ABSTRACT

The purpose of this study was to compare the expression of O-acetylated sialic acids on normal colonic epithelial cells to that on primary and metastatic human adenocarcinoma of the colon and rectum. In 24 cases, the relative percentages of biosynthetically labeled mono-, di-, and tri-O-acetylated sialic acids were measured after hydrolytic release, separation, and identification by paper chromatography. In one case, the presence of di- and tri-O-acetylated sialic acids was confirmed by fast atom bombardment-mass spectral analysis. Differences were observed in the expression of sialic acids between normal colonic epithelium, "uninvolved" colon mucosa remote to a colon adenocarcinoma, and colonic adenocarcinoma. The levels of mono- and tri-O-acetylated sialic acids accounted for the difference in the ratios of sialic acids expressed between normal and "uninvolved" colon mucosa, while the total amount of O-acetylation was unchanged. However, no difference was observed in the relative amounts of non- and O-acetylated sialic acids between either fresh and tissue culture-established colon carcinomas, or fresh and tissue culture-established liver metastasis derived from carcinoma of the colon. The relative expression of these O-acetylated sialic acids molecules appears to vary according to tissue type. This study suggests that individuals with adenocarcinoma of the colon express a field defect resulting in abnormal ratios of O-acetylated sialic acids.

INTRODUCTION

A number of N- and O-substituted derivatives of neuraminic acid (see Fig. 1) have been identified in biological samples (1). The presence of O-substituted sialic acid molecules in human colonic epithelial mucins was first suggested by the resistance of normal human colonic epithelial mucins to periodic acid-Schiff histochemical staining without prior saponification (2-4) as well as their resistance to bacterial neuraminidases (5-8). The degree of O-substitution of sialic acids on human colonic epithelium appears to be a sensitive indicator of early malignant change (9-16). Using histochemical methods, these studies demonstrated a loss of O-substituted sialic acid molecules in colon-derived mucins at the onset of malignancy. In a few cases, sialomucins from normal and malignant tissue of human colon were fractionated and analyzed chemically (17-23). These investigations, however, were unable to prove unequivocally the O-acetylated nature of these molecules or to quantitate the relative amounts of each sialic acid species.

This study was designed to quantitate the relative amounts of the sialic acids on normal, malignant, primary, and metastatic human colonic epithelial cells from both fresh and cultured specimens. Biosynthetically radiolabeled sialic acids from various human tissues were purified and analyzed by ascending paper chromatography. We demonstrate that mono-, di- and tri-O-acetylated sialic acid molecules can be separated in this system and confirm the chemical nature of these molecules by FAM-MS. Significant differences were noted in the relative expression of non- and O-acetylated sialic acids on normal colonic mucosa, "uninvolved" mucosa remote to colonic adenocarcinoma, adenocarcinoma of the colon, and hepatic metastases from primary colon lesions.

MATERIALS AND METHODS

Cell Lines and Patient Samples. The HT-29 colon carcinoma lines R and F were maintained as separate cell lines, originating from the same source (24). The HT-29 (lung) line, isolated from a lung metastatic lesion, and the HT-29 (liver) line, isolated from a liver metastatic lesion in nude mice (25), HC 84, CL 3.5, FI 81 fetal intestinal cells, and colonic fibroblast cell lines (4014, 4961, and HCF20), were maintained as monolayer cultures in Eagle's MEM (M. A. Bioproducts). The monolayers were supplemented with 10% FBS, sodium pyruvate, nonessential amino acids, l-glutamine, and a 2-fold vitamin solution (GIBCO, Grand Island, NY). The HC 1410 and HC 1544 cell lines were derived from liver metastases of patients with primary rectal carcinoma, and HC 8589 was derived from a primary rectal carcinoma. These cell lines were established in culture after s.c. growth of the tumor in nude mice (26). The HC 2998 was established in vitro directly from surgically resected adenocarcinoma of the colon. These cell lines were maintained in Ham's F12 (M. A. Bioproducts) medium supplemented with 10% FBS, 1-glutamine, 5 µg/ml insulin, and 2 µg/ml transferrin (Sigma Chemical Co., St. Louis, MO). Isoenzyme and karyotype analysis verified the human origin of tumor lines. All of the experiments were performed within 20 passages in vitro.

Fresh normal colon tissue was obtained from patients who underwent surgery at the University of Texas Medical School at Houston. Specimens were also obtained from patients who underwent surgical resection of primary colorectal carcinomas or hepatic metastases at the University of Texas M. D. Anderson Hospital and Tumor Institute. All tissues were obtained in accordance with institutional and federal guidelines and were obtained from patients who underwent similar bowel preparation consisting of tap water enemas and p.o. cathartic. The following noncolon human tissues and cell lines were examined for the expression of O-acetylated sialic acids. Human melanoma cell lines GRD, M40, and PHIL were originally established by Dr. W. A. Cassel of Emory University, Atlanta, GA, and the SKMel 40, 23, and 93DX6 melanoma cell lines were provided by Dr. G. Nicolson, University of Texas MDAH, Houston, TX. The human melanoma cell line A375P and its metastatic variant A375M were provided by Dr. I. J. Fidler, MDAH. The human breast carcinoma cell lines 231 and 435 were provided by Dr. R. Cailleau, MDAH. Lymphoblastoid cell lines Fo and Hu, established by Epstein-Barr virus transformation of purified B-lymphocytes as well as purified B- and T-lymphocytes from peripheral blood were provided by Dr. A. J. Roome, MDAH, and peripheral blood monocytes purified by counterflow elutriation, by J. Turpin, MDAH. The T-cell line CEM and B-cell lines Daudi, REH, and Raji were provided by Dr. G. Spitzer, MDAH. Human adult lung fibroblast GM1610, adult skin fibroblast GM5659 and GM6969A, and fetal lung fibroblast GM1604 cell lines were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, and J- (connective tissue) and K- (fetal skin) fibroblasts were established.
O-ACETYLATED SIALIC ACIDS ON HUMAN COLON CELLS

Fig. 1. Nature and position of the N- and O-substituents of sialic acids. The parent sialic acid molecule is in the chair conformation with the individual carbons numbered 1-9. Sialic acids are glycosidically linked at the anomeric C-2 position to galactose, N-acetylgalactosamine, N-acetylgalactosiose, or sialic acid. R₂, an acetyl substitution at the nitrogen group; R₃, R₄, R₅, possible O-acetyl substitutions.

by Dr. M. Pollack, Baylor College of Medicine, Houston, TX. Human hemangioblastoma P and its clone CI-6, human hepatocytes, DSS 972 kidney cells, and splenic endotheloid cells were provided by P. Belloni, MDAH. Human acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, and chronic myelogenous leukemia cells were obtained by pheresis and provided by Dr. J. Hester, MDAH. GRD, M40, and PHL cells were cultured in Leibovitz L-15 medium (GIBCO) containing 10% FBS (Irvine Scientific, Santa Ana, CA), SKMel 40, 23, and 93DX6 cells in Dulbecco’s modified Eagle medium (GIBCO) containing 10% FBS, and all other cell lines and fresh samples in RPMI 1640 medium (GIBCO) containing 10% FBS. Adherent cell lines were maintained by harvesting monolayer cultures with 0.025% trypsin and 0.02% EDTA (Irvine Scientific).

Clinical Characteristics of Patients. Surgical specimens were obtained from 11 patients with primary or metastatic disease. Median age of the patients (4 males, 7 females) was 56 years (range, 36-65). Eight of eleven patients had elevated levels of carcinoembryonic antigen (greater than 2.5 ng/ml) before surgery.

Preparation of Fresh Samples. Tumor specimens from primary human colon carcinoma or liver metastases including the respective uninvolved tissues were obtained immediately after surgery and were dissociated with collagenase and DNase, according to the technique of Giavazzi et al. (26). In brief, each tissue was dissected free of necrotic areas, connective tissue, and blood clots, then rinsed twice in cold (4°C) MEM containing antibiotics. With some tissues, the specimen was cut into 3- to 5-mm³ fragments using a sterile scalpel, pressed through a sterile 50-mesh stainless steel screen, and washed 5 times in MEM containing antibiotics at 4°C. Cell concentrations were adjusted to 2 x 10⁶ cells/ml and cultured in alpha-MEM (M. A. Bioproducts) containing 10% FBS.

The remaining infiltrating cells of 10 cell suspensions were analyzed for indirect immunoperoxidase with murine monoclonal antibodies to a pan T-cell marker (T101; Atlantic Antibodies, Davis, CA), a B-cell marker (a rabbit anti-human immunoglobulin IgM plus IgG plus IgA (Miles Laboratories, Naperville, IL), a marker of NK cells (Leu-7; Becton Dickinson, Mt. View, CA), and monocytes-macrophages with MAC-1. The results showed that primary tumors contained 3.5 ± 0.6% (SE) T-cells, 4.2 ± 1.1% B-cells, 0% NK cells, and 7.5 ± 1.2% monocytes-macrophages. The results showed that primary tumors contained 3.5 ± 0.6% (SE) T-cells, 4.2 ± 1.1% B-cells, 0% NK cells, and 7.5 ± 1.2% monocytes-macrophages.

Metabolic Labeling. Cell lines and freshly processed tissue samples were cultured in medium containing 10 μCi/ml of D-[6-3H]glucosamine hydrochloride (specific activity, 45 Ci/mmol; ICN Pharmaceuticals, Irvine, CA) at 37°C in a humidified 5% CO₂ atmosphere in 75-cm² tissue culture flasks. Cell lines were cultured until they were confluent and harvested by gentle scraping. Freshly processed human tissue samples were cultured for 3 days and harvested by gentle scraping.

In kinetic studies not detailed in this paper, we found that after continuous labeling for 72 h the specific activity of the radiolabeled species reached equilibrium. We found no difference in the relative amounts of radioactivity incorporated in the non- and O-acetylated species of sialic acid from 24-72 h of labeling. In experiments where cells were labeled for only 1 h prior to harvesting, the relative levels of di- and tri-O-acetylated sialic acids were slightly higher than the 72-h labeling.

Purification of Sialic Acids. Sialic acids were purified from human cells and tissues according to a modification of the procedure of Varki and Diaz (27). In brief, biosynthetically labeled sialic acids were released from cells by mild-acid hydrolysis in 3 ml of 2 M acetic acid (pH 2.5) at 80°C for 3 h. After cooling on ice, insoluble material was removed by centrifugation. The clear supernatant fraction was passed through a 1-m1 AG50W-X8 (200-400 mesh, hydrogen form; Bio-Rad, Richmond, CA) cation exchange column and washed with 4 ml of water. The column effluent was lyophilized, resuspended in 3 ml of 0.01 m sodium formate (pH 5.5), and applied to a 1-m1 AG2-X4A (100-200 mesh, formate form; Bio-Rad) anion exchange column equilibrated with 0.5 ml 0.01 m sodium formate (pH 5.5). The column material was washed with 8 ml of 0.01 m formic acid, after which sialic acids were eluted with 10 ml of 1 M formic acid and lyophilized. The lyophilized material was resuspended in water and stored at -20°C before analysis by paper chromatography. Using this procedure, sialic acids were separated from other biosynthetically radiola Beled molecules (such as the biosynthetic precursors glucosamine, N-acetylgalactosamine, and N-acetylgalactosamine) of the basis of the negative change of sialic acids (Fig. 1).

In some cases, another round of mild-acid hydrolysis was performed to measure the percentage of radiolabeled sialic acids resistant to removal. In all cases tested the second round of mild acid hydrolysis yielded less than 1% of the total radiolabeled sialic acids recovered in the first round of hydrolysis. The recovery of biosynthetically labeled sialic acids was assessed by repeating the purification procedure on purified material, yielding 87% of the starting material with no change in the relative percentages of non- and O-acetylated biosynthetically labeled sialic acids.

Paper Chromatographic Identification and Analysis. Biosynthetically labeled sialic acids from normal and malignant human cells were identified by ascending paper chromatographic analysis in a solvent system of n-butanol:n-propanol:0.1 N HCl (1:2:1, v/v) (28). Prior to analysis, Whatmann 3MM paper (Fisher Scientific, Houston, TX) was washed sequentially with 0.1 N hydrochloric acid, distilled water, and chloroform:methanol (2:1, v/v). Carrier Neu5Ac (5 μg) containing 1.5 nCi of [14C]Neu5Ac (specific activity, 244 mCi/mM; Amersham, Arlington Heights, IL) was added to each sample as an internal standard. The chromatogram was developed for 6 h (solvent migration; 20 cm), air-dried at room temperature, and cut sequentially into 0.2-cm strips (1 cm wide) beginning at the origin. Each strip was placed in a 5-ml liquid scintillation vial and soaked in 100 μl of water overnight. Four ml of liquid scintillation cocktail (Formula 963; New England Nuclear, Boston, MA) were added to each vial and assayed for levels of 3H and 14C incorporation. Using this chromatographic system, all biosynthetically radiolabeled carbohydrates other than sialic acids (N-acetylgalactosamine, N-acetylgalactosamine, and glucosamine) had slower rates of migration when compared to the migration of non- and O-acetylated species of sialic acids. Relative percentages of the separated sialic acids were determined by integrating the amount of 3H material in each peak. For each given cell type, zero values indicated the expression of less than 0.1% of the total sialic acids expressed. Nonparametric statistical tests were used to determine the significance of observed differences in the amounts of non- and O-acetylated sialic acid species. Differences were considered statistically significant at P values of less than 0.05.

Synthetic O-Acetylation of Neu5Ac. Paper chromatographic standards of mono-, di-, and tri-O-acetylated sialic acids were prepared by per-O-acetylation of Neu5Ac to form N-acetyl-4,7,9,11-tetra-O-acetylneuraminic acid (Neu5Ac4,7,9,11-acetate) (4,5,7,8,9,11-acetate) (Neu5Ac4,7,9,11-acetate) (Neu5Ac4,7,9,11-acetate) (Neu5Ac4,7,9,11-acetate) (Neu5Ac4,7,9,11-acetate), followed by partial de-O-acetylation with mild acid and base. In brief, 500 μg of Neu5Ac (Sigma) containing 0.5 μCi of [3H]radiolabeled Neu5Ac were resuspended in 200 μl of dry pyridine:acetic anhydride (1:1) and incubated overnight at room temperature. The acetylated material was dried under a stream of nitrogen, resuspended in water, and lyophilized. An aliquot of this material was
treated with 2 N acetic acid at 80°C for 3 h, lyophilized, treated with 0.1 N ammonium hydroxide at 25°C for 1 h, and lyophilized. This partially de-O-acetylated material was resuspended in water and identified by paper chromatography.

FAB-MS Analysis. Cryopreserved nonradio-labeled normal colon tissue (5 × 10⁶ cells) was pressed through a 50-mesh stainless steel screen and washed three times in Hank’s balanced salt solution prior to release and purification of the associated sialic acids as described above. The purified sialic acids were separated by paper chromatography, and material from the region corresponding to the migration of biosynthetically radiolabeled di- and tri-O-acetylated sialic acids (from the same tissue) was eluted in water and lyophilized. Material eluted from the paper directly adjacent to the region described above served as a control. Each sample was reconstituted in glycerol:water (1:1, v/v) and 5 µl were placed on the probe plate. Positive-ion FAB-MS spectra were recorded on a Kratos MSS50 RF mass spectrometer (acceleration voltage, 8 kV) with a 3000 ppm sweep and 3.0-s scan time. The FAB gun used xenon (emission current, 0.15 mA; acceleration voltage, 8 kV). The glycerol served as a matrix for the sample material and an internal standard. Mass equivalents in daltons were determined by standardizing the spectrophotometer with triazine and counting signal peaks representing single mass units. Analysis was confined to the 185-500 region of the mass spectrum, since we were only interested in identifying the monosaccharides released from the human colon cells.

RESULTS

Separation of Synthetic O-Acetylated Sialic Acids by Paper Chromatography. The paper chromatographic migration profile of O-acetylated sialic acids was standardized using synthetic 14C-labeled O-acetylated sialic acids after partial de-O-acetylation of Neu4,5,7,8,9Ac5. In this chromatographic system, sialic acid molecules were separated according to their degree of oxygen substitution (Fig. 1). Fig. 2 represents the migration of these O-acetylated sialic acids expressed as a ratio of their migration divided by the migration of Neu5Ac. The Neu5Ac peak is referred to as 1.0, the mono-O-acetylated sialic acid peak as 1.24, the di-O-acetylated sialic acid peak as 1.40, the tri-O-acetylated sialic acid peak as 1.64, and the tetra-O-acetylated peak as 1.80. The migration of all radiolabeled sialic acid material (Fig. 4; Tables 1–3) was found to vary no more than ±0.02 units from the recorded peak values shown in Fig. 2.

To confirm that the synthetic O-acetylated sialic acids migrating at the 1.64 peak were isomers of tri-O-acetylated sialic acid, sialic acid material was eluted from the region for FAB-MS analysis. From this material, a positive ion molecular weight peak (436 mass units) was identified as the molecular weight peak of tri-O-acetylated sialic acid (data not shown).

FAB-MS Analysis of O-Acetylated Sialic Acids from Normal Colon. Sialic acid material released and purified from unlabeled, cryopreserved human colon cells (NC2) migrating on paper to the region corresponding to the migration of biosynthetically radiolabeled di- and tri-O-acetylated sialic acids from the same tissue (Fig. 3A) was analyzed using FAB-MS. Ion molecular weight peaks generated in the positive-ion mode that were not present in the paper control (paper directly adjacent to the migration of di- and tri-O-substituted sialic acids) are shown in Fig. 3B. By this subtractive analysis, molecular ions of 394 and 436 were the only masses detected, corresponding to the ion molecular weights of di-O-acetylated sialic acids and tri-O-acetylated sialic acids, respectively. The 416 and 458 peaks correspond to di- and tri-O-acetylated sialic acids, respectively, each complexed with a sodium ion, while the 185, 277, and 369 peaks correspond to the di-, tri-, and tetraglycerol peaks, respectively. Unfortunately, salt contamination causing signal suppression limited the quantitative aspect of this analysis.

Sialic Acids on Normal and Malignant Colon Cells. Biosynthetically radiolabeled sialic acids expressed on normal and malignant colon cells were separated and identified by paper chromatography. Table 1 represents the relative expression of non-(1.00), mono- (1.24), di- (1.40), and tri- (1.64) O-acetylated sialic acids on normal colon tissue, fresh "uninvolved" tumor mucosa (the excised end of colon tissue in resections for carcinoma), and fresh and cultured colon carcinomas. The uninvolved tumor mucosa and carcinoma tissue were taken from the same patient. In Fig. 4, paper chromatographic profiles of HC 10700 (A) and NC2 (B) demonstrate that the relative expression of tri-O-acetylated sialic acid (1.64) was high in the fresh normal colon tissue (45.2%) compared with carcinoma of the colon (1.1%). In the case of NC2, the spent medium was analyzed for secreted biosynthetically labeled sialic acids (non-, 22.9%; mono-, 13.1%; di-, 5.4%; and tri-, 58.6%) demonstrating similar expression compared with NC2 tissue (Table 1). The fresh uninvolved colon mucosa tissue expressed a greater proportion of mono-O-acetylated sialic acid compared to other tissues. In one case (HC 9434), the macroscopically normal mucosa resected adjacent to the frank colon carcinoma ("adjacent") was analyzed for the relative amounts of non- and O-acetylated sialic acids (non-, 82.8%; mono-, 13.1%; di-, 3.5%; and tri-, 0.6%). The relative amounts of sialic acids expressed on the adjacent tumor mucosa were similar to the sialic acids expressed on fresh colonic adenocarcinoma (Table 1).

Statistical analysis comparing the tissue types in Table 1 demonstrated a significant difference in the expression of non-, di-, and tri-O-acetylated sialic acids on fresh normal colon
compared with fresh colon carcinoma. A statistical difference was also observed in the expression of mono- and tri-O-acetylated sialic acids on normal colon from patients without tumors compared to uninvolved tumor mucosa; the expression of non- and di-O-acetylated sialic acids was statistically the same in the two groups. Comparison of uninvolved tumor mucosa and colon carcinoma from the same patient demonstrated statistically significant differences in the relative expression of all sialic acids (Table 1), with the greatest differences in the non- and mono-O-acetylated forms. No significant difference was observed in the relative expression of sialic acids on fresh and tissue culture established colon carcinomas.

Silicic Acids on Secondary Colon Tumors. Table 2 represents the relative expression of non-, mono-, di-, and tri-O-acetylated sialic acids expressed on fresh and established secondary (metastatic) hepatic adenocarcinomas and uninvolved liver tissue from the same patient. There was no significant difference in the relative expression of each species of non- and O-acetylated sialic acid on the following tissues: fresh liver metastasis; established metastatic cell lines; uninvolved liver adjacent to tumor; fresh colon carcinoma (Table 1); and established colon carcinoma cell lines (Table 1). The fresh normal colon and uninvolved colon mucosa were significantly different from the rest of the samples in Table 1 and in the whole of Table 2 in the relative expression of all non- and O-acetylated species of sialic acid.

Silicic Acids on Other Human Tissues. The molecular species of biosynthetically radiolabeled sialic acid molecules expressed on a variety of fresh and cultured human cells were also identified by ascending paper chromatography. The relative percentages of non-, mono-, di-, and tri-O-acetylated sialic acids expressed on noncolonic cells are shown in Table 3. This survey demonstrates the presence of O-acetylated sialic acids on human cells with relative differences in the percentages of non- and O-acetylated species of sialic acid found on fresh tissues and established cell lines.

The levels of O-acetylation of sialic acids expressed on peripheral blood mononuclear cells (B- and T-cells and monocytes) and fibroblasts were different from those expressed on normal colon or uninvolved colon tissue (Table 1). However, the levels of O-acetylation of sialic acids expressed on fresh uninvolved (Table 2) and normal liver (Table 3) were similar.

DISCUSSION

The chemical identification of enzymatically modified sialic acids has been limited in the past because of purification, analysis, and labeling techniques that either destroy or fail to measure O-acetylated sialic acids. To avoid these problems, we cultured human established cell lines and fresh tissue samples in the presence of radiolabeled [6-3H]glucosamine and measured biosynthetically radiolabeled sialic acids after separation by paper chromatography, on the basis of the number of O-substitutions on each sialic acid molecule (Fig. 1). By this technique, we have separated and identified varying proportions of non-, mono-, di-, and tri-O-acetylated sialic acids expressed on glycoproteins and glycolipids of human normal and neoplastic colon tissue as well as a variety of other human tissues.

By FAB-MS analysis (Fig. 3), we provide chemical evidence that these human cells express di- and tri-O-acetylated sialic acids. In other studies we have demonstrated that biosynthetically radio-

Table 1 Sialic acids released from normal and malignant colon cells

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Species of sialic acid (%)</th>
<th>Non-OAc</th>
<th>Mono-OAc</th>
<th>Di-OAc</th>
<th>Tri-OAc</th>
</tr>
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<tbody>
<tr>
<td>Fresh normal colon mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NC1</td>
<td></td>
<td>59.1</td>
<td>3.9</td>
<td>3.6</td>
<td>33.4</td>
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<tr>
<td>NC2</td>
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<td>31.2</td>
<td>9.3</td>
<td>14.3</td>
<td>45.2</td>
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<tr>
<td>NC3</td>
<td></td>
<td>65.8</td>
<td>9.9</td>
<td>7.8</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.0 ± 10.6</td>
<td>7.7 ± 1.9</td>
<td>8.6 ± 3.1</td>
<td>31.7 ± 8.3</td>
</tr>
<tr>
<td>Fresh uninvolved colon mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC 7091</td>
<td></td>
<td>68.8</td>
<td>19.0</td>
<td>9.8</td>
<td>2.4</td>
</tr>
<tr>
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<td>64.0</td>
<td>25.1</td>
<td>8.4</td>
<td>2.5</td>
</tr>
<tr>
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<td></td>
<td>66.4</td>
<td>26.1</td>
<td>5.4</td>
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</tr>
<tr>
<td>HC 10139</td>
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<td>63.8</td>
<td>25.6</td>
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<td>69.9</td>
<td>22.0</td>
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<td></td>
<td></td>
<td>66.6 ± 1.2</td>
<td>23.6 ± 1.3</td>
<td>8.0 ± 0.8</td>
<td>1.8 ± 0.3</td>
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<td>Fresh colon adenocarcinoma</td>
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<td>HC 7091 (rectum)</td>
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<td>87.5</td>
<td>9.3</td>
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<tr>
<td>HC 9445 (sigmoid)</td>
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<td>86.2</td>
<td>10.3</td>
<td>2.6</td>
<td>0.9</td>
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<tr>
<td>HC 9434 (sigmoid)</td>
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<td>91.7</td>
<td>6.8</td>
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<td>86.7</td>
<td>11.3</td>
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<td>84.0</td>
<td>12.6</td>
<td>1.3</td>
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<td>87.2 ± 1.3</td>
<td>10.3 ± 1.1</td>
<td>1.9 ± 0.2</td>
<td>0.6 ± 0.3</td>
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<td>Colon adenocarcinoma cell lines</td>
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<td></td>
<td></td>
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<tr>
<td>HT 29 (R)</td>
<td></td>
<td>88.8</td>
<td>8.8</td>
<td>2.3</td>
<td>0.1</td>
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<tr>
<td>HT 29 (F)</td>
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<td>88.4</td>
<td>9.3</td>
<td>2.1</td>
<td>0.2</td>
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<tr>
<td>HC 2998</td>
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<td>94.2</td>
<td>5.6</td>
<td>0</td>
<td>0.2</td>
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<tr>
<td>HC 8589</td>
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<td>91.1</td>
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<td>90.8</td>
<td>8.6</td>
<td>0.6</td>
<td>0</td>
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<tr>
<td>CL 3.5</td>
<td></td>
<td>86.0</td>
<td>11.7</td>
<td>1.0</td>
<td>1.3</td>
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<tr>
<td></td>
<td></td>
<td>89.9 ± 1.1</td>
<td>8.6 ± 0.8</td>
<td>1.3 ± 0.4</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>

*OAc, O-acetylated.

† Migration ratio values relative to the migration of Neu5Ac on Whatman 3MM paper.

‡ Mean ± SE.

§ Anatomical location of adenocarcinoma.
O-acetylated sialic acids from human cells were converted to the non-O-acetylated form by saponification and crude mouse liver esterase treatments. Previous work by Reid et al. (20) provided indirect evidence for the expression of O-acetyl groups on sialic acids on rat colonic epithelial tissue by measuring the production of methyl acetate liberated from O-acetylated sialic acids in sodium methoxide by gas-liquid chromatography.

To estimate patterns and relative amounts of O-acetylated sialic acids, most chemical studies (18–23) were limited to measuring formaldehyde formation after periodate oxidation and colorimetric estimations of sialic acid concentration. Barker et al. (17), however, reported a change in the paper chromatographic mobility of sialic acids from a malignant gastric tumor, suggesting the presence of di-O-acetylated sialic acid. Another study (22) used thin-layer chromatography to separate five types of non- and O-substituted sialic acids from normal and malignant human colon epithelium. These and many other studies (2, 8, 9, 12–16, 29) have suggested that the loss of O-acetylation on sialic acid molecules expressed in human colon is a sensitive indicator of early malignant change, since side chain-substituted sialic acids of normal colonic mucosa (3, 4, 8, 11, 30) are predominantly di- and tri-O-substituted (31). Our data support these findings and demonstrate lower levels of O-acetylation in the colon carcinomas compared to normal colon mucosa (Fig. 4, Table 1) and differences between the ratios of O-acetylated sialic acids expressed on uninvolved tumor mucosa and normal colonic mucosa (Table 1). Furthermore, the relative amount of tri-O-acetylated sialic acid expressed on these tissues appears to be a sensitive indicator of premalignant change (Table 1). Fetal intestinal cells expressed levels of non- and O-acetylated sialic acids similar to colon carcinomas (Table 3). These findings support histochemical studies by Greaves et al. (16) who observed a significant difference in the histochemical staining pattern of O-acetylation of sialic acids in normal, “transitional” mucosa, benign adenomas, and adenocarcinoma of the colon.

The histochemical and chemical patterns of O-acetylated sialic acids on adjacent and uninvolved mucosa appear to be unique when compared to the autochthonous colon carcinoma or normal colon (10, 15). We have found decreased amounts of O-acetylated sialic acids on adjacent and uninvolved tumor mucosa compared to normal mucosa (Table 1). This is in concert with previous histochemical analysis (14) that graded the levels of O-acetylated sialic acids on transitional and far tumor mucosa in between carcinomas of the colon and normal control colon mucosa. Our findings and those of others (16, 32, 33) that adjacent and uninvolved tumor mucosa express abnormal patterns of O-acetylated sialic acids provide evidence of a premalignant field defect, rather than a local secondary effect of tumor growth. Reduced levels of O-acetylation in cases of ulcerative colitis and Crohn's disease (34) support this suggestion.

When the relative amounts of each species of sialic acid were compared between colon carcinoma and uninvolved mucosa in the same patient, significant differences were observed (Table 1). However, this same analysis on hepatic metastasis derived from a primary colon adenocarcinoma and uninvolved liver tissue from the same patient demonstrated little difference in the expression of each species of sialic acid (Table 2). Furthermore, no significant difference was observed in the expression of non- and O-acetylated sialic acids between primary adenocarcinomas of the colon and liver metastasis derived from a primary colon adenocarcinoma (Tables 1 and 2). These findings

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**Table 2 Sialic acids released from secondary colon tumors**

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Specie of sialic acid (%) of total sialic acids</th>
<th>Non-OAc</th>
<th>Mono-OAc</th>
<th>Di-OAc</th>
<th>Tri-OAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh uninvolved liver</td>
<td>HC 7617</td>
<td>85.0</td>
<td>10.3</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Fresh hepatic metastasis (from sigmoid colon)</td>
<td>HC 7617</td>
<td>87.9</td>
<td>8.2</td>
<td>2.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Metastatic cell lines (from colon primary)</td>
<td>Ht 29 (Lung)</td>
<td>83.2</td>
<td>14.1</td>
<td>2.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Ht 29 (Liver)</td>
<td>91.5</td>
<td>8.1</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hc 1410 (Liver)</td>
<td>89.3</td>
<td>9.7</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hc 1544 (Liver)</td>
<td>90.6</td>
<td>9.4</td>
<td>0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

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*OAc, O-acetylated.*

3-MM paper.

Mean ± SE.

Anatomical location of metastatic lesion.
support earlier work by Gad (35) that showed that mucosubstance found in the secondary deposits in lymph nodes were similar to the main type of mucosubstance in the primary tumor as well as later work (36) histochemically demonstrating the expression of O-acetylated sialic acids in cases of gallbladder, pancreatic, and gastric cancer.

The relationship of total cellular sialic acid levels in normal, malignant, and metastatic tissues has been a long-standing observation detailed in histochemical analysis (10, 29, 32) and biochemical studies (37). In particular, cell surface sialic acid has been shown to be positively correlated with metastatic potential in animal models (38, 39). Alternatively, the increase in sialic acid levels, as detected by colorimetric assays, may be a function of the modulation of O-acetylated sialic acid species to the non-O-acetylated form (Neu5Ac). It is quite apparent that sialic acids may play an important role in antigen masking, glycoconjugate turnover, and cellular differentiation in many biological systems (1). The ability to identify abnormal ratios of O-acetylated sialic acids represents a possible diagnostic tool to evaluate patients who are genetically and culturally predisposed to the development of adenocarcinoma of the colon. However, the regulation and functional significance of O-acetylation on the sialic acid molecule remains to be determined.

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Distribution of Mono-, Di-, and Tri-O-Acetylated Sialic Acids in Normal and Neoplastic Colon

Jeff T. Hutchins, Christopher L. Reading, Raffaella Giavazzi, et al.


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