Expression of Glycolipids Bearing Lewis Phenotypes in Tissues and Cultured Cells of Human Gynecological Cancers

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Transformation-associated expression of Leb (Lewis antigen-b) or LeY in human colorectal carcinomas has been well described. To examine the expression of glycosphingolipids (GSLs) bearing Lewis-phenotypes in human gynecological carcinoma-derived cells, we determined the concentrations of all GSLs. Although neither Leb nor LeY was present in HEC-108 cells established from the poorly differentiated type of endometrial adenocarcinoma, other cell lines from moderately or well-differentiated types expressed either Leb or LeY, or both, at concentrations of 0.01 to 0.03 µg per mg of dry cells, which comprised 0.3 to 1.3% of the total GSLs. In the cervical and ovarian carcinoma-derived cell lines, Lewis phenotypes tended to be carried by nLc4Cer, which was accumulated in the cells without sialylation or fucosylation. These results indicated that expression of Leb- or LeY-phenotypes was strongly dependent on the metabolic ability to supply the precursor GSLs. Both Leb and LeY were successfully detected by monoclonal antibody MSN-1, which was a useful probe for the simultaneous detection of Leb and LeY. On application of MSN-1, either Leb or LeY was detected in tissues from patients with well- and moderately differentiated types of endometrial adenocarcinoma at concentrations of 0.01 to 0.04 µg per mg of dry tissues, but not in the tissues of poorly differentiated type. Normal endometria at the follicular and luteal phases also contained the antigens, but the concentrations and the frequency of antigen expression were lower than those in the well- and moderately differentiated types of endometrial adenocarcinoma.

Key words: Glycosphingolipid — TLC-immunostaining — Fast atom bombardment mass spectrometry — Uterine endometrial adenocarcinoma — Lewis antigen

The plasma membrane of nearly all mammalian cells bears a glycocalyx layer, consisting of glycolipids, glycoproteins and proteoglycans, whose carbohydrate structures have been well defined in cells at various stages of proliferation and differentiation to determine the histo-blood group antigenicity and several carbohydrate-mediated functions.1 During the transformation of cells, alterations in the cell surface carbohydrates have been frequently observed, mainly due to the aberrant expression of glycosyl transferases, and among the transformation-associated carbohydrates, CA19-9, with a sialylated lacto-N-fucopentaose (sialyl Lea) structure, has been successfully applied for the clinical diagnosis of cancer patients.2, 3) In addition, on the basis of the finding that sialylated or sulfated Lewis carbohydrates are involved in the ligands for selectins,4, 5) cancer cells expressing sialylated or sulfated Lewis structures have been shown to exhibit high dissemination and metastatic potentials through selectins, and the concentrations of these molecules in cancer cells and tissues, and in sera of patients have provided useful clues for predicting metastasis.6, 7) Also, the expression of Leb, LeY, H and mucin core antigen-1 has been reported to be related to the grade of dysplasia and malignancy in cancer patients, and can be used to predict prognosis.8, 9) However, such carbohydrate structures applicable to clinical diagnosis are synthesized through multi-step reactions by several glycosyltransferases, the activities of which are influenced by availability of the respective sugar nucleotides, acceptor carbohydrate chains, and other factors including divalent cations, indicating that tumor-associated carbohydrates are dependent upon various epigenetic factors. To understand the metabolic alterations causing the expression of histo-blood group antigens in cancer cells, quantitative determination of all molecular species of carbohydrates including metabolic precursors is necessary. For this purpose, analysis of the glycosphingolipid (GSL) composition should provide useful information because GSLs with carbohydrate moieties of different chain lengths can be readily separated by means of a convenient procedure. In our previous study, human uterine...
endometrial carcinoma-derived cells were shown to express high specific activity of β1,3-galactosyltransferase for the synthesis of lacto-N-tetraosyl ceramide as an important precursor of Lewis-GSL. We have now extended this study to determine the entire GSL compositions in various gynecological cancer-derived cells and to compare the expression of Lewis-GSLs in tissues from patients with various grades of endometrial carcinomas.

MATERIALS AND METHODS

Materials Glycolipids were purified from various sources in our laboratory: I3SO3-GalCer, GM2, GD3, GM1 and GD1a from bovine brain, GlcCer, LacCer, Gb3, Gb4, GM3 and IV3NeuAc-nLc4Cer from human erythrocytes, Lc4Cer, IV3NeuAc-Lc4Cer, Lea (Lewis antigen-a) (III4Fuc-Lc4Cer) and Leb (IV2Fuc, III4Fuc-Lc4Cer) from human meconium, Lea (III4Fuc-nLc4Cer) and Leb (IV2Fuc, III4Fuc-nLc4Cer) from human umbilical cord, Lc4Cer, IV3NeuAc-nLc4Cer, Lea (Lewis antigen-a) from rectal carcinoma tissues, and II3SO3-LacCer and II3SO3-Gg3Cer from human kidneys. Asialo GM1 and nLc4Cer were prepared by treatment with Arthrobacter ureafaciencs sialidase as reported previously, and N-stearoyl derivatives of GlcCer, LacCer and Gb3 were synthesized by acylation of glucosyl, lactosyl and globotetraosyl sphingosine, prepared with sphingolipid ceramide N-desacylase (Pseudomonas sp. TK4), with stearoyl chloride.

Carbohydrate-specific antibodies Rabbit polyclonal antibodies toward GM1, asialo GM1 and asialo GM2 were generated by immunizing rabbits intradermally with an emulsion prepared with 1 mg of purified glycolipid and 0.5 mg of BSA (bovine serum albumin) in 1 ml of PBS (phosphate-buffered saline), and 1 ml of Freund’s complete adjuvant (Sigma, St. Louis, MO), and by monitoring the antibody titer by means of an enzyme-linked immunosorbent assay, the titers being 1:100 000 for anti-GM1, 1:600 000 for anti-asialo GM1, and 1:300 000 for anti-asialo GM2 antibodies. No cross reaction with structurally related glycolipid derivatives of individual antigens was observed. Human monoclonal anti-Lc3,Cer (HMST-1), mouse monoclonal anti-Le6 plus Le7 (MSN-1), and anti-GM2 (YHD-06) antibodies were established in our laboratory. Monoclonal antibodies against Le6 (NCC-LU-279) and Le7 (NCC-ST-433 and H18A) were kindly donated by Dr. S. Hirohashi, National Cancer Center (Tokyo). Monoclonal anti-Le6 (73-30), anti-sialyl Le6 (2D3), and sialyl Le6 (KM-93) were obtained from Seikagaku Co. (Tokyo).

Cell lines derived from human gynecological cancers The cell lines used in this experiment were established from patients with the following cancers: SNG-II, HHUA and Ishikawa cells, SNG-M cells, and HEC-108 cells from the well-, moderately and poorly differentiated types of uterine endometrial adenocarcinoma, respectively, SKG-II, SKG-IIIa and TCS cells from the non-keratinizing type of uterine cervical carcinoma, and MCAS and HKMCO cells, HAC-2, HUOAC and RMG-I cells, and HTBOA cells from a mucinous cystadenocarcinoma, clear cell carcinoma and undifferentiated carcinoma of the ovary, respectively.

Human tissues Cancerous tissues from patients suffering from uterine endometrial adenocarcinomas and normal endometria were obtained from the Departments of Pathology, and Obstetrics and Gynecology, Keio University Hospital, and National Saitama Hospital after pathological examination, and were used according to the guidelines of the Committee for Informed Consent.

Separation and quantitation of GSLs The cultured cells were harvested with a scraper and then lyophilized. Total lipids were extracted from the lyophilized cells with chloroform/methanol/water (20:10:1, 10:20:1 and 1:1, by vol.), and then the combined extracts were fractionated into neutral and acidic lipids on a DEAE-Sephadex column (A-25, acetate form; Pharmacia, Uppsala, Sweden). The neutral GSLs were separated from unabsorbed neutral lipid fractions by acetylation, separation of the acetylated GSLs, deacetylation and desalting, whereas the acidic GSLs were prepared from the absorbed acidic lipid fractions by cleavage of the ester-containing lipids, followed by dialysis. The acidic and neutral GSLs thus obtained were then detected on TLC (thin-layer chromatography) plates with chloroform/methanol/0.5% CaCl2 in water (55:45:10, by vol.) and chloroform/methanol/water (65:35:8, by vol.), respectively, and then visualized with orcinol-H2SO4 reagent. The density of spots was determined at an analytical wavelength of 420 nm and a control wavelength of 710 nm using a dual-wavelength TLC densitometer (CS-9000; Shimadzu, Kyoto). Standard curves were essentially obtained by using the same GSLs as those detected in the cells: 0.1 to 1.5 µg of GalCer (18:0), Lac-Cer (18:0), Gb3, Gb4, Gb5, Lc4Cer, nLc4Cer, Le6, Le7, II3SO3-LacCer, II3SO3-Gg3Cer, GM3, IV3NeuAc-nLc4Cer, GM2, GD3, GM1 and GD1a, on the same plate. The concentration of lipid-bound sialic acid was also determined densitometrically after visualization of the spots with resorcinol-HCl reagent, and the density of the spots was determined at an analytical wavelength of 580 nm and a control wavelength of 710 nm.

TLC-immunostaining The neutral and acidic GSLs prepared from cultured cells and total lipid extracts from the cancerous tissues were applied on plastic-coated TLC plates, which were then developed successively with n-hexane/diethyl ether (4:1, by vol.) and chloroform/methanol/0.5% CaCl2 in water (55:45:10, by vol.). Each plate was incubated with a blocking buffer (1% PVP (polyvinylpyrrolidone) and 1% ovalbumin in PBS) at 4°C overnight and then with anti-carbohydrate antibodies in 3% PVP in PBS at 37°C for 2 h. Mouse monoclonal antibodies were used at the concentration of approximately.
0.2 µg/ml, and rabbit antisera were usually diluted to 1:500 (by vol.). Afterward, the plates were washed 5 times with 0.1% Tween 20 in PBS, and the bound antibodies were detected using peroxidase-conjugated anti-rabbit IgG+M or anti-mouse IgG+M antibodies (Cappel Laboratories, Cochranville, PA), diluted 1:1000 (by vol.) with 3% PVP in PBS, and with enzyme substrates H2O2 and 4-chloro-1-naphthol, as described previously. The density of spots was also determined using 10 to 100 ng of the respective GSLs as standards for quantitation with a TLC densitometer as described above, and the limit of detection was 5 ng of GSLs.

**Structural analysis and quantitation of GSLs** The individual GSLs were purified using a silica gel (Iatrobeads 6RS8060; Iatron Lab., Tokyo) column, with gradient elution with chloroform/isopropyl alcohol/water (85:15:0.2 and 40:60:0.2, by vol.) for mono- to trihexaosyl ceramides in the neutral GSLs and for sulfatides in the acidic GSLs, followed by a gradient of chloroform/methanol/water (70:30:4 and 10:90:4, by vol.) for polar GSLs. Then, characterization and quantitation of individual GSLs were performed as follows. Ceramide monohexosides: Purified ceramide monohexosides were chromatographed on a borate-impregnated plate with chloroform-methanol-water-15 M ammonia (280:70:6:1, by vol.) to separate and quantify GlcCer and GalCer. Ceramide trihexosides: Gb3Cer closely migrated with Lc3Cer, but was separated from Gg3Cer on a silica gel column, and after quantitation of Gb3Cer plus Lc3Cer by TLC-densitometry, Lc3Cer was removed by enzymatic hydrolysis with β-glucosaminidase (bovine kidney; Sigma), followed by determination of the product, LacCer from Lc3Cer, and the remaining Gb3Cer as above. Gg3Cer was determined by TLC-immunostaining with anti-Gg3Cer antibodies. Ceramide tetrahexosides: By silica gel column chromatography, Gb3Cer was separated from the mixture of Lc3Cer and nLc3Cer, in which the concentration of Lc3Cer was determined by TLC-immunostaining with anti-Lc3Cer antibodies, and the amount of nLc3Cer was determined by TLC-immunostaining with anti-Gg3Cer antibodies. The results are compiled in Table I. Cholesterol was present in various cell lines at concentrations of 36.7 µg (105.9 nmol) per mg of dry weight, and total GSLs amounted to 3 to 4 µg in eight out of 14 cell lines.

**RESULTS**

**GSLs in human gynecological cancer-derived cell lines** To determine the precise concentrations of GSLs in the cultured cells, standard curves for quantitation by TLC-densitometry and TLC-immunostaining were prepared using the same purified GSLs as those detected in the cell lines, as described under “Materials and Methods.” The results are compiled in Table I. Cholesterol was present in various cell lines at concentrations of 36.7 µg (64.8 nmol) to 41.0 µg (105.9 nmol) per mg of dry weight, and total GSLs amounted to 3 to 4 µg in eight out of 14 cell lines, corresponding to approximately 4 mol% of GSLs with respect to cholesterol. Fig. 1 shows TLC chromatograms of neutral and acidic GSLs in 0.5 mg of dried cells. On chromatograms, at least 10 neutral and 7 acidic GSLs, which were separated according to the differences in the carbohydrate moieties, were detected, but the relative amounts of individual GSLs were distinct among the cells. In no case was an identical GSL composition found in two lines. The ubiquitous GSLs present at concentrations of more than 0.02 µg per mg of dry weight were GlcCer, LacCer, Gb3Cer, Lc3Cer, Gb3Cer and GM3, indicating that the metabolic pathways leading to Gb3Cer and GM3 are always active in cell lines established from various human gynecological cancers.
Table I. Glycosphingolipid Compositions in Human Gynecological Carcinoma-derived Cells

| No. | Cell line | GlcCer | LacCer | GalCer | Le3Cer | GM3 | Gb3Cer | Gb4Cer | Gg4Cer | IV%-NeuAc-Lc4Cer | GM2 | IV%-NeuAc-Lc3Cer | GM1 | GD3 | GD1a |
|-----|-----------|--------|--------|--------|--------|-----|--------|--------|--------|------------------|-----|------------------|-----|-----|-----|
| 1   | HHUA      | 0.26±0.01 | 0.20±0.01 | 0.2       | 0.06±0.02 | —   | 0.05 | —       | —       | 0.74±0.02 | t.r. | —       | 0.16 | 0.06 | —   |
| 2   | SNG-M     | 0.42±0.01 | 0.18±0.01 | 1.19       | 0.24±0.02 | 0.20 | 0.20 | 0.04 | 0.01 | 0.02 | 0.28±0.09 | 0.22±0.03 | 0.26±0.07 | t.r. | 0.11 | 0.02 |
| 3   | HEC-108   | 0.86±0.03 | 0.06±0.01 | 1.29       | 0.24±0.01 | 0.31 | 0.02 | 0.12 | —       | 0.22±0.07 | 0.06±0.01 | 0.60±0.06 | 0.15 | 0.07 | —   |
| 4   | SNG-II    | 1.64±0.05 | 0.26±0.01 | 0.18       | 0.12±0.02 | 0.15 | 0.05 | 0.02 | 0.03 | 1.94±0.06 | 2.04±0.05 | 3.4±0.05 | t.r. | 0.07 | 0.16 |
| 5   | Ishikawa  | 1.34±0.04 | 0.28±0.02 | 0.12       | 0.14±0.01 | —   | 0.02 | 0.03 | —       | 0.02±0.02 | —       | —       | —     | —    | —    |
| 6   | TCS       | 0.10±0.01 | 0.06±0.01 | 0.08       | 0.08±0.01 | —   | 0.02 | —       | —       | 0.06±0.02 | 0.05 | 0.04   | —    | —    | —    |
| 7   | SKG-IIIa  | 0.65±0.02 | 0.12±0.02 | 2.17       | 0.75     | 3.77 | t.r. | —       | —       | 0.46±0.02 | 1.25 | 1.16   | 2.34 | —    | —    |
| 8   | SKG-II    | 0.06±0.01 | 0.04±0.01 | 0.2        | 0.06     | 0.44 | 0.02 | 0.1   | —       | 1.30±0.06 | —     | —      | —    | —    | —    |
| 9   | MCAS      | 0.78±0.03 | 0.09±0.02 | 0.3        | 0.12     | 0.20 | 0.05 | —       | —       | 2.24±0.03 | —     | 0.08 | 0.06 | —    | —    |
| 10  | HMKOA     | 1.66±0.05 | 0.22±0.02 | 0.13       | 0.05     | 0.32 | 0.03 | —       | —       | 0.16±0.04 | t.r. | 0.22   | 0.02 | 0.02 | 0.08 |
| 11  | HUOCA     | 1.34±0.03 | 0.62±0.02 | 0.35       | 0.11     | 0.07 | 0.64 | 0.03   | —       | 0.16±0.05 | 0.38 | 0.06 | 0.10 | 0.10 | —    |
| 12  | RMG-1     | 0.72±0.02 | 1.44±0.02 | 1.4        | 0.82     | 1.42 | 0.02 | 0.05   | t.r.     | 0.20±0.05 | —     | 0.06 | 0.08 | 0.10 | —    |
| 13  | HTBOA     | 0.60±0.02 | 0.28±0.01 | 0.31       | 0.03     | 0.07 | 0.34 | 0.03   | —       | 0.20±0.05 | 0.96 | 0.13 | 0.31 | 0.30 | —    |
| 14  | HAC-2     | 1.88±0.01 | 1.00±0.01 | 1.57       | 0.02     | 0.96 | 0.07 | 0.02   | 0.02±0.05 | 0.02±0.02 | 0.02 | —     | —    | —    | —    |

t.r., trace amount; —, not detected.

Values with standard deviation were obtained by TLC-densitometry after visualization of the spots with orcinol-H2SO4 reagent, and those without standard deviation were obtained by TLC-immunostaining, as described under “Materials and Methods.”

Fig. 1. TLC of neutral (A) and acidic (B) GSLs from human gynecological cancer-derived cell lines. GSLs, corresponding to 0.5 mg of dry weight, were chromatographed with chloroform/methanol/water (65:35:8, by vol.) for A and with chloroform/methanol/0.5% CaCl2 in water (55:45:10, by vol.) for B, and then the spots were visualized with orcinol-H2SO4 reagent. St-N, standard neutral GSLs, LacCer, Gb3Cer, Gb4Cer and Gg4Cer from the top; St-A, standard acidic GSLs, II3SO3-GalCer, GM3, II3NeuAc-nLc4Cer, GM1 and GD1a from the top; 1, HHUA; 2, SNG-M; 3, HEC-108; 4, SNG-II; 5, Ishikawa; 6, TCS; 7, SKG-IIIa; 8, SKG-II; 9, MCAS; 10, HMKOA; 11, HUOCA; 12, RMG-1; 13, HTBOA; 14, HAC-2.

Fig. 2. TLC-immunostaining of acidic GSLs from human gynecological cancer-derived cell lines. Acidic GSLs, corresponding to 0.2 mg of dry weight, were chromatographed with chloroform/methanol/0.5% CaCl2 in water (55:45:10, by vol.), and then the spots were visualized by immunostaining with anti-GM3 (A), and anti-αGM1 (B) antibodies. The numbers for the cell lines are the same as in Fig. 1.

GSLs with trihexose or shorter carbohydrate chains
GlcCer was the major ceramide monohexoside and was maintained at a higher concentration than that of LacCer in all cells, probably due to the kinetic properties of β-galactosyl transferase as to the synthesis of LacCer. The subsequent metabolic steps from LacCer, involving sialyl, sulfo, α-galactosyl, β-N-acetylgalcosaminyl and β-N-acetylgalactosaminyl transferases to afford GM3, II3SO3-LacCer, Gb4Cer, Lc3Cer and Gg3Cer, respectively, determine the extension of the carbohydrate chains. Among them, Gb4Cer, Lc3Cer and GM3 were ubiquitous at concentrations of more than 0.02 µg per mg of dry weight, providing a sufficient supply of precursors for the globo-, lacto- and ganglio-series GSL pathways (Fig. 2). The ratios of GM3 to LacCer, indicating a gradient of GM3 synthetic potential with appropriate synthetic activity of LacCer as a substrate and consumption of GM3 for the
following ganglio-series GSLs in ovarian carcinoma-derived cells (6 out of 7 cell lines), were less than 1.0, but the endometrial and cervical carcinoma-derived cells exhibited ratios of higher than 1.0. The ability to sulfate LacCer for the synthesis of II\(^3\)SO\(_3\)-LacCer was observed in 3 endometrial carcino-ma-derived cell lines and one ovarian carcinoma-derived cell line. Although the amount of II\(^3\)SO\(_3\)-LacCer was lower than that of LacCer in ovarian carcinoma-derived HAC-2 cells, the ratios of II\(^3\)SO\(_3\)-LacCer to LacCer were 2- to 7-fold in 3 endometrial-derived cell lines, all of which exhibited further synthesis of II\(^3\)SO\(_3\)-Gb\(_3\)Cer by GalNAc transferase (Fig. 1). Among the neutral trihexosyl ceramides, Gb\(_3\)Cer and Lac\(_4\)Cer were present at concentrations of 0.02 to 5.77 \(\mu\)g in all cells, and the former was always at a higher concentration than the latter. Gg\(_4\)Cer was only detected in 3 ovarian carcinoma-derived cell lines.

**GSLs with tetrahexose or longer carbohydrate chains**

In all cells, Gb\(_4\)Cer was present at concentrations of more than 0.06 \(\mu\)g per mg of dry weight, and extended structures of Gb\(_4\)Cer, such as N-acetylhexosamine-Gb\(_3\)Cer, hexose-Gb\(_3\)Cer and NeuAc-hexose-Gb\(_3\)Cer, were not detected at all on negative ion fast atom bombardment mass spectrometry (FABMS), indicating that Gb\(_3\)Cer is the terminal structure of the globo-series GSLs. However, the concentrations of Gb\(_3\)Cer, as well as the ratio of Gb\(_3\)Cer to Gb\(_4\)Cer, were different among the cells, probably due to the activity of Gb\(_3\)Cer:GalNAc-transferase, and were not correlated with the type of cancer. In contrast to the globo-series GSLs, further modification by fucosyl and sialyl transferases of Lac\(_4\)Cer and nLc\(_4\)Cer occurred in all the cell lines except SKG-II, in which nLc\(_4\)Cer was present as the terminal structure of the lacto-series GSLs at significantly high concentrations, and accordingly the concentrations of Lac\(_4\)Cer and nLc\(_4\)Cer in the other cells were less than one-tenth of that in SKG-II. Coincident with the previous finding that \(\beta\)1,3-Gal transferase involved in the synthesis of Lac\(_4\)Cer was frequently activated in endometrial carcinoma-derived cells, SNG-II, SNG-M, HEC-108 and Ishikawa cells expressed Lac\(_4\)Cer and its sialylated derivatives (Fig. 3). Although IV\(^3\)NeuAc-Lac\(_4\)Cer was detected in 5 cell lines, IV\(^3\)NeuAc-nLc\(_4\)Cer was detected in 13 cell lines other than SKG-II. Also, the rate of sialylation of nLc\(_4\)Cer was higher than that of Lac\(_4\)Cer, when the ratios of sialylated derivatives to Lac\(_4\)Cer and nLc\(_4\)Cer were compared. On the other hand, expression of the ganglio-series GSLs, Gg\(_4\)Cer, GM1 and GD1a, was rare in human gynecological cancer-derived cells.

**Expression of Lewis-phenotypes in human gynecological cancer-derived cells**

As shown in Fig. 1 and Table I, GSLs with longer carbohydrate chains, which were present at concentrations of more than 0.02 \(\mu\)g per mg of dry weight, comprised lacto-series GSLs, which were modified with fucose to confer the Lewis-phenotypes. Since the synthetic potential for Lac\(_4\)Cer was highly enhanced in the endometrial carcinoma-derived cells, as described above, Lac\(_4\)Cer-based Le\(^a\) or Le\(^b\) was detected in

![Fig. 3. TLC-immunostaining of neutral GSLs from human gynecological cancer-derived cell lines.](image)

Fig. 3. TLC-immunostaining of neutral GSLs from human gynecological cancer-derived cell lines. Neutral GSLs, corresponding to 0.2 mg of dry weight, were chromatographed with chloroform/methanol/water (65:35:8, by vol.), and then the spots were visualized by immunostaining with anti-Lac\(_4\)Cer (A), anti-Le\(^a\) (B), anti-Le\(^a\)+Le\(^b\) (C), and anti-Le\(^b\) (D) antibodies. The numbers for the cell lines are the same as in Fig. 1.

![Fig. 4. TLC-immunostaining with monoclonal antibody MSN-1 of lipid extracts of tissues of patients suffering from gynecological carcinomas.](image)

Fig. 4. TLC-immunostaining with monoclonal antibody MSN-1 of lipid extracts of tissues of patients suffering from gynecological carcinomas. Numbers under the TLC plate correspond to the numbers of cancer patients in Table II.
The Lewis-phenotype was preferentially on the nLc4Cer backbone to express LeY. Only HEC-108 cells established from the poorly differentiated type of endometrial adenocarcinoma expressed neither Leb nor LeY. Both of these phenotypes based on Lc4Cer and nLc4Cer were detected by monoclonal antibody MSN-1. On the other hand, the Lewis-phenotypes in cervical and ovarian carcinomas were mainly on nLc4Cer. In the case of SKG-II cells, Lewis-GSLs were scarcely detected, but a compensatory increase in nLc4Cer was observed, probably due to the decreased activities of α1,3-fucosyltransferase, for LeX, and α2,3-sialyltransferase, for IV3NeuAc-nLc4Cer. Thus, expression of Lewis-phenotypes largely depended on the synthesis of either Lc4Cer or nLc4Cer. In summary, αFuc, IV2αFuc-Lc4Cer (Leb), and III3αFuc, IV2αFuc-nLc4Cer (LeY) were detected in 11 out of 14 cell lines at concentrations of 0.01–0.13 µg per mg of dry weight, and comprised 0.20–3.92% of the total GSLs. Also, monoclonal antibody MSN-1 was found to be a useful probe for the simultaneous detection of Leb and LeY.

Expression of Leb- and LeY-GSLs in the tissues of gynecological cancers

By use of monoclonal antibody MSN-1, expression of Leb and LeY in tissues from the patients was examined, as shown in Fig. 4, and antigens were found to be detected sufficiently with 0.2 mg dry weight. Antigens were present in normal endometria at the follicular and luteal phases and in the well- and moderately differentiated types of endometrial carcinoma, but not in the poorly differentiated type (Table II). The amounts of Leb and LeY in the well-differentiated type (0.01–0.04 µg/mg of dry weight) were higher than those in the moderately differentiated type (0.01–0.02 µg/mg of dry weight) and normal endometria (less than 0.01 µg/mg of dry weight). Also, the frequency of antigen expression in the well- and moderately differentiated types of endometrial adenocarcinoma was higher than that in the normal endometria tested.

DISCUSSION

Transformation-associated carbohydrate antigens provide a useful means of diagnosis and prediction of the prognosis for patients suffering from cancers. However, their expression on cancerous cells and tissues is not always observed, and their intensity, as detected with specific monoclonal antibodies, is variable in the tissues of individual patients with the same type of cancer and in cell lines during the subculture process. These phenomena are thought to be mainly due to the multi-step metabolism required for the synthesis of carbohydrate antigens, which are composed of plural carbohydrates in both lipid- and protein-conjugated forms. In fact, although all cell lines used in this experiment contained globo-series GSLs up to Gb,Cer, the relative concentrations of GlcCer, LacCer, Gb,Cer and Gb,Cer were entirely different among the cell lines, indicating that the metabolic flow determining the expression of GSL-bearing epitopes is flexibly regulated.

Table II. Concentrations of Leb plus LeY in Human Normal Endometria and Cancerous Tissues, as Detected with Monoclonal Antibody MSN-1

| Tissue | Patient No. | Leb plus LeY (µg/mg of dry weight) |
|--------|-------------|-----------------------------------|
| Normal endometria | follicular phase N-1 | 0.01 |
| | N-2 | — |
| | N-3 | t.r. |
| | N-4 | — |
| | N-5 | 0.01 |
| luteal phase N-6 | — |
| | N-7 | t.r. |
| | N-8 | — |
| | N-9 | 0.01 |
| | N-10 | 0.01 |
| | N-11 | — |
| | N-12 | — |
| | N-13 | — |
| Endometrial adenocarcinoma | well-differentiated 3 | 0.04 |
| | 5 | — |
| | 7 | 0.02 |
| | 8 | 0.03 |
| | 11 | 0.03 |
| | 14 | 0.01 |
| | 21 | 0.01 |
| | 22 | 0.04 |
| | 26 | — |
| moderately differentiated 9 | 0.01 |
| | 10 | 0.01 |
| | 15 | — |
| | 18 | 0.02 |
| poorly differentiated 2 | — |
| | 13 | — |
| | 16 | — |
| | 23 | — |
| | 24 | — |
| | 25 | — |
| unknown 1 | 0.01 |
| | 4 | t.r. |
| | 17 | — |
| | 19 | — |
| Endometrial carcinoma (clear cell) 20 | 0.01 |
| cervical carcinoma 6 | 0.01 |
| ovarian cancer 12 | — |

t.r., trace amount; —, not detected.

4 endometrial cell lines other than HHUA, whose Lewis-phenotype was preferentially on the nLc4Cer backbone to express LeY. Only HEC-108 cells established from the poorly differentiated type of endometrial adenocarcinoma expressed neither Leb nor LeY. Both of these phenotypes based on Lc4Cer and nLc4Cer were detected by monoclonal antibody MSN-1. On the other hand, the Lewis-phenotypes in cervical and ovarian carcinomas were mainly on nLc4Cer. In the case of SKG-II cells, Lewis-GSLs were scarcely detected, but a compensatory increase in nLc4Cer was observed, probably due to the decreased activities of α1,3-fucosyltransferase, for LeX, and α2,3-sialyltransferase, for IV3NeuAc-nLc4Cer. Thus, expression of Lewis-phenotypes largely depended on the synthesis of either Lc4Cer or nLc4Cer. In summary, IIIαFuc, IVαFuc-Lc4Cer (Leb), and IIIαFuc, IVαFuc-nLc4Cer (LeY) were detected in 11 out of 14 cell lines at concentrations of 0.01–0.13 µg per mg of dry weight, and comprised 0.20–3.92% of the total GSLs. Also, monoclonal antibody MSN-1 was found to be a useful probe for the simultaneous detection of Leb and LeY.

Expression of Leb- and LeY-GSLs in the tissues of gynecological cancers

By use of monoclonal antibody MSN-1, expression of Leb and LeY in tissues from the patients was examined, as shown in Fig. 4, and antigens were found to be detected sufficiently with 0.2 mg dry weight. Antigens were present in normal endometria at the follicular and luteal phases and in the well- and moderately differentiated types of endometrial carcinoma, but not in the poorly differentiated type (Table II). The amounts of Leb and LeY in the well-differentiated type (0.01–0.04 µg/mg of dry weight) were higher than those in the moderately differentiated type (0.01–0.02 µg/mg of dry weight) and normal endometria (less than 0.01 µg/mg of dry weight). Also, the frequency of antigen expression in the well- and moderately differentiated types of endometrial adenocarcinoma was higher than that in the normal endometria tested.

DISCUSSION

Transformation-associated carbohydrate antigens provide a useful means of diagnosis and prediction of the prognosis for patients suffering from cancers. However, their expression on cancerous cells and tissues is not always observed, and their intensity, as detected with specific monoclonal antibodies, is variable in the tissues of individual patients with the same type of cancer and in cell lines during the subculture process. These phenomena are thought to be mainly due to the multi-step metabolism required for the synthesis of carbohydrate antigens, which are composed of plural carbohydrates in both lipid- and protein-conjugated forms. In fact, although all cell lines used in this experiment contained globo-series GSLs up to Gb,Cer, the relative concentrations of GlcCer, LacCer, Gb,Cer and Gb,Cer were entirely different among the cell lines, indicating that the metabolic flow determining the expression of GSL-bearing epitopes is flexibly regulated.
and can change rapidly. For example, the ratio of Gb$_3$Cer to Gb$_4$Cer, the substrate to product ratio for GalNAc trans-
ferase at the final step of Gb$_4$Cer synthesis, was not identi-
cal among the cells, as shown in Table I. Six cell lines exhib-
ted values higher than 1.00, and the other 8 cell lines gave values lower than 1.00, probably due to not only differences in the expression of the GalNAc trans-
ferase gene and its enzymatic activity, but also the meta-
bolic equilibrium throughout the synthetic pathways for the glo-
bo- and all other series GSLs, because LacCer is also utilized as a common precursor for lacto- and gan-
glio-series GSLs. In the case of the lacto-series GSLs, LeCer and nLeCer were further converted to sialylated and fucosylated derivatives, and accordingly the concentra-
tions of GSLs in the metabolic route seem to be regu-
lated in complex ways. Actually, the amounts of GSLs belonging to the lacto-series were clearly different among the cell lines, as shown in Table I. Some cell lines con-
tained sialylated and fucosylated nLeCer-derivatives with-
out precursor nLeCer, suggesting metabolic potential for the complete conversion of nLeCer to derivatives. In the case of SKG-II cells, which did not contain fucosyl-
ated or sialylated nLeCer, precursor nLeCer was found to be accumulated at a significantly high concentration, and this supports the idea that modification of nLeCer is a major metabolic pathway in human gynecological carci-
noma-derived cells. As for LeY and Lea, they have been proven to be colorectal carcinoma-associated phenotypes useful as markers for diagnosis, and to be synthesized by α1,2-fucosyltransferase from LeX and Lea, respecti-
vately. Considering the enzymatic properties of α1,2-fucosyltrans-
ferase, which catalyzes the fucosylation of LeX and Lea together, if cancer cells possess the activity, the expression of either LeX- or Lea-phenotypes should be determined by the supply of the precursor structure, LeX or Lea. Therefore, the best way to detect the transformation-associated alterations in the Lewis-phenotypes would be the simultaneous detection of LeX and Lea, which could be performed by use of monoclonal antibody MSN-1. As shown in this paper, the MSN-1 antibody was successfully applied to discriminate Lewis-positive cells from negative ones, a distinction that is related to the differentiation types of endometrial adenocarcinomas. HHUA and SNG-
II cells preferentially expressed LeY and Lea, respectively, SNG-M and Ishikawa cells had both structures, and nei-
ther structure was expressed in HEC-108 cells, indicating that either LeY or Lea is expressed in well- and moderately differentiated types of endometrial adenocarcinoma. The results obtained for the cell lines were similar to those for the cancerous tissues, either LeY or Lea being expressed in the tissues of the well- and moderately differentiated types, but not in the poorly differentiated type. The mode of antigen expression in the cancerous tissues was also in accord with the previous observations using immunohis-
tochemical and flow cytometric procedures with the MSN-
1 antibody, whose reactivity was significantly higher in the well-differentiated type than in the poorly differenti-
atated type. From the findings that Lewis-GSLs in endo-
metrial adenocarcinoma-derived cells comprised 0.7 to 3.4% of the total GSLs, and their concentrations were maintained despite the additional sulfating pathway from LacCer to give sulfated GSLs amounting to 7 to 53% of the total GSLs, the synthetic potential for LeX or Lea was judged to be mainly governed by the supply of the precur-
sor GSLs, resulting in the higher expression of Lewis-phe-
otypes in the well-differentiated types. Thus, to assess the metabolic background of the expression of tumor-assoc-
ipated Lewis-phenotypes in relation to clinical diagnosis, immunochemical detection of antigens, together with analyses of the genes for α1,3/4- and α1,2-fucosyltrans-
ferases are essential, and the MSN-1 antibody should pro-
vide a powerful tool for probing endometrial adeno-
carcinoma-associated antigens.

(Received June 20, 2002/Revised July 30, 2002/Accepted August 2, 2002)

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