Sphingolipids and Response to Chemotherapy

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Contents

Abstract Chemotherapy is frequently used to treat primary or metastatic cancers, but intrinsic or acquired drug resistance limits its efficiency. Sphingolipids are important regulators of various cellular processes including proliferation, apoptosis, differentiation, angiogenesis, stress, and inflammatory responses which are linked to various aspects of cancer, like tumor growth, neoangiogenesis, and response to chemotherapy. Ceramide, the central molecule of sphingolipid metabolism, generally mediates antiproliferative and proapoptotic functions, whereas sphingosine-1-phosphate and other derivatives have opposing effects. Among the variety of enzymes that control ceramide generation, acid or neutral

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sphingomyelinases and ceramide synthases are important targets to allow killing of cancer cells by chemotherapeutic drugs. On the contrary, glucosylceramide synthase, ceramidase, and sphingosine kinase are other targets driving cancer cell resistance to chemotherapy. This chapter focuses on ceramide-based mechanisms leading to cancer therapy sensitization or resistance which could have some impacts on the development of novel cancer therapeutic strategies.

Keywords chemotherapy • ceramide • sphingomyelinases • ceramide kinase • sphingosine kinase

1 Introduction

Sphingolipids are ubiquitous components of eukaryotic cell membranes known to be involved in a variety of cellular processes including proliferation, growth, differentiation, apoptosis, and membrane structure. Dysregulation of their metabolism is evident in various pathological conditions including metabolic disorders and cancer. Thus, sphingolipids represent interesting targets for the development of novel chemotherapy. Studies performed over the past decade demonstrate that chemotherapy induces the death of cancer cells by apoptosis via two major death pathways. The first one is called the extrinsic death receptor pathway that begins with ligation of cell surface death receptors like CD95 by CD95L (Suda et al. 1993) or via CD95 clustering at the cell surface independently of its ligand (Micheau et al. 1999; Shao et al. 2001). Activation of CD95 death receptor leads to the recruitment of the protein Fas-associated death domain (FADD) and procaspase-8 to form a death-inducing signaling complex (DISC). Within the DISC, the procaspase-8 is transactivated to release mature caspase-8 initiating directly the apoptotic cascade (Muzio et al. 1996) or indirectly via the cleavage of Bid in t-Bid (truncated Bid) that consequently activates the mitochondrial death pathway (Li et al. 1998; Luo et al. 1998). The second one is named the intrinsic mitochondrial death pathway regulated by members of the Bcl-2 family (Green 2000). Most chemotherapeutic drugs induce disruption of the outer mitochondrial membrane and the release of proapoptotic molecules such as cytochrome c, Smac/DIABLO, HtrA2/Omi, apoptosis-inducing factor (AIF), and endonuclease G (Endo G) from the mitochondrial intermembrane space (Ravagnan et al. 2002). In the cytosol, cytochrome c forms a complex with apoptosis protease activating factor-1 (Apaf-1), which induces via caspase-9 activation of caspase-3 leading to cell death. Since exogenous treatment with ceramide (Obeid et al. 1993) or endogenous production of ceramide following many apoptotic stimuli (Hannun 1994; Lin et al. 2006) induces apoptosis, ceramide is considered as an important mediator in both intrinsic and extrinsic death pathway. Ceramide levels are significantly decreased in human colon (Selzner et al. 2001), glial (Riboni et al. 2002), ovarian (Rylova et al. 1998), and head and neck cancers (Koybasi et al. 2004), and malignant cells with low level of ceramide are resistant to apoptosis (Chmura et al. 1997). Moreover, absence of generation of ceramide is associated with chemoresistance (Wang et al. 1999,

2003). In this context, modulation of ceramide content may favor apoptosis and targeting altered sphingolipid metabolism in cancer could contribute to potentiate chemotherapy and counteract chemoresistance. This chapter describes the current knowledge about sphingolipids and their roles in response to chemotherapy.

2 Sphingolipid Metabolism

Sphingolipids are membrane lipids containing a sphingoid base (sphingosine or sphinganine) which is in most cases acylated with a fatty acid. The resulting ceramides can carry hydrophilic headgroups such as phosphorylcholine in sphingomyelin (SM), carbohydrate residues in glycosphingolipids, and a phosphate moiety in ceramide-1-phosphate. Sphingolipids (like sphingomyelin or glycosphingolipids) with glycerophospholipids and cholesterol are characteristic components of cell membranes. As structural lipids, they are mainly found in the plasma membrane and to a lesser extent in intracellular membranes (Van Meer and Hoetzl 2010). Plasma membrane is characterized by the presence of distinct microdomains enriched in sphingolipids and cholesterol, termed lipid rafts (Brown and London 2000; Simons and Van Meer 1988; Simons and Ikonen 1997). These membrane domains have the properties to be insoluble in nonionic detergents at 4 °C, of weak density on sucrose gradient, and destabilized by cholesterol-depleting agents. Moreover, these structures actively participate to metabolic and signal transduction processes (Verkleij and Post 2000), especially in the CD95 death receptor pathway (Hueber et al. 2002) but also in response to chemotherapy (Bezombes et al. 2003; Dimanche-Boitrel et al. 2005). Then, sphingolipids play a role as mediator lipids and are involved in the regulation of cellular functions. Their biosynthesis and catabolism involve a large number of intermediate metabolites with distinct biological activities (Fig. 1).

Intracellular ceramide can be formed either by de novo synthesis that requires the action of serine palmitoyltransferase (SPT) (Kang et al. 2010) and/or ceramide synthase (Bose et al. 1995) or through the SMase-dependent catabolism of SM, in various separate cellular compartments. In response to chemotherapy, SMase activation is the predominant pathway to generate ceramide (Rizzieri and Hannun 1998; Pettus et al. 2002; Ogretmen and Hannun 2004) besides the activation of de novo synthesis (Bose et al. 1995). SMases are phospholipase C-like enzymes that mediate the hydrolysis of SM to phosphocholine and ceramide. Today, three classes of SMases have been described: the acid, the neutral, and the alkaline form, according to their optimum pH, cation dependency, and subcellular location (Levade and Jaffrézou 1999; Goni and Alonso 2002). Acid sphingomyelinase (ASMase), a soluble glycoprotein, was the first described. It was originally identified in the lysosomes with an optimum pH at 4.5–5, but this isoform could be translocated to the plasma membrane, particularly in lipid rafts after activation by ligand binding to specific receptor (Gulbins and Grassmé 2002) or chemotherapy (Lacour et al. 2004; Dimanche-Boitrel et al. 2005; Carpinteiro et al. 2008),

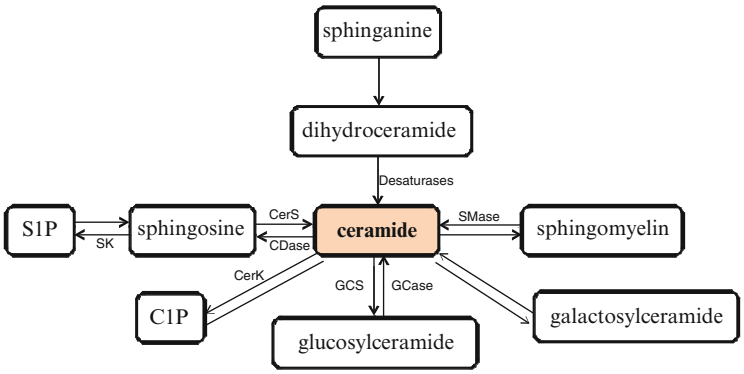

Fig. 1 Overview of sphingolipids metabolism. Ceramide generation can arise from the de novo synthesis pathway and/or the hydrolysis of membrane sphingomyelin by various sphingomyelinases. Ceramide can be transformed in different metabolic intermediates such as ceramide-1-phosphate, glucosylceramide, galactosylceramide, or sphingosine-1-phosphate. *CDase* ceramidase, *CerK* ceramide kinase, *GCS* glucosylceramide synthase, *GCase* glucosylceramidase, *SK* sphingosine kinase, *SMase* sphingomyelinase

promoting subsequently receptor clustering. The secretory sphingomyelinase (SSMase) arises from the ASMase gene through differential protein trafficking of a common precursor localized either in lysosomes or in Golgi (Schissel et al. 1998). This protein is activated by physiological concentrations of Zn^{2+} (Schissel et al. 1996; Spence et al. 1989). Different forms of neutral sphingomyelinases (NSMase) are characterized with a pH optimum at 7.4: a plasma membrane, Mg^{2+}- or Mn^{2+}-dependent form and a cytosolic, Mg^{2+}-independent form as well as nuclear and mitochondrial forms (Goni and Alonso 2002; Birbes et al. 2002; Tomiuk et al. 2000; Wu et al. 2010). Several ceramide synthases (CerS1–6) have been described (Pewzner-Jung et al. 2006). These enzymes locate in microsomes, are integral membrane proteins of the endoplasmic reticulum, and synthesize ceramides with different fatty acid chain lengths (Mizutani et al. 2005). As reported above, many inductors of apoptosis generate ceramide, suggesting a role of this sphingolipid in programmed cell death (Pettus et al. 2002). Additionally, increasing the levels of endogenous ceramide results in apoptosis and growth arrest (Abe et al. 1995; Bielawska et al. 1996). Most of studies suggest that ceramide levels decrease in cancers, notably in ovarian, head and neck, colon, and brain tumors. However, this notion must be seen with caution because it seems that it is the content in specific ceramides which is important rather than their global level. For example, only C_{18}-ceramide and no other ceramide species is significantly lower in tumor tissues of HNSCC patients when compared with controls, which correlates with lymphovascular invasion and nodal metastasis (Koybasi et al. 2004; Karahatay et al. 2007), suggesting that this ceramide inhibits tumor growth. By contrast, recent data suggest that elevated C_{16}-ceramide is associated with a positive lymph node status in breast cancer patients (Schiffmann et al. 2009).

Since a network of specialized and compartmentalized enzymes regulates the levels of ceramide, ceramide metabolites are produced in distinct localization and have different functions. For example, an important metabolite of ceramide is ceramide-1-phosphate (C1P). This metabolite is described as mitogenic and antiapoptotic. It is formed from ceramide by the action of a specific ceramide kinase (CerK), which is distinct from the sphingosine kinases (SK1 and SK2) that synthesize sphingosine-1-phosphate (S1P). CerK is localized in three major compartments: the Golgi complex, the plasma membrane, and cytoplasmic vesicles (Bornancin 2011). C1P blocks apoptosis in bone-marrow-derived macrophages through inhibition of ASMase, thereby reducing ceramide generation (Gómez-Muñoz et al. 2004). CerK mRNA level is upregulated in estrogen receptor (ER)-negative breast cancer tumors in comparison with ER-positive ones (Ruckhäberle et al. 2009). Moreover, CerK is upregulated in several hepatoma cell lines and knockdown of CerK increases susceptibility to UV-induced apoptosis (Hsieh et al. 2009). Specific inhibition of CerK by NVP-231 in combination with tamoxifen increases ceramide levels and reduces cell growth (Graf et al. 2008).

Like many products of sphingomyelin, S1P exhibits a wide range of biological activities. It is a pleiotropic lipid mediator that has been shown to regulate cell growth, cell survival, cell invasion, vascular maturation, and angiogenesis, processes that are important for cancer progression (Olivera and Spiegel 1993; Cuvillier et al. 1996). However, S1P may have opposing roles depending on synthesis via SK1 and SK2 and subcellular localization, since SK1 is localized mainly in the cytosol, whereas SK2 is present in several intracellular compartments (nucleus, mitochondria, and intracellular membranes). In fact, S1P synthesized by SK1 is involved in proliferative signaling (Taha et al. 2006), whereas S1P synthesized by SK2 is described as an antiproliferative and apoptotic mediator (Maceyka et al. 2005).

In summary, ceramide and sphingosine are very often involved in apoptosis, cell-cycle arrest, and cell senescence, whereas sphingosine-1-phosphate and ceramide-1-phosphate promote cell survival, proliferation, and inflammation. The balance between these sphingolipids may affect the fate of the cell.

3 Sphingolipids and Sensitivity to Chemotherapy

Ceramide is composed of sphingosine linked to a fatty acyl chain varying in length from 16 to 26 carbon atoms (Mimeault 2002; Pettus et al. 2002; Ogretmen and Hannun 2004; Zheng et al. 2006). These distinct ceramides have different role in apoptosis, differentiation, and cell growth depending on stimulus and cell context. A number of cytotoxic agents appear to be effective because of their ability to activate ceramide-mediated pathways in cancer cells. Daunorubicin and 1-beta-D arabinofuranosylcytosine (Ara-C) are the first two anticancer agents to induce apoptosis via the generation of ceramide (Bose et al. 1995; Strum et al. 1994; Jaffrézou et al. 1996). Many other chemotherapeutic drugs are shown to produce

ceramide: vincristine (Zhang et al. 1996), vinblastine (Cabot et al. 1999), etoposide (Tepper et al. 1999), paclitaxel (Charles et al. 2001), irinotecan (Suzuki et al. 1997), mitoxantrone (Bettaieb et al. 1999), and cisplatin (Lacour et al. 2004). And more recently, it was shown that arsenic trioxide induces accumulation of cytotoxic levels of ceramide in acute promyelocytic leukemia and adult T-cell leukemia/lymphoma cells (Dbaibo et al. 2007). As reported in many studies, chemotherapy can impact ceramide metabolism by promoting ceramide synthesis de novo, by activating sphingomyelinase, and/or by blocking glucosylceramide formation. In each case, the result is an increase in ceramide-induced cytotoxic response.

4 Ceramide Synthases

The role of ceramide synthases as targets for chemotherapeutic drugs is currently emerging. Ceramide synthases (CerS) differ by their specificity for the generation of endogenous ceramides with distinct fatty acid chain lengths (Spassieva et al. 2006). Particularly, CerS1/4 mainly generates ceramide with a C_{18}-containing fatty acid chain (C_{18}-ceramide) (Venkataraman et al. 2002), CerS2 rather generates very long chain ceramides (C_{24}-ceramide) (Laviad et al. 2008; Mizutani et al. 2005), whereas CerS5/6 preferentially mediates the generation of C_{16}-ceramide and, to a lesser extent, C_{12}- and C_{14}-ceramides (Riebeling et al. 2003). Myeloid leukemia cells treated with daunorubicin exhibit ceramide accumulation via activation of CerS and inhibition of ceramide synthase with fumonisin B1 prevents daunorubicin-induced apoptosis (Bose et al. 1995). Activation of CerS is also observed in response to various cytotoxic agents including lymphotoxin, TNF, camptothecin, doxorubicin, Taxol, oxidative stress, and androgen ablation (Plo et al. 1999; Xu et al. 1998; Rath et al. 2009; Ueda et al. 2001; Eto et al. 2003). Expression of CerS1 sensitizes cancer cells to several chemotherapeutic agents including cisplatin, gemcitabine, doxorubicin, imatinib, and vincristine (Min et al. 2007; Senkal et al. 2007; Baran et al. 2007), and small interfering RNA directed against CerS1 reduces the effects of these drugs. CerS1 mRNA and enzymatic activity is increased in HSNNC cells upon treatment with gemcitabine and doxorubicin leading to C_{18}-ceramide generation and cell death (Senkal et al. 2007). In addition, CerS5 seems to increase the sensitivity of mammalian cells to doxorubicin and vincristine but not to cisplatin and carboplatin (Min et al. 2007). And overexpression of CerS6 in resistant cells resensitized them to TRAIL-induced apoptosis via increased C_{16}-ceramide (White-Gilbertson et al. 2009). Cannabinoids lead to transcriptional induction of CerS3 and CerS6 and generation of C_{16}, C_{18}, C_{24}, and $C_{24:1}$-ceramides inducing cell death in lymphoma cells (Gustafsson et al. 2009), and knockdown of CerS6 expression abolishes activation of CD95 and significantly reduces toxicity of vorinostat combined with sorafenib in hepatoma and pancreatic carcinoma cells (Park et al. 2010). On the other hand, CerS2 and CerS4 levels seem to have no effects on cell response to cytotoxic drugs.

5 Sphingomyelinases

Several studies indicate a role of ASMase in chemotherapy-induced ceramide generation (Pettus et al. 2002; Ogretmen and Hannun 2004), particularly after treatment with gemcitabine, fenretinide, and paclitaxel (Modrak et al. 2004; Lovat et al. 2004). The hydrolysis of SM by ASMase produces ceramide in specific membrane domains termed lipid rafts (Liu and Anderson 1995), resulting in the formation of large ceramide-enriched membrane platforms where membrane receptors are clustered (Grassmé et al. 2001, 2002). Sub-toxic doses of doxorubicin result in ASMase activation, release of ceramide, and formation of ceramide-enriched membrane platforms that facilitate DR5 clustering after treatment with very low doses of TRAIL in BJAB Burkitt lymphoma cell line and murine T splenocytes (Dumitru et al. 2007). In addition, ceramide and ASMase are also important in the induction of apoptosis by other antineoplastic agents such as rituximab (Bezombes et al. 2004) and TRAIL (Dumitru and Gulbins 2006), and overexpression of ASMase sensitizes glioma cells to doxorubicin and gemcitabine (Grammatikos et al. 2007). Similar mechanisms are described after treatment with cisplatin which can induce a redistribution of CD95 death receptor in lipid rafts of human colon cancer cells (Lacour et al. 2004). Few minutes after treatment, the sodium-proton exchanger-1 NHE1 located at the plasma membrane is inhibited, leading to an intracellular acidification which facilitates the activation of ASMase. Then, this enzyme hydrolyzes membrane sphingomyelin into ceramide, allowing the aggregation of lipid rafts into large signaling platforms in which CD95 death receptors are oligomerized, inducing cell death (Rebillard et al. 2007). Concomitantly, an increase in membrane fluidity is measured early after cisplatin treatment by electron paramagnetic resonance which could be related to the ability of ceramide to induce membrane fusion/fission (Cremesti et al. 2002; Dimanche-Boitrel et al. 2005; Rebillard et al. 2008a). A pretreatment with imipramine, an inhibitor of ASMase, or reduced expression of ASMase by RNA interference strongly reduces cisplatin-induced apoptosis in human colon cancer cells, suggesting a main role of ceramide in cell death. In the same way, cisplatin early activates ASMase in breast cancer cells, leading to ceramide production (Zeidan et al. 2008). These data confirm the important involvement of ceramide pathway in apoptosis induction (Gulbins and Grassmé 2002; Gulbins and Kolesnick 2003; Gulbins and Li 2006). Moreover, reactive oxygen species (ROS) seem to be involved in ASMase activation by fenretinide, doxorubicin, and TRAIL (Lovat et al. 2004; Dumitru and Gulbins 2006; Grammatikos et al. 2007), as demonstrated by the use of ROS scavengers. Consistent with this, a mechanism implicating ROS in ASMase activation is characterized in vitro with the oxidation of the cysteine residue 629 in purified ASMase, leading to activation and dimerization of the enzyme (Qiu et al. 2003). However, the role of ASMase in response to chemotherapy has been mainly studied on in vitro cellular models, and the precise

mechanisms of its action in vivo remain not well defined. A recent work points out that in a Niemann–Pick disease (NPD; type B) patient who developed a marginal zone lymphoma, rituximab is still acting, suggesting that ASMase is dispensable for rituximab efficacy (Sabourdy et al. 2011). Further studies are needed to confirm the role of ASMase in response to chemotherapy in cancer patients. The role of ASMase is not limited to tumors since this enzyme is also involved in deleterious effects induced by anticancer drugs in normal tissues. Doxorubicin induces ASMase-dependent oocyte lethality, which is responsible for sterility (Morita et al. 2000). In the same way, as irradiation (Paris et al. 2001), cisplatin induces apoptosis of endothelial cells in small intestine leading to the gastrointestinal (GI) syndrome which is not observed in ASMase-knockout mice (Rebillard et al. 2008b). Moreover, cisplatin triggers dendritic cells (DC) apoptosis through increased expression and activation of ASMase, limiting the use of chemoimmunotherapy in cancer treatment. However, the ex vivo nitric oxide (NO) donors treatment protects DC from cisplatin toxicity and enhances tumor regression in B16 mouse melanoma model and animal survival following cisplatin treatment (Perrotta et al. 2007). As previously described, daunorubicin triggers the release of ceramide in leukemic cells through the activation of NSMases (Mansat et al. 1997; Mansat-de Mas et al. 1999) and this activation is mediated by both serine proteases, protein kinase C, and ROS, resulting in the consecutive activation of Jun-N-terminal kinases (JNK). Another chemotherapeutic agent, 1-beta-D-arabinofuranosyl-cytosine (Ara-C), is shown to rapidly enhance NSMases activation in leukemia cells (Whitman et al. 1997; Strum et al. 1994) by altering the cellular redox status. Ceramide generation activates the Src-like tyrosine kinase Lyn and JNK to mediate apoptosis (Bezombes et al. 2001; Grazide et al. 2002). Consistent with the proposed proapoptotic role of NSMases and NSMase-generated ceramide, NSMase3 expression is induced upon Adriamycin treatment and its overexpression sensitizes cells to Adriamycin (Corcoran et al. 2008). Further, daunorubicin transcriptionally regulates *neutral sphingomyelinase 2* in human breast cancer cell lines, leading to an increased ceramide production and cell death (Ito et al. 2009).

6 Sphingolipid and Chemoresistance

As well known, the main obstacle against cancer therapy is the development of drug resistance resulting in chemotherapy failure. A possible mechanism to overcome this drug resistance is modulation of the sphingolipid metabolism.

Among the sphingolipids, S1P seems to be a key regulator of chemoresistance. As previously described, S1P is generated by the conversion of ceramide to sphingosine by ceramidase and the subsequent rapid phosphorylation of sphingosine to S1P, which is catalyzed by sphingosine kinase.

7 Sphingosine Kinases

High expression of sphingosine kinase-1 SK1 and S1P is observed in many types of cancers such as gastric, lung, colon, breast, uterus, and kidney (Kawamori et al. 2009; Visentin et al. 2006). SK1 activity is increased 2.5-fold in endometrial tumors compared with healthy sections, and S1P levels are 1.6-fold higher in cancer tissues. In breast tumors samples, elevated SK1 expression is correlated with poor prognosis and promotion of metastasis (Ruckhäberle et al. 2008). Moreover, SK1 increases RAS V12-dependent transformation of NIH3T3 fibroblasts to form fibrosarcoma cells, demonstrating for the first time the role of SK1 in cancer transformation (Xia et al. 2000). Targeting SK1 induces apoptosis and suppresses growth of human glioblastoma cells and xenografts (Kapitonov et al. 2009). S1P and SK1 are involved in resistance to apoptosis induced by CD95, ceramide, (Bektas et al. 2005) and a myriad of other stimuli such as camptothecin, gemcitabine, imatinib, and Taxol. Cancer cell lines that are resistant to chemotherapeutic agents have a high expression of SK1 and SP1, such as prostate cancer cells that are resistant to camptothecin (Akao et al. 2006; Pchejetski et al. 2005), pancreatic cancer cells resistant to gemcitabine (Guillermet-Guibert et al. 2009), and chronic myeloid leukemia (CML) cells that are resistant to imatinib (Baran et al. 2007). Imatinib-sensitive CML cells and daunorubicin-sensitive leukemia cells have a higher ceramide/S1P ratio than their chemotherapeutic resistant counterparts (Baran et al. 2007; Sobue et al. 2008). The involvement of SK1 in drug resistance is also established in breast cancer cells. SK1 overexpression causes promotion of cell proliferation and resistance to tamoxifen-induced apoptosis, and the inhibition of SK1 by a specific inhibitor induces the resensitization of breast cancer cells to tamoxifen-induced apoptosis (Sukocheva et al. 2009). In contrast, the role of SK2 is much less known. Whereas, endogenous SK2 also promotes survival by direct involvement of S1P (Hait et al. 2009), its enforced overexpression suppresses cell growth and enhances apoptosis and sensitivity to doxorubicin (Liu et al. 2003; Sankala et al. 2007). However, colon cancer cells with high SK1 and SK2 expression were resistant to oxaliplatin (L-OHP), and inhibition of both SK isoenzymes renders the colon cancer cells sensitive to L-OHP (Nemoto et al. 2009).

8 Ceramidases

Due to their ability to break down ceramide to regulate sphingosine and S1P levels, acid, neutral, and alkaline ceramidases (Canals et al. 2011) are important regulators of cell survival (Mao and Obeid 2008). Human acid ceramidase is overexpressed in prostate cancer (Seelan et al. 2000). Overexpression of acid ceramidase in prostate cancer cell line DU145 or in fibrosarcoma cell line L929 elevates resistance to chemotherapy (Saad et al. 2007) or TNF-α (Strelow et al. 2000), respectively. On the contrary, downregulation of acid ceramidase sensitizes A375 melanoma cells to dacarbazine (Bedia et al. 2011). Moreover, addition of acid ceramidase inhibitors,

B13 or N-oleoyl ethanolamine, induces apoptosis in prostate and colon cancer cell lines and xenografts (Samsel et al. 2004; Holman et al. 2008; Selzner et al. 2001) or overcomes TNF-α resistance (Strelow et al. 2000), respectively. In the same way, overexpression of neutral ceramidase confers resistance of primary hepatocytes to TNF-α and protection against TNF-α-induced liver damage (Osawa et al. 2005).

9 Glucosylceramide Synthase and Gangliosides

Other dysfunctions in ceramide metabolism also contribute to multidrug resistance. Specifically, ceramide glycosylation by the glucosylceramide synthase (GCS), which forms the metabolite glucosylceramide, may be an important pathway for bypassing apoptosis. Tumors from patients who fail to respond to chemotherapy express elevated glucosylceramide levels (Lucci et al. 1998) as well as a human ovarian adenocarcinoma cell line established from a patient resistant to doxorubicin, melphalan, and cisplatin. A number of drug-resistant cancer cell lines accumulate this noncytotoxic metabolite (Lavie et al. 1996). Drug-resistant breast cancer cells and cutaneous cancer cells have higher levels of glucosylceramide than their drug-sensitive counterparts. The level of GCS activity may determine the multidrug resistance phenotype in cancer cells. The introduction of GCS gene into drug-sensitive breast cancer cell lines results in an 11-fold higher level of GCS activity, leading to resistance to doxorubicin, exogenous ceramide (Liu et al. 1999a), and TNF-α-induced cell death (Liu et al. 1999b). This process seems to be related to hyperglycosylation of ceramide and not to changes in the levels of P-glycoprotein, Bcl-2, or TNF receptor-1 expression. However, recent data demonstrated that knockdown of GCS expression significantly inhibits the expression of MDR1, a gene encoding for P-glycoprotein (P-gp), and reverses drug resistance (Gouazé et al. 2005; Gouazé-Andersson et al. 2007). On the contrary, overexpression of GCS increases P-gp expression and resistance acquisition in breast cancer cells (Gouazé et al. 2004; Liu et al. 2010). Interestingly, P-gp is proposed as a specific transporter for glucosylceramide, translocating this molecule across the Golgi to deliver it for the synthesis of glycosphingolipids (De Rosa et al. 2004). Thus, P-gp and GCS appear to function in the same pathway of ceramide/GlcCer metabolism, and this may provide an important link for the function of GCS in drug resistance. These data are consistent with an earlier study demonstrating that the inhibition of P-gp prevents GCS activity and alters glucosylceramide levels (Goulding et al. 2000). Additionally, P-gp overexpressing cells have an increased accumulation of glucosylceramide (Gouazé et al. 2004; Morjani et al. 2001) which is the precursor for the generation of complex glycosphingolipids and gangliosides (Futerman and Hannun 2004). Most gangliosides are known to protect cells from apoptosis (Bektas and Spiegel 2004). For example, GM1 could prevent cell death in growth factor-deprived neuronal cells (Ferrari et al. 1995), could enhance S1P production in rat heart fibroblasts through the activation of sphingosine kinase, and could protect cells from C2-ceramide or staurosporine-induced cell death (Cavallini et al. 1999).

Moreover, gangliosides GM2 and GM3 have been associated with multidrug resistance phenotype in cancer cells (Gouazé-Andersson and Cabot 2006).

10 Conclusion and Future Directions

Experimental evidence suggests that there is an alteration of ceramide contents and of the expression of enzymes involved in sphingolipid metabolism in several cancers that contributes to cancer therapy resistance. In fact, the roles of sphingolipids in the regulation of response to chemotherapy are demonstrated in various cellular models but need to be further studied in cancer patients. As described, chemotherapy induces ceramide generation via activation of several enzymes in different subcellular localizations (plasma membrane, reticulum endoplasmic, nuclear membrane, and mitochondria-associated membranes). Moreover, different ceramide species could be generated with distinct biological properties showing the complexity of cell response to chemotherapy. Therefore, ceramide analogues, modulators of sphingolipids metabolism, inhibitors of SK or CerK, might be exploited for the development of new therapeutic cancer strategies via the increase in ceramide levels. Such therapeutic strategies based on the modulation of ceramide level in tumors have already been the subjects of novel patents (Dimanche-Boitrel et al. 2011). However, further studies are needed to better understand the role of sphingolipid metabolism in drug resistance in patients and to elaborate new therapeutic strategies by comparing their toxicity in malignant and normal tissues using conventional methods of biochemistry and molecular biology and also more complex approaches such as lipidomics and bioinformatics.

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