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Development and Characterization of a Novel Anti-fucosylated Antigen Monoclonal Antibody YB-2 and Its Usefulness in the Immunohistochemical Diagnosis of Colorectal Cancer

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A novel monoclonal antibody, YB-2 was obtained after immunization of mice with fucosylated antigens isolated from human saliva. The antibody was demonstrated to react with Y (Fucα1→2Galβ1→4[Fucα1→3]GlcNAcβ), Le^b (Fucα1→2Galβ1→3[Fucα1→4]GlcNAcβ) and H type 2 (Fucα1→2Galβ1→4GlcNAcβ) antigens, but not with H type 1 (Fucα1→2Galβ1→3GlcNAcβ), Le^a (Galβ1→3[Fucα1→4]GlcNAcβ), X (Galβ1→4[Fucα1→3]GlcNAcβ) or with non-fucosylated antigens. Inhibition assays of YB-2 antibody with such reactive antigens showed that YB-2 antibody preferentially reacted with Y antigen. Formalin-fixed and paraffin-embedded sections prepared from normal and malignant colorectal tissues were examined immunohistochemically with YB-2. The positive rates of staining with YB-2 antibody were 88.6% in malignant and 12.0% in normal tissues. The expression of fucosylated antigens detected by YB-2 antibody seemed to be correlated with survival among patients with primary colorectal cancer. Therefore, YB-2 antibody could be useful as an immunochemical tool for diagnosis and evaluation of the prognosis of colorectal cancer.

Key words: Anti-fucosylated antigen antibody — Tumor-associated antigen — Colorectal cancer — Immunohistochemical diagnosis

Changes of the expression of glycoconjugates have been described in a variety of cancers,[1,2] and the presence of aberrant glycoproteins and glycolipids which are absent or present at only low levels in normal tissues has been widely observed in various tumor tissues.[1-3] A series of fucosylated glycoconjugates has been demonstrated to be accumulated in various tumor tissues and some of them are involved in blood group specificities.[2] There are three different types of backbone structures: type 1 (Galβ1→3GlcNAcβ)[7]; type 2 (Galβ1→4GlcNAcβ); and type 3 (Galβ1→3GalNAcα) (see Table I).

With the development of hybridoma techniques, many monoclonal antibodies against glycoconjugates have been obtained and carbohydrate antigens can be defined specifically with the aid of such monoclonal antibodies.[1-5] On the other hand, some monoclonal antibodies show relatively broad patterns of reactivities and cross-react with closely related or even distantly related structures.[6-8] As mentioned in the cited reports, it is important to understand precisely the specificities and reactivities of monoclonal antibodies if they are to be used as reagents for structural and immunohistochemical analysis.

In colorectal carcinoma tissues, the expression of blood group substances and related antigens has been reported to be different from that in normal colon.[9-13] The expression of ABH and Le^b antigens is almost lost from normal distal tissue but preserved in proximal tissue, while Le^a antigen appears to be more uniformly distributed throughout the proximal and distal colon tissues. The ABH and Le^b antigens are re-expressed in colorectal tumors, and in particular, accumulation of Y, Le^b and H antigens in colon cancer has been shown by immunohistochemical means.

In this study, we developed and characterized a monoclonal antibody, designated YB-2, that specifically reacts with Y, Le^b and H type 2 antigens, and used it to investigate the expression and accumulation of such fucosylated antigens in colorectal carcinoma.

MATERIALS AND METHODS

Materials Chemically synthesized oligosaccharides (Le^c, sialyl-Le^c, H type 1, Le^a, sialyl-Le^a, Le^b, LacNAc, sialyl-

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[7] The abbreviations used are: Gal, D-galactose; GlcNAc, N-acetyl-D-glucosamine; GalNAc, N-acetyl-D-galactosamine; Fuc, L-fucose; LacNAc, N-acetyllactosamine; NANA, N-acetylneuraminic acid; BSA, bovine serum albumin; AAL, *Aleuria aurantia* lectin; PBS, 0.01 *M* phosphate-buffered saline, pH 7.0.

Table I. Antigenic Determinants of Carbohydrate Antigens Carried by Type 1, Type 2 and Type 3 Structures

Type 1	
Le^c	Galβ1→3GlcNAcβ
sialyl-Le^c	NANAα2→3Galβ1→3GlcNAcβ
H type 1	Fucα1→2Galβ1→3GlcNAcβ
Le^a	Galβ1→3[Fucα1→4]GlcNAcβ
sialyl-Le^a	NANAα2→3Galβ1→3[Fucα1→4]GlcNAcβ
Le^b	Fucα1→2Galβ1→3[Fucα1→4]GlcNAcβ
Type 2	
LacNAc	Galβ1→4GlcNAcβ
sialyl-LacNAc	NANAα2→3Galβ1→4GlcNAcβ
H type 2	Fucα1→2Galβ1→4GlcNAcβ
X	Galβ1→4[Fucα1→3]GlcNAcβ
sialyl-X	NANAα2→3Galβ1→4[Fucα1→3]GlcNAcβ
Y	Fucα1→2Galβ1→4[Fucα1→3]GlcNAcβ
Type 3	
Tn	GalNAcα
sialyl-Tn	NANAα2→3GalNAcα
T	Galβ1→3GalNAcα
sialyl-T	NANAα2→3Galβ1→3GalNAcα
H type 3	Fucα1→2Galβ1→3GalNAcα

LacNAc, H type 2, X, sialyl-X, Y, Tn, sialyl-Tn, T, sialyl-T and H type 3) (Table I) attached to BSA (Syntagen) and H disaccharide (Fucα1→2Galβ) attached to silica beads (Synsorb) and anti-Le^a and anti-X monoclonal antibodies were obtained from Chembiomed (Edmonton, Canada). The amounts of oligosaccharides contained in Syntagen and in Synsorb were estimated to be 10 to 20 mol per mole of BSA and 0.414 μmol per gram of silica beads, respectively. BSA, Tween 20, Dulbecco's PBS (−), diaminobenzene tetrahydrochloride and L-fucose were purchased from Sigma Chemical Co. (St. Louis, MO). Affi-Gel-10 and Protein Assay kit were from Bio-Rad Laboratories (Richmond, CA). Horseradish peroxidase-conjugated anti-mouse (IgG+IgA+IgM) goat IgG was obtained from Zymed Laboratories (South San Francisco, CA). Polyethylene glycol 1500 was from Boehringer Mannheim (Indianapolis, IN). E-RDF culture medium containing RD-1 was obtained from Kyokuto Pharmaceutical Inc. (Tokyo). Anti-Le^b (TT42) monoclonal antibody was obtained after immunization with stomach adenocarcinoma tissues (D. Chia, manuscript in preparation). Anti-Y (BM-1),[14] and anti-H type 2 (OSK 16) monoclonal antibodies were kindly supplied by Japan Immunoresearch Laboratories (Takasaki) and Osaka Red Cross Blood Center (Osaka), respectively. Tissues from primary colorectal carcinoma (n=44) and normal colon (n=25) were obtained from the First Department of Surgery, Gunma University. Surgically resected tissues were fixed in 10% formalin and embedded in paraffin.

Preparation of immunogen and monoclonal antibody Human saliva samples were collected from OLe(a−b+) secretor individuals, pooled, boiled for 20 min and then centrifuged. The supernatant was applied to an AAL-Affi-Gel 10 column[15] equilibrated with 0.01 *M* PBS (pH 7.0). The column was washed with PBS until the absorbance at 280 nm of the effluent returned to the background level, then the bound materials were eluted with 20 m*M* L-fucose in PBS. The eluted fractions were pooled, dialyzed (Spectrum No. 6, MW cut-off 2000) (Spectrum Medical Industries, Los Angeles, CA), lyophilized and used as an immunogen.

Two 6-week-old Balb/c mice were immunized intraperitoneally three times with 5 μg of the immunogen in Freund's complete adjuvant (Difco Lab., Detroit, MI) once ever other week. Three days after the final immunization the spleens were removed were from the immune donors and the splenocytes were isolated. Splenocytes were fused with P3U1 Balb/c myeloma cells at a ratio of 5:1 with the aid of polyethylene glycol 1500. After the fusion, cell pellets were resuspended in RPMI-1640 medium supplemented with 2 m*M* glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, 15% fetal calf serum, 10 m*M* hydroxanthine, 0.04 m*M* aminopterin and 1.6 m*M* thymidine and then distributed into 96-well plates (Falcon). The plates were incubated at 37°C in a humid atmosphere of 5% CO_2 in air and the medium was periodically replaced with fresh medium. The supernatant of wells showing growth of hybrids was screened for the presence of antibodies against various oligosaccharides attached to BSA by ELISA as described below. The antibody-producing cells were cloned by the limiting dilution technique with Balb/c thymocytes as feeder cells in 96-well microtiter plates.

Enzyme-linked immunosorbent assay (ELISA) ELISA was used to screen the supernatant for the presence of the antibody and also to characterize the antibody. One hundred microliters of each oligosaccharide attached to BSA solution at a concentration of 1 μg/ml was used per well to coat 96-well microtiter plates (Costar) and the plates were incubated for 12 h at 25°C. They were washed 3 times with washing buffer, Dulbecco's PBS (−) containing 0.05% Tween 20, and exposed to the same buffer containing 2% BSA and 5% sorbitol for 12 h at 25°C to block uncoated sites on the wells. The buffer was removed by aspiration, and the plates were dried for 12 h at 25°C then stored at 4°C until use. Plates were washed twice with washing buffer, then 100 μl of the supernatant or purified antibody was added to each well and the plates were incubated for 2 h at 25°C. They were washed with washing buffer 3 times, then 100 μl of horseradish peroxidase-conjugated anti-mouse Ig at a dilution ratio of one to 3000 was added. The plates were incubated for 2 h at 25°C, then substrate solution (*o*-

phenylenediamine and H_2O_2 in citrate phosphate buffer, pH 5.0) was added for color development after removal of unbound components from the plates by washing. Optical densities were read by a Titerteck Multiscan Colorimeter (Flow Lab.) with a 492 nm filter. Inhibition assays of the antibody were also carried out in the same ELISA after adding various oligosaccharides attached to BSA as inhibitors at a concentration of 0.1 to 10,000 μg per well.

Characterization of YB-2 monoclonal antibody Two liters of culture in E-RDF medium containing RD-1 at 37°C in a humid atmosphere of 5% CO_2 in air was prepared and the supernatant was applied to a column packed with 10 g of H disaccharide Synsorb equilibrated with PBS. The column was washed with PBS and the bound antibody was eluted with 20 m*M* L-fucose in PBS as described previously.[16] The eluate was dialyzed, concentrated by ultrafiltration, applied to a TSK G5000PW column (Tohso, Tokyo) and eluted with PBS. The fractions containing the main peak were pooled (YB-2 antibody) and the protein concentration was measured by using a Protein Assay kit. This solution was used for the following experiments.

Immunostaining of tissues The deparaffinized sections were stained by the indirect immuno-peroxidase method. The sections were treated with 100% methanol containing 0.3% hydrogen peroxide to destroy endogenous peroxidase activity, and were preincubated in 10% normal goat serum for 30 min. Then they were incubated for 30 min with YB-2 antibody and anti-fucosylated antigen antibodies. They were stained with biotinylated anti-mouse IgM (Vector Laboratories, Burlingame, CA) and treated with the Vectastain ABC kit (Vector Laboratories) according to the manufacturer's instructions. Color was developed with a solution containing 0.05% diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 *M* Tris-Cl buffer (pH 7.6), then the sections were counterstained with hematoxylin, washed with distilled water and mounted. The 44 patients were classified into three groups based upon the intensity of staining with YB-2 antibody as follows; Grade I, negative; Grade II, positive with intratumor heterogeneity; Grade III, uniformly positive.

RESULTS

Selection of monoclonal antibody Fused cells were plated into 384 original wells. Supernatant from the culture was first assayed for the presence of antibody to fucosylated antigens by ELISA. The supernatant from some wells showed the presence of anti-fucosylated antigen antibodies. Fused cells which produced antibody to H, Le^b and Y antigens were cloned by limiting dilution and several fused cells retained antibody production. These clones secreted IgM antibody.

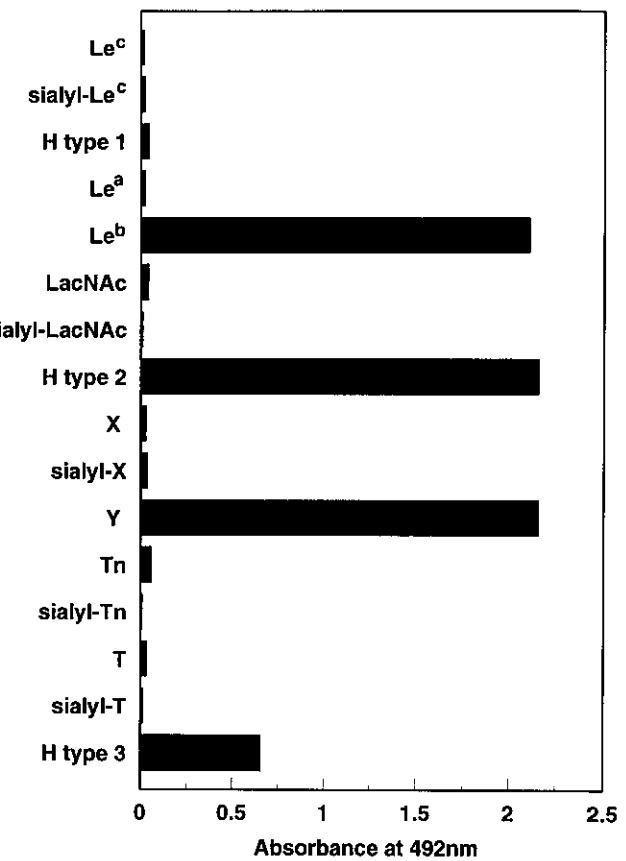

Fig. 1. Reactivities of YB-2 antibody with carbohydrate antigens attached to BSA.

Specificity analysis Preliminary tests were done by ELISA using culture supernatant from the final clone to analyze the specificity. As shown in Fig. 1, YB-2 monoclonal antibody showed cross-reactivities with Le^b, H type 2, Y and H type 3 antigens. No cross-reactivity with other fucosylated antigens such as H type 1, Le^a and X antigens, or non-fucosylated antigens such as Le^c, LacNAc, T, Tn, sialyl-T, sialyl-Tn, sialyl-Le^c and sialyl-LacNAc antigens was observed.

Purification of YB-2 antibody YB-2 antibody was purified by affinity chromatography using an H disaccharide (Fucα1→2Galβ) Synsorb column.[16] Approximately 6 mg of the antibody was retrieved from two liters of cultured supernatant from the final cloned cell line. The purified YB-2 antibody was shown to react with Y, Le^b and H type 2 antigens, respectively, but the reactivity against H type 3 antigen was clearly lower than that against these three antigens (data not shown).

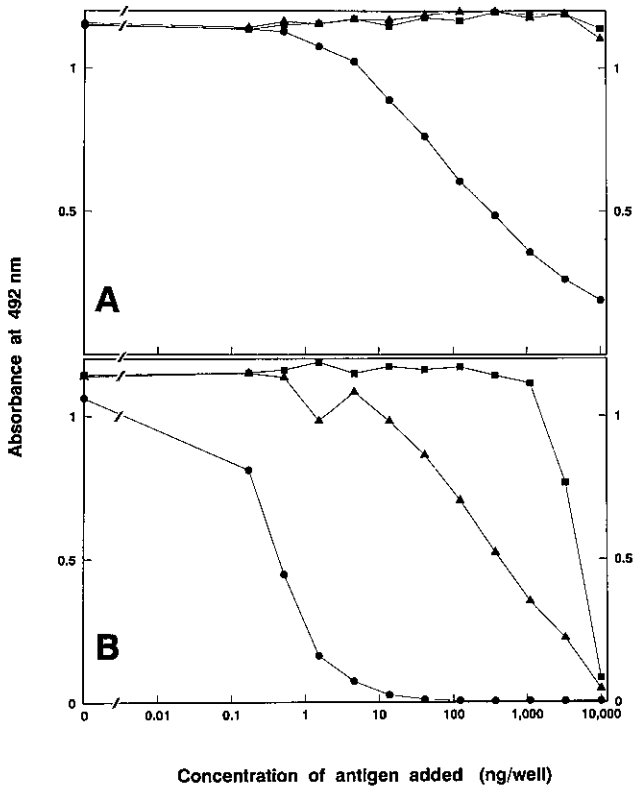

Fig. 2. Inhibition of binding of YB-2 antibody to Y (A) and Le^b (B) antigen coated plates by Y, Le^b and H type 2 antigen. Antigens used were Y-BSA (●), Le^b-BSA (▲) and H type 2 (■).

Inhibition assay of YB-antibody When various amounts of Y, Le^b and H type 2 antigen were added to the purified YB-2 antibody, the reactivity of the antibody to Y antigen-coated trays was inhibited only by Y antigen, but not by Le^b or H type 2 antigen, even when their concentrations added were hundred times higher than that of Y antigen coated (Fig. 2-A). In contrast, the reactivity of the antibody to Le^b antigen-coated trays was inhibited by not only Le^b antigen but also Y antigen, and the inhibitory activity of Y antigen was found to be much stronger than that of Le^b antigen (Fig. 2-B). Furthermore, H type 2 antigen was found to inhibit the same reaction at high concentrations. These results demonstrated that the rank order of inhibiting activity for YB-2 antibody was $Y \gg Le^b > H$ type 2 antigen.

Immunostaining of colorectal carcinoma and normal tissues Sections of colorectal carcinoma and normal colon tissues were examined immunohistochemically with YB-2 and some other monoclonal antibodies. The incidence of positive staining of colorectal caricnoma and normal tissues was 88.6% and 12.0%, respectively

Table II. Immunoreactivity of Monoclonal Antibodies in Colorectal Tumors and Normal Tissues

Antibody	Number positive/number tested (%)		
	Specificity (Antigen)[a]	Tumor	Normal
YB-2	Y, Le^b, H type 2	39/44 (88.6)	3/25 (12.0)
BM-1	Y	40/44 (90.9)	18/25 (76.2)
TT42	Le^b	27/44 (61.4)	2/25 (8.0)
OSK 16	H type 2	23/44 (6.8)	0/25 (0.0)
anti-X	X	19/42 (45.2)	2/25 (8.0)
anti-Le^a	Le^a	29/42 (69.0)	20/23 (87.0)

a) Specificities of BM-1, TT42 and OSK 16 monoclonal antibodies were confirmed by ELISA as described in the text.

(Table II). All five patients whose tumors did not react with YB-2 antibody were classified[17] as Dukes' A. No tumor tissues from Dukes' B and C patients gave negative staining with YB-2 antibody. There was no significant correlation between blood group ABO status of the specimens and the intensity of immunostaining with YB-2 (data not shown). The expression of Y, Le^b, H type 2, X and Le^a antigens in the same specimens was also determined using the respective monoclonal antibodies (Table II). The incidence of positive staining of carcinoma was very high (90.9%) for BM-1 antibody and there was no significant difference in the intensity of staining between YB-2 and BM-1 antibodies. However, positive staining with BM-1 antibody was also observed frequently in normal tissues (76.2%) (Fig. 3). In contrast, Le^b and X antigens detected by TT42 and anti-X antibody, respectively, were frequently expressed in carcinoma and H type 2 antigen detected by OSK16 was expressed only in a small number of carcinomas. The incidence of positive staining with anti-Le^a increased in normal tissues as compared with that in carcinomas.

Survival of patients with colorectal cancer Five-year survival according to the grade of immunostaining with YB-2 antibody was investigated among 38 patients who were followed-up for five years. The three patients (Duke's A) whose tumors did not react with YB-2 antibody (Grade I) had 100% survival. However, the survival rate of patients whose tumors were positive with YB-2 antibody decreased according to the grade of immunostaining. The average survival rate of patients with Grade II (n=14) (7 patients with Duke's A, 3 patients with Duke's B and 4 patients with Duke's C) was 85.7%, and that of patients with Grade III (n=21) (1 patient with Duke's A, 10 patients with Duke's B and 10 patients with Duke's C) was 50.0%. The difference between Grade II and III was significant ($P<0.01$). Similar trends in the survival rate of patients were also observed according to the Duke's stages, but were not statistically significant (data not shown).

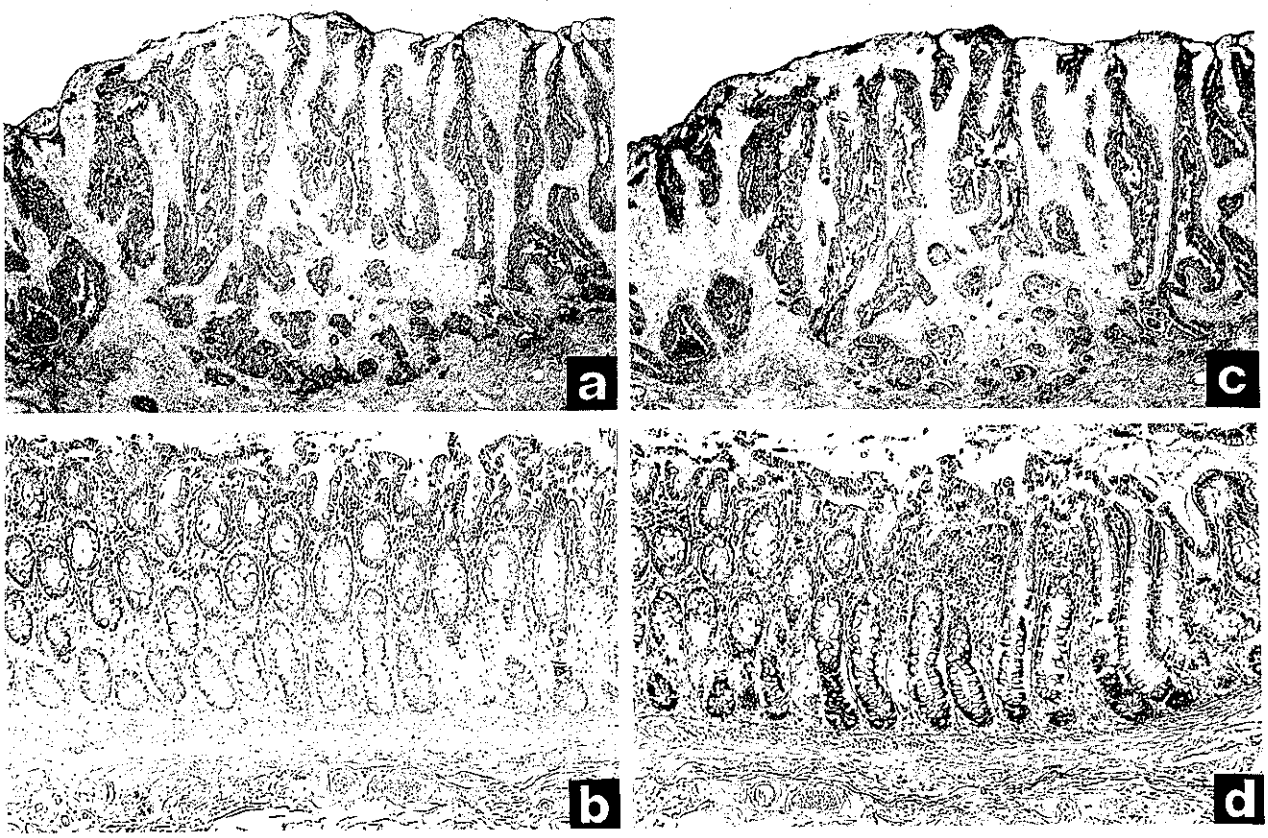

Fig. 3. Immunohistochemical staining of colorectal tissues with YB-2 (a and b) and BM-1 (c and d) antibody. Malignant (a and c) (×35) and normal (b and d) (×88) tissues.

DISCUSSION

Accumulation of fucosylated antigens in a variety of human cancers has been regarded as a cancer-associated phenomenon, and levels of some fucosylated antigens are elevated in sera and tissues from cancer patients.[4, 18-22] Therefore, the use of highly specific monoclonal antibodies which react with such antigens selectively would allow us to detect each antigen as a putative cancer-associated antigen. In our previous studies, we have developed assays for fucosylated type 1 and type 2 antigens using a series of appropriate structure-specific monoclonal antibodies and assayed cancer-associated antigens in sera from patients with various cancers.[19, 22] We have also demonstrated that fucosylated glycoconjugates including some tumor-associated antigens could be isolated by one-step affinity chromatography on an *Aleuria aurantia* lectin (AAL) column.[15] Since human saliva has been reported to contain many fucosylated antigens with different fucosyl linkages,[23] fucosylated glycoconjugates were isolated from human saliva by use of the AAL column and used as an immunogen to develop anti-fucosylated antigen antibodies.

Many well-defined monoclonal antibodies are now available to detect the aforementioned fucosylated and related antigens. However, the epitopes of some antibodies have not yet been identified since the antibodies were raised against crude immunogens and screened with crude antigens. In addition, we found that some monoclonal antibodies raised against fucosylated glycoconjugates, which are now commercially available, did not show restricted specificities but rather cross-reacted with distantly related structures (unpublished results). As shown in this study, chemically synthesized oligosaccharides attached to BSA are useful and convenient antigens for precise analysis of the reactivity and the specificity of such antibodies.

Previously, most of the anti-H monoclonal antibodies were found to react with H type 2 antigen and cross-react with Y antigen.[8] It was also demonstrated that the lectin IV of *Griffonia simplicifolia*[24] and some anti-Le^b antibodies[7, 25] could react with both Le^b and Y antigens, as

predicted by studies on the stereospatial conformation. On the other hand, monoclonal antibodies directed to a specific carbohydrate structure, Fucα1→2Galβ1→R, were obtained recently.[26] Even though strict definition of the antigen is still in progress, the epitope of YB-2 antibody can not be involved in this H disaccharide structure, which is common in Y, Le^b and H type 2 antigens, because the antibody did not react with H type 1 (Fucα1→2Galβ1→3GlcNAcβ) antigen at all. Specificity analysis of the antibody clearly showed that YB-2 reacts with Y, Le^b and H type 2 antigens. The titration curves of the antibody against these antigens were very similar and suggested that the affinities of YB-2 antibody for the antigens were almost the same (data not shown). However, it is of particular interest that YB-2 antibody reacted preferentially with Y antigen and then Le^b antigen when Y, Le^b and H type 2 antigens were competitively present, as demonstrated in the inhibition study. It remains to be seen whether the specificity and reactivity of YB-2 antibody may be affected by not only the carrier structures beyond Y, Le^b and H type 2 but also the stereospatial conformation of these antigens.

In colorectal tissues, Y, Le^b and H antigens are expressed much more strongly in cancer tissues than in normal tissues[4-9] and it was suggested that α1→2fucosylation to synthesize these fucosylated antigens was enhanced in cancer. In fact, elevated activities of α1→2fucosyltransferase were found in human colon cancer.[13] The expression of Y, Le^b and H type 2 antigens in colorectal carcinoma was confirmed in our present study on immunohistochemical staining with anti-Y, Le^b and H type 2 monoclonal antibodies, and therefore, the lesions stained with YB-2 antibody must contain these antigens. The incidence of positive staining of colorectal carcinoma for anti-H antibody was found to be low in this study in comparison with the previous results. The anti-H antibody used in this study was demonstrated to react only with H type 2 structure (unpublished results), whereas anti-H antibodies previously used tended to react not only with H type 2 but also with Y structures, as demonstrated before.

The intensity and patterns of staining with YB-2 and BM-1 antibodies were very similar in colorectal carcinoma but significantly different in normal tissues. Although it is difficult to explain the difference in the Y reactivity of these two antibodies, the epitope of YB-2 antibody may involve some other Y-related structures such as an extended Y antigen, which may be rather specific to colorectal carcinomas, and hardly be present in normal tissues. More recently, the monoclonal antibody (1MH2) was obtained and demonstrated to react more strongly with extended Le^b (Fucα1→2Galβ1→3[Fucα1→4]GlcNAcβ1→3Galβ1→3[Fucα1→4]GlcNAcβR) or Y (Fucα1→2Galβ1→4[Fucα1→3]GlcNAcβ1→3Galβ1→4[Fucα1→3]GlcNAcβR) antigen than with Le^b or Y antigen itself and further to react with specimens from colorectal carcinomas but not from normal colonic mucosa.[27] Possible reactivities of YB-2 antibody with Y-related structures or a common structure to Y, Le^b and H type 2 antigen are under consideration, and chemical and enzymatic syntheses of fucosylated antigens are in progress in order to define precisely the epitope of YB-2 antibody. One case of colorectal carcinoma was exceptional in that it was stained by BM-1 but not by YB-2 antibody. Through the specificity analysis of antibodies used in this study it was demonstrated that BM-1 antibody cross-reacted weakly with X antigen (unpublished results). The positive staining might be caused by the presence of X antigen in the sample, and in fact, the sample was also stained by anti-X antibody.

Monoclonal antibodies whose epitopes were claimed to be Y-related structures have been reported and their usefulness for the diagnosis of cancer was demonstrated.[27-29] The results of immunohistochemical staining of colorectal tissues and the correlation with the survival rate suggested that YB-2 antibody could be useful for immunodiagnosis and evaluation of the prognosis of colon cancer.

In our previous studies,[30-32] elevated activities of α1→3-L-fucosyltransferase, which should be responsible for the synthesis of X-related antigens, were found in sera from patients with cancer. The incompatible presence of α1→4-L-fucosyltransferase, which might be related to the synthesis of CA19-9 or Le^a antigen, was also demonstrated in various types of cancers.[33] The fucosylated antigens detected in colon tissues by YB-2 antibody seemed to be significantly correlated with the presence of tumors. The biosynthesis of such fucosylated antigens from their precursors has been reported to depend on at least three different α-L-fucosyltransferases, i.e., α1→2-, α1→3- and α1→4-L-fucosyltransferases.[34] Therefore, α-L-fucosyltransferases responsible for the synthesis of the fucosylated antigens in colon cancer should be investigated to demonstrate how aberrant fucosylated antigens are synthesized and accumulated in colorectal carcinomas. Extremely elevated activities of α1→2-fucosyltransferase, presumably related to the synthesis of the aforementioned fucosylated antigens, were also observed in the tumor tissues regardless of their blood group types and secretor status. In contrast, α1→2-fucosyltransferase related to the synthesis of Y antigen was hardly detected in normal colorectal mucosa (these results will be published elsewhere).

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REFERENCES

1) Hakomori, S. Aberrant glycosylation in cancer cell membranes as focused on glycolipid: overview and perspective. *Cancer Res.*, **45**, 2405–2414 (1985).
2) Hakomori, S. Tumor-associated glycolipid antigens, their metabolism and organization. *Chem. Phys. Lipids*, **42**, 209–233 (1986).
3) Dabelsteen, E., Clausen, H. and Mandel, U. Aberrant glycosylation in oral malignant and premalignant lesions. *J. Oral Pathol. Med.*, **20**, 361–368 (1991).
4) Sakamoto, J., Furukawa, K., Cordon-Cardo, C., Yin, B. W. T., Rettig, W. J., Oettgen, H. F., Old, L. J. and Lloyd, K. O. Expression of Lewis^a, Lewis^b, X and Y blood group antigens in human colonic tumors and normal tissue and in human tumor-derived cell lines. *Cancer Res.*, **46**, 1553–1561 (1986).
5) Kjeldsen, T., Clausen, H., Hirohashi, S., Ogawa, T., Iijima, H. and Hakomori, S. Preparation and characterization of monoclonal antibodies directed to the T associated O-linked sialosyl-2→6α-N-acetylgalactosaminyl (sialosyl-Tn) epitope. *Cancer Res.*, **48**, 2214–2220 (1988).
6) Blaszczyk-Thurin, M., Thurin, J., Hindsgaul, O., Karlsson, K. A., Steplewski, Z. and Koprowski, H. Y and blood group B type 2 glycolipid antigens accumulation in a human gastric carcinoma cell line as detected by monoclonal antibody. *J. Biol. Chem.*, **262**, 372–379 (1987).
7) François, A., Sansonetti, N., Mollicone, R., Le Pendu, J., Galton, J., Jaulmes, B. and Oriol, R. Heterogeneity of Lewis antibodies. A comparison of the reaction of human and animal reagents with synthetic oligosaccharides. *Vox Sang.*, **50**, 227–234 (1986).
8) Furukawa, K., Welt, S., Yin, B. W. T., Feickert, H. J., Takahashi, T., Ueda, R. and Lloyd, K. O. Analysis of the fine specificities of 11 mouse monoclonal antibodies reactive with type 2 blood group determinants. *Mol. Immunol.*, **27**, 723–732 (1990).
9) Hakomori, S. and Andrews, H. D. Sphingoglycolipids with Le^b activity, and the co-presence of Le^a-, Le^b-glycolipids in human tumor tissues. *Biochim. Biophys. Acta*, **202**, 225–228 (1970).
10) Yuan, M., Itzkowitz, S. H., Palekar, A., Shamsuddin, A. M., Phelps, P. C., Trump, B. F. and Kim, Y. S. Distribution of blood group antigens A, B, H, Lewis^a, and Lewis^b in human normal, fetal, and malignant colonic tissue. *Cancer Res.*, **45**, 4449–4511 (1985).
11) Itzkowiz, S. H., Yuan, M., Ferrell, L. D., Palekar, A. and Kim, Y. S. Cancer-associated alteration of blood group antigen expression in human colorectal polyps. *Cancer Res.*, **46**, 5976–5984 (1986).
12) Dahiya, R., Itzkowitz, S. H., Byrd, J. C. and Kim, Y. S. ABH blood group antigen expression, synthesis, and degradation in human colonic adenocarcinoma cell lines. *Cancer Res.*, **49**, 4550–4556 (1989).
13) Ørntoft, T. F., Greenwell, P., Clausen, H. and Watkins, W. M. Regulation of the oncodevelopmental expression of type 1 chain ABH and Lewis^b blood group antigens in human colon by α-2-L-fucosylation. *Gut*, **32**, 287–293 (1991).
14) Adachi, M., Hayashi, M., Kashiwagi, N., Mizuta, T., Ohta, Y., Gill, M. J., Matheson, D. S., Tamaoki, T., Shiozawa, C. and Hakomori, S. Expression of Le^y antigen in human immunodeficiency virus-infected human T cell lines and in peripheral lymphocytes of patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC). *J. Exp. Med.*, **167**, 323–331 (1988).
15) Yazawa, S., Kochibe, N. and Asao, T. A simple procedure for isolation of tumor-associated antigens by affinity chromatography using fucose-specific *Aleuria aurantia* lectin. *Immunol. Invest.*, **19**, 319–327 (1990).
16) Yazawa, S., Hosomi, O. and Takeya, A. Isolation and characterization of anti-H antibody from egg yolk of immunized hens. *Immunol. Invest.*, **20**, 569–581 (1991).
17) Dukes, C. E. The classification of cancer of the rectum. *J. Pathol. Bacteriol.*, **35**, 323–331 (1932).
18) Chia, D., Terasaki, P. I., Suyama, N., Galton, J., Hirota, M. and Katz, D. Use of monoclonal antibodies to sialylated Lewis^x and sialylated Lewis^a for serological tests of cancer. *Cancer Res.*, **45**, 435–437 (1985).
19) Kannagi, R., Fukushi, Y., Tachikawa, T., Noda, A., Shin, S., Shigeta, K., Hiraiwa, N., Fukuda, Y., Inamoto, T., Hakomori, S. and Imura, H. Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing fucosyl or sialyl-fucosyl type 2 chain polylactosamine. *Cancer Res.*, **46**, 2619–2626 (1986).
20) Itzkowitz, S. H., Yuan, M., Fukushi, Y., Palekar, A., Phelps, P. C., Shamsuddin, A. M., Trump, B. F., Hakomori, S. and Kim, Y. S. Lewis^y- and sialylated Lewis^x-related antigen expression in human malignant and nonmalignant colonic tissues. *Cancer Res.*, **46**, 2627–2632 (1986).
21) Abe, K., Hakomori, S. and Ohshiba, S. Differential expression of difucosyl type 2 chain (Le^Y) defined by monoclonal antibody AH6 in different locations of colonic epithelia, various histological types of colonic polyps, and adenocarcinomas. *Cancer Res.*, **46**, 2639–2644 (1986).

22) Kannagi, R., Kitakura, A., Itai, S., Zenita, K., Shigeta, K., Tachikawa, T., Noda, A., Hirano, H., Abe, M., Shin, S., Fukushi, Y., Hakomori, S. and Imura, H. Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing epitopes consisting of sialyl or sialy-fucosyl type 1 chain. *Cancer Res.*, **48,** 3856–3863 (1988).
23) Klein, A., Carnoy, C., Wieruszeski, J. M., Strecker, G., Strang, A. M., Halbeek, H., Roussel, P. and Lamblin, G. The broad diversity of neutral and sialylated oligosaccharides derived from human salivary mucins. *Biochemistry,* **31,** 6152–6165 (1992).
24) Sphor, U., Hindsgaul, O. and Lemieux, R. U. Molecular recognition. II. The binding of the Lewis^b and Y human blood group determinants by the lectin IV of *Griffonia simplicifonia. Can. J. Chem.*, **63,** 2644–2652 (1985).
25) Good, A. H., Yan, O., Lamontogne, L. R. and Oriol, R. Serological and chemical specificities of twelve monoclonal anti-Le^a and anti-Le^b antibodies. *Vox Sang.*, **62,** 180–189 (1992).
26) Miyake, M. and Hakomori, S. A specific cell surface glycoconjugate controlling cell motility: evidence by functional monoclonal antibodies that inhibit cell motility and tumor cell metastasis. *Biochemistry,* **30,** 3328–3334 (1991).
27) Ito, H., Tashiro, K., Stroud, M. R., Ørntoft, T. F., Meldgaard, P., Singhal, A. K. and Hakomori, S. Specificity and immunobiological properties of monoclonal antibody IMH2, established after immunization with Le^b/Le^a glycosphingolipid, a novel extended type 1 chain antigen. *Cancer Res.*, **52,** 3739–3745 (1992).
28) Hellström, I., Garrigues, H. J., Garrigues, H. and Hellström, K. E. Highly tumor-reactive, internalizing, mouse monoclonal antibodies to Le^y-related cell surface antigens. *Cancer Res.*, **50,** 2183–2190 (1990).
29) Pastan, I., Lovelace, E. T., Gallo, M. G., Rutherford, A. V., Magnani, J. L. and Willingham, M. C. Characterization of monoclonal antibodies B1 and B3 that react with mucinous adenocarcinomas. *Cancer Res.*, **51,** 3781–3787 (1991).
30) Yazawa, S., Madiyalakan, R., Izawa, H., Asao, T., Furukawa, K. and Matta, K. L. Cancer-associated elevation of $\alpha(1\rightarrow3)$-L-fucosyltransferase activity in human sera. *Cancer,* **62,** 516–520 (1988).
31) Yazawa, S., Asao, T., Nagamachi, Y. and Matta, K. L. Elevated activities of serum $\alpha(1\rightarrow3)$-L-fucosyltransferase in human cancer. *J. Tumor Marker Oncol.*, **4,** 355–362 (1989).
32) Tachikawa, T., Yazawa, S., Asao, T., Shin, S. and Yanaihara, N. Novel method for quantifying $\alpha(1\rightarrow3)$-L-fucosyltransferase activity in serum. *Clin. Chem.*, **37,** 2081–2086 (1991).
33) Yazawa, S., Asao, T., Izawa, H., Miyamoto, Y. and Matta, K. L. The presence of CA19-9 in serum and saliva from Lewis blood-group negative cancer patients. *Jpn. J. Cancer Res.*, **79,** 538–543 (1988).
34) Salmon, C., Cartron, J. P. and Rouger, P. "The Human Blood Groups," pp. 54–80 (1984). Masson, New York.