Journal of Cellular Biochemistry 37:193–202 (1988)
Altered Glycosylation in Tumor Cells 167–176

Ulex Europeus Type I Agglutinin Detects Carcinoembryonic Antigen in Extracts of Human Colorectal Carcinoma

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Recent interest has focused on fucosylated epitopes expressed on human neoplasms. The plant lectin *Ulex europus* agglutinin, Type I (UEA) binds fucosylated oligosaccharides, while UEA-reactive substances have a tissue distribution similar to carcinoembryonic antigen (CEA). We sought to determine if UEA reacted with CEA in extracts of fresh primary and metastatic colorectal carcinomas and paired normal tissues. The extracts were electrophoretically transferred to nitrocellulose membranes after the proteins were separated by SDS-PAGE in 10% polyacrylamide gels. The transfer membranes were then stained with peroxidase-conjugated UEA (UEA-P) or antibody to CEA (CEA-P). UEA-P reacted with a 170–190-kDa band in extracts of 22 of 30 primary tumors, 10 of 12 metastases, but only 1 of 5 villous adenomas. UEA-P generally did not react with normal colon or liver extracts. UEA-P also did not bind to 170–190-kDa molecules in Western transfers of a breast carcinoma metastatic to bowel and a focal nodular hyperplasia of liver. CEA-P displayed similar reactivity and detected CEA in a tumor extract negative for UEA. Fucose blocked binding of UEA-P to Western transfers of tumor extracts. CEA-P reacted with a 170–190-kDa substance in tumor extracts eluted with fucose from a column of immobilized UEA. Thus, UEA reacts with fucosylated oligosaccharides on most, but not all, species of CEA and may be a useful adjunct to anti-CEA immunohistochemistry.

Key words: colorectal carcinoma, carcinoembryonic antigen, UEA

Abbreviations: CEA, carcinoembryonic antigen; CEA-P, peroxidase-conjugated antibody to CEA; MEM, minimal essential medium; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; UEA, *Ulex europeus* agglutinin, Type I; UEA-P, peroxidase-conjugated UEA.

Received April 21, 1987; revised and accepted November 6, 1987.

INTRODUCTION

Carcinoembryonic antigen (CEA) is a heterogeneous substance of approximately 180 kDa, some of whose determinants may be shared with several other epitopes, including blood group determinants [1]. Since the lectin *Ulex europeus* agglutinin, Type I (UEA) binds oligosaccharides on glycoproteins and glycolipids that contain terminal alpha-*L*-fucose in an alpha 1----2 bond to galactose, UEA reacts with H blood group substance, the precursor for the A and B blood group isotypes. Several histochemical studies [2–6] demonstrated that UEA binds molecules in primary human colorectal carcinomas and normal colonic epithelial cells in the proximal colon. Although Irimura et al. [7] reported that the masses of UEA-binding proteins in adenocarcinomas of the colon and rectum are greater than 300 kDa, Matsushita et al. [8] showed that UEA-binding molecules in primary human colorectal carcinomas have a tissue distribution that is similar to that of CEA. Further, they demonstrated that UEA binds a 180-kDa molecule in Western transfers of extracts of colorectal carcinoma. In contrast, Denk et al. [9] found that blood group substances were distributed in colorectal tissues differently from CEA. Simmons and Perlmann [10] were not able to demonstrate H, A, or B blood group substances in preparations of CEA. Thus, it is not clear whether UEA binds to a subset of CEA molecules or to other molecules in colorectal carcinomas.

The purpose of this study was to determine if UEA binds to CEA in primary and metastatic human colorectal carcinoma. We performed Western enzyme-linked lectin analysis instead of histochemistry [11]. Western transfers were made of detergent extracts of neoplastic and normal colorectal tissues and reacted with horseradish peroxidase-conjugated UEA (UEA-P). UEA-P bound a 170–190-kDa substance that was also detected with a peroxidase-conjugated polyvalent antibody to CEA. Lectin affinity chromatography then identified CEA as a UEA-binding glycoprotein.

MATERIALS AND METHODS

Patients

The patients in this study were admitted to the general surgery service of the University of Texas M.D. Anderson Hospital and consented to participate in accordance with institutional and Federal guidelines. The age, sex, and other characteristics of each patient are described (Table I–III). Stage of disease is that described by Astler and Coller [12], where a B1 lesion is a primary tumor confined to the submucosa of the bowel without lymph nodal or other metastases; a B2 lesion is a primary tumor that has penetrated the full thickness of the bowel wall without metastasis; C1 and C2 lesions are similar in depth of penetration to B1 and B2, but the regional lymph nodes contain metastasis; and a D lesion has metastasis to a distant site. The origin of the primary tumor is in the right colon if it arose in the cecum, ascending colon, or hepatic flexure, and in the left colon if it arose in the transverse, descending, or sigmoid colon. Focal nodular hyperplasia of the liver is a benign proliferative disorder of the liver.

Preparation of Extracts

Tumors were resected according to standard techniques and the operative specimens were immediately examined by a surgical pathologist who placed samples of primary tumor and normal colon mucosa or metastasis and normal liver tissue in cold minimal

TABLE I. UEA Lectin Binding to Extracts of Primary Large Bowel Carcinomas

Extract	Sex	Age	Blood group	CEA[a]	Grade	Site[b]	Stage	UEA (170–190 kDa) Tumor	UEA (170–190 kDa) Normal
27	M	67	B	21.3	2	LC	C2	–	–
30	M	53	O	<1.5	2	LC	C2	+	–
38	M	57	O	6.7	3	RC	B2	+	–
45	M	66	A	<1.5	1	RC	C2	+	–
54	M	76	O	<1.5	1	LC	B2	+	–
61	M	40	A	ND	1	R	B2	+	–
64	F	84	A	<1.5	1	LC	B1	+	–
71	M	56	AB	<1.5	1	LC	B2	–	–
83	M	48	O	469.0	2	R	D	+	–
87	F	63	A	<1.5	2	LC	B2	–	–
90	M	84	A	3.0	2	LC	B1	+	–
93	F	65	O	3.6	2	LC	C2	+	–
103	F	36	A	1.5	2	LC	B1	–	–
105	M	69	A	ND	2	LC	B1	+	–
112	F	45	B	<1.5	2	RC	B2	+	–
118	F	71	B	14.7	2	RC	C2	+	–
120	M	68	A	6.0	2	RC	D	+	–
139	M	67	A	2.3	1	RC	B2	+	–
141	F	50	O	21.1	2	LC	B2	+	–
151	F	70	A	8.4	2	LC	D	+	–
153	F	69	O	ND	2	RC	B2	+	+
158	M	79	O	ND	2	LC	C2	+	+
162	M	76	O	7.5	2	LC	B2	+	–
165	M	75	O	3.2	2	LC	B2	+	–
167	M	61	O	<1.5	2	LC	B2	+	–
182	M	48	A	<1.5	1	LC	C2	–	–
184	M	52	O	281.0	2	LC	D	–	–
191	F	54	O	15.7	1	LC	C2	–	–
192	M	86	–	ND	2	RC	C2	–	–
194	F	31	O	48.3	2	LC	D	+	–

[a]ND, not done.
[b]RC, right colon; LC, left colon; R, rectum

essential medium (MEM, Grand Island Biological Co., Grand Island, NY). Normal colon mucosa was taken 10 cm from the nearest encroachment of tumor. Normal liver tissue was taken at least 3 cm from gross tumor. Extracts were prepared essentially as described by Reading and Hickey [11]. Cell suspensions were prepared from 1–2 g of tumor or normal tissue disaggregated by passage through a fine mesh screen after mincing with scissors. The cells were washed twice with 0.15 M sodium chloride, 0.01 M sodium phosphate, pH 7.2. Ten volumes (v/v) of extraction buffer (0.5% Triton X-100, 0.5 M mannitol, 5.0 mM $CaCl_2$, 0.02% NaN_3, 100 μM phenylmethylsulfonylfluoride; all chemicals unless stated otherwise from Sigma Chemical Co., St. Louis, MO) was added to the cell pellet, which was extracted for 20 min on ice. The supernatant was collected after the extracts were centrifuged at 13,000 × g for 3 min, one-third volume of three-fold concentrated sodium dodecylsulfate (SDS) sample buffer (2% SDS, 0.063 M Tris-phosphate, pH 6.7, 1% 2-mercaptoethanol, 0.5 mM EDTA, 10% glycerol, 0.003% bromophenol blue) was added, and the sample was heated for 5 min at 100°C. Extracts in SDS sample buffer were stored at −20°C until sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Aliquots of samples in extraction buffer

TABLE II. UEA Lectin Binding to Extracts of Metastatic Large Bowel Carcinomas

Extract	Sex	Age	Blood group	CEA[a]	Site	UEA (170–190 kDa) Tumor	UEA (170–190 kDa) Normal
57	F	32	O	4.9	Liver	+	–
59	F	59	O	ND	Liver	+	–
81	M	58	A	22.9	Liver	+	+
91	M	54	B	2.0	Liver	+	–
101	M	61	A	ND	Liver	–	–
108	F	60	O	6.2	Liver	+	–
132	M	76	O	308.0	Liver	+	–
118	F	71	B	14.7	Mesentery	+	–
142	F	50	O	21.1	Mesentery	–	–
121	M	68	A	6.0	Lymph node	+	
127	F	32	O	4.1	Lymph node	+	
133	F	49	A	36.0	Lymph node	+	

[a]ND, not done.
Peroxidase-conjugated UEA was reacted with transfers of Triton-mannitol extracts of colorectal carcinomas and other tissues, as described in "Materials and Methods."

TABLE III. UEA Lectin Binding to Extracts of Villous Adenomas and Other Neoplasms

Extract	Sex	Age	Blood group	CEA[a]	Neoplasm	Site[b]	UEA(170–190kDa) Tumor	UEA(170–190kDa) Normal
49	M	73	AB	<1.5	Villous adenoma	LC	–	–
107	M	69	A	2.0	Villous adenoma	LC	–	–
114	F	45	B	<1.5	Villous adenoma	RC	–	–
130	M	80	O	2.3	Villous adenoma	RC	–	–
155	F	69	O	ND	Villous adenoma	RC	+	+
69	F	63	O	2.8	Breast cancer		–	–
80	F	28	B	ND	Focal nodular hyperplasia		–	–

[a]ND, not done.
[b]RC, right colon: LC, left colon

were analyzed for protein content by the Bio-Rad microcolorimetric assay (Bio-Rad Laboratories, Inc., Richmond, CA).

SDS-PAGE and Western Enzyme-Linked Lectin Analysis

Molecules were separated in single dimension SDS-PAGE in a 10% polyacrylamide gel with a Tris/glycine discontinuous buffer system [13,14] in a vertical slab gel cell (LKB Instruments, Inc., Gaithersburg, MD) maintained at 15°C. After electrophoresis at 50 mA constant current for 3–5 hr, molecules were then electrophoretically transferred to nitrocellulose membranes in 24 mM Tris-192 mM glycine/20% (vol/vol) methanol, pH 8.3 buffer in an Electroblot apparatus (E-C Apparatus Corp., Philadelphia, PA) with a current setting of 50–300 mA [15]. Membranes were stained for protein using 2% amido black in 2% acetic acid for 15 min at 25°C followed by destaining with 2% acetic acid. Prestained high molecular weight standards (Bethesda Research Laboratories, Inc., Gaithersburg, MD) were included in each transfer to assess adequacy of transfer, as well as to estimate the mass of molecules transferred. Quantities of samples applied to

each lane were normalized so that there was 30–50 μg protein per lane in the SDS-PAGE.

Transfer membranes were air dried and then incubated with blocking buffer (1% bovine serum albumin, 1% polyvinylpyrrolidone in phosphate-buffered saline [PBS]) for 30 min at 25°C. Each batch of UEA-horseradish peroxidase conjugate (UEA-P, E-Y Laboratories, San Mateo, CA) was dissolved in blocking buffer and titrated for optimal reactivity, which was usually 10 μg/ml. The blocking solution was decanted, replaced with UEA-P in blocking buffer sufficient to cover the transfer membrane completely, and gently rocked for 60 min at 25°C. The transfers were than washed 10 times with 0.05% Triton X-100 and bands visualized by incubating the membranes for 5–15 min in 0.06% 4-chloro-1-naphthol in 20% methanol-PBS buffer containing 0.02% hydrogen peroxide. When the bands were adequately developed, the reaction was stopped by washing with deionized water. Specificity of binding was assessed by incubating UEA-P with 0.2 M alpha-*L*-fucose prior to applying the lectin to the transfer membrane. Confirmation of the identity of CEA was sought with horseradish peroxidase-conjugated polyclonal anti-CEA (CEA-P, Accurate Surgical and Scientific Inst. Co., Westbury, NY). CEA-P was substituted for UEA-P at a dilution of 1:100 in blocking buffer. The remainder of the staining process was the same.

Lectin Chromatography

UEA (E-Y Laboratories) was conjugated to Affi-Gel 10 (Bio-Rad Laboratories) according to the instructions supplied by the manufacturer. UEA-Affi-Gel conjugate (2 ml gel) was packed in Pasteur pipettes and washed with three bed volumes of PBS. Extracts (0.5 ml) in extraction buffer were applied to the column that was then washed with three bed volumes of extraction buffer. The column was eluted with three bed volumes of extraction buffer containing 2 mM alpha-*L*-fucose. SDS sample buffer was added to the filtrates and eluates, which were then analyzed with the Western enzyme-linked lectin analysis described above.

RESULTS

UEA-Binding Glycoproteins in Extracts of Colorectal Carcinomas

UEA-binding glycoproteins were identified in extracts of normal and neoplastic colorectal tissues. The dominant band is 170–190 kDa and is present in the extracts of tumor but not normal tissue (Fig. 1). Other molecules bound UEA to a lesser extent. Binding of UEA-P to the 170–190-kDa substance was specific for fucosylated glycoproteins, since incubation of the lectin with 0.2 M alpha-*L*-fucose abolished this binding (Fig. 2). Thus, UEA binds a fucosylated molecule of 170–190 kDa in Western transfers that is preferentially expressed in extracts of colorectal carcinoma.

Distribution of UEA-Binding Glycoproteins

UEA reacted with a 170–190-kDa band in 22 of 30 primary (Table I) and 6 of 7 colorectal carcinomas metastatic to liver (Table II). The UEA-binding substance was also detected in extracts of three lymph nodes containing metastatic tumor and in one

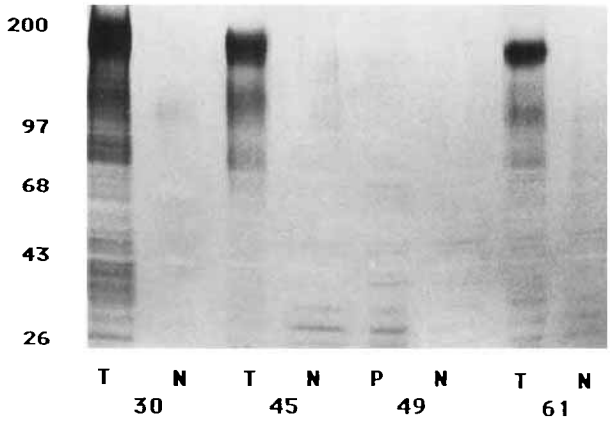

Fig. 1. Identification of UEA-binding proteins in representative extracts of neoplastic (but not normal) colon. Triton–mannitol extracts of colorectal tissues were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and then incubated with the peroxidase-conjugated lectin from *Ulex europeus* (UEA). **T** is an extract of a primary colorectal carcinoma, **N** is an extract of normal colorectal mucosa, and **P** is an extract of a polyp, a villous adenoma.

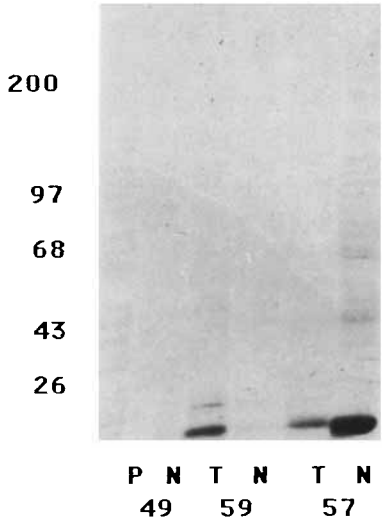

Fig. 2. A solution of 0.2 M alpha-*L*-fucose blocks the binding of peroxidase-conjugated UEA to the 170–190-kDa UEA-binding protein in extracts of colorectal carcinoma. Extracts from tumor (**T**), polyp (**P**), or normal tissue (**N**) were transferred to nitrocellulose membranes after SDS-PAGE and then reacted with UEA-P that had been incubated with 0.2 M alpha-*L*-fucose. Extracts 57 and 59 are from metastases, and extract 49 is of a villous adenoma that did not contain UEA-binding proteins. Specific binding to the 170–190-kDa protein is blocked and nonspecific binding to low molecular weight substances is not blocked.

of two extracts of mesenteric metastases (Table II). The 170–190-kDa UEA-binding band was detected in only 2 of 30 extracts of normal colon mucosa, 1 of 7 extracts of normal liver, and 1 of 5 villous adenomas (Tables I–III). The villous adenomas are benign polyps that have the potential to become malignant. Interestingly, neither an

extract from a patient whose breast cancer metastasized to colon (extract 64), nor an extract of a benign proliferative disorder of hepatocytes (fibronodular hyperplasia, extract 80) contained the UEA positive band.

Isolation of CEA by UEA Lectin Affinity Chromatography

CEA was identified by UEA lectin affinity chromatography in an extract of a metastatic colorectal carcinoma. Triton–mannitol extracts from a metastatic colon carcinoma (extract 57) that contained the 170–190-kDa UEA-binding protein were passed over UEA attached to a solid phase and eluted with Triton–mannitol extraction buffer containing 0.2 M alpha-*L*-fucose. The eluates and filtrates were then analyzed by the transfer technique after SDS-PAGE. There was very little 170–190-kDa glycoprotein present in either the eluate or the filtrate as detected by amido black staining (Fig. 3). However, both anti-CEA antibody and UEA-P reacted with a 170–190-kDa substance in the eluate and the filtrate (Fig. 3). Interestingly, the substance in the eluate had a higher mass than the substance in the filtrate that bound both UEA and antibody to CEA. This suggests that species of CEA have different affinities for UEA.

Comparison of CEA- and UEA-Binding Proteins

CEA was probed in seven Western transfers of extracts of tumor tissue with a horseradish peroxidase-conjugated polyvalent anti-CEA antiserum, and the results com-

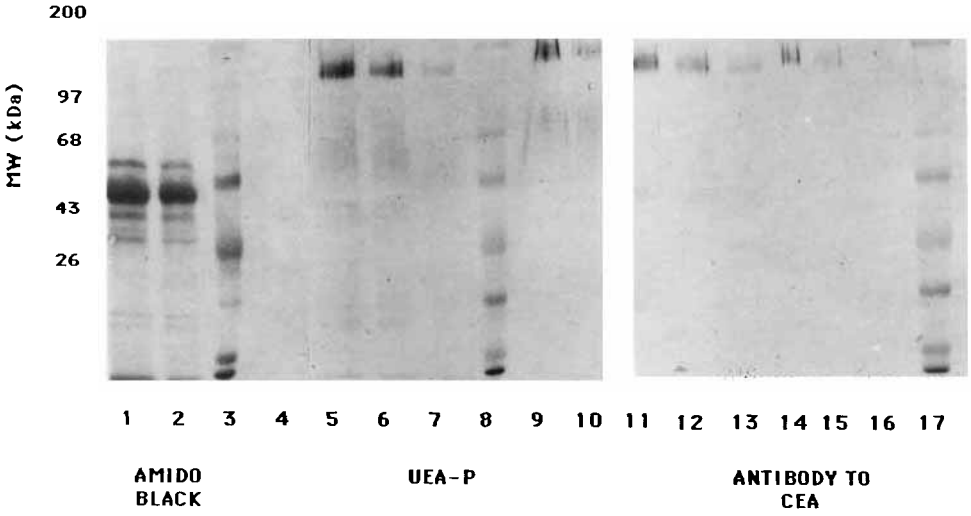

Fig. 3. The UEA-binding protein is a species of CEA. Extract from a liver metastasis (case 57) was passed over UEA attached to a solid phase. The column was washed with three bed volumes of Triton –mannitol buffer and the filtrate collected. Bound material was eluted with three bed volumes of Triton–mannitol buffer containing 0.2 M alpha-*L*-fucose. The eluate and filtrate were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and then incubated with peroxidase-conjugated UEA and antibody to CEA. The molecular weight standards are 200, 97, 68, 43, 26, 17, and 14 kDa in **lanes 3**, **8**, and **17**. The filtrate contains proteins detected by amido black staining (**lanes 1** and **2**), while the eluate does not (**lane 4**). There was not enough material to do a direct protein determination. The filtrate (**lanes 5–7** and **11–13**; 50, 25, 12.5 μl of sample/lane) and eluate (**lanes 9**, **10** and **14–16**; 50, 25, and 12.5 μl sample/lane [lane 16]) contained a 170–190-kDa substance that reacted with UEA-P and antibody to CEA. The mass of the UEA-binding substance in the eluate is higher than the substance in the filtrate, suggesting that the material in the eluate has a different affinity for UEA.

pared with similar transfers that had been incubated with UEA-P. A 170–190-kDa band that reacted with both UEA and antibody to CEA was observed in six extracts (Table IV). One extract did not contain any 170–190-kDa glycoprotein, and one extract contained CEA that did not react with UEA (Table IV). Thus, many but not all CEA species contain fucosylated oligosaccharides that react with UEA.

DISCUSSION

This study demonstrates that UEA binds to fucosylated epitopes of CEA in extracts of human colorectal carcinoma. The lectin from *Ulex europeus* has been used to detect blood group H determinants in tissues. Thus, the most likely oligosaccharide expressed on CEA that binds UEA is the H oligosaccharide, the precursor for A and B blood group substances. Although Simmons and Perlmann [10] did not find H substance in preparations of CEA on direct testing in enzyme-linked immunoassays, Holborn et al. [16] reported that H substance weakly inhibited anti-CEA antibody binding to CEA in a radioimmune assay. Gold et al. [17] demonstrated blood group A substance on CEA. Matsushita et al. [8] demonstrated a similarity between the molecules bound by anti-CEA antibody and UEA in Western transfers of extracts of colorectal carcinoma, but did not prove that the molecules were identical. We have taken their findings a step further by demonstrating that molecules isolated by affinity chromatography on immobilized UEA also react with antibodies to CEA.

Although the primary UEA-binding glycoprotein was 170–190 kDa, several smaller glycoproteins also bound UEA (Figs. 1, 3) and may be the antigens TEX, NCA, or other molecules that are related to CEA and express similar oligosaccharide epitopes [18–20]. We also did not identify the high molecular weight UEA-binding substance reported by Irimura et al. [7], but that may be because our transfer method may not be optimal for detection of molecules greater than 300 kDa.

The H blood group substance is not the only fucosylated oligosaccharide expressed in CEA. Di- and tri-fucosylated oligosaccharides are present in CEA isolated from colorectal carcinoma metastases [21]. Since the terminal sugars of Lewis Y structure contain fucose in an alpha 1——2 bond to galactose, it is possible that UEA may bind this structure as well as the monofucosylated H oligosaccharide.

UEA-binding CEA was extracted from primary carcinomas and hepatic, mesenteric, and lymph node metastases, but not from the majority of extracts of normal colon mucosas of villous adenomas. Histochemical studies demonstrate that UEA binds to epithelial cells in the normal mucosa of the right colon and to adenomatous polyps, but

TABLE IV. Comparison of Binding of the Lectin UEA and Anti-CEA Antibody to Extracts of Human Colorectal Carcinomas

Extract	Tissue	UEA	CEA
90	Primary	+	+
81	Metastasis	+	+
83	Primary	+	+
87	Primary	−	+
103	Primary	−	−
108	Metastasis	+	+
116	Primary	+	+
118	Metastasis	+	+

not epithelial cells in the normal mucosa of the left colon. The polyps in this study were well differentiated without dysplasia or other characteristics to suggest malignant change. As a result, the polyps may not express CEA or contain UEA-binding proteins, since Greaves et al. [22] reported that expression of CEA occurred only when a polyp was severely dysplastic or malignant. Similarly, the concentration of CEA may be too low to permit detection of UEA-binding proteins in extracts of normal right colon mucosa. CEA is a heterogeneous molecule whose terminal oligosaccharides may be sialylated rather than fucosylated. Thus, the technique may not be sensitive enough to detect fucosylated molecules that are present in low concentration in extracts of normal colon.

Although seven of the eight negative extracts of primary carcinomas were from left colon cancers, there was not a significant difference in the incidence of UEA-binding proteins between right colon carcinomas (7 of 8 were UEA positive; 88%) compared to left colon and rectal carcinomas (15 of 22 were UEA positive; 68%). A larger series might demonstrate that the decreased expression of fucosylated CEA in left colon carcinomas is significant. However, Kim and his co-workers [3] have not demonstrated significant decreases in the expression of blood group H substance in carcinomas of the left colon.

In summary, UEA binds to fucosylated oligosaccharides expressed on CEA. This finding may have several uses. First, the function of CEA remains unknown two decades after its original description [23]. Byrn et al [24] have reported that different species of CEA are cleared from the circulation at different rates and that clearance is directly proportional to the degree of fucosylation. The degree of fucosylation of CEA may have practical significance because the fucosylated species has been isolated from colorectal carcinoma metastatic to liver while sialylated CEA (slowly cleared CEA) has been isolated from the ascites of well-differentiated colorectal carcinoma. Affinity chromatography with UEA may help isolated cells or molecules expressing fucosylated CEA for functional analysis. Finally, UEA may help distinguish colorectal neoplasms from other neoplasms when the source of the neoplasm is in doubt.

ACKNOWLEDGMENT

This work supported by a contract from Smith Kline and French Laboratories and by grant RO1CA42587 from the Department of Health and Human Services.

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