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Levels of Blood Group Synthetic Enzymes in Human Colonic Carcinoma[1] (40065)

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In colorectal carcinoma, the reappearance of carcinoembryonic antigen (1) and the loss of organ specific antigen (2, 3) suggest a change associated with dedifferentiation of the malignant cells. Loss of ABH isoantigen was observed in gastrointestinal carcinoma and was postulated to be due to a similar mechanism (4). In this study we report on the activity of enzymes responsible for the synthesis of A or B isoantigen in the colorectal cancerous tissues and its adjacent normal tissues. Preliminary results of this study were previously presented (5).

Materials and methods. Eighteen patients with colorectal carcinoma were included in this study. Seven patients belonged to blood group (O); 6 (A); 3 (B); and 2 (AB). The location of tumor varied from cecum to rectum (Table I). Normal and cancerous colonic tissues were frozen immediately in dry ice after surgical removal and stored at −80° until used for assay. Colonic mucosa was obtained by scraping the mucosal layer from the strip of resected colon. The normal mucosal layer was obtained from the tissues at least 5 cm away from the tumor in the tumor-free margin. At that distance the tissue was free of abnormal cells as judged by microscopic examination.

UDP-D-galactose:2′-fucosyllactose galactosyl transferase (Gal transferase) and UDP-*N*-Acetyl-D-galactosamine:2′-fucosyllactose *N*-Acetyl-galactosaminyl transferase (GalNAc transferase) activities were measured by the method of Kobata, Grollman and Ginsburg (6). Approximately 100 mg of tissue were homogenized in 1 ml of 0.05 *M* Tris, 0.005 *M* mercaptoethanol (pH 6.8) at 0°. After centrifugation at 1000*g* for 15 min, 0.1 ml of supernatant was incubated with 16 m*M* Tris–Cl pH 7.5, 8 m*M* $MnCl_2$, 0.24 mM 2′-fucosyl lactose [O-α-L-fucopyranosyl-(1 → 2)-O-β-D-galactopyranosyl-(1 → 4)-D-glucose] (7), 0.24 m*M* UDP-D-galactose (galactose ^{14}C[G] 0.4 Ci/mole) or UDP-*N*-acetyl-D-galactosamine (galactosamine-1-[^{14}C], 1.5 Ci/mole) (both from New England Nuclear, Boston) (total aqueous volumne 125 μl) and 25 μl toluene at 37° for 20 h. The time course of the sugar incorporations was nonlinear from the outset in the cases where the entire time course was studied. The ratio of incorporation (tumor/normal) was essentially constant from the beginning to the end. We chose the long incubation time to be certain that very low levels of activity would not be overlooked. In fact, the sugar incorporation at 4 hr was about 80% of that at 20 hr. Under the conditions of the assay, the amount of product formed was proportional to the amount of tissue homogenate used. At the end of the incubation, the mixture was heated at 100° for 3 min and applied to a 1 ml column of Dowex-1 × 8-Cl previously treated by washing with 0.02 *M* lactose followed by water. This treatment reduces the nonspecific absorption of the neutral sugar products. The unabsorbed products were washed from the columns with 5 ml H_2O and the eluates concentrated to dryness *in vacuo* at 30°. The residue was dissolved in 100 μl of water, spotted on Whatman 3 mm paper and chromatographed for 30 hr using ethyl acetate–pyridine–H_2O (2:1:2) as the solvent. After drying, the chromatogram was scanned for radioactivity. The magnitude of radioactivity in the tetrasaccharide peak was estimated by graphical integration with the resulting value converted into micromoles of galactose or galactosamine incorporated per gram of tissue in 20 hr using appropriate standards.

Three control systems, one without tissue homogenate, one with homogenate which had been heated to inactivate the enzyme and

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TABLE I. SITE OF COLORECTAL CANCER IN 18 PATIENTS STUDIED.

Sites of cancer	Patient study numbers
Cecum	20
Ascending	4
Transverse	1, 7, 9
Descending	13
Sigmoid	8, 16, 17, 19, 21
Rectosigmoid	5, 12, 14
Rectal	2, 3, 6, 22

a third without 2′-fucosyl-lactose, were included and performed simultaneously in each experiment. The enzyme activities were also examined in whole blood samples from normal individuals.

The α-galactosidase activity, a possible degradative enzyme for B substance, was measured by the method of Levvy and Conchie (8), using *P*-nitrophenol-α-D-galactopyranoside as the substrate.

Results. The radioactive "tetrasaccharide" peak, which was not found in the three control systems as described in the previous section, was observed in the complete assay system (Fig. 1). The tetrasaccharide peak was further characterized to be α-D-galactosyl 2′-fucosyllactose by graded acid hydrolysis and coffee bean α-galactosidase to yield the appropriate mono- and oligosaccharides.

The activities of Gal transferase in normal and tumor tissues are shown in Table II. The transferase was found in tissues from patients of blood group B or AB. Four (No. 16, 17, 1 and 7) of five tumors had increased activity in cancerous tissue by 1.6- to 6.9-fold. The normal or tumor tissues of group A or O did not contain this enzyme.

Nonspecific α-galactosidase activities were studied in nine tumors. They were elevated by 1.3- to 2.7-fold in A, B, and AB tumors, and decreased in three of four group 0 tumors. When preformed tetrasaccharide from the biosynthetic experiments of B tumor were incubated with A or B tumor homogenate, there was no galactose released. This suggests that the nonspecific α-D-galactosidase in tissue can not hydrolyze galactosyl fucosyllactose and thus does not influence the result of α-D-galactosyl transferase measurements *in vitro*. The activity of galactosyl transferase in AB or B blood was negligible at the level of 0.048 and 0.061 μmoles/ml of blood in 20 hr respectively.

The activity of GalNAc transferase was found only in normal or cancerous colorectal tissues from patients with blood group A or AB (Table III). The tumor/normal ratio varied widely from patient to patient

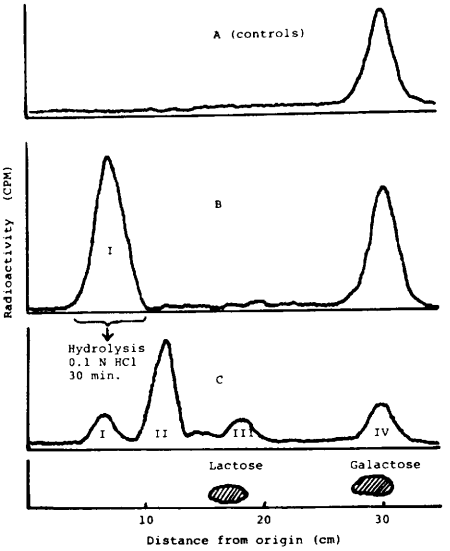

FIG. 1. Chromatography of radioactive oligosaccharides formed by a galactosyl transferase of human colonic cancer tissue. The formation of tetrasaccharide peak (I) was found after incubation of tissue homogenate with UDP-D-Gal-[^{14}C] and its acceptor fucosyllactose (Graph B); Peak (I) was not observed in three sets of control, one without tissue homogenate, one without fucosyllactose, and the third with heated tissue homogenate (Graph A); when the Peak I in graph B was subjected to acid hydrolysis and rechromatographed, Tri-, di-, and monosaccharides were found (Peaks II, III and IV in Graph C).

TABLE II. α-D-GALACTOSYL TRANSFERASE ACTIVITIES IN NORMAL AND CANCEROUS COLON.

Blood group	Patient study number	UDP-Gal transferase activities μmoles/g tissue/20 hr		
		Normal	Tumor	Ratio (tumor/normal)
B	8	0.70	0.07[a]	—
	16	0.99	6.85	6.92
	17	2.55	4.70	1.84
AB	1	0.38	1.08	2.84
	1 (repeated)	(0.32)	(1.06)	(3.31)
	7	0.29	0.45	1.55
A	3, 9, 12, 14, 20, 22	0	0	—
O	2, 4, 5, 6, 13, 19, 21	0	0	—

[a] Necrotic tumor.

TABLE III. Gal NAc Transferase Activity in Normal and Cancerous Colon.

Blood group	Patient study number	UDP-Gal NAc Transferase Activity μmoles/g tissue/20 hr		
		Normal	Tumor	Ratio (tumor/normal)
A	9	0.073	0.052	0.71
	12	0.438	0.037	0.08
	14	0.279	0.300	3.58
	20	0.025	0.337	13.5
AB	1	0.014	0.071	5.07
	1 (repeated)	(0.017)	(0.059)	(3.47)
	7	0.156	0.115	0.74
B	8	0	0	
O	2, 4, 5, 6, 13	0	0	

(0.08–13.48). The enzyme activity in cancerous tissue increased in three of the six patients.

Discussion. The levels of galactosyl transferases have previously been studied in various tissues from patients with intestinal tumors (9–11). Galactosyl transferase isoenzyme II was detected in serum in 13 of 17 patients with colorectal adenocarcinoma, but was observed only in two of 39 patients with nonmalignant gastrointestinal disorders (9). Concentration of galactosyl transferase II decreased in colonic cancerous tissue (10), but increased in tumors of small intestine (11). It should be noted that the substrate used for transferase assay was not 2′-fucosyllactose, and the serum or tumor tissues were from blood group-nonspecified patients; therefore, the enzymatic activity may not represent the blood group B specific enzyme, α-D-galactosyl transferase. The methods used for determination of the activity of Gal transferase or GalNAc transferase in this study were blood group specific, since Group B synthetic enzyme was not detected in Group A or O patients and vice versa.

The variation in transferase activities among the normal tissues was large but not unexpected. Using 2′-fucosyllactose as the acceptor, Kobata and Ginsburg found a fifteenfold range in the *N*-acetyl-D-galactosaminyl transferase activities in the milk of type A women (12). Race *et al.* found an elevenfold variation in the galactosyl transferase activity in the stomach mucosa of type B baboons using the same acceptor (13). It is known that antigenic properties varied among subgroups of type A. Activity of α-GalNAc transferases in sera of A_1 human subjects was considerably higher than that of A_2 (14). In a Caucasian population of blood donors with normal B phenotype, 16% of population has α-D-galactosyl transferase activity 1.94 times higher than the rest of the population (15). These may reflect the variant gene expression. Because of this wide variation of blood group related transferase activities in normal colon tissue, the activities in cancerous tissue were expressed as tumor/normal ratio in each individual. Tumors from types B or AB patients invariably had higher galactosyl transferase activities than adjacent non-tumorous tissues. In one-half (3 of 6) of the patients with A or AB blood group, the level of acetyl-D-galactosamine transferase was increased in the tumor. It is conceivable that the higher density of epithelial cells in the tumor tissue may be responsible for the increased activity. When the results were re-stated on the basis of activity per mg of protein in the homogenate, there was no qualitative difference in the pattern of enzyme activity changes.

The possibility of bacterial contamination as the cause of increased B antigen should be considered. Marsh has found a factor in a bacterial filtrate which produced B antigens in O and A red cells *in vitro* (16). Since galactosyl transferase activity was never found in either normal or cancerous tissues from 13 patients with blood group A or O, the possibility is highly remote that any of the enzyme activity found in B or AB tissue might be derived from bacterial contamination.

From the results of this study, it is apparent that loss of ABH isoantigens in colorectal cancer as documented in fluorescein-conjugated antiserum technique (4), can not be ascribed to a general lack of A or B isoantigen synthetic enzymes. It was recently demonstrated that human feces contain enzymes produced by enteric bacteria that degrade the A, B, and H blood group antigens (17). However, it will be difficult to explain why the bacterial-producing enzymes should act differently between normal and cancerous tissues.

Although we have documented that the transferases involved in A or B isoantigen synthesis are not deficient in cancerous tissue, there could still be a defect on the synthetic

pathway prior to transferase step leading to a loss in the ABH isoantigens. However, a more likely reason in our opinion is that the changes are due to an accumulation of mucoprotein in the colonic cancer (18) which may mask the expression of surface antigen(s) (19) and cause a negative result for the immunofluorescent stain technique for detecting ABH isoantigen. In leukemic patients, loss of blood group antigen in red blood cells has also been observed (20), but the expression of the antigen could be recovered with neuraminidase treatment (21).

Summary. Homogenates of tumorous and adjacent non-tumorous colorectal tissues from 18 patients were tested for the activities of blood group synthetic enzymes, namely α-D-galactosyl transferase for B isoantigen and α-*N*-acetyl-D-galactosamine transferase of A isoantigen.

The galactosyl transferase activity in non-tumorous intestinal tissue was high in patients with blood group B, intermediate in group AB and was absent in group A or O. As compared with adjacent non-tumorous tissue, the Gal transferase activity increased in tumors by 1.6- to 6.9-fold in four of five patients with blood group B or AB, and the α-*N*-acetyl-D-galactosaminyl transferase activity increased in three- of six-tumor tissues from patients of blood group A or AB.

The results suggest that the reported losses of ABH isoantigen in colorectal cancer are not due to deficiencies of Gal or GalNAc transferase activities involved in the synthesis of blood group antigens.

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