Aberrant $\alpha 1 \rightarrow 2$ Fucosyltransferases Found in Human Colorectal Carcinoma Involved in the Accumulation of Le^b and Y Antigens in Colorectal Tumors

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Evidence indicates that the presence of aberrant $\alpha 1 \rightarrow 2$ fucosylation pathways is responsible for the accumulation of large quantities of Le^b and Y antigens in human colorectal carcinoma. Significantly higher activities of $\alpha 1 \rightarrow 2$ as well as $\alpha 1 \rightarrow 3$ and $\alpha 1 \rightarrow 4$ fucosyltransferases were found in most of the tissues from carcinoma than in the adjacent normal tissues and in healthy subjects. $\alpha 1 \rightarrow 2$ Fucosyltransferases associated with the synthesis of Le^b (Fuc$\alpha 1 \rightarrow 2$Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβ) and Y (Fuc$\alpha 1 \rightarrow 2$Gal$\beta 1 \rightarrow 4$[Fuc$\alpha 1 \rightarrow 3$]GlcNAcβ) structures from Le^a (Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβ) and X (Gal$\beta 1 \rightarrow 4$[Fuc$\alpha 1 \rightarrow 3$]GlcNAcβ) ones, respectively, were demonstrated in colorectal carcinomas and in colorectal carcinoma cell lines (COLO201, LS174T and SW1116). The activation of $\alpha 1 \rightarrow 2$ fucosyltransferase with such new substrate specificities in colorectal carcinoma might result in the preferential synthesis of Le^b and Y structures from Le^a and X rather than from H type 1 and H type 2 structures.

Key words: $\alpha 1 \rightarrow 2$ Fucosyltransferase — Colorectal cancer — Le^b and Y antigen — Tumor-associated antigen

Changes in the expression of blood group antigens and related antigens have been observed in various epithelial cells and in sera, and some of these antigens were identified as tumor-associated antigens.[1] Blood group Le^b antigen and its isomer Y antigen have been demonstrated to accumulate in many cancers, and in particular, in colorectal tissues, and the presence of Le^b and Y antigens seems to be closely related to malignancy.[2-9] Since these two antigens, as well as ABH antigens, are hard to detect in the same tissues from normal subjects, much interest has been focused on the biosynthetic pathways for such antigens in colorectal carcinoma.

The biosynthesis of carbohydrate antigenic structures is under gene control, residing in sequential actions of various glycosyltransferases, which are the primary gene products.[10] The Le^b antigenic determinant (Fuc$\alpha 1 \rightarrow 2$Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβ)[6] is formed by the sequential action of an $\alpha 1 \rightarrow 2$ and $\alpha 1 \rightarrow 4$ fucosyltransferase through H type 1 (Fuc$\alpha 1 \rightarrow 2$Gal$\beta 1 \rightarrow 3$GlcNAcβ) but not through Le^a (Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβ) struc-

ture. Likewise, the Y antigenic determinant is generated by the sequential action of $\alpha 1 \rightarrow 2$ and $\alpha 1 \rightarrow 3$ fucosyltransferases through H type 2 (Fuc$\alpha 1 \rightarrow 2$Gal$\beta 1 \rightarrow 4$GlcNAcβ) but not through X (Gal$\beta 1 \rightarrow 4$[Fuc$\alpha 1 \rightarrow 3$]GlcNAcβ) structure.[10] Recently, tumor-associated glycosyltransferases have been observed in tissues and serum samples and the elevation of some glycosyltransferase activities has also been demonstrated in the presence of tumors.[11-15] Previously, we demonstrated the presence of $\alpha 1 \rightarrow 4$ fucosyltransferase and CA19-9 in some cancer patients who lacked Le gene and were typed as Le(a−b−), and suggested the presence of cancer-associated $\alpha 1 \rightarrow 4$ fucosyltransferase related to the synthesis of sialyl-Le^a (Nue5Ac$\alpha 2 \rightarrow 3$Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβR) structure of CA19-9.[13] A gastric cancer-associated $\alpha 1 \rightarrow 2$ fucosyltransferase synthesizing Le^b glycolipid from Le^a glycolipid was also reported in a gastric cancer cell line.[16] In this study, we have demonstrated, for the first time, the presence of aberrant $\alpha 1 \rightarrow 2$ fucosyltransferase which converts not only Le^a to Le^b but also X to Y antigens and could cause the accumulation of Le^b and Y antigens in colorectal carcinoma.

MATERIALS AND METHODS

Materials GDP-L-[^{3}H]Fucose (85.1 GBq/mmol) and Aquasol-2 were purchased from Du Pont (Boston, MA).

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[6] The abbreviations used are: Fuc, L-fucose; Gal, D-galactose; GlcNAc, N-acetyl-D-glucosamine; Neu5Ac, N-acetylneuraminic acid; LacNAc, N-acetyllactosamine; Bn, benzyl; BSA, bovine serum albumin; Con A, concanavalin A; PBS, 0.01 M phosphate-buffered saline, pH 7.0.

GDP-Fucose, phenyl β-D-galactoside and Triton X-100 were from Sigma (St. Louis, MO). Sep-Pak plus C_{18} reverse-phase cartridges were obtained from Waters (Milford, CT). The protein assay kit was from Bio-Rad (Richmond, CA). Galβ1→3[Fucα1→4]GlcNAcβ-O(CH$_2$)$_8$COOCH$_3$, Galβ1→4[Fucα1→3]GlcNAcβ-O(CH$_2$)$_8$COOCH$_3$ and purified anti-Le^a and anti-Le^b monoclonal antibodies were obtained from Chembiomed (Edmonton, Canada). Galβ1→3[Fucα1→4]-GlcNAcβBn, Galβ1→4[Fucα1→3]GlcNAcβBn, 2'OMe-Galβ1→4GlcNAcβBn (K. L. Matta, manuscript in preparation) and 2'OMeGalβ1→3GlcNAcβBn[17] were synthesized as described. Mouse monoclonal antibody, YB-2, which reacts with Y, Le^b and H type 2 antigens but not with other fucosylated and nonfucosylated antigens[18] was from Otsuka Pharmaceutical Company (Tokushima). An LA-Con A column (0.46×15 cm, Lot No. 301291), on which Con A lectin was immobilized (14.5 mg/mg gel), was obtained from Honen (Tokyo).

α-Fucosyltransferase assay The standard reaction mixture in a final volume of 100 μl contained 0.1 nmol of GDP-[^{3}H]fucose (62,000 dpm); 5 μmol of Tris-HCl, pH 7.2; 1 μmol of NaN$_3$; 1 μmol of MnCl$_2$; 0.5 μmol of ATP; 500 μg of Triton X-100; 10 μl of enzyme preparation and 10 μl of each acceptor. After incubation at 37°C for five hours, the reaction was terminated by the addition of an equal volume of ethanol. The enzyme activities of the supernatant were measured using Sep-Pak plus C_{18} by the method described previously.[19] Enzyme activity was defined as pmol of fucose incorporated into acceptor per hour per mg enzyme preparation.

Enzyme preparation Colorectal tissues: carcinoma tissues were obtained from 7 patients with colorectal cancer undergoing surgical resection at Gunma University Hospital, Maebashi. Normal mucosa distinct from the tumor lesion was obtained from the same patients. Normal mucosa was also obtained from healthy subjects at autopsy. One gram of sample was solubilized in 2 ml of PBS containing 2% Triton X-100 by sonication at 4°C and the mixture was centrifuged at 10,000 rpm for 30 min. The supernatant was used for the assays. Colorectal cell lines: COLO201, LS174T and SW1116 cells were grown in RPMI 1640 medium containing 10% fetal calf serum. The cells were harvested, washed with PBS and stored until use at −80°C. One gram of each cell sample was thawed and added to 10 ml of PBS containing 2% Triton X-100. The enzyme preparation was obtained as described above.

Determination of blood group Lewis phenotype Determination of Lewis phenotypes of samples was carried out by the hemagglutination test and hemagglutination inhibition test of red cells and saliva.

Determination of protein Protein was determined with a DC protein assay kit (Bio-Rad) using BSA as a standard.

Identification of oligosaccharide products by affinity chromatography The positional linkages of fucosylated products were established by affinity chromatography using a monoclonal antibody-immobilized Con A column by the method of Dakour et al.[20] The purified YB-2 monoclonal antibody (500 μg/500 μl)[18] was immobilized on an LA-Con A column (0.46×15 cm) equilibrated with 0.05 M Tris-HCl (pH 8.0) including 0.15 M NaCl. The products formed by transfer of ^{3}H-labeled Fuc to Le^a (Galβ1→3[Fucα1→4]GlcNAcβ)Bn and X (Galβ1→4[Fucα1→3]GlcNAcβ)Bn were applied to a Sep-Pak plus C_{18} and radioactive products were eluted with methanol and dried as described previously.[19] The residue was taken up with a small amount of water, and an aliquot (about 300 μl) was applied to the YB-2 antibody-immobilized Con A column and eluted with the same buffer at 0.3 ml/min at 20°C using a Shimadzu LC-6A liquid chromatography apparatus. After 20 min the column temperature was increased to 42°C. Radioactivity of each tube (1 ml/tube) was measured as described above.

RESULTS

α1→2Fucosyltransferase activities were investigated in the cell extracts from colorectal carcinomas (n=7) and normal (n=10) tissues and three colorectal cancer cell lines using phenylβ-D-galactoside, Galβ1→3[Fucα1→4]-GlcNAcβ-O-R and Galβ1→4[Fucα1→3]GlcNAcβ-O-R (R=(CH$_2$)$_8$COOCH$_3$ and CH$_2$C$_6$H$_5$) as acceptors. The incorporation of [^{3}H]fucose into the three different acceptors was demonstrated to be high in all the tumors compared with those in normal lesions from carcinomas, irrespective of their ABO, Lewis blood group phenotypes and secretor status (Table I). On the other hand, α1→2fucosyltransferase activities were very low in normal mucosa from healthy subjects of both secretors (n=6) and non-secretors (n=4). It is of particular interest that both Le^a and X active oligosaccharides are good acceptors for α1→2fucosyltransferase from colorectal carcinomas and their cell lines, and that the enzyme activity in normal lesions obtained from cancer patients was somewhat elevated compared to that in normal mucosa from healthy subjects, even though the tissues were pathologically free from tumors. No significant difference of acceptor activity was found between 8-methoxycarbonyl-glycoside ((CH$_2$)$_8$COOCH$_3$) and benzyl (CH$_2$C$_6$H$_5$) Le^a or X active oligosaccharides (data not shown).

In order to obtain further evidence for the linkages of the fucosylated products from Le^a and X active oligosaccharides, the products were structurally analyzed with the aid of immunochemical reaction with monoclonal antibodies. [^{3}H]Fucosylated Le^a and X active oligo-

Table I. $\alpha 1 \rightarrow 2$Fucosyltransferase Activities in Colorectal Tissues and Cell Lines from Colorectal Cancer

Sample	Blood type	Tissue[a]	$\alpha 1 \rightarrow 2$Fucosyltransferase activity (Fuc, pmol/mg/h)		
			phenylβGal	Le^a-R[b]	X-R[c]
#1	OLe(a−b+)	N	7.0	26.3	12.4
		T	10.4	31.0	14.6
#2	OLe(a+b−)	N	8.9	14.7	9.0
		T	17.4	23.4	18.9
#3	OLe(a+b−)	N	27.3	35.3	16.3
		T	72.8	34.9	17.8
#4	ALe(a−b−)	N	7.2	13.5	6.5
		T	8.1	22.7	11.9
#5	ALe(a−b−)	N	34.6	38.4	29.3
		T	49.4	59.1	42.7
#6	BLe(a−b+)	N	15.1	14.1	11.0
		T	18.0	13.8	13.8
#7	BLe(a−b+)	N	18.9	32.4	20.3
		T	41.1	69.7	28.6
Normal tissues (n=10)[d]			6.1±3.2	6.1±3.1	4.9±2.5
Cell lines	COLO201		19.9	45.4	39.5
	LS174T		224.5	54.1	62.0
	SW1116		209.1	28.6	80.2

a) N, normal tissue from tumor patient; T, tumor lesion.
b) Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβ-O-(CH$_2$)$_8$COOCH$_3$ and
c) Gal$\beta 1 \rightarrow 4$[Fuc$\alpha 1 \rightarrow 3$]GlcNAcβ-O-(CH$_2$)$_8$COOCH$_3$ were used as acceptors.
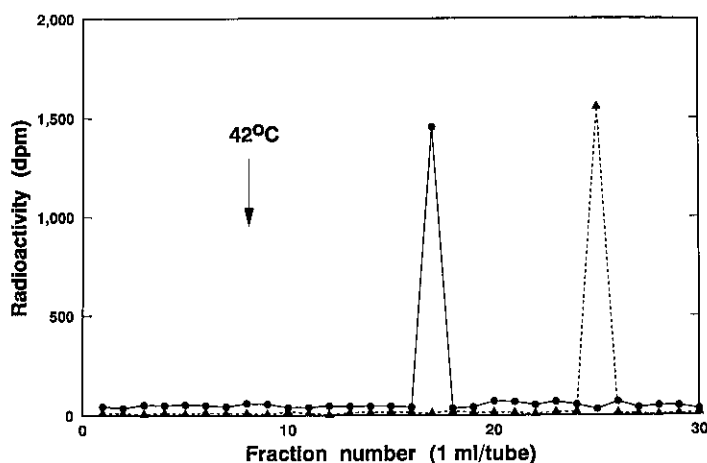d) Six samples with Le(a−b+) and 4 samples with Le(a+b−).

Fig. 1. Affinity chromatography of the fucosylated oligosaccharides on an LA-Con A column containing immobilized YB-2 monoclonal antibody. Fucosylated Gal$\beta 1 \rightarrow 3$[Fuc$\alpha 1 \rightarrow 4$]GlcNAcβBn (●) and Gal$\beta 1 \rightarrow 4$[Fuc$\alpha 1 \rightarrow 3$]GlcNAcβBn (▲) were separately applied to the column.

saccharides were prepared as described above with enzyme from COLO201 cells and then applied to a YB-2 antibody-immobilized affinity column, separately (Fig. 1). No radioactivity was detected in the eluates when the column was eluted at 20°C, but a single peak which contained most of the radioactivity applied to the column was obtained when the column was eluted at 42°C. The same chromatograms were also observed when the tissue extracts from colorectal cancer were used as an enzyme

source (data not shown). In our recent study, YB-2 antibody was found to show a higher affinity to the Y antigen than to the Le^b antigen.[18] The different elution profiles obtained from the two products, therefore, seemed to reflect the specificity of the antibody. These results confirmed that the products could be Le^b and Y active structures, and that $\alpha 1 \rightarrow 2$fucosyltransferase in colorectal carcinoma could synthesize Le^a and Y antigens from Le^a and X structures, respectively.

Table II. $\alpha1\rightarrow3$ and $\alpha1\rightarrow4$Fucosyltransferase Activities in Colorectal Tissues and Cell Lines

Sample	Blood type	Tissue[a]	Fucosyltransferase activity (Fuc, pmol/mg/hr)	
			$\alpha1\rightarrow3$[b]	$\alpha1\rightarrow4$[c]
#1	OLe(a−b+)	N	121.3	282.4
		T	216.4	408.3
#2	OLe(a+b−)	N	190.5	210.4
		T	254.1	578.8
#3	OLe(a+b−)	N	297.3	502.9
		T	351.9	661.2
#4	ALe(a−b−)	N	181.6	357.9
		T	485.0	599.8
#5	ALe(a−b−)	N	514.7	430.1
		T	615.6	915.4
#6	BLe(a−b+)	N	99.8	207.2
		T	160.3	272.0
#7	BLe(a+b−)	N	745.9	1008.1
		T	1466.1	2186.6
Normal tissues (n=10)[d]			28.5±6.7	44.3±8.6
Cell lines	COLO201		345.0	1054.3
	LS174T		871.3	1186.0
	SW1116		433.7	471.1

a) N, normal tissue from tumor patient; T, Tumor lesion.
b) N-Acetyl-2'-O-methyllactosamineβBn and
c) 2'-O-methyllacto-N-biose IβBn were used as acceptors.
d) Six samples with Le(a−b+) and 4 samples with Le (a+b−).

$\alpha1\rightarrow3$ and $\alpha1\rightarrow4$Fucosyltransferase activities were also measured in the same colorectal tissues and cell lines. Extremely high levels of $\alpha1\rightarrow3$ and $\alpha1\rightarrow4$fucosyltransferase activities were found in the colorectal carcinomas and the cell lines compared with those in normal tissues, which consisted of 6 samples with Le(a−b+) and 4 samples with Le(a+b−) (Table II). Furthermore, two samples from Le(a−b−) subjects (No. 4 and 5) showed significantly elevated activities of $\alpha1\rightarrow4$ as well as $\alpha1\rightarrow3$fucosyltransferase.

DISCUSSION

Recently, two distinct $\alpha1\rightarrow2$fucosyltransferases encoded by two structural genes, *H* and *Se*, have been proposed to be present in humans.[21] The expression of the ABH and related antigens in human tissues has also been suggested to follow the embryological origin of the tissue and the tissues of ecto- and mesodermal origin express antigens mainly under the control of the *H* gene coding $\alpha1\rightarrow2$fucosyltransferase, whereas those of endodermal origin express antigens under the control of the *Se* gene coding $\alpha1\rightarrow2$fucosyltransferase.[22] $\alpha1\rightarrow2$Fucosyltransferases encoded by these two genes have been purified[23–25] and demonstrated to show different affinities for various acceptor substrates and GDP-fucose.[24, 25]
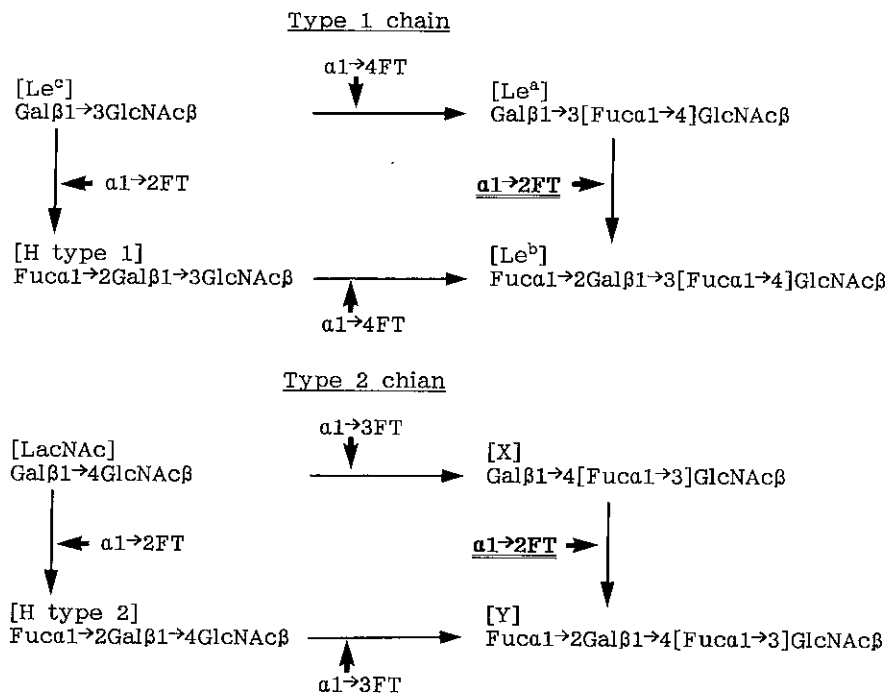
Fig. 2. Biosynthetic pathways for H, Lewis and related antigens carried by type 1 and type 2 chains. FT, fucosyltransferase; $\alpha1\rightarrow2$FT with two-underling, $\alpha1\rightarrow2$fucosyltransferase found in colorectal carcinoma.

Furthermore, it has been reported that $\alpha 1 \rightarrow 2$fucosylated antigens such as H and Le^b antigens are hardly detected in normal colorectal tissues from secretor and Lewis-positive individuals even though such antigens are commonly present in other tissues of the same individuals.[10] $\alpha 1 \rightarrow 2$Fucosyltransferase responsible for the synthesis of these antigens in the epithelial tissues is therefore suggested to be encoded by *Se* gene and absent in colorectal tissues. On the other hand, the presence of Y and Le^b antigens seems to be a tumor-associated phenomenon in colorectal carcinomas.[2, 6-9] In fact, our immunohistochemical studies showed that all the tumor tissues from colorectal carcinomas used in this study were stained positively with anti-Le^b and anti-Y antibodies (data not shown).

The difucosylated type 1 and type 2 such as Le^b and Y antigens have been proposed to be biosynthesized only from H type 1 and H type 2 antigens, respectively, but not from Le^a or X antigens, respectively[10] (Fig. 2). Previously, an aberrant $\alpha 1 \rightarrow 2$fucosyltransferase which could synthesize Le^b active glycolipid from Le^a active glycolipid was demonstrated in the cell extract from a gastric cancer cell line (KATO III), while the extract did not contain any activity which could synthesize Y active glycolipid from X active glycolipid and hence Y glycolipid was formed exclusively from H type 2 active glycolipid.[16] On the other hand, it was also shown that the Le^b antigen was formed only from the H type 1 antigen using the cell extract from SW1116 colorectal cancer cell line.[26] However, our present study clearly showed that $\alpha 1 \rightarrow 2$fucosyltransferase obtained not only from tumors and other cell lines, but also from SW1116 cells, could synthesize Le^b and Y from Le^a and X structures, respectively. These differences may be caused either by the different structures of the acceptor substrates used in each study or the different assay conditions used.

Since the Le^a antigen was commonly present in both normal and cancer tissues while the H antigen was hardly detected in the normal colorectal tissues,[27] the formation of Le^b antigen in tumors must proceed via the fucosylation of Le^a antigen. Likewise, the formation of Y antigen must proceed via the fucosylation of X antigen, since the X antigen was also found to be present in both colorectal tissues and cell lines. Phenyl β-D-galactoside has been used as a specific acceptor for $\alpha 1 \rightarrow 2$fucosyltransferase,[28] but it is not clear whether it is generally available for this purpose. The presence of $\alpha 1 \rightarrow 2$fucosyltransferase in colorectal carcinomas operating such new biosynthetic pathways was demonstrated, for the first time, in the present study with the aid of chemically synthesized substrates, Le^a and X active oligosaccharides. The use of these substrates attached to hydrophobic aglycones is advantageous not only to detect such a novel $\alpha 1 \rightarrow 2$fucosyltransferase but also to simplify the assay method, as described in our previous studies.[17, 19]

Interestingly, activities of $\alpha 1 \rightarrow 2$ as well as $\alpha 1 \rightarrow 3$ and $\alpha 1 \rightarrow 4$fucosyltransferases in normal tissue from colorectal carcinoma patients were demonstrated to be higher than those in healthy controls. Because the normal tissue from patients was pathologically free from tumor, such an elevation of α-fucosyltransferase activities might occur in advance of tumor formation in colorectal tissue. Furthermore, significantly elevated activities of the latter two enzymes could be helpful for the diagnosis of colorectal cancer, irrespective of the ABO and Lewis blood types and secretor status of the patients.

Recently, α-L-fucosyltransferases have been investigated based upon molecular genetic analysis, and the isolation of cDNAs encoding each enzyme has been reported.[29-35] It remains to be seen whether $\alpha 1 \rightarrow 2$fucosyltransferase described in this study is encoded by a new cancer-associated gene, or whether *Se* gene is changed by cancerous transformation to induce an aberrant $\alpha 1 \rightarrow 2$fucosyltransferase which is responsible for the synthesis of Le^b and Y antigens from Le^a and X antigens, respectively, in colorectal carcinomas. Studies are in progress to purify and characterize aberrant $\alpha 1 \rightarrow 2$fucosyltransferase from colorectal carcinoma.

ACKNOWLEDGMENTS

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