Ganglioside GD3 Sensitizes Human Hepatoma Cells to Cancer Therapy*

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Ganglioside GD3 (GD3) has emerged as a modulator of cell death pathways due to its ability to interact with mitochondria and disable survival pathways. Because NF-κB activity contributes to cancer therapy resistance, this study was undertaken to test whether GD3 modulates the response of human hepatoblastoma HepG2 cells to radio- and chemotherapy. NF-κB was activated in HepG2 cells shortly after therapeutic doses of ionizing radiation or daunorubicin treatment that translated into up-regulation of κB-dependent genes. These effects were accompanied by minimal killing of HepG2 cells by either ionizing radiation or daunorubicin. However, GD3 pretreatment blocked the nuclear translocation of active κB members, without effect on Akt phosphorylation, induced by either treatment. The suppression of κB-dependent gene induction by GD3 was accompanied by enhanced apoptotic cell death caused by these therapies. Furthermore, the combination of GD3 plus ionizing radiation stimulated the formation of reactive species followed by the mitochondrial release of cytochrome c and Smac/Diablo and caspase 3 activation. Pretreatment with cyclosporin A before radiotherapy protected HepG2 cells from the therapeutic combination of GD3 plus ionizing radiation. These findings underscore a key role of mitochondria in the response of tumor cells to cancer therapy and highlight the potential relevance of GD3 to overcome resistance to cancer therapy by combining its dual action as a mitochondria-interacting and NF-κB-inactivating agent.

The resistance of tumor cells to current cancer therapy-induced cell death underscores a major problem that limits the successful treatment of human cancer (1, 2). Among the different molecular strategies that contribute to the resistance of tumor cells to radio- and chemotherapy, the role played by transcription factor NF-κB has been well defined. Indeed, the prior inactivation of NF-κB rendered different tumor cell types sensitive to cancer therapy (3–7). Although ionizing radiation and chemotherapeutic agents activate apoptosis pathways, the impact of these is often antagonized by the early activation of NF-κB-dependent survival pathways induced by these treatments (8, 9).

NF-κB is usually kept inactive in the cytoplasm through association with an endogenous inhibitor protein of the IκB (inhibitor of NF-κB) family. The most common pathway leading to NF-κB activation by a wide variety of stimuli, including cancer therapy involves the phosphorylation of IκB at specific serine residues that targets its subsequent degradation by the proteasome (3–9). The released subunits of NF-κB then translocate to the nuclei where they bind to specific sites in the promoter/enhancer region of target genes. NF-κB is known to induce the expression of many different genes involved in the regulation of immune or stress response and apoptosis (3). Since its first recognition as an anti-apoptotic factor in mice lacking the p65 component of NF-κB (10), increasing evidence has further documented the anti-apoptotic activity of NF-κB against a variety of stimuli (11–13). The central role of NF-κB in the prevention of apoptosis is mediated through the induction of antiapoptotic genes, including Bcl-αL, c-IAP1, c-IAP2, A1/Bfl1, or antioxidant enzymes such as Mn-SOD (14–17).

Glycosphingolipids (GSLs), carbohydrate-bearing lipid components of biological membranes, participate in the regulation of various cellular functions, including cell adhesion and signal transduction (18, 19). In particular, ganglioside GD3, a sialic acid-containing GSL, has been identified as a lipid death effector due to its ability to interact with and recruit mitochondria to apoptotic pathways, contributing to the mitochondria-dependent apoptosome activation and subsequent apoptosis triggered by death ligands (20–26). In addition to the direct apoptosis-promoting activity of GD3 through mitochondrial interaction, this GSL species counteracts survival signals by suppressing the NF-κB activation and subsequent κB-dependent gene induction (27, 28). This dual function of GD3 has been shown to render rat hepatocytes susceptible to tumor necrosis factor-mediated cell death through the inactivation of NF-κB (27). Yet, despite the available evidence supporting the proapoptotic function of GD3, the role of glycolipids in apoptosis is controversial. The inhibition of glycolipid synthesis has been shown to enhance apoptosis, reversing multidrug resistance in certain tumor cells (29, 30).

AMC, Ac-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin; CsA, cyclosporin A; DCF, dichlorofluorescein; EMSA, electrophoretic mobility shift assays; GSL, glycosphingolipid; GD3, ganglioside GD3; LDH, lactate dehydrogenase; MPT, mitochondrial permeability transition; ROS/RNS, reactive oxygen/nitrogen species; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-propanol.
Hence, in light of these findings and due to the pivotal role of NF-κB in mediating the resistance of tumor cells to cancer therapy, the purpose of the present work was to test the hypothesis that GD3 pretreatment sensitizes human hepatoblastoma HepG2 cells to ionizing radiation and chemotherapy by its combined function as a mitochondria-recruiting and NF-κB-disabling agent.

EXPERIMENTAL PROCEDURES

Materials—Daunorubicin and CsA were purchased from Sigma (Madrid, Spain). Hoescht 33258 was obtained from Molecular Probes (Eugene, OR). GD3, 99% purity by thin-layer chromatography (TLC), was a gift from Dr. Nirianz (Pleasant Gap, IN). Phos-tag (Nippon Genetics, Tokyo, Japan) was prepared by dissolving Phos-tag in 2× Tris-glycine SDS-PAGE gel electrophoresis running buffer, and the solution was clarified by centrifugation. GD3, 99% purity by thin-layer chromatography (TLC), was from Ac-DVE-AMC (Madrid, Spain). Hoescht 33258 was obtained from Molecular Probes (Eugene, OR). GD3, 99% purity by thin-layer chromatography (TLC), was from Ac-DVE-AMC (Madrid, Spain). Hoescht 33258 was obtained from Molecular Probes (Eugene, OR). GD3, 99% purity by thin-layer chromatography (TLC), was from Ac-DVE-AMC (Madrid, Spain).

Cell Culture and Treatments—The human hepatoblastoma cell line, HepG2, was obtained from the European Collection of Animal Cell Cultures (Salisbury, Wilts, UK) and grown at 37 °C in 5% CO_2 in Dulbecco’s modified Eagle’s medium containing high glucose levels. Culture medium was supplemented with 10% heat-inactivated fetal bovine serum, 2 mm L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml). Subconfluent HepG2 cells were irradiated in a linear accelerator (IDS Siemens) at room temperature using an electron beam of 18 MeV as described previously (31). Doses between 2 and 20 Gy were applied at a rate of 3 Gy/min. Estimate errors on dose have been calculated to be below 1%. Alternatively, cells were treated with daunorubicin (1–10 μM) for 2–72 h. In some cases, cells were preincubated with GD3 (5 μM in ethanol) 4 h before radiation or daunorubicin treatment and examined for cell survival (2–72 h). The final concentration of the carrier solvent did not exceed 0.1% nor affected any of the parameters determined.

NF-κB Activation—NF-κB DNA binding activity in nuclear extracts was assessed by EMSA using NF-κB consensus oligonucleotide (5'-AGTTGAGGGACTTTCCTCAAGGC-3') as described previously (32). After radiation or daunorubicin treatment, cells were washed twice with ice-cold phosphate-buffered saline, collected with a rubber policeman, and lysed with Nonidet P-40 (10%). The nuclear pellet was recovered by spinning (15,000 × g at 4 °C for 30 s), resuspended in ice-cold 20 mM Hepes, pH 7.4, containing 0.4 μM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10 μg/ml 1-chloro-3-tosylamide-2-aminohexahydronaphthalene and stored at −80 °C. Binding reaction contained 7 μg of nuclear extracts, 5 μl of incubation buffer (10 mM Tris-HCl, 40 mM NaCl, 1 mM EDTA, and 4% glycerol) and 1 μg of poly(dI-dC). After 15 min on ice, the labeled oligonucleotide (30,000 cpm) was added, and the mixture was incubated for 20 min at room temperature. The mixture was electroeluted from an acrylamide gel for 90 min at 150 V.

Transactivation of NF-κB—HepG2 cells were transfected with the plasmid pNF-κB-Luc containing four tandem copies of the κB enhancer upstream of the herpes simplex virus thymidine kinase gene fused to the luciferase reporter using LipofectAMINE reagent as described previously (32). In each experiment, six 35-mm wells containing 5 × 10^5 cells were transfected with 5 μg of a DNA and 5 μg of the pSV-β-galactosidase vector as control to monitor transfection efficiency. After 24–48 h, transfection cells were irradiated (4 Gy) or treated with daunorubicin (1–10 μM), and 6 h later, they were harvested in a reporter lysis buffer and supernatants were used to determine luciferase activity. Results are expressed as the mean ± S.D. and averages of three to five experiments. Statistical analysis of mean values for multiple comparisons were may be one-way analysis of variance.

RESULTS

Activation of NF-κB by Cancer Therapy and Inhibition by GD3—NF-κB activation has been reported in different tumor cell lines after chemotherapeutic treatments and ionizing radiation and thought to function as a defense mechanism to counteract the cell death cascades generated by these cancer therapies (3–6). To study the effect of ionizing radiation or chemotherapy on the DNA binding activity of NF-κB in human hepatoblastoma cells, EMSA was performed using nuclear extracts obtained from HepG2 cells after exposure to ionizing radiation (2–20 Gy). As shown (Fig. 1A), ionizing radiation enhanced in a dose-dependent fashion the DNA binding activity of several complexes of NF-κB. Although these complexes were displaced by molar excess of unlabeled κB oligonucleotide (not shown), indicating their specificity for κB binding sites, only the two upper bands were identified as RelA/p52 and p52/p50 dimers by supershifting assays using antibodies against individual NF-κB components (RelA, p52, and p50). The activation of these dimers by ionizing radiation occurred within 30 min post-treatment and lasted for 6–8 h (not shown). Treatment of HepG2 cells with daunorubicin (1 μM) resulted in a similar pattern of NF-κB DNA binding activity (Fig. 1B). The level of activation of both RelA/p52 and p52/p50 dimers ranged from 3- to 4-fold with respect to untreated control cells (Fig. 1B).

Because previous findings in cultured rat hepatocytes revealed that GD3 suppressed the activation of NF-κB induced by tumor necrosis factor (27), we next examined whether GD3 pretreatment abolished the activation of NF-κB by ionizing radiation and daunorubicin treatments. As observed (Fig. 1B), GD3 preincubation prevented the activation of NF-κB induced by ionizing radiation (4 Gy) or daunorubicin (1 μM), decreasing the intensity of RelA/p52 and p52/p50 complexes to control levels. Thus, these findings confirm the activation of NF-κB upon cancer therapy treatment of HepG2 cells, which is blocked by pretreatment of cells with GD3.

for 15 min. Upon centrifugation at 14,000 × g the supernatant and the mitochondria-containing pellet were resolved by SDS-PAGE (15% gels). Proteins were transferred to nitrocellulose, and the blots were incubated with anti-cytochrome c antibody (PharMingen) and anti-Smac/Diablo antibody (Calbiochem) followed by ECL-based detection. To monitor the specificity of cytochrome c and Smac/Diablo release, parallel aliquots were immunoblotted with human monoclonal antibody anti-cytochrome c oxidase subunit II (Molecular Probes).

Caspase Activation—Cytosolic extracts were used to measure caspase 3 activity from the release of 7-amino-4-trifluoromethyl coumarin from Ac-DEVD-AMC, and fluorescence was continuously recorded with excitation at 380 nm and emission at 480 nm as described previously (23).

Reactive Species Determination—Reactive species formation were determined using chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (Molecular Probes, Eugen, OR), which becomes highly fluorescent upon oxidation by peroxides (17, 31) as well as pentyroxinite (33). DCF formation was continuously recorded in a fluorometer with excitation at 380 nm and emission at 460 nm. Relative fluorescence units were normalized per milligram of cellular protein.

Cell Viability and Apoptosis—Cell death was determined by the measurement of the release of lactate dehydrogenase (LDH) into the medium and in the remaining cell monolayer after lysis with 5% Triton X-100, which expresses the percentage of LDH release in the medium as the fraction of LDH (medium plus cells). Results were confirmed by the MTT assay (34). Antibodies for caspase 3, caspase 8, and anti-κB antibodies were from Cell Signaling Technology (Beverly, MA).

Cell Signaling Technology (Beverly, MA). Membranes were then stripped and incubated with anti-Akt antibody (Cell Signaling Technology, Beverly, MA) and anti-phospho-Akt (Ser-473) antibody (Cell Signaling Technology, Beverly, MA) and subsequently incubated with horseradish peroxidase-conjugated antibodies. Blots were visualized by ECL detection (Amersham Biosciences, Piscataway, NJ). The distribution of NF-κB p65 subunit, HepG2 cells were fixed with Hoescht 33258 and analyzed by a Leica TCS-NT confocal microscope. More than 200 cells per condition were examined for apoptotic features, including the presence of chromatin condensation or fragmentation.

Statistical Analyses—Results are expressed as the mean ± S.D. and averages of three to five experiments. Statistical analysis of mean values for multiple comparisons were may be one-way analysis of variance.

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**Fig. 1.** NF-κB activation by cancer therapy and effect of GD3 treatment.

A, HepG2 cells were exposed to increasing doses of ionizing radiation (IR), and EMSA was performed 4 h after treatment to assess activation of NF-κB. The arrows denote the activation of relA/p52 and p52/p50. B, HepG2 cells were preincubated 4 h with GD3 (5 μM) before ionizing radiation (4 Gy) or daunorubicin (D) treatment (1 μM). Nuclear extracts from 2 × 10⁶ cells were isolated 2 h after treatments and assayed for DNA binding activity. Both are representative EMSA gels out of four to five individual experiments showing similar results. The right panel shows the quantitative activation of RelA/p52 (closed bars) and p52/p50 (open bars) by cancer therapy and the inactivation induced by GD3 pretreatment. Results are the mean ± S.D. of five to six independent experiments. #, p < 0.05 versus control; *, p < 0.05 versus IR or D alone.

GD3 Blocks the Nuclear Translocation of NF-κB—To confirm if NF-κB-active complexes from GD3-pretreated cells remained in the cytosol after exposure to radiotherapy, we determined the cellular localization of NF-κB p65 subunit. As seen (Fig. 2), compared with control HepG2 cells, which display a diffuse fluorescence pattern, ionizing radiation induced the localization of p65 into the nuclei. This shift in the cellular distribution of p65 induced by ionizing radiation was blunted by GD3 pretreatment (Fig. 2). Moreover, EMSA assays confirmed the presence of DNA binding activity of NF-κB in cytosolic extracts (not shown). Similar findings were observed when HepG2 cells were exposed to daunorubicin with or without GD3 pretreatment (not shown).

Because the blocking effect of GD3 on the nuclear translocation of competent DNA-binding NF-κB members may be translated at the gene level, we next examined the transactivation of NF-κB using a luciferase reporter gene construct controlled by four κB-binding sites. Ionizing radiation and daunorubicin treatment enhanced the κB-dependent luciferase expression by 8- to 10-fold (Fig. 2). However, the preincubation of HepG2 cells with GD3 abolished the inducible expression of luciferase by ionizing radiation or daunorubicin. Thus, taken collectively, these findings underscore the impact of GD3 pretreatment in suppressing the inducible expression of κB-regulated genes.

**GD3 Sensitizes HepG2 Cells to Cancer Therapy**—Because NF-κB is known to induce the expression of antiapoptotic genes that counteract apoptosis pathways, we next assessed the consequences of the inactivation of NF-κB by GD3 on the survival of HepG2 cells following cancer therapy. As shown (Fig. 3A), HepG2 cells were relatively resistant to a therapeutic dose of ionizing radiation or daunorubicin treatment displaying slight signs of cell injury after 3 days post-treatment (17 ± 7% and 22 ± 6%, respectively) (Fig. 3B). Of note, when HepG2 cells were pretreated with GD3 at a sublethal dose, it rendered HepG2 cells vulnerable to both ionizing radiation and daunorubicin treatment (Fig. 3). This sensitization was observed as early as 24 h after GD3 pretreatment (17 ± 4% and 19 ± 6% cell death after ionizing radiation or daunorubicin treatment, compared with 5 ± 3% and 7 ± 4%, without GD3, respectively). Similar findings were observed when cell survival was quantitated by the MTT assays (not shown).

To investigate if the cell death caused by these treatments after GD3 preincubation was accompanied by apoptotic features, HepG2 cells were exposed to the DNA-binding fluorochrome H-33258, and chromatin morphology was visualized by fluorescence microscopy. As shown (Fig. 4) the combination of GD3 plus ionizing radiation or daunorubicin resulted in increased presence of cells displaying chromatin disruption, indicative of apoptosis. Indeed, the estimation of apoptotic cell death indicated a significant sensitization 24 h after the combination of ionizing radiation or daunorubicin after GD3 compared with either therapy alone (Fig. 4B). Clearly, these findings indicate that the pretreatment of HepG2 cells with GD3 increases the efficiency of therapies intended to kill human tumor cells.

**GD3 Does Not Interfere with Akt Signaling**—Akt, a serine/threonine protein kinase, has been shown to regulate cell survival signals by rendering key components of the apoptotic cascade inactive (34–36). Therefore, in view of the preceding findings, we next assessed whether GD3 disabled the Akt signaling pathway thus contributing to the susceptibility of HepG2 cells toward cancer therapy. To study the contribution of this pathway, we determined the level of Akt and its phosphorylated form (at serine 473) using cell extracts from ionizing...
radiation-exposed cells with or without GD3 preincubation. As seen, compared with the phosphorylation induced by insulin, ionizing radiation did not induce the phosphorylation of Akt (Fig. 5). The phosphorylated form of Akt was determined at different times (from 15 min to 6 h) after ionizing radiation without observing a significant increase in the active form induced by ionizing radiation (4 Gy) with or without the presence of GD3. Similar results were obtained with daunorubicin (not shown). Thus, these findings indicate that Akt-dependent survival signals do not play a role either in the resistance of HepG2 cells to cancer therapy or in the sensitization by GD3.

The Combination of GD3 Plus Radiotherapy Activates the Mitochondria-dependent Apoptosome—Mitochondria function as a strategic cell death control center (37, 38). In this role the commitment to cell death is mediated by a regulated release of specific proteins from the intermembrane space that assist in the assembly of the apoptosome. Because reactive species have been implicated as early mediators of cancer therapy-induced cell death (31, 39), we next assessed the regulation of ROS generation by cancer therapy with or without GD3 pretreatment. A significant increase in DCF fluorescence was observed in HepG2 cells within 30 min after exposure to ionizing radiation that declined gradually over time (Fig. 6). Because this fluorescent probe is sensitive to both peroxides and peroxynitrite (33), these findings indicate that ionizing radiation generates a modest burst of reactive oxygen (ROS) and nitrogen (RNS) species insufficient to cause cell death. Treatment of HepG2 cells with GD3 increased the DCF fluorescence to a level similar to that caused by ionizing radiation that did not decline as long as GD3 was present. This outcome reflects the ability of GD3 to stimulate mitochondrial ROS generation (23, 27). However, when HepG2 cells were pretreated with GD3 and then irradiated there was an overgeneration of ROS/RNS compared with either of the treatments alone (Fig. 6). To examine whether the mitochondrial permeability transition (MPT) modulates the generation of reactive species induced by GD3 and ionizing radiation, we investigated the effect of CsA, an inhibitor of MPT. Although CsA by itself did not affect the DCF fluorescence, preincubation of cells with CsA before exposure to ionizing radiation abolished the ROS/RNS formation (Fig. 6).

Because MPT allows the release of apoptosis-promoting proteins from mitochondria, we examined the mitochondrial release of cytochrome c and Smac/Diablo in HepG2 cells. Consistent with the findings on ROS/RNS generation, the combination of GD3 plus ionizing radiation resulted in enhanced release of both cytochrome c and Smac/Diablo compared with either stimuli alone (Fig. 7A). Furthermore, CsA pretreatment abrogated the release of both factors, further supporting the involvement of MPT in the release of mitochondrial proapoptotic proteins. Finally, we examined the activation of effector caspases and the consequences on cell survival and the effect of CsA. As seen, GD3 plus ionizing radiation stimulated the activation of caspase 3 respect to either treatment alone (Fig. 7B). Pretreatment of GD3-exposed HepG2 cells with CsA blunted the acti-
vation of caspase 3 (Fig. 7B) resulting in protection of against GD3 plus ionizing radiation-induced cell death (55 ± 7% cell death versus 6 ± 3% at 72 h post-treatment, p < 0.05). Similar findings were observed when cells were treated with daunorubicin instead of ionizing radiation in terms of ROS/RNS formation, cytochrome c and Smac/Diablo release, and caspase 3 activation (not shown).

**DISCUSSION**

Ganglioside GD3 has emerged as an apoptosis lipid effector through its mitochondrial-interacting (20–26) and NF-κB-suppressing function (27, 28). Based on this dual function, exogenous GD3 may actually modulate cancer resistance, a phenomenon characterized by the activation of pathways that circumvent the toxicity of current cancer therapies. To test this hypothesis we have used the human hepatoblastoma cell line HepG2 because of their absence of endogenous GD3 (40) and because hepatocellular carcinoma is a highly resistant tumor to currently available chemotherapeutics and radiotherapy (41). The present study shows that exogenous GD3 sensitizes HepG2 cells to both ionizing radiation and daunorubicin-mediated cell death. Because survival pathways contribute to the resistance of tumor cells to cancer therapy, we examined...
whether exogenous GD3 interfered with survival pathways signaling. Our data show that pretreatment of HepG2 cells with GD3 disrupts the pathway leading to NF-κB activation induced by ionizing radiation and daunorubicin, resulting in the suppression of inducible κB-dependent gene expression. These findings are consistent with previous results reported in cultured rat hepatocytes (27), thus indicating that the anti-

NF-κB function of GD3 is not cell-specific. Our findings, in addition, discard the involvement of Akt, a protein kinase that has been shown to promote cell survival through the inactivation of key elements of apoptosis pathways (34–36). Thus, rather than inactivating proapoptotic factors, GD3 seems to interfere with the expression of survival genes controlled by NF-κB. Several κB-regulated antiapoptotic proteins have been described (14–17), and further work will be required to identify the specific survival of one or more genes responsible for the resistance of HepG2 to cancer therapy.

An important aspect of our findings is the mechanism of NF-κB inactivation by GD3. Unlike other strategies that target the degradation step of IκB, the inhibitor protein family of NF-κB (4–7), GD3 acts as a late step in the pathway preventing the translocation of active κB members to the nuclei. Although the exact mechanism whereby GD3 prevents this vital step in NF-κB activation is presently unknown, it offers the advantage of ensuring the inactivation of NF-κB regardless of the mechanism leading to the release of the active, DNA-binding competent members of NF-κB. Further work is currently underway to address whether GD3 interferes with the nuclear localization sequence that directs the translocation of NF-κB members to the nuclei (42).

Our findings define a vital role for the burst of ROS/RNS in the sensitization by GD3 of HepG2 cells to radiotherapy. The level of those potentially harmful species generated by the combination of GD3 plus ionizing radiation was greater that with either treatment alone, and the deadly cooperation between both strategies resulted in the additive generation of ROS/RNS. Although ionizing radiation may signal the stimulation of ROS/RNS as an early event as shown recently (39), it is conceivable that the induction of anti-oxidant enzymes such as γ-glutamylcysteine synthase (43), the inactivation of this transcription factor by GD3 would contribute to the enhanced ROS/RNS generation induced by GD3 plus ionizing radiation, resulting in the killing of cells. One of the consequences of this synergistic ROS/RNS stimulation by GD3 and ionizing radiation is the onset of MPT that functions as a gateway to apoptosis (44, 45). Consistent with these events, GD3 plus ionizing radiation stimulates the release of cytochrome c and Smac/Diablo from mitochondria and the pretreatment of cells with CsA before exposure to ionizing radiation diminishes the burst of ROS/RNS induced by GD3 and ionizing radiation. Consequently, the resulting assembly of the mitochondrial-dependent apoptosome and inactivation of survival genes dependent on NF-κB by GD3 culminates in an efficient demise of tumor cells following a therapeutic dose of ionizing radiation.

Although the present study contributes to the current evidence supporting a proapoptotic function of GD3, the role of glycolipids in apoptosis of cancer cells is controversial. Previous studies have shown that down-regulation of glycolipid synthesis enhances apoptosis (29, 30). Much for this evidence has derived from the inhibition of glucosylceramide synthase, the enzyme responsible for the glucosylation of ceramide. In par-
multidrug resistance of cancer cells (29, 30, 46), because this strategy is accompanied by enhanced ceramide levels. Ceramide itself has been shown to cause ROS/RNS from mitochondria (47–49), contributing to the apoptotic cell death of cancer therapies. However, as recently described, the chemosensitizing effect of PDMP appears to be independent of its role as an inhibitor of ceramide glucosylation (46). In fact, it was shown that PDMP significantly inhibited the activity of P-glycoprotein, a member of the membrane proteins of the ATP-binding cassette family that contributes to multidrug cancer resistance (50).

Our data, however, are in agreement with recent findings describing that ganglioside GM3 overexpression induces apoptosis and reduces malignant potential in murine bladder cancer (51). Thus, although the multidrug resistance in cancer cells is a complex phenomenon involving the interplay of different molecular mechanisms and multistep alteration of the sphingolipid metabolism (52), our present study contributes to the emerging evidence suggesting that gangliosides may function as sensitizing agents enhancing the anti-cancer properties of currently used cancer therapy.

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