ABSTRACT

Sulfated macromolecules synthesized in tumor and mucosa tissues derived from colorectal cancer patients were labeled with $[^{35}S]$sulfate and separated into two fractions on DEAE-Sephaclone: the slightly acidic peak (peak I) was eluted with 0.2 M NaCl and the highly acidic peak (peak II) was eluted with 0.5 M NaCl. A total of 40 specimens, which included primary colon cancer, liver metastases, and normal mucosa obtained at surgery (16 patients), were examined regarding the amount of peak I and peak II. The amount of peak I significantly decreased in the order of normal mucosa > primary tumors > metastases, while the amount of peak II did not significantly change among the tissues. Peak I was mostly resistant to chondroitinase ABC and nitrous acid treatment under acidic conditions, whereas combined chondroitinase-sensitive materials and nitrous acid-sensitive materials were greater than 80% of the radioactivity in peak II. The major radioactive component of peak I migrated at a position corresponding to $M_r > 300,000$ by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and became $M_r < 40,000$ after alkaline borohydride treatment. The major component of peak I was likely to be a sulfated glycoprotein containing sulfate groups on alkaline labile carbohydrate chains. Peak II consisted of a mixture of heparan sulfate proteoglycans and chondroitin sulfate proteoglycans. Differential incorporation of $[^{35}S]$sulfate into peak I among normal mucosa, primary colon carcinoma, and colon carcinoma metastasis was observed. Therefore, decreased peak I production may be a biochemical change associated with colorectal cancer progression and metastasis.

INTRODUCTION

The prognosis for cancer is often directly related to presence or absence of metastases. This is apparently true with colorectal cancer in which the 5-year survival rate is greater than 60% for patients with localized disease (Dukes' stage B) and smaller than 5% for those with disseminated metastases (Dukes' stage D) (1, 2). Dukes' classification is a good indicator of survival (3, 4). However, stage of disease is not sufficient for prediction of recurrence or development of metastasis in individual patients, especially in those without apparent metastases at the time of surgical removal of primary colorectal tumors. Search for a specific marker for those tumor cells most likely to metastasize may lead to the development of a new prognostic indicator.

Recent work in experimental animals has shown that tumor cell subpopulations with different metastatic potentials may exist within the same primary tumor (5–7). In these experimental systems, metastases appeared to result from the selective growth of subpopulations with a higher tendency to metastasize (6, 7). These highly metastatic cells possess a variety of specific biochemical properties, e.g., differences in cell surface glycoproteins (8–10), differences in cell adhesiveness (11, 12), presence of specific enzymes for the degradation of basement membranes, and extracellular constituents (11–14). However, little is known about biochemical properties that are associated with human colorectal carcinoma cells having higher metastatic potentials. We are interested in examining whether biochemical changes known to occur on cell surface and extracellular molecules during malignant transformation also influence the metastatic potential of colorectal cancer. We have already shown that the expression of fucosylated high $M_r$ glycoproteins tends to decrease upon progression of carcinomas in the distal colon and rectum (15). There are quantitative and qualitative changes in the expression of acidic glycoconjugate components in colon cancer. For example, Felipe (16) histochemically demonstrated a decrease of sulfomucins and an increase in sialylated mucins in colon tumors as well as in transitional mucosa. Gold and Miller (17) suggested that a mucin-like molecule purified from colon tumors has a lower density of carbohydrate moieties on the peptide core than those from normal mucosa. However, little information is available on sulfated glycoconjugates concerning the differential expression between primary colon carcinoma and metastasis and the chemical properties. Izoz et al. showed that glycosaminoglycans isolated from colon tumors contain higher amounts of chondroitin sulfate than those from normal mucosa (18) and that colon carcinoma cells release a factor that stimulates fibroblasts to produce chondroitin sulfate (19). The involvement of these molecules in determining metastatic potentials was not known. Furthermore, little biochemical or functional information was available regarding sulfated glycoproteins of colorectal tumors.

The purpose of this study was to examine the differences among normal mucosa, primary colon tumors, and metastases for the expression of acidic substances metabolically labeled by $[^{35}S]$sulfate. The results indicate that the three tissues are significantly different in the production of slightly acidic $^{35}S$-sulfated, large $M_r$ components, presumably sulfated mucin-like glycoproteins.

MATERIALS AND METHODS

Tissues Studied. Tissues were obtained from patients undergoing surgery at M. D. Anderson Hospital and Tumor Institute, Houston, TX. Sixteen colon cancer patients were selected for this study. Those who had undergone previous radiotherapy or chemotherapy or those who had a previous colon carcinoma were excluded. Fourteen colorectal adenocarcinomas, four liver metastases, one lymph node metastasis, and 14 normal mucosa specimens were studied.

Processing of Tissues. Tumor specimens of approximately 0.5–1 g were obtained from the luminal edge of colorectal tumors (superficial regions). In some cases, tumor specimens were also obtained from areas of bowel wall invasion (deep regions). Liver metastases and lymph node metastases were also obtained when available. Necrotic portions of the tumors were excluded. Normal mucosa was obtained at approximately 5 cm from the primary carcinoma. All tissues were processed immediately. One hundred milligrams of the tissue were rinsed with DPBS.

The abbreviations used are: DPBS, Dulbecco's phosphate buffered saline; $M_r$, relative molecular mass; NaDodeSO$_4$, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]1-propanesulfonate.
containing 50 units/ml of penicillin, 50 μg streptomycin, and 1.25 μg/ml of amphotericin B, minced with scalpel blade into small pieces, and incubated in 1 ml of a half-and-half mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium containing 10% fetal bovine serum, 50 units/ml of penicillin, 50 μg/ml streptomycin, 1.25 μg/ml of amphotericin B, and 50 μCi/ml of [35S]Na2SO4 under humidified conditions with 5% CO2 at 37°C for 48 h. The tissue was removed by centrifugation and subjected to the following procedures or stored at −70°C until use.

Extraction of 35S-Sulfated Materials. 35S-Sulfated materials were extracted according to the method described by Kimura et al. (20), with slight modifications. The tissue incubated with [35S]Na2SO4 was mixed with 1 ml of 4 M guanidine hydrochloride containing 4% Zwittergent 3-12 (Calbiochem, La Jolla, CA), 0.1 M sodium acetate (pH 6.0), and protease inhibitors: 10 mM EDTA, 10 mM benzamidine, 25 mM E-aminoacapric acid, 5 mM phenylmethylsulfonyl fluoride, 10 μg/ml of N-tosyl-L-phenylalanine chloromethyl ketone, 10 μg/ml of N-acetyl-p-tosyl-L-lysine chloromethyl ketone, 20 μM/ml of aprotinin, and 2 μM/ml of leupeptin. After ultrasonication on ice for 10 s (Heat System Ultrasonic Cell Disruptor; Branson Sonic Power Co., Plainview, NY), the mixture was incubated on ice for 18 h. Supernatant was collected by centrifugation at 13,000 × g for 10 min and applied to a Sephadex PD-10 column (Pharmacia, Piscataway, NJ) equilibrated with 50 mM sodium acetate buffer (pH 6.0) containing 50 mM NaCl, 8 mM urea, 1 mM phenylmethylsulfonyl fluoride, and 0.02 U/ml of aprotinin and eluted with the same buffer. Each 0.1 ml fraction was collected, and radioactivity was measured after small aliquots of each fraction were mixed with Liquiscint (National Diagnostics, Sommerville, NJ). Radioactive materials eluted at the void volume fractions were pooled and used for further analysis.

Fractionation of 35S-Sulfated Materials by DEAE-Sephacel Ion-Exchange Chromatography. For the characterization of 35S-sulfated materials, a 50 μl aliquot of tissue extract was diluted with 450 μl of 50 mM sodium acetate buffer (pH 6.0), 50 mM NaCl in 8 mM urea, and 0.2% CHAPS (buffer A), then applied to an analytical DEAE-Sephacel column (0.6 × 2 cm) equilibrated with the same buffer. The column was washed with about 5 ml of buffer A, and the 35S-sulfated molecules were eluted first with 4 ml of 0.1 M sodium acetate buffer (pH 6.0), 0.2 M NaCl in 8 mM urea, and 0.2% CHAPS (buffer B), and second with 4 ml of 0.23 M acetate buffer (pH 6.0), 0.5 M NaCl in 8 mM urea, and 0.2% CHAPS (buffer C). Radioactivity in each 0.5 ml effluent fraction was measured. Preparative chromatography was also carried out, in some cases with a larger size DEAE-Sephacel column (1.1 × 3.5 cm). The fractions eluted with buffers B and C were respectively pooled, dialyzed against distilled water, and lyophilized. The samples were redissolved in 0.5 ml of distilled water and subjected to the following analyses.

Characterization of 35S-Sulfated Materials. For chondroitinase ABC (ICN Biochemicals, Irvine, CA) treatment, 60 μl of sample solution was mixed with 20 μl of enzyme solution (10 U/ml dissolved in water) and 20 μl of 0.25 M Tris-HCl (pH 8.0) containing 0.3 μM sodium acetate, 0.25 M NaCl, 0.05% bovine serum albumin, and 0.1% sodium azide and incubated at 37°C for 18 h. The incubation mixture was then mixed with 100 μl of buffer A and applied to an analytical Bio-Gel P-10 column (0.7 × 20 cm) equilibrated with the same buffer. Radioactivity in each 0.5 ml effluent fraction was measured. Percentage of degradation was calculated based on the increase of radioactivity eluted at the inclusion volume compared to the control incubation without chondroitinase ABC. Nitrous acid deamination under acidic conditions was carried out according to Shively and Conrad (21). A sample solution (60 μl) was mixed with freshly prepared 1 M nitrous acid (40 μl) and incubated at 25°C for 1 h. The reaction mixture was analyzed by gel filtration on Bio-Gel P-10 as described above. Some of the 35S-sulfated materials were subjected to alkaline borohydride degradation. Lyophilized samples containing 3000 cpm were dissolved in a 100 μl solution of 0.1 M NaOH and 0.3 M NaBH4 incubated for 48 h at 25°C. The excess borohydride was destroyed by neutralization with 20 μl of 2 M acetic acid, and the sample was diluted with 100 μl of buffer A, then applied to an analytical column (0.7 × 20 cm) of Sepharose CL-6B equilibrated with buffer A. Radioactivity in each 0.5 ml effluent fraction was measured. An aliquot of neutralized samples, each containing a radioactivity of 2000 cpm, were mixed with a half volume of 6% NaDodSO4, 9% 2-mercaptoethanol, 1.5 mM EDTA, 30% glycerin, 187.5 mM Tris-HCl buffer (pH 6.8) and then analyzed by NaDodSO4-PAGE. NaDodSO4-PAGE was carried out according to Laemmli (22) on 7.5% gels, as described previously (9). 35S-Sulfated materials were treated with 10 mg/ml of either trypsin (Sigma, St. Louis, MO) or pronase (Calbiochem) in 0.05 M Tris-HCl (pH 8.0), containing 0.01 M calcium chloride, and 0.02% sodium azide. The trypsin digestion mixture (100 μl) was incubated at 37°C for 24 h. The pronase digestion mixture was incubated under identical conditions except that the temperature was at 50°C. For neuraminidase treatment, sample was incubated at 37°C for 1 h in 100 μl of 25 mM acetate buffer (pH 5.5) containing 0.1 U/ml of neuraminidase from Vibrio cholerae (Calbiochem) and 1 mM calcium chloride. The mixture was subsequently diluted with 10 times volume of buffer A and applied to an analytical DEAE-Sephasil column, then analyzed as described above. The neuraminidase digestion mixture was also analyzed by NaDodSO4-PAGE. Protein was determined by the method of Lowry (23) with bovine serum albumin as a standard. Neutral sugar was determined according to Dubois et al. (24) using glucose as a standard. Sulfate was determined by Silverstein's method (25).

RESULTS
Analysis of 35S-Sulfated Materials by DEAE-Sephasil Anion-Exchange Chromatography. 35S-Sulfated macromolecules produced by colon cancer and normal colonic mucosa were applied to DEAE-Sephasil chromatography after solubilization. Preliminary experiments using gradient elution demonstrated that sulfated materials from colonic tissues were found to elute as two major peaks at approximate sodium chloride concentrations of 0.15 and 0.35 M. Therefore, we obtained these fractions by stepwise NaCl elutions (0.2 M and 0.5 M NaCl). Typical elution profiles are shown in Fig. 1. Radioactivity eluted in the pass through fractions (before peak I) varied from one specimen to another, but all the radioactivity eluted in the pass through fraction had small molecular weight. This material was probably derived from incomplete separation of free 35S-sulfate during the PD-10 column chromatography of the previous step. We therefore compared the ratio of peak II to peak I (peak II/I ratio) in normal colonic mucosa, colon tumors, and metastases. Fourteen normal mucosa, 14 primary colorectal tumors, four liver metastases, and one lymph node metastasis were subjected to this analysis. As shown in Fig. 2, the peak II/I ratio of the...
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Fig. 2. Comparison of peak II/I ratio among normal mucosa, primary tumors, and metastases. The incorporation of $^{35}$S-sulfate to peak I (A) and peak II (B) was determined based on the protein amount that was recovered in the PD-10 void volume. Spots, single specimens; bars, mean values.

Table 1  Significance of the change in peak II/I ratio among normal mucosa, primary tumors, and metastases

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No.</th>
<th>2rank*</th>
<th>Mean rank*</th>
<th>H#</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mucosa</td>
<td>14</td>
<td>147</td>
<td>10.5</td>
<td>15.191</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>14</td>
<td>267</td>
<td>19.071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>5</td>
<td>147</td>
<td>29.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The statistical analysis was based on the rank order of peak II/I ratio.

# Kruskal-Wallis test.

* Estimated from $\chi^2$ table.

We further examined intratumoral regional differences of the peak II/I ratio, i.e., differences among superficial portion representing the growing luminal edge of tumors and deep portion representing the invasive edge of tumors. Each peak II/I ratio of the specimen obtained from the superficial portion of the tumor was significantly lower than that in the corresponding deep portion of the tumor (Fig. 4).

Characterization of $^{35}$S-Sulfated Materials. Peak I and peak II obtained from normal mucosa and primary colon tumor of one 62-year-old male patient were analyzed by NaDodSO₄-PAGE. He had an adenocarcinoma of right colon classified as stage D. The tumor specimen was obtained from the superficial portion of the tumor; the normal mucosa was obtained at approximately 5 cm from the tumor. Fig. 5 shows that the major components of peak I from normal mucosa and those from primary tumor were observed in the similar high molecular weight region (> $M_r$ 300,000). The major components of peak II were also observed in a high molecular weight region. There was no difference in the migration profiles between normal mucosa and primary tumor.

Fig. 4. A comparison of the peak II/I ratio between superficial tumors and deep tumors. Peak II/I ratio was significantly lower in the superficial portions of primary tumors than in the deep portions (P < 0.05 by Wilcoxon signed-rank test).

Table 2  Significance of the differential production of peak I and peak II among normal mucosa, primary tumors, and metastases

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No.</th>
<th>2rank*</th>
<th>Mean rank*</th>
<th>H#</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak I</td>
<td></td>
<td></td>
<td></td>
<td>10.892</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Normal mucosa</td>
<td>14</td>
<td>325</td>
<td>23.214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary tumor</td>
<td>14</td>
<td>191</td>
<td>13.643</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>5</td>
<td>45</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The statistical analysis was based on the rank order of production of peak I or peak II (cpm/mg protein).

# Kruskal-Wallis test.

* Estimated from $\chi^2$ table.

* Not significant.

Fig. 5. Comparisons of the production of peak I or peak II among normal mucosa, primary tumors, and metastases. The incorporation of $^{35}$S-sulfate to peak II obtained from normal mucosa and primary colon tumor of one 62-year-old male patient were analyzed by NaDodSO₄-PAGE. He had an adenocarcinoma of right colon classified as stage D. The tumor specimen was obtained from the superficial portion of the tumor; the normal mucosa was obtained at approximately 5 cm from the tumor. Fig. 5 shows that the major components of peak I from normal mucosa and those from primary tumor were observed in the similar high molecular weight region (> $M_r$ 300,000). The major components of peak II were also observed in a high molecular weight region. There was no difference in the migration profiles between normal mucosa and primary tumor.

Peak I and peak II from the tissues obtained from six patients were subjected to chondroitinase ABC and nitrous acid deamination under acidic conditions (Table 3). The results of these degradation experiments revealed that the major components of peak II were heparan sulfate proteoglycan and chondroitin sulfate proteoglycan. The percentage of these two components fluctuated, and the values from normal mucosa and primary colon tumors were not significantly different. Superficial portions of the primary tumors and the corresponding deep portions did not show significant differences in the composition of
peak II materials. The major portions of peak I of all six patients were resistant to these degradative reactions. The amount of heparan sulfate proteoglycan estimated by nitrous acid deamination reaction in peak I was approximately 10% on an average, and the amount of chondroitin sulfate proteoglycan estimated by chondroitinase ABC was less than 5%. Therefore, heparan sulfate proteoglycan and chondroitin sulfate proteoglycan are apparently minor components of peak I.

Further Biochemical Analysis of Peak I. Since the major part of peak I was resistant to nitrous acid deaminative cleavage and chondroitinase ABC treatment, peak I fractions obtained from some of the specimens were subjected to alkaline borohydride treatment, and one of the peak I fractions was further treated with trypsin, pronase, or neuraminidase. The reaction mixtures were then analyzed by NaDodSO₄-PAGE and/or column chromatography on Sepharose CL-6B or DEAE-Sephacel. The major component of peak I migrated at a high molecular weight region in NaDodSO₄-PAGE as described above (Fig. 5), and also eluted at the void volume fractions of the Sepharose CL-6B analytical column (Fig. 6). Alkaline borohydride treatment, which is supposed to cleave glycoside linkages between carbohydrates and serine or threonine of the polypeptide backbone of peak I, resulted in complete degradation of the high molecular weight major component (Figs. 6a and 7). Very little change was observed in the Sepharose CL-6B elution profile after trypsin treatment (Fig. 6b). Pronase reduced the size of the major component (Fig. 6c), but the elution profile indicated that the degradation was not as intensive as with alkaline borohydride treatment. Neuraminidase treatment did not affect the elution profile of the peak I on DEAE-Sephacel column eluted with 0.05, 0.2, and 0.5 M NaCl, respectively, nor did it affect the electrophoretic mobility of the major components of peak I on 7.5% gels (data not shown). These data suggest that the major radioactive components (>85%) of this peak consisted of high $M_r$ glycoproteins possessing sulfate groups on their alkaline labile residues. Preliminary compositional analysis indicated that peak I from normal mucosa contains sulfate (as $SO_4$) at a weight ratio 1:4.76 to protein, and 1:4.43 to total neutral sugar. This latter value is very similar to the data by Nemoto and Yoshizawa on the analysis of sulfated glycopeptide isolate from rabbit intestinal mucosa (27). We are currently investigating the purification and biochemical characterization of the sulfomucin molecule from normal colonic mucosa and colorectal carcinoma.

### Table 3 Chemical and enzymatic analysis of $^{35}$S labeled materials

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type of tissue</th>
<th>Peak I</th>
<th>Peak II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HSPG (%)</td>
<td>CSPG (%)</td>
</tr>
<tr>
<td>A</td>
<td>Normal mucosa</td>
<td>11.2</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (superficial portion)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (deep portion)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>B</td>
<td>Liver metastasis</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>C</td>
<td>Normal mucosa</td>
<td>2.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (superficial portion)</td>
<td>22.4</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (deep portion)</td>
<td>32.8</td>
<td>0.2</td>
</tr>
<tr>
<td>D</td>
<td>Primary tumor (superficial portion)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (deep portion)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>E</td>
<td>Normal mucosa</td>
<td>11.8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (superficial portion)</td>
<td>2.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (deep portion)</td>
<td>6.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymph node metastasis</td>
<td>5.9</td>
<td>3.0</td>
</tr>
<tr>
<td>F</td>
<td>Normal mucosa</td>
<td>4.2</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Primary tumor (superficial portion)</td>
<td>8.6</td>
<td>0</td>
</tr>
</tbody>
</table>

| Mean of normal mucosa | 7.5 ± 4.7<sup>a</sup> | 2.8 ± 2.7 | 37.3 ± 19.2 | 47.3 ± 11.4 |
| Mean of primary tumor | 14.5 ± 12.7<sup>a</sup> | 0.6 ± 1.1 | 40.1 ± 17.4 | 54.2 ± 20.5 |

<sup>a</sup> HSPG, heparan sulfate proteoglycan. Determined on the degradation by nitrous acid deamination.

<sup>b</sup> CSPG, chondroitin sulfate proteoglycan. Determined on the degradation by chondroitinase ABC.

<sup>c</sup> NT, not tested because of a low yield of purified $^{35}$S labeled materials.

<sup>d</sup> Mean ± SD.
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DISCUSSION

By using DEAE-Sephadex ion-exchange chromatography, we have separated $^{35}$S-sulfated macromolecules produced by normal colonic mucosa, primary colon tumors, and metastases into two major constituents (peak I and peak II) and demonstrated that the amount of peak I significantly decreases in the following order: normal mucosa > primary tumors > metastases, while the amount of peak II does not significantly change. Sulfated glycoproteins have been isolated from colonic mucosa (26–28), and these components may represent a class of compounds that is histochemically defined as sulfomucins (16). Mucins are operationally defined as large molecular weight and highly glycosylated glycoproteins. They usually contain a high content of threonine and serine, to which carbohydrate chains consisting of galactose, glucosamine, galactosamine, fucose, and sialic acid are attached. The sulfated compounds in peak I, in the present study, were mostly resistant to chondroitinase ABC and nitrous acid treatment, suggesting that these glycosaminoglycans are minor components of this peak. The major component of peak I had a large molecular weight ($M_r > 300,000$), and the $^{35}$S-sulfate-labeled component was converted into small molecules by alkaline borohydride reduction. In addition, the component was partially digested with pronase but barely digested by trypsin. The results are similar to those presented by Gold et al. for the sensitivity of human colonic mucins to these enzymes (29). Based on our results, we tentatively concluded that the major component of peak I was a sulfated glycoprotein likely to be identical to the one sometimes called as a sulfomucin. The molecule was known to be abundant in normal colonic mucosa but lower in colon carcinoma (17, 30–32). For example, Filipe (16) showed a decrease of sulfomucin in normal mucosa adjacent to colon carcinoma (transitional mucosa). Reduction of sulfomucin was also observed as an early change seen during chemical carcinogenesis in the colons of experimental animals (32). These observations suggested that a decrease in sulfomucins was associated with malignant transformation of colonic epithelial cells. A decrease in peak I in primary tumor compared with normal mucosa appeared consistent with these previous histological observations, provided that peak I represents sulfomucin.

It is not easy to definitely determine whether the sulfation groups are linked to the carbohydrate chains, but the following preliminary observation strongly suggested that they are. We prepared peak I which was metabolically double-labeled with $^{[35]}$S-sulfate and either $^{[3]H}$serine or $^{[3]H}$glucosamine. They were degraded by alkaline borohydride as described in “Materials and Methods,” and desalted by gel filtration on Bio-Gel P-2. The radioactive sulfate passed through CM-cellulose columns under the condition where $^{[3]H}$serine-labeled material was completely absorbed. On the contrary, more than 90% of $^{[3]H}$glucosamine-labeled material passed through the columns. Therefore, the sulfate groups were not associated with the protein portion of the molecule. Furthermore, the peak of $^{[35]}$S sulfate was associated with $^{[3]H}$glucosamine when the peak passed through fraction from CM-cellulose column was loaded on DEAE-cellulose column and eluted with a concentration gradient of sodium chloride solution. Details in the characterization of sulfated carbohydrate chains will be published elsewhere.

We have shown that the amount of peak I in metastases is lower than that in primary colon tumors. Although the difference was statistically significant, peak I production by some of the primary tumors were as low as those by metastases. This may be due to the difference in the degree of intratumoral heterogeneity in the sulfomucin production. Recent work on metastases strongly suggested that the primary tumor consisted of cell populations with different metastatic potentials and that metastases result from the selective growth of those subpopulations having higher metastatic potentials (6, 7). For colon cancer, considerable evidence now exists that the primary colon tumors of mouse (33–35) and human (36, 37) consist of heterogeneous cell populations with different metastatic potentials.
It is conceivable that the highly metastatic cells in the primary colon tumor might be those cells that produce lesser amounts of peak I materials. The possibility, however, that peak I production was halted or lost during metastasis or that peak I production is modulated by the surrounding microenvironment at the secondary sites, cannot be excluded. There was a significant difference in peak II/1 ratio between superficial and deep portions of primary tumors. It will be interesting to assess whether these differences are related to invasiveness or desmplasia of the carcinoma at the deeper portions of the tumors.

The mechanism(s) governing the decrease in peak I is/are not known at the present time. Histochemically, a decrease in sulfation of peak II have been accompanied by an increase in sialomucins (16, 30–32). Filipe and Cooke (38) demonstrated increased amounts of hexosamine and sialic acid in transitional mucosa adjacent to carcinoma. However, these observations were not substantiated at the molecular level. We have previously reported that the amount of a Ulex europeus agglutinin I-reactive high molecular weight glycoprotein is higher in carcinoma of the rectum and sigmoid colon than in normal mucosa and is also different among the carcinoma tissues derived from colon cancer patients at different stages (15). Reactivity of various lectins with different specificity to the high Mr region varied, suggesting an alteration in the glycosylation process. Similar mechanisms may explain the increased sulfation of peak I. Wesley et al. (39) indicated that the polypeptide structure of intestinal fucosylated mucin and sialylated mucin are different. Sulfated mucin might represent another class of intestinal mucin, and the change in peak I may have resulted from changes independent of alterations in sialylated or fucosylated glycoproteins and may be attributed to decreased numbers of copies of whole sulfated mucin molecules.

In contrast to peak I, the amount of peak II was not significantly different among samples of normal mucosa, primary tumors, and metastases. Biochemical analyses of peak II revealed that the major components of peak II are heparan sulfate proteoglycan and chondroitin sulfate proteoglycan. Proteoglycans are a family of cell and extracellular matrix-associated molecules that are involved in cell adhesion and other cellular interactions (40). Changes in proteoglycans were suggested to be related to malignant transformation, progression, and metastasis (41). In the present study, however, neither the content of heparan sulfate nor chondroitin sulfate was significantly different between normal mucosa and primary tumors. Although the content of chondroitin sulfate in metastases tended to be higher than that in normal mucosa and primary tumors, available data did not permit us to assess the significance of this change. However, Ioizzo et al. (18) found a considerable increase in chondroitin sulfate content in human colon carcinoma when compared with normal colon. This discrepancy might be due to the differences in sample preparations.

In conclusion, this study has demonstrated for the first time the quantitative changes in slightly acidic sulfated macromolecules (peak I on DEAE-cellulose ion-exchange chromatography) among normal colonic mucosa, primary colon tumors, and metastases. The major component of this peak I is likely to be a mucin-like sulfated glycoprotein and the amount of peak I could be a potential indicator for the prognosis of colon cancer. It will be important to identify the molecular structure of the sulfated component and identify the molecular basis for its differential expression.

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We thank Dr. Garth L. Nicolson for suggestions and encouragement on this work; Dr. Peter A Steck for helping us to obtain preliminary data related to this study; Ms. Carolyn P. Cooke and Deborah A. Carlson for their technical assistance; Ms. Eleanor Felonia and Susan Lyman for their help in the preparation of this manuscript; and Ms. Tania Busch for her help in the preparation of illustrations.

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SULFATED GLYCOPROTEINS IN COLORECTAL CANCER METASTASIS


Differential Production of High Molecular Weight Sulfated Glycoproteins in Normal Colonic Mucosa, Primary Colon Carcinoma, and Metastases
