

# Expression of $\alpha$ 2,6-sialic acid-containing and Lewis-active glycolipids in several types of human ovarian carcinomas

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**Abstract.** To identify glycolipid antigens associated with histologically defined types of ovarian carcinomas, we determined the amounts of  $\alpha$ 2,6-sialyl and Lewis-active glycolipids, the specific activities of the  $\alpha$ 2,3- and  $\alpha$ 2,6-sialyltransferases, and the gene expression of sugar transferases in mucinous and serous cystadenocarcinoma, clear cell adenocarcinoma and endometrioid carcinoma tissues and cell lines derived from them.  $\alpha$ 2,6-sialyl glycolipid IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer detected with a newly developed monoclonal antibody, Y916, was present in 5/7 serous cystadenocarcinoma cases in relatively higher amounts than those in the other carcinoma tissues. On the other hand, the amounts of Lewis-active glycolipids in serous cystadenocarcinoma tissues were lower than those in the other carcinoma tissues. No correlation was observed between the structures of Lewis glycolipids and the histological classification. The gene expression of  $\alpha$ 2,3- and  $\alpha$ 2,6-sialyltransferases and  $\alpha$ 1,3/4-fucosyltransferase for the synthesis of Lewis-active glycolipids was not positively correlated with the amounts of the respective glycolipids, probably due to the epigenetic regulation of transferases in the overall metabolic pathways for lacto-series glycolipids. However, the amounts of GM3 and GD3 with short carbohydrate chains correlated with the relative intensities of GM3 and GD3 synthase gene expression, respectively. Among ovarian carcinoma-derived cell lines, the serous cystadenocarcinoma-derived ones exhibited a lower frequency of Lewis-active glycolipid expression than the other carcinoma-derived ones, which was similar to that

in the respective tissues. Thus, malignancy-related Lewis-active glycolipids were shown to be regulated in different modes in ovarian serous cystadenocarcinomas and the other carcinomas.

## Introduction

Transformation-associated alteration of the carbohydrate structures in cellular glycoconjugates, including glycolipids and glycoproteins, occurs frequently in various types of cancer, mainly due to the aberrant expression of glycosyltransferases (1). Detection of these structures, including sialyl lacto-N-fucopentaose (CA19.9, sialyl Lewis a), in sera was successfully applied for the clinical diagnosis of epithelial cancer in gynecologic tissues and the digestive tract (2). However, in comparison to CA125, whose frequency in ovarian carcinomas is higher than that of CA19.9, and the level of which is used for preoperative surgical counseling and planning, the clinical usefulness and cell biological properties of ovarian cancers with CA19.9-carbohydrates have not been clearly elucidated yet (3). Since a number of carbohydrate structures were shown to play a role in the ligands of animal lectins, such as NeuAc $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc for CD22 (4), sialyl Le<sup>x</sup> for P-selectin (5) and sialyl 6-sulfo-Le<sup>x</sup> for L-selectin (6), the expression of these structures and their modifications may affect the lectin-mediated adhesion related to the invasion and metastasis of cancer cells. Transfection of the  $\alpha$ 1,2-fucosyltransferase gene into RMG-1 cells resulted in increases in Le<sup>x</sup> and H-1 glycolipids, and a concomitant decrease in sialylated glycolipids. The transfectants exhibited increased adhesion with mesothelial cells and resistance against an anticancer drug, 5-fluorouracil, in comparison to those of RMG-1 cells (7,8). In addition, significant changes in glycolipids including Lewis-active ones were observed in ovarian carcinoma-derived KF28 cells exhibiting anticancer drug-resistance to cisplatin and taxol, probably due to an alteration of the activities of transporter proteins in regard to the excretion of drugs in glycolipid-rich membrane rafts (9,10). These findings showed that the expression of fucosylated glycolipids exhibiting

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Lewis- and H-antigenicities is closely correlated to the malignancy of cancer cells, including increased dissemination, metastatic potential and anticancer drug-resistance. However, since Lewis-active glycolipids are constructed of more than five carbohydrates, synthesis occurs through more than five glycosyltransferase reactions, whose activities are regulated by various epigenetic factors, including the concentrations of sugar nucleotides and acceptor glycolipids, pH and divalent cations. Notably, glycolipids in each step of the sequential multi-step reaction serve as substrates for the following step, suggesting that glycosyltransferase reactions determine the overall profile of glycolipids, including cancer-associated ones. Accordingly, the glycolipids in tissues from patients with ovarian carcinomas and cell lines derived from them were quantitatively determined in order to clarify their histologic classification-associated alterations, including Lewis-active ones, and to apply them as molecular markers for determining the malignancy of ovarian carcinomas, similar to those for colorectal carcinomas (11,12).

## Materials and methods

**Tissue specimens.** Histologic classification of the ovarian cancers was performed using criteria defined by the World Health Organization. The serous (7 cases) and mucinous (6 cases) cystadenocarcinoma, clear cell adenocarcinoma (3 cases) and endometrioid carcinoma (3 cases) tissues were obtained from the National Saitama Hospital. Written informed consent to use the specimens in this study was obtained from the patients, and the experimental protocol was approved by the local ethics committee.

**Cell lines derived from ovarian cancers.** The cell lines used in this experiment were obtained from patients with the following ovarian cancers: HAC-2 and RMG-1 from clear cell adenocarcinomas, 2008 and KF28 from serous cystadenocarcinomas, HMKOA from mucinous cystadenocarcinomas and HNOA from endometrioid carcinomas. The cell lines were cultured in Dulbecco's modified Eagle's medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Nichirei Biosciences Inc., Tokyo, Japan) under a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C.

**Materials.** The glycolipids used in this experiment were purified from various sources in our laboratory: GM3 and IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer from human erythrocytes, GD3 from bovine brain, Le<sup>x</sup> from human fetal brain and Lc<sub>4</sub>Cer, Le<sup>b</sup> and IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer from human meconium (9,10).

**Antisera.** Monoclonal antibodies, Y916 and 5h6, were prepared by the immunization of mice with gangliosides from bovine milk and IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer from human erythrocytes, respectively, and the hybridization of lymphocytes with myeloma P3-X63-Ag8.653 in our laboratory (13,14). As shown in Fig. 1, Y916 reacted with GD3 and IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer, and 5h6 with GM3, GD3 and IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer, indicating that structural isomers, IV<sup>6</sup>NeuAc $\alpha$ - and IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer, are identified with Y916 and 5h6. Moreover, GM3 and GD3 were identified by their mobility on thin layer chromatography (TLC) and their reactions with Y916 and 5h6. The following

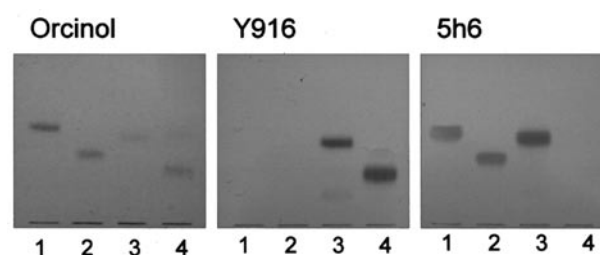


Figure 1. Thin layer chromatography (TLC) and TLC-immunostaining of glycolipids with monoclonal antibodies Y916 and 5h6. (1) GM3, (2) IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer, (3) GD3 and (4) IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer were developed on plastic-coated TLC plates with chloroform/methanol/0.5% CaCl<sub>2</sub> in water (55:45:10, by volume). Spots were visualized with orcinol-H<sub>2</sub>SO<sub>4</sub> reagent and monoclonal antibodies Y916 and 5h6.

monoclonal antibodies were kindly donated: NCC-LU-279 for Le<sup>x</sup> and NCC-ST-433 for Le<sup>y</sup> by Dr S. Hirohashi, National Cancer Center, Tokyo, Japan, MSN-1 for Le<sup>b</sup> by Dr S. Nozawa, Keio University, Tokyo, Japan, and 3C11 for sialyl Le<sup>a</sup> by Dr K. Matsumoto, Mikuri Immunol. Lab., Kyoto, Japan.

**Analysis of lipids.** The neutral and acidic glycolipids derived from the tissues and cells were examined by TLC and TLC-immunostaining with the development solvents, chloroform/methanol/water (65:35:8, by volume) for neutral glycolipids and chloroform/methanol/0.5% CaCl<sub>2</sub> (55:45:10, by volume) for gangliosides, as previously described (10,13). Known amounts (0.1–1.5  $\mu$ g) of glycolipids, such as GM3, Lc<sub>4</sub>Cer, IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer, IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer and GD3, were developed on the same TLC plates for the preparation of standard curves. The densities of spots on TLC plates were determined by image analysis using NIH image.

**$\alpha$ 2,3- and  $\alpha$ 2,6-sialyltransferases.** The cancer tissues were homogenized using a homogenizer (Polytron; Kinematica, Luzern, Switzerland) in 0.25 M sucrose, and the microsomal fractions were prepared by centrifugation as previously described (15). The standard assay mixture for microsomal  $\alpha$ 2,3- and  $\alpha$ 2,6-sialyltransferases comprised 7.6 nmol nLc<sub>4</sub>Cer, 10 mM MgCl<sub>2</sub>, 5 mM CaCl<sub>2</sub>, 10 mM CMP-sialic acid, 0.3% Triton CF54, 50 mM 4-morpholinoethane sulfonic acid-NaOH buffer (pH 6.4), and 50  $\mu$ g enzyme protein, in a final volume of 50  $\mu$ l (16,17). After incubation at 37°C for 3 h, the reaction was terminated with 100  $\mu$ l of ethanol. Then, 50  $\mu$ l aliquots of the solution were developed on two TLC plates with chloroform/methanol/0.5% CaCl<sub>2</sub> in water (55:45:10, by volume), detection being performed with 5h6 for one and with Y916 for the other. Known amounts of IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer and IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer (5–100 ng) were stained on the same plates, and the densities of the spots were determined by image analysis using NIH image. The amounts of endogenous gangliosides in the microsomes were subtracted from the values after the enzyme reactions.

**RT-PCR analysis.** Total RNA extracted from the tissues with Isogen (Nippongene, Toyama, Japan) was reverse-transcribed to cDNA with reverse transcriptase (M-MuLV; Takara, Kyoto, Japan) and oligo dT-primers, and then subjected to PCR with 0.5 units of Taq DNA polymerase (GoTaq; Promega,

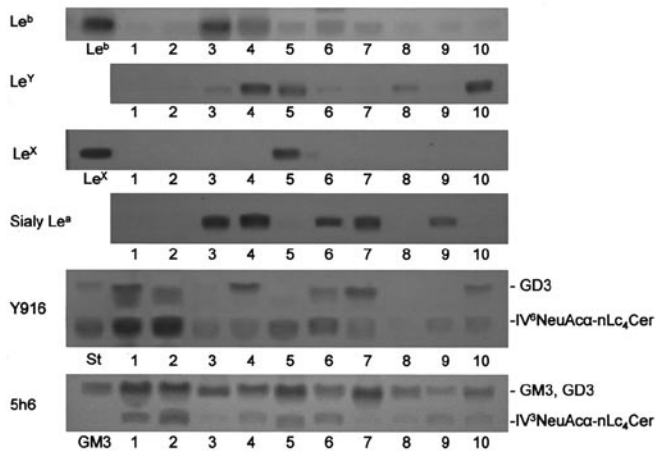


Figure 2. TLC-immunostaining of glycolipids from human ovarian carcinoma tissues. Glycolipids, corresponding to 0.2 mg dry weight, were developed with chloroform/methanol/0.5%  $\text{CaCl}_2$  in water (55:45:10, by volume). Spots were visualized by TLC-immunostaining with anti- $\text{Le}^b$ , anti- $\text{Le}^Y$ , anti- $\text{Le}^X$ , anti-sialyl  $\text{Le}^a$ , and Y916 and 5h6 antibodies. The specimen numbers are the same as those shown in Table I. Standard glycolipids for Y916 was a mixture of GD3 and  $\text{IV}^6\text{NeuAca-nLc}_4\text{Cer}$ .

Kyoto, Japan) under the following conditions: LacCer sialyltransferase (GM3 synthase, AB018356), sense primer, attgagcacaggtatagc, antisense primer, gatgtcaaaggcagtctct; GM3 sialyltransferase (GD3 synthase, D26360), sense primer, acaaatggaagactgctgcga, antisense primer, tggctctgtctctgtctcat;  $\alpha 2,6$ -sialyltransferase (BC031476), sense primer, tgcgtctctgtctcttctct, antisense primer, tctgcactgaactgatgcc;  $\alpha 2,3$ -sialyltransferase (BC010645), sense primer, atctcccg ggaagacaggtg, antisense primer, ccatgaagaaggggttgaga; and  $\alpha 1,3$ -fucosyltransferase 3 (FUT3, NM1097640), sense primer, tgggtgctgtgtgttcttc, antisense primer, ggetccaagtgaaccagat; 35 cycles of 95°C for 15 sec, 54-64°C for 30 sec and 72°C for 40 sec. The primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as controls. The resulting PCR products were electrophoresed on a 1.5% agarose gel, stained

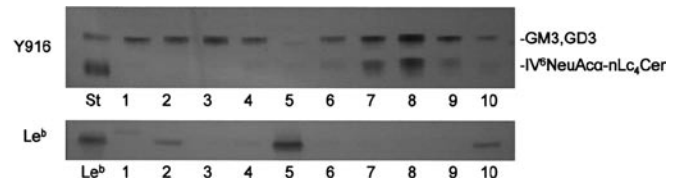


Figure 3. TLC-immunostaining of glycolipids from human ovarian mucinous and serous cystadenocarcinoma tissues. Glycolipids were obtained from an additional five specimens, respectively, i.e., other than those in Fig. 2 and Table I. The glycolipids, corresponding to 0.1 mg dry weight, were used for TLC-immunostaining with monoclonal Y916 and anti- $\text{Le}^b$  antibodies as described in Materials and methods. Standard glycolipids for Y916 was a mixture of GD3 and  $\text{IV}^6\text{NeuAca-nLc}_4\text{Cer}$ .

with ethidium bromide, and examined using a UV trans-illuminator (15).

## Results

**Glycolipids in ovarian carcinoma tissues.** Fig. 2 shows TLC-immunostaining of lipids from a number of ovarian carcinoma and uterine endometrial carcinoma tissues. In agreement with our previous results (7),  $\text{Le}^b$  in mucinous cystadenocarcinomas was present in higher amounts than those in the other carcinomas (Table I). Among the Lewis glycolipids examined,  $\text{Le}^b$ ,  $\text{Le}^Y$ ,  $\text{Le}^X$  and sialyl  $\text{Le}^a$ , whether one or a number of them, were detected in all tissues other than ovarian serous cystadenocarcinoma ones, in which they were not present or only in a trace amount. Alternatively, serous cystadenocarcinomas contained  $\text{IV}^6\text{NeuAca-nLc}_4\text{Cer}$  in significantly higher amounts than in the other carcinomas (Fig. 2). The amounts of  $\text{IV}^6\text{NeuAca-nLc}_4\text{Cer}$  in serous cystadenocarcinomas were >9 times higher than those in the other carcinomas, while no significant differences were observed in the amounts of  $\text{IV}^3\text{NeuAca-nLc}_4\text{Cer}$  among the various types of ovarian carcinomas (Fig. 2 and Table I). The expression of  $\text{IV}^6\text{NeuAca-nLc}_4\text{Cer}$  and  $\text{Le}^b$  was further examined in an additional 5 cases of ovarian serous and mucinous cystadenocarcinomas, respectively. As shown in Fig. 3 and Table II,

Table I. Glycolipids in human ovarian carcinoma tissues.

No.	Histological classification	Specimen Case	$\text{Le}^X$	$\text{Le}^Y$	$\text{Le}^b$	Sialyl $\text{Le}^a$	GM3	GD3	$\alpha 2,6$	$\alpha 2,3$
1	Ovarian serous cystadenocarcinoma	1	—	—	—	—	1.19	0.11	0.45	0.04
2		2	—	—	—	—	1.25	0.08	0.45	0.06
3	Ovarian mucinous cystadenocarcinoma	1	—	0.01	0.53	0.04	0.54	—	0.01	—
4	Endometrial adenocarcinoma	1	—	0.30	0.06	0.04	0.48	0.08	0.02	0.02
5	Ovarian endometrioid carcinoma	1	0.09	0.09	0.02	—	1.33	—	0.04	0.03
6		2	—	tr	0.03	0.02	0.50	0.01	0.05	0.02
7		3	—	—	0.02	0.04	0.90	0.07	0.01	tr
8	Ovarian clear cell adenocarcinoma	1	—	0.01	0.01	—	0.37	—	—	tr
9		2	—	tr	0.01	0.01	0.15	tr	0.02	0.01
10		3	—	0.20	tr	—	0.22	0.02	0.02	0.01

The amounts of glycolipids are expressed as  $\mu\text{g}$  per mg dry tissue and are the means of three determinations by TLC-densitometry with the respective standard glycolipids.  $\alpha 2,6$ ,  $\text{IV}^6\text{NeuAca-nLc}_4\text{Cer}$ ;  $\alpha 2,3$ ,  $\text{IV}^3\text{NeuAca-nLc}_4\text{Cer}$ ; tr, trace amount. Sample numbers correspond to those in Fig. 2.

Table II. Amounts of IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer and Le<sup>b</sup> in ovarian serous and mucinous cystadenocarcinomas.

No.	Histological classification	Specimen Case	α2,6	Le <sup>b</sup>
1	Ovarian serous cystadenocarcinoma	3	0.06	0.01
2		4	0.23	—
3		5	0.37	—
4		6	0.11	—
5		7	0.01	0.13
6	Ovarian mucinous cystadenocarcinoma	2	—	0.01
7		3	—	0.12
8		4	—	0.02
9		5	0.01	0.08
10		6	—	0.48

The amounts of glycolipids are expressed as  $\mu\text{g}$  per mg dry tissue and are the means of three determinations by TLC-densitometry with the respective standard glycolipids. Sample numbers correspond to those in Fig. 3.

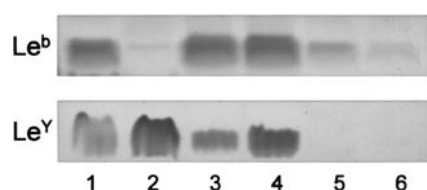


Figure 4. TLC-immunostaining of glycolipids from ovarian carcinoma-derived cells. Glycolipids, corresponding to 0.2 mg dry weight, were used for TLC-immunostaining with anti-Le<sup>b</sup> and anti-Le<sup>Y</sup> antibodies. Lanes: 1, RMG-1; 2, HAC-2; 3, HMkoa; 4, HNOA; 5, 2008 and 6, KF28.

IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer was present in 3/5 serous cystadenocarcinomas in amounts  $>0.1 \mu\text{g}$  per mg of dry weight, but not in mucinous cystadenocarcinomas. Conversely, Le<sup>b</sup> was detected in 4/5 mucinous cystadenocarcinomas and in one of the serous cystadenocarcinomas. Thus, the frequencies of expression of IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer and Lewis-active glycolipids were significantly high in serous cystadenocarcinomas and the other ovarian carcinoma tissues, respectively.

**Glycolipids in ovarian carcinoma-derived cells.** The expression of IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer and Lewis-active glycolipids was examined in cell lines established from various types of ovarian carcinomas. Although IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer was not detected in any cell line, Lewis-active glycolipids were present in clear cell adenocarcinoma-derived RMG-1 and HAC-2, mucinous cystadenocarcinoma-derived HMkoa and endometrioid carcinoma-derived HNOA, in amounts higher than those in serous cystadenocarcinoma-derived 2008 and KF28. Their presence shows that relatively low and high amounts of Lewis-active glycolipids in serous cystadenocarcinomas and the other ovarian carcinoma tissues, respectively, are retained in the respective cell lines (Fig. 4).

**Enzyme activities and gene expression of α2,3- and α2,6-sialyltransferases in ovarian carcinoma tissues.** To examine the enzymatic and genetic backgrounds of the expression of

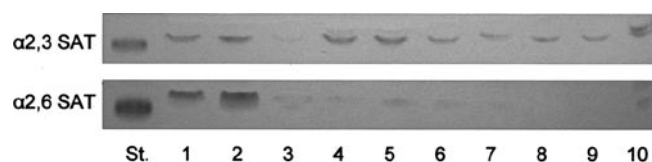


Figure 5. TLC-immunostaining of products following reactions of α2,3- and α2,6-sialyltransferases (SAT). The products following the reactions with 50  $\mu\text{g}$  of enzyme proteins were developed with chloroform/methanol/0.5% CaCl<sub>2</sub> in water (55:45:10, by volume), and detected by TLC-immunostaining with monoclonal antibodies 5h6 for IV<sup>3</sup>NeuAca-nLc<sub>4</sub>Cer (α2,3SAT) and Y916 for IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer (α2,6SAT), respectively. Standard glycolipids IV<sup>3</sup>NeuAca-nLc<sub>4</sub>Cer for α2,3SAT and IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer for α2,6SAT, respectively. The specimen numbers are the same as those in Fig. 2 and Table I.

IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer and Lewis-active glycolipids, the specific activities of α2,3- and α2,6-sialyltransferases were determined by detection of the products, IV<sup>3</sup>NeuAca-nLc<sub>4</sub>Cer and IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer, and the gene expression by RT-PCR.

As shown in Fig. 5 and Table III, although the specific activities of α2,3-sialyltransferase with nLc<sub>4</sub>Cer as the substrate in the tissues were not correlated with the amounts of IV<sup>3</sup>NeuAca-nLc<sub>4</sub>Cer, or with the histological classification, those of α2,6-sialyltransferase were positively correlated with the relative amounts of IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer in the tissues, indicating that the high amounts of IV<sup>6</sup>NeuAca-nLc<sub>4</sub>Cer in serous cystadenocarcinomas are due to the higher specific activity of α2,6-sialyltransferase. However, the α2,3- and α2,6-sialyltransferase genes were ubiquitously expressed in all of the tissues examined and their relative intensities were not positively correlated with the enzymatic activities, or with the amounts of glycolipids (Fig. 6). Similarly, expression of the FUT3 gene encoding an α1,3/4 fucosyltransferase responsible for the synthesis of Lewis antigen was not correlated with the amounts of Le<sup>b</sup>, Le<sup>x</sup>, Le<sup>y</sup> and sialyl Le<sup>a</sup>. In contrast to the gene expression of sugar transferases for neolacto-series glycolipids, the relative intensities of the GM3 and GD3 synthase genes were positively correlated with the amounts of GM3 and GD3 (Fig. 6).

Table III. Specific activities of  $\alpha$ 2,3- and  $\alpha$ 2,6-sialyltransferases with nLc<sub>4</sub>Cer as the substrate.

No.	$\alpha$ 2,3-sialyltransferase	$\alpha$ 2,6-sialyltransferase
1	7.4	57.2
2	8.5	105.2
3	1.0	21.7
4	11.6	12.4
5	12.0	7.5
6	5.5	10.9
7	5.3	nd
8	5.2	nd
9	3.9	nd
10	11.0	16.1

Specific activities (pmol/100  $\mu$ g of enzyme protein/h) are calculated from the amounts of products by TLC-immunostaining as shown in Fig. 5. Microsomes that served as the enzyme source were prepared from the same tissues as those in Table I and Fig. 2. nd, not detected.

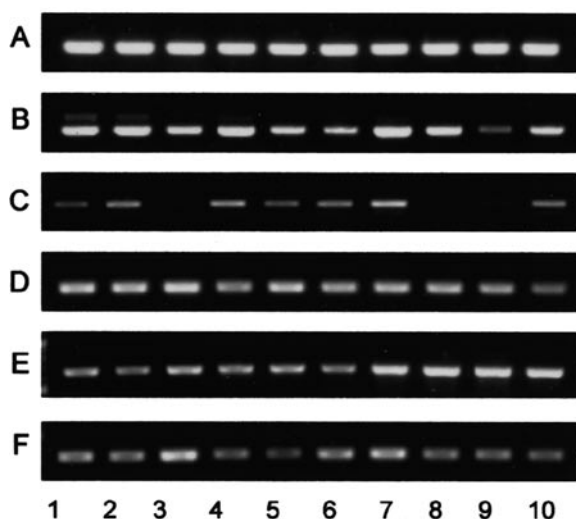


Figure 6. RT-PCR analysis of the glycosyltransferase genes in human ovarian carcinoma tissues. A, GAPDH; B, GM3 synthase; C, GD3 synthase; D,  $\alpha$ 2,3-sialyltransferase; E,  $\alpha$ 2,6-sialyltransferase and F,  $\alpha$ 1,3/4-fucosyltransferase 3. The specimen numbers are the same as those in Fig. 2 and Table I.

## Discussion

Among ovarian carcinoma tissues with different histologic classifications, serous cystadenocarcinomas have been shown to express IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer at a higher frequency than the other carcinomas, and in compensation, the expression of Lewis-related glycolipids in serous cystadenocarcinomas was lower than in the other carcinomas. As shown in Fig. 7, since the syntheses of IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer and Lewis-active glycolipids occur with the same substrate at the branch of the lacto-series pathway, our findings suggest that the synthesis of lacto-series glycolipids is influenced by the availability of substrate glycolipids in individual steps of glycosyltransferase reactions. As reported in our previous paper (8), when the

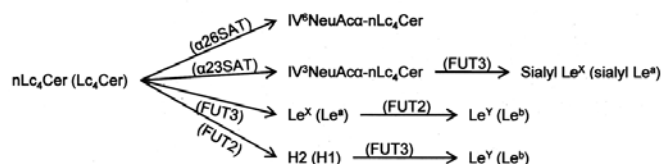


Figure 7. Metabolic pathways for the syntheses of IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer and Lewis-active glycolipids. SAT, sialyltransferase; FUT2,  $\alpha$ 1,2-fucosyltransferase; FUT3,  $\alpha$ 1,3/4-fucosyltransferase; H, blood group H antigen.

activity of  $\alpha$ 1,2-fucosyltransferase in ovarian carcinoma-derived RMG-1 cells increased to 20-30 fold that in the original cells on transfection with the  $\alpha$ 1,2-fucosyltransferase gene, the amount of Le<sup>Y</sup> increased to 10-fold of the original level. On the other hand, sialylated glycolipids in the original cells, including sialyl Le<sup>X</sup> and IV<sup>3</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer, were absent in the transfectants, suggesting that the enhanced fucosylation of Le<sup>X</sup> and nLc<sub>4</sub>Cer at the terminal step of glycosylation inhibits their sialylation through deprivation of the substrate. Similarly, the high specific activity of  $\alpha$ 2,6-sialyltransferase in serous cystadenocarcinomas was considered to cause the increased amount of IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer (Lc<sub>4</sub>Cer) and the absence of the Lewis antigen due to the consumption of nLc<sub>4</sub>Cer (Lc<sub>4</sub>Cer) for the syntheses of Le<sup>X</sup> (Le<sup>a</sup>) by  $\alpha$ 1,3/4-fucosyltransferase (FUT3), blood group H-glycolipid by  $\alpha$ 1,2-fucosyltransferase (FUT2) and IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer by  $\alpha$ 2,3-sialyltransferase ( $\alpha$ 2,3SAT), whose mRNAs were expressed in all of the tissues examined (18). Therefore, the epigenetic regulation of enzymatic activities in the individual steps of the neolacto- and lacto-series pathways may be involved in the determination of the mode of expression of sialylated and fucosylated glycolipids, including Lewis antigens, irrespective of the expression of glycosyltransferase mRNA. In contrast, the amounts of GM3 with shorter carbohydrate chains were closely correlated to the relative intensities of GM3- and GD3-synthase mRNAs, whose expression may directly lead to the active syntheses of GM3 and GD3 with a sufficient supply of substrate LacCer in proportion to their enzymatic activities. However, among ovarian carcinoma-derived cells, Lewis-related glycolipids were present in mucinous cystadenocarcinoma-, clear cell adenocarcinoma- and endometrioid carcinoma-derived cells in significantly higher amounts than in serous cystadenocarcinoma-derived cells, suggesting that the synthetic potential as regards to Lewis glycolipids in the tissues is maintained in the cultured cell lines. On the other hand, IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer was present in 5/7 serous cystadenocarcinoma tissues in amounts of more than 0.1  $\mu$ g per mg dried tissue, while the amounts in the other carcinomas, if present, were less than 0.05  $\mu$ g per mg dry weight, indicating that the expression of IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer in serous cystadenocarcinomas occurs at a higher frequency than in the other carcinomas. In agreement with our results, the frequency of detection of sialyl Le<sup>a</sup> (CA19.9) in sera of patients with ovarian serous cystadenocarcinomas was reported to be low in comparison to those in the other carcinomas (3). In the case of a murine lymphoblastoid cell line, cells with IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer were shown to exhibit a low metastatic potential in comparison to those without it, probably due to the attenuated expres-

sion of adhesion-related Lewis structures, due to enhanced  $\alpha 2,6$ -sialyltransferase activity (19). Transfection of the  $\alpha 2,6$ -sialyltransferase gene into cell lines with Lewis glycolipids is currently under investigation to demonstrate the modification of the Lewis glycolipid expression. In addition, since serous cystadenocarcinomas generally show a favorable prognosis, it can be suggested that IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer is a useful marker for the benign properties of cancer cells. To demonstrate the value of screening for IV<sup>6</sup>NeuAc $\alpha$ -nLc<sub>4</sub>Cer and Lewis-active glycolipids for the diagnosis of ovarian carcinomas, amounts of these glycolipids in sera of patients with serous cystadenocarcinomas are now being determined in comparison to those in tissues in our laboratory.

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