Ganglioside G\textsubscript{D2} in Small Cell Lung Cancer Cell Lines: Enhancement of Cell Proliferation and Mediation of Apoptosis\textsuperscript{1}

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ABSTRACT

Expression levels of gangliosides and glycosyltransferase genes responsible for their synthases in human lung cancer cell lines and a normal bronchial epithelial cell line were analyzed. Both non-small cell lung cancers and small cell lung cancers (SCLCs) mainly expressed G\textsubscript{M2} and G\textsubscript{M1}, whereas only SCLCs expressed b-series gangliosides, such as G\textsubscript{D2}, G\textsubscript{D1b} and G\textsubscript{T14}. Accordingly, many SCLC cell lines showed up-regulation of the G\textsubscript{D3} synthase gene. Consequently, we introduced G\textsubscript{D3} synthase cDNA into a SCLC line with low expression of b-series gangliosides and analyzed the effects of newly expressed gangliosides on tumor phenotypes. The transfectant cells expressing high levels of G\textsubscript{D3} and G\textsubscript{D2} exhibited markedly increased growth rates and strongly enhanced invasion activities. Addition of anti-G\textsubscript{D2} monoclonal antibodies into the culture medium markedly increased growth rates and strongly enhanced invasion activities. The transfectant cells expressing high levels of G\textsubscript{D2} and G\textsubscript{D3} exhibited partly inhibited by a caspase inhibitor, z-Val-Ala-Asp-fluoromethyl ketone.

INTRODUCTION

Acidic glycosphingolipids and gangliosides are widely expressed in many tissues and organs in vertebrates (1) and have been suggested to be involved in the regulation of development and differentiation as recognition molecules or signal modulators (2). However, there are many tissues and organs in vertebrates (1) and have been suggested to specifically found in SCLC cells with up-regulation of the GD\textsubscript{3} synthase gene and GD\textsubscript{M2}/GD\textsubscript{M1} were used as a target molecule in antibody immunotherapy (9). The finding that anti-GD\textsubscript{2} antibodies suppressed the cell growth of GD\textsubscript{2}-expressing SCLC cells strongly suggested the usefulness of GD\textsubscript{2} as a target for the therapy of disastrous cancer, although the precise mechanisms for apoptosis remain to be clarified.

RESULTS

MATERIALS AND METHODS

Cell Lines. All human lung cancer cell lines were provided by Dr. T. Takahashi (Aichi Cancer Center Research Institute, Aichi, Japan) and were maintained in RPMI 1640 containing 10% FBS in a humidified 5% CO\textsubscript{2} atmosphere at 37°C. The immortalized human bronchial epithelial cell, BEAS-2B, was obtained from the American Type Culture Collection and was cultured on human fibronectin-coated plastic dishes in serum-free small airway cell growth medium (Clonetics) in a humidified 5% CO\textsubscript{2} atmosphere at 37°C.

Flow Cytometry. Cell surface expression of gangliosides was analyzed by FACScan (Becton Dickinson) using various antiganglioside mAbs. The mAbs used were as follows: anti-GD\textsubscript{M3}, M2590 (mouse IgM; Japan Biotest Research). 2

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\textsuperscript{3} The abbreviations used are: NSCLC, non-small cell lung cancer; mAb, monoclonal antibody; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MAPK, mitogen-activated protein kinase; PI, propidium iodide; RT-PCR, reverse transcription-PCR; FAM, 6-carboxyfluorescein; TAMRA, 6-carboxy-6-N,N,N′-tetramethylethridamine; z-VAD-fmk, z-Val-Ala-Asp-fluoromethyl ketone.

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Institute, Tokyo, Japan); anti-G M2, 10-11 (mouse IgM; provided by Dr. P. O. Livingston, Memorial Sloan-Kettering Cancer Center, New York, NY); anti-G D1a, 92-22 (mouse IgM); anti-G D3, R-24 (mouse IgG3; provided by Dr. L. J. Old, Memorial Sloan-Kettering Cancer Center); anti-G D2, 220-51 (mouse IgG3); anti-G D1b, 370 (mouse IgM); anti-G T1b, 549 (mouse IgM); anti-G A2, 2D4 (mouse IgM; American Type Culture Collection); and anti-G A1, 229 (mouse IgM). Unless described otherwise, the antibodies were generated in our laboratory (28). The cells were incubated with mAbs for 45 min on ice and then stained with FITC-conjugated goat antimouse IgM or IgG (Cappel, Durham, NC). To analyze GM1, cells were incubated with a biotin-conjugated cholera toxin B subunit (List Biological Laboratories, Campbell, CA) for 45 min on ice and then stained with FITC-conjugated avidin (EY Laboratories, San Mateo, CA). Control cells for flow cytometry were prepared using the second antibody alone. For quantification of positive cells, the CELLQuest program was used.

Quantitative Real-Time RT-PCR Analysis. The quantitative RT-PCR analysis was performed using TaqMan One-Step RT-PCR Master Mix Reagents kit (PE Biosystems). The TaqMan probe contained a reporter dye at the 5’ end (FAM) and a quencher dye at the 3’ end (TAMRA; PE Biosystems). Target-specific PCR amplification results in cleavage and release of the reporter dye from the quencher-containing probe by nuclease activity of AmpliTag Gold (PE Biosystems). Thus, the fluorescence signal generated from released reporter dye is proportional to the amount of PCR product. To compare the relative expression levels of glycosyltransferase genes in various lung carcinoma cells, total RNA was extracted from each cell line with the guanidine isothiocyanate/cesium chloride ultracentrifugation method, and real-time RT-PCR was performed using gene-specific primers in each sample according to the manufacturer’s protocol. The primers used for α2,8-sialyltransferase (G D3 synthase; Ref. 29) were a forward primer (GenBank L32867, nucleotides 456–476), 5’-CATTCCAGCTGCCATTGAAGA-3’, and a reverse primer (nucleotides 582–561), 5’-CTTGACAAAGGAGGGAGATTGC-3’. The primers for β1,4N-acetylgalactosaminyltransferase (G M2 synthase; Ref. 30) were a forward primer (GenBank NM-001478, nucleotides 1163–1184), 5’-GGACATGAGGCTGCTTTCACTA-3’, and a reverse primer (nucleotides 1306–1286), 5’-CCGATCATACAAGGAGGAAGGT-3’.

Fig. 1. Ganglioside typing of lung cancer cell lines. Cell surface expression of gangliosides in 44 lung cancer cell lines (A, lung small cell carcinoma; B, lung adenocarcinoma; C, lung squamous cell carcinoma; and D, lung large cell carcinoma) and a bronchial epithelial line, BEAS-2B (E), were analyzed by flow cytometry using antiganglioside mAbs as described in “Materials and Methods.” The ganglioside expression level was classified into five groups based on the percentages of positive cells (A, bottom). Data not determined.

Fig. 2. Synthetic pathway of gangliosides. *, point where G M2 synthase works. Lac-Cer, lactosyl ceramide; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; SA, sialic acid.

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Table 1. Relative Expression Levels of Glycosyltransferase Genes in Various Lung Carcinoma Cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Level</th>
</tr>
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<tbody>
<tr>
<td>α2,8-sialyltransferase (G D3 synthase)</td>
<td>100%</td>
</tr>
<tr>
<td>β1,4N-acetylgalactosaminyltransferase (G M2 synthase)</td>
<td>75%</td>
</tr>
</tbody>
</table>

Fig. 2. Synthetic pathway of gangliosides. *, point where G M2 synthase works. Lac-Cer, lactosyl ceramide; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; SA, sialic acid.
Fig. 3. Correlation between the expression levels of gangliosides and those of glycosyltransferases in the lung cancer cell lines. Surface expression of gangliosides was analyzed as described in Fig. 1. Left, numbers are individual cell lines presented in Fig. 1. Percentages of positive cells are shown. Expression levels of the GD3 synthase gene and the GM2/GD2 synthase gene were measured with a quantitative real-time RT-PCR analyzer system as described in “Materials and Methods.” A: a, SCLC; b, NSCLC. Percentages of b-series positive cells (left) were the sum of those of GD3-, GD2-, and GT1b-positive cells. The relative expression level of the GD3 synthase gene (right) is presented as a percentage of that of melanoma SK-MEL-37 cells, which were regarded as 100%. B: a, SCLC; b, NSCLC. The percentages of complex ganglioside-positive cells (left) were the sum of those of GM3-, GM1-, GD1a-, GD2-, and GT1b-positive cells. The relative expression level of the GM2/GD2 synthase gene (right) is presented as a percentage of that of the SCLC line ACC-LC-171 cells, which were regarded as 100%.

Antibody Purification. The anti-GD2 mAb (220-51) was purified using a protein G affinity column, and the concentration of the protein was determined by Lowry’s method (32).

MTT Assay. For cell proliferation assay, transfectant cells and control cells (1 × 10^4 cells/well) were prepared in 48-well plates in serum-containing medium and cultured for 6 days. For cell growth inhibition assay, transfectant cells and control cells (5 × 10^4 cells/well) were seeded in 48-well plates in serum-containing medium and treated with various anti-GD2 mAbs diluted to the indicated concentrations for 3 days. Freshly prepared medium containing antibodies was used for medium exchange on days 1 and 2.

Fig. 4. Remodeling of ganglioside expression by gene manipulation. A, surface expression of gangliosides in transfectant cells. A vector control cell line (C-8; top) transfected with pM1Kneo vector alone and a GD3 synthase gene transfectant cell line (D-18; bottom) transfected with pM1Kneo/D3T-31 were used for flow cytometry as described in “Materials and Methods.” Filled gray lines, the controls with the second antibody alone. B, TLC of glycolipids extracted from SK-LC-17 cells (LC-17), C-8, and D-18 are presented. TLC was performed as described in “Materials and Methods.”

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were added. The color reaction was quantitated using an automatic plate reader, Immuno-Mini NJ-2300 (Nihon InterMed, Tokyo, Japan), at 590 nm with a reference filter of 620 nm as reported previously (33). MTT assays were carried out in triplicate. To analyze the growth-suppression effects of mAb 220-51 in SCLC lines, cells (4 × 10^4 cells/well) were prepared in 96-well plates in serum-containing medium and treated with 40 μg/ml of mAb 220-51 for 3 days and then used for the MTT assay. In this case, lysis buffer (n-propylalcohol with 0.1% NP40 and 4 mM HCL) was added to each well without aspiration of the medium.

**In Vitro Invasion Assay.** The Boyden chamber in vitro invasion assay was performed as described previously (34) with some modifications. In brief, Matrigel (Becton Dickinson) was diluted with ice-cold PBS (250 μg/ml), added to each filter (190 μg/filter; polyethylene terephthalate membrane, 8-μm pore size, 23.1 mm in diameter; Falcon 3093) and left to polymerize and dry overnight. The polymerized and dried Matrigel membranes were reconstituted with serum-free medium. The lower chamber (6-well plate; Falcon 3502) was filled with serum-free medium (3 ml) before the chamber was assembled. Cells (1 × 10^6 cells/well) were added to serum-free medium in the upper chamber and incubated for 18 h in a humidified 5% CO2 atmosphere at 37°C. After incubation, the cells on the upper surface of the filter were removed completely by wiping with a cotton swab. The filters were fixed in ethanol and stained with Giemsa (Wako). The number of cells migrating to the lower surface of the filter was counted in 10 fields under ×200 magnification, and the mean of the number in each field was calculated. Assays were carried out in triplicate.

**Western Immunoblotting.** Cells (8 × 10^5 cells) were plated in 60-mm tissue culture plates in serum-containing medium and then treated with either 40 or 60 μg/ml of mAb 220-51 for the appropriate times in the individual experiments. After treatment, cells were harvested with 0.02% EDTA, washed twice with ice-cold PBS, and solubilized in lysis buffer [20 mM Tris-HCl (pH 7.4), 1% NP40, 10% glycerol, 50 mM NaF, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, and 20 units of aprotinin]. Soluble proteins (50 μg/lane) were subjected to either 10 or 15% SDS-PAGE and then transferred onto a polyvinylidene difluoride membrane (Millipore). The membrane was incubated with 5% dry milk in PBS overnight at 4°C, washed with PBS containing 0.05% Tween 20, and then incubated with antibodies reactive with either MAPK, phospho-MAPK (New England Biolabs), or active caspase-3 (Pharmingen). Bands were detected with peroxidase-conjugated antirabbit IgG (New England Biolabs) combined with an ECL kit (Amersham Pharmacia Biotech).

**Analysis of Apoptotic Cells.** Cells (6 × 10^5 cells) were plated in 60-mm tissue culture plates in serum-containing medium and then treated with either 20 μg/ml of various anti-GD2 mAbs or 20 μg/ml of irrelevant mAbs (10-11 and R24) for 24 h at 37°C. For analysis of the time course and dose dependency of the antibody effects, cells were treated with mAb 220-51 diluted appropriately. After treatment, cells were harvested with trypsin/EDTA, resuspended in binding buffer [10 mM HEPES/NaOH (pH 7.4), 140 mM NaCl, and 2.5 mM CaCl2], and then incubated with FITC-conjugated Annexin V and 1 μg/ml PI (Annexin V-FITC kit; Bender Medsystems), according to the manufacturer’s protocol. The numbers of apoptotic cells were monitored with a FACScan and determined using the CELLQuest program. For analysis of sub-G1 DNA content, cells (1.5 × 10^6 cells) were cultured with or without 100 μM z-VAD-fmk (Peptide Institute, Inc., Osaka, Japan) in the presence of mAb 220-51 (30 μg/ml). After 48 h, cells were fixed with 70% ethanol and incubated with 4 μM phosphate-citrate buffer (4 μM citric acid, 192 mM Na2HPO4) for 30 min at room temperature. After centrifugation, cells were resuspended in PBS containing PI/RNase (10 μg/ml each) and incubated for 20 min at room temperature. Quantification of sub-G1 DNA content was determined using the CELLQuest program.

**DNA Fragmentation Assay.** Cells (3 × 10^6 cells) were treated with 60 μg/ml of mAb 220-51. After 48 h, cells were harvested and lysed in 100 μl of lysis buffer [10 mM Tris-HCl (pH 7.4), 10 mM EDTA, and 0.5% Triton X-100] for 10 min at 4°C. After centrifugation, the supernatants were collected, and 2 μl of RNase (10 mg/ml) and 2 μl of Proteinase K (10 mg/ml) were added. After incubation for 1 h at 37°C, the fragmented DNA was precipitated in 2-propanol and electrophoresed at 50 V for 1.6 h on a 2% agarose gel containing 0.2 μg/ml ethidium bromide in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). The gel was observed under UV light.

**Statistical Analysis.** Significance was determined using the Student t test.
RESULTS

Ganglioside Typing of Lung Cancer Cell Lines. Expression of gangliosides in 44 lung cancer cell lines was analyzed by flow cytometry (Fig. 1, A–D). G_{M2} and G_{M1}, belonging to the a-series gangliosides, were expressed at significant levels in almost all of the lung cancer cell lines, whereas the b-series gangliosides, such as GD2, GD1b, and GT1b, were found characteristically in SCLC lines. These b-series gangliosides were not expressed or were expressed at very low levels, if present, in non-SCLC lines. A bronchial epithelial cell line, BEAS-2B, expressed no or very low levels of gangliosides except for G_{M1} (Fig. 1E).

Gene Expression of Glycosyltransferases in the Lung Cancer Cell Lines. To analyze the correlation between the expression levels of gangliosides and those of glycosyltransferase genes, expression levels of the GD3 synthase gene and the G_{M2}/GD2 synthase gene were measured with a quantitative real-time RT-PCR analyzer system (PE Biosystems). The expression level of the GD3 synthase gene was well correlated with that of b-series gangliosides, i.e., high levels of the G_{M3} Synthase gene expression were shown in the SCLC cell lines (Fig. 2 and Fig. 3A). As for the G_{M2}/GD2 synthase gene, its expression levels were well correlated with those of complex gangliosides (Fig. 3B). This gene was broadly expressed in all of the lung cancer lines examined, whereas SCLC lines showed slightly higher expression levels.

Remodelling of Ganglioside Expression by Gene Manipulation. To analyze the effect of b-series gangliosides dominantly expressed in the SCLC cell lines on malignant cancer phenotypes, a cDNA expres-
sion vector of G_{D3} synthase was introduced into a SCLC line, SK-LC-17, which did not express b-series gangliosides. Newly established transfectant cell lines showed definite and high expression of b-series gangliosides in the flow cytometric analysis (Fig. 4A). In contrast, G_{M2} and G_{M1} levels were reduced dramatically because of the shift of the synthetic flow from a-series to b-series. Control lines transfected with a vector alone showed no change in the ganglioside profile compared with the parent cell, i.e., G_{M2} and G_{M3} expressions were observed (Fig. 4A). These findings were confirmed by TLC of ganglioside fractions extracted from the individual cell lines (Fig. 4B).

Changes in Phenotypes of G_{D3} Synthase Gene Transfectant Cells. In the MTT assay, the transfectant cells expressing b-series gangliosides exhibited significantly enhanced proliferation compared with control lines (Fig. 5A). Cell growth, as presented by absorbance at 590 nm, was ~2-fold or greater after 4–6 days of culture. Invasion activity as analyzed by the Boyden chamber method was markedly increased in the transfectant lines, i.e., 483 ± 117 cells were found for the transfectants and 98.7 ± 26.3 cells in the controls, which had penetrated the Matrigel layer and come out of the reverse side of the filter (Fig. 5, B and C).

Suppression of Cell Growth by Anti-G_{D2} Antibodies. Effects of anti-G_{D2} mAbs on cell growth were then examined by adding antibodies to the culture medium. The increased cell growth after G_{D3} synthase gene transfection was strongly suppressed in the presence of an anti-G_{D2} mAb 220-51. The suppression effects were dependent on the concentration of the added antibody and became significant, even at 5 μg/ml on day 3 (Fig. 6A). Activation (phosphorylation) levels of MAPK were also reduced along with the time course after antibody addition (Fig. 6B). In vector control cells, neither suppression of cell proliferation nor reduction in MAPK activation were observed in MAPK activation (Fig. 6, A and B). These suppression effects of anti-G_{D2} mAb on the cell growth were also detected in SCLC lines (NCI-N417 and ACC-LC-171; Fig. 6C), which primarily expressed high levels of G_{D2} but not in a non-G_{D2}-expressing line, ACC-LC-170 (data not shown). Other anti-G_{D2} mAbs than 220-51, including KM666 (mouse mAb) and KM1138 (mouse-human chimeric mAb), also showed significant and similar growth suppression activity for the transfectant cells (Fig. 7). The nonspecific mouse IgG3 fraction showed no effects (data not shown).

Apoptosis Induction by Anti-G_{D2} mAbs. To clarify the induction of apoptosis during the growth suppression of SCLC cell lines with anti-G_{D2} mAbs, double staining of cells with Annexin V (FITC) and PI was performed. Transfectant lines treated with anti-G_{D2} mAb were 72.2% positive for Annexin V binding and 76.9% positive for PI staining at 24 h after the addition of mAbs (Fig. 8A), indicating the induction of apoptotic cell death. A vector control line, C-8, showed no staining for both reagents. The transfectant cells treated with anti-G_{D2} mAb showed marked shrinkage of cytoplasm and formation of spaces between cells, whereas vector control cells showed no change (Fig. 8B). When other anti-G_{D2} mAbs were added, similar morphological changes (data not shown) and double staining with Annexin V and PI were found (Fig. 9A). However, nonrelevant antibodies, such as anti-G_{M2} mAb or anti-G_{M3} mAb, did not induce these changes (Fig. 9B). During the time course, the Annexin V-positive population increased after 0.5 h, and then the PI-positive fraction expanded after 1 h incubation (Fig. 10, A and B). This apoptosis induction depended on the antibody concentration (Fig. 10C). To investigate the sensitivity to the apoptosis induction with the anti-G_{D2} mAb in cell lines with various levels of G_{D2} expression, G_{D2} expression levels and the percentage of positive Annexin V and PI after the antibody treatment were compared in six lung cancer cell lines. Not only high G_{D2} expressants such as ACC-LC-171 and NCI-N417 but also those with moderate G_{D2} levels showed apparent apoptosis (Fig. 11). To confirm the DNA degradation in the anti-G_{D2} mAb-treated cells, cytoplasmic DNA was prepared from 3 x 10^6 cells and used for agarose gel electrophoresis. Only cells treated with the mAb showed definite DNA ladder formation (Fig. 12A) and also activation of caspase-3 (Fig. 12B). This apoptotic process was partially inhibited by a caspase inhibitor, z-VAD-fmk (Fig. 12C), which suggested that apoptosis induced by anti-G_{D2} mAb was mediated via the caspase-3-dependent pathway and some independent pathway.

DISCUSSION

Despite much effort to elucidate the biological functions of glycosphingolipids, few definite molecular functions have been demonstrated to date. Current ways to modulate carbohydrate structures on cells have provided only indirect information about the roles of glycoconjugates, because methods to modulate carbohydrate chains harbor substantial limitations in the specificity of their effects. Successful cloning of glycosyltransferase genes dramatically changed this situation and improved the limitations in the analysis of carbohydrate functions. In particular, application of gene knockout technology has clearly demonstrated in vivo functions of products of individual glycosyltransferases (35), although the expected outcome has not necessarily been obtained because of various unknown reasons.
Remodeling of ganglioside profiles in cultured cells has shown their roles in the regulation of cell proliferation and differentiation (36, 37). Then, gene targeting of galactosyl-ceramide synthase revealed GalCer functions in the stability of myelin (38) and spermatogenesis (39). GM2/GD2 synthase gene knockout mice lacking complex gangliosides exhibited their roles in the regulation of the nervous system (40), spermatogenesis (41), and T-cell functions (42). However, roles of gangliosides in various malignant phenotypes in tumors have never been clearly demonstrated by glycosyltransferase gene manipulation, although many glycolipid antigens have been considered as tumor markers or have been used as target molecules of antibody therapy (9, 10).

GD2 studied in this paper is one of the melanoma-associated glycolipid antigens (8). In particular, GD2 is up-regulated in advanced and vertically metastatic melanoma cells (43), suggesting that GD2 plays roles in malignant features of melanomas such as metastasis (44). Actually, GD2 and GD3 have been considered to modify integrin functions (45). Moreover, an anti-GD2 immune reaction against melanoma cells in melanoma patients was reported by Watanabe et al. (46) and Cahan et al. (47). These findings suggest that GD2 is one of the target molecules for immunotherapy of melanoma patients. In addition to melanoma, GD2 has been considered to be a neuroblastoma-associated antigen, and anti-GD2 mAb has been tried in a therapy for neuroblastoma patients (48). We also reported previously the specific expression of GD2 on human T-cell lymphotrophic virus type I-infected lymphocytes under regulation of p40ras transactivator (14). Despite a number of studies indicating the tumor-specific expression or malignant phenotype-associated expression of GD2, no definite studies to demonstrate the significant roles of GD2 in tumor cell proliferation have been reported. The findings demonstrated in the
present study strongly suggest that GD2 actually exerts important roles to enhance cellular proliferation signals as shown in Fig. 6B.

Kasahara et al. (49) reported recently that GD3 on primary cultured cerebellar neurons mediated activation of a Src family tyrosine kinase, lyn, in cooperation with the TAG-1 molecule. Fukumoto et al. (37) reported that introduction of the GD3 synthase gene into the rat pheochromocytoma cell line PC12 resulted in the continuous activation of nerve growth factor receptor TrkA and its downstream signal molecule, MAPK. These alterations in the signal molecules appeared to induce a marked enhancement of cell proliferation. These findings suggest that GD2 on the cell surface of SK-LC-17 is associated with some growth factor receptors and modulates their functions, resulting in an observed increase in cell growth. Identification of GD2-associated receptors or signal molecules remains to be investigated.

Apoptosis induced by anti-GD2 mAbs is very interesting and provides a possibility for the application in immunotherapy of SCLC. Gangliosides, including GD2, are expressed on the outer layer of plasma membrane; therefore, it is unclear how GD2 can mediate the apoptosis signal triggered by the binding of anti-GD2 mAb. Binding of anti-GD2 mAb to GD2 might cause clustering of GD2 molecules, resulting in the modulation of unknown neighboring molecules and in the activation of subsequent intracellular molecules that emit a signal for apoptosis. No studies have been performed on the induction of apoptosis of tumor cells using anticycolipid mAbs except for anti-GM2 mAb by Nakamura et al. (50). However, they showed just a decrease of tumor cell layer volume in multicellular heterosperoids after treatment with anti-GM2 mAb. Apoptosis of SCLC cells induced by anti-GD2 mAb, as demonstrated in the present study, should be the first example in which apoptosis induction was definitely shown using anticycolipid mAbs. The molecular mechanisms for this apoptosis are now under investigation in our laboratory and should provide information necessary for the application of anti-GD2 mAbs in therapies against drug-resistant malignant tumors.

Although this study was performed with lung cancer cell lines, GD2 expression in tumor specimens of SCLC has been reported (23), and successful in vivo detection of SCLC tumor sites with anti-GD2 mAbs was also reported (27), suggesting that GD2 in SCLC is worth being focused on as a target of chemoinmunotherapy. Of course, many devices in the modification of antibodies, in the modes of antibody treatment, e.g., in combination with vaccines in the adjuvant setting (11), or in the controlling of side effects (51) remain to be achieved.

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REFERENCES


Fig. 12. Apoptosis induction by caspase-3. A, cytoplasmic DNA prepared from D-18 after treatment with (Lane 2) or without (Lane 1) 60 μg/ml of mAb 220-51 for 48 h was analyzed by agarose gel electrophoresis. B, activation of caspase-3 after treatment with anti-GD2 mAb. D-18 was treated with 60 μg/ml of mAb 220-51 for the time indicated and then used for 15% SDS-PAGE and immunoblotting with an antiaactive caspase-3 antibody, as described in “Materials and Methods.” C, partial inhibition of apoptosis by a caspase inhibitor. D-18 was cultured with (Lane 2) or without (Lane 1) 100 mM z-VAD-fmk in the presence of mAb 220-51 (30 μg/ml) for 48 h. After treatment, the sub-G1 DNA content was measured by flow cytometry, as described in “Materials and Methods.”


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