Glycoconjugate Journal 20, 157–168, 2004

Apoptosis of human carcinoma cells in the presence of inhibitors of glycosphingolipid biosynthesis: I. Treatment of Colo-205 and SKBR3 cells with isomers of PDMP and PPMP

Subhash Basu[1], Rui Ma[1], Brian Mikulla[1], Mathew Bradley[1], Christopher Moulton[1], Manju Basu[1], Sipra Banerjee[2] and Jin-ichi Inokuchi[3]

[1] *Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA,* [2] *Department of Cancer Biology, Cleveland Clinic Foundation, Cleveland, OH 44129, USA,* [3] *Department of Biomembranes, Hokkaido University, Sapporo 060-0812, Japan*

Apoptosis, or programmed cell death, plays an important role in many physiological and diseased conditions. Induction of apoptosis in cancer cells by anti-cancer drugs and biosynthetic inhibitors of cells surface glycolipids in the human colon carcinoma cells (Colo-205) are of interest in recent years. In our present studies, we have employed different stereoisomers of PPMP and PDMP (inhibit GlcT-glycosyltransferase (GlcT-GLT)) to initiate apoptosis in Colo-205 cells grown in culture in the presence of ^{3}H-TdR and ^{3}H/or ^{14}C-L-Serine. Our analysis showed that the above reagents (between 1 to 20 μM) initiated apoptosis with induction of Caspase-3 activities and phenotypic morphological changes in a dose-dependent manner. We have observed an increase of radioactive ceramide formation in the presence of a low concentration (1–4 μM) of these reagents in these cell lines. However, high concentrations (4–20 μM) inhibited incorporation of radioactive serine in the higher glycolipids. Colo-205 cells were treated with L-threo-PPMP (0–20 μM) and activities of different GSL: GLTs were estimated in total Golgi-pellets. The cells contained high activity of GalT-4 (UDP-Gal: LcOse3Cer β1-4galactosyltransferase), whereas negligible activity of GalT-3 (UDP-Gal: GM2 β1-3galactosyltransferase) or GM2-synthase activity of the ganglioside pathway was detected. Previously, GLTs involved in the biosynthetic pathway of SA-Le^{x} formation had been detected in these colon carcinoma (or Colo-205) cells (Basu M *et al. Glycobiology* 1, 527–35 (1991)). However, during progression of apoptosis in Colo-205 cells with increasing concentrations of L-PPMP, the GalT-4 activity was decreased significantly. These changes in the specific activity of GalT-4 in the total Golgi-membranes could be the resultant of decreased gene expression of the enzyme.
Published in 2004.

Introduction

Trillions of cells of the normal healthy body live in a complex, interdependent atmosphere, regulating another's proliferation, formation, and death. Normal cells reproduce only when introduced to do so by other cells in their activity. Such collaboration ensures each tissue to maintain its size, differentiation and structure formation until death (Figure 1). Apoptosis occurs when cells have sufficient time to organize and participate in their own demise [1]. It is the cellular process where death occurs in a selective and developmentally regulated process. The process is involved in embryological molding, normal cell turnover, immune regulation, and hormone-dependent atrophy [2]. Tumor or cancer cell death can be triggered by necrosis or apoptosis induced by anti-cancer drugs [3–9]. A potent synthetic anti-estrogenic agent, Tamoxifen [citrate or 4-hydroxy-(z)-), induces apoptosis in human malignant glioma cell lines [6,7] and prostate cancer [8,9] cells by indication of p21 protein. Programmed cancer cell death (PCCD) results from signals generated within the cells and has been the subject of intensive investigation in recent years. Pathways of apoptotic signals are being investigated in recent years [10–21]. However, during chemotherapy [22], how a specific anti-cancer drug induces apoptosis in a specific cancer cell is known very little and needs to be explored carefully.

To whom correspondence should be addressed: Dr. Subhash Basu, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA. Tel: 574-631-5759; Fax: 574-631-7520; E-mail: sbasu@nd.edu

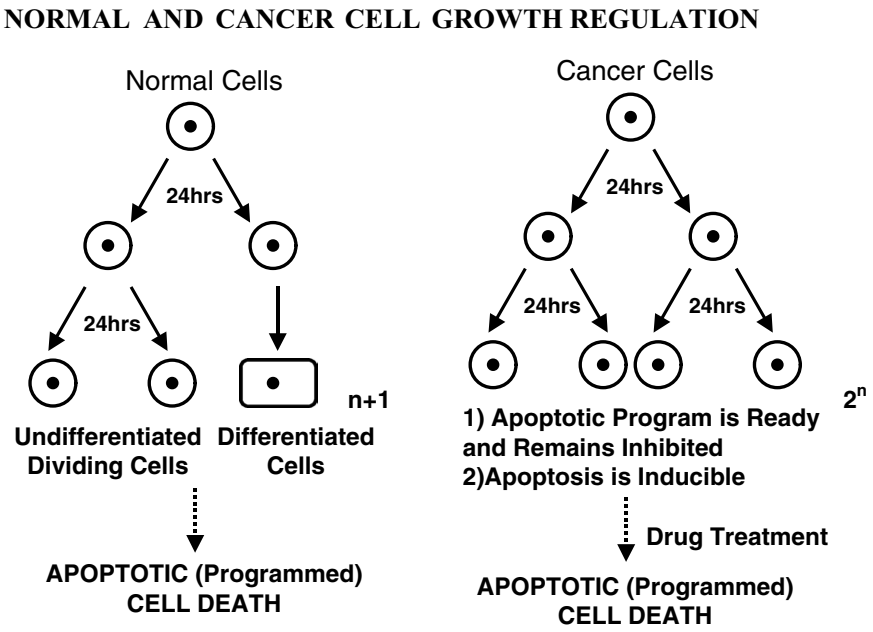


Figure 1. Normal, tumor, and cancer cell growth regulation.

The presence of sphingolipids: sphingomyelin (Ceramide-phosphoryl choline) [23–27] and glycosphingolipids (oligo-glycosylceramides) [28–32] on the outer leaflet of both normal and cancer cells has been reported. Until recently, very little is known about their functional roles in cell adhesion [33], in metastasis [34] or during the apoptotic process [35–37]. The role of ceramide [38–52] and disialosyl-gangliosides [53–62] has been suggested as apoptotic reagents in normal and cancer cells. On the other hand, SA-Le^x(SAα2,3Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ1,4-Glc-Ceramide) and SA-Le^a(SAα2,3Galβ1,3(Fucα1,4)GlcNAcβ1,3-Galβ1,4Glc-Ceramide) have been suggested as markers for certain metastatic carcinoma cells [63–69]. Biosyntheses *in vitro* of SA-Le^X and related GSLs have been studied in recent years [70–78] in metastatic colon carcinoma cells (Figure 2).

Ceramide is a transmembrane sphingolipid composed of an *N*-acylated (C_{14}-C_{26}-fatty acids) sphingosine (C_{18}-erythro-4, 5-trans unsaturated amino alcohol) in most of the eukaryotic

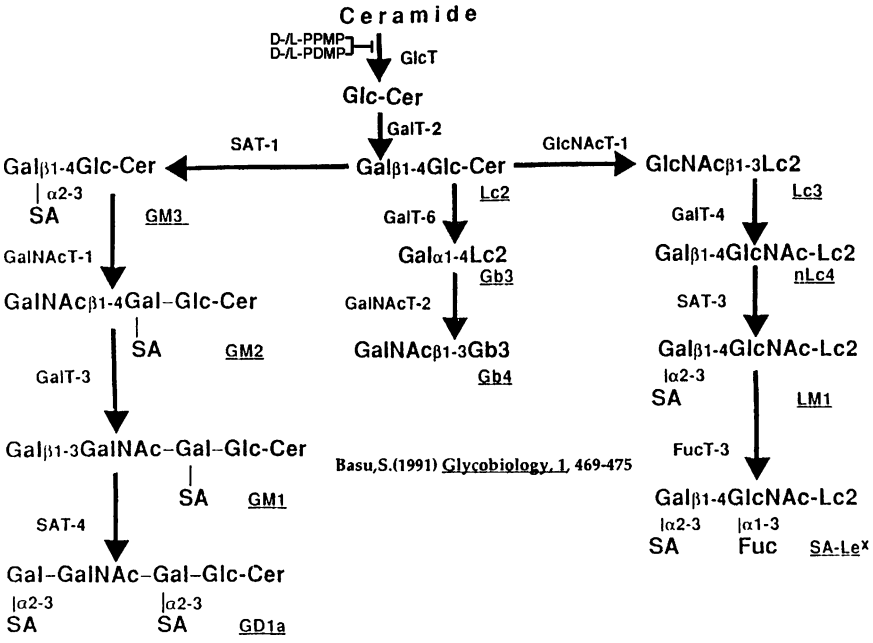
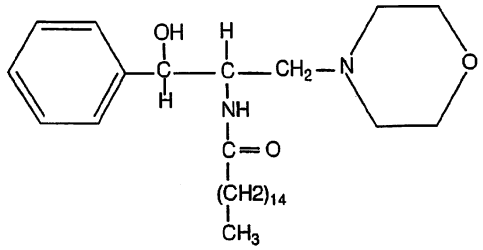


Figure 2. Biosynthetic pathways for gangliosides, globooside and SA-Le^X.

animal cells. In yeast cells [79,80] and squid cells [81], polyunsaturated sphingosine has been reported. Ceramides containing phyto-sphingosines are quite common in plant cells [82–84]. Hydrolysis of sphingomyelin (SphM) to generate ceramide in HL-60 human leukemia cells in response to the action of 1,25-dihydroxyvitamin D_3 led to the suggestion that sphingolipid metabolism is regulated in response to extracellular agents [25,26]. Ceramide-induced cell death is also correlated with DNA-fragmentation in the apoptotic programmed cell death [27]. Several comprehensive reviews have been published recently on the role of exogenously added ceramide in the apoptosis of cultured carcinoma cells [38–42].

In spite of almost 1100 papers published on this subject, very little is known on the generation of ceramide during apoptosis by anti-cancer drugs in the cancer cells. Our present goal [85–88] is to correlate the changes in the expression of cancer cell surface glycosphingolipids with apoptosis induced by anti-cancer drugs, inhibitors of glycosphingolipid biosynthesis (L-/D-PPMP; L-/D-PDMP) and disialosylgangliosides (GD3 and GD1b). The stepwise biosynthesis *in vitro* of SA-Le^x in human carcinoma, Colo-205 cells has been achieved in our laboratory during the last two decades. Individual steps for its biosynthesis from ceramide and the enzymes catalyzing the reactions (Figure 1) have been characterized in our laboratory [70–76,89–91] and in the other laboratories also [77,78]. The GalT-4 (UDP-Gal: LcOsc3Cer β1,4galactosyltransferase) [75,76,90,92–96] and the SAT-3 (CMP-NeuAc: α2,3sialyltransferase) [70,71,97–99] have also been cloned from Colo-205 cells [73,74]. Formation of radioactive ceramide during induction of apoptosis using inhibitors (L-/D-PPMP; L-/D-PDMP) of GlcT (UDP-Glc: Ceramide β1,1glucosyltransferase) [89,100,101] and some added disialosyl- gangliosides (GD3 or GD1b) in these Colo-205 cells have been studied. The DNA-laddering [102,103] caspase-3 activity [104–106] and outer-leaflet dye-binding [107] assays were used to monitor apoptosis.



D-/L- PPMP

(1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol)

Figure 3. Structure of D-/L-PPMP (1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol).

Materials and methods

Materials

Colo-205 colonic cancer cell line was purchased from ATCC and SKBR3 breast cancer cell line was a gift from Dr. Sipra Banerjee of Cleveland Clinic, Cleveland, OH. Cell culture medium powder RPMI-1640 and DMEM were from Gibco/Invitrogen Corporation (Carlsbad, CA). Penicillin, streptomycin, and L-glutamine were from Gibco. Fetal bovine serum was purchased from Intergen (Purchase, NY) and Gibco. ^{3}H-L-serine and ^{14}C-L-Serine was from American Radiochemical (St. Louis, MO) and Moravek Biochemicals (Brea, CA), respectively. L-PPMP, L-PDMP, and D-PDMP were obtained as gift samples from Dr. J-i. Inokuchi of Hokkaido University, Japan. GF/A glass filters were from Fisher Scientific (Pittsburgh, PA). Pierce BCA Micro Protein Assay kit is from Pierce Biotechnology, Inc. (Rockford, IL). Rabbit anti-caspase3 polyclonal antibody was from BioMol Research Lab, Inc. (Plymouth Meeting, PA). Goat anti-rabbit IgG antibody–ALP (alkaline phosphatase) conjugate, NBT (nitro blue tetrazolium)/BCIP (5-bromo-4-chloro-3-indolyl-phosphate) ALP developing dye, and all other regular reagents were from Sigma (St. Louis).

Cell culture

Human colonic cancer cell line Colo-205 and breast cancer cell line SKBR3 were grown in RPMI 1640 and DMEM media, respectively, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, and 50 mM L-glutamine. When cells were 90% confluent ($\sim 5 \times 10^6$ per 25 cm^2 T flask) they were used for passage or harvested for biochemical work. Treating the semi-confluent cells twice with 0.5 mM hydroxyurea for 24 h under the same culturing condition performed the cell-synchronization. Hydroxyurea was removed, and cells were treated with different apoptotic reagents, L-PPMP, L- and D-PDMP (Figure 3), and 0.5 μCi ^{3}H-L-serine or ^{14}C-L-Serine per 25 cm^2 T flask under the following indicated conditions.

Harvest of cells

After incubation, cells were scraped off and transferred into 12 ml conical tubes followed by 2 times of phosphate-buffered saline (PBS) wash. Finally all the cells were suspended in 5 ml PBS and cell counts were performed. These cells were used for further analysis of incorporation of radioactivity of L-serine in radioactive total sphingolipid, glycosphingolipids, and ceramide following protocol as indicated in Figure 4. GF/A Filtering Assay–0.5 ml suspending cells were loaded onto a GF/A glass filter, which had been treated with 50 mM sodium pyrophosphate. Then the sample on each GF/A disc was washed twice with 5% TCA followed by 2 times chloroform/methanol (2:1) wash plus 2 times acetone wash, or 2 times acetone wash only. Each cell sample was repeated twice. After that, the GF/A discs were fully dried and counted in a toluene scintillation solution.

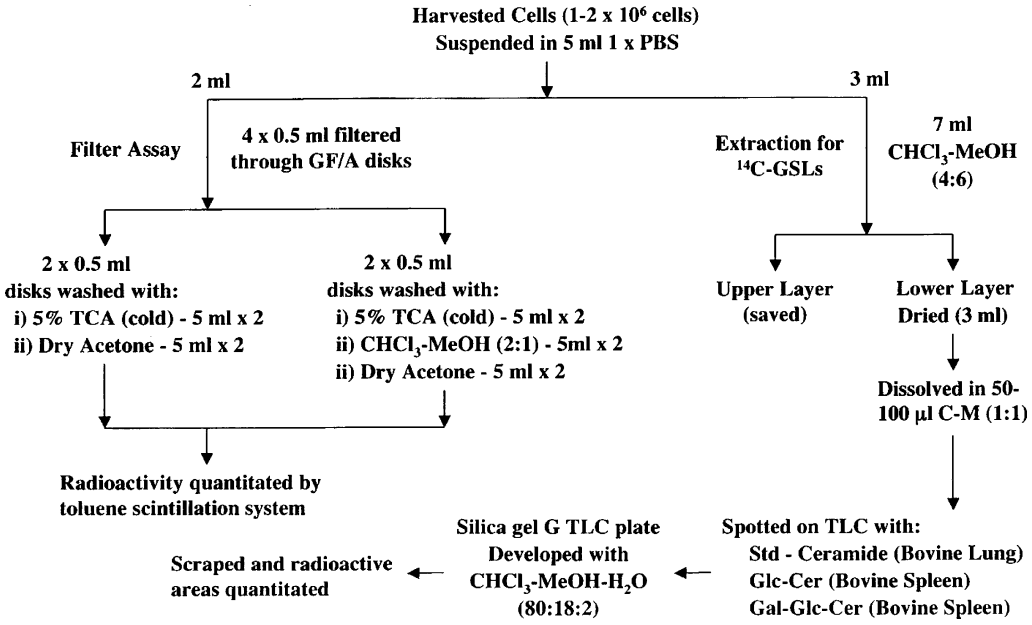


Figure 4. Analysis of L-^{14}C-serine incorporation in radioactive sphingolipids in Colo-205 cells.

Extraction and analysis of radioactive of glycosphingolipids

Cells (0.5 ml) were centrifuged and resuspended with 200 μl 0.1 M NaOH and 500 μl chloroform/methanol (2:1) and incubated at 37°C for 1 h. Then 50 μl of the upper layer or lower layer was spotted onto a 4 cm^2 3 mm Whatman paper and quantitated by a toluene scintillation system. For further analysis of radioactive glycosphingolipids and ceramide. Larger quantities of cells were extracted as above, concentrated and purified by silicic acid columns, and further analyzed by TLC plates as published previously [70–74,89–92,97].

Western blot for identification of activation of caspases

Cells (0.5 ml aliquots) were pelleted and resuspended with 100μl lysis buffer (62.5 mM Tris-HCl pH6.8, 2% w/v SDS, 10% glycerol, and 50 mM DTT) followed by homogenization with 3 × 10 sec sonication. Then the homogenized samples were incubated at 37°C for 1 h before 5 min of denaturation at 95°C and loading onto SDS-PAGE. The protein mixture in the amount of 20–25 μg was loaded for each sample and blotted to nitrocellulose membranes [106]. Nonspecific binding was blocked by incubation in Tris-buffered saline containing 5% bovine serum albumin, and 0.1% Tween 20 for 1 h at room temperature. The blots were then incubated overnight at 4°C in blocking buffer containing the primary antibody. Antibodies used were a rabbit polyclonal anti-caspase-3 antibody raised against full-length human caspase-3 diluted 1:1,000. Afterward, membranes were washed and incubated with anti-rabbit IgG-Alkaline phosphatase conjugate (1:3,000; Sigma). Antibody-Alkaline phosphatase activity was visualized using the NBT-BCIP reagent (Sigma) in AP buffer (100 mM Tris-HCl pH9.5, 100 mM NaCl, and 5 mM $MgCl_2$) [106].

DNA laddering analysis

Cells (0.5 ml) were pelleted and resuspended with 100 μl lysis buffer (20 mM Tris-HCl pH8.0, 20 mM NaCl, 20 mM EDTA, and 10% w/v SDS) followed by sonication for 3 × 10 sec. The homogenized samples were incubated at 37°C for 1 h before adding 400 μg deionized water. After that Phenol/chloroform (1:1), 500 μl, was added to the homogenate, vortexed, and placed in ice for 15 min. The samples were centrifuged at 4°C (5,000 × g) for 20 min. The upper layer was then transferred to a new tube and 500 μl of chloroform was added to it. The sample was vortexed and spun (5,000 × g) at 4°C . The upper layer was collected and mixed with 40 μl of 3M NaAc and 800 μl of absolute ethanol. All the samples were incubated at–20°C for 1 h, centrifuged at 4°C, and 10,000 × g for 30 min. The DNA pellet was washed with 70% ethanol and dissolved in 50 μl deionized sterile water before being loaded onto the 1% low-melting point (DNA-free) argrose-ethidium bromide gel and subjected to electrophoresis [102,103]. The laddering bands in the gel were visualized by UV (Figure 5).

Assay of β1,4GalT-4 (UDP-Gal: LcOse3Cer β-galactosyl-transferase)

Enzymatic assays for GalT-2, GalT-3 and GalT-4 were carried out as described previously [92], with some modifications to provide the optimum conditions for all those galactosyltransferase activities with Golgi membrane fractions isolated from human Colo-205 or SKBR3 cells (before and apoptosis). The incubation mixtures contained the following components in a final incubation volume of 35 μL; acceptor glycolipids, 1.2 mM; HEPES buffer (pH 6.8), 10 mM containing 10 mM $MnCl_2$; total Golgi membrane fraction, 20 μL-containing 100–200 μg

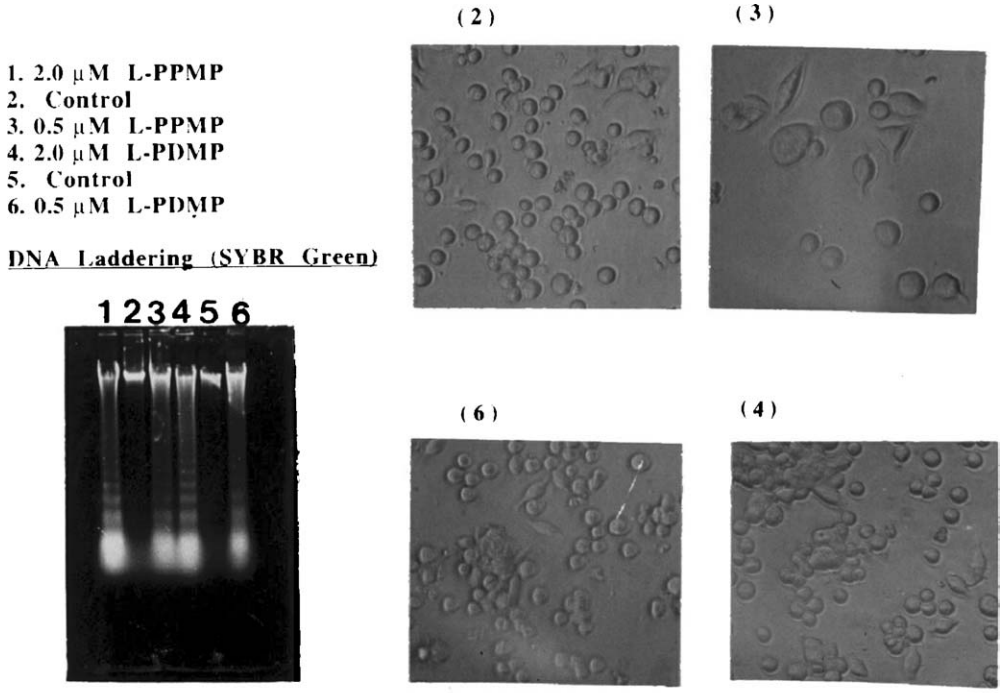


Figure 5. Phase contrast microscopic pictures of apoptotic colon carcinoma (Colo-205) cells with L-PPMP/L-PDMP; DNA-laddering analysis of treated cells by 1% agarose gel electrophoresis.

protein (estimated by Peterson *et al.* [108]); UDP- [^{3}H] galactose (30,000 to 50,000 cpm of specific activity 1.5 to 2 $\times$ 10^6 cpm per micromole) Triton X-100 at a ratio of 1:6 (protein to: detergent) was used in mixed micelle form during drying of the substrate glycolipids dissolved in chloroform-methanol (2:1). Incubations were carried at 37° between 1 to 2 h and final results were obtained in the range where the reaction rate remained constant within the incubation time and was proportional to the added protein concentrations. The reactions were stopped with 25 mM EDTA and 20 μL chloroform-Methanol (1:1). The whole mixture was quantitatively transferred on Whatman 3 MM or 1 MM chromatographic paper followed by descending chromatography with 1% sodium tetraborate (pH 9.1). The appropriate areas of each chromatogram were cut and the amounts of radioactive galactose transferred to the potential glycolipids were determined by a toluene scintillation system. For identification of the products, the radioactivity was eluted from the papers by chloroform-Methanol-water (60:35:8) and further analyzed by TLC using proper standards and solvent systems as described previously [89–97,109].

Results

DNA laddering analysis with the apoptotic carcinoma cells initiated by L-PPMP

Treatment of human colon carcinoma (Colo-205) (Figure 5) and breast (SKBR3) (Figure 6) cells with varying concentrations (0.5 to 20 μM) L-/D-PPMP and D-PDMP led to observed cell blebbing and DNA condensation, while the control cells remained uniformly round with no damaged DNA negative staining from Propidium iodide [86] (will be published in the 3rd issue of the series in this journal). The DNA laddering resulted in altering UV fluorescent bright and dark bands, and increased concentration of L-PPMP (Figure 3) led to brighter banding, indicating a greater degree of systematic DNA fragmentation. Similar results were observed with the other isomers of D-PPMP or -PDMP (data not shown here). The observed blebbing of both the Colo-205 and the SKBR3 cells with these isomers of GSL: GlcT (glucosyltransferase) inhibitors (L-/D-PPMP or L-/D-PDMP) suggested that these inhibitors were inducing apoptosis in these carcinoma cells after 6 h and damaging DNA between 24 and 48 h after treatment. Fluorescent dye-binding pictures will be published ([86]; in the third issue of this series by Ma *et al.*)

Incorporation of ^{14}C-Serine in ceramide in apoptotic carcinoma cells

Synchronized Colo-205 cells were treated with L-PPMP (Figure 7(a)), L-PDMP (Figure 7(b)) and SKBR3 cells with L-PPMP (Figure 7(c)) in the presence of uniformly labeled L-^{14}C-Serine (Figure 8). Incorporation of radioactivity in total sphingolipids (Figures 7(a), (b) and (d)) and ceramide (Figure 7(c)) were estimated in live cells as well as in total cells and were determined and quantitated per 10^6 cells. The extraction procedure followed is explained on the chart (Figure 4). Once the cells were harvested and suspended in 1 $\times$ PBS, part

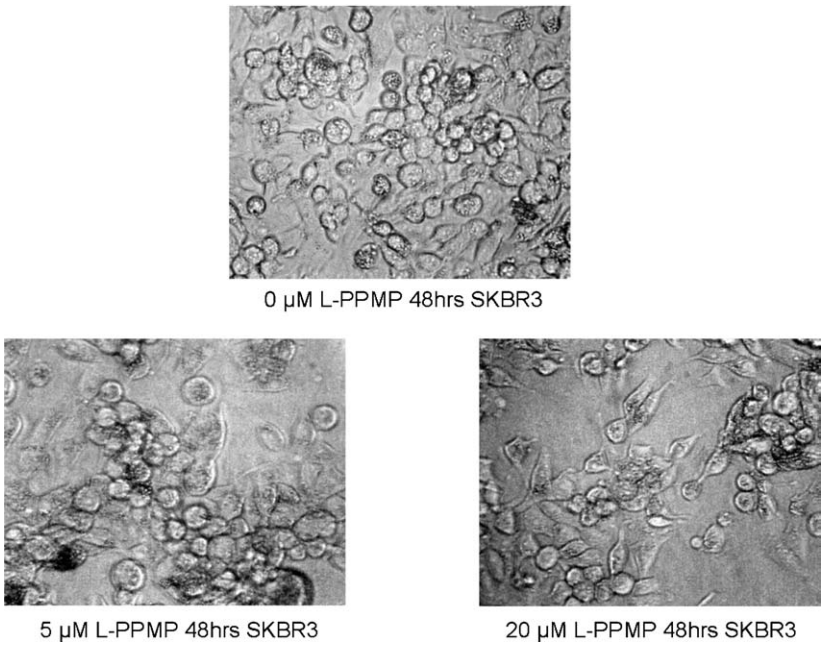


Figure 6. Phase contrast microscopic pictures of apoptotic breast carcinoma (SKBR3) cells treated with L-PPMP.

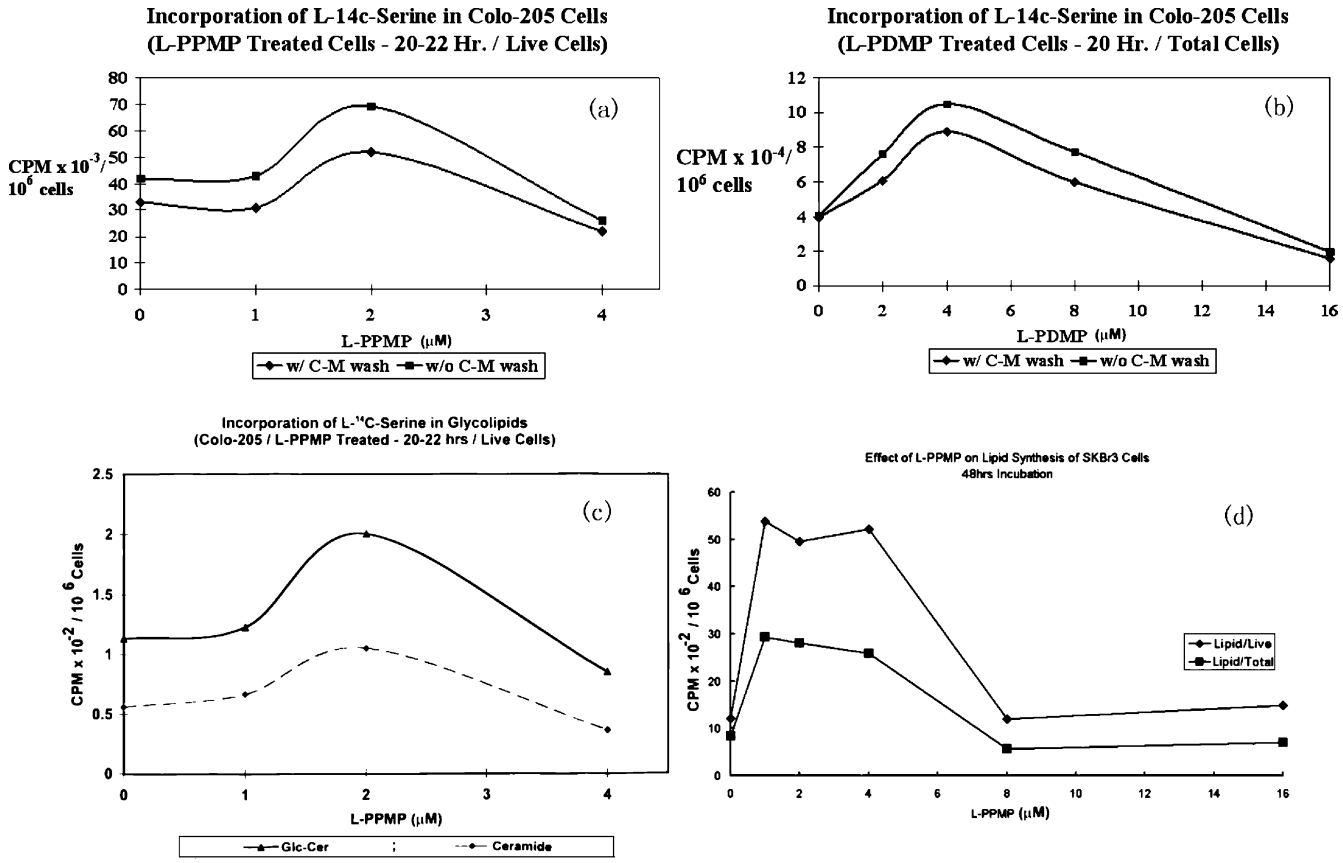


Figure 7. Incorporation of L-^{14}C-Serine in Colo-205 and SKBR3 Cells: (a) Colo-205 cells (live) after 22 h treatment with indicated amount of L-PPMP; (b) Colo-205 cells (total) after 22 h treatment with indicated amount of L-PPMP; (c) incorporation of radioactivity in radioactive ceramide after 22 h treatment of synchronized Colo-205 cells (live only); (d) incorporation of radioactive serine in total sphingolipids in SKBR3 cells after 22 h treatment with indicated amount of L-PPMP. Each point is average of data obtained from four sets of experiments.

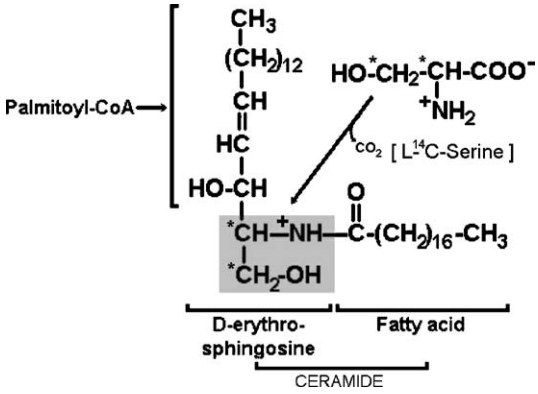


Figure 8. ^{14}C-carbons and nitrogen atoms incorporated from L-[U-^{14}C] serine into the ceramide moiety of the sphingolipids.

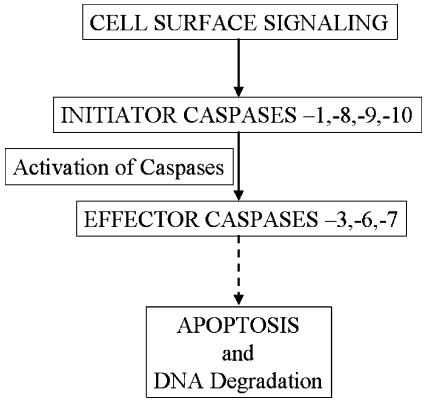


Figure 9. Proposed interaction between different caspases during apoptosis.

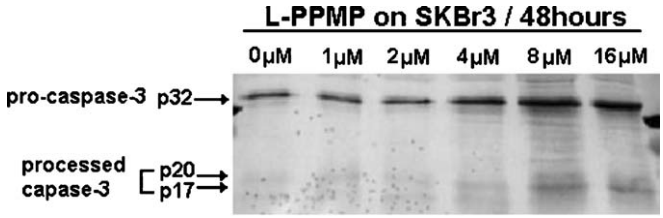


Figure 10. Western-blot identification of Caspase-3 activation by indicated amount of L-PPMP.

of this suspension was used for the filter assay as explained in the method section. In this assay, 4 samples were filtered through GF/A glass fiber filter discs. The first two were washed with cold 10% TCA, followed by washings with water and acetone. The other two were washed with cold 10% TCA, followed by washes with Chloroform-Methanol (1:1) and then with water and acetone. The radioactivity on these dried disks were quantitated with a toluene scintillation sytem. The rest of the harvested cells were then used for extraction of ^{14}C-GSLs by extraction with 5–10 ml of chloroform: Methanol (2:1) and 0.2 volume of 0.1 N NaOH. The upper layer and the lower layers were separated and dried before further analysis by TLC using specific solvent systems. Incorporation of radioactivity in ceramide was quantitated by TLC using the solvent system chloroform-Methanol-water (80:18:2)). Migration of radioactivity in standard ceramide or GSLs areas were scraped and quantitated by the toluene liquid scintillation system. Maximum incorporation was observed with the inhibitor concentrations between 2 and 4 micromolar. As expected, inhibition of incorporation was observed in higher concentrations of the inhibitors (data not shown). However, inhibitions of incorporation in the radioactive ceramide at higher concentrations of L/D-PPMP or L-/D-PDMP are not well understood as yet (Figure 7(c)).

Westernblot analysis of Caspase-3 activation in apoptotic carcinoma cells by GSL biosynthesis inhibitors (L-/D-PPMP)

Caspases make up part of the cascade that a cell follows in order to undergo apoptosis. Caspase-3 is one of the effector Caspases (Figure 9). When activated it cleaves proteins by recognizing the amino acid sequence DEVD. Upon recognition of the target proteins, the nucleus is broken down, starting with the disassembly of the chromosomes (Figure 10). We have studied extensively the Caspase-3 activation in the presence of all the isomers of the inhibitor of GlcT (UDP-Glc: ceramide β1,1 glucosyltransferase [100,101]). A typical activation profile of Caspase-3 activation as evidenced in breast cancer SKBR3 cells in the presence of L-PPMP (1 to 16 mM) is shown in Figure 11. Appearance of both p17 and p20 peptide fragments from the pro-caspase-3 p32 were observed when tested by Westernblot analysis (see Method section). These results suggested the involvement of Caspase-3 activation during apoptosis of the human carcinoma cells from breast (SKBR3) and colon (Colo-205) ([86]; this will be published in the next issue of this journal).

Modulation of β1,4GalT-4 activity in apoptotic carcinoma cells induced by L-PPMP

In vitro biosynthetic steps of Sialo-Le^X in Colo-205 cells have been established in this laboratory previously [70–76]. Very little is known about the biological roles of SA-Le^X in metastasis and apoptosis of human carcinoma cells. A systematic study on the activities of different GSL:glycosyltransferases involved in the biosynthesis of SA-Le^X (Figure 2) has begun in our laboratory. Previously, we have observed that the β1,4GalT-4 activity is overexpressed in several tumors in rats induced by adenocarcinoma cells [110]. Presently we have tested the activity of β1,4GalT-4 (UDP-Gal: LcOse3Cer β1,4Galactosyltransferase) in apoptotic SKBR3 cells treated with L-PPMP (5 to 20 μM). It appeared (Table 1) that the β1,4GalT-4 activity was lowered by 35% when L-PPMP was added at a concentration of 20.0 μM. A gradual reduction of the enzyme activity was observed between 5 and 20 μM concentration (Table 1). It is concluded that the inhibition is not due to the presence of any L-PPMP in the incubation mixture. The Golgi membranes were isolated from the L-PPMP- treated cells before being tested for GalT-4 activity (UDP-Gal:LcOse3Cerβ1,4Galactosyl-transferase). Perhaps this is due to the lowering of the total expression of the enzyme

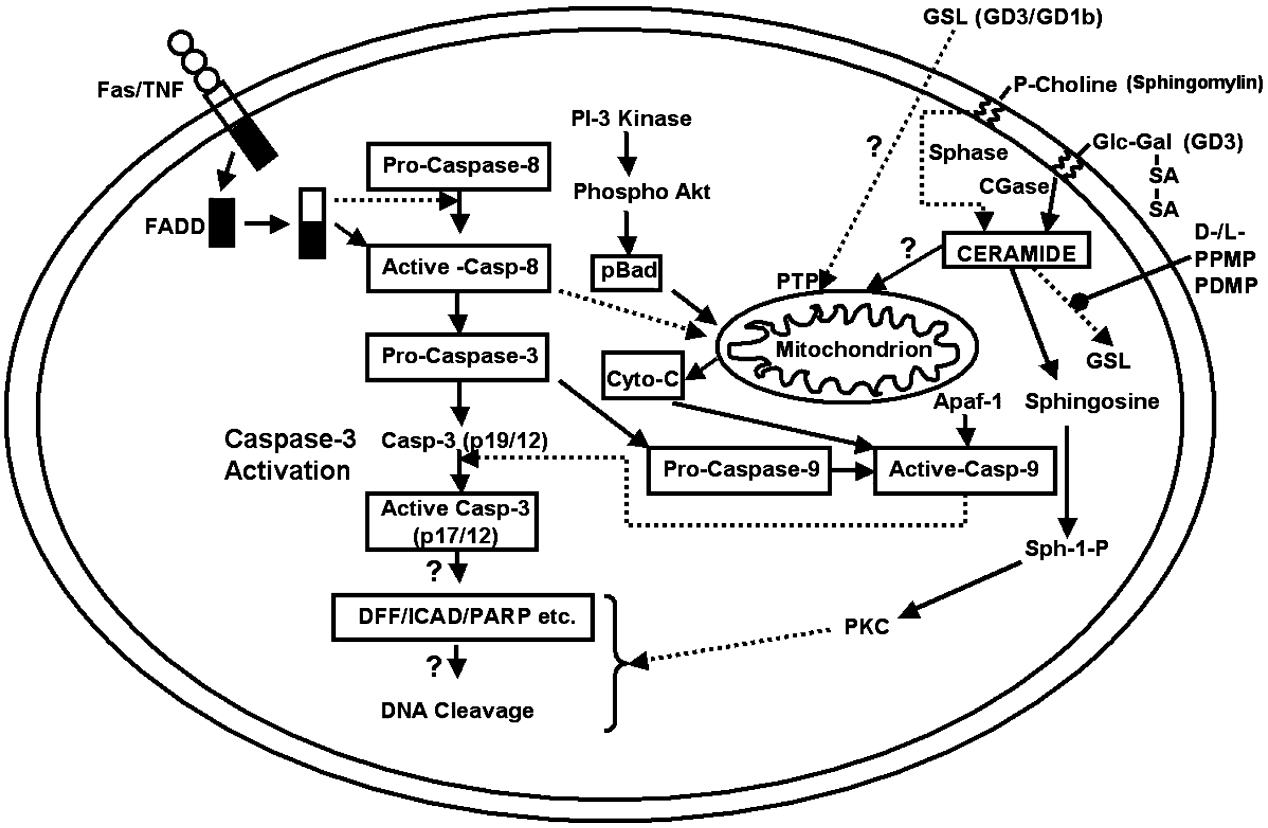


Figure 11. Activation cascade of Caspase-3 by Cytochome-c pathway induced by ceramide or inhibitors of the glycosphingolipids syntheses (L-PPMP).

or its post-translational regulation or degradation. Quantitative expression of GalT-4 mRNA [111] in the total mRNA is under study using a cDNA clone isolated from human Colo-205 in this laboratory [95,96].

Discussion

High expression of SA-Le^x, SA-Le^a, and Le^X type epitopes promote invasion metastasis of colon [32,62,66] and breast carcinoma cells [68]. Expression of O-linked epitopes in Le^X, SA-Le^X, or SA-Le^a on the cell surfaces is the resultant expression of glycosyltransferase genes [73–76,112]. However, little is known about their regulation during cancer cell invasiveness.

Table 1. Decrease in β1,4GalT-4 (UDP-Gal: LcOse3Cer β1,4-Galactosyltransferase) in SKBR3 cells treated with L-PPMP for 48 h

(L-Thero-PPMP) Cell treatment	*% Activity*	
	Total	*Net change*
Control (none)	100	–
5.0 μM	93.5	6.5 ± 0.4
20.0 μM	64.5	35.5 ± 4.0

Our present studies on the apoptosis initiation in colon Colo-205 and breast SKBR3 cells in the presence of L-/D-PPMP or L-/D-PDMP clearly indicated that the death-programming is initiated with the inhibition of GSL-biosynthesis and concomitant increase of ceramide concentration in these cell lines.

It has been shown recently [113] that ganglioside change is caused by enhanced expression of ganglioside-specific sialidase in colorectal cancer. Resulting lactosylceramide (Galβ1,4Glcβ1,1Ceramide) is claimed to inhibit apoptosis, mainly through increased Bcl-2 and decreased Caspase expressions [32,113]. How lactosylceramide is signaling to enhance Bcl-2 is not known.

There have been many studies on the effect of gangliosides on various growth factor receptor tyrosine kinases [114]. If it is believed that colon and breast carcinoma cell metastasis are dependent on the higher oligosaccharide-containing lacto-series glycosphingolipids (such as Le^X, SA-Le^X or SA-Le^{a}type GSLs), then changes in their biosynthesis may cause apoptosis of these cells or an anti-metastatic effect.

Apoptosis is associated with Le^Y expression in various types of cancer cells [115]. It is also observed that GD3 (disialosyl-lactosyl-ceramide; NeuAcα2,8NeuAcα2,3Galβ1,4Glcβ1,1-Ceramide) may affect some yet unknown process to enhance tumor growth, invasiveness, and metastasis [116]. Addition of GD3 or GD1b to both Colo-205 or SKBR3 cells caused

apoptosis in these cells within 6 h and initiated Caspase-3 activation within 16 to 24 h [86,87]; a complete study will be published in later issues of this journal). Our present observation on the decrease of β1, 4GalT-4 expression in SKBR3 cells after treatment with the GSL biosynthesis inhibitor may suggest a regulation of these pathways during cancer cell metastasis or apoptosis induced by cancer drugs [87] (a complete paper will be published in the "Glycoconjugates and Drugs" No. 1 issue of this journal).

Acknowledgments

This article was written based on research supported by grants NS-18005 (NIH), CA-14764 (NCI) and a grant-in-aid from the Bayer Corporation, Elkhart, IN to SB. Our special thanks to Mrs. Dorisanne Nielsen for her help in the preparation of the final draft of the manuscript.

References

1 Wyllie AH, Apoptosis, cell death in tissue regulation, *J Pathol* **153**, 313–6 (1987).
2 Arends MJ, Wyllie AH, Apoptosis: Mechanisms and roles in pathology, *Int Rev Exp Pathol* **32**, 223–54 (1991).
3 Pisha E, Chai H, Lee IS, Chagwedera TE, Farnsworth NR, Cordell GA, Beecher CW, Fong HH, Kinghorn AD, Brown DM, *et al.*, Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis, *Nat Med* **1**, 1046–51 (1995).
4 Ritke MK, Rusnak JM, Lazo JS, Allan WP, Dive C, Heer S, Yalowich JC, Differential induction of etoposide-mediated apoptosis in human leukemia HL-60 and K562 cells, *Mol Pharmacol* **46**, 605–11 (1994).
5 Schmidt ML, Kuzmanoff KL, Ling-Indeck L, Pezzuto JM, Betulinic acid induces apoptosis in human neuroblastoma cell lines, *Eur J Cancer* **33**, 2007–10 (1997).
6 Couldwell WT, Hinton DR, He S, Chen TC, Sebat I, Weiss MH, Law RE, Protein kinase C inhibitors induce apoptosis in human malignant glioma cell lines, *FEBS Lett* **345**, 43–6 (1994).
7 Issandou M, Faucher C, Bayard F, Darbon JM, Opposite effects of tamoxifen on *in vitro* protein kinase C activity and endogenous protein phosphorylation in intact MCF-7 cells, *Cancer Res* **50**, 5845–50 (1990).
8 Rohlff C, Blagosklonny MV, Kyle E, Kesari A, Kim IY, Zelner DJ, Hakim F, Trepel J, Bergan RC, Prostate cancer cell growth inhibition by tamoxifen is associated with inhibition of protein kinase C and induction of p21(waf1/cip1), *Prostate* **37**, 51–9 (1998).
9 Gundimeda U, Chen ZH, Gopalakrishna R, Tamoxifen modulates protein kinase C via oxidative stress in estrogen receptor-negative breast cancer cells, *J Biol Chem* **271**, 13504–14 (1996).
10 Wajant H, The Fas signaling pathway: More than a paradigm, *Science* **296**, 1635–6 (2002).
11 Neves SR, Ram PT, Iyengar R, G protein pathways, *Science* **296**, 1636–9 (2002).
12 Singer AL, Koretzky GA, Control of T cell function by positive and negative regulators, *Science* **296**, 1639–40 (2002).
13 Gauld SB, Dal Porto JM, Cambier JC, B cell antigen receptor signaling: Roles in cell development and disease, *Science* **296**, 1641–2 (2002).
14 Moon RT, Bowerman B, Boutros M, Perrimon N, The promise and perils of Wnt signaling through beta-catenin, *Science* **296**, 1644–6 (2002).
15 Attisano L, Wrana JL, Signal transduction by the TGF-beta superfamily, *Science* **296**, 1646–7 (2002).
16 Vaudry D, Stork PJ, Lazarovici P, Eiden LE, Signaling pathways for PC12 cell differentiation: Making the right connections, *Science* **296**, 1648–9 (2002).
17 Cantley LC, The phosphoinositide 3-kinase pathway, *Science* **296**, 1655–7 (2002).
18 Parent CA, Devreotes PN, A cell's sense of direction, *Science* **284**, 765–70 (1999).
19 Artavanis-Tsakonas S, Rand MD, Lake RJ, Notch signaling: Cell fate control and signal integration in development, *Science* **284**, 770–6 (1999).
20 Burgering BM, Kops GJ, Cell cycle and death control: Long live Forkheads, *Trends Biochem Sci* **27**, 352–60 (2002).
21 Ashkenazi A, Dixit VM, Death receptors, signaling and modulation, *Science* **281**, 1305–8 (1998).
22 Devita VT, Hellman S, Rosenberg SA, *Biologic Therapy of Cancer*, 2nd edition (1995).
23 Kolesnick R, The therapeutic potential of modulating the ceramide/sphingomyelin pathway, *J Clin Invest* **110**, 3–8 (2002).
24 Kolesnick RN, Sphingomyelin and derivatives as cellular signals, *Prog Lipid Res* **30**, 1–38 (1991).
25 Okazaki T, Bell RM, Hannun YA, Sphingomyelin turnover induced by vitamin D3 in HL-60 cells. Role in cell differentiation, *J Biol Chem* **264**, 19076–80 (1989).
26 Okazaki T, Bielawska A, Bell RM, Hannun YA, Role of ceramide as a lipid mediator of 1 alpha,25-dihydroxyvitamin D3-induced HL-60 cell differentiation, *J Biol Chem* **265**, 15823–31 (1990).
27 Hannun YA, Luberto C, Argraves KM, Enzymes of sphingolipid metabolism: From modular to integrative signaling, *Biochemistry* **40**, 4893–903 (2001).
28 Hakomori S, Tumor-associated carbohydrate antigens, *Annu Rev Immunol* **2**, 103–26 (1984).
29 Fukushima K, Hirota M, Terasaki PI, Wakisaka A, Togashi H, Chia D, Suyama N, Fukushi Y, Nudelman E, Hakomori S, Characterization of sialosylated Lewisx as a new tumor-associated antigen, *Cancer Res* **44**, 5279–85 (1984).
30 Phillips ML, Nudelman E, Gaeta FC, Perez M, Singhal AK, Hakomori S, Paulson JC, ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x, *Science* **250**, 1130–32 (1990).
31 Hakomori S, Handa K, Glycosphingolipid-dependent cross-talk between glycosynapses interfacing tumor cells with their host cells: Essential basis to define tumor malignancy, *FEBS Lett* **531**, 88–92 (2002).
32 Hakomori S, Glycosylation defining cancer malignancy: New wine in an old bottle, *Proc Natl Acad Sci USA* **99**, 10231–3 (2002).
33 Ugorski M, Laskowska A, Sialyl Lewis(a): A tumor-associated carbohydrate antigen involved in adhesion and metastatic potential of cancer cells, *Acta Biochim Pol* **49**, 303–11 (2002).
34 Fuster MM, Brown JR, Wang L, Esko JD, A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells, *Cancer Res* **63**, 2775–81 (2003).

35 Bose R, Verheij M, Haimovitz-Friedman A, Scotto K, Fuks Z, Kolesnick R, Ceramide synthase mediates daunorubicin-induced apoptosis: An alternative mechanism for generating death signals, *Cell* **82**, 405–14 (1995).

36 Liao WC, Haimovitz-Friedman A, Persaud RS, McLoughlin M, Ehleiter D, Zhang N, Gatei M, Lavin M, Kolesnick R, Fuks Z, Ataxia telangiectasia-mutated gene product inhibits DNA damage-induced apoptosis via ceramide synthase, *J Biol Chem* **274**, 17908–17 (1999).

37 Cuvillier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind S, Spiegel S, Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate, *Nature* **381**, 800–3 (1996).

38 Pettus BJ, Chalfant CE, Hannun YA, Ceramide in apoptosis: An overview and current perspectives, *Biochim Biophys Acta* **1585**, 114–25 (2002).

39 Tilly JL, Kolesnick RN, Sphingolipids, apoptosis, cancer treatments and the ovary: Investigating a crime against female fertility, *Biochim Biophys Acta* **1585**, 135–8 (2002).

40 Cuvillier O, Sphingosine in apoptosis signaling, *Biochim Biophys Acta* **1585**, 153–62 (2002).

41 Radin NS, Killing tumours by ceramide-induced apoptosis: A critique of available drugs, *Biochem J* **371**, 243–56 (2003).

42 Bieberich E, Hu B, Silva J, MacKinnon S, Yu RK, Fillmore H, Broaddus WC, Ottenbrite RM, Synthesis and characterization of novel ceramide analogs for induction of apoptosis in human cancer cells, *Cancer Lett* **181**, 55–64 (2002).

43 Goswami R, Kilkus J, Scurlock B, Dawson G, CrmA protects against apoptosis and ceramide formation in PC12 cells, *Neurochem Res* **27**, 735–41 (2002).

44 Luberto C, Kraveka JM, Hannun YA, Ceramide regulation of apoptosis versus differentiation: A walk on a fine line. Lessons from neurobiology, *Neurochem Res* **27**, 609–17 (2002).

45 Toman RE, Movsesyan V, Murthy SK, Milstien S, Spiegel S, Faden AI, Ceramide-induced cell death in primary neuronal cultures: Upregulation of ceramide levels during neuronal apoptosis, *J Neurosci Res* **68**, 323–30 (2002).

46 Akao Y, Kusakabe S, Banno Y, Kito M, Nakagawa Y, Tamiya-Koizumi K, Hattori M, Sawada M, Hirabayasi Y, Ohishi N, Nozawa Y, Ceramide accumulation is independent of camptothecin-induced apoptosis in prostate cancer LNCaP cells, *Biochem Biophys Res Commun* **294**, 363–70 (2002).

47 el Bawab S, Mao C, Obeid LM, Hannun YA, Ceramidases in the regulation of ceramide levels and function, *Subcell Biochem* **36**, 187–205 (2002).

48 Jaffrezou JP, Laurent G, Levade T, Ceramide in regulation of apoptosis. Implication in multitoxicant resistance, *Subcell Biochem* **36**, 269–84 (2002).

49 Ahn EH, Schroeder JJ, Sphingoid bases and ceramide induce apoptosis in HT-29 and HCT-116 human colon cancer cells, *Exp Biol Med (Maywood)* **227**, 345–53 (2002).

50 Smyth MJ, Obeid LM, Hannun YA, Ceramide: A novel lipid mediator of apoptosis, *Adv Pharmacol* **41**, 133–54 (1997).

51 Hannun YA, Obeid LM, Mechanisms of ceramide-mediated apoptosis, *Adv Exp Med Biol* **407**, 145–9 (1997).

52 Kolesnick RN, Kronke M, Regulation of ceramide production and apoptosis, *Annu Rev Physiol* **60**, 643–65 (1998).

53 Colell A, Morales A, Fernandez-Checa JC, Garcia-Ruiz C, Ceramide generated by acidic sphingomyelinase contributes to tumor necrosis factor-alpha-mediated apoptosis in human colon HT-29 cells through glycosphingolipids formation. Possible role of ganglioside GD3, *FEBS Lett* **526**, 135–41 (2002).

54 Malisan F, Testi R, GD3 in cellular ageing and apoptosis, *Exp Gerontol* **37**, 1273–82 (2002).

55 Paris R, Morales A, Coll O, Sanchez-Reyes A, Garcia-Ruiz C, Fernandez-Checa JC, Ganglioside GD3 sensitizes human hepatoma cells to cancer therapy, *J Biol Chem* **277**, 49870–6 (2002).

56 Watanabe R, Ohyama C, Aoki H, Takahashi T, Satoh M, Saito S, Hoshi S, Ishii A, Saito M, Arai Y, Ganglioside GM3 overexpression induces apoptosis and reduces malignant potential in murine bladder cancer, *Cancer Res* **62**, 3850–4 (2002).

57 Simon BM, Malisan F, Testi R, Nicotera P, Leist M, Disialoganglioside GD3 is released by microglia and induces oligodendrocyte apoptosis, *Cell Death Differ* **9**, 758–67 (2002).

58 Copani A, Melchiorri D, Caricasole A, Martini F, Sale P, Carnevale R, Gradini R, Sortino MA, Lenti L, De Maria R, Nicoletti F, Beta-amyloid-induced synthesis of the ganglioside GD3 is a requisite for cell cycle reactivation and apoptosis in neurons, *J Neurosci* **22**, 3963–8 (2002).

59 Kristal BS, Brown AM, Apoptogenic ganglioside GD3 directly induces the mitochondrial permeability transition, *J Biol Chem* **274**, 23169–75 (1999).

60 Scorrano L, Petronilli V, Di Lisa F, Bernardi P, Commitment to apoptosis by GD3 ganglioside depends on opening of the mitochondrial permeability transition pore, *J Biol Chem* **274**, 22581–5 (1999).

61 Rippo MR, Malisan F, Ravagnan L, Tomassini B, Condo I, Costantini P, Susin SA, Rufini A, Todaro M, Kroemer G, Testi R, GD3 ganglioside directly targets mitochondria in a bcl-2-controlled fashion, *Faseb J* **14**, 2047–54 (2000).

62 Kristal BS, Brown AM, Ganglioside GD3, the mitochondrial permeability transition, and apoptosis, *Ann NY Acad Sci* **893**, 321–4 (1999).

63 Kannagi R, Carbohydrate-mediated cell adhesion involved in hematogenous metastasis of cancer, *Glycoconj J* **14**, 577–84 (1997).

64 Farmer RW, Richtsmeier WJ, Scher RL, Identification of sialyl Lewis-x in squamous cell carcinoma of the head and neck, *Head Neck* **20**, 726–31 (1998).

65 Ugorski M, Laskowska A, Sialyl lewis a: A tumor-associated carbohydrate antigen involved in adhesion and metastatic potential of cancer cells, *Acta Biochimica* **49**, 303–11 (2002)

66 Nakagoe T, Sawai T, Tsuji T, Jibiki MA, Nanashima A, Yamaguchi H, Yasutake T, Kurosaki N, Ayabe H, Arisawa K, Preoperative serum levels of sialyl Lewis(a), sialyl Lewis(x), and sialyl Tn antigens as prognostic markers after curative resection for colorectal cancer, *Cancer Detect Prev* **25**, 299–308 (2001).

67 Paganuzzi M, Bobbio B, Marroni P, Filiberti R, Secco GB, Grossi CE, Prognostic role of serum sialyl Lewisx (CD15s) in colorectal cancer, *Oncology* **65**, 52–9 (2003).

68 Renkonen J, Paavonen T, Renkonen R, Endothelial and epithelial expression of sialyl Lewis(x) and sialyl Lewis(a) in lesions of breast carcinoma, *Int J Cancer* **74**, 296–300 (1997).

69 O I, Otvos L, Kieber-Emmons T, Blaszczyk-Thurin M, Role of SA-Le(a) and E-selectin in metastasis assessed with peptide antagonist, *Peptides* **23**, 999–1010 (2002).

70 Basu M, Hawes JW, Li Z, Ghosh S, Khan FA, Zhang BJ, Basu S, Biosynthesis *in vitro* of SA-Le^x and SA-diLe^x by alpha 1-3

fucosyltransferases from colon carcinoma cells and embryonic brain tissues, *Glycobiology* **1**, 527–35 (1991).

71 Basu M, Basu SS, Li Z, Tang H, Basu S, Biosynthesis and regulation of Le(x) and SA-Le(x) glycolipids in metastatic human colon carcinoma cells, *Indian J Biochem Biophys* **30**, 324–32 (1993).

72 Basu M, Khan FA, Das KK, Zhang BJ, Biosynthesis *in vitro* of core lacto-series glycosphingolipids by N-acetyl-D-glucosaminyltransferases from human colon carcinoma cells, Colo 205, *Carbohydr Res* **209**, 261–77 (1991).

73 Basu SS, Basu M, Li Z, Basu S, Characterization of two glycolipid: Alpha 2-3sialyltransferases, SAT-3 (CMP-NeuAc:nLcOse4Cer alpha 2-3sialyltransferase) and SAT-4 (CMP-NeuAc:GgOse4Cer alpha 2-3sialyltransferase), from human colon carcinoma (Colo 205) cell line, *Biochemistry* **35**, 5166–74 (1996).

74 Basu SS, Basu M, Dastgheib S, Ghosh S, Basu S, Cloning and expression of SAT-3 involved in SA-Le(x) biosynthesis: Inhibition studies with polyclonal antibody against GST-SAT-3 fusion protein, *Indian J Biochem Biophys* **34**, 97–104 (1997).

75 Basu S, Basu M, Dastgheib S, Haws JW, Biosynthesis and regulation of glycosphingolipids. In *Comprehensive Natural Products Chemistry*, edited by Barton D, Nakanishi K, Meth-Cohen O, Pinto BM (Pergamon Press, New York, 1999), Vol. 3, pp. 107–128.

76 Basu S, Das, K, Basu M, Glycosyltransferase in oligosaccharides in chemistry and biology—A comprehensive handbook, *Glycosyltransferases in Glycosphingolipid Biosynthesis*, edited by Ernst B, Sinay P, Hart G (Wiley-VCH Verlag GmbH, Germany, 2000), pp. 329–347.

77 Holmes EH, Ostrander GK, Hakomori S, Biosynthesis of the sialyl-Le^x determinant carried by type 2 chain glycosphingolipids (IV3NeuAcIII3FucnLc4, VI3NeuAcV3FucnLc6, and VI3NeuAcIII3V3Fuc2nLc6) in human lung carcinoma PC9 cells, *J Biol Chem* **261**, 3737–43 (1986).

78 Kannagi R, Levery SB, Hakomori S, Lea-active heptaglycosylceramide, a hybrid of type 1 and type 2 chain, and the pattern of glycolipids with Le^a, Le^b, X (Le^x), and Y (Le^y) determinants in human blood cell membranes (ghosts). Evidence that type 2 chain can elongate repetitively but type 1 chain cannot, *J Biol Chem* **260**, 6410–5 (1985).

79 Kaufman B, Basu S, Roseman S, Isolation of glucosylceramides from yeast (Hansenula ciferri), *J Biol Chem* **246**, 4266–71 (1971).

80 Dickson RC, Lester RL, Metabolism and selected functions of sphingolipids in the yeast *Saccharomyces cerevisiae*, *Biochim Biophys Acta* **1438**, 305–21 (1999).

81 Ohashi Y, Tanaka T, Akashi S, Morimoto S, Kishimoto Y, Nagai Y, Squid nerve sphingo-myelin containing an unusual sphingoid base, *J Lipid Res* **41**, 1118–24 (2000).

82 Carter HE, Hendry RA, Mojima S, Stanacer NF, *Biochim Biophys Acta* **45** 402–16 (1960)

83 Lester RL, Dickson RC, Sphingolipids with inositolphosphate-containing head groups, *Adv Lipid Res* **26**, 253–74 (1993).

84 Sperling P, Heinz E, Plant sphingolipids: Structural diversity, biosynthesis, first genes and functions, *Biochim Biophys Acta* **1632**, 1–15 (2003).

85 Basu S, Ma R, Mikulla B, Boyle P, Basu M, Bose R, Apoptosis of human colon and breast carcinoma cells by inhibitors of DNA, Glycolipid and Glycoprotein biosynthesis. *Proceedings of FASEB Summer Research Meeting on* "*Apoptosis and Immonity*" (July 13–18 2002), p. 9.

86 Basu S, Ma R, Milulla B, Boyle P, Basu M, Bose R, Apoptosis of human colon and breast carcinoma cells by inhibitors of glycoconjugate biosynthesis. In *Proceedings of XVIIth International Meeting on Glycoconjugate in Bangulore*, India (Jan. 11–15, 2003), p. 42.

87 Basu S, Ma R, Milulla B, Boyle P, Moulton C, Campbell B, Basu M, Bose R, Role of cancer cell surface glycoconjugate in the anti-cancer drug discorvery. In *Proceedings of 6th International Cell Surface Meeting in Kolkata*, India. (Jan. 16–18, 2003), p. 1.

88 Ma R, Daus B, Hesketh A, Galindo E, Basu M, Banerjee S, Basu S, Apoptosis induction in colon (Colo-205) and breast (SKBr3) carcinoma cells by L-PPMP and regulation of gene expression of a glycolipid:glycosyltransferase. Cell Biol. Abstract-, vol. (2003).

89 Basu S, Kaufman B, Roseman S, Enzymatic synthesis of glucocerebroside by a glucosyltransferase from embryonic chicken brain, *J Biol Chem* **248**, 1388–94 (1973).

90 Basu M, Basu S, Enzymatic synthesis of a tetraglycosylceramide by a galactosyltransferase from rabbit bone marrow, *J Biol Chem* **247**, 1489–95 (1972).

91 Basu M, Basu S, Enzymatic synthesis of a blood group B-related pentaglycosylceramide by an alpha-galactosyltransferase from rabbit bone marrow, *J Biol Chem* **248**, 1700–6 (1973).

92 Basu M, De T, Das KK, Kyle JW, Chon HC, Schaeper RJ, Basu S. Glycosyltransferases involved in glycolipid biosynthesis. *Methods in Enzymol*. edited by Ginsburg V. vol. 138 (Academic Press, New York, 1987) pp. 575–607.

93 Narimatsu H, Sinha S, Brew K, Okayama H, Qasba PK, Cloning and sequencing of cDNA of bovine N-acetylglucosamine (beta 1-4)galactosyltransferase, *Proc Natl Acad Sci USA* **83**, 4720–4 (1986).

94 Shaper NL, Shaper JH, Meuth JL, Fox JL, Chang H, Kirsch IR, Hollis GF, Bovine galactosyltransferase: Identification of a clone by direct immunological screening of a cDNA expression library, *Proc Natl Acad Sci USA* **83**, 1573–7 (1986).

95 Ghosh S, Basu SS, Basu S, Isolation of a cDNA clone for beta 1–4 galactosyltransferase from embryonic chicken brain and comparison to its mammalian homologs, *Biochem Biophys Res Commun* **189**, 1215–22 (1992).

96 Basu SS, Dastgheib S, Ghosh S, Basu M, Kelly P, Basu S, Purification and characterization of avian glycolipid: Beta-galactosyltransferases (GalT-4 and GalT-3): Cloning and expression of truncated betaGalT-4, *Acta Biochim Pol* **45**, 451–67 (1998).

97 Basu M, Basu S, Stoffyn A, Stoffyn P, Biosynthesis *in vitro* of sialyl(alpha2-3)neolactotetraosylceramide by a sialyltransferase from embryonic chicken brain, *J Biol Chem* **257**, 12765–9 (1982).

98 Kitagawa H, Mattei MG, Paulson JC, Genomic organization and chromosomal mapping of the Gal beta 1,3GalNAc/Gal beta 1,4GlcNAc alpha 2,3-sialyltransferase, *J Biol Chem* **271**, 931–8 (1996).

99 Lee YC, Kojima N, Wada E, Kurosawa N, Nakaoka T, Hamamoto T, Tsuji S, Cloning and expression of cDNA for a new type of Gal beta 1,3GalNAc alpha 2,3-sialyltransferase, *J Biol Chem* **269**, 10028–33 (1994).

100 Radin NS, Chemotherapy by slowing glucosphingolipid synthesis, *Biochem Pharmacol* **57**, 589–95 (1999).
101 Manning LS, Radin NS, Effects of the glucolipid synthase inhibitor, P4, on functional and phenotypic parameters of murine myeloma cells, *Br J Cancer* **81**, 952–8 (1999).
102 Eldadah BA, Yakovlav AG, Faden AL, A new approach for the electrophoretic detection of apoptosis, *Nucleic Acids Res* **24**, 4092–3 (1996).
103 Gullicksen PS, Dean RG, Baile CA., Detection of DNA fragments and apoptotic proteins and quantification of uncoupling protein expression by real-time RT-PCR in adipose tissue, *J Biochem Biophgys Methods* **58**, 1–13 (2004).
104 Thornberry NA, Lazebnik Y, Caspases: Enemies within, *Science* **281**, 1312–6 (1998).
105 Green DR, Reed JC, Mitochondria and apoptosis, *Science* **281**, 1309–12 (1998).
106 Wang L, Ma R, Flavell RA, Choi ME, Requirement of mitogen-activated protein kinase kinase 3 (MKK3) for activation of p38alpha and p38delta MAPK isoforms by TGF-beta 1 in murine mesangial cells, *J Biol Chem* **277**, 47257–62 (2002).
107 Koulov AV, Stucker KA, Lakshmi C, Robinson JP, Smith BD, Detection of apoptotic cells using a synthetic fluorescent sensor for membrane surfaces that contain phosphatidylserine, *Cell Death Differ* **10**, 1357–9 (2003).
108 Peterson GL, A simplification of the protein assay method of Lowry *et al.* which is more generally applicable, *Anal Biochem* **83**, 346–56 (1977).
109 Das KK, Basu M, Basu S, A rapid preparative method for isolation of neutral and acidic glycosphingolipids by radial thin layer chromatography, *Anal Biochem* 125–134 (1984).
110 Jenis DM, Basu S. Pollard M. Increased activity of a b-galactosyltransferase in tissues of rats bearing prostate and mammary adenocarcinomas, *Cancer Biochem Biophys* **6**, 37–45 (1982).
111 Wang AM, Doyle MV, Mark DF, Quantification of mRNA by the polymerase chain reaction, *Proc Natl Acad Sci USA* **86**, 9717–21 (1989).
112 Taniguchi N., Honke K, Fukuda M, *Handbook of Glycosyltransferases and their Related Genes* (Springer, Tokyo, 2002).
113 Kakugawa Y, Wada T, Yamaguchi K, Yamanami H, Ouchi K, Sato I, Miyagi T, Up-regulation of plasma membrane-associated ganglioside sialidase (Neu3) in human colon cancer and its involvement in apoptosis suppression, *Proc Natl Acad Sci USA* **99**, 10718–23 (2002).
114 Yates AJ, Ramprosad A, in *Sphingolipids as Signaling Modulators in the Nervous System*, edited by Ledeen RW, Hakomori S, Yates AJ, Schneider JS, Yu RK (Ann. N.Y. Acad. Sci., New York, 1998), Vol. 845. pp. 57–71.
115 Hiraishi K, Suzuki K, Hakomori S, Adachi M, Le(y) antigen expression is correlated with apoptosis (programmed cell death), *Glycobiology* **3**, 381–90 (1993).
116 Zeng G, Gao L, Birkle S, Yu RK, Suppression of ganglioside GD3 expression in a rat F-11 tumor cell line reduces tumor growth, angiogenesis, and vascular endothelial growth factor production, *Cancer Res* **60**, 6670–6 (2000).