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REVIEW ARTICLE

Therapeutic potential of targeting ceramide/glucosylceramide pathway in cancer

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Abstract Sphingolipids including ceramides and its derivatives such as ceramide-1-phosphate, glucosylceramide (GlcCer), and sphingosine-1-phosphate are essential structural components of cell membranes. They now recognized as novel bioeffector molecules which control various aspects of cell growth, proliferation, apoptosis, and drug resistance. Ceramide, the central molecule of sphingolipid metabolism, generally mediates anti-proliferative responses such as inhibition of cell growth, induction of apoptosis, and/or modulation of senescence. There are two major classes of sphingolipids. One of them is glycosphingolipids which are synthesized from the hydrophobic molecule, ceramide. GlcCer, generated by glucosylceramide synthase (GCS) that transfers the glucose from UDP-glucose to ceramide, is an important glycosphingolipid metabolic intermediate. GCS regulates the balance between apoptotic ceramide and antiapoptotic GlcCer. Downregulation or inhibition of GCS results in increased apoptosis and decreased drug resistance. The mechanism underlying the drug resistance which develops with increased glucosylceramide expression is associated with P-glycoprotein. In various types of cancers, overexpression of GCS has been observed which renders GCS a good target for the treatment of cancer. This review summarizes our current knowledge on the structure and functions of glucosylceramide synthase and glucosylceramide and on the roles of glucosylceramide synthase in cancer therapy and drug resistance.

Keywords Glucosylceramide synthase · Cancer therapy · Glucosylceramide · Drug resistance · Ceramide · Sphingolipid

Introduction

Sphingolipids (SLs) are a family of lipids that play essential roles as structural cell membrane components that contribute to the regulation of the fluidity and the subdomain structure of the lipid bilayers [1–4]. These membrane lipids do not only function as structural components of the cell membrane, but they also possess important roles in signal transduction as second messengers and in vital cellular processes such as differentiation, migration, apoptosis, cell proliferation, cell cycle arrest, senescence, and inflammation [5–8]. The basic structure of all sphingolipids consists of up to three components: a sphingoid backbone (such as sphingosine, 1,3-dihydroxy-2-aminoalkane and its derivatives), an amide-linked long-chain fatty acid tail, and several distinct modifications of the head group [9, 10]. The head groups define the various sphingolipid classes, with a hydroxyl group found in ceramides; phosphorylcholine, in sphingomyelin (SM); and carbohydrates, in the various glycosphingolipids [11, 12]. The sphingoid backbone is an aliphatic 2-amino-1,3-diol. From this basic lipid, addition of fatty acids that are typically 16–26 carbon atoms in length, phosphate/sulfate groups, and carbohydrates results in a large group of lipids with numerous physiological roles [10, 11, 13, 14].

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Briefly, sphingolipids are synthesized de novo from serine and palmitate, which condense serine and palmitoyl CoA to form 3-keto-dihydrosphingosine through the action of serine palmitoyltransferase (SPT) [15–17]. This is then reduced to produce dihydrosphingosine (sphinganine), which is then acylated by dihydroceramide synthases (also known CerS) (Fig. 1) [15, 17, 18].

Bioactive sphingolipids including ceramide, ceramide-1-phosphate (C1P), dihydroceramide (dhCer), sphingosine, and sphingosine-1-phosphate (S1P) play important roles in malignant growth [6, 19]. Ceramide is the central molecule in sphingolipid and glycosphingolipid biosynthesis, and there are three metabolic pathways leading to ceramide: the sphingomyelinase pathway, the de novo pathway, and the exogenous ceramide-recycling pathway [11, 18]. These metabolic pathways occur in different cellular compartments [3].

Ceramide is an intracellular lipid that has been shown to regulate the activity of various biochemical and molecular targets involved in anti-proliferative responses and in cellular responses including oxidative stress and apoptosis [20, 21]. The biological effects of ceramide depend on many parameters, such as cell type, nature of cell receptors, and their concentration [22].

Ceramide consists of a long-chain amino alcohol (sphingoid base) carboamidically linked to a fatty acid, most commonly with a long chain. The primary alcoholic group of ceramide serves as the attachment site for different moieties such as phosphate, phosphocholine, and saccharides, producing ceramide-1-phosphate, sphingomyelin and glycosphingolipids, respectively [4]. Glycosphingolipids (GSL) are membrane components composed of a group of membrane lipids in which the lipid portion is embedded in the outer leaflet of the plasma membrane with the sugar chain extending to the extracellular space [23–25]. GSLs are involved in many fundamental cellular processes, including growth, differentiation, morphogenesis, sensitivity, and response to exogenous compounds [26]. These molecules may also modulate cell signaling by controlling the assembly and specific activities of the plasma membrane

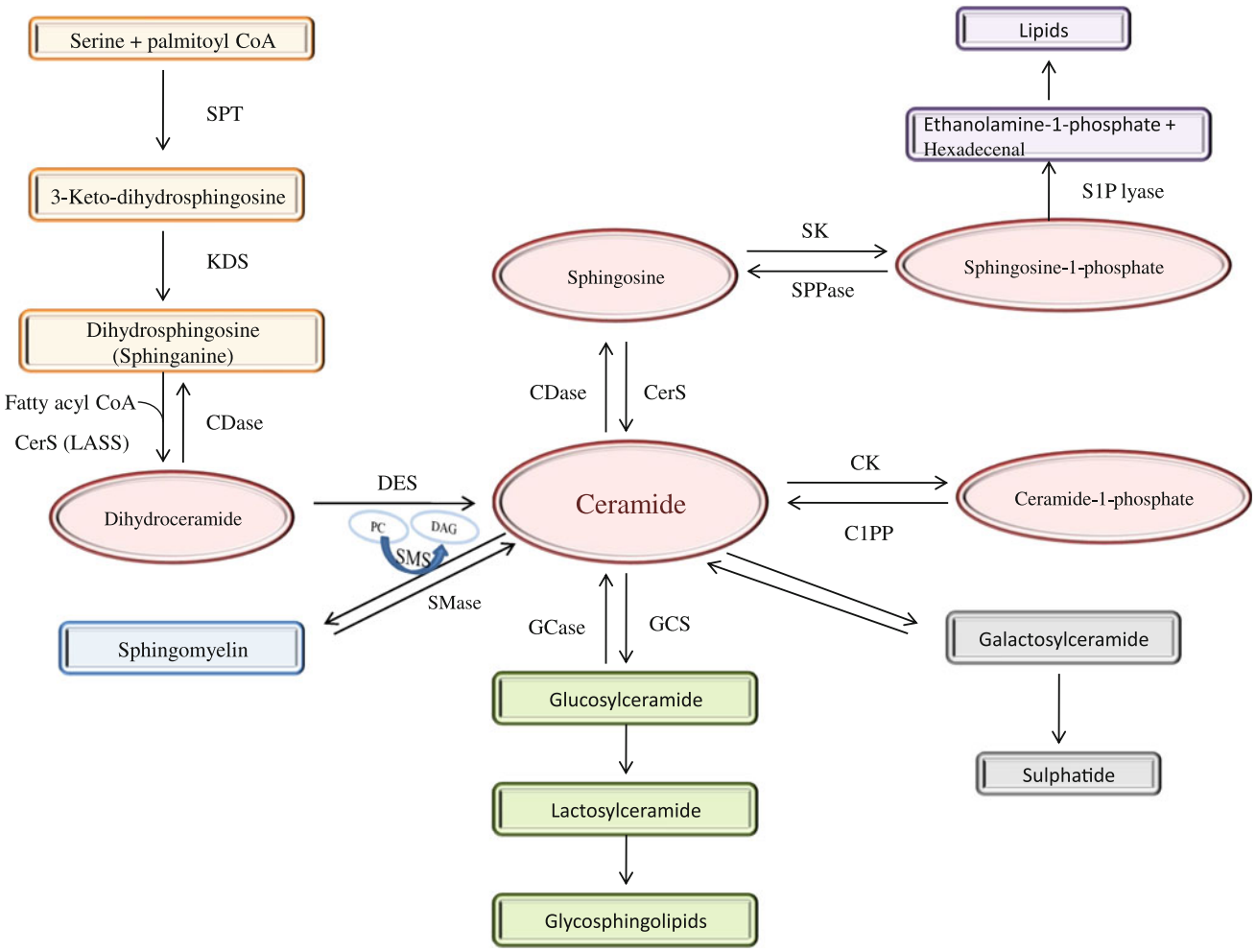

Fig. 1 Pathways of sphingolipid metabolisms

proteins [27]. GSLs are composed of a sphingoid base and a long, mostly saturated amide-linked acyl chain. The structure of the polar head group may vary significantly, ranging from one neutral monosaccharide residue to big assemblies of carbohydrates and sialic acid [28, 29].

Glucosylceramide (GlcCer) is an important glycosphingolipid metabolic intermediate [6, 30, 31] which serves as the starting point in the biosynthesis of a wide variety of GSLs [32]. The synthesis and organization of lipids take place at the endoplasmic reticulum (ER) and the Golgi complex and are precisely regulated. Ceramide is synthesized at the ER and transported to other locations. It either undergoes vesicular trafficking to the *cis*-Golgi, where it is converted to GlcCer, or gets transported to the *trans*-Golgi for conversion to sphingomyelin (SM) [33]. GlcCer is the product of the transfer of glucose by glucosylceramide synthase (GCS) from UDP-glucose to ceramide [6, 34]. Studies have shown that GluCer has proliferative functions on various cells. Therefore, it is important in the chemotherapeutic drug resistance [6].

This review will focus on the structure and functions of glucosylceramide synthase and glucosylceramide, and the roles of glucosylceramide synthase in treatment and drug resistance of cancer. It will also discuss targeting the glucosylceramide synthase/glucosylceramide pathway for the treatment of cancer.

Structure and functions of glucosylceramide synthase and glucosylceramide

Glucosylceramides are present in almost all eukaryotic organisms and in a few bacteria, and they play a key role in the synthesis of hundreds of different GSLs [29, 35, 36]. GSLs are characteristic constituents of plasma membranes of mammalian cells. They may modulate cell proliferation, differentiation, and cell–cell interaction [37] and play an important role in the metastatic spread of tumor cells since GSLs on the cell membrane have been implicated as functionally important molecules in tumor cell attachment [38]. They are glycolipids that contain a hydrophilic head group sugar, D-glucose, and a hydrophobic lipid moiety [26]. The structures of the sugar head groups and the ceramide backbones of many GlcCer from animals, plants, fungi, and bacteria have demonstrated variety [35]. The biosynthesis of GlcCer results of biochemical events leading to complex structures. The above structures are embedded at the surface of cells by non-covalent interactions between phospholipids and the ceramide part of the glycolipids. The carbohydrate is endowed of recognition properties, modulated by the nature of the lipid moiety responsible for the self-assembling properties of the whole [39]. GlcCers have been degraded both by a glucocerebrosidase in lysosomes and by a non-lysosomal glucocerebrosidase in the cytosol [29].

GlcCers have been found to be involved in many cellular processes such as cell proliferation, oncogenic transformation, differentiation, and tumor metastasis, and more recently, they have been implicated in venous thrombosis and in the anticoagulant activity of protein C [26]. GlcCer functions have been listed as (1) contributing to the physical properties and physiological functions of membranes, (2) serving as the basic precursor for over 300 species of glycosphingolipids found in different mammalian cell types, and (3) GlcCer synthesis and degradation are believed to contribute to the control of the level of ceramide [35, 40].

GlcCers are formed from ceramide and UDP-glucose by the microsomal enzyme, UDP-glucose: ceramide d-glucosyltransferase also known as GCS (EC 2.4.1.80), that is, a transmembrane protein localized in the cis/medial Golgi, with an N-terminal signal-anchor sequence and a C-terminal catalytic domain located in the cytoplasm [14, 23, 41–43]. The rate of reaction under physiological conditions may depend on the tissue level of UDP-glc, which in turn depends on the level of glucose in a particular tissue [44]. This enzyme does not possess similarity to other known glycosyltransferases. The structure of the enzyme is quite unique since all other glycosyltransferases involved in GSL synthesis are localized to the lumenal side of the Golgi apparatus or ER [45]. It catalyzes the transfer of a glucose moiety from UDP-glucose to the primary hydroxyl group of ceramide to yield GlcCer with inversion of the anomeric configuration for synthesis of glucosylceramide [46–48].

The catalytic activity of GSC interferes with the function of both GlcCer and higher GSLs. These functions include two phenomena of medical importance. First, turnover of higher GSLs requires their continuous but matching degradation. Inhibition of GlcCer biosynthesis by drugs can reduce the accumulation of higher sphingolipid. Second, the development of cancer cells toward apoptosis or proliferation and their level of multidrug resistance depend on the ratio of ceramide to glycosphingolipids [42, 43]. The activity of GCS was first determined in 1968 and since then different enzymatic assays have been developed [49]. This enzyme is located on the cytosolic membrane leaflet of the Golgi apparatus. From here, GlcCer can reach the plasma membrane by direct transport, or it can be modified by further glycosylation in the Golgi apparatus [37, 50, 51]. Glucose is first glycosylated with the C1 hydroxyl group of ceramide, and then, the GlcCer unit serves as a common coupling partner for the oligosaccharide donors [52]. This enzyme is also available in the ER and microsome [53].

It has been shown that human GCS is a glycoprotein containing 394 amino acids encoded from 1182

nucleotides, including a G1C rich, 59 untranslated regions of 290 nucleotides [34]. GCS is composed of 38-kDa monomers organized as heterodimers or heterooligomers with both C and N termini present in the final enzyme form. The active site of the enzyme is present on the cytosolic face of the Golgi membrane with some epitopes shielded by proximity to other parts of the enzyme or Golgi membrane. In addition, there is an associated protein of 15 kDa found in conjunction with the enzyme normally found in Golgi membranes whose specific structure is currently unknown [38]. Enzyme protein is both tightly membrane bound and is a minor component of the Golgi membrane. Therefore, purification of GCS is very difficult [45].

GCS activity is stimulated by a number of means that increase ceramide concentrations, such as the addition of a short-chain ceramide and treatment with bacterial sphingomyelinase, endoglycoceramidase, or inhibitors of GCS synthase [7].

Glucosylceramide synthase in drug resistance

Despite of the presence of many therapeutic approaches developed continuously for cancer therapy, in clinic, resistance cases against these approaches are arising at the same time. For this reason, resistance development is one of the major obstacles in the struggle between the cancer cells and the therapeutics.

As well as the known mechanisms for many years, abnormal sphingolipid metabolism has also been seen as an effective drug resistance mechanism, recently [27, 54]. Conversion of ceramide into glucosylceramide by the activity of GCS and therefore intracellular aggregation of glucosylceramide is generally hold responsible for this type of mechanism in drug resistance [1, 27, 55]. In 1996, adriamycin resistance has been reported to be associated with increased glucosylceramide levels in MCF-7 breast cancer cells for the first time. This effect of glucosylceramide on drug resistance has been then confirmed in many other types of cancer cells such as melanoma, leukemia, and neuroblastoma [27, 47, 55, 56]. However, downregulation or inhibition of GCS results in increased levels of intracellular ceramide and decreased drug resistance, that is, reversion of drug resistance [57].

The mechanism underlying the drug resistance that gets developed with increased glucosylceramide expression is associated with P-glycoprotein (P-gp) overexpression of cancer cells [58, 59]. In leukemia, melanoma, colon, and breast cancer cells, overexpression of GCS causes an increment in the expression levels of P-gp that results in drug resistance [60]. P-gp has been also reported to prevent human AML cells from C8:ceramide-mediated apoptosis, and inhibition of P-gp resulted in the sensitization of the cells to apoptosis via C8:ceramide [54]. P-gp expression has been reported to be responsible for the development of resistance against the apoptotic effects of C6:ceramide on HeLa cells [52].

Interestingly, in addition to increased levels of glucosylceramide, increased levels of ceramide also results in the overexpression of P-gp in breast cancer cells. In 2007, it has been reported that long-term treatment of MDA-MB-231 cells with C8:ceramide induces P-gp overexpression [61]. Moreover, under the stress conditions generated by the doxorubicin treatment, ceramide has been observed to trigger the overexpression of GCS and cause the arise of doxorubicin resistance [62].

High levels of GCS also cause the overexpression of Bcl-2 gene, an apoptosis suppressor. In adriamycin-resistant K562/AO2 cells, increased levels of Bcl-2 have been reported rather than that in sensitive K562 cells. Following the inhibition of GCS by PPMP or downregulation by siRNA transfection, decreased Bcl-2 gene expression levels have been reported [63]. Likewise, β-catenin- and cSrc signaling are responsible for the development of drug resistance due to increased levels of GCS in colon, cervical, breast, and ovary cancer cells that resist doxorubicin. In OVCAR-8 human ovarian cancer cells, GCS upregulation has been reported to result in a significant increase in the levels of cSrc- and β-catenin signaling and also in a significant decrease in intracellular paclitaxel levels, showing increased activity of P-gp. In contrast, downregulation of GCS causes suppression of these signaling molecules and also of P-gp [64].

The resistance generated as a result of the overexpression of GCS can be reversed by the inhibition of GCS, P-gp, or any other responsible genes through the particular inhibitors, by siRNA transfection, or via using nanoparticles [65]. Siddigui et al. [66] reported that using oligonucleotide nanoparticles that have been loaded with antisense GCS reversed resistance against adriamycin in NCI/ADR-RES cells. The use of these mixed-backbone oligonucleotides suppressing selectively GCS overexpression have been also reported to significantly enhance the sensitivity of human NCI/ADR-RES cells and also of EMT6/AR1 murine breast cancer cells against doxorubicin. Furthermore, these oligonucleotides decrease the size of the tumors by increasing the levels of C18:ceramide and apoptosis mediated by caspases [67]. In breast cancer, in vivo and in vitro suppression of GCS by GCS siRNA also decreases P-gp expression and the tumor size; therefore, it reverses multidrug resistance [68]. Transfection of adriamycin-resistant MCF-7/ADM human breast cancer cells with both GCS and MDR1 siRNAs results in significant, and more importantly, efficient reversal of multidrug resistance [69]. Our group recently showed that there were significant increases in expression levels of imatinib resistant K562 cells as compared to parental sensitive cells [70].

Considering all these data, GCS is mainly responsible for MDR in almost all types of cancer, and targeting and inhibiting glucosylceramide synthase lead to the reversal of this MDR, and thus sensitization, and even totally removal of cancerous cells.

Glucosylceramide synthase in cancer therapy

GCS regulates the balance between ceramide and GlcCer, meaning that GCS regulates drug sensitivity or resistance to anticancer drugs [46]. Intracellular accumulation of ceramide or exogenous ceramides cause anti-proliferative responses, since ceramide is a strong apoptotic molecule [51]. There is evidence that ceramide mediates cell death by apoptotic and non-apoptotic mechanisms in several systems [27]. Ceramide mediates cell death, while its detoxification by conversion to glucosylceramide can inhibit this process [51]. Because GSLs on the cell membrane have been implicated as functionally important molecules in tumor cell attachment, GluCers play an important role in the metastatic spread of tumor cells [38].

Because of the propagator effects of GCS in cancer cells, many researchers have studied targeting GCS in cancer therapy in order to trigger apoptosis and render MDR [69]. Previously, Radin has reported that the inhibition of GCS with PDMP, a glucosylceramide analog, results in an increase in the levels of ceramide and sphingosine and also a decrease in protein kinase C levels in mouse Ehrlich ascites carcinoma cells and in rat glioma cells, that is, GCS inhibition enhances the anti-proliferative effects of chemotherapeutics on cancer cells [71, 72]. Maurer et al. [73] have also reported that GCS inhibition increases the intracellular ceramide levels and enhances the cytotoxic effects of 4-HPR and safingol in tumor cells. Not only in vitro but also in vivo studies have shown that progression of melanoma cells decreases in response to GCS suppression, and thus P-gp inhibition [74, 75]. In neuroepithelioma cells, it has been reported that antisense- and PDMP-mediated inhibition of GCS decreases the p53-independent apoptotic effects of the antineoplastic reagent, retinoid [76]. Furthermore, Gouazé et al. [77] have reported that GCS inhibition leads to increased sensitivity and thus enhanced effects of paclitaxel, doxorubicin, and vinblastine via increasing intracellular ceramide levels in MCF-7-AdrR cells. In 2006, PDMP, the GCS inhibitor, has been reported to render neuroblastoma cells sensitive against paclitaxel. This sensitization results in abnormal progression of cell cycle rather than the induction of apoptosis [78]. In addition, apoptotic effects of a caspase-dependent apoptosis inducer peptide molecule, lactoferricin, have been reported to increase in response to the treatment of CCRF-CEM and Jurkat T cell leukemia cells with PPMP, a GCS inhibitor. It has also been shown that the combined use of lactoferricin and tamoxifen, due to its known inhibitory effect on GCS function, enhances the apoptotic effects of lactoferricin, and thus, it leads to increased levels of apoptosis in Jurkat T cell leukemia cells [79]. The imino sugar OGT2378 that is also an inhibitor of GCS reduced the tumorigenic capability of MEB4 melanoma cells [80]. It has been shown that GCS is overexpressed in many multidrug-resistant cancer cell lines in leukemia, breast cancer, and renal cell cancer [64]. Treatment of various kinds of cancer cells with several GCS inhibitors affects basic cellular functions such as growth, death, and adhesion. Also, recent studies have demonstrated a direct correlation between the development of multidrug resistance and increased levels of GC [81]. The effects of GCS on cancer therapy have also been revealed in follicular thyroid carcinoma cells. In this type of cancer cells, the inhibition of GCS enhances the anti-cancer effects of camptothecin and doxorubicin via increasing the level of ceramide synthesis [82]. Recently, it has been also reported that in tumor cells bearing p53 mutant alleles, GCS inhibition activates the phosphorylation of p53 and also activates the genes related with p53-mediated apoptosis, such as Puma, p21Waf1/Cip1, and Bax. These p53-mutant cancer cells have also become more sensitive against doxorubicin in response to GCS inhibition [83].

Moreover, our group has revealed that the suppression of GCS by PDMP synergistically increases the anti-proliferative and apoptotic effects of resveratrol on human acute and chronic myeloid leukemia cell lines [84, 85]. Our group has also shown that the inhibition of GCS with PDMP increased anti-proliferative and apoptotic effects of imatinib [86], nilotinib [87], and dasatinib [88] on chronic myeloid leukemia cells synergistically. GCS inhibition causes enhanced cytotoxic and apoptotic responses in human prostate cancer cells in response to docetaxel treatment [89]. It was also demonstrated that inhibition of GCS reversed resistance to doxorubicin and vincristine in leukemia [90, 91].

Accumulating literature in this area strengthen the importance of bioactive sphingolipid metabolism for the diagnostic and therapeutic applications in various cancers.

Conflict of interest The authors do not have any kind of conflict of interest affecting the compilation of the current knowledge in this area for writing this review. We apologize to the ones whose elegant studies are not included here because of space limitations.

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