Jpn. J. Cancer Res. 88, 669–677, July 1997

High Expression of Uridine Diphosphate-galactose: Lc_3Cer β1-3 Galactosyltransferase in Human Uterine Endometrial Cancer-derived Cells as Measured by Enzyme-linked Immunosorbent Assay and Thin-layer Chromatography-immunostaining

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We have developed a new procedure for the selective determination of β1-3 and β1-4 galactosyltransferases with Lc_3Cer as the substrate and the microsomes of fetal and adult porcine livers as the enzyme sources. This method was based on the detection of such products as Lc_4Cer for β1-3 galactosyltransferase (β1-3GT) and nLc_4Cer for β1-4 galactosyltransferase (β1-4GT), with monoclonal anti-Lc_4Cer and anti-nLc_4Cer antibodies, respectively. This method thus enabled us to differentiate the activity of β1-3GT from that of β1-4GT with a high degree of sensitivity. The method was then used to determine the activities of both enzymes in human gynecological carcinoma-derived cells. Four of the five cell lines derived from uterine endometrial cancer expressed significantly high levels of specific activity of β1-3GT among the cell lines examined, while their β1-4GT activities were less than 20% of that for β1-3GT in the endometrial carcinoma-derived cells. On the other hand, a higher specific activity of β1-4GT than that of β1-3GT was detected in the cell lines derived from uterine cervical and ovarian cancers. These findings were thus found to correlate closely with the rate of expression of Lc_4Cer- and nLc_4Cer-based carbohydrate chains in the cell lines based on the results of immunohistochemical staining.

Key words: Galactosyltransferase — TLC-immunostaining — ELISA — Uterine endometrial cancer — Carbohydrate chain

Glycosphingolipids are ubiquitous in various types of mammalian tissue and cells, and their carbohydrate chains are classified into the following five series according to their metabolic pathways: gala-, globo-, ganglio-, lacto-, and neolacto-series. Among these series, the lacto- and neolacto-series glycosphingolipids frequently function as antigens related to the blood group, and their expression is also known to be characteristically altered with the malignant transformation of cells, thus providing a useful molecular marker for the diagnosis of cancer.[1–9] The structural difference between lactotetraose and neolactotetraose is the linkage position of the non-reducing terminal galactose at the *N*-acetylglucosamine residue, with the former and the latter having β1-3 galactose and β1-4 galactose linkages, respectively. Glycosphingolipids, which belong to the lacto- and neolacto-series, express different antigenicities due to their distinct three-dimensional structures. In our project to characterize the biochemical and biological properties of human gynecological cancers, we previously studied the abnormal expression of blood group-related antigens in uterine endometrial cancer. We found the expression of lacto-series carbohydrate chains to be relatively higher than that of the neolacto-series carbohydrate chains in uterine endometrial cancer in comparison to that in uterine cervical and ovarian cancers,[10] which exhibit an antigen which distinguishes uterine endometrial cancer from other types of gynecological cancer. The key enzymes responsible for the synthesis of lacto- and neolactotetraoses are β1-3GT and β1-4GT, respectively, and their selective measurement has thus been thought to be essential for the analysis of the metabolic background regarding the expression of cancer-related lacto- and neolacto-glycosphingolipids. The only way to determine selectively the activities of β1-3GT and β1-4GT is to measure quantitatively the products, Lc_4Cer and nLc_4Cer respectively, which have been detected by TLC-immunostaining and ELISA with mAbs. This approach has thus been used to clarify the mechanism of the abnormal expression of lacto- and neolacto-series carbohydrate chains in cultured cell lines derived from various gynecological cancers.

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Abbreviations used: Lc_3Cer, GlcNAcβ1-3Galβ1-4Glcβ1-1Cer; Lc_4Cer, Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer; nLc_4Cer, Galβ1-4 GlcNAcβ1-3Galβ1-4Glcβ1-1Cer; ELISA, enzyme-linked immunosorbent assay; TLC, thin-layer chromatography; PBS, phosphate-buffered saline; BSA, bovine serum albumin; UDP, uridine diphosphate; β1-3GT, UDP-Gal: Lc_3Cer β1-3 galactosyltransferase; β1-4GT, UDP-Gal: Lc_3Cer β1-4 galactosyltransferase; sLe^a, sialyl Le^a; sLe^x, sialyl Le^x; mAb, monoclonal antibody.

MATERIALS AND METHODS

Glycolipids Lc_4Cer and $IV^3NeuAc\alpha$-nLc_4Cer were purified in our laboratory from human meconium and human placenta, respectively. $IV^3NeuAc\alpha$-nLc_4Cer was treated with *Arthrobacter ureafaciens* neuraminidase (Marukin Shoyu, Kyoto),[11] then Lc_3Cer was prepared from nLc_4Cer by incubation with *Diplococcus pneumoniae* β-galactosidase in 200 m*M* citrate-phosphate buffer (pH 7.1), containing sodium taurocholate (0.5 mg/ml) at 37°C for 18 h.[12–15] The Lc_3Cer thus obtained was then purified by Iatrobeads (6RS8060, Iatron Lab., Tokyo) column chromatography with a gradient of chloroform/methanol (9 : 1 and 2 : 8, by vol).

mAbs A human monoclonal anti-Lc_4Cer antibody, HMST-1, was established by hybridizing the lymphocytes from a patient with endometrial carcinoma and murine myeloma cells[16] and then monitoring the reactivity with endometrial carcinoma-derived cells. The antibody was an IgM, and was proven to react exclusively with Lc_4Cer, but not with nLc_4Cer. The murine monoclonal anti-nLc_4Cer antibody, H-11, was kindly donated by Dr. T. Taki (Tokyo Medical and Dental University, Tokyo).[17] mAb against Le^a was obtained from the Green Cross Corporation (Osaka). A hybridoma producing mouse mAb to Le^b, MSN-1, established in our laboratory, was used as the Le^b probe.[18] mAb against Le^y (NCC-ST-433) was provided by Dr. S. Hirohashi (National Cancer Center Research Institute, Tokyo). mAbs against Le^x (73–30),[19] sialyl Le^a (2D3)[20] and sialyl Le^x (KM-93)[21] were obtained from the Seikagaku Corporation (Tokyo).

Preparation of the microsomal fraction from the tissue and cells as enzyme sources In a separate experiment, we found that the livers of fetal and adult pigs preferentially contained Lc_4Cer and nLc_4Cer, respectively. Accordingly, we prepared the microsomal fractions of fetal (7 months gestation) and adult (2 years old) pig livers as sources of β1-3 and β1-4 galactosyltransferases to establish the analytical conditions. The tissue specimens were homogenized with 9 volumes of 0.25 *M* sucrose in a Potter homogenizer, and centrifuged at 1,000*g* for 10 min to remove the cell debris. The supernatant was then centrifuged at 100,000*g* for 60 min to obtain the microsomal fraction, which was suspended in 50 m*M* cacodylate buffer (pH 6.8) containing 0.01% Triton CF-54 with the aid of sonication. In a similar way, the microsomal fractions were prepared from the following cultured cell lines: uterine endometrial adenocarcinoma-derived cells, HEC108 (poorly differentiated type),[22] SNG-M (moderately differentiated type),[23] SNG-II (well differentiated type),[24] HHUA (well differentiated type),[25] and Ishikawa (well differentiated type);[26] uterine cervical carcinoma-derived cells, SKG-II (large cell, non-keratinizing type),[27] SKG-IIIb (large cell, non-keratinizing type),[28] and HKTUS (small cell, non-keratinizing type);[29] and ovarian carcinoma-derived cells, RMUG-S (mucinous type)[30] and RMG-II (clear cell type).[31] The protein concentration of the fractions was measured by the protein-dye binding method with bovine serum albumin as the standard.[32]

Assay of β1-3GT and β1-4GT by TLC-immunostaining The standard assay mixture for β1-3GT and β1-4GT comprised 25 μg of Lc_3Cer, 50 m*M* cacodylate buffer (pH 6.8) containing 0.01% Triton CF-54, 0.2 *M* KCl, 20 m*M* $MnCl_2$, 1.56 m*M* UDP-galactose, and enzyme in a final volume of 50 μl. After incubation at 37°C for 2 h, the reaction was terminated by the addition of 100 μl of chloroform/methanol (2 : 1, by vol) and the lower phase was then applied to a plastic TLC plate (Sigma, St. Louis, MO). For a quantitative determination of the products, various known amounts (0.01–1.0 μg) of Lc_4Cer or nLc_4Cer were spotted on the same plate and then chromatographed with chloroform/methanol/water (65 : 35 : 8, by vol). The plate was next blocked overnight at 4°C with 1% BSA in PBS, and incubated at 37°C for 2 h with either anti-Lc_4Cer antibody (1 : 10,000 dilution) for the assessment of the β1-3GT activity or anti-nLc_4Cer antibody (1 : 5,000 dilution) for the assessment of β1-4GT activity. The plate was washed five times with 0.1% Tween 20 in PBS, then incubated at 37°C for 1 h with horseradish peroxidase-conjugated antibody, anti-human IgM antiserum (1 : 500, Jackson Lab, Westgrove, PA) in the assays using anti-Lc_4Cer antibody or anti-murine IgM antiserum (1 : 500, Cappel Lab, Cochraville, PA) in the assays using anti-nLc_4Cer antibody, and again washed five times as above. The antibody bound on the plate was then detected by incubation with 2.8 m*M* 4-chloro-1-naphthol and 0.02% H_2O_2 in 50 m*M* Tris-HCl (pH 7.4) containing 200 m*M* NaCl at 37°C for 15 min and the bands were quantitated densitometrically at 420 nm with a dual-wavelength TLC densitometer (CS-9000; Shimadzu, Kyoto). The standard curves were linear up to 1 μg of both Lc_4Cer and nLc_4Cer, and the detection limit was 1 ng for Lc_4Cer and 10 ng for nLc_4Cer. Enzymatic reactions without substrate and with heat-denatured enzymes were also carried out as controls.

Assay of β1-3GT and β1-4GT by ELISA An analysis of β1-3GT and β1-4GT by ELISA was done according to the procedure reported by Taki *et al.*[33,34] In brief, 4 μg of Lc_3Cer in ethanol (1 mg/ml) and 100 μl of 0.08% polyisobutylmethacrylate in chloroform/hexane/methanol (1 : 5.25 : 25, by vol) were added to each well of a 96-well microtiter plate. After evaporation of the solvent using a hair dryer, 100 μl of 1% BSA in PBS was added as a blocking solution and the plate was left overnight at 4°C. Next, the plate was washed once with PBS, and 25 μl of 50 m*M* cacodylate buffer (pH 6.8) containing

0.01% Triton CF-54, 0.2 *M* KCl, 20 m*M* $MnCl_2$, and 1.56 m*M* UDP-galactose, and 25 μl of enzyme (10–80 μg protein) were added to each well. After incubation at 37°C for 2 h, the plate was washed three times with PBS and 50 μl of either monoclonal anti-Lc_4Cer (1 : 10,000) or anti-nLc_4Cer (1 : 5,000) antibodies diluted with 1% BSA in PBS was added to detect the products. The plate was left at 37°C for 2 h and washed three times with PBS. The plate was then incubated at 37°C for 2 h with 50 μl of horseradish peroxidase-conjugated anti-human IgM or anti-mouse IgM antibody diluted 1 : 500 with 1% BSA in PBS. After washing three times with PBS, the peroxidase reaction was carried out at 37°C for 15 min with 100 μl of 0.3% *O*-phenylenediamine and 0.01% H_2O_2 in 10 m*M* acetate buffer (pH 5.5), and was stopped with 10 μl of 8 *N* H_2SO_4. The optical density was measured at 450 nm with an ELISA reader. Reactions using heat-denatured enzyme were simultaneously carried out as the control.

Immunohistochemical examination of Le^a, Le^b, Le^x, Le^y, sialyl Le^a, and sialyl Le^x in various cultured cell lines Immunohistochemical staining was performed by the avidin-biotin complex method as follows.[10] The cells were incubated with monoclonal antibody (1 : 100) directed to Le^a, Le^b, Le^x, Le^y, sialyl Le^a, or sialyl Le^x at room temperature for 1 h and then treated with biotinylated horse anti-mouse IgG antibody and peroxidase-conjugated avidin (Vector Laboratory Inc., Burlingame, CA). The peroxidase on the sections was developed with 0.56 m*M* 3,3′-diaminobenzidine tetrahydrochloride and 0.02% H_2O_2 in PBS, and the sections were counterstained with hematoxylin. The staining patterns were evaluated on the basis of both the staining intensity and the incidence of positive cells. The intensity was graded as weak, moderate, or strong, and the incidence was classified into three grades, <10%, 10% to 50%, and 50%< according to the percentage of positive cells. By combining both the intensity and incidence, we classified the reactivity of the antibodies as (±), (+), (++) or (+++).[10]

RESULTS

Selective determination of β1-3GT and β1-4GT by ELISA Since we found Lc_4Cer to be exclusively contained in the livers of fetal pigs, and nLc_4Cer in adult pigs, we prepared the microsomal fractions of these livers as enzyme sources to establish the reaction conditions for the selective determination of β1-3GT and β1-4GT by ELISA. As reported by Taki *et al.*,[33,34] 4 μg of Lc_3Cer as the substrate per well was required to achieve the maximum production of Lc_4Cer and nLc_4Cer, when the reaction products were detected with anti-Lc_4Cer and anti-nLc_4Cer antibodies, and the optimum pH for both β1-3GT and β1-4GT was 6.8. No production of Lc_4Cer and nLc_4Cer was observed after incubation with 50 μg of microsomes from either fetal or adult livers in wells without Lc_3Cer, as well as with 50 μg of heat-denatured microsomes in wells with Lc_3Cer, thus indicating that endogenous Lc_3Cer, Lc_4Cer and nLc_4Cer do not interfere with the enzyme reaction. Under the above conditions, the synthesis of Lc_4Cer was linear up to 50 μg protein of fetal liver microsomes, and the synthesis of nLc_4Cer was linear up to 80 μg protein of adult liver microsomes (Fig. 1). The specific activities of β1-3GT in the microsomes of

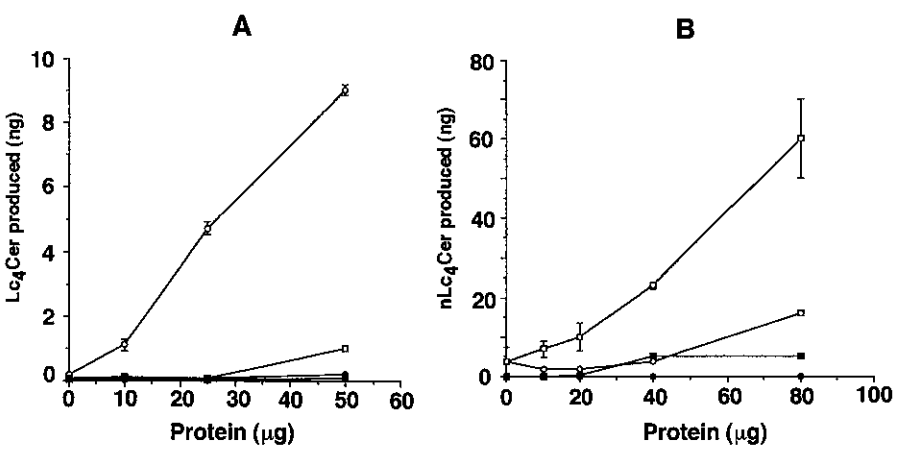

Fig. 1. The activities of β1-3GT(A) and β1-4GT(B) in the microsomes of fetal and adult porcine livers measured by ELISA. The background value was measured with heat-denatured enzymes. The amounts of Lc_4Cer and nLc_4Cer produced were measured by coating known amounts of Lc_4Cer and nLc_4Cer on the wells of an ELISA plate and then letting them react with monoclonal antibodies as described in the text. ○, microsomes of fetal liver; ●, heat-denatured microsomes of fetal liver; □, microsomes of adult liver; ■, heat-denatured microsomes of adult liver. Bars show the mean±SD.

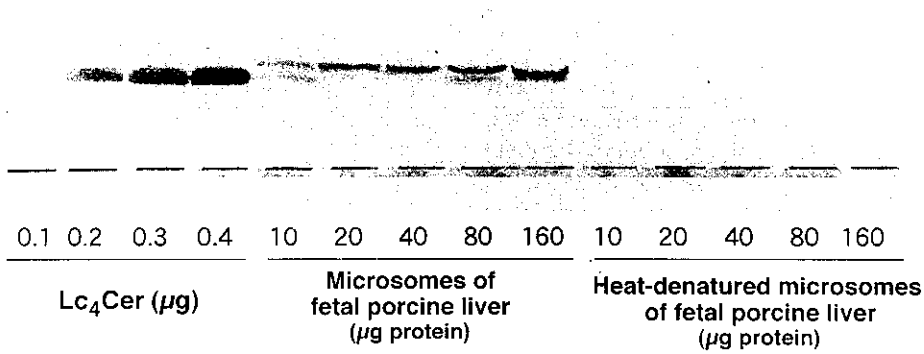

Fig. 2. TLC-immunostaining of the products after reaction of β1-3GT in fetal porcine liver microsomes. Known amounts of Lc_4Cer and the products reacted with various protein concentrations of microsomes and heat-denatured microsomes were chromatographed on a TLC plate with chloroform/methanol/water (65 : 35 : 8, by vol) and visualized by immunostaining with a monoclonal anti-Lc_4Cer antibody as described in the text.

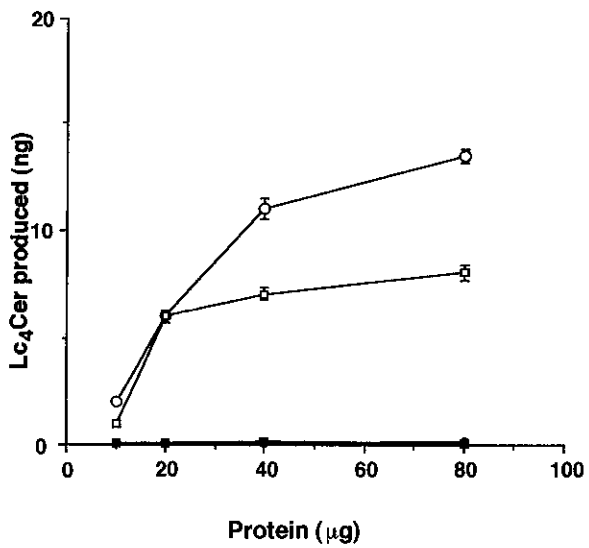

Fig. 3. The activity of β1-3GT in the microsomes of fetal and adult porcine livers measured by TLC-immunostaining. The amounts of Lc_4Cer produced by the reaction of β1-3GT with the microsomes of fetal (○) and adult (□) porcine livers, and the heat-denatured microsomes of fetal (●) and adult (■) livers as controls, were obtained by measuring the density of the spots visualized after immunostaining with a TLC densitometer. Bars show the mean±SD.

fetal and adult livers were 90.0 ng/mg protein/h and 1.5 ng/mg protein/h, respectively, whereas those of β1-4GT were 33.3 ng/mg protein/h and 312.5 ng/mg protein/h, respectively. Therefore, the specific activity of β1-4GT was higher than that of β1-3GT in the adult liver (the ratio of specific activity of β1-3GT to that of β1-4GT in adult liver being 0.005), while in the fetal liver, that of β1-3GT was higher than that of β1-4GT (the ratio being 2.7), thus indicating that the expression of β1-3GT and β1-4GT is developmentally regulated in the porcine liver and the high activity of β1-3GT is a marker of fetal liver.

Determination of β1-3GT by TLC-immunostaining To characterize further the properties of β1-3GT, the product Lc_4Cer was separated on a TLC plate and the amount was quantitated by applying a known amount of Lc_4Cer to the same plate and detecting with anti-Lc_4Cer antibody (Fig. 2). The amount of Lc_4Cer produced increased in proportion to the protein concentration used for the enzyme reaction, and the synthesis of Lc_4Cer was not observed with heat-denatured enzymes. After incubation with 160 μg protein of fetal porcine liver microsomes at 37°C for 2 h, 0.6% of Lc_3Cer as the substrate was converted to Lc_4Cer. As shown in Fig. 3, the specific activities of β1-3GT in the fetal and adult porcine liver microsomes were 137.5 and 46.8 ng/mg protein/h, respectively. The difference in the specific activity evaluated by TLC-immunostaining from that by ELISA was thought to be due to the difference of the substrates, that is, solubilized Lc_3Cer for TLC-immunostaining, and coated Lc_3Cer for ELISA. Thus, a higher specific activity of β1-3GT relative to that of β1-4GT seems to be a characteristic of the fetal porcine liver and may be related to the expression of fetal antigens containing Lc_4Cer.

Application of the selective determination of β1-3GT and β1-4GT for the characterization of several cultured cells derived from human gynecological cancers The procedures established above were applied to characterize the following cell lines: HEC108, SNG-M, SNG-II, HHUA, and Ishikawa from uterine endometrial cancer; SKG-II,

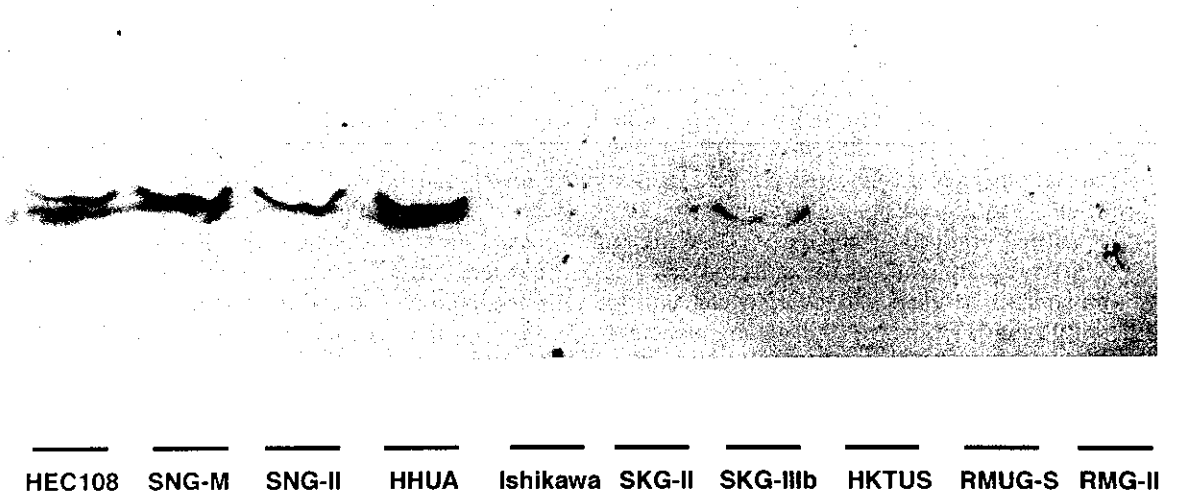

Fig. 4. TLC-immunostaining of the products after the reaction of β1-3GT with anti-Lc_4Cer antibody in various cultured cell lines. After reaction with the enzyme preparations from various gynecological cancer-derived cells at 37°C for 2 h, the products were developed with chloroform/methanol/water (65 : 35 : 8, by vol) and quantitatively detected with the anti-Lc_4Cer antibody.

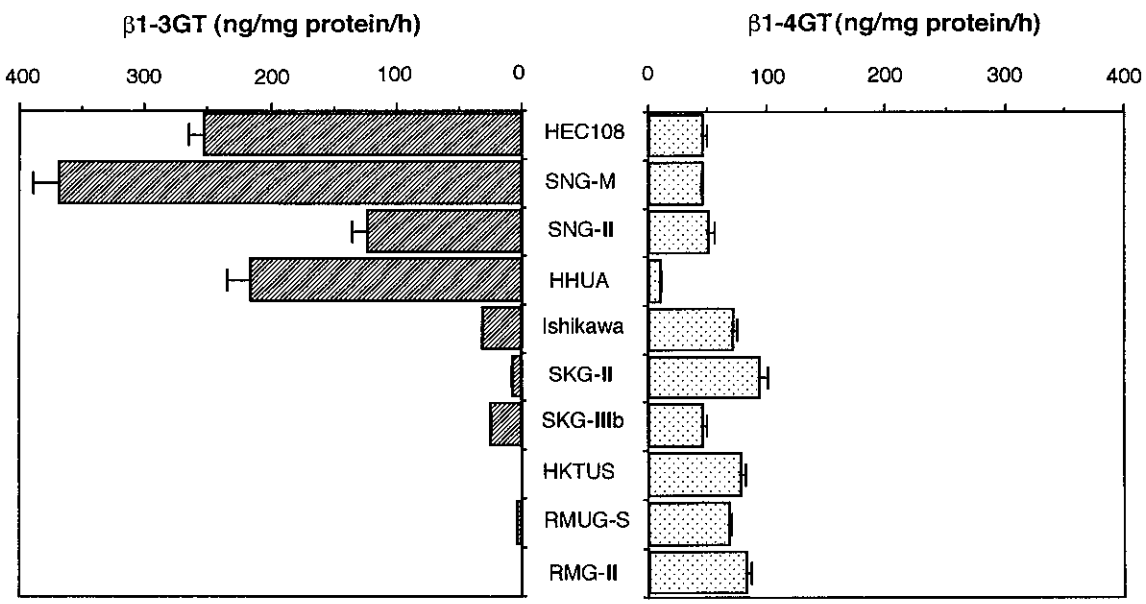

Fig. 5. A comparison of the specific activities of β1-3GT and β1-4GT in various cell lines derived from human gynecological cancers. The specific activities of β1-3GT and β1-4GT measured by TLC-immunostaining and ELISA, respectively, are shown. The standard deviations were all within 10%.

SKG-IIIb, and HKTUS from uterine cervical cancer, and RMUG-S and RMG-II from ovarian cancer. As shown in Fig. 4, the activity of β1-3GT measured by TLC-immunostaining clearly differed among the cell lines, and the uterine endometrial carcinoma-derived cells had relatively high levels of activity. Accordingly, we compared the specific activities of β1-3GT and β1-4GT in these cell lines. Four of the five cell lines derived from uterine endometrial cancer expressed significantly high specific activities of β1-3GT among the cell lines

examined, while their activities of β1-4GT were less than 20% that of β1-3GT in the endometrial carcinoma-derived cells. On the other hand, a higher specific activity of β1-4GT than that of β1-3GT was detected in the cultured cell lines derived from uterine cervical and ovarian cancers (Fig. 5). In cultured cell lines derived from uterine endometrial cancer, the β1-3GT activity ranged from 29.73 to 251.64 ng/mg protein/h, whereas the β1-4GT activity ranged from 10 to 71.25 ng/mg protein/h. On the other hand, in the cultured cell lines derived from uterine cervical and ovarian cancers, the β1-3GT activity ranged from 0 to 24.1 ng/mg protein/h, whereas the β1-4GT activity ranged from 44.38 to 93.13 ng/mg protein/h. The ratio of the specific activity of β1-3GT to β1-4GT ranged from 2.4 to 21.5 in uterine endometrial cancer, whereas it ranged from 0 to 0.08 in uterine cervical and ovarian cancers, thus indicating that endometrial cancer-derived cells have the potential to produce more Lc_4Cer than cervical and ovarian cancer-derived cells.

Comparison of specific activities of β1-3GT and β1-4GT, and immunoreactivities of Lc_4- and nLc_4-based carbohydrate chains in several cultured cell lines Since the concentrations of Lc_4Cer and nLc_4Cer in these cell lines were low, probably due to the rapid modification of these carbohydrate chains to longer carbohydrate chains, and since β1-3GT and β1-4GT were also expected to synthesize glycoprotein-associated carbohydrate chains, we investigated the correlations between the activities of β1-3GT and β1-4GT, and the immunoreactivities of the blood group-related lacto (Lc_4)- and neolacto (nLc_4)-series carbohydrate chains for the characterization of cultured cell lines derived from gynecological cancers. As summarized in Table I, the expression rate of Le^a, sialyl Le^a and Le^b with Lc_4Cer was higher than that of Le^x, sialyl Le^x and Le^y with nLc_4Cer in uterine endometrial cancer-derived cells, whereas the nLc_4-based carbohydrate chains were more highly expressed than the Lc_4-based carbohydrate chains in the cervical and ovarian cancers-derived cells. Furthermore, the findings closely correlated with the specific activities of β1-3GT and β1-4GT, which are closely involved in the expression of blood group-related antigens containing Lc_4- and nLc_4-carbohydrate chains. In the cultured cell lines derived from uterine endometrial cancer, the expression of Le^y antigen was also observed, thus suggesting the presence of Le^y-epitope on the glycoproteins in these cells.[35,36] Similarly, the Lc_4-based carbohydrate chains in HKTUS and RMG-II cells without β1-3GT activity were thought to be mainly expressed on glycoproteins.

Table I. Comparison of the Specific Activities of β1-3GT and β1-4GT, and the Expression of Blood Group-related Antigens in Various Cell Lines Derived from Human Gynecological Cancers

Cell lines	Relative intensity of expression of carbohydrate chains					
	Lacto-series			Neolacto-series		
	Le^a	sLe^a	Le^b	Le^x	sLe^x	Le^y
HEC108	++	++	+++	+	++	+
SNG-M	±	−	++	±	−	++
SNG-II	++	+	++	−	+	++
HHUA	++	++	+++	−	+	++
Ishikawa	++	++	++	±	+	+++
SKG-II	−	++	±	++	+	+++
SKG-IIIb	±	++	±	−	+	++
HKTUS	++	+++	+	++	++	+
RMUG-S	−	±	−	−	+	+
RMG-II	±	++	+	+++	−	++

The relative intensity of the expression of several carbohydrate antigens is shown according to the categories described in the text.

DISCUSSION

The synthesis of carbohydrate chains mainly occurs by the sequential addition of monosaccharides at the non-reducing terminal of the carbohydrate chain, and the rate-limiting step for the synthesis of lacto- and neolacto-series glycosphingolipids is known to be β1-3 *N*-acetylglucosaminyl transferase for the synthesis of Lc_3Cer,[37] in which galactose addition is mediated by β1-3GT and β1-4GT. Therefore, β1-3GT and β1-4GT are considered to be important enzymes determining the carbohydrate chain structures of the lacto- and neolacto-series. The metabolic flow is also regulated by β1-3 *N*-acetylglucosaminyl transferase. In our previous study for the characterization of several gynecological cancers on the basis of antigenicity and the metabolism of cancer-associated carbohydrate antigens, we found that Le^a, Le^b, and Lc_4 antigens, built on the lacto-series carbohydrate chains showed a higher specificity for endometrial cancer than their corresponding positional isomers, Le^x, Le^y, and nLc_4 antigens, built on the neolacto-series carbohydrate chains.[10] Furthermore, this tendency was similar to that observed by staining for Le^a and Le^b antigens in the distal colon. We also found that human endometrial cancers frequently tend to express α1-2 and α1-4 fucosyltransferases,[35] which have low specific activities in the normal human endometrium, thus suggesting that the expression of lacto-series glycolipids and their related enzymes is an endometrial cancer-associated phenomenon. The increase in the expression of Lc_4 antigens in endometrial cancers may be explained by the promotion of galactosyltransferase activity. Taki *et al.* reported that UDP-Gal:GlcNAc glycoprotein β1-4 galactosyltransferase

activity is elevated in the serum of ovarian cancer patients.[33] To our knowledge, few studies have been made on the selective determination of β1-3 and β1-4 galactosyltransferases, which bind galactose to the terminal GlcNAc to form Lc_4- and nLc_4-structures, respectively, and are also the key enzymes in the expression of blood group-related antigens with Lc_4- and nLc_4-structures in association with neoplastic transformation. Clinically, sigmoid colon, rectum, and endometrial cancers frequently tend to express lacto-series carbohydrate chains, and also show a better prognosis compared to the ascending colon, lung, and stomach cancers, which express neolacto-series carbohydrate chains. This might be due to the fact that lacto-series carbohydrate chains are frequently expressed in human fetal tissue, but to a lesser extent in human adult tissue,[38] thus indicating that Lc_4-based carbohydrate chains in human tumors are the oncofetal antigens for the human immune system. In fact, we were able to establish a monoclonal anti-Lc_4Cer antibody from lymphocytes of a lymph node adjacent to the tumor tissue of human uterine endometrial carcinoma, and the antibody was shown to react preferentially with the tumor, but not with normal tissue in the same patient.[16] Consequently, a selective determination of β1-3GT and β1-4GT was required to clarify further the metabolic basis of cancer-associated carbohydrate antigens. In the present study, we developed an assay which enabled us to determine selectively β1-3 and β1-4 galactosyltransferases by detecting their products and we also confirmed that the cell lines derived from endometrial cancer cells have an activity to produce more lacto-series carbohydrate chains, compared to those derived from uterine cervical and ovarian cancers. Therefore, along with the expression of blood group-related carbohydrates, the activity of β1-3GT involved in the synthesis of lacto-series carbohydrate chains was shown to be a useful marker for discriminating endometrial cancer-derived cells from other gynecological cancer-derived cells. We also found that the ratio of specific activity of β1-3GT to that of β1-4GT was closely correlated to the rate of expression of Lc_4Cer-based blood group-related antigens in the cell lines from various gynecological cancers. Further studies are in progress in our laboratory to evaluate these cancerous tissue findings and the relationship between the prognosis after surgical operation and chemotherapy, and the lacto-series carbohydrate-bearing cells.

ACKNOWLEDGMENTS

The authors are very grateful to Dr. S. Hirohashi, Chief of the Pathology Division and Deputy President of the National Cancer Center Research Institute of Japan, and Associate Prof. T. Taki, Tokyo Medical and Dental University, for providing monoclonal antibodies against Le^y and nLc_4Cer antigens and for their helpful advice. The authors also thank Yuko Yoshida for her expert technical assistance. This work was supported in part by a Grant-in-Aid for General Scientific Research (No. 08771364) from the Ministry of Education, Science, Sports and Culture, Japan, and by grants from the Keio University Medical Research Foundation.

(Received March 19, 1997/Accepted May 26, 1997)

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