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Ceramide Transfer Protein and Cancer

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Abstract

Sphingolipids are important structural components of membranes, and play an equally important role in basic cellular processes as second messengers. Recently, sphingolipids are receiving increasing attention in cancer research. Ceramide is the central molecule that regulates sphingolipid metabolism forming the basic structural backbone of sphingolipids and the precursor of all complex sphingolipids. It is been proposed to be an important regulator of tumor cell death following exposure to stress stimuli. The increase or decrease of ceramide levels leading to change in sensitivity of cancer cells to stress stimuli provides support for a central role of ceramide signaling in cell death. In this review, we have focused on ceramide transfer protein (CERT) as a major regulator of ceramide flux in the cell.

Keywords

Ceramide; Ceramide transfer protein; cancer; shingolipids

1. INTRODUCTION

Sphingolipids comprise a wide array of specialized lipids found in all eukaryotic organisms. For about a century after their discovery, sphingolipids were still considered mostly as structural components of the membrane. However, our understanding of the functions of sphingolipids in the past two decades has dramatically expanded and convincing evidences from extensive studies indicate that sphingolipids have the ability to mediate and regulate basic cellular processes, such as proliferation, apoptosis, transformation, differentiation and motility [1–3]. These important findings have enabled researchers to design novel therapeutic strategies targeting sphingolipid signaling. Ceramide forms the basic structural backbone of sphingolipids and is the precursor of all complex sphingolipids. Recent advances in our understanding of the role of sphingolipids in mammalian cell signaling reveal an emerging picture of a complex signaling system with second messenger functions for ceramide, sphingosine and sphingosine 1-phosphate (S1P) and structural roles for glycosphingolipids, sphingomyelin and ceramide [4, 5]. This system is involved in regulating cellular processes from embryonic development to cell senescence and death.

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Sphingolipids can be synthesized from simpler building blocks by the *de novo* biosynthetic pathway or can be assimilated by dietary intake or uptake from the circulating blood by the salvage pathway. The *de novo* biosynthetic pathway is integral for the developmental viability of eukaryotes since studies done in several model systems have shown that disruption of this pathway is incompatible with life or in the least leads to early embryonic lethality [6–8]. The biosynthesis of sphingolipids is compartmentalized in that not all components are synthesized in one organelle of the cell. A relay of sorts orchestrates the biosynthetic pathway. The early steps of the biosynthetic pathway leading to the generation of ceramide occur in the endoplasmic reticulum (ER). The subsequent steps of sphingolipid biosynthesis including the generation of sphingomyelin and other cell surface glycosphingolipids require the transport of ceramide out of the ER to the Golgi complex [9, 10]. Intuitively, one could imagine that the vesicular transport machinery involved in mobilizing proteins between ER and the Golgi complex could easily accommodate the nascent sphingolipids synthesized in ER within their membranes and help transfer them from one compartment to the other. This notion was questioned in the early nineties when studies showed that ceramide transfer was independent of vesicular transport [11–14]. These discoveries led to several studies in different experimental systems to define the ceramide transport out of the ER to the Golgi complex. Eventually, different experimental observations of a decade were coalesced into a concrete phenomenon with the discovery of the Ceramide Transfer Protein (CERT) by Hanada and colleagues in 2003 [15].

A direct role, as observed for a carcinogen like ethylmethane sulfonate or as initiated by a mutant protein such as Ras in the MAPK pathway, has not been established either for a sphingolipid metabolite or an enzyme/protein of the sphingolipid metabolic pathway in the aetiopathogenesis of cancer in human patients. However, hundreds of studies point towards sphingolipid metabolic pathway as a target of metabolic reprogramming during oncogenesis. The findings reported in these studies have led to the evaluation of sphingolipids as diagnostic, prognostic and even therapeutic tools in the field of oncology [2, 16].

This review will focus on ceramide transfer protein (CERT) mediated trafficking of ceramide from the ER to the Golgi complex and potential role for CERT in regulating ceramide levels at the ER, Golgi complex and mitochondria. We will also evaluate the functional role of ceramide in organelle homeostasis in malignancies.

2. OVERVIEW OF STRUCTURE AND BIOSYNTHESIS OF SPHINGOLIPIDS

All sphingolipids contain Long Chain Base (LCB) as the primary structural backbone and it is commonly referred to as the sphingoid base. The structure of ‘sphingoid base’ might vary in different species comprising a broad range of 2-amino-1,3-dihydroxyalkanes or –enes [17]. In yeast there are two types of LCBs, dihydrosphingosines (DHS) and phytosphingosine (PHS), which are of 18 to 20 carbon (C18 and C20) chain length. Mammals have sphingosine (predominantly C18), as the major sphingoid base although they contain small amounts of DHS and PHS [18, 19]. In *Drosophila* and other dipterans, the sphingoid bases are not C18, they are rather C14 and C16 [20, 21]. A long fatty acid chain attached to LCB through amide linkage forms ceramide. Ceramide can be further esterified

with different head groups to form complex sphingolipids. The chemical nature of the head groups defines different class of complex sphingolipids.

De novo biosynthesis of sphingolipids begins in the ER with the condensation of serine with acyl CoA resulting in the formation of 3-keto dihydrosphingosine (Fig. 1). The subsequent reactions lead to the formation of ceramide. This general scheme of reactions can be thought to be essentially same in yeast, mammals and *Drosophila* although the intermediates in the biosynthetic steps vary in their chain length and degree of saturation and hydroxylation between the species [1, 22, 23]. Through ceramide transfer protein (CERT)-mediated transport, and other as yet ill-understood mechanisms ceramides reach the lumenal side of Golgi apparatus, where they serve as substrate for the synthesis of complex sphingolipids [24–26].

Once transported to Golgi complex, various head groups are enzymatically added to ceramide resulting in the formation of different classes of complex sphingolipids. The nature of the head group varies between different species and is the basis for classification of sphingolipids. In yeast, the major sphingolipids are inositol phosphoceramide (IPC), mannose inositol phosphoceramide and mannose inositol (P2) ceramide (M(IP)2C) [19]. Mammals have Sphingomyelin (SM) (phosphocholine moiety as the head group), glycosphingolipids such as glucosylceramide (with glucose as the head group) and gangliosides (which contain different sugar residues including sialic acid linked by specific types of glycosidic linkages) [27, 28].

3. CERAMIDE METABOLISM AND CANCER

A central molecule of the sphingolipids metabolic pathway is ceramide, since it is a major precursor and the basic structural unit of all complex sphingolipids. Increased cellular levels of ceramide accompany apoptosis and cell death in response to stress factors, including anti-cancer agents, and often, perturbation in ceramide metabolism results in tumor resistance to therapy. Various tumor models are associated with impaired ceramide metabolism and signaling suggesting that ceramide has a key function in tumor development and progression [29–32].

Ceramide in general is perceived as an important regulator of tumor cell death following exposure to stress stimuli such as hypoxia, starvation, or chemotherapy [33]. Decreasing the intracellular levels of ceramide by impaired ceramide generation or rapid enzymatic degradation results in resistance to cell death signals [34, 35]. On the contrary, restoration of intracellular ceramide levels increases sensitivity of cancer cells to stress stimuli providing support for a central role of ceramide signaling in cell death [36].

Ceramides can be generated in membrane compartments by either the *de novo* biosynthesis, or generated from sphingomyelin by the action of sphingomyelinases or formed during the break-down of other complex sphingolipids such as glucosylceramide. Unless either sequestered by binding to specific proteins or metabolically converted to other products, the ceramides thus formed have a tendency to self-aggregate into domains within the membranes. Sphingomyelin and cholesterol normally intercalate to form liquid-ordered

domains within membrane bilayers. When sphingomyelin is hydrolyzed to ceramide, the liquid ordered structure of membranes generated by sphingomyelin/cholesterol interaction is converted to rigid areas of ceramide self aggregation [37]. Ceramide enriched domains interfere with membrane protein interactions, lateral segregation of proteins and affect the phase behavior of the membranes. Thus ceramide generation from sphingomyelin physically affects the membrane properties and consequently affects the behavior of these membranes [38, 39].

As signaling entities, studies have reported that ceramides activate cathepsin D, PKC-ζ, PP2A, KSR, c-Raf, among others [40–42]. Activation of cathepsin D has been proposed to mediate some of the cell death effects noted in studies involving ceramide generation [43, 44]. Likewise, activation of PKC-ζ would result in activation of stress-activated protein kinase (SAPK) and growth suppression [45]. It is very clear that not all increase in ceramides leads to cell death since activation of the ERK-MAPK cascade by activation of KSR and Raf should result in growth advantages. The subcellular location of ceramide generation, the state of the cell (differentiated *vs* proliferating) and the species of ceramides generated will contribute to the final outcome of a specific instance of ceramide production. For example, recently it was shown that increasing C16 ceramide levels generated in reactions catalyzed by ceramide synthase 6 increased proliferation of head and neck squamous cell carcinoma cells (HNSCC) in SCID mice whereas increasing C18 ceramide levels by expressing ceramide synthase 1 suppressed HNSCC xenograft tumor growth [46].

3.1. Sphingolipid Rheostat

A variety of cytokines, anticancer drugs and other stress-causing agonists cause an increase in endogenous ceramide levels through *de novo* synthesis and/or the hydrolysis of sphingomyelin, resulting finally in aberrant signaling of ceramide and activation of cell death. Since ceramide levels are regulated by the activity of numerous enzymes located in different organelles, a collateral impact of impaired ceramide signaling is perturbed interaction between these enzymes and consequently altered sphingolipid homeostasis.

Sphingolipid metabolites can have antagonistic effect on the cellular fate, thus relative intracellular concentrations of different species of sphingolipids together with their localization have to be tightly regulated under physiological conditions. In general ceramide and sphingosine are considered to be ‘pro apoptotic’ whereas sphingosine-1-phosphate and ceramide-1-phosphate are ‘pro growth’ and anti-apoptotic [4, 47, 48]. In this context, a concept of “sphingolipid rheostat” that regulates opposing signaling pathways by adjusting the levels of individual sphingolipid species has gained major clinical interest. According to this concept, organisms operate a sphingomyelin cycle, in which bioactive sphingolipid molecules such as ceramide, sphingosine and sphingosine 1-phosphate continuously interconvert in order to regulate the vital cellular processes and decide survival or death of the cell [48, 49]. Since a high percent of the cellular sphingomyelin is at the plasma membrane, it is not surprising that many biological functions are associated with sphingomyelin metabolism/production at the plasma membrane [50].

The emerging specific functions for individual biologically active sphingolipids have underscored the importance of the mechanisms that regulate their formation and clearance.

These mechanisms are based on highly regulated and synchronized functioning of the enzymes acting on ceramide pathway and balance between the different metabolic products. Today, it is generally accepted that enzymes involved in ceramide metabolism represent critical regulators of cancer cell growth and/or survival. Two major metabolic routes clear excess ceramide formed during stress: ceramide can be hydrolyzed to sphingosine by ceramidases or metabolized to complex sphingolipids. Ceramide can be phosphorylated by ceramide kinase yielding ceramide phosphate, glycosylated by glucosyl- or galactosylceramide synthases to glycosphingolipids, or it can be consumed in the biosynthesis of sphingomyelin through the action of sphingomyelin synthases. Hence, all these enzymes and proteins can influence the cell fate at physiological and pathological levels.

4. CERAMIDE TRANSFER PROTEIN

In order for ceramide synthesized in the ER to reach the Golgi, it has to first traverse the cytosolic compartment. The extreme hydrophobic nature of the molecule precludes diffusion as a method of transport. This necessitates an efficient machinery to transport ceramide form ER to Golgi. By late eighties it was clear that sphingolipid transport from the Golgi complex to the plasma membrane was mediated by vesicular transport. The same could not be said of ceramide transport from the ER to the Golgi complex. It was believed that ceramide was transported from the ER to the Golgi complex mostly by the vesicular transport mechanism. Reports in the early nineties began to suggest that perhaps ceramide was transported from the ER to the Golgi complex independent of vesicular transport. Warren and Collins working with mitotic HeLa cells showed that during mitosis when vesicular trafficking from the ER to the Golgi complex was inhibited ceramide was still being trafficked to the Golgi complex and underwent some modifications within the Golgi [12]. Morre and colleagues working with purified fractions of ER, Golgi, transitional ER and vesicles were able to show that bulk of ceramide transfer occurred by a non-vesicular transport mechanism [14]. Kendler and Dawson working with rat oligodendrocytes also observed that transfer of ceramide from ER to the Golgi complex was dependent on supply of ATP [51]. The systematic analysis of ceramide transport was taken up by Hanada and colleagues in the nineties and culminated in their discovery of ceramide transfer protein as the major carrier of ceramide from the ER to the Golgi complex *via* a non-vesicular mode of transport [15, 52–55]. Hanada and colleagues performed a genetic screen looking for mutants that would be defective in sphingomyelin synthesis and thus render them resistant to the cytotoxic effects of the peptide lysenin. Lysenin is a hemolytic protein derived from the earthworm *Eisenia foetida* and causes cytolysis of Chinese hamster ovary cells through its high affinity binding to sphingomyelin. One of the mutant cell lines identified in this screen LY-A, was resistant to treatment with lysenin. When grown in sphingolipid deficient media LY-A cells produced only 20% of the levels of sphingomyelin as that produced in wild type Chinese hamster ovary cells. Metabolic labeling, brefeldin-A treatment studies and cell free reconstitution experiments demonstrated a defect in transport of ceramide from the ER (site of ceramide biosynthesis) to the Golgi complex (site of synthesis of majority of sphingomyelin). These studies also suggested that at least under tissue culture conditions this transport pathway was more important for sphingomyelin as opposed to glycosphingolipid biosynthesis.

Subsequently, using a human cDNA library to rescue the defects of ceramide transport in the mutant LY-A cells Hanada's group identified Ceramide Transfer Protein (CERT) as the protein responsible for the transport process. This protein had been earlier identified as the Goodpasture antigen-binding protein (GPBP). It was identified as a protein that could bind the carboxyl terminus of human collagen α3 (IV) *in vitro* (hence also called Col4A3BP protein).

The human COL4A3BP gene (as annotated) is localized to chromosome 5 (5q13.3) and spans about 140 Kb. It encodes for two alternatively spliced proteins of 624 and 598 amino acid isoforms. Both isoforms efficiently transfer ceramide between two membrane systems. An N-terminal PH domain exhibits a preference to bind to phosphatidylinositol 4-phosphate (PI4P) and thus directs the protein to the PI4P enriched Golgi complex. A ceramide binding START domain in the C-terminus of the proteins mediates the transfer of ceramide between ER and the Golgi complex. An FFAT motif (two phenylalanines in an acid rich tract) in the middle interacts with the ER resident VAP protein [56]. The protein is regulated by phosphorylation at multiple sites and phosphorylation generally attenuates the transfer process [57, 58]. The step of ATP hydrolysis, the molecular details of the transfer phenomenon, its regulation and movement of ceramide from the cytosolic surface of the ER to the lumenal surface of the Golgi all need to still be worked out.

Our studies, using animal model systems, have further authenticated the importance of this event in the development and well being of eukaryotic organisms. In *Drosophila* loss of this protein function does not affect the development or growth of the organism [7]. Instead, flies lacking a functional CERT protein show classic signs of accelerated aging and die within 20–30 days of life while normal wild type flies live between 90–120 days. CERT deficiency leads to more than 80% decrease in the levels of ceramide phosphoethanolamine (CPE) the sphingomyelin analog in *Drosophila*. Lack of CPE changes the physical characteristics of the plasma membrane in mutant cells including, decreased viscosity and increased fluidity. The increased fluidity renders the unsaturated fatty acids within the plasma membrane bilayer susceptible to repeated attacks by reactive oxygen species (ROS) generated as a byproduct of metabolic reactions. The end products of fatty acid oxidation are reactive aldehydes that can initiate further rounds of oxidation of the lipid molecules within the bilayer. The lipid peroxidation products thus generated diffuse to the cytosol and cause further oxidative damage to proteins, organelles and DNA. The progressive oxidative damage to cellular components compromises metabolic functions and affect key parameters of homeostasis such as glucose regulation, metabolic pathways and mitochondrial function. The animal thus shows progressive oxidative damage and die of accelerated aging. In mammals, CERT function is critical for embryonic development. In mice, we showed that *cert* mutant embryos went through embryonic development until E10.5 reaching all macroscopic milestones expected in normal embryos, although their development was delayed by about 12 hours compared to wild type sibling [6]. However, organogenesis is compromised in the mutant embryos and they die of catastrophic cardiac failure around E11.5. Our studies show that lack of CERT function leads to accumulation of ceramide in the ER. Unexpectedly, ceramides also accumulate in the mitochondria perhaps transported *via* the mitochondria associated membrane (MAM) fraction of the ER. The accumulating ceramide compromises ER function and causes mitochondrial dysfunction and degeneration.

While these changes do not seem to affect survival of individual cells they do interfere with the process of organogenesis that accompanies embryonic development. Heart is one of the first organs that begin to function in a developing embryo. Indeed the process of embryogenesis is dependent on a fully functioning heart around E10.5. Since organ development is compromised in the CERT mutant embryos they eventually die of cardiac failure around E11.5. Lack of CERT also compromises sphingomyelin levels in the mutant embryos. However, they are reduced to only about 42% of the levels seen in wild type embryos. This could be due to the fact that the embryos are developing in a heterozygous mother that could provide for some of the sphingolipids through the maternal blood. Sphingomyelin synthase 2 present in the plasma membrane could be a source of non-Golgi sphingomyelin in this situation. Also, the plasma membrane fluidity is not affected in the *cert* mutant mice. Similarly, knock down of CERT in zebra fish also leads to embryonic death of these perhaps due to accumulating ceramide [59]. The mutant embryos showed defects in the brain with reduction in myelinated tracts and hydrocephaly of the fourth ventricle. There were damage to the somites and degeneration of the muscle fibers. It is worth noting here that Riezman and colleagues using the yeast *Saccharomyces cerevisiae* have also shown that a non-vesicular route that is independent of vesicular protein trafficking can transfer ceramide from the ER to the Golgi complex, although they do not encode for a high homology CERT sequence and a definitive analog of CERT has yet to be identified in yeast [11].

Most studies have shown that CERT mediates bulk of the ceramides transferred from the endoplasmic reticulum to the Golgi complex. However, studies also reveal that a minor but significant fraction of ceramide seems to be available at the Golgi complex even in the absence of functional CERT protein. In one of the early studies Slomiany and colleagues showed that transport vesicles containing apomucin contained ceramide [60]. Using *in vitro tra*nsport assays they showed that more than half of radiolabeled oleate added to endoplasmic reticular membranes used in the transport assays was incorporated into ceramide contained in the transport vesicles, thus raising the possibility that ceramide was indeed a component of the vesicular system and could be transported to the Golgi complex. Using semi-intact cell system, cytosolic extracts prepared from wild type and a CHO cell line defective in a cytosolic factor required for ceramide transfer (before the identification of this factor as CERT) and monitoring the conversion of radiolabeled ceramide to radiolabeled sphingomyelin as an efficient read out for transfer, Hanada and colleagues showed that CERT independent pathway accounted for about 15–20% of net ceramide transport from the endoplasmic reticulum to the Golgi complex [55]. Riezman and colleagues reported that both vesicular and non-vesicular routes were utilized for ceramide transport in *Saccharomyces cerevisiae* [11]. They showed that sec18 mutants that are defective in vesicular transport from the endoplasmic reticulum to the Golgi complex were defective in ceramide transport, implying that vesicular transport could deliver ceramide to the Golgi complex. It is worth noting that *in vitro* experiments done in the same study showed that a non-vesicular route mediated by a heat labile cytosolic factor was contributing to a significant fraction of the ceramide transfer. Our own studies in *Drosophila* and mouse show that synthesis of ceramide phosphoethanolamine in the former and sphingomyelin in the latter, events downstream of ceramide transport, is not completely abolished in the absence

of ceramide transfer protein, implying other mechanism(s) could be operating to transfer ceramide from the endoplasmic reticulum to the Golgi complex.

Holthuis and colleagues [61] have argued for the existence of a ceramide rheostat in the endoplasmic reticulum. Their studies in human and *Drosophila* S2 cell culture showed that down regulation of a sphingomyelin synthase related protein (SMSR) results in ceramide accumulation in the ER and perturbation of the structures of ER exit sites and the Golgi complex. They have proposed that together with CERT perhaps SMSR would act as a ceramide sensor in the ER complex each one of which is capable of monitoring and regulating the ceramide levels within the organelle [3].

5. CERT IN CANCER

A novel approach to cancer chemotherapy is pharmacological manipulation of sphingolipid metabolism aiming to enhance cell ceramide, as a pro-apoptotic molecule.

During *de novo* synthesis, ceramide transfer from ER to Golgi is the rate-limiting step in conversion of ceramide to anti-apoptotic metabolites, such as sphingomyelin and glucosylceramide, which protect cells from ceramide-induced apoptosis. Since CERT represents the main exit gate of ceramide from ER, it is likely that its manipulation will modulate ceramide levels in the cells, making it an appealing target for chemotherapy. Since, recent studies indicate that increasing ceramide in the ER can increase ceramide levels in the mitochondria it is alluring to pharmacologically increase ceramide levels in these organelles of malignant cells to induce ER/mitochondrial disruption and apoptosis. As many chemotherapeutic agents increase ceramide levels in cells, due to increased *de novo* synthesis of ceramide, presence or lack of CERT could modulate the response to these drugs (Fig. 2).

In a recent study, Swanton and colleagues showed that CERT down regulation sensitized cancer cells to various cytotoxic agents, by potentiating endoplasmic reticulum stress [62]. The authors used an RNA interference (RNAi) screen in three cancer cell lines (MDA-MB-231, HCT-116, and A549) in order to evaluate the contribution of all human protein kinases and proteins involved in ceramide metabolism to the sensitivity/resistance of cancer cells to a number of common chemotherapeutic agents, including paclitaxel. Paclitaxel belongs to taxane group of anticancer drugs, which, through stabilization of microtubules, cause mitotic arrest, and, ultimately, apoptosis. The authors identified a set of genes that confer resistance to paclitaxel, some of them being also involved in mitotic spindle assembly checkpoint. Knock down of these genes potentiated a mitotic arrest and induced the initiation of endoplasmic reticulum stress, resulting in increased sensitivity to taxane action. COL4A3BP (CERT) was unique among these genes that when knocked down, it sensitized all cell types to paclitaxel and additionally, to doxorubicin in MDA-MB-231, 5-FU in HCT-116, and cisplatin in A549. In the context of involvement of sphingolipids in cytotoxic drug resistance, previous studies had shown that overexpression of glucosylceramide synthase (UGCG) promotes resistance to paclitaxel [63, 64]. However, comparison between UGCG and CERT showed the latter as more favorable target for the reversal of multidrug resistance. Moreover, CERT expression is increased in drug-resistant cell lines and in

residual tumor following paclitaxel treatment of ovarian cancer, suggesting that it could be a target for chemotherapy-resistant cancers.

These results were used in a more recent study by Swanton and colleagues, where they developed a metagene to predict paclitaxel response in patients with triple-negative breast cancer [65]. In a clinical study these patients were subjected to T-FAC therapy, containing paclitaxel in combination with other three drugs (paclitaxel followed by fluorouracil, doxorubicin and cyclophosphamide), or to a regimen without paclitaxel. Based on their previous work they defined the paclitaxel metagene as being composed of two gene modules related to the paclitaxel response: one four-gene mitotic module (*BUB1B, CDC2, AURKB, TTK*), where higher expression means sensitivity to the drug and a two-gene ceramide module {*UGCG, COL4A3BP (CERT)*}, where higher expression indicates resistance to the drug. The difference in the average expression of the two gene modules was correlated to extent of sensitivity to paclitaxel. The finding of the study was that the metagene correlated with pathological complete response (pCR) of the patients treated with paclitaxel, making it a potential drug-specific biomarker.

Although the studies described above are able to define a clear connection between ceramide metabolism and taxane-induced apoptosis, several questions remain unanswered.

Despite the fact that, ER would be theoretically the site of ceramide increase as a consequence of CERT knockdown, the final topology of accumulating ceramide remains unknown. Also, it is not clear the mechanism that relates the increase in ceramide levels produced by CERT knockdown to cell sensitivity to taxane.

Our studies with CERT knockout mice, show that lack of CERT function causes abnormal ceramide accumulation not only in the ER, but also in the mitochondria of CERT-deficient embryos and that leads to severe mitochondrial dysfunction. Thus, our data supports the notion that some mechanism exists for the transfer of ceramide from the ER to the mitochondria, either by physical continuity between the organelles or by a transfer protein. The existence of a physical interaction between ER and mitochondria has also been demonstrated by numerous studies. These tethers are called mitochondria-associated membranes (MAMs) and have been implicated in the integration of several aspects of ER and mitochondrial function [66].

Extensive studies show that paclitaxel induces mitochondrial stress and loss of mitochondrial membrane potential [67, 68]. An alternative explanation is that subsequent to CERT knockout in the breast cancer cells, overloading of ER with ceramide causes its flow through MAMs in mitochondria and exacerbates mitochondrial damage and dysfunction. The final outcome would still be apoptosis and sensitivity to paclitaxel, however ER stress response alone may not be the sole contribution. Is ER stress that is augmented by CERT knockdown the main reason for sensitization to taxane action or are there other biochemical phenomena that accompany the silencing of spindle assembly checkpoint genes, arrest of mitotic checkpoint and mitotic slippage? The latter scenario seems more likely since ablation of the two genes belonging to ceramide pathway (COL4A3BP and UGCG) has also been shown to improve response to doxorubicin treatment.

Finally, ceramide and sphingosine-1-phosphate are related sphingolipid metabolites that can be generated through a *de novo* biosynthetic route or derived from the recycling of membrane sphingomyelin. Both these lipids regulate cellular responses to stress, with generally opposing effects. Sphingosine-1-phosphate functions as a growth and survival factor, acting as a ligand for a family of G protein-coupled receptors, whereas ceramide activates intrinsic and extrinsic apoptotic pathways through receptor-independent mechanisms. A growing body of evidence has implicated ceramide, sphingosine-1-phosphate and the genes involved in their synthesis, catabolism and signaling in various aspects of oncogenesis, cancer progression and drug- and radiation resistance. This may be explained in part by the finding that both lipids impinge upon the PI3K/ AKT pathway, which represses apoptosis and autophagy. In addition, sphingolipids influence cell cycle progression, telomerase function, cell migration and stem cell biology. Considering the central role of ceramide in mediating physiological as well as pharmacologically stimulated apoptosis, ceramide can be considered a tumor-suppressor lipid. In contrast, sphingosine-1-phosphate can be considered a tumor-promoting lipid, and the enzyme responsible for its synthesis functions as an oncogene. Not surprisingly, genetic mutations that result in reduced ceramide generation, increased sphingosine-1-phosphate synthesis or which reduce steady state ceramide levels and increase sphingosine-1-phosphate levels have been identified as mechanisms of tumor proression and drug resistance in cancer cells. Pharmacological tools for modulating sphingolipid pathways are being developed and represent novel therapeutic strategies for the treatment of cancer.

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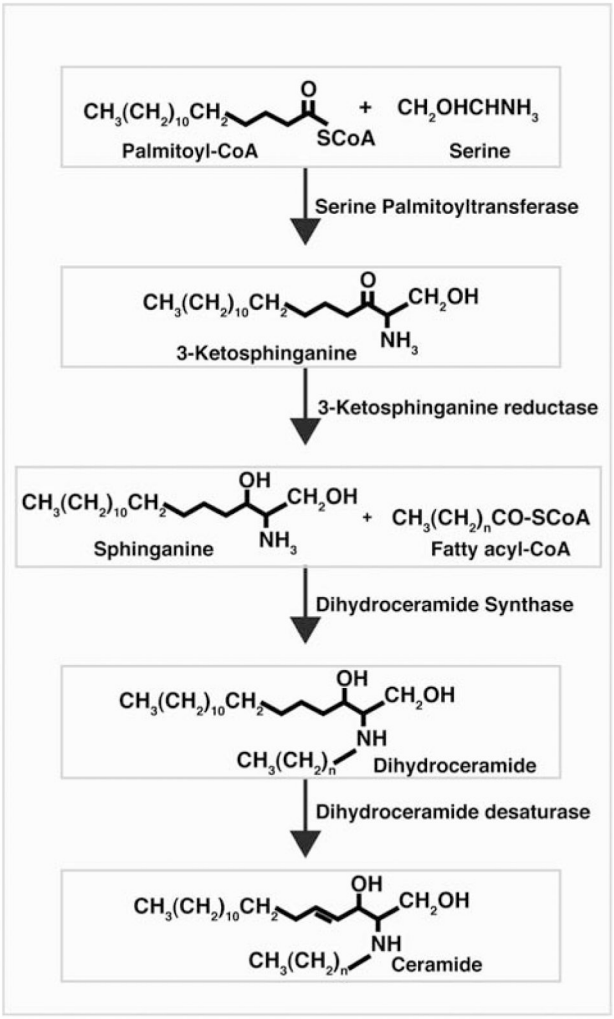

Fig. (1).

de novo sphingolipid biosynthetic pathway leading to the generation of ceramide. Sphingolipid biosynthesis is initiated in the endoplasmic reticulum resulting in the generation of ceramide. Subsequent reactions that generate sphingomyelin, complex sphingolipids and other reactions require the transfer of ceramide from the ER to the Golgi complex. The ceramide transfer protein mediates a great fraction of this transfer.

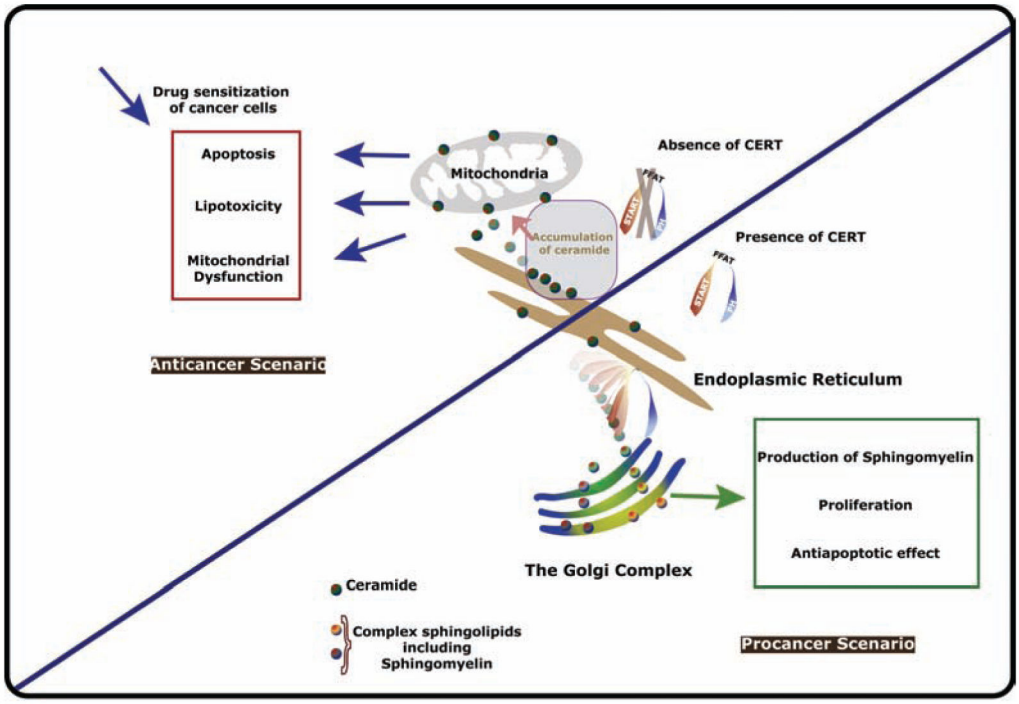

Fig. (2).

CERT regulates ER-mitochondrial ceramide levels. CERT function results in reduction of steady state level of ceramides in the ER and consequently in the mitochondria. The facile transport of ceramide to the Golgi complex is critical for regulated biosynthesis of sphingomyelin and other complex sphingolipids in the post-ER compartments. This function is enhanced during tumorigenesis to facilitate expansion of the membrane compartments fundamental for rapidly proliferating cells. When CERT function is abrogated either by pharmacological intervention or by genetic manipulation ceramide accumulates in the ER and is tracked to the mitochondria *via* the mitochondria associated membranes (MAMS) or by an unidentified carrier protein. This results in ER stress and mitochondrial dysfunction. The resulting unfolded protein response can initiate apoptosis if the program is unable to overcome the toxic effects of ER stress. The increasing ceramide in mitochondria can initiate apoptosis or lead to mitochondrial dysfunction. Eventually these changes compromise the viability of these cells and cause death of the tumor cells.