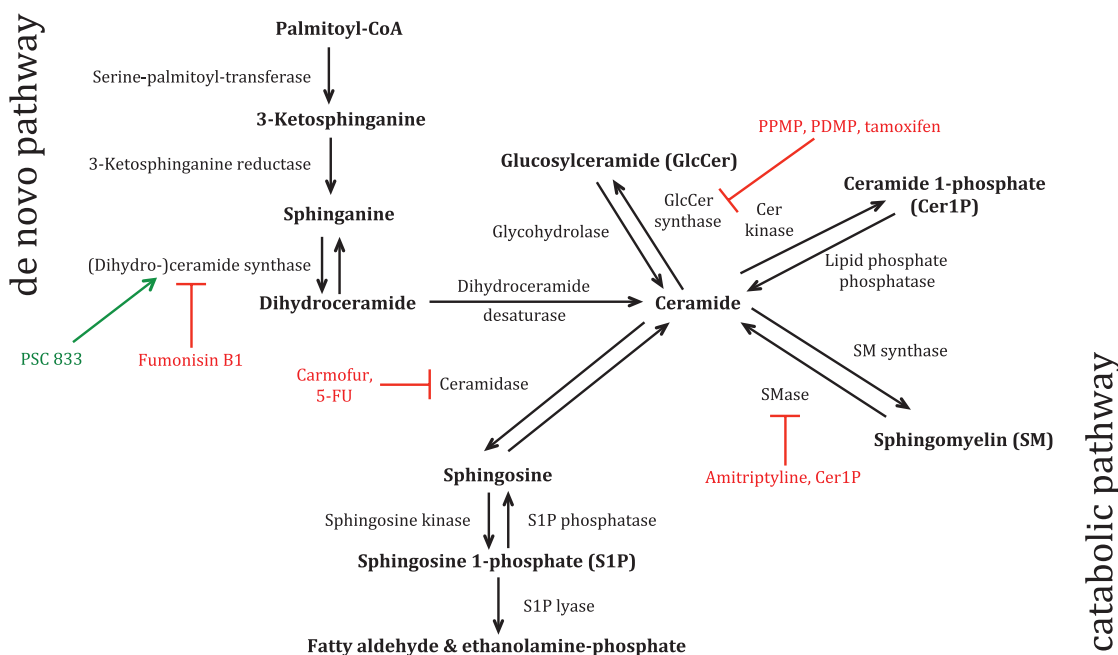


Figure 1: Pathways of ceramide generation.

Ceramide can be synthesized *de novo*, released from sphingomyelin or generated from sphingosine, glucosylceramide or ceramide 1-phosphate. 5-FU, 5-fluorouracile; Cer, ceramide; Cer1P, ceramide 1-phosphate; GlcCer, glucosylceramide; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PPMP, 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol; S1P, sphingosine 1-phosphate; SM, sphingomyelin; SMase, sphingomyelinase.

involves sequential events that ultimately lead to activation of calcium- and magnesium-dependent endonucleases that fragment the nuclear chromatin at specific internucleosomal linker sites. Elevation of ceramide with exogenous ceramide analogs was shown to be enough for induction of apoptosis in bovine aortic endothelial cells (Haimovitz-Friedman et al., 1994). Moreover, protein kinase C (PKC) activation blocked both radiation-induced sphingomyelin hydrolysis and apoptosis, and apoptosis was reinstated by exogenously added ceramide analog (Haimovitz-Friedman et al., 1994).

In addition to inducing apoptosis, ceramides are also involved in autophagy by down-regulating nutrient transporters (Guenther et al., 2008). Autophagy refers to a survival pathway responsible for the breakdown of damaged organelles, protein aggregates and long-lived proteins. This process is initiated by the generation of double-membrane vacuoles, the autophagosomes, which engulf these cellular components. Subsequent fusion of the autophagosomes with the lysosomes results in the formation of single membrane autolysosomes in which the cellular contents are degraded by hydrolytic lysosomal enzymes. Autophagy is therefore a catabolic survival pathway that plays a role in cancer suppression (Klionsky, 2007).

It was shown that senescent fibroblasts are more resistance to $\text{TNF}\alpha$ -induced apoptosis as the result of, at least in part, interrupted ceramide signaling, which suggests that senescence may be another way to escape apoptosis (Wright and Shay, 2001; DeJesus et al., 2002). In cancer cells, senescence can be induced by two distinct mechanisms: replicative senescence which involves inhibition of telomerase, and senescent-like state induced through the overexpression of cell-cycle-inhibitory proteins or DNA damage. Senescent cells present a barrier to the effective treatment of cancer because they might be capable of subsequently re-entering active cell cycling or could provide support to other cancer cells, including stem cells (Modrak et al., 2009).

Lastly, short-chain ceramide (C_8 -ceramide) was shown to be capable of inducing both senescence and apoptosis in a dose-dependent manner (Modrak et al., 2009). C_8 -ceramide-induced senescence occurred at lower concentrations, whereas apoptosis was observed at higher concentrations.

Mechanisms of drug resistance

In addition to other mechanisms described, many cancer cells have been shown to resist treatment by

limiting ceramide generation or rapidly removing ceramide (Truman et al., 2014). There are different possibilities to metabolize the pro-apoptotic ceramide. The de-acetylation of ceramide to generate sphingosine is catalyzed by the acid ceramidase (AC), an enzyme primarily located in lysosomes. Sphingosine itself can be further modified by a sphingosine kinase to generate sphingosine 1-phosphate (S1P), which antagonizes ceramide signaling by initiating a pro-survival signaling pathway (Bonnaud et al., 2010). S1P is therefore a product of ceramide deacylation and phosphorylation of sphingosine. S1P promotes survival by activating the PI3K (phosphoinositide 3-kinase)/AKT pathway (Bonnaud et al., 2010). S1P and ceramide have opposing properties and the balance between them will determine cell fate, in what is known in the field as the ‘S1P/Ceramide rheostat’ (Figure 1). Sphingosine kinase has also been significantly implicated in cellular survival pathway via generation of S1P (Liu et al., 2000b). Moreover, ceramide glycosylation, which is catalyzed by glycosylceramide synthase, leads to the conversion of ceramide to glycosylceramide. Ceramide glycosylation swiftly eliminates ceramide, which consequently protects cancer cells from apoptosis (Liu et al., 2000a, 2013; Dumitru et al., 2009a,b).

Ceramide kinase phosphorylates ceramide to produce ceramide 1-phosphate, which inhibits ASMase, thus protecting cells against apoptotic death (Gomez-Munoz et al., 2004; Gangoiti et al., 2008). In addition, using an unbiased screen of RNAi, Swanton et al. (Kolesnick et al., 2007; Swanton et al., 2007) identified CERT, whose downregulation sensitizes cancer cells to multiple cytotoxic agents, potentiating endoplasmic reticulum stress. Furthermore, CERT was found to be overexpressed in drug-resistant cell lines and in residual ovarian cancer tumors treated with paclitaxel. Therefore, targeting chemotherapy-resistant cancers with tumor specifically tailored treatment will require addressing in addition to other possible mechanisms, one or more ceramide metabolism pathways possibly involved.

The effect of sphingolipids on the systemic treatment of cancer

Anthracyclines

Anthracyclines – including daunorubicin, used in the treatment of leukemia – induce cell death by acting on mammalian cells through multiple mechanisms involving

cell-membrane effects, intercalation into DNA, and inhibition of topoisomerase II. Anthracyclines were shown to induce apoptosis both *in vitro* and *in vivo*. Studies published by our group provided evidence that generation of ceramide mediates daunorubicin-induced apoptosis in both leukemia P388 and lymphoma U937 cell lines, and that ceramide generation precedes the onset of apoptosis (Bose et al., 1995). Moreover, the dose-dependencies for ceramide elevation and apoptosis in response to daunorubicin correlated closely. Daunorubicin-stimulated ceramide elevation however did not result from sphingomyelin hydrolysis but rather from *de novo* synthesis (Figure 1). The stimulation of ceramide synthesis by the enzyme CerS appeared obligatory for daunorubicin-induced apoptosis as its specific inhibition by fumonisin B1 (a specific natural inhibitor of CerS) blocked daunorubicin-induced ceramide elevation and apoptosis. Fumonisin B1 did not block TNF-induced ceramide generation, which occurred by sphingomyelinase activation, nor TNF-initiated apoptotic death. Therefore, it was concluded that mechanism of cell death involving CerS activation was specific for daunorubicin in these cells (Bose et al., 1995). Other groups demonstrated that fumonisin B1 can also abrogate daunorubicin-induced apoptosis in granulosa cells (Witty et al., 1996) and CPT11-induced apoptosis in fibrosarcoma L929 cells (Suzuki et al., 1997).

Cabot et al. examined the impact of chemotherapy using agents that elicit ceramide formation (like the cyclosporine A analog SDZ, PSC 833) combined with agents that block ceramide glycosylation (e.g., tamoxifen, Figure 1). A 3-component regimen comprised of tamoxifen, doxorubicin, and PSC 833 increased ceramide levels 26-fold and dropped cell viability to zero (Lucci et al., 1999a). Subsequently, it was shown that ceramide-governed glucosylceramide synthase gene expression drives cellular resistance to doxorubicin (Liu et al., 2008). Treatment of Adriamycin-resistant MCF-7 breast cancer cells with Adriamycin promoted an increase in ceramide only if tamoxifen was present, in which case ceramide levels increased 7-fold, and cell viability decreased to 50% (Lucci et al., 1999b). Moreover, uncoupling ceramide glycosylation by transfection of glucosylceramide synthase antisense reverses Adriamycin resistance (Liu et al., 2000a). In addition, Zhang and coworkers demonstrated that doxorubicin could modulate the expression of glucosylceramide (GlcCer) through the Sp1 site of GlcCer promoter in ER-positive breast cancer cells (Zhang et al., 2012).

Doxorubicin can also switch protective autophagy in sphingosine 1-phosphate phosphohydrolase 1 (SPP-1)-depleted cells to apoptosis by calpain-mediated Atg5 cleavage (Lepine et al., 2011). Unfortunately, cardiotoxicity of

doxorubicin is also mediated by the generation of ceramide. Delpy et al. demonstrated that doxorubicin induces ceramide accumulation and apoptosis in cardiac myocytes (Delpy et al., 1999). Pretreatment of cardiac myocytes with L-carnitine, a compound that is known for its protective effects on cardiac injuries (Andrieu-Abadie et al., 1999), inhibited ceramide generation within these cells. Work by the same group showed that L-carnitine blocked the doxorubicin induced activation of the ASMase, the enzyme that catalyzes the hydrolysis of sphingomyelin to generate ceramide, within this system (Andrieu-Abadie et al., 1999). Further investigations have to analyze whether L-carnitine negatively influences the drug effect on tumor cells.

Fluoropyrimidines

The first fluoropyrimidine, 5-fluorouracil (5-FU), was synthesized in 1957 (Heidelberger et al., 1957; Heidelberger, 1981). It is a pyrimidine analog used in the treatment of anal, breast, colorectal, esophageal, stomach, pancreatic and skin cancers. Inhibition of thymidylate synthase in Molt-4 human T-cell leukemia cells using folate analog GW1843 was shown to increase the activity of both ASMase and neutral sphingomyelinase, leading to apoptosis (Laethem et al., 1998). Pretreatment of head and neck squamous cell carcinoma cell lines with deguelin increased ceramide levels, which in turn sensitized the cells to further 5-FU treatment (Yang et al., 2013). Moreover, the addition of exogenous sphingomyelin also enhanced the sensitivity of human colonic xenografts to 5-FU (Modrak et al., 2002). Carmofur, a drug that can intra-cellularly release 5-FU, inhibited AC activity and subsequently increase ceramide levels in human colon adenocarcinoma SW403 (Figure 1). This in turn sensitized these cells to treatment with 5-FU (Realini et al., 2013). Whether the toxic side effects of 5-FU and other fluoropyrimidines, on erythrocytes and spleenocytes for example, are also mediated by activation of ceramide pathways, remains to be elucidated.

Gemcitabine

As 5-FU, gemcitabine is a nucleoside analog. Beside its effects on DNA replication, it was shown that gemcitabine triggers the release of ceramide in glioma cells, not always correlating with tumor response (Dumitru et al., 2009a,b). The drug increased ceramide levels via activation of ASMase in these cells. In contrast to cells being sensitive to the treatment with gemcitabine,

drug-resistant cells failed to accumulate ceramide by rapidly consuming ceramide via activation of ceramide glucosyltransferases. Sensitivity of resistant glioma cells was restored by pharmacological or genetic inhibition of the glucosyltransferase (Dumitru et al., 2009b). Experiments published from the same group demonstrated that gemcitabine induced apoptotic death in the glioma cells. The activation of ASMase results in lysosomal accumulation of ceramide, the activation of Cathepsin D and insertion of the pro-apoptotic protein Bax into mitochondria. Pharmacological inhibition or genetic deficiency of the ASMase abolished the apoptotic effect of gemcitabine in glioma cells, while overexpression of the enzyme increased sensitivity of these cells to the drug (Dumitru et al., 2009a).

Because of its anti-proliferative effect on tumor cells, ceramide has been used to treat various human cancers, both *in vitro* and *in vivo*. However, this approach was challenging as ceramide has very low water solubility, moderate/low cellular uptake, intracellular metabolism to complex sphingolipids, and uncontrolled delivery, release, and intracellular targeting. To overcome these problems, pyridium ceramide analogs with increased water solubility and bioavailability have been used in combination with gemcitabine in the treatment of head and neck squamous cell carcinoma tumors (Senkal et al., 2006). Gemcitabine is frequently used to treat various cancers including, but not limited to, non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer. Moreover, these novel cationic ceramide analogs were shown to inhibit growth and telomerase activity in various cell lines of head and neck cancer (Rossi et al., 2005). The therapeutic efficacy of the combination Safingol/gemcitabine, when compared to that of 5-FU/cisplatin, was 2.5-fold better (Senkal et al., 2006).

Interestingly, ceramide was also used as a biomarker of chemotherapy response in patients with head and neck cancer. In a phase II clinical trial, patients with head and neck squamous cell carcinoma were treated with gemcitabine and doxorubicin (Saddoughi et al., 2011). After two treatment cycles, patients were assessed radiographically, and serum samples were taken for sphingolipid measurements. Significant differences in patterns of C_{18} -ceramide elevation were noted in patients with complete response, partial response and stable disease, in comparison to patients whose disease progressed after chemotherapy, indicating the reconstitution of tumor suppressor ceramide generation by this chemotherapeutic regimen. The authors concluded that serum C_{18} -ceramide elevation might be a novel serum biomarker of chemotherapy response.

The same group described the upregulation of AC in 60% of primary prostate cancer tumors and showed that AC upregulation generated tumor cells that are resistant to normal homeostatic control mechanisms, have an increased number of metastatic characteristics and exhibit resistance to chemotherapy (doxorubicin, cisplatin, etoposide and gemcitabine), hypothesizing potential benefit from targeting AC in combination with chemotherapy to increase tumor response (Saad et al., 2007).

Modrak et al. showed that synergistic interaction between sphingomyelin and gemcitabine potentiates ceramide-mediated apoptosis in pancreatic cancer (Modrak et al., 2004). Gemcitabine also induces senescence in pancreatic cancer cells and sphingomyelin-enhanced chemosensitivity is achieved through reducing the induction of senescence by redirecting the cell to enter the apoptotic pathway (Modrak et al., 2009).

Mitomycin C

As the majority of chemotherapeutic agents, Mitomycin C (MMC) achieves its effects on cancer cells by inducing apoptosis initiated by DNA damage. Typically, MMC induces an apoptotic cascade in which p53 plays a central role. Interestingly, Haynes et al. showed that MMC induces the downregulation of the UDP-glucose ceramide glucosyltransferase (UGCG) in a p53-deficient osteosarcoma cell line. UGCG catalyzes the synthesis of GlcCer from ceramide and therefore the inhibition of UGCG by MMC results in increasing ceramide concentrations and subsequent apoptosis (Haynes et al., 2012). Induction of cell death via ceramide accumulation in response to MMC in these cells only occurs in cells lacking a functional p53 protein.

Safingol, a synthetic L-threo-stereoisomer of endogenous sphinganine, is a potent inhibitor of protein kinase C (PKC) and sphingosine kinase *in vitro* and has been shown to induce ceramide generation and apoptosis in several cell types (Coward et al., 2009). Used alone, Safingol has a minimal effect on tumor cell growth, but when used in combination with conventional chemotherapeutic agents it dramatically potentiates the antitumor effects by inducing apoptosis. MMC is a potent DNA cross-linker used in the treatment of esophageal, anal and breast cancer. Schwartz and coworkers demonstrated that Safingol potentiates apoptosis in MMC-treated gastric cancer cells. In this study, SK-GT-5 (p53-deficient and MMC-resistant) and MKN-74 (p53 wild-type and MMC-sensitive) gastric cancer cells were exposed to either no drug, Safingol alone, MMC alone, or a combination of MMC and Safingol. In the SK-GT-5 cells, Safingol alone induced apoptosis in

2%±1% of the cells, MMC increased that level to 18±1%, and the combination Safingol and MMC induced apoptosis in 39±1% of the cells ($p<0.001$, for the drug combination vs. MMC alone). In the MKN-74 cells, Safingol alone induce apoptosis in 8±3% of the cells, MMC increased that level to 40±4%, and the combination Safingol and MMC induced apoptosis in 83±4% of the cells ($p<0.001$, for the drug combination vs. MMC alone). The induction of apoptosis occurred regardless of the p53 status or the drug-resistance status of the cells (Schwartz et al., 1995).

Retinamide

The synthetic retinoid N-(4-hydroxyphenyl)-retinamide (4-HPR or fenretinide) was demonstrated to have potent chemotherapeutic and antimetastatic effects in several animal models (Green et al., 1999; Shaker et al., 2000; Raffaghello et al., 2003). It was shown that 4-HPR increased the level of intracellular ceramide (up to approximately 10-fold) in a dose-dependent manner in two retinoblastoma cell lines and induced cell death under oxygenated and hypoxic conditions (Maurer et al., 1999). Combination of retinamide and modulators of ceramide metabolism was also shown to have a synergistic cytotoxic effect in other solid tumor cell lines (lung, melanoma, prostate, colon, breast and pancreas, including p53 mutant and alkylator-resistant cell lines) (Maurer et al., 2000). Conversely, Messner and Cabot (2011) showed that human pancreatic cancer cell lines MIA PaCa-2 and PANC-1 treated with retinamide died by apoptosis, whereat sensitivity appeared to be mediated by reactive oxygen species and not by ceramide. Subsequently, the same group showed that nanoliposomal ceramide was effective as anti-pancreatic cancer therapeutics in combination with gemcitabine (Jiang et al., 2011). The influence of AC inhibition was tested on the effects of PSC 833 in pancreatic cancer cell lines. The authors concluded that AC played a significant role in conversion of cytostatic to cytotoxic endpoint in pancreatic cancer cell lines (Morad et al., 2013b). In human colorectal cancer cell lines, tamoxifen was also shown to enhance the therapeutic impact of ceramide (Morad et al., 2013a).

Cisplatin

Cisplatin is a DNA crosslinking agent, widely used to treat a variety of cancers. Studies demonstrated that cisplatin induces ceramide generation in tumor cells via activation of the ASMase (Lacour et al., 2004). In addition, S1P has

been implicated in regulating sensitivity to cisplatin and modulation of the S1P lyase can alter cisplatin sensitivity (Min et al., 2004). Moreover, members of the sphingosine kinase (SphK1 and SphK2) and CerS (CerS1, CerS4, and CerS5) enzyme families each have a unique role in regulating sensitivity to cisplatin and other drugs (Min et al., 2007). Expression of SphK1 decreases sensitivity to cisplatin, carboplatin, doxorubicin, and vincristine, whereas expression of SphK2 increases sensitivity; expression of CerS1 increases the sensitivity to all the drugs tested, whereas expression of CerS5 only increases sensitivity to doxorubicin and vincristine; and CerS4 expression has no effect on the sensitivity to any drug tested. Cisplatin was shown to cause a specific translocation of CerS1, but not CerS4 or CerS5, from the endoplasmic reticulum to the Golgi apparatus, which is mechanistically involved in the response to cisplatin. Min and colleagues demonstrated that expression of SphK1, but not SphK2, explains both increased cisplatin sensitivity in cells stably expressing CerS1 and translocation of the CerS1. Sensitivity to the widely used drug cisplatin can be improved via the manipulation of enzymes of the sphingolipid metabolic pathway (Min et al., 2007).

In another study, C6 rat glioma cells were treated with various concentrations of cisplatin. Cisplatin induced a dose-dependent increase in ceramide levels and the glioma cells became apoptotic (Noda et al., 2001). Blocking the activity of the neutral sphingomyelinase prior to cisplatin administration resulted in reduced ceramide formation and concomitantly reduced cell death. Conversely, pretreatment of the cells with a ceramidase inhibitor potentiated apoptosis induced by cisplatin in these cells, indicating that, whereas ceramide is generated by neutral sphingomyelinase, a portion of it is concomitantly converted by ceramidase to sphingosine and the non-apoptotic S1P (Noda et al., 2001).

In contrast, Sassa et al. showed that the generation of ceramide in cisplatin-induced apoptosis is not important in HeLa cells (Sassa et al., 2012), at least under the tested experimental conditions. Nevertheless, their results demonstrated that a shift from C₂₄ to C₁₆ sphingolipids affects cisplatin-induced apoptosis.

One side effect of cisplatin is nephrotoxicity, whereby DNA damage of the renal tubular cells plays a critical role (Siddik, 2003; Wang et al., 2006). Interestingly, recently published experiments demonstrated that S1P receptor 1 (S1P1) is involved in cisplatin-induced acute kidney injury. Administration of the S1P receptor agonist FTY720 prevents cisplatin toxicity in renal tubular cells (Bajwa et al., 2014). In contrast, another side effect of cisplatin, eryptosis (followed by anemia), is not mediated by

cisplatin-induced ceramide generation within erythrocytes (Mahmud et al., 2008).

Vinblastine and Vinorelbine

Vinblastine is an antimicrotubule drug. Early work from Miller-Prodraza and Fishman showed that the administration of vinblastine resulted in changes within the sphingolipid composition of treated cells (Miller-Prodraza and Fishman, 1984). PSC 833, a derivative of cyclosporine and a powerful multidrug resistance modulator, increased cellular ceramide levels via activation of the CerS (Cabot et al., 1998, 1999) (Figure 1). The increase of ceramide levels was accompanied by decreased cell survival of the cells. Combined administration of PSC 833 with vinblastine resulted in the synergistically increase of ceramide synthesis not only in human wild-type epidermoid carcinoma cells, but also in the multidrug resistance population of these cells. As a matter of fact, Cabot et al. demonstrated the capacity of PSC 833 to reverse drug resistance with vinblastine. When used as a single agent at a concentration of 1.0 μ M, PSC 833 and vinblastine reduced cell survival by approximately 20%. When these two drugs were co-administered, cell viability dropped to zero. In addition, this study showed that this combination synergistically increased cellular ceramide levels. Moreover, PSC 833 in wild-type KB-3-1 cells intensified vinblastine toxicity, and this was accompanied by enhanced ceramide generation (Cabot et al., 1999; Goulding et al., 2000).

More recently, *in vitro* and *in vivo* experiments showed a synergistic antitumor activity of nanoliposomal C_6 -ceramide and vinblastine (Adiseshaiah et al., 2013). Whereas nanoliposomal C_6 -ceramide acts as an autophagy inducer, vinblastine inhibits the maturation of autophagosomes (Kochl et al., 2006; Guenther et al., 2008; Xie et al., 2010). In combination, the drugs induced cell death by inducing apoptosis. In human liver and colon cancer cells, this combination treatment increased autophagic vacuole accumulation and decreased autophagy maturation, without reducing the autophagy flux protein P62. *In vivo*, a single intravenous injection of this combination significantly decreased colon tumor growth in comparison to the single agent treatments (Adiseshaiah et al., 2013).

In patients with non-small cell lung cancer, a decrease in GlcCer was positively correlated with the cytotoxic effects of vinorelbine when given concurrently with radiation treatment. Pharmacologically inhibiting glucosylceramide synthase facilitated vinorelbine and concurrent chemoradiotherapy-induced apoptosis by activating the JNK pathway (Chiu et al., 2014).

Taxanes

Taxanes are microtubules inhibitors that induce disruption of microtubule function. Myrick et al. demonstrated that simultaneous treatment of leukemic Jurkat cells with the taxane paclitaxel and ceramide enhanced paclitaxel-induced cell growth inhibition via a significant increase in apoptosis (Myrick et al., 1999). Subsequently, Mehta et al. published a study that showed exogenous ceramide augmented paclitaxel-induced apoptosis in Tu138 head and neck squamous carcinoma cells *in vitro* when added simultaneously in combination with the paclitaxel (Mehta et al., 2000). In human breast cancer cells, it was suggested that taxol-induced apoptosis is, in part, secondary to ceramide and sphingoid bases generation (Charles et al., 2001). In pancreatic cancer cells, the combination of paclitaxel and ceramide also induced cell death synergistically. The cell death mechanism was mediated through differential activation of EGFR-mediated MAP kinases (Qiu et al., 2006). Short-carbon chain C_2 -ceramide was also shown to effectively sensitize paclitaxel-induced senescence in human lung cancer cells via both p21 (waf1/cip1)- and p16 (ink4)-independent pathways (Chen et al., 2010). Functional genomic screens identified ceramide as a key regulator of the taxane-mediated spindle assembly checkpoint and taxane-induced cell death. Kolesnick et al. therefore concluded that ceramide metabolism serves as a legitimate target for modulation of taxane effect on tumors (Kolesnick et al., 2007). Multiple groups have worked on developing formulations to effectively deliver paclitaxel and ceramide to tumors. Oil-in-water nanoemulsions have been designed with paclitaxel and ceramide combination therapy for enhancement of cytotoxic effect in brain tumor cells (Desai et al., 2008). Multifunctional polymer-blend nanoparticle formulations were also used in drug-resistant breast cancer model (van Vlerken et al., 2008). Finally, it was shown that S1P receptor activation is required for the development and maintenance of paclitaxel-induced neuropathic pain (Janes et al., 2014). Additional work is necessary to analyze whether taxane-induced neuropathy can be circumvented by blocking the S1P/S1P receptor signaling without negatively influencing the effects of the drug on tumor cell death.

Targeted therapy agents: Sorafenib and Sunitinib

Treatment of several diverse cell lines (including multidrug resistant prostate cancer cell line DU-145) with

Sunitinib, which is a multi-targeting-tyrosine kinase inhibitor, inhibited ASase activity and led to lysosomal destabilization and cell death (Ellegaard et al., 2013). Conversely, treatment of implanted hepatocellular carcinoma cells with both sorafenib (a multi-serine/threonine kinase inhibitor) and recombinant ASase increased cell death relative to sorafenib alone (Savic et al., 2013).

Tetradecanoylphorbol 13-acetate

Although PKC activation is often anti-apoptotic, activation of PKC α by 12-O-tetradecanoylphorbol 13-acetate (TPA) in LNCaP prostate cancer cells induces apoptosis. Garzotto et al. (1998) provided the first description of a mechanism by which activation of PKC α pathway can signal apoptosis and showed that it is mediated by ceramide generation detectable by 1 h and increased linearly for 12 h. TPA-induced apoptosis was measurable by 12 h and was progressive for 48 h. Ceramide generation was caused by an increase in CerS enzyme activity that persisted for at least 16 h. Pretreatment with fumonisin B1 abrogated both ceramide production and TPA-induced apoptosis. Therefore, it was concluded that ceramide appears to be a necessary signal for TPA-induced apoptosis in LNCaP cells (Garzotto et al., 1998).

Conclusion

Multiple groups involved in sphingolipid cancer research have challenged the mechanisms of action of commonly used chemotherapeutic agents. It is now widely accepted that most of these agents used in cancer treatments are also implicated in cancer cell death via activation of the ceramide pathways. Moreover, resistance to some of these agents can be explained by the disruption of these pathways, either by limiting ceramide generation or by increasing ceramide clearance. In addition, CERT down-regulation was shown to sensitize cancer cells to multiple cytotoxic agents. Novel strategies are now targeting the sphingolipid metabolism pathway both to synergistically increase the tumor sensitivity to chemotherapy and to overcome resistance to treatment. Furthermore, ceramide species have also been shown to be valuable biomarkers to predict response to treatment. Transferring this knowledge from bench to bedside is warranted.

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