RAPID COMMUNICATION Jpn. J. Cancer Res. 86, 131–134, February 1995

Expression of Sialylparagloboside in a Case of Liposarcoma: Aberrant Glycosylation in Tumors Arising in Adipose Tissues

Yoshitugu Kubo,[1] Sumiko Hamanaka,[2] Ikuo Kawashima,[3] Tadashi Tai,[3] Susumu Kawamura[4] and Kiwamu Okita[1]

[1]Department of First Internal Medicine, Yamaguchi University School of Medicine, 1144 Kogushi, Ube-shi, Yamaguchi 755, Departments of [2]Dermatology and [4]Internal Medicine, Yamaguchi Rosai Hospital, 1315-4 Onoda, Onoda-shi, Yamaguchi 756 and [3]Department of Tumor Immunology, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113

Gangliosides of liposarcoma, lipoma and lipids from omental tissues were analyzed. By immunostaining after thin layer chromatography, gangliosides of liposarcoma were identified as GM3, sialylparagloboside and GD3, whereas those of lipoma were GM3 and GD3, and those of fat in omental tissues were GM3, GD1a, GD3 and some unknown ones. Expression of sialylparagloboside is thought to be very rare.

Key words: Ganglioside — Liposarcoma — Lipoma — Adipose tissue — Monoclonal antibody

Since the introduction of hybridoma technology, many monoclonal antibodies (MAbs) have been produced.[1)] Among them, considerable numbers of MAbs against tumor cells detect glycosphingolipids expressed on the cell membrane.[2)] The detection of those glycosphingolipids is of interest to cancer researchers and clinicians as these antigens are tumor-associated. Gangliosides,[3)] glycosphingolipids containing sialic acid, were identified as tumor-associated antigens in melanoma,[4)] neuroblastoma.[5)] leukemia,[6)] gastic cancer,[7)] hepatocellular carcinoma,[8)] pancreatic cancer,[9)] colon carcinomas,[10)] lung cancer[11)] and teratocarcinoma.[12)] With the identification of the ganglioside antigens using MAbs, marked structural differences have been found to characterize many gangliosides of tumor origin, in comparison with gangliosides of normal tissues.[13)] Expression in tumors of gangliosides that are not found in normal tissues suggests that such gangliosides may be tumor-associated antigens.

Liposarcoma is one of the primary retroperitoneal tumors, which are very rare, with a reported frequency of approximately 0.1–0.3% of all tumors; the rate of malignancy is quoted as being at 70–90%.[14, 15)] Because of its rarity and the difficulty of total resection, the biological characteristics of liposarcoma have not yet been investigated. We were able to obtain a liposarcoma mass from which we could purify and analyze gangliosides. At the same time, we analyzed gangliosides from lipoma, a benign tumor arising from adipose tissue, and fat in the omentum as a normal control. We report here the significant alteration of ganglioside patterns in adipose tissue as malignant or benign tumorigenesis develops, and the expression in liposarcoma of sialylparagloboside, which is not found in lipoma or omental tissue.

A retroperitoneal tumor (83.9 g) was obtained operatively from a 65-year-old Japanese male. Histological diagnosis identified liposarcoma, which was classified as well-differentiated type. Lipomas were resected from the subcutaneous region of 4 male and 5 female Japanese aged between 45 and 63 years old. Each tumor weighed from 5 to 10 g, and tumors were combined to give 146.0 g of tissue after histological diagnoses had been confirmed. Omental tissue was resected from 2 Japanese males aged 60 and 37 years old, on the occasion of surgical operation, and 192.1 g of normal lipids was obtained. These specimens were stored at −80°C until use.

Lipids were extracted from the tissues with 20 volumes of chloroform-methanol, 2:1, 1:1 and 1:2 (v/v), at room temperature overnight for each extraction. All extracts were combined, evaporated to dryness, suspended in water, dialyzed against distilled water and evaporated, affording 37.6 g of crude lipids from liposarcoma, 73.7 g from lipoma and 27.4 g from omental tissue. The crude lipids thus obtained were dissolved in 500 ml of chloroform-methanol-water, 30:60:8 (v/v/v) and subjected to DEAE-Toyopearl column (acetate form, 1.5 cm × 27 cm) chromatography according to the method of Ledeen *et al.*[16)] The neutral lipids were eluted, then the acidic lipids were obtained with 500 ml of chloroform-methanol-0.8 *M* sodium acetate, 30:60:8 (v/v/v). The acidic lipid fraction was evaporated to a small volume, dialyzed against distilled water, and evaporated to dryness. Then, the acidic lipids were dissolved in 100 ml of 0.1 *N* KOH in methanol and incubated at 40°C for 1 h.

[2] To whom correspondence should be addressed.

After neutralization with concentrated acetic acid, the acidic lipids were dialyzed against distilled water, and evaporated to dryness. The gangliosides thus obtained were analyzed by thin layer chromatography (TLC).

TLC was performed on Silica Gel 60 HPTLC plates (Merck, Darmstadt) with a solvent system of chloroform-methanol-0.2% $CaCl_2$ solution, 55:45:10 (v/v/v). After development, spots were visualized by spraying with resorcinol reagent, followed by heating of the plates at 95°C for 30 min. The relative intensities of the detected bands on TLC were converted to percentages using a chromatoscanner (Shimadzu CS-9000 dual-wavelength flying-spot scanner; Kyoto). For use as standards, GM3 from bovine brain, GD3 from bovine milk and bovine brain gangliosides were purchased from Iatron (Tokyo). After chromatography of the gangliosides, the plates were soaked for 1 h in phosphate-buffered saline (PBS) containing 1% bovine serum albumin. After drying, the plates were incubated with MAb solution (1:10 diluted) for 2 h at room temperature. The plates were washed by dipping in five successive changes of PBS containing 0.05% Tween 20, and incubated with horseradish peroxidase-conjugated goat antimouse immunoglobulins for 2 h at room temperature. After washing of the plates, color development was performed by the addition of a solution of 400 μg/ml of *o*-phenylenediamine in 80 m*M* citrate phosphate buffer, pH 5.0, containing 0.12% H_2O_2 at room temperature, and stopped after 15 min by dipping of the plate into PBS. In order to identify the gangliosides, six MAbs were used. GMR6 (anti-GM3) reacts predominantly with GM3, but moderately with four other gangliosides, GM1b, GD1a, GT1b and sialylparagloboside, and weakly with GM4.[17] GMR17 (anti-GD1a) reacts strongly with GD1a, and moderately with GM1b and GT1b.[17] GMR1 reacts with GM3 and GD3 (unpublished data). GMB28 (anti-GM2),[17] GMB16 (anti-GM1)[17] and GMR7 (anti-GD2)[18] show restricted binding specificities, reacting only with the gangliosides used as immunogens.

TLC of the gangliosides from liposarcoma gave one distinct band and two indistinct bands (Fig. 1A). The gangliosides of liposarcoma were GM3 and small amounts of two other gangliosides (Table I). One of the latter reacted with GMR6, and since the *Rf* value of this ganglioside lay between those of GM3 and GM1 (Fig. 1B), it was identified as sialylparagloboside (Fig. 2A). The other minor ganglioside from liposarcoma reacted with GMR1 and was identified as GD3 (Fig. 2C). Gangliosides of lipoma were GM3, which reacted with GMR6, and GD3, which reacted with GMR1 (Fig. 2C). Lipids from omental tissue contained a relatively large variety of gangliosides (Fig. 1A). On immunostaining, GM3 reacted with GMR6, GD1a reacted with GMR17, GD3 reacted with GMR1, and an unknown ganglioside

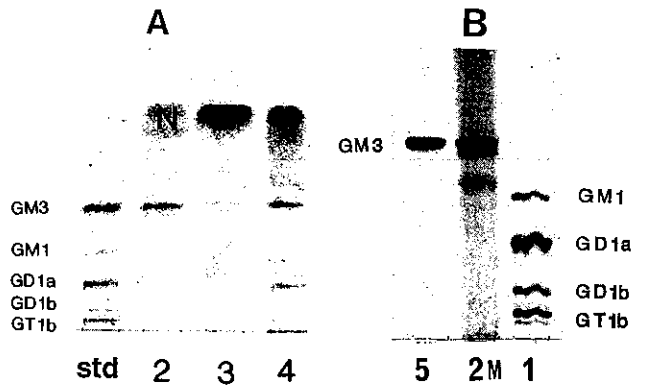

Fig. 1. Thin layer chromatogram of liposarcoma, lipoma and omental fat. Lanes: std, standard GM3 and bovine brain gangliosides; 1, bovine brain gangliosides; 2, liposarcoma; 3, lipoma; 4, omental fat; 5, standard GM3; 2M, monosialoganglioside fraction of 2, eluted with chloroform/methanol/0.02 *M* sodium acetate in water from the DEAE-Toyopearl column. The solvent used for TLC was chloroform/methanol/0.2% $CaCl_2$ (55:45:10, by volume), and resorcinol reagent was used for the detection of bands. N; non specific color.

Table I. Ganglioside Compositions of Liposarcoma, Lipoma and Omental Fat

Ganglioside (%)	Liposarcoma	Lipoma	Omental fat
GM3	86.4	46.9	51.6
Sialylparagloboside	4.2	—	—
GD1a	—	—	25.9
GD3	9.4	53.1	5.2
Unknown	—	—	17.3

(Fig. 2) did not react with these or the other MAbs used, GMB16, GMB28 and GMR7. The percentage compositions of gangliosides from liposarcoma, lipoma and lipids from omental tissue are summarized in Table I. Liposarcoma contained 86.4% GM3, 4.2% sialylparagloboside and 9.4% GD3. Lipoma contained 46.9% GM3 and 53.1% GD3. Omental fat contained 51.6% GM3, 25.9% GD1a, 5.2% GD3 and 17.3% unknown ganglioside.

The ubiquity of tumor-associated changes has resulted in the assignment of the term "aberrant glycosylation," to the metabolic process presumed to underlie tumor-associated oligosaccharide changes in gangliosides. However, these changes were observed in carcinomas. In sarcoma, only the gangliosides of leiomyosarcoma have been reported.[19] Histopathologically, liposarcoma often resembles leiomyosarcoma, when the former contains proliferating lipoblasts or fibroblast-like spindle cells arranged in fascicles on a myxoid background. In the case reported here, such an appearance was also detected

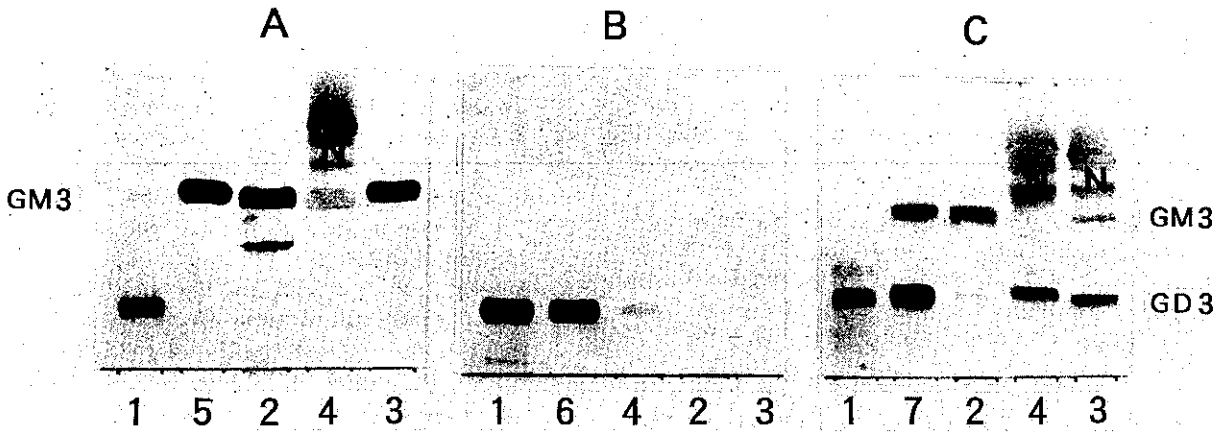

Fig. 2. TLC immunostaining of gangliosides from liposarcoma, lipoma and omental tissue. (A), (B) and (C) were stained with GMR6, GMR17 and GMR1, respectively. Lane 1, bovine brain gangliosides; 2, liposarcoma; 3, lipoma; 4, omental fat; 5, standard GM3; 6, standard GD1a; 7, standard GM3 and GD3. N; non specific reaction.

to a small extent and was diagnosed as the myxoid type of liposarcoma, although the major part was well-differentiated liposarcoma. In leiomyosarcoma, the accumulation of globotriaosylceramides has been reported.[19] In liposarcoma, no accumulation of globotriaosylceramides was found (data not shown) but large amounts of GM3 were detected, as reported here. The disparity in accumulated glycosphingolipid between liposarcoma and leiomyosarcoma indicated that the tumor cells of these two sarcomas have different characteristics, despite the similarity in histopathological figures.

Omental fat contained various gangliosides, especially disialogangliosides. The benign tumor, lipoma, did not contain GD1a. On malignant transformation, almost all expressed gangliosides were monosialogangliosides. Therefore, in fatty tissue, malglycosylation occurs when the tumor arises and the malignant transformation promotes it. It should be noted that sialylparagloboside was newly expressed in liposarcoma, and could be a tumor-associated ganglioside. As paragloboside was not detected in the neutral fraction of the liposarcoma (data not shown), it is suggested that the sialyltransferase activity would be high. Sialylparagloboside is a component of human erythrocytes and the precursor of sialyl Le^X. There are no reports of sialylparagloboside accumulation in malignancy, although sialyl Le^X is known to be a cancer-associated antigen recognized by MAb in stomach adenocarcinoma, colon adenocarcinoma, lung tumors, esophagus tumors, pancreas adenocarcinomas, breast tumors and ovary tumors.[20]

Recently, MAbs against gangliosides were reported to be useful in clinical diagnosis and treatment.[21] Considering that early diagnosis and treatment of liposarcoma are very difficult, the generation of liposarcoma ganglioside MAbs could be helpful.

The authors wish to thank Prof. T. Takemoto (Tokyo Women's Medical College) for his encouragement and valuable suggestions.

(Received August 26, 1994/Accepted November 25, 1994)

REFERENCES

1) Kohler, G. and Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature,* **256,** 495–497 (1975).
2) Hakomori, S. and Kannagi, R. Glycosphingolipids as tumor-associated and differentiation markers. *J. Natl. Cancer Inst.,* **71,** 231–251 (1983).
3) Svennerholm, L. The gangliosides. *J. Lipid Res.,* **5,** 145–155 (1964).
4) Houghton, A. N., Mintzer, D., Cordon-Cardo, C., Welt, S., Fliegel, B., Vadhan, S., Carswell, E., Melamed, M. R., Oettgen, H. F. and Old, L. J. Mouse monoclonal antibody detecting GD3 ganglioside: a phase I trial in patients with malignant melanoma. *Proc. Natl. Acad. Sci. USA,* **82,** 1242–1246 (1985).
5) Mujoo, K., Cheresh, D. A., Yang, H. M. and Reisfeld, R. A. Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. *Cancer Res.,* **47,** 1098–1104 (1987).
6) Huang, L. C., Civin, C. I., Magnani, J. L., Shaper, J. H. and Ginsburg, V. My-1, the human myeloid-specific antigen detected by mouse monoclonal antibodies, is a sugar

sequence found in lacto-N-fucopentaose III. *Blood,* **61,** 1020–1023 (1983).
7) Brockhause, M., Magnani, J. L., Herlyn, M., Blaszczyk, M. and Ginsburg, V. Monoclonal antibodies directed against the sugar sequence of lacto-N-fucopentaose III obtained from mice immunized with human tumors. *Fed. Proc.,* **41,** 897 (1982).
8) Hiraiwa, N., Iida, N., Ishizuka, I., Itai, S., Shigeta, K., Kannagi, R., Fukuda, Y. and Imura, H. Monoclonal antibodies directed to a disulfated glycosphingolipid, SB1a (GgOse4Cer-II3IV3-bis-sulfate), associated with human hepatocellular carcinoma. *Cancer Res.,* **48,** 6769–6774 (1988).
9) Falk, K.-E., Karlsson, K.-A., Larson, G., Thurin, J., Blaszczyk, M., Steplewski, Z. and Koprowski, H. Mass spectrometry of a human tumor glycolipid antigen defined by mouse monoclonal antibody NS-19-9. *Biochem. Biophys. Res. Commun.,* **110,** 383–391 (1983).
10) Magnani, J. L., Nilson, B., Brockhouse, M., Zopf, D., Steplewski, Z., Koprowski, H. and Ginsburg, V. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *J. Biol. Chem.,* **257,** 14365–14369 (1982).
11) Lloid, K. O., Larson, G., Stromberg, N., Thurin, J. and Karlsson, K.-A. Mouse monoclonal antibody F-3 recognizes the difucosyl type-2 blood group structure. *Immunogenetics,* **17,** 537–541 (1983).
12) Kannagi, R., Nudelman, E., Levery, S. and Hakomori, S. A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. *J. Biol. Chem.,* **257,** 14865–14874 (1982).
13) Hakomori, S. Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and prospectives. *Cancer Res.,* **45,** 2405–2414 (1985).
14) Kinne, D. W., Chu, F. C. M., Huvos, A. G., Yagoda, A. and Fortner, J. G. Treatment of primary and recurrent retroperitoneal liposarcoma. *Cancer,* **31,** 51–64 (1973).
15) Bek, V. Primary, retroperitoneal tumours. *Neoplasma,* **17,** 253–263 (1970).
16) Ledeen, R. W., Yu, R. K. and Eng, L. F. Gangliosides of human myelin: sialosylgalactosylceramide (G7) as a major component. *J. Neurochem.,* **21,** 829–839 (1973).
17) Kotani, M., Ozawa, H., Kawashima, I., Ando, S. and Tai, T. Generation of one set of monoclonal antibodies specific for a-pathway ganglio-series gangliosides. *Biochim. Biophys. Acta,* **1117,** 97–103 (1992).
18) Ozawa, H., Kotani, M., Kawashima, I. and Tadashi, T. Generation of one set of monoclonal antibodies specific for b-pathway ganglio-series gangliosides. *Biochim. Biophys. Acta,* **1123,** 184–190 (1992).
19) Li, S., Kundu, S. K., Degasperi, R. and Li, Y. Accumulation of globotriaosylceramide in a case of leiomyosarcoma. *Biochem. J.,* **240,** 925–927 (1986).
20) Fukushima, K., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, H., Chia, D., Suyama, N., Fukushi, Y., Nudelman, E. and Hakomori, S. Characterization of sialosylated Lewis^x as a new tumor-associated antigen. *Cancer Res.,* **44,** 5279–5285 (1984).
21) Steffens, T. A., Bajorin, D. F. and Houghton, A. N. Immunotherapy with monoclonal antibodies in metastatic melanoma. *World J. Surg.,* **16,** 261–269 (1992).