[CANCER RESEARCH 56, 5165–5171, November 15, 1996]

Gangliosides Expressed in Human Breast Cancer

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ABSTRACT

Breast tumors that were histopathologically diagnosed as invasive ductal carcinoma were examined in relation to their abnormal expression of gangliosides. Total ganglioside levels that were expressed as lipid-bound sialic acids were significantly higher in breast tumor tissues than in normal mammary tissues. Two kinds of unusual gangliosides were found to be expressed in many cases of breast tumors. One was a group of *O*-acetylated gangliosides, such as *O*-acetyl-GD3 and *O*-acetyl-GT3. They are known as fetal gangliosides, which appear in fetal brains. The other was an *N*-glycolylneuraminic acid-containing ganglioside, *N*-glycolyl-GM3, which had not been previously found in normal human tissues. The finding that unusual gangliosides are expressed in breast tumors may provide the basis for their immunological diagnosis and vaccine therapy.

INTRODUCTION

Gangliosides are mostly localized on plasma membranes with their sialylated sugar chains protruding out of cells. They serve as specific antigens in immunoreactions and receptors for ligands in cell adhesion. It has been demonstrated by Hakomori (1) that the expression of gangliosides is affected in various ways by malignant transformation. This is very relevant to uncontrolled cell growth because some species of gangliosides have been shown to modulate growth factor receptors (2). From this point of view, many studies have revealed tumor-specific expression of gangliosides in various cancers. Gangliosides expressed in melanoma have been studied the most intensively (3–7), and those in others, such as gastrointestinal cancers (8–10), lung cancers (11, 12), lymphomas (13), and neuroblastomas (14–17), also have been examined. On the other hand, gangliosides expressed in breast cancer have received little attention except for the studies by Dyatlovitskaya *et al.* (18) and Wiesner and Sweely (19). They reported the composition of major gangliosides in breast tumor tissues and sera of breast cancer patients but did not report tumor-specific gangliosides. The present study was undertaken to search for any unusual gangliosides that are expressed in breast tumors that have been identified as IDC.[2] In this study, in addition to higher levels of total gangliosides, two unusual species of sialic acids in gangliosides, *N*-glycolylneuraminic acid and *O*-acetyl-*N*-acetylneuraminic acid, have been found in the tumor tissues by chemical and immunochemical methods.

MATERIALS AND METHODS

Human Tissues. Breast cancer tissues were obtained from patients who were operated on at the Department of Breast Oncology, National Oncology Institute, Havana, Cuba. Among these tissues, malignant tumors (n = 37) histopathologically diagnosed as IDC were subjected to the present examination. Normal mammary tissues (n = 6) were obtained from healthy women who died in accidents. These were autopsied at the Institute of Legal Medicine, Havana, Cuba. Pooled breast tumors identified as IDC were provided by the Hormonal Receptors Department, National Oncology Institute, Havana, Cuba.

Preparation of Gangliosides. The extraction of total lipids and the preparation of gangliosides were carried out according to our previous method applied to brain tissues (20). The present procedure for mammary tissues is described below. A tissue sample (0.3–6.7 g) was homogenized with 2 volumes of water. The homogenate was mixed with 5 volumes of chloroform/methanol (2:1) and incubated at 37°C for 1 h. The ratio of the chloroform/methanol in the mixture was adjusted to 1:1 by addition of methanol, and the suspension was centrifuged at 1000 × g for 10 min. After the supernatant was removed as the extract, the residue was reextracted with 10 volumes of chloroform/methanol/water (1:2:0.8) at 37°C for 2 h. The two extracts were combined, and the solvent was evaporated to give total lipids. The lipidic residue was redissolved in 5 ml of chloroform/methanol (9:1) with sonication and applied to a phenyl-Sepharose CL-4B column (Pharmacia, Uppsala, Sweden; bed volume, 2 ml). The column was filled with a suspension of phenyl-Sepharose in chloroform/methanol (1:2) and then equilibrated with chloroform/methanol (9:1). Most of neutral lipids and phospholipids were washed out with 6 ml of chloroform/methanol (9:1) and 6 ml of chloroform/methanol (85:15). Gangliosides were then eluted with 10 ml of chloroform/methanol (1:1) and 10 ml of methanol. The solvent was removed by a stream of nitrogen. The samples were redissolved in a known volume of chloroform/methanol (1:1). An aliquot of the fraction was assayed for sialic acid content by means of GC/MS. To further purify the ganglioside fractions for TLC, the samples were redissolved in 0.3 ml of chloroform/methanol/water (5:5:1) and applied to a Toyopearl HW-40C column (Tosoh, Japan; bed volume, 10 ml). After the removal of a void volume (3 ml), gangliosides were recovered in the next 3 ml. The samples were redissolved in a known volume of chloroform/methanol (1:1) and stored at −20°C.

Sialic Acid Determination by GC/MS. A ganglioside fraction was methanolized with 1 ml of 0.5% methanolic HCl at 100°C for 2 h to liberate sialic acid as its derivative. The reaction mixture was dried under a nitrogen stream. A known amount of phenyl-α-*N*-acetylglucosaminide (Sigma Chemical Co., St. Louis, MO) was added as an internal standard, and the sample was peracetylated with 0.1 ml of a mixture of pyridine/acetic anhydride (1:1, v/v) at 100°C for 30 min. Excess acetic anhydride was degraded by adding methanol. After evaporation, the sample was redissolved in 100 μl of chloroform, and then a few μl of the sample were applied to GC/MS. A JMS-DX 304/304 mass spectrometer (JEOL, Tokyo, Japan) and a gas chromatograph 5890A (Yokogawa Hewlett-Packard, Tokyo, Japan) equipped with an OV-17 capillary column (0.25 mm internal diameter × 10 m; Quadrex, New Haven, CT) were used. The SIM method was used to measure the fragment ions derived from the specific molecules using the electron impact ionization mode. Ionization voltage and current were 70 electron volt (eV) and 100 μA, respectively. Column oven temperature was 228°C. The ion strengths of fragments m/z 446 and m/z 330 were traced to detect sialic acid and the internal standard, respectively. Calibration curves were drawn using an authentic ganglioside mixture consisting of GM1, GD1a, GD1b, GT1b, and GQ1b (20).

Determination of Sialic Acid Species by GC/MS. An aliquot of total gangliosides was taken and was hydrolyzed in 100 μl of 2 M acetic acid at 80°C for 1 h. The sample was lyophilized, and a known amount of phenyl-α-*N*-acetylglucosaminide was added as an internal standard. The sialic acid released was trimethylsilylated. The sample was subjected to GC/MS analysis using SIM by the electron impact ionization mode. An OV-17 capillary column (0.25 mm internal diameter × 10 m) was used, and column oven temperature was maintained at 200°C. Fragment ions of m/z 356, m/z 388, and m/z 420 were

Received 6/10/96; accepted 9/19/96.

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[2] The abbreviations used are: IDC, invasive ductal carcinoma; GC/MS, gas chromatography-mass spectrometry; SIM, selected ion monitoring; HPTLC, high-performance TLC; aq., aqueous; TBS, Tris-HCl-buffered saline; H-D, Hanganutziu and Deicher; FAB/MS, fast atom bombardment mass spectrometry; DEAE, diethylaminoethyl.

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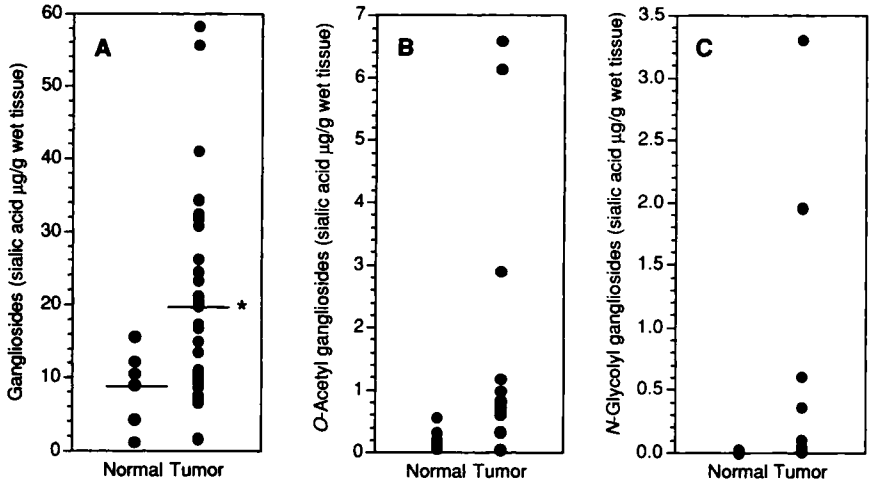

Fig. 1. Contents of total gangliosides (*A*), *O*-acetyl gangliosides (*B*), and *N*-glycolyl gangliosides (*C*). Gangliosides were prepared from total lipids using a phenyl-Sepharose column. Ganglioside contents are expressed as lipid-bound sialic acid. The levels of sialic acid were determined using SIM by the GC/MS method. ●, individual measurements. *A*, total ganglioside contents. The mean values of normal and tumor tissues (*horizontal lines*) are 8.8 ± 5.3 μg/g of wet tissue (mean ± SD, $n = 6$) and 19.7 ± 13.0 μg/g of wet tissue ($n = 37$), respectively. *, statistical significance ($P < 0.05$, two-tailed Student's *t* test). *B*, levels of *O*-acetyl gangliosides (normal tissues, $n = 6$; tumor tissues, $n = 12$). *C*, levels of *N*-glycolyl gangliosides (normal tissues, $n = 6$; tumor tissues, $n = 12$).

used to quantitate *O*-acetyl-*N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, and the internal standard, respectively. Calibration curves were made using an authentic ganglioside mixture containing *N*-acetylneuraminic acid, *O*-acetyl-*N*-acetylneuraminic acid, and *N*-glycolylneuraminic acid.

Quantitative TLC of Gangliosides. Ganglioside composition was determined by TLC-densitometry (21). Ganglioside samples were applied on a HPTLC plate (E. Merck, Darmstadt, Germany) using an automatic applicator (Automatic TLC Sampler III; CAMAG, Muttenz, Switzerland). The plate was developed with chloroform/methanol/0.2% aq. $CaCl_2$ (50:40:11) or chloroform/methanol/2.5 N ammonium hydroxide (65:35:8). Standard samples containing known amounts of gangliosides were developed in parallel with the test samples. Gangliosides were visualized with the resorcinol-HCl reagent (22) and then determined at 580 nm using a Shimadzu CS-9000 flying spot scanner (Shimadzu, Kyoto, Japan).

TLC-Immunostaining of Gangliosides Containing *O*-Acetyl Sialic Acid and *N*-Glycolylneuraminic Acid. Immunostaining of gangliosides on HPTLC plates was performed by a modification of the method of Saito *et al.* (23). A HPTLC plate was developed with the chloroform/methanol/0.2% aq. $CaCl_2$ (50:40:11) for staining *O*-acetylated gangliosides or with chloroform/methanol/2.5 N ammonium hydroxide (65:35:8) for staining *N*-glycolylneuraminic acid-containing gangliosides. The developed plate was dried *in vacuo* for 20 min and dipped in 0.4% polyisobutylmethacrylate in a solution of chloroform/*n*-hexane (16:84) for 30 s, and then the organic solvent was removed from the plate. For detecting *O*-acetylated gangliosides, the plate was covered with monoclonal antibodies GMR2 (24) or 493D4 (established by immunizing mice with mouse embryonic membranes) and incubated for 3 h at room temperature. The antibodies were diluted with TBS, pH 7.4, containing 0.3% gelatin. The plate was washed with TBS and then covered with a solution of horseradish peroxidase-conjugated antimouse IgM. The enzyme-linked second antibodies were diluted with TBS containing 0.3% gelatin and 5% skim milk. The plate was incubated for 90 min and washed with TBS. The plate was then incubated with ECL Western blotting detection reagents (Amersham, Buckinghamshire, England), and chemiluminescence was recorded on an X-ray film. For detecting *N*-glycolylneuraminic acid-containing gangliosides, the samples were reacted with H-D antibody (25, 26) or monoclonal antibody P3 (27) raised against *N*-glycolyl-GM3 and then treated with horseradish peroxidase-conjugated antichicken IgG against H-D antibody or horseradish peroxidase-conjugated antimouse IgM against P3 in the same way as described above.

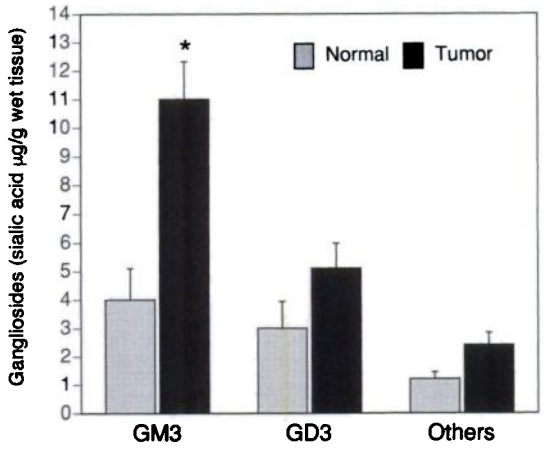

Fig. 2. Distribution of lipid-bound sialic acid in the major gangliosides. Values are means ± SE (normal tissues, $n = 6$; tumor tissues, $n = 29$). $P < 0.05$ compared to normal tissues (two-tailed Student's *t* test). *Others*, minor gangliosides, including GD1a and GT1b.

Isolation of *O*-Acetylated Gangliosides from Pooled Breast Tumor Tissues. Total lipids were extracted from 82.6 g of tumor tissues with chloroform/methanol (2:1), followed by chloroform/methanol/water (1:2:0.8). After evaporation of solvent, total lipids were suspended in a small volume of methanol and water and dialyzed against water for 3 days. The lyophilized sample was dissolved in chloroform/methanol/water (30:60:8), and applied to a DEAE-Toyopearl 650M column (Tosoh, Tokyo, Japan; bed volume, 150 ml; Ref. 28). Neutral lipids were washed out with chloroform/methanol/water (30:60:8). Acidic lipids were eluted with chloroform/methanol/0.4 M aq. sodium acetate (30:60:8), followed by chloroform/methanol/0.8 M aq. sodium acetate (30:60:8). The sample was dialyzed and then lyophilized. GD3 and *O*-acetyl-GD3 were isolated from the acidic lipid fraction using a Q-Sepharose column (Pharmacia, Uppsala, Sweden; bed volume, 10 ml) with a solvent system of chloroform/methanol/aq. sodium acetate solution (30:60:8; Ref. 29). The salt concentration in the aq. part was linearly increased from 0 to 3 M. The samples were desalted by Toyopearl HW-40C column chromatography.

Isolation of a Ganglioside Containing *N*-Glycolylneuraminic Acid from Pooled Breast Tumor Tissues. Total gangliosides were prepared from total lipids of tumor tissues (13.7 g) by phenyl-Sepharose column chromatography (bed volume, 15 ml; Ref. 20). The ganglioside fraction was subjected to a mild base treatment and then purified using a Toyopearl HW-40 column (bed volume, 10 ml; Ref. 20). *N*-Glycolyl-GM3 and *N*-acetyl-GM3 were isolated by preparative TLC. The sample was developed on a HPTLC plate with a solvent system of chloroform/methanol/2.5 N ammonium hydroxide containing 0.2% NaCl (50:40:10). The portions of silicic acid containing the gangliosides were scraped off from the plate and then gangliosides were extracted from silicic acid with chloroform/methanol/water (40:55:5) three times. The isolated samples were applied to a Toyopearl HW-40 column to remove salts and impurities.

FAB/MS. Gangliosides isolated from pooled tumor tissues were subjected to FAB/MS analyses in the negative mode using a JMS-DX 304/304 mass spectrometer. The sample was dissolved in DMSO to give a concentration of 5 μg/μl. A sample of 1 μl was mixed with triethanolamine as a matrix. Mass spectra were measured in the accumulation mode at 3 kV of acceleration voltage.

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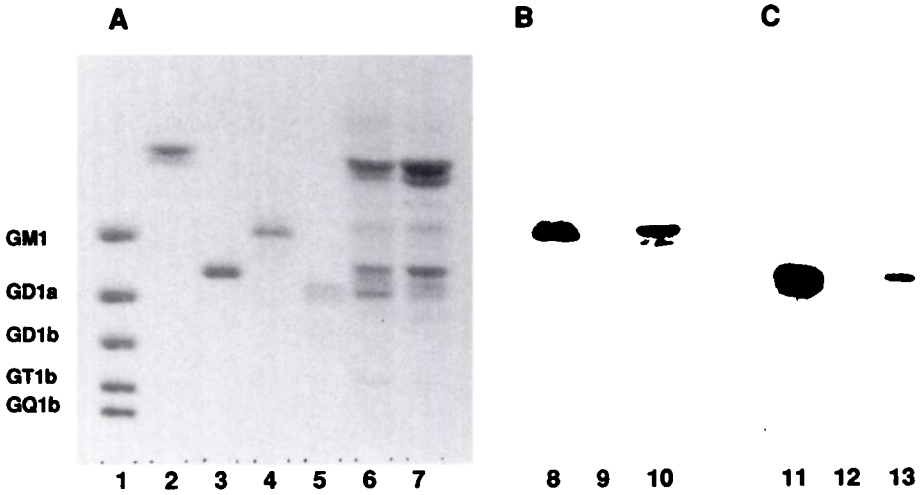

Fig. 3. Detection of *O*-acetyl ganglioside by TLC-immunostaining. A HPTLC plate was developed with chloroform/methanol/0.2% aq. $CaCl_2$ (50:40:11). After development, the plate was divided into three parts. *A*, gangliosides were visualized with resorcinol-HCl reagent. *B* and *C*, gangliosides were detected using GMR2 and 493D4, respectively. *Lane 1*, standard ganglioside mixture; *Lane 2*, GM3; *Lane 3*, GD3; *Lanes 4* and *8*, authentic *O*-acetyl-GD3; *Lanes 5* and *11*, authentic *O*-acetyl-GT3; *Lanes 6, 9*, and *12*, gangliosides of a normal tissue; *Lanes 7, 10*, and *13* are gangliosides of a tumor tissue.

RESULTS

Ganglioside Contents and Distributions in Normal and Cancerous Breast Tissues. Sialic acids were liberated from gangliosides by methanolysis and peracetylated. The sialic acid derivatives were determined by GC/MS using SIM. As shown in Fig. 1*A*, the ganglioside contents in cancerous breast tissues were found to be significantly higher than those in normal tissues (19.7 ± 13.0 μg sialic acid/g of wet tissue *versus* 8.8 ± 5.3 μg; $P < 0.05$). Eighteen of 37 tumor cases showed levels of gangliosides that were more than 2 SD higher than the mean in normal tissues.

The distribution of the lipid-bound sialic acid is shown in Fig. 2. GM3 and GD3 were the major gangliosides in mammary tissues and they accounted for 85–90% of the lipid-bound sialic acid in both normal tissues and tumor tissues. The levels of GM3 and GD3 in tumor tissues were 2.8-fold and 1.7-fold greater than those in normal tissues, respectively.

Occurrence of *O*-Acetyl-*N*-Acetylneuraminic Sialic Acids and *N*-Glycolylneuraminic Acid in Breast Cancer Gangliosides. *O*-Acetyl-*N*-acetylneuraminic acid-containing gangliosides or *O*-acetyl gangliosides were characterized by TLC-immunostaining using anti-*O*-acetyl ganglioside monoclonal antibodies. *O*-Acetyl gangliosides were detected by two different antibodies, GMR2 and 493D4, as shown in Fig. 3. *N*-glycolylneuraminic acids were shown to occur in the breast cancer gangliosides by positive reactions with H-D antibody and monoclonal antibody P3 (Fig. 4).

The gangliosides containing *O*-acetyl-*N*-acetylneuraminic acids or *N*-glycolylneuraminic acids were attempted to be definitely characterized. To obtain large enough amounts of samples to be analyzed by FAB/MS, *O*-acetyl-*N*-acetylneuraminic acid and *N*-glycolylneuraminic acid-containing gangliosides were isolated from pooled tumor tissues. One of them comigrated with authentic *O*-acetyl-GD3 on a HPTLC plate. This compound was stained with anti-*O*-acetyl-GD3 antibody (GMR2; Fig. 5*B*). It was easily converted by a base treatment to a compound that comigrated with authentic GD3 (Fig. 5*A*), suggesting the presence of an *O*-acetyl group. This compound was further examined by FAB/MS analysis. The quasi-molecular ion of this compound at *m/z* 1594 (Fig. 6*B*) was 42 mass units higher than that of GD3 isolated from pooled tumor tissues (Fig. 6*A*). This difference corresponds to 1 mol of acetate. Loss of the terminal sugar residue from this ganglioside [*i.e.*, −333 mass units (*O*-acetyl-*N*-acetylneuraminic acid)] yielded a fragment ion at *m/z* 1261, which was identical with monosialyl-dihexosylceramide. This spectrum indicates the presence of *O*-acetyl-*N*-acetylneuraminic acid situated at the terminal position of the disialyl residue. Another ganglioside containing an unusual neuraminic acid was isolated from pooled tumor tissues. This compound comigrated with authentic *N*-glycolyl-GM3 and was stained with H-D antibody and P3 (Fig. 7). This ganglioside has three molecular species, and their quasi-molecular ions in a FAB/MS spectrum were *m/z* 1279, 1251, and 1167 (Fig. 8*B*). They were 16 mass units larger than quasi-molecular ions of *N*-acetyl-

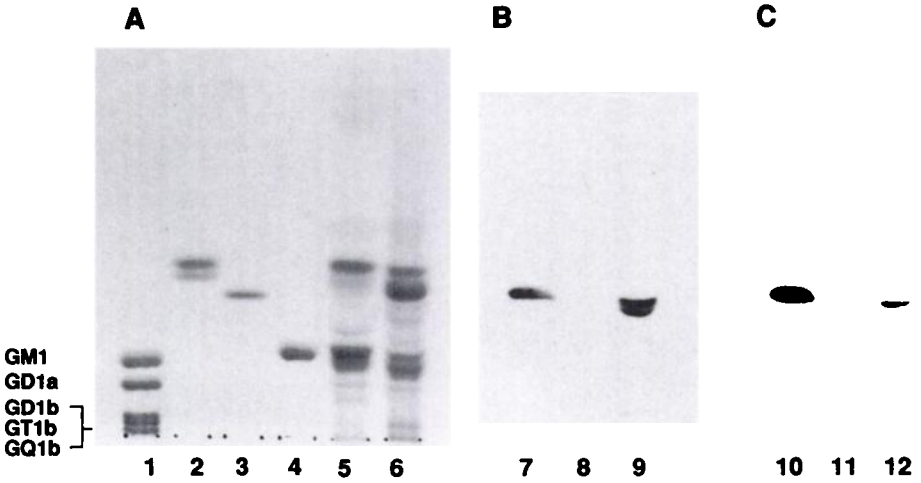

Fig. 4. Detection of *N*-glycolylneuraminic acid-containing ganglioside by TLC-immunostaining. A HPTLC plate was developed with chloroform/methanol/2.5 N ammonium hydroxide (65:35:8). After development, the plate was divided into three parts. *A*, gangliosides were visualized with resorcinol-HCl reagent; *B* and *C*, gangliosides were detected using H-D antibody and P3, respectively. *Lane 1*, standard ganglioside mixture; *Lane 2*, *N*-acetyl-GM3; *Lanes 3, 7*, and *10*, *N*-glycolyl-GM3; *Lane 4*, GD3; *Lanes 5, 8*, and *11*, gangliosides of a normal tissue; *Lanes 6, 9*, and *12*, gangliosides of a tumor tissue.

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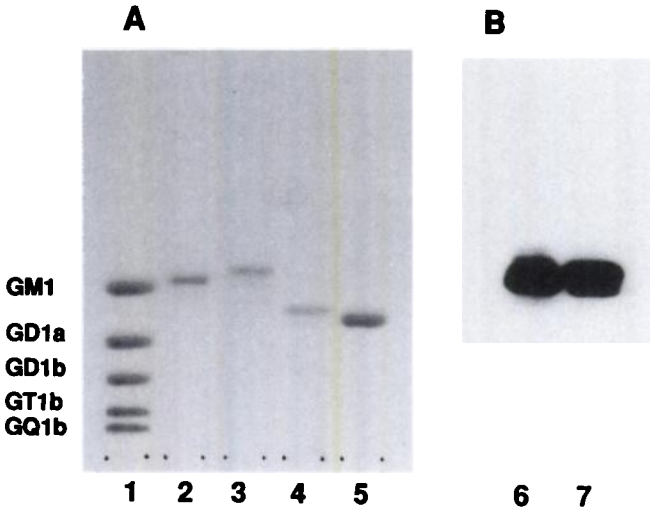

Fig. 5. Isolation of *O*-acetyl-GD3 from tumor tissues. An *O*-acetyl-ganglioside was isolated by Q-Sepharose column chromatography. *A*, gangliosides were visualized with resorcinol-HCl reagent; *B*, gangliosides were detected with GMR2. *Lane 1*, standard ganglioside mixture; *Lanes 2* and *6*, authentic *O*-acetyl-GD3; *Lane 5*, authentic GD3; *Lanes 3 and 7*, *O*-acetyl ganglioside isolated from tumor tissues; *Lane 4*, deacylated product of the tumor ganglioside.

GM3 isolated from pooled tumor tissues (Fig. 8*A*). Removal of the terminal sugar unit from this compound yielded *m/z* 972, 944, and 860, which were all identical to each of dihexosylceramides from *N*-acetyl-GM3. This spectrum indicates the presence of an *N*-glycolyl residue instead of an *N*-acetyl group.

The contents of sialic acid species found in breast tumor gangliosides are shown in Fig. 1 as compared with those in normal mammary tissue gangliosides. Three species of sialic acids, *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, and *O*-acetyl-*N*-acetylneuraminic acid, were determined by GC/MS. Some samples from tumor cases were not subjected to this measurement because of their limited sample sizes. Most samples of breast tumors contained levels of *O*-acetyl sialic acid (10 of 12 cases) and *N*-glycolylneuraminic acid (8 of 12 cases) that were higher than their normal ranges.

DISCUSSION

Various types of tumors have been identified on the basis of histopathological diagnosis. The existence of various types of tumors indicates that many different mechanisms are involved in malignant cellular differentiations. Therefore, biochemical data on tumor tissues that have not been histologically identified do not seem to be very meaningful. In this study, only breast cancer tissues that were histopathologically diagnosed as IDC, a major type of malignant breast tumor, were examined to determine whether there was a correlation between a particular type of cancer and the expression of cell surface ganglioside antigens.

As gangliosides are minor components in mammary tissues, good methods for their quantitative isolation and sensitive analysis are needed. We previously established a facile method for the isolation of gangliosides from brain tissues using a phenyl-Sepharose column (20). This method has successfully been applied to the present study on breast cancer gangliosides. The detailed conditions of phenyl-Sepharose column chromatography were modified for the case of mammary tissue because the ganglioside contents were extremely low compared with those in brain tissues. To quantitate gangliosides as the lipid-bound sialic acid, a reliable method using GC/MS was used (20). Colorimetric methods that have been widely used in the literature

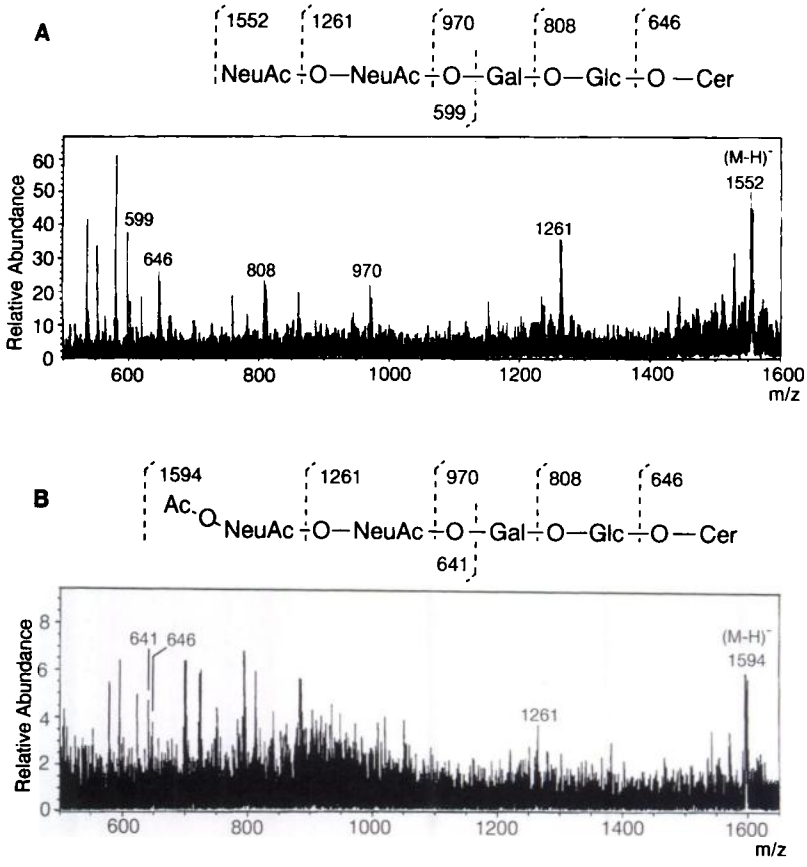

Fig. 6. FAB/MS spectra of *O*-acetylated ganglioside. Gangliosides were isolated from pooled tumor tissues. *A*, GD3; *B*, *O*-acetyl-GD3.

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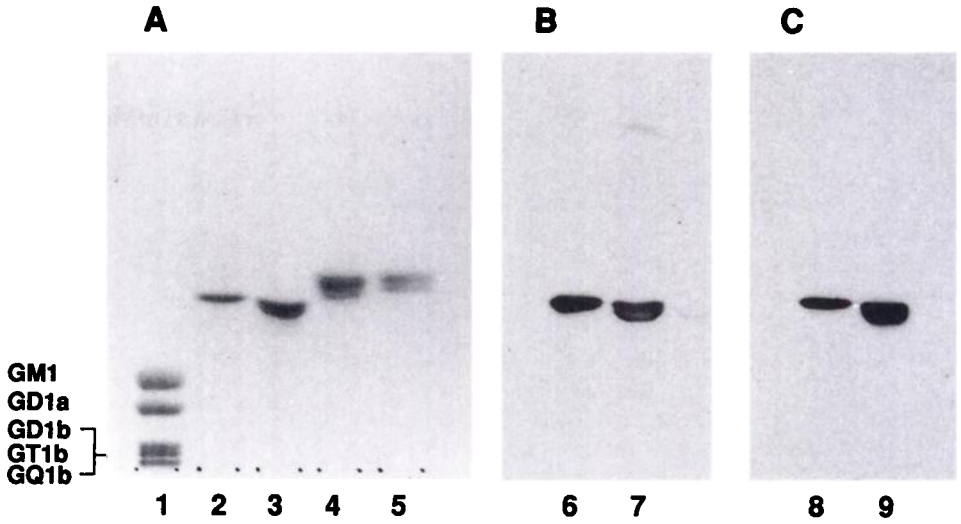

Fig. 7. Isolation of *N*-glycolyl-GM3 from tumor tissues. *N*-glycolyl-GM3 was isolated from tumor tissues by preparative TLC. *A*, gangliosides were visualized with resorcinol-HCl reagent; *B* and *C*, gangliosides were detected with H-D antibody and P3, respectively. *Lane 1*, standard ganglioside mixture; *Lanes 2, 6,* and *8*, authentic *N*-glycolyl-GM3; *Lane 5*, authentic *N*-acetyl-GM3; *Lanes 3, 7,* and *9*, *N*-glycolyl-GM3 isolated from tumor tissues; *Lane 4*, *N*-acetyl-GM3 isolated from tumor tissues.

were not applicable to this purpose because they easily produced false color and were not sufficiently sensitive. Our analytical method made it possible to specifically detect sialic acids in the presence of many other components because of the high specificity of the SIM method. Another method for the determination of three different sialic acid species at once using GC/MS was developed in this study. The present method enables us to analyze *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, and *O*-acetyl-*N*-acetylneuraminic acid in one run. Kawai *et al.* (30) reported a method that was similar to ours, but their method could measure only the former two neuraminic acids because *O*-acetyl group was eliminated by methanolysis.

The quantitation of gangliosides revealed that the average level was significantly higher in breast cancer tissues than in normal tissues (Fig. 1*A*). However, ganglioside levels in one-half of the tumor cases remained in the normal range, whereas levels in the other cases were far above that. Merritt *et al.* (31) and Kloppel and Morré (32) reported increased levels of gangliosides in rat hepatomas compared with normal liver tissues. Kawai *et al.* (30) indicated that the ganglioside contents in glycosphingolipid fractions were elevated in liver, lung and gastric cancers. An increase in the ganglioside content seems to be a characteristic of transformed cells.

The major gangliosides in breast cancer tissues were GM3 and GD3, as in normal tissues. The average levels of GM3 and GD3 in tumor tissues were 2.8-fold and 1.7-fold higher than the normal levels (Fig. 2). Our results were similar to those of Dyatlovitskaya *et al.* (18). In melanoma, it is known that GD3 and GD2 are expressed to greater

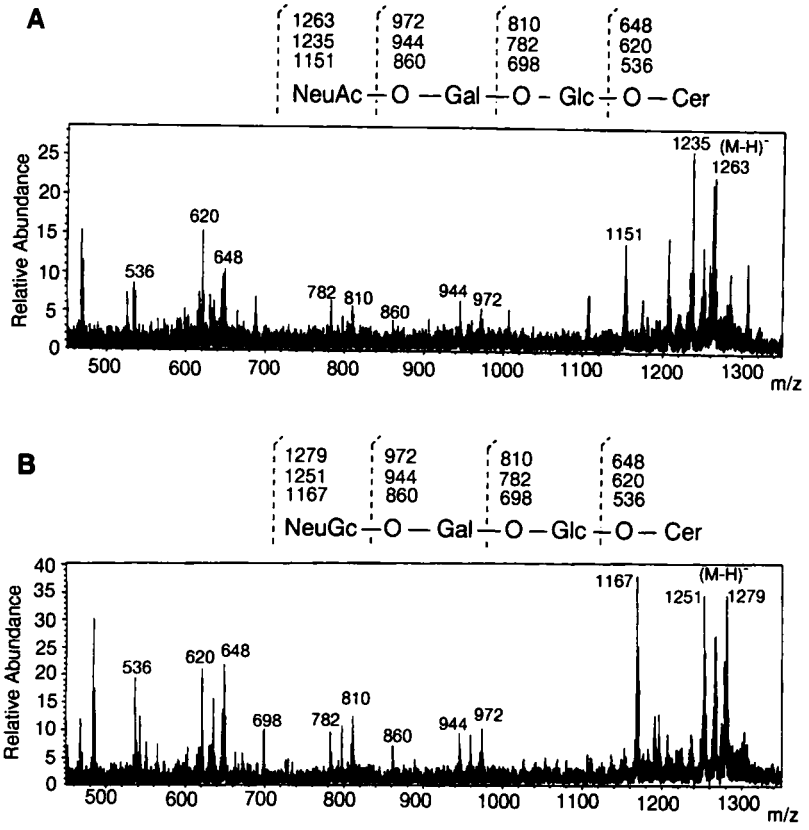

Fig. 8. FAB/MS spectra of *N*-glycolylneuraminic acid containing ganglioside. Gangliosides were isolated from pooled tumor tissues. *A*, *N*-acetyl-GM3; *B*, *N*-glycolyl-GM3.

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extents than in melanocytes (4–7). Ravindranath *et al.* (6) reported that a decrease in the GM3/GD3 ratio was correlated with the prognoses of melanoma patients. Zheng *et al.* (33) showed that GM3 modulated an integrin receptor in a mouse mammary carcinoma cell line, and Cheresh *et al.* (34) revealed that the vitronectin receptor formed a complex with GD3 or GD2. High expressions of GM3 and GD3 in breast cancers might affect the adhesive property of transformed cells.

In the present study, two ganglioside species containing unusual sialic acid derivatives were found in breast tumor tissues. One was the *O*-acetyl ganglioside species. *O*-Acetyl-GD3 and *O*-acetyl-GT3 expressed in breast tumor tissues were detected by monoclonal antibodies as shown in Fig. 3. The chemical structures of both gangliosides from other sources were already established by three groups, including us (28, 35, 36). *O*-Acetyl-GD3 isolated from pooled breast tumor tissues was analyzed by FAB/MS and TLC after a base treatment as well as by its immunoreactivity with a specific antibody against *O*-acetyl-GD3. In the present study, using a newly developed method, we determined the contents of *O*-acetylated sialic acid as shown in Fig. 1*B*. *O*-Acetyl gangliosides have been reported to exist in other tumors: *O*-acetyl-GD3 in melanomas (3, 6, 7) and *O*-acetyl-GD2 in neuroblastomas (17). *O*-acetylated gangliosides have been reported to occur in fetal mammalian brains as a fetal antigen (37–39). Hirabayashi *et al.* (38) and Mendez-Otero *et al.* (37) revealed that these gangliosides were expressed at high levels in murine embryonic brain and decreased in the postnatal period. *O*-Acetylation of gangliosides in tumor tissues may show retrogenetic expression to a fetal developmental stage. The other unusual ganglioside species contained *N*-glycolylneuraminic acid. In breast tumor tissues, *N*-glycolyl-GM3 was detected by its reactions with specific antibodies as shown in Fig. 4. The chemical nature of this ganglioside isolated from tumor tissues was characterized by FAB/MS. The occurrence of this species in other cancer tissues was reported in several cases such as colon cancer (8), melanoma (40), germ cell tumor (41), and others (32). Quantitative data on the contents of *N*-glycolylneuraminic acid in the total gangliosides are shown in Fig. 1*C*.

Breast tumor tissues that were diagnosed as IDC, were shown to be distinct from normal mammary tissues in their ganglioside expression. Total ganglioside levels were significantly higher on average in the tumor tissues than in normal tissues. However, not all tumor tissues had ganglioside levels that were higher than those in normal tissues. On the other hand, the unusual ganglioside species containing *O*-acetyl-*N*-acetylneuraminic acid or *N*-glycolylneuraminic acid were expressed in most tumor cases to extents that exceeded the normal range (Fig. 1). Thus, the abnormal occurrence of these unusual gangliosides seems to be characteristic of IDC of mammary glands.

ACKNOWLEDGMENTS

We thank Drs. Ines Perez, Luis Moreno, Guido Carrillo, and Celia Pereda for providing tumor samples and the members of the Department of Pathology of the National Oncology Institute and the Institute of Legal Medicine, Havana, Cuba, especially Drs. Sonia Franco and Daysi Ferrer for histopathological examinations. We are grateful to Drs. Tadashi Tai (Tokyo Metropolitan Institute of Medical Science) for providing monoclonal antibody GMR2, Shinobu Fujita (Mitsubishi Kasei Institute of Life Sciences) for providing monoclonal antibody 493D4, and Ana Maria Vazquez (Center of Molecular Immunology, Havana, Cuba) for providing monoclonal antibody P3. We express much appreciation to Dr. Yoshio Hirabayashi (Institute of Physical and Chemical Research) for characterizing monoclonal antibody 493D4 and for supplying H-D antibody.

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