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Human Cervical Epidermal Carcinoma-associated Intracellular Localization of Glycosphingolipid with Blood Group A Type 3 Chain

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A monoclonal antibody, MRG-1, was produced by immunizing a mouse with a human ovarian mucinous cyst adenocarcinoma-derived cell line, RMUG-L. By immunohistochemical staining, the antigen was found to be exclusively localized in the intracellular structures of the cells used as the antigen and of the epithelial cells in normal human cervical glands. However, although the antigen was predominantly detected in the plasma membrane and the intercellular structure of the middle layer of normal human cervical squamous epithelium (92%), it was also contained in the intracellular structure of cervical epidermal carcinoma at a high frequency (80%). The striking difference in the distribution of the MRG-1 antigen between normal and cancerous tissues was found to be a cervical carcinoma-associated phenomenon and a useful tumor marker for immunohistochemical examination. Since the antigen was found to be of a blood group A-related nature by immunohistochemical staining of the tissues and to be a glycosphingolipid, it was purified from human erythrocytes of blood group A, and the structure was concluded to be GalNAcα1-3Gal(2-1αFuc)β1-3GalNAcα1-3Gal(2-1αFuc)-β1-4GlcNAcβ1-3Galβ1-4Glcβ1-1′Cer, blood group A type 3 chain-containing glycosphingolipid, by NMR, negative ion FABMS and permethylation analysis. In the subcellular localization analysis of the antigen, type 3-A glycosphingolipid antigen was detected in the Golgi body and the microsomes of RMUG-L cells, and the distribution coincided with the finding by immunohistochemical staining. In addition, in cervical epidermal carcinoma, although the blood group A, mainly type 2-A chain, was localized in the plasma membrane and the intercellular structure, the blood group A type 3 chain was selectively found in the perinuclear structure. Also, the blood group A type 3 chain in cervical dysplasia as well as that in normal cervix was predominant in the plasma membrane. Thus, the selective intracellular localization of blood group A type 3 chain was a phenomenon characteristic of cervical epidermal carcinoma and the carcinoma *in situ*.

Key words: Blood group A type 3 chain — Blood group A type 2 chain — Cervical epidermal carcinoma — Immunohistochemistry

Dramatic change in blood group phenotypes is frequently associated with neoplastic transformation. The altered phenotypes include (a) a loss of carbohydrate-chain elongation, (b) neosyntheses of oncofetal antigen, alloantigen and inappropriate tissue antigen and (c) enhanced synthesis of blood group antigens. So far, Le^a, Le^b, Le^x. Le^y, sialyl Le^a and sialyl Le^x, as well as ABH antigens, have been shown to be altered in several types of human cancer.[1–5] These antigens have been detected by utilizing monoclonal antibodies, which have been prepared by immunizing cancer cells or tissues and examining the reactivity, and some of them have been applied to the diagnosis of cancer by detection of the antigens in patients' sera.[6] The genetic background causing the phenotypic alteration is unclear, but the structural polymorphism of the core structures with the same antigenicity is quite interesting, because of their characteristic distribution in different tissues and cells. In the case of blood group A antigen, there are four different structures, namely type 1 to 4 structures[7,8] (Table I), whose distributions in human tissues are quite characteristic. For example, blood group A glycolipids in adult human kidney and the ureter are predominantly composed of the type 4 structure,[9,10] and those in human erythrocytes and granulocytes have the type 2 chain.[11,12] In the mucosa of adult human intestine, type 1 chain A and ALe^b are detected in the columnar and the goblet cells, respectively, and

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Abbreviations used: Le^a, $III^4Fuc\alpha$-Lc_4Cer; Le^b, $IV^2Fuc\alpha$, $III^4Fuc\alpha$-Lc_4-Cer; Le^x, $III^3Fuc\alpha$-nLc_4Cer; Le^y, $IV^2Fuc\alpha$, $III^3Fuc\alpha$-nLc_4Cer; TLC, thin-layer chromatography; PVP, polyvinylpyrrolidone; PBS, phosphate-buffered saline, DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; FABMS, fast atom bombardment mass spectrometry; GLC, gas liquid chromatography.

Table I. Structural Polymorphism of Blood Group A

Blood group A	Structure
Type 1	GalNAcα1-3Gal(2-1αFuc)β1-(3GlcNAcβ1-3Galβ1)$_n$-4Glcβ1-1'Cer
Type 2	GalNAcα1-3Gal(2-1αFuc)β1-(4GlcNAcβ1-3Galβ1)$_n$-4Glcβ1-1'Cer
Type 3	GalNAcα1-3Gal(2-1αFuc)β1-3GalNAcα1-3Gal(2-1αFuc)β1-(4GlcNAcβ1-3Galβ1)$_n$-4Glcβ1-1'Cer
Type 4	GalNAcα1-3Gal(2-1αFuc)β1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1'Cer
ALe^b	GalNAcα1-3Gal(2-1αFuc)β1-3GlcNAc(4-1αFuc)β1-3Galβ1-4Glcβ1-1'Cer

type 2 and type 3 chain A structures are absent. By contrast, A antigens with type 1, type 2 and type 3 chains are expressed in distal colon adenocarcinoma as well as in fetal colonic mucosa.[3] Thus, expression of blood group A antigens with different basic structures is characteristic of different tissues and cells, and is applicable for the diagnosis of cancer and the characterization of cells in different differentiation stages. We also observed a characteristic change in the distribution of blood group A antigens with different structures, which was associated with human cervical epidermal carcinoma.

MATERIALS AND METHODS

Monoclonal antibodies Murine monoclonal antibody MRG-1 was prepared by immunizing mouse Balb/c with a cultured cell line, RMUG-L, which was established from human ovarian mucinous cyst adenocarcinoma, and monitoring the antibody-producing hybridoma with RMUG-L as the antigen. The antibody MRG-1 established was IgM. Murine monoclonal anti-human blood group A antibody was purchased from Dako, Copenhagen.

Cells and tissues Cell lines from pathologically well-defined gynecological cancer were established in the Department of Obstetrics and Gynecology, Keio University School of Medicine.[13] Tissues from patients suffering from uterine endometrial adenocarcinoma, cervical adenocarcinoma and ovarian mucinous cyst adenocarcinoma were obtained from the University Hospital of Keio University. Also, human blood group A erythrocytes were obtained from the Japanese Red Cross Blood Center.

Extraction and preparation of glycosphingolipids Cells and tissues were lyophilized and the lipids were extracted successively with chloroform:methanol:water (20:10:1, 10:20:1 and 10:10:1, by volume) at 40°C.[14] The combined lipid extracts were fractionated into neutral and acidic lipids by DEAE-Sephadex column chromatography. Then neutral and acidic glycosphingolipids were prepared from the neutral and acidic lipid fractions by acetylation. Florisil column chromatography and deacetylation, and by mild alkaline hydrolysis and dialysis, respectively. Individual neutral glycosphingolipids were purified on a column (1.8 cm internal diameter × 55 cm) packed with Iatrobeads (6RS8060, Iatron Lab., Tokyo) with a gradient formed from chloroform-methanol-water, 70:30:0.5 and 10:90:4 (by volume). Also, purification of octahexaosyl ceramide (blood group A type 2 chain) from nonahexaosyl ceramide (blood group A type 3 chain) was achieved by Iatrobeads column chromatography with a gradient formed from *n*-propanol-2.5 *M* ammonia in water, 90:10 and 50:50 (by volume).

TLC and TLC-immunostaining Neutral glycosphingolipids were chromatographed on silica gel 60 HPTLC plates (E. Merck Co., Darmstadt, Germany) with solvent systems (a) chloroform-methanol-0.5% calcium chloride in water (55:45:10, by volume), (b) chloroform-methanol-water (65:35:8, by volume) and (c) *n*-propanol-2.5 *M* ammonia in water (70:30, by volume), and were detected with orcinol-H_2SO_4 reagent. The mobilities on TLC of glycosphingolipids were compared with those of blood group A-glycosphingolipids, IV^{2}Fucα,IV^{3}GalNAcα-nLc$_4$Cer and VI^{2}Fucα,VI^{3}GalNAcα-nLc$_6$Cer, and the amounts were determined densitometrically with a dual-wavelength TLC-densitometer (CS-9000, Shimadzu, Kyoto) at 420 nm analytical wavelength and 710 nm control wavelength. For TLC-immunostaining, a plastic-coated TLC plate (Polygram, Macherey Nagel, Duren, Germany) was used for development of glycosphingolipids with solvent (a). After development, the plate was incubated with blocking buffer (1% PVP, 1% ovalbumin and 0.02% NaN_3 in PBS) at 37°C for 1 h and was reacted with about 0.2 μg protein of murine monoclonal antibody in 3% PVP in PBS at 37°C for 2 h. The plate was washed 5 times with 0.1% Tween 20 in PBS, and the antibody bound on the plate was detected with peroxidase-conjugated anti-mouse IgG+M antiserum (Cappel Lab., Cochranville, PA) diluted 1:1000 with 3% PVP in PBS, and the enzyme substrate (H_2O_2 and 4-chloro-1-naphthol) as described previously.[15] The density of the spots was determined with a TLC-densitometer as described above.

Structural analysis of the glycosphingolipid reacted with monoclonal antibody The homogeneity of the glycosphingolipid antigens purified by Iatrobeads column

chromatography, as above, was monitored by TLC with orcinol-H_2SO_4 reagent and by TLC-immunostaining with monoclonal antibody MRG-1. The structure of the purified glycosphingolipids was determined by NMR, negative ion FABMS and permethylation analysis. Prior to NMR analysis, the protons of about 1 mg of glycosphingolipid were replaced with deuterons by incubating the sample with DMSO-d_6-^2H_2O (2:1, by volume) at 60°C for 10 min. The NMR spectrum was obtained in DMSO-d_6-^2H_2O (98:2, by volume) with a 500 MHz NMR spectrometer (GSX-500, JEOL, Tokyo) at 60°C with TMS as an internal reference. Then, about 5 μg of glycosphingolipid in 5 μl of triethanolamine was analyzed by negative ion FABMS with a neutral xenon beam (JMS HX-110, JEOL).[4,16] Assignment of the mass numbers was performed with a computer system (JMA-3500, JEOL) by comparing the spectrum with that of perfluoroalkyl phosphazine (Ultra mark, PCR, FL). In addition, for determination of the linkage positions of the carbohydrate moiety of glycosphingolipids, the partially methylated aldohexitol acetates and N-methylacetamido aldohexitol acetates were prepared from the permethylated derivative of glycosphingolipid,[17] and were analyzed by GLC on 3% OV-275 at 190°C and on 2% OV-17 with a programmed temperature increase of 1°C/min from 170 to 240°C, respectively.

Immunohistochemical detection of antigen in tissues and cells A 4 μm section of formaldehyde-fixed tissues, of which the endogenous peroxidase had been inactivated by preincubation with H_2O_2, was incubated with murine monoclonal antibody and subsequently treated with peroxidase-conjugated anti-mouse IgG+M antibody, followed by 3,3-diaminobenzidine tetrahydrochloride plus H_2O_2 solution.[18] Cells grown on a chamber slide (Lab-Tek, Nunc, Naperville, IL) were also fixed with formaldehyde and stained with monoclonal antibody, as described above.

Subcellular localization of antigen in cells Cells derived from human ovarian mucinous cyst adenocarcinoma, RMUG-L, were homogenized in 0.25 *M* sucrose with a Potter-Elvehjem homogenizer and the homogenate was centrifuged at 700*g* for 10 min. The nucleus fraction was prepared from the resultant pellet by centrifugation in 2.4 *M* sucrose containing 1 m*M* $MgCl_2$ at 50,000*g* for 60 min. The 700*g* supernatant was centrifuged at 2,000*g* for 20 min and the resultant pellet was centrifuged in discontinuous sucrose solution (0.5 *M* to 1.25 *M*) at 100,000*g* for 30 min to obtain the Golgi bodies, followed by discontinuous centrifugation of the pellet with 0.8 *M* to 1.2 *M* sucrose to obtain the mitochondria and the lysosome fractions. Microsomes were obtained from the supernatant by centrifugation at 105,000*g* for 100 min. After dialysis against water, each fraction was lyophilized and the lipids were extracted as described above. The lipid extracts, corresponding to 1 μg of protein, were chromatographed on a plastic-coated TLC plate with solvent (a) and stained with monoclonal antibody MRG-1.

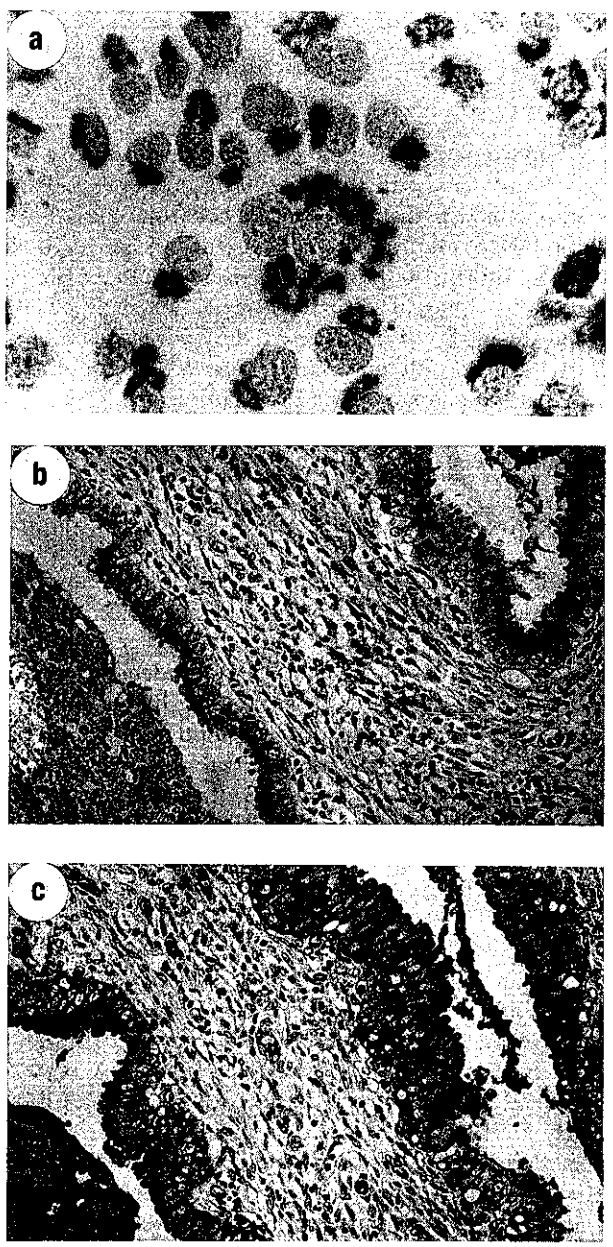

Fig. 1. Indirect immunohistochemical staining of human ovarian mucinous cyst adenocarcinoma-derived RMUG-L cells (a) and normal human cervical gland (b and c) with murine monoclonal antibody MRG-1 (a and b) and murine monoclonal anti-blood group A antibody (c). The second antibody was peroxidase-conjugated anti-mouse IgG+M antibody.

RESULTS

Immunohistochemical staining with monoclonal antibody MRG-1 Murine monoclonal antibody MRG-1 was strongly reactive with cell line RMUG-L, which was derived from human ovarian mucinous cyst adenocarcinoma and was used as an immunogen. However, the antigen was found to be distributed in the intracellular structure, but not in the plasma membrane of the cell line RMUG-L (Fig. 1-a). Furthermore, the structure giving a positive reaction with antibody MRG-1 seemed to be located in the perinuclear structure. The same distribution of the antigen was observed in the intracellular structure of the epithelial cells in the cervical glands (Fig. 1-b), but the positive staining with antibody MRG-1 was restricted to the normal human cervical glands of blood groups A and AB, suggesting that the antigen is related to the blood group A antigen. Then, the same tissue section was stained with anti-blood group A antibody (Dako)(Fig. 1-c). The A-antigen was only observed in the epithelial cells of the cervical gland, like the MRG-1 antigen, but was distributed in both the cell surface and the cytoplasm including the perinuclear structure. As clearly shown in Fig. 1-c, the intracellular structure of epithelial cells of the cervical gland was diffusely stained with anti-blood group A antibody and the staining pattern was completely different from that with monoclonal antibody RMG-1. Thus the intracellular distribution of MRG-1 antigen was different from that of A-antigen. On the other hand, the plasma membrane and the intercellular space of the normal cervical squamous epithelium, particularly the cells in the middle layer of the epithelium close to the basal layer, but not the cells in the surface region, were strongly positive with monoclonal antibody MRG-1, and no antigen was detected in the intracellular structure of the cervical epithelium (Fig. 2-a). As shown in Fig. 2-b, the staining pattern with monoclonal antibody MRG-1 of the cervical epithelium was the same as that with anti-blood group A antibody. However, in tissues from the patients with cervical epidermal carci-

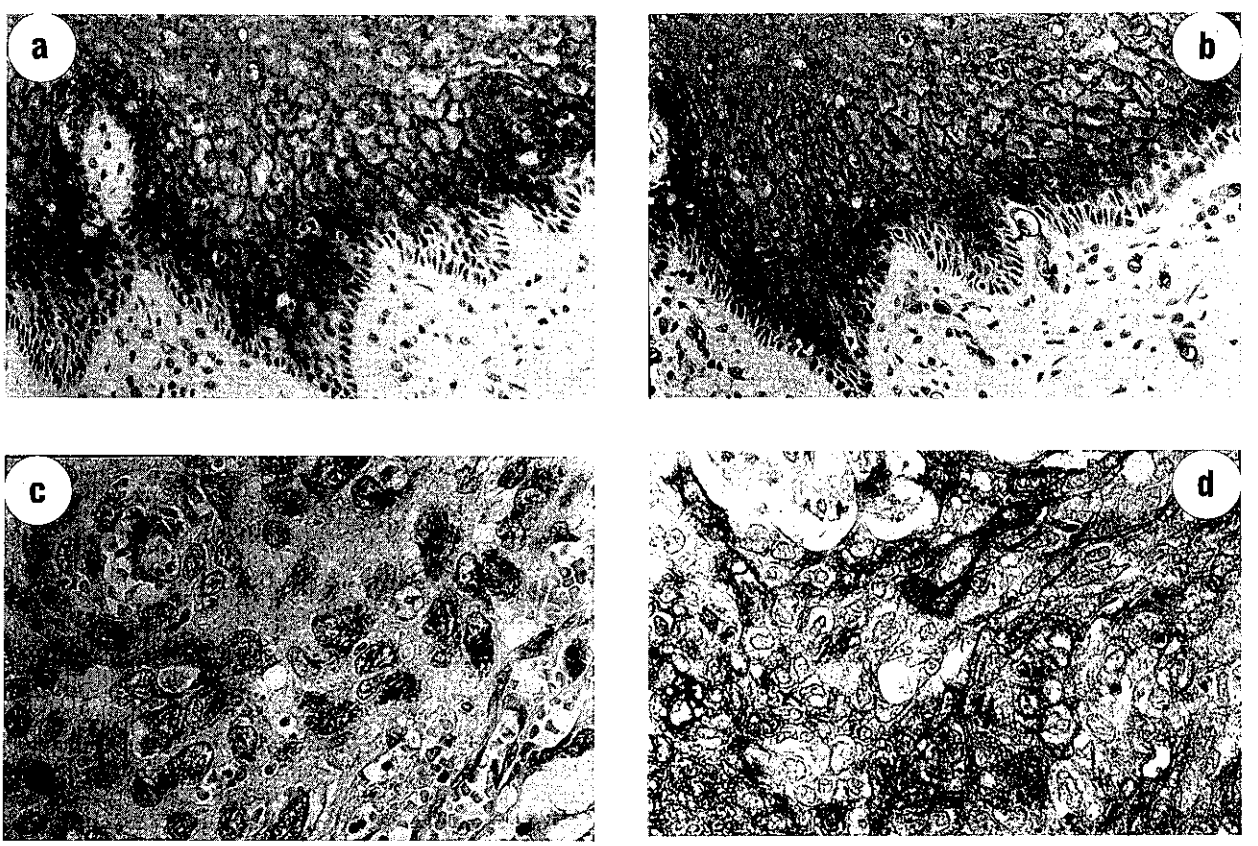

Fig. 2. Indirect immunohistochemical staining of normal human cervical squamous epithelium (a and b) and cervical epidermal carcinoma (c and d) with murine monoclonal antibody MRG-1 (a and c) and murine monoclonal anti-blood group A antibody (b and d).

noma, MRG-1 antigen was only distributed in the intracellular space as a particle close to the nucleus (Fig. 2-c), and the antigen reactive with anti-blood group A antibody was abundantly distributed in the plasma membrane (Fig. 2-d). In the case of cervical dysplasia, distribution of the MRG-1 antigen was frequently observed in the plasma membrane (Fig. 3). The MRG-1 antigen was detected in all normal cervical epithelia from the patients of the blood groups A and AB, and the high rate of positive staining was maintained in the tissues from cervical epidermal carcinoma *in situ* (72.7%) and cervical epidermal carcinoma (71.4%) (Table II). Also, the antigen was detected in cervical epidermal carcinoma of blood group B and O individuals. Then, we compared the distribution of the MRG-1 antigen in the plasma membrane and the intracellular space of the cells (Table III). In normal cervical epithelium, the antigen was predominantly distributed in the plasma membrane and the intercellular space (92%), but the cells in cervical epidermal carcinoma *in situ* and cervical epidermal carcinoma exclusively contained the MRG-1 antigen in the intracellular space (72.7% for cervical epidermal carcinoma *in situ* and 82.3% for epidermal carcinoma), indicating that the intracellular distribution of the MRG-1 antigen is a human cervical cancer-associated phenomenon.

Characterization of the MRG-1 antigen The antigen reactive with monoclonal antibody MRG-1 was found to be predominantly expressed in the glycosphingolipid fraction of the ovarian mucinous cyst adenocarcinoma-

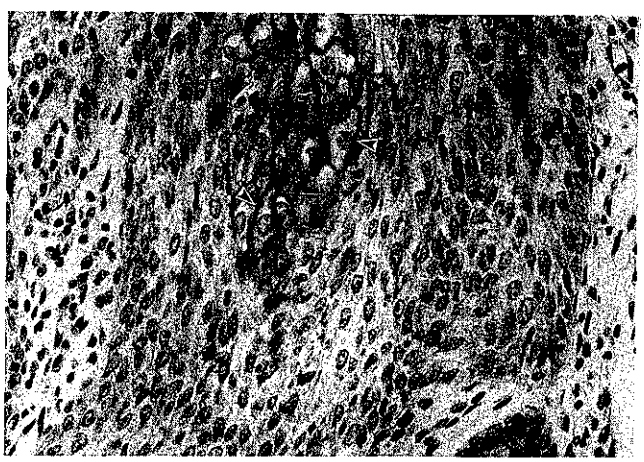

Fig. 3. Indirect immunohistochemical staining of human cervical dysplasia with murine monoclonal antibody MRG-1.

Table II. Positive Rate of Staining with Monoclonal Antibody MRG-1 in Human Cervical Dysplasia and Cervical Epidermal Carcinoma

Blood group	Cervical dysplasia	Cervical epidermal carcinoma *in situ*	Cervical epidermal carcinoma
A	8/9	6/7	6/10
B	0/1	0/2	1/4
O	2/5	3/4	5/10
AB	4/4	2/4	4/4

Table III. Distribution of the MRG-1 Antigen in Mormal Human Cervical Epithelium, Cervical Dysplasia and Cervical Epidermal Carcinoma

Tissue	Regions reactive with monoclonal antibody MRG-1	
	Plasma membrane and intercellular space	Intracellular space
Normal cervical epithelium	23/52 (92.0%)	2/25 (8.0%)
Cervical dysplasia	5/6 (83.3%)	1/6 (16.7%)
Cervical epidermal carcinoma *in situ*	6/22 (27.3%)	16/22 (72.7%)
Cervical epidermal carcinoma	3/17 (17.6%)	14/17 (82.3%)

Tissue sections giving a positive reaction with monoclonal antibody MRG-1 were used for examination.

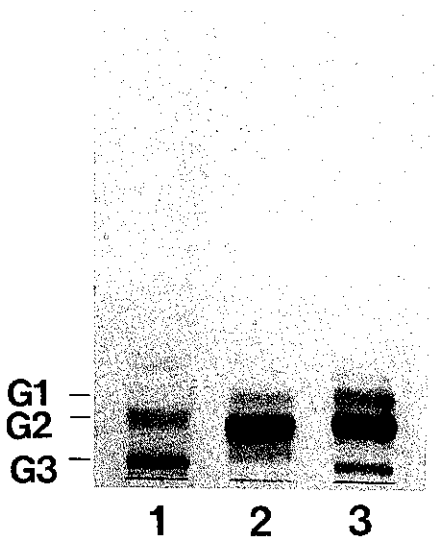

Fig. 4. TLC-immunostaining of neutral glycosphingolipids obtained from human cervical epidermal carcinoma (lane 1), ovarian mucinous cyst adenocarcinoma-derived RMUG-L cells (lane 2) and human blood group A erythrocytes (lane 3) with murine monoclonal antibody MRG-1. Neutral glycosphingolipids were chromatographed on a plastic-coated TLC plate with solvent (b) and were stained with murine monoclonal antibody MRG-1 as described in the text.

derived cell line RMUG-L, and tissues from normal cervix and cervical epidermal carcinoma, and was also detected in erythrocytes of blood group A, but not blood group B. As shown in Fig. 4, the major band stained with antibody MRG-1 of glycosphingolipids from blood group A erythrocytes was also detected in the tissue of cervical epidermal carcinoma. The concentration of the major band in the cancerous tissues was estimated to be less than 5 pmol/g dry weight and detection with orcinol-H_2SO_4 reagent was difficult due to the limited amount present. Therefore, the antigen was purified from human blood group A erythrocytes by monitoring with orcinol-H_2SO_4 reagent, anti-blood group A antibody and antibody MRG-1. As shown in Fig. 5, five glycosphingolipids having positive reaction with the antibodies were obtained in pure form (glycosphingolipids in lanes 1 to 5 were designated as glycosphingolipids 1 to 5, respectively). Glycosphingolipids 2, 3 and 5 were positive with both antibodies, whereas glycosphingolipids 1 and 4 were positive with anti-blood group A antibody, but not with antibody MRG-1. Separation of glycosphingolipid 3 from 4 was achieved only with solvent systems of *n*-propanol-ammonia, as described in "Materials and Methods." Glycosphingolipid 3 was identical with the antigen in cancerous cells and tissues in terms of the mobilities with different solvent systems, the negative ion FABMS and the reactivity with anti-blood group A antibody and antibody MRG-1, and was the antigen for monoclonal antibody MRG-1. In the negative ion FABMS of glycosphingolipid 3, the molecular and sequential fragment ions were clearly detected, indicating that the carbohydrate sequence is HexNAc-Hex(Fuc)-HexNAc-Hex(Fuc)-HexNAc-Hex-Hex-ceramide (Fig. 6). The NMR spectrum (Fig. 7) also showed that the glycosphingolipid was nonahexaosyl ceramide, as above. Among the anomeric protons, two GalNAc residues were found to be α-anomers and one of them was located inside the carbohydrate chain. On permethylation analysis, the glycosphingolipid gave 2,3,6-tri-O-methyl-1,4,5-tri-O-acetylglucitol, 2,4,6-tri-O-methyl-1,3,5-tri-O-acetyl-

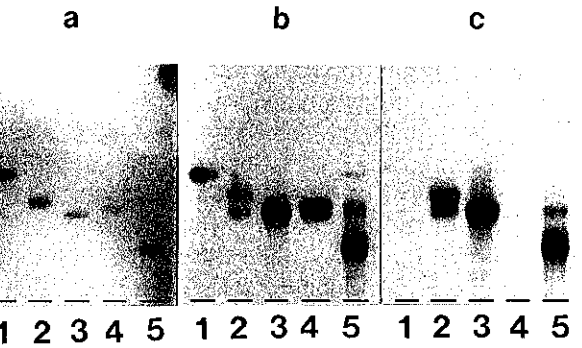

Fig. 5. TLC of neutral glycosphingolipids purified from human blood group A erythrocytes. Neutral glycosphingolipids were chromatographed on a plastic-coated TLC plate with solvent a and visualized with orcinol-H_2SO_4 reagent (a), murine monoclonal anti-blood group A antibody (b) and murine monoclonal antibody MRG-1 (c). Glycosphingolipids G1, G2 and G3 in Fig. 4 correspond to those in lanes 2, 3 and 5.

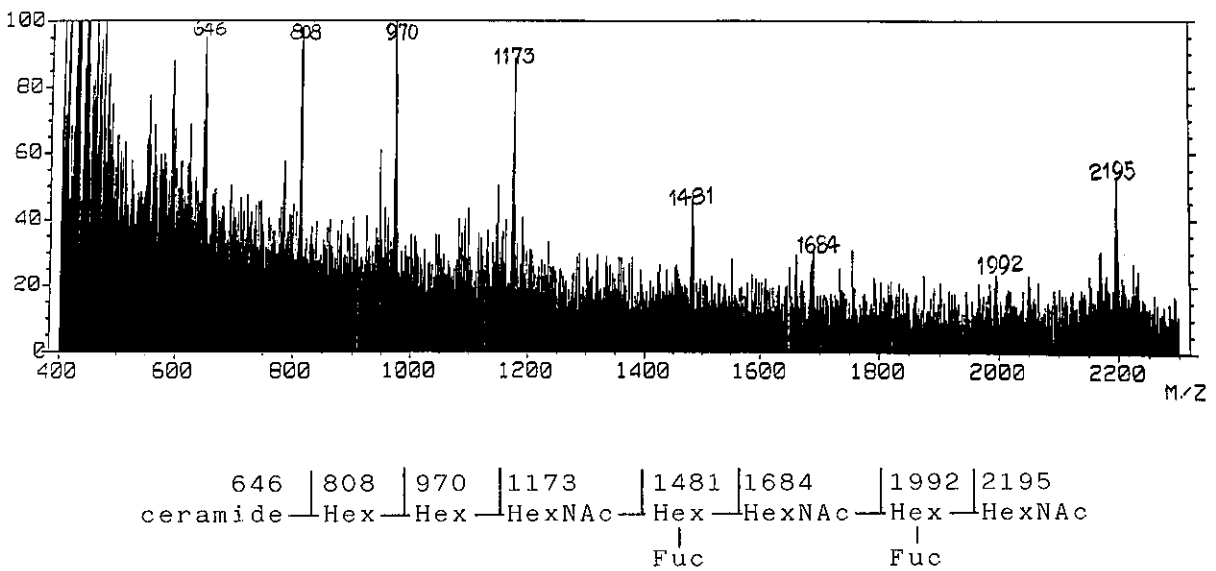

Fig. 6. Negative ion FABMS of neutral glycosphingolipid 3 in Fig. 5.

galactitol, 4,6-di-O-methyl-1,2,3,5-tetra-O-acetylgalactitol, 2,3,4-tri-O-methyl-1,5-di-O-acetylfucitol, 3,6-di-O-methyl-2-deoxy-2-(N-methylacetamido)-1,4,5-tri-O-acetylglucitol, 4,6-di-O-methyl-2-deoxy-2-(N-methylacetamido)-1,3,5-tri-O-acetylgalactitol and 3,4,6-tri-O-methyl-2-deoxy-2-(N-methylacetamido)-1,5-di-O-acetylgalactitol in a ratio of 1:1:2:2:1:1:1. Thus, glycosphingolipid 3 was concluded to be a blood group A type 3 chain-containing molecule, GalNAcα1-3Gal(2-1α-Fuc)β1-3GalNAcα1-3Gal(2-1αFuc)β1-4GlcNAcβ1-3Galβ1-4Glcβ1-1′ceramide. By the same analytical procedures, glycosphingolipids 1, 4 and 5 were shown to be GalNAcα1-3Gal(2-1αFuc)β1-4GlcNAcβ1-3Galβ1-4Glcβ1-1′Cer (blood group A type 2 chain), GalNAcα1-3Gal(2-1αFuc)β1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1′ceramide (blood group A type 2 chain) and GalNAcα1-3Gal(2-1αFuc)β1-3GalNAcα1-3Gal(2-1αFuc)β1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1′ceramide (blood group A type 3 chain), respectively. The complete structure of glycosphingolipid 2 with blood group A type 3 antigenicity could not be determined due to the limited amount available for analysis. Thus, monoclonal anti-blood group A antibody, available commercially, was found to recognize the nonreducing terminal of blood group A glycosphingolipid, GalNAcα1-3Gal(2-1αFuc)β1-, whereas monoclonal antibody MRG-1 required the repeated structure of the blood group A determinant, GalNAcα1-3Gal(2-1αFuc)-β1-3GalNAcα1-3Gal(2-1αFuc)β-.

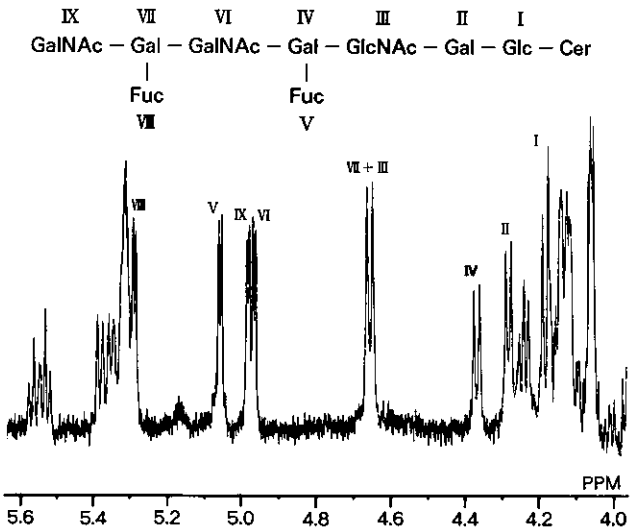

Fig. 7. NMR spectrum of neutral glycosphingolipid 3 in Fig. 5.

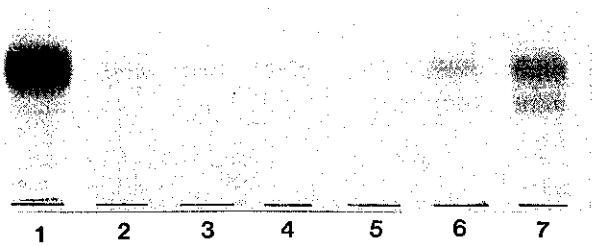

Fig. 8. TLC-immunostaining with murine monoclonal antibody MRG-1 of the lipid extracts from total homogenate (lane 2), nucleus (lane 3), plasma membrane (lane 4), mitochondria (lane 5), microsomes (lane 6) and Golgi body (lane 7) of human ovarian mucinous cyst adenocarcinoma-derived RMUG-L cells. Neutral glycosphingolipid with blood group A type 3 chain (lane 1) was cochromatographed on a plastic-coated TLC plate with solvent (a). Total lipid extracts, corresponding to 1 μg of protein, were spotted on the plate.

Localization of glycosphingolipid with blood group A type 3 chain in subcellular fraction To determine whether the antigen detected in the intracellular structure of cancerous cells by the immunohistochemical technique is glycosphingolipid or not, glycosphingolipid with a blood group A type 3 chain in several subcellular fractions of the RMUG-L cells was measured by means of the TLC-immunostaining procedure. As shown in Fig. 8, the glycosphingolipid antigen was detected in the microsome and Golgi body fractions of the cells at concentrations very much higher than those in the homogenate and the other fractions, reflecting the characteristic distribution profile of the antigen in the perinuclear structure of the cells found by immunohistochemical staining (Fig. 1-a).

DISCUSSION

As clearly shown in this paper, in normal human cervical gland, the distribution of the blood group A type 3 chain was restricted to the perinuclear structure of the epithelial cells of the gland, whereas it was found in the plasma membrane and the intercellular space of the middle layer of the cervical squamous epithelium. In addition, although the blood group A materials were distributed diffusely in the intracellular space and the plasma membrane of the epithelial cells of the cervical gland (Fig. 1), the distributions of blood group A materials and type 3-A chain in the cervical glands were clearly different from each other. A similar differential distribution of blood group A type 2 and 3 chains has

been reported in adenocarcinoma of human distal colon.[3] Our monoclonal antibody MRG-1 seems to recognize the same antigenic determinant as does the monoclonal antibody TH-1 reported previously.[17] In the case of colonic adenocarcinoma, expression of blood group A type 2 and 3 chains was a tumor-associated phenomenon, because no blood group A chain was found in the mucosa of the sigmoid colon and rectum. However, in normal cervix uteri, blood group A (mainly type 2 chain) and type 3-A chain were distributed in the same manner as in the middle layer of the epithelium, and their distribution was significantly altered in cervical epidermal carcinoma, with type 3-A chain being selectively localized in the intracellular space with high frequency. Two genes, A-1 and A-2, were recently clarified to be responsible for the synthesis of blood group A types 1-4 chains,[18] but their expression is regulated by several factors. For example, Se and Le genes affect the espression of type 1 chains in tissues of endodermal origin, but not the expression of type 2 chain in tissues of ectodermal and mesodermal origin.[19] This regulation causes a distinct difference in the expression of glycosphingolipids, such as selective expression of the ALe^b chain in human kidney artery and the blood group A type 3 chain in kidney vein.[20] A key enzyme for the synthesis of blood group A type 3 chain is β1-3 galactosyl transferase to the αGalNAc residue of blood group A type 2 chain,[21] which is a factor regulating the expression of blood group A type 3 chain. The characteristic expression of blood group A type 3 chain in the middle layer of normal cervical epithelium also seem to be regulated by the above factors. Thus, the biosynthetic potential for blood group A type 3 chain was retained in the cervical epidermal carcinoma, but a factor for regulating transfer of blood group A type 3 chain, but not type 2 chain, from the Golgi body to the plasma membrane was thought to be inactive due to the neoplasmic transformation. Since the distribution of the type 3 A chain in cervical epidermal carcinoma was clearly different from that in normal cervical epithelium, the monoclonal antibody MRG-1 should be a useful reagent for determining the change in distribution of blood group A type 3 chain by immunohistochemical examination.

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