Regulation of Intracellular Ceramide Content in B16 Melanoma Cells

BIOLOGICAL IMPLICATIONS OF CERAMIDE GLYCOSYLATION

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We previously reported that ceramide released from glycosphingolipids ( GSLs) by endoglycosylceramidase was directly metabolized to GSLs, and thus the content of GSLs was constantly maintained in B16 melanoma cells (Ito, M., and Komori, H. (1996) J. Biol. Chem. 271, 12655–12660). In this study, the metabolism of ceramide released from sphingomyelin ( SM) by bacterial sphingomyelinase ( SMase) was examined using B16 cells and their GSL-deficient mutant counterpart GM95 cells. Treatment of B16 melanoma cells with bacterial SMase effectively hydrolyzed SM on the plasma membrane. Under these conditions, NeuAcO2,3Galβ1,4Glcβ1,1ceramide was significantly increased. Interestingly, UDP-glucose:ceramide glucosyltransferase-1 ( GlcT-1) activity and GSL synthesis, but not SM synthesis or sphingosine generation, were found to be up-regulated by SMase treatment. The up-regulation of GSL synthesis seemed to occur at both the transcriptional and post-translational steps of GlcT-1 synthesis. Accumulation of ceramide by bacterial SMase was much higher in GM95 cells than in the parental cells. When the enzyme was removed from the culture medium, the intracellular ceramide level in B16 cells, but not that in the mutant cells, normalized. No rapid restoration of SM in either of the cell lines was observed after removal of the enzyme. SMase treatment strongly inhibited DNA synthesis in GM95 cells but not that in B16 cells. In the presence of threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, an inhibitor of GlcT-1, SMase treatment markedly increased the ceramide content and thus inhibited DNA synthesis in B16 cells. Our study provides the first evidence that GlcT-1 functions to regulate the level of intracellular ceramide by glycosylation of the ceramide when it is present in excess.

Glycosphingolipids ( GSLs) and sphingomyelin ( SM) are characteristic components of vertebrate plasma membranes and have the same hydrophobic residue, ceramide, which consists of a sphingosine and a fatty acid. GSLs have been defined as tumor antigens, receptors for microbes and their toxins, and possible modulators of cell proliferation, differentiation, and cell-cell interactions (1–3). Recently, ceramide has emerged as a novel second messenger for intracellular signaling pathways responding to various cytokines and stress (4). Several lines of evidence indicate that a signaling ceramide is produced from SM by the action of endogenous neutral (5) and acid sphingomyelinase ( SMase; EC3.1.4.12) (6), or by de novo synthesis (7). However, little is known about the mechanism of regulation of intracellular level of ceramide, which should be strictly regulated within cells.

Endoglycosylceramidase ( EC3.2.1.123) is a GSL-specific enzyme from Rhodococcus sp. that hydrolyzes the glycosidic linkage of ceramide and sugar chains of various GSLs (8). The cell surface GSLs of various erythrocytes (9) and cultured mammary cells (10) were hydrolyzed by the purified rhodococcal endoglycosylceramidase (11) with the assistance of its protein activator (12). We found that treatment of B16 melanoma cells with a microbial endoglycosylceramidase activated GSL synthesis via transient up-regulation of UDP-glucose:ceramide glucosyltransferase-1 ( GlcT-1, glucosylceramide synthase; EC2.4.1.80) (13). As a result, cell surface NeuAcO2,3Galβ1,4Glcβ1,1ceramide (GM3), the end product of GSL synthesis in B16 cells, was restored quickly when the enzyme was removed from the culture medium (13).

In this study, we examined the effects of bacterial SMase on the metabolism of GSLs and SM using B16 cells and their GSL-deficient mutant counterpart GM95 cells, which lack GlcT-1 (14). Although GSLs were quickly restored after endoglycosylceramidase treatment, restoration of SM was not observed after treatment with bacterial SMase in B16 melanoma cells. Interestingly, ceramides generated from not only GSLs but also SM by the microbial enzymes were primarily glucosylated by GlcT-1, metabolized to GM3, and then transported to the plasma membrane. Ceramide was accumulated during SMase treatment in GM95 or B16 cells in the presence of threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol ( PDMF) (15) or N-butyldeneoxygirimycin (16), potent inhibitors of GlcT-1. These results suggest a biological role of GlcT-1 in the regulation of intracellular ceramide content.

Because excess generation of ceramide is toxic to cells, GlcT-1 seems to function for expulsion of ceramide from the cell. This regulation, regarded as a putative detoxification mechanism, may function as a defense against an unexpected increase of ceramide, which could be caused by various forms of

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The abbreviations used are: GSL, glycosphingolipid; C6-ceramide, N-butyldeneoxygirimycin; CMI, ceramide monohexoside; GlcT-1, UDP-glucose:ceramide glucosyltransferase-1; GM3, NeuAcO2,3Galβ1,4Glcβ1,1ceramide; MEM, minimum essential medium; PBS, phosphate-buffered saline; PDMF, threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; SM, sphingomyelin; GM3, sphingomyeline; TLC, thin layer chromatography; FBS, fetal bovine serum.
stress, e.g., infections with pathogenic microorganisms that produce SMase or endoglycoceramidase. This paper also indicates the biological role of ceramide as a modulator of the overall synthesis of GSLs by regulating GlcT-1 at both the transcriptional and post-translational levels.

**EXPERIMENTAL PROCEDURES**

*Materials—* Monoclonal antibody M2590 and fluorescein isothiocyanate-conjugated goat anti-mouse IgM were purchased from Cosmo Bio Co., SMases from *Staphylococcus aureus* and *Bacillus cereus* were obtained from Sigma and Funakoshi Co., respectively. D-Threo-PDMP and

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**FIG. 1.** Effects of SMase treatment on sphingolipid metabolism in B16 cells. A, two-dimensional TLC of 14C-labeled sphingolipids of B16 cells. B16 cells (1 × 10^6) were incubated in MEM supplemented with 5% FBS containing 1 µCi of [14C]serine for 24 h, washed with fresh MEM, and recultured in MEM containing 20 milliunits of SMase for 18 h in the presence or absence of Fumonisin B1 (100 µM). Total 14C-labeled lipids were extracted, separated by TLC, and analyzed with an imaging analyzer as described under “Experimental Procedures.” A, results in the absence of Fumonisin B1. B, de novo synthesis of GSLs in B16 cells during SMase treatment. B16 cells (1 × 10^6) were metabolically labeled with 1 µCi of [14C]Gal in the presence or absence of 20 milliunits of SMase in 200 µl of MEM supplemented with 5% FBS at 37 °C for 18 h. 14C-labeled GSLs were extracted, separated by TLC, and analyzed with an imaging analyzer. Values are the means ± S.D. for triplicate determinations. PSL, photo-stimulated luminescence/mm^2. C, cytofluorometric analysis of cell surface GM3. B16 cells (1 × 10^6) were incubated with 100 milliunits of SMase in 1 ml of MEM supplemented with 5% FBS at 37 °C for 18 h. Cells with or without (Control) SMase treatment were incubated with M2590 monoclonal antibody followed by a second incubation with fluorescein isothiocyanate-conjugated goat anti-mouse IgM and analyzed by flow cytometry as described under “Experimental Procedures.”
N-hexanoylphosphosine (C6-ceramide) were purchased from Matreya, and thin layer chromatography (TLC) plates (Silica Gel 60) were from Merck. [3H]-Labeled Gal, serine, choline, and [32P]ATP were obtained from DuPont NEN, and [3H]thymidine, [3H]palmitic acid, and [14C]UDP-glucose were from American Radiolabeled Chemicals. sn-1,2-Diacylglycerol kinase was kindly provided by Dr. T. Okazaki (Kyoto University, Kyoto, Japan). All other chemicals were of the highest grade available.

Cell Culture and SMase Treatment—All experiments using B16 melanoma and the GltT-1-deficient mutant GM9 cells (14) were conducted at 37 °C in minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS) in a humidified 95% air, 5% CO2 incubator. SMase from S. aureus was used for all experiments except that shown in Fig. 2, in which the enzyme from B. cereus was used.

Metabolic Labeling—Cells were incubated in 200 μl of MEM supplemented with 5% FBS containing 1 μCi of [3H]Gal, [3H]serine, or [14C]choline for the time indicated.

Extraction and Determination of Radiolabeled Sphingolipids—Cells (1 × 10^6) were harvested by centrifugation (2000 rpm for 5 min) and washed twice with phosphate-buffered saline (PBS). Sphingolipids were extracted with 750 μl of i-propanol/hexane/water (55:35:10, v/v/v) in a sonicator for 20 min. After centrifugation at 13,000 rpm for 5 min, the supernatants obtained were dried under N2 gas, dissolved in 25 μl of chloroform/methanol (2:1, v/v), and applied to TLC plates, which were developed with chloroform/methanol/acetic acid/H2O (50:30:8:5, v/v) for SM. Radio-labeled sphingolipids separated on TLC plates were analyzed and quantified using a BAS1000 imaging analyzer (Fuji Film). Identification of C6-ceramide GSLs was done by the method described by Komori and Ito (17).

Two-dimensional TLC—The radiolabeled total lipid extracts from cells were spotted onto TLC plates (10 × 10 cm). The first chromatographic run was performed with chloroform/methanol/formic acid/H2O (65:25:8:9:1.1, v/v/v/v/v). The second run was performed with chloroform/methanol/5% NH4OH (50:40:10, v/v/v) perpendicular to the original direction. Then the third run was performed with diethyl ether in the opposite direction to the second run to separate ceramides from other neutral lipids. Spots on the TLC plates were identified using authentic standards (18).

Determination of SM Content—Total lipids, extracted from 5–7 × 10^6 cells with a mixture of i-propanol/hexane/water (55:35:10, v/v/v), were applied to TLC plates, which were then developed with chloroform/methanol/acetic acid/H2O (50:30:8:5, v/v/v/v). SM was visualized with Coomasie Brilliant Blue (19) and quantified using a Shimadzu CS-9300 Chromatoscanner with reflection mode set at 580 nm.

Determination of Ceramide Content—Ceramide was extracted from cells (2 × 10^6) with 3 ml of chloroform/methanol (1:2, v/v), and 0.8 ml of water was added and mixed well. The organic and aqueous phases were subsequently separated by addition of 1 ml of chloroform and 1 ml of water followed by vigorous shaking and centrifuged at 3000 rpm. The organic phase was carefully removed and transferred to a new tube, and water followed by vigorous shaking and centrifuged at 3000 rpm. The organic and aqueous phases were subsequently separated by addition of 1 ml of chloroform and 1 ml of water followed by vigorous shaking and centrifuged at 3000 rpm. Then, the third run was performed with diethyl ether in the opposite direction to the second run to separate ceramides from other neutral lipids. Spots on the TLC plates were identified using authentic standards (18).

Measurement of DNA Synthesis—Cells (2 × 10^4) were incubated in MEM supplemented with 5% FBS in 96-well plates at 37 °C for 14 h. After incubation, 10 μl of PBS containing 0.1 μCi of [3H]thymidine was added to the medium. After incubation at 37 °C for 4 h, cells were collected using a Combi 11025 cell harvester (Skatron), and the incorporation of [3H]thymidine into DNA was quantified by liquid scintillation counting.

Assay of GltT-1—GltT-1 activity was determined according to the method of Basu et al. (22) with slight modifications. To prepare cell lysates, cells were washed with 1 ml of PBS and suspended in 50 μl of 10 mM Tris-HCl, pH 7.5, containing 2 mM KCl and 5 mM MgCl2. Standard incubation mixture (50 μl) contained 0.5% Triton X-100, 500 μM [3H]UDP-glucose (0.02 μCi/μl), 0.3 mM ceramide (type III), and cell lysate (500 μg as protein) in 20 mM Tris-HCl, pH 7.5. After incubation at 32 °C for 2 h, 100 μl of chloroform/methanol (2:1, v/v) was added to terminate the reaction, and the lower layer was applied to TLC plates, which were then developed with chloroform/methanol/12
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mm MgCl₂ (65:25:4, v/v). The NBD-SM produced was determined with a Shimadzu CS-9300 chromatocorder (excitation, 475 nm; emission, 525 nm). The activity measured by this method appeared to show the total activity of phosphatidylcholine-specific phospholipase C and CDP-choline:ceramide cholinephosphotransferase.

Protein Determination—Protein contents of cell extracts were determined by the BCA method (Pierce) with bovine serum albumin as the standard.

Isolation of Total RNA and Northern Blotting Analysis—Total RNA was isolated from cells with an RNeasy mini kit (Qiagen). Aliquots of 30 μg of total RNA were subjected to electrophoresis on 1% agarose gels containing 18% formaldehyde, and transblotted onto nylon membranes (Hybond-N, Amersham Pharmacia Biotech). The 1.2-kb BamH-I-XhoI cDNA fragment of the mouse GlcT-1 gene was labeled with [α-32P]dCTP (6000 Ci/μmol, Amersham Pharmacia Biotech) by the Multiprime DNA labeling system (Amersham Pharmacia Biotech) and used as a probe.

Hybridization was carried out at 42 °C for 24 h in 5 × SSPE (750 mM NaCl, 43.3 mM NaH₂PO₄, 6.25 mM EDTA) containing 50% formamide, 4% SDS, 5 × Denhardt’s solution, salmon sperm DNA (100 μg/ml), and 32P-labeled probe (23). After hybridization, the membrane was washed continuously with 2 × SSPE containing 0.5% SDS, 1 × SSPE containing 0.5% SDS, and 0.1 × SSPE containing 0.5% SDS at 50 °C for 40 min at each step. The membranes were also hybridized by the method described above but with a β-actin cDNA probe for normalization of mRNA levels. mRNA levels were quantified using an imaging analyzer (BAS1000, Fuji Film).

Determination of Fluorescent C6-NBD Sphingolipids—NBD-labeled C6-ceramide was added to cells (1 × 10⁶) and incubated at 37 °C in a CO₂ incubator for the times indicated. Cells were harvested by centrifugation (2000 rpm for 5 min) and washed twice with PBS. Sphingolipids were extracted with 750 μl of i-propanol/hexane/water (55:35:10, v/v) in a sonicator for 20 min. After centrifugation at 13,000 rpm for 5 min, the supernatants obtained were dried under N₂ gas, dissolved in 25 μl of chloroform/methanol (2:1, v/v), and applied to TLC plates, which were then developed with chloroform/methanol/12% MgCl₂ (65:25:4, v/v). The NBD-sphingolipids produced were determined with a Shimadzu CS-9300 chromatocorder (excitation, 475 nm; emission, 525 nm).

RESULTS

Effects of SMase Treatment on Sphingolipid Metabolism in B16 Cells—We previously reported that endoglycoceramidase treatment of B16 melanoma cells stimulated GSL synthesis (13). Because ceramide is the common lipid backbone of GSLs and SM, we examined the metabolism of SM-derived ceramide using bacterial SMase instead of endoglycoceramidase. The ceramide portions of SM and GSLs of B16 cells were metabolically labeled with [14C]serine, washed with fresh MEM, and then treated with SMase from S. aureus. Two-dimensional TLC revealed that SMase treatment markedly reduced the content of [14C]SM by 90% of that in controls, whereas that of [14C]GM3 simultaneously increased by 197% of the control level (Fig. 1A). To avoid the influence of de novo synthesis of ceramide, the same experiment was conducted but in the presence of Fumonisin B1, an inhibitor for acyl-CoA:ceramide 1-acyltransferase (24). Even in the presence of Fumonisin B1, the increase in GM3 concomitant with the decrease in SM was observed after SMase treatment (Fig. 1A), indicating that the increase in GM3 level was not attributable to an increase in de novo synthesis of ceramide. During SMase treatment, [14C]Gal uptake into ceramide monohexoside (CMH) and GM5 was increased in B16 cells (Fig. 1B). However, the level of sphingosine was not changed after SMase treatment (data not shown). These results strongly suggest that increased GM3 was metabolized from SM-derived ceramide by direct glycosylation but not from sphingoid base salvaged from hydrolysis of ceramide. The increase of GM3 was also observed in not only B16 cells but also other cell lines, as shown by the synthesis of CMH in HL60 human myelocytic leukemia cells, which increased by not less than 2-fold during SMase treatment.

Effects of SMase on GlcT-1 and SM Synthase Activities, GSL Synthesis, and GlcT-1 mRNA—The GlcT-1 activity and de novo synthesis of GSLs in B16 cells were examined with or without SMase treatment. Interestingly, GlcT-1 activity in the cell lysate increased by ~2-fold by the enzyme treatment, whereas SM synthase activity was not affected (Fig. 2A). It was confirmed that GM95 cells completely lacked GlcT-1 activity (data not shown). The activation of GlcT-1 seems to occur post-translationally, because even in the presence of cycloheximide an increase in GSL synthesis was observed after SMase treatment (Fig. 2B). On the other hand, Northern blotting analysis indicated that SMase treatment slightly activated transcription of the GlcT-1 gene (Fig. 2C). Treatment of B16 cells with C6-ceramide also increased GlcT-1 mRNA expression in a concentration-dependent manner (Fig. 2D). These results indicate that up-regulation of GSL synthesis by SMase treatment occurs at the level of both transcription and post-translation of the GlcT-1 gene, and also that ceramide could modulate the overall synthesis of GSLs via regulation of GlcT-1.

SM Restoration—The content of GM3 in B16 cells was reduced by endoglycoceramidase treatment but recovered quickly after the enzyme was removed from the culture medium (13). However, no restoration in SM content was observed after removal of SMase when B16 cells were prelabeled with [14C]choline (Fig. 3). GM95 cells also showed no rapid restoration of SM (Fig. 3). It should be noted that this restoration was estimated by a base exchange reaction between ceramide and phosphatidylcholine, which is considered to be the major pathway of SM synthesis (25). However, a portion of SM (~25%) appeared to recover in B16 cells after removal of the enzyme and reculture for 9 h when the SM mass was estimated by Coomassie Brilliant Blue staining. This increase in SM seemed to be derived from de novo synthesis of ceramide and not from the recycling of ceramide.

Accumulation of Ceramide after SMase Treatment—The ceramide content of B16 cells, which was measured by sn-1,2-diacylglycerol kinase assay, increased by ~2-fold after SMase treatment for 3 h but gradually decreased after removal of the enzyme and returned to the basal level after 6–9 h. In contrast, in GlcT-1-deficient mutant GM95 cells the accumulation of ceramide by SMase treatment was much higher than in the

![Fig. 3. SM content after SMase treatment.](Image)
parental cells and was maintained after the enzyme was removed from the culture medium (Fig. 4A). Interestingly, SMase treatment of B16 cells in the presence of PDMP, a potent inhibitor of GlcT-1, markedly increased the accumulation of ceramide (Fig. 4B). Ceramide generation in B16 cells by bacterial SMase was also enhanced 2.6 times by addition of N-butyldeoxynojirimycin at 200 μM, which is another inhibitor of GlcT-1 (16). These results suggest that GlcT-1 functions to remove the excess ceramide generated by bacterial SMase.

**Effects of Ceramide on DNA Synthesis**—Although ceramide functions as a novel class of second messenger (4), the excess generation of ceramide must be harmful to the cell. As shown in Fig. 5A, DNA synthesis of GM95 cells measured by the incorporation of [3H]thymidine was significantly inhibited by bacterial SMase treatment, whereas this enzyme treatment seemed to have no effect on that of B16 cells (Fig. 5A). However, in the presence of PDMP, SMase treatment appeared to be harmful for B16 cells (Fig. 5A). The degree of inhibition of DNA synthe-
sis was consistent with that of accumulation of ceramide (Fig. 4). Hidari et al. (26) reported that the treatment of GM95 cells with the bacterial SMase disrupted cell-substratum adhesion, and the cells were peeled off from dishes. However, in this experiment a much lower concentration of SMase was used to avoid disrupting the adhesion. The effects of the short chain ceramide, C6-ceramide, on DNA synthesis of B16 and GM95 cells were also examined. Incubation with 50 μM C6-ceramide for 3 h strongly suppressed DNA synthesis in GM95 cells but not in B16 cells (Fig. 5B). This result is consistent with that using SMase (Fig. 5A).

**Conversion of Short Chain Ceramide to GSLs and SM—**

When C6-ceramide was added to B16 cell cultures in the presence of [14C]Gal, C6-CMH and C6-GM3 were generated, both of which were also released into the medium (Fig. 6A). However, glycosylation of the short chain ceramide was not found in cultures of GM95 cells (Fig. 6A). To examine the ratio for conversion of ceramide to GSLs and SM, NBD-C6-ceramide was added to cultures of B16 and GM95 cells. The NBD-C6-ceramide was exhausted in B16 cells much more rapidly than in GM95 cells (Fig. 6B). In B16 cells, NBD-C6-ceramide was converted to NBD-C6-CMH and NBD-C6-SM at the same rate by 15 min, but thereafter the generation of NBD-C6-CMH was much faster than that of NBD-C6-SM. In GM95 cells, NBD-C6-ceramide was converted to NBD-C6-SM, but not to NBD-C6-GSLs, and the conversion reached a plateau by 15 min when 70% of NBD-C6-ceramide still remained in the cells. These results indicated that the exclusion of ceramide in B16 cells was mainly achieved by glycosylation. The preference for glycosylation of ceramide seems to be restricted to ceramide generated from GSLs (13) and SM on the plasma membrane, because the de novo synthesis of GM3 and SM in B16 cells was almost 1:1 when [14C]serine was used as a precursor and chased for 30 min and also 18 h.

In conclusion, this study has clearly demonstrated the biological role of GlcT-1 in regulation of the intracellular ceramide content; i.e., genetic and pharmacological blockade of GlcT-1 results in accumulation of ceramide and inhibition of DNA synthesis after SMase treatment in B16 melanoma cells.

**DISCUSSION**

Recently, Zhang et al. (27) reported the expression of a recombinant *B. cereus* SMase in Molt-4 leukemia cells. After the transfection of the gene and stable expression of the SMase, intracellular ceramide content increased, resulting in induction of apoptosis. However, exogenously added *B. cereus* SMase, despite causing a greater elevation of ceramide level, did not induce apoptosis in Molt-4 cells (27). This result suggested the existence of two distinct SM pools, one of which is accessible by endogenous SMase and the other by exogenous SMase. The former seems to be responsible for transduction of the apoptotic signal, and the turnovers of these two pools might be somewhat different. On the other hand, some reports indicated that exogenous bacterial SMase had biological effects on cells. For example, the *Streptomyces* SMase enhanced the action of subthreshold vitamin D₃ in inducing HL60 cell differentiation (28), and the enzyme from *S. aureus* induced apoptosis of U937 cells (29). In the present study, treatment of B16 cells with bacterial SMase inhibited DNA synthesis under conditions of genetic or pharmacological blockade of GlcT-1. These results suggest that the localization and topology of SM and their susceptibility to SMase differ according to cell type.

Luberto and Hannun (30) reported that treatment of human lung fibroblast WI38 cells with bacterial SMase resulted in a decrease in SM level and concomitant generation of ceramide. This ceramide level decreased very slowly in the cells, but there was little restoration of SM content. In contrast, SV40-transformed cells, in which the activity of SM synthase (phosphatidylcholine-specific phospholipase C) was found to be 3-fold higher than that in parental cells, cleared ceramide much more rapidly and regenerated SM. The authors argued the potential significance of SM synthase for regulation of intracellular ceramide levels in the fibroblasts. We showed, on the other hand, that in B16 melanoma cells the ceramides generated from SM as well as GSLs (13) by microbial enzymes were primarily glucosylated by GlcT-1 and metabolized to GM3. This discrepancy may be attributable to the balance between GlcT-1 and SM synthase activities in cells, which is genetically defined depending on the origin of cells or their phenotype and might be affected by other environmental factors.

The present study revealed that ceramide generated on the
outer leaflet of the plasma membrane by bacterial SMase was directly, but not via the sphingoid-base salvage pathway (31), metabolized to GSLs in B16 melanoma cells. Because the catalytic domain of GlcT-1 is located on the cytosolic side of the Golgi membrane (32), the generated ceramide must be transported to the outer leaflet of the Golgi membrane before it becomes accessible to the enzyme. Although the transport of ceramide to the Golgi membrane remains unclear, our findings suggest that the transport of ceramide in protein-directed (33) and vesicle-independent manners (34) is significant.

Many pathogenic and opportunistic microbes produce SMases, some of which have been identified as hemolysins and cytotoxins (35). These observations indicate that cell surface SM of vertebrates might be exposed to the action of microbial SMase, which may result in the elevation of the intracellular ceramide level. Because the excess generation of ceramide must be toxic for the cell, the exclusion of ceramide from the cell by glycosylation can be regarded as a mechanism of defense against infection by SMase-producing pathogens. It is interesting to note that many opportunistic pathogens can also produce endoglycoceramidase extracellularly (13).

Lavie et al. (36, 37) reported that multidrug-resistant human breast cancer cells exhibited marked accumulation of glucosylceramide compared with the parental cells. The reverse multidrug resistance drug tamoxifen was found to inhibit GlcT-1, resulting in a decrease in the level of glucosylceramide and an increase in that of ceramide. This drug as well as 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol, an inhibitor of GlcT-1, sensitized the multidrug-resistant cells to some anticancer drugs. These results suggested that GlcT-1 is involved in regulation of ceramide levels, which may affect the sensitivity of cancer cells to anticancer drugs.

We conclude that GlcT-1, distributed ubiquitously in vertebrate cells, functions to regulate the level of intracellular ceramide by glycosylation of the ceramide when it is present in excess.

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