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Expression of H type 1 antigen of ABO histo-blood group in normal colon and aberrant expressions of H type 2 and H type 3/4 antigens in colon cancer

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We have immunohistochemically examined the distribution of the H antigens of type 1, type 2 and type 3/4 chains of the ABO(H) histo-blood group system in human normal colon and in colon cancer using three monoclonal antibodies specific for each of the H type 1/2, H type 2, and the H type 3/4 chain. We unexpectedly found that mucosa of the normal colon from secretors but not that from nonsecretors expressed only H type 1 and did not express H type 2 or H type 3/4. The H type 1 was expressed in goblet cells. Positive goblet cells expressing H type 1 were decreased in number progressively from the proximal colon to the rectum. In tumors, 4 (57%) of 7 cancer tissues of the proximal colon from secretors expressed no H type 1, whereas all 8 cancer tissues of the distal colon from secretors expressed H type 1. The aberrant expressions of H type 2 and H type 3/4 (47 and 67%, respectively) were found in cancer tissues from both the proximal and the distal colon. Tumors from nonsecretors did not express any H antigens. Our results suggested that the expression of H type 1 in the normal colon and the aberrant expressions of H type 2 and H type 3/4 in colon cancer tissues were regulated by *FUT2*-encoded Se type α(1,2)fucosyltransferase. However, UEA-I-positive substance(s) rather than H type 2 were uniquely expressed throughout the normal colon and in colon cancers from both secretors and nonsecretors.

Keywords: **colon, colon cancer, H antigen, secretor, ABO, anti-H antibody**

Introduction

The histo-blood group ABO-related antigens are carried by at least four core structures (type 1 chain, Galβ1-3GlcNAcβ1-; type 2 chain, Galβ1-4GlcNAcβ1-; type 3 chain, Galβ1-3GalNAcα1-; and type 4 chain, Galβ1-3GalNAcβ1-) [1,2]. The ABO(H) antigens are expressed not only in erythrocytes but also in the gastrointestinal tract and in secretions. All the H antigens (H type 1 ∼ H type 4) are synthesized by both H type (H enzyme) and Se type (Se enzyme) α(1,2)fucosyltransferases *in vitro*. However, it is believed that the H enzyme regulates the expression of the H antigen mainly in erythrocytes, and that the Se enzyme regulates the expression of the H antigen mainly in gastrointestinal epithelial cells and in secretions.

There are many studies on the immuno- and lectin-histochemical distributions of the ABH antigens in normal colon and in colon cancer tissues [3–17]. The use of highly specific monoclonal antibodies (MAbs) which react selectively with such carbohydrate antigens would allow us to detect each antigen as a putative cancer-associated antigen. However, few well-defined monoclonal antibodies are available to detect the histo-blood group-related antigens. Earlier immunohistochemical techniques using *Ulex europaeus* I lectin (UEA-I) or monoclonal anti-H antibodies have demonstrated the expression of the H type 2 chain in the normal proximal colon, and the accumulation of H type 2 chain in distal colon cancers [10,13,15,17], while later techniques have not found H type 2 in the normal colon mucosa or in the transitional mucosa adjacent to colon cancer tissues [8,14,16]. However, it has

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been reported that UEA-I and most of the anti-H MAbs, believed to be specific for H type 2, cross-reacted with the Y antigen and with several oligosaccharides [18,19].

Recently, we have discriminated among the distributions of the H type 1, H type 2, and H type 3/4 in cell types of the human submandibular gland immunohistochemically using well defined MAbs for the H antigens [20–22]. In the present study, we systematically investigated the distribution of H type 1 ~ 4 antigens in the normal colon and in colon cancers using a panel of monoclonal anti-H antibodies, the specificity of which have been identified with the H oligosaccharides [23–25], because some controversial results concerning the expression of types of core structures of ABH antigens in normal colon and colon cancer have been reported [8,10,13–17]. We found unexpectedly that the normal colon mucosa expressed only the H type 1 antigen and not the H type 2 or H type 3/4 antigens, and that the H type 1 antigen expression was regulated by Se type α(1,2)fucosyltransferase (*FUT2*). We also found that some cancer tissues (47–67%) of the proximal colon and the distal colon from secretors but not that from nonsecretors expressed aberrantly the H type 2 and/or H type 3/4 antigens. However, UEA-I-positive substances were expressed in the normal colon and in cancer tissues throughout the colon independent from the secretor status.

Materials and methods

Tissue specimens and secretor-nonsecretor phenotypes

Normal colorectal tissues were obtained from six forensic autopsy cases of blood group O individuals. The ABO and Lewis phenotypes in the autopsy cases were determined by the hemagglutination method. The *Secretor* genotype was determined as described previously after preparation of DNA from peripheral leukocytes [26]. Three cases were secretors Le(a−b+), whose genotypes were homozygous for the functional allele of the *Se* gene (*FUT2*), and 3 were nonsecretors Le(a+b−), whose genotypes were homozygous for the inactive allele ($se^{357,385}$) [26,27]. From each individual, sections of the ascending colon, the transverse colon, the descending colon, and the rectum were obtained.

Primary colorectal carcinoma tissues were obtained from a further 19 patients with blood group O undergoing surgical resection at Kurume University Hospital. Of these, 8 were from the proximal colon (4 from the ascending colon and 4 from the transverse colon) and 11 were from the distal colon (4 from the descending colon, 4 the sigmoidal colon and 3 from the rectum). Of these, 3 were a papillary adenocarcinoma, 13 a well differentiated adenocarcinoma, and 3 were a poorly differentiated adenocarcinoma. The secretor status of the colon cancer patients was estimated according to a staining pattern by MAb 1E3 in well-oriented transitional mucosa adjacent to the cancer. As a result, we concluded there were 7 secretors and 1 nonsecretor having proximal colon cancer, and 8 secretors and 3 nonsecretors having distal colon cancer.

Antibodies

MAbs specific for blood group Le^a and Le^b (Ortho Diagnostic, Tokyo, Japan) and *Ulex europaeus* agglutinin I (UEA-I) conjugated with horseradish peroxidase (EY Laboratories, San Mateo, CA) were purchased. Three anti-H MAbs—3A5, 1E3 and MBr1—were used in the present study. Anti-H MAb 3A5 specific for H type 2 was prepared in our laboratory using human erythrocyte membranes as the antigen [24]. Anti-H MAb 1E3, that was produced using mixed saliva from individuals with blood group O as the antigen, was a kind gift from Drs. Ken Kurukawa and Shin Yazawa (Department of Legal Medicine, Gunma University School of Medicine). MAb 1E3 has been demonstrated to be reactive with synthetic H type 1 ~ H type 4 oligosaccharides [25], but predominantly to be reactive with H type 1 and H type 2 antigens *in situ* [20,21]. In addition, we demonstrated that anti-H 1E3 became specific to H type 1 after absorption with red cell ghosts [20]. The anti-H MAb MBrl specific for H type 3/4 structures [23,28–30] was a kind gift from Dr. Maria Ines Colnaghi (Division of Experimental Oncology E, National Institute for Tumor, Milan, Italy). MAb MBrl was raised against cell membranes of the breast cancer cell line MCF7.

Immunohistochemistry

Deparaffinized sections of 10% formalin-fixed paraffin-embedded normal colon from autopsy cases and colon cancer tissues were immunostained using the streptavidin-biotin complex immunoperoxidase method, as described previously [20–22]. After pretreatment with 3% H_2O_2 and then 10% normal rabbit serum, primary monoclonal antibodies (all 500-fold diluted) in PBST (0.01 M phosphate buffer, pH 7.4, 0.15 M NaCl, and 0.5% Tween 20) and UEA-I (10 mg/ml) in PBS containing 0.1% BSA were reacted at 4°C overnight in a moist chamber. After washing with PBST, the sections were incubated sequentially with biotinylated rabbit anti-mouse immunoglobulins (Nichirei, Tokyo, Japan) and then peroxidase-conjugated streptavidin (Nichirei). Positive sites were developed with 3,3′-diaminobenzidine and H_2O_2 in PBS, and finally the sections were counterstained by hematoxylin. Control staining without the first antibody showed negative staining.

Since it is known that the expression of ABH antigens in tissues shows a mosaic pattern, two parameters to describe the positive results were set up, one of which was the percentage of positive crypts and the other was the percentage of positive goblet cells in a crypt. The former indicated that the percentage of crypts containing positively stained goblet cells or luminal surfaces counted in a low field of vision, and the latter indicated the percentage of positive goblet cells counted in a crypt (the mean average from examining five crypts). The positive result was presented as (the percentage of crypts that were positive) × (the percentage of positive goblet cells in a crypt).

Results

Normal colon mucosa expressed H type 1 but not H type 2 and H type 3/4

In autopsy cases, anti-H MAb1E3, which recognizes H type 1 and H type 2 chains *in situ*, stained moderately and regularly goblet cells in normal colon mucosa from secretor individuals but not from nonsecretors, while the other anti-H antibodies, 3A5 specific for H type 2 chain and MBr1 specific for H type 3/4 chains, did not stain mucosa of either proximal or distal colon at all (Fig. 1). However, microvessels in tissue samples were well stained by MAbs 1E3 and 3A5 (Fig. 1A and B). Accordingly, we concluded that normal colon mucosa expressed only H type 1 chain but neither H type 2 nor H type 3/4 chains. The staining by 1E3 in the normal colon was found mainly in goblet cells (Figure 1A). The positive staining (% positive crypts $\times$ % positive goblet cells in a crypt) in the ascending segment, transverse segment, descending segment, and in the rectum from the same subject were (100×50), (100×33), (100×20), and (5×2), respectively (Table 1). All crypts in mucosa of ascending, transverse, and of descending colon contained positive goblet cells, but positive goblet cells were diminished progressively from the proximal segment to the distal segment, and a few positive crypts were observed in the rectum (Fig. 2). Sections throughout the colon from nonsecretors were not stained at all by any of the three anti-H MAbs (not shown). However, positive stainings with UEA-I were observed in the cytoplasm of goblet cells, luminal surfaces or luminal contents throughout the colon, regardless of the secretor status (Fig. 1D and E), but the rectum showed a negative reaction.

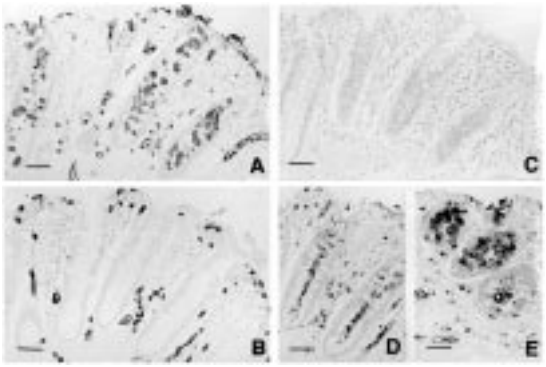

Figure 1. Staining of serial sections of normal mucosa of the ascending colon from a secretor (A $\sim$ D) and from a nonsecretor (E) by anti-H 1E3 (A), anti-H 3A5 (B), anti-H MBr1 (C) and UEA-I (D and E). Only goblet cells from the secretors were stained by 1E3 but not by 3A5 and MBr1. Microvessels and red blood cells were well stained by anti-H 1E3 (A) and by anti-H 3A5 (B). Luminal surfaces, luminal contents and goblet cells of crypts from secretors (D) and from nonsecretors (E) showed a positive reaction with UEA-I. Bars indicate 50 μm.

Antigenic expression in cancer tissues

First, we investigated the expression of H type 1 in the transitional mucosa adjacent to the cancer tissue to determine the secretor status. The pattern of staining of transitional mucosa by anti-H antibodies was similar to that of the normal mucosa of the autopsy cases. Neither 3A5 nor MBr1 stained all samples in agreement with normal colon from autopsy cases. Only 1E3 revealed a regular staining in the goblet cells. Seven of 8 proximal and 8 of 11 distal colon cancers showed a positive reaction with 1E3 and were concluded to be secretors. One proximal and three distal colon cancers showed no staining by 1E3 in their transitional mucosa and were concluded to be nonsecretors. One (with rectal cancer) of 4 nonsecretors did not express Le^a and Le^b antigens, and was assumed to be a nonsecretor with the Lewis-negative phenotype.

When we examined the cancer tissues, the staining patterns varied among individuals. Even if in the same individual, only a part of neoplastic glands showed a positive reaction with one or two anti-H antibodies or UEA-I, while the other parts were not stained at all. While the normal colon mucosa from autopsy cases expressed the H type 1 antigen with a proximal-distal gradient in antigen expression, four of the 7 proximal cancer tissues from secretors deleted the expression of H type

Table 1. The expression of the H antigens and of UEA-I-reactive substances in human normal colon mucosa.

		Colon segments			
Reagent	*Secretor status*	*Ascending*	*Transverse*	*Descending*	*Rectum*
MAb 1E3	Secretor	100×50[a]	100×33	100×20	5×2
	Nonsecretor	0	0	0	0
MAb 3A5	Secretor	0	0	0	0
	Nonsecretor	0	0	0	0
MAb MBr1	Secretor	0	0	0	0
	Nonsecretor	0	0	0	0
UEA-I	Secretor	>80	>80	>80	0
	Nonsecretor	>80	>80	>80	0

Anti-H MAb 1E3 is specific for H type 1/2 chains, anti-H MAb 3A5 for H type 2 chain and anti-H MAb MBr1 for H type 3/4 chains.
[a](percentage of positive crypts) $\times$ (percentage of positive goblet cells in a crypt).

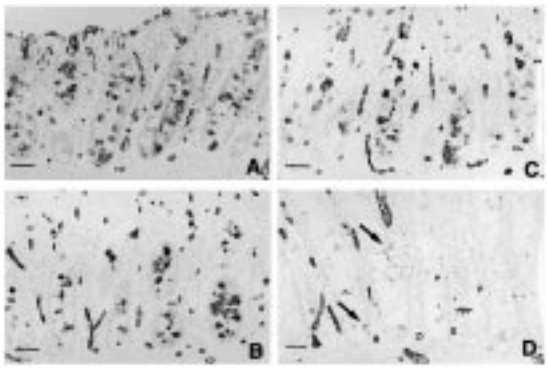

Figure 2. Staining of normal mucosa of the ascending (A), transverse (B), and the descending (C) colon and of the rectum (D) from a secretor individual by anti-H MAb 1E3. Goblet cells from the secretor individual reacted with 1E3, and the number of positive goblet cells was decreased progressively form the ascending colon to the rectum. Arrows in D indicate positive goblet cells in rectum. Bars indicate 50 μm.

1 and all 8 distal colon cancers from secretors expressed H type 1. Some cancer tissues from secretors were stained by anti-H 3A5 (7/15, 47%) and/or by anti-H MBr1 (10/15, 67%), suggesting aberrant expressions of H type 2 and of H type 3/4 antigens in cancer tissues (Table 2). The expressions of H type 2 and H type 3/4 antigens were not correlated to the expression of H type 1 or to the differentiation of the carcinomas (Table 3) [13], although papillary adenocarcinoma from secretor patients was well stained by all three anti-H MAbs. Cancer tissues from nonsecretors were not stained by any anti-H antibodies, but stained by UEA-I. Staining by UEA-I of the distal colon was of interest. Staining of transitional mucosa of distal colon cancer tissues by UEA-I was negative except in one case from the descending colon, whereas 10 of 11 distal colon cancer tissues showed positive reactions by UEA-I irrespective of the secretor status (Table 2). Sections of one poorly differentiated carcinoma from rectal cancer of a nonsecretor with Lewis-negative phenotype showed no staining by any of the three anti-H antibodies, by the anti-Lewis antibodies or by UEA-I.

Generally, in well differentiated adenocarcinoma, 1E3, 3A5 and MBr1 each stained luminal surfaces and contents, and accidentally stained cytoplasm of cancer cells, while UEA-I stained strongly luminal surfaces and contents and cytoplasm of cancer cells (Fig. 3). Neoplastic glands of well differentiated adenocarcinoma showed strong staining by UEA-I, but not by any one of the anti-H antibodies (Fig. 4). In contrast, cribriforms of well-differentiated adenocarcinoma showed a positive reaction with UEA-I and with 1E3 on luminal surfaces, but not in cytoplasm of the cancer cells (Fig. 5). Unpolarized cells in a cancer cell-invaded area or in poorly differentiated cancer tissues with mucin production showed diffuse staining of cytoplasm of some cancer cells by UEA-I, 1E3, 3A5, and by MBr1, but staining by MBr1 was moderate, and staining by 3A5 was weak (Fig. 6). Mucin produced was strongly stained by UEA-I, moderately by 1E3, and weakly by 3A5 and by MBr1 (Fig. 6).

Discussion

The expression of ABO(H) antigens by colon epithelial cells undergoes certain regional variation associated with the animal development and the transformation of the malignancy. In the fetus, the ABH antigens are expressed by colonocytes along the entire length of the colon [5,14]. However, at birth and persisting through adulthood, the ABH antigen expression by colonocytes in the distal colon disappears, resulting in a proximal-distal gradient of the antigen expression [5,6,10,13,14]. It is reported that the expression of Le^b, H type 2 and Le^y determinants was associated with tumor progression in the distal colon and with that in the rectum [3,4,7–14,16]. The data presented here concerning the H type 1 antigen expression in the colon were generally consistent with previous reports [4,8,10–13,15,17]. There was 1) a proximal-distal gradient in the H type 1 expression in the

Table 2. The expression of H antigens and of UEA-I-reactive substances in transitional mucosa adjacent to colon cancer and in cancer tissues.

H antigen (reagent)	*Tissue*	*Proximal colon*		*Distal colon*	
		Secretor n = 7	*Nonsecretor n = 1*	*Secretor n = 8*	*Nonsecretor n = 3*
H type 1 (MAb 1E3)	Transitional mucosa	7	0	8	0
	Cancer tissues	3	0	8	0
H type 2 (MAb 3A5)	Transitional mucosa	0	0	0	0
	Cancer tissues	2	0	5	0
H type 3/4 (MAb MBr1)	Transitional mucosa	0	0	0	0
	Cancer tissues	4	0	6	0
UEA-I-reactive substances (UEA-I)	Transitional mucosa	7	1	1	0
	Cancer tissues	7	1	8	2

Table 3. Correlation of the expressions of H type 1, H type 2, H type 3/4 antigens and UEA-I-reactive substances to differentiation of cancer in 19 cases of colon cancer.

Well differentiated adenocarcinoma				*Poorly differentiated adenocarcinoma*			
1E3	*3A5*	*MBr1*	*UEA-I*	*1E3*	*3A5*	*MBr1*	*UEA-I*
(+)	(+)	(+)	(+)[1]	(+)	(+)	(+)	(+)
(+)	(+)	(+)	(+)[1]	(+)	(+)	(−)	(+)
(−)	(−)	(−)	(+)[1,2]	(−)	(−)	(−)	(−)[3]
(−)	(−)	(+)	(+)				
(−)	(−)	(+)	(+)				
(−)	(−)	(−)	(+)				
(+)	(−)	(−)	(+)				
(−)	(−)	(+)	(+)				
(+)	(−)	(−)	(+)				
(+)	(+)	(+)	(+)				
(+)	(+)	(+)	(+)				
(+)	(+)	(+)	(+)				
(+)	(−)	(−)	(+)				
(+)	(−)	(+)	(+)				
(−)	(−)	(−)	(+)[2]				
(−)	(−)	(−)	(+)[2]				

[1]papillary adenocarcinoma; [2]nonsecretor phenotype; [3]nonsecretor and Lewis-negative phenotype.

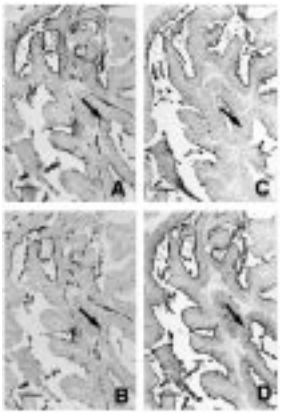

Figure 3. Staining of serial sections of papillary adenocarcinoma from a secretor by a panel of anti-H MAbs, 1E3 (A), 3A5 (B), MBr1 (C), and UEA-I (D). Luminal surfaces showed a strong reaction with UEA-I and anti-H antibodies, while cytoplasm (arrows) showed a positive reaction only with 1E3 (A) and UEA-I (D), but not with 3A5 or MBr1. Bars indicate 100 μm.

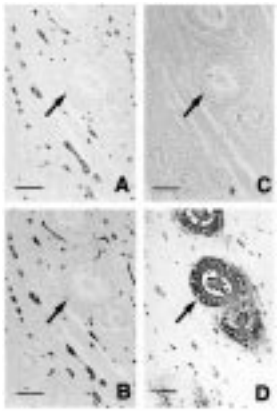

Figure 4. Serial sections of neoplastic gland formation of well differentiated adenocarcinoma from a secretor were stained by a panel of anti-H MAb, 1E3 (A), 3A5 (B) and MBr1 (C), and UEA-I (D). Luminal contents and cytoplasm of cells of neoplastic glands (arrows) were strongly stained by UEA-I (D), but not by anti-H 1E3, 3A5 or MBr1, although 1E3 and 3A5 stained erythrocytes and microvessels. Bars indicate 100 μm.

normal colon, and 2) a deletion of H type 1 expression in the proximal colon cancer and no change in the H type 1 expression in the distal colon cancer. However, many differences from other reports were observed. The normal colon expressed only H type 1 but neither H type 2 nor H type 3/4, while colon cancer tissue aberrantly expressed H type 2 and/or H type 3/4. The expressions of H type 1 in the normal colon and of H type $1 \sim 4$ chains in colon cancer tissues were dependent on the secretor status, that is, regulated by Se type α(1,2)fucosyltransferase (*FUT2*). The staining by UEA-I was different from the results described previously by others [10,15,17]. We observed positive staining by UEA-I in normal colon and in colon cancer irrespective of the secretor status (Tables 1 and 2). Schoentag et al. [13] also found UEA-I-positive substances in colorectal carcinoma from nonsecretors.

Most of our knowledge about the H expression in normal and cancer tissues has come from immunohistochemical studies using UEA-I lectin and commercial anti-H MAbs (most of which are described to be H type 2-specific). Recently, Mollicone et al. [19] and Baldus et al. [18] have

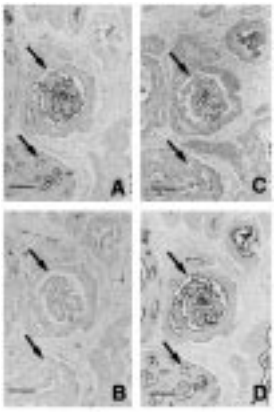

Figure 5. Serial sections of cribriforms of well differentiated adenocarcinoma from a secretor were stained by anti-H, 1E3 (A), 3A5 (B) and MBr1 (C), and UEA-I (D). Luminal surfaces (arrows) revealed a strong reaction with 1E3 (A) and UEA-I (D), while the cytoplasm of the cancer cells showed a negative reaction with neither anti-H nor UEA-I. Bars indicate 100 μm.

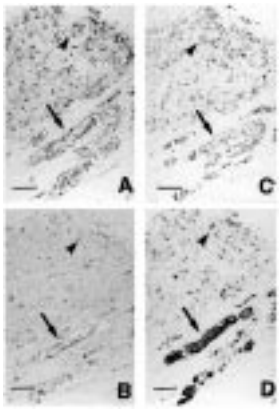

Figure 6. Serial sections of poorly differentiated cancer tissues with mucin production from a secretor were stained by anti-H 1E3 (A), 3A5 (B) and MBr1 (C), and UEA-I (D). Diffuse cytoplasm staining (arrowheads) with 1E3, 3A5 and MBr1 was observed. Mucin (arrows) showed a strong reaction with UEA-I (D), but a weak reaction with 1E3, 3A5 and MBr1. Bars indicate 100 μm.

systematically characterized the binding specificity of UEA-I and 28 anti-H MAbs, that were reported to be specific against H type 2. They found that only one of the 28 anti-H antibodies was specific for H type 2 structure without cross-reaction with any similar structures, and that the other 27 MAbs cross-reacted with Le^y or others similar to H type 2 in structures. They also found that UEA-I reacted with H type 2, Le^y and others. By means of a sensitive immunohistochemical technique with a panel of well-characterized anti-H MAbs, 1E3, 3A5 and MBr1, we simultaneously assessed the expression of the H antigens in different segments of the colon from the same individual. We unexpectedly found that the normal colon expressed only the H type 1 antigen [2,14,31], and that the expression of H type 1 in normal colon was regulated by Se type α(1,2)fucosyltransferase. In contrast to previous results which showed the presence of H type 2 in normal colonal mucosa [10,13,15,17,32,33], we did not find any expression of H type 2 in the normal colon (Fig. 1 and Table 1). Yazawa et al. [16] also demonstrated no staining for H type 2 and a low incidence of positive staining for Le^x (type 2 structure) in normal colon using specific MAbs. Our present results were also in good agreement with those reported by Dabelsteen et al. [14] who demonstrated the presence of A type 1 chain and the absence of A type 2 and A type 3 chains in normal colon, and the expression of A type 2 and A type 3 chains in colon adenocarcinoma and in fetal colon. The Le^a antigen (type 1 structure) appears to be uniformly distributed throughout the normal proximal and distal colon of adults, and the expression of the Le^b antigen often parallels that of the ABO antigens [5,9,10,13], suggesting the predominant presence of the type 1 chain of ABO- and Lewis-related carbohydrates in colon.

Studies by Holmes et al. [34,35] have indicated the expression of type 1 chain-based blood group structures in normal colon with only trace amounts of type 2 chain structures [31] despite the presence of both *β*(1,3)- and *β*(1,4)-galactosyltransferases, and they have suggested that preferential synthesis of type 1 chain-based antigens was correlated with a favorable membrane distribution of *β*(1,3)-galactosyltransferase and efficient transport of glycolipid precursors to it. The accumulation in type 2 chain antigens such as H type 2, Le^x and Le^y in colon cancer may be correlated to differences in precursor transport between competing glycosyltransferases or uneven distributions of glycosyltransferases in subcellular membranes. There are some reports on α(1,2)fucosyltransferase activity in colon cancer tissues. Orntoft et al. [36] found a significantly higher activity of α(1,2)fucosyltransferase in rectal cancer tissues than that in normal rectum from both secretors and nonsecretors. From studies on acceptor substrate specificity, they suggested that both the H gene (*FUT1*)- and the Se gene (*FUT2*)-dependent α(1,2)fucosyltransferases were increased in rectal cancer tissues. Yazawa *et al*. [37] also reported significantly higher activities of α(1,2)-, α(1,3)-, and α(1,4)-fucosyltransferases in colorectal cancer tissues irrespective of their secretor and Lewis phenotypes, and suggested that an increased activity of α(1,2)fucosyltransferase in colorectal carcinoma may be due to an increased activity of Lewis gene (*FUT3*)-encoded α(1,3/4)fucosyltransferase activity [38, 39]. Sun et al. [40] also reported 40-fold, and 340-fold, increases in levels of the mRNA of H gene (*FUT1*)-encoded α(1,2)fucosyltransferase in surgically resected samples of colon adenocarcinoma, and in colon tumor cell lines, respectively. However, we found no expression of any H antigens in the colon and cancer tissues from nonsecretors (Table 2). We previously reported a trace amount of α(1,2)fucosyltransferase activity in the gastric mucosa from nonsecretors [41]. Accordingly, the expressions of the H antigens in normal colon and in colon cancer tissues were suggested to be dependent on the Se gene (*FUT2*)-encoded α(1,2)fucosyltransferase. An immunohistochemical study by

Mariani-Costantini *et al*. [42] showed that about 80% of patients with breast cancer had the MBr1 antigen in breast cancer tissues. Their results that about 20% of breast cancer tissues were negative for MBr1 reactivity may be due to the nonsecretor phenotype.

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