Sulfated Lewis X Determinants as a Major Structural Motif in Glycans from LS174T-HM7 Human Colon Carcinoma Mucin*

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This article describes oligosaccharide structures of mucin isolated from nude mouse xenograft tumors produced by LS174T-HM7 cells, a subline of the human colon carcinoma LS174T with higher metastatic tendency and higher mucin production. A striking feature of the oligosaccharides of the LS174T-HM7 xenograft tumor mucin was a predominance of sulfated Lewis X determinants: HS\textsubscript{0}Gal\textsubscript{2}GlcNAc. In addition to one previously known saccharide with one sulfated Lewis X determinant, the HM7 xenograft tumor mucin contained multiple novel structures containing one, two, or three sulfated Lewis X determinants. This determinant, known to act as a selectin ligand, has been found previously in minor saccharide components of human milk as well as mucins, but never before as a predominant structure in one mucin source.

Mucins are large glycoproteins containing serine- and/or threonine-rich domains with multiple O-linked glycans, and occur as soluble secreted and membrane-bound forms. As major components of the mucous coat, mucins are thought to have a mechanical protective role on mucosal surfaces. They may also have more specific activities, such as regulation of solute transport or providing attachment sites for commensal and pathogenic microbes, and in endothelial cells a mucin may form attachment sites for targeting of leukocytes (1–4). Many epithelial cancers produce mucins. Biochemical and immunohistochemical studies indicate that well to moderately differentiated adenocarcinoma of the colon and cell lines derived from them synthesize and secrete varying amounts of mucins (5–9). In animal models, human colon carcinoma cells with high mucin production were found to have more liver metastasizing activity than those with low mucin production (10). When the high mucin variant colon cancer cells were treated with O-glycosylation inhibitor benzyl-α-N-acetylgalactosamine, marked reduction in liver metastasis was observed.

Saccharide structures are frequently altered in cancer cells compared with the corresponding normal cell (11, 12). Numerous carbohydrate epitopes detected by monoclonal antibodies or lectins have been studied with the hope of finding specific tumor cell markers (13). Some have become useful in diagnostic procedures (14) or promising for antibody-mediated tumor imaging and drug targeting (14, 15). However, only a few studies have characterized the carcinoma mucin saccharides at the chemical structural level (5, 16), undoubtedly due to the relative scarcity of such mucins.

Here we describe the structural analysis of saccharides from mucin produced by nude mouse xenografts of the human colon carcinoma LS174T-HM7: a subline of LS174T cells that produces more mucin (6) and more metastasis than the parent cell line (10, 17). To our surprise, we found that this mucin had an abundance of an otherwise relatively unusual structural motif (the sulfated Lewis X determinant) that is not detected by the available lectins or monoclonal antibodies.

** MATERIALS AND METHODS

Cell Line, Tumor Xenografts, and Mucin Purification—LS174T-HM7 cells (6) were grown as subcutaneous xenografts in nude mice (18). Mucin was purified and quantitated essentially as described previously for mucin from xenografts of the parent LS174T cell line (18).

Release of Oligosaccharide Alditols from Mucin by Alkaline Borohydride Treatment—The colonic mucina were subjected to β-elimination under reductive conditions (0.1 M KOH, 1 M KBH\textsubscript{4}) for 24 h at 45 °C (19). The reaction was stopped by addition of ion exchange resin (Dowex 50WX8, H\textsuperscript{+} form) until pH reached 5.0. After filtration, the filtrate was dried under vacuum and boric acid removed by repeated evaporation with methanol. The mixture of oligosaccharide alditols was purified further by size exclusion chromatography on a column of Bio-Gel P6 (2 × 85 cm, 400 mesh, Bio-Rad) equilibrated with 0.5% acetic acid, and eluted at 15 ml/h at room temperature. The oligosaccharide fractions, detected by UV absorption at 206 nm and eluted at about 165 ml, were pooled for further fractionation and structural analysis.

Separation of Neutral (FN) and Acidic Fractions (FI–III) by Anion Exchange Chromatography—The purified oligosaccharide alditols were fractionated on quartenary amine-bonded silica (20) using a 10-μm Micro-Pak-10 column (50 × 0.8 cm; Varian, Walnut Creek, CA) on a Spectra-Physics model 8700 liquid chromatograph (Spectra-Physics, San Jose, CA) equipped with a LDC variable wavelength detector (Spectro Monitor D, Milton Roy, Riviera Beach, FL) connected to a Spectra-Physics model 4100 computing integrator. The solvents and gradients are given in Fig. 1. Before further analysis, the acidic fraction was desalted by ion exchange chromatography on a column of Bio-Gel P6 (2 × 85 cm, 400 mesh, Bio-Rad) equilibrated with 0.5% acetic acid, and eluted at 15 ml/h at room temperature. The oligosaccharide fractions, detected by UV absorption at 206 nm and eluted at about 165 ml, were pooled for further fractionation and structural analysis.

The abbreviations used are: F, fraction; HPAGEC, high pH anion exchange chromatography; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight mass spectrometry; HPLC, high performance liquid chromatography.
tions, eluted with high salt, were desalted by size exclusion chromatography on a Bio-Gel P2 column (size of column, 200–400-mesh) using water as eluant at a flow rate of 8 ml/h.

Fractionation of Neutral Oligosaccharide Alditol Mixture (FN) on Primary Amine-bonded Silica—The neutral oligosaccharide fraction (FN) was dried, dissolved in 40 μl of initial solvent (acetoni-trile-water, 85:15), and injected onto a 5-μm Sphero-5 column (250 × 46 mm, inner diameter; Brownlee Labs, Santa Clara, CA) equilibrated in initial solvent. The saccharides were eluted with a gradient of decreasing concentrations of acetonitrile in water (Fig. 2). The flow rate was 1 ml/min

Fractionation of the Acidic Oligosaccharide Alditol Mixtures (FI-III) by Anion Exchange Chromatography (HPAEC)—Fractions FI, FII, and FIII obtained after anion exchange chromatography were desalted, dried, and dissolved in 200 μl of water, and then fractionated by HPAEC on Dionex HPLC system equipped with a model PAD2 pulsed amperometric detector (Dionex Corp., Sunnyvale, CA). The neutral oligosaccharide composition of the mucin and oligosaccharide alditol fractions were determined by gas chroma-tography on a silicone OV 101 capillary column (25 m × 0.32 mm) after methanolysis (0.5 M HCl-methanol for 24 h at 80 °C), N-reacylation, and trimethylsilylation as described by Kamering et al. (23) and modified by Montreuil et al. (24).

Matrix-assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF)—Molecular weights of oligosaccharide alditos were measured on a MALDI-TOF instrument (Vision 2000, Finnegan-MAT) equipped with a 337-nm UV laser. The mass spectra were acquired in reflectron mode under 6 kV accelerating voltage and positive or negative ion detection. The sample was dissolved in water at about 100 pmol/μl. Two μl of sample solution was mixed with an equal volume of matrix solution: either 2,5-dihydroxy benzoic acid (10 mg/ml in methanol-water, 80:20) for neutral or monoacetic acid fractions or 2-aminoquinoline (10 mg/ml in acetonitrile-water, 80:20) for the di- and triacidic fractions analyzed in negative ion mode. External calibration was done with angiotensin I (M., 12967.5; Sigma).

Nuclear Magnetic Resonance (NMR) Spectroscopy—Prior to 1H NMR spectroscopic analysis, the oligosaccharide-alditol fractions were exchanged twice by 2H2O at room temperature and pD 6.5. After each exchange (1 h) the materials were lyophylized. Finally each sample was redissolved in 0.5 ml of 2H2O (99.96 atom % 2H, CEA, France). The samples were analyzed at 27 °C on a Bruker DMX-600 600-MHz NMR spectrometer equipped with a triple resonance (1H/13C/15N) self shielded z-gradient probe head. Chemical shifts are expressed in ppm downfield from sodium 4,4-dimethyl-4-silapentanoate sodium salt, but were measured by reference to internal acetone (d = 2.225 ppm). The NMR spectra were interpreted by comparison to spectral data published for a large number O-linked glycans and other relevant saccharides. Overviews are given by Kamering and Vliegenthart (25) and in Sugabase. Specific pertinent references are given under “Results” and “Discussion.”

Permethylolation Analysis—Fraction FII-7 and FII-11 were first desulfated and defucosylated by incubation in 50 μl of 0.1 M NaOH containing varying amounts of NaOAc (Fig. 3). Each fraction collected was immediately neutralized with 30% acetic acid and then desalted on a Bio-Gel P2 column.

Sulfate Analysis—An aliquot of native mucin (50 μg) was hydrolyzed with 1 M HCl for 5 h at 100 °C (21), and the amount of released sulfate was measured by HPAEC as described by Lo-Guidice et al. (22).

Carbohydrate Analysis—The carbohydrate composition of the mucin and oligosaccharide alditol fractions were determined by gas chroma-tography on a silicone OV 101 capillary column (25 m × 0.32 mm) after methanolysis (0.5 M HCl-methanol for 24 h at 80 °C), N-reacylation, and trimethylsilylation as described by Kamerling et al. (23) and modified by Montreuil et al. (24).

RESULTS

Isolation of LS174T-HM7 Mucin and Preparation of Oligosaccharide Alditols—LS174T-HM7 was grown as xenografts under the skin of nude mice. Mucin (22 mg) was isolated by a procedure that included gel filtration followed by CsCl centrifugation and a final purification by gel filtration again (18). The mucin contained 4 mg of protein and about 18 mg of carbohydrate (estimated from hexose content of 6.6 mg). The yield of oligosaccharide alditols obtained by base-borohydride treatment followed by ion exchange chromatography and desalting was 6.5 mg (about 40% of total saccharides). The carbohydrate and sulfate composition of the intact mucin and the oligosaccharide fractions are shown in Table I. The published (18) sulfate content and carbohydrate composition of mucin from the parent LS174T cell line is included for comparison. It is evident that the LS174T-HM7 mucin has relatively more sulfate and less NeuAc. Moreover, the relatively larger amounts of GlcNAc and Fuc suggests on average more complex saccharides in the HM7 mucin compared with the parent cell line.

Overview of Fractionation and Structural Characterization of Oligosaccharide Alditols—The oligosaccharide alditols were initially fractionated by quartenary amine anion exchange chromatography (Fig. 1) into one neutral (FN) and three acidic

| Intact mucin | Released saccharide fractions |
|-------------|-------------------------------|
| LS174T      | LS174T-HM7                    |
| A            | FN  | FI  | FII | FIII |
| Yield (mg)   | NA  | 22  | 6.5 | 1.3 | 0.85 | 0.8 |
| HSO3        | 0.15 | 0.45 | ND  | ND  | ND  | ND |
| NeuAc       | 1.1  | 0.33 | 0.6 | 0.48 | 0.46 | 0.4 |
| Fuc         | 0.26 | 0.67 | 2.3 | 1.0 | 0.85 | 1.5 |
| Gal         | 1.0  | 1.3  | 3.3 | 1.3 | 2.5 | 3.7 |
| GlcNAc      | 0.62 | 0.85 | 2.5 | 1.2 | 0.83 | 1.6 |
| GalNAc-ol   | 1.0  | 1.0  | 1.0 | 1.0 | 1.0 | 1.0 |
| Man         | 0.04 | 0.1  | 0.1 | 0.1 | 0.1 | 0.1 |
| GalNAc      | 1.0  | 1.0  | 0.5 | 0.4 | 0.5 | 0.05 |

a Molar ratios with GalNAc taken as 1.
b Molar ratios with GalNAc-ol taken as 1.
c Data for parent cell line as reported by Byrd et al. (18).
d NA, not applicable.
e ND, not done.
fractions (FI–III). Further fractionation of FN by HPLC on amino-bonded silica (Fig. 2) and FI–III by high pH anion exchange chromatography (Fig. 3, a–c) revealed multiple components. Fractions were collected and subjected to combinations of compositional analysis (Table I and data not shown), MALDI-TOF mass spectrometry (Tables II–IV; Fig. 4), and NMR spectroscopy (Tables V and VI). The following description of the fractions is organized based on structural themes rather than order of isolation by chromatography.

**Nonsulfated Dipentasaccharides**—Structures 1–7 (Table VII) were deduced based on their composition (Tables II and III) and NMR spectra (Table V) being identical to already published structures as given in footnote a of Table VII.

**Sulfated Lewis X Determinants**—All the remaining structures were both fucosylated and sulfated. The NMR analysis (Tables V and VI) indicated that most Fuc residues were linked α1–3 to GlcNAc as in a Lewis X-determinant; the signal from Fuc H-1 was in the range of 5.09–5.14, Fuc H-5 in the range of 4.79–4.82, and Fuc CH₃ at 1.17–1.18 (30, 31). Less prominent signals from Fucα1–4 to GlcNAc, as in a Lewis a determinant, cannot be ruled out, but no other positions of Fuc were evident. The Fuc-CH₃ signal from Fucα1–4GlcNAc (δ = 1.17–1.18 ppm) would overlap with the Fucα1–3GlcNAc, and the H-5 signal (about 5.89 ppm) may be hidden in the downfield slope of the Fuc H-5 of Fucα1–3GlcNAc and/or the HDO peak. However, the Fuc H-1 signal of Fucα1–4GlcNAc (typically found at 5.00–5.05 ppm) should have been easily detected if present. Indeed, a small signal was detected at 5.03 ppm in some of the fractions (e.g. Fig. 6, arrowhead) but not in others (Figs. 7 and 8).

All sulfate, with one exception, was attached to the 3-position of Gal, as evidenced by a characteristic strong downfield shift of the signals from Gal H-3 and Gal H-4 to about 4.32 and 4.27 ppm, respectively (32). It was clear that most Galβ1–4GlcNAc residues would have to have both sulfate and Fuc attached, forming a sulfated Lewis X determinant, simply based on stochiometric considerations. However, additional shifts in the NMR spectra due to cross-interaction of the sulfate and Fuc within this determinant (22, 32) gave further support for this conclusion. The signal from H-4 of the sulfated Gal is particularly diagnostic since it is a tall (small coupling constant) peak in a relatively uncrowded area of the spectrum. In the absence of Fuc on the nearby GlcNAc, this signal is found slightly below 4.29 ppm, whereas, in the presence of Fuc, it is found slightly below 4.27 ppm. H-1 and H-3 of the sulfated Gal are also shifted downfield by about Δδ = 0.02 due to the nearby Fuc, and Fuc H-5 is shifted upfield by Δδ = −0.02 due to the nearby sulfate, but these shifts are sometimes hard to identify because of obscuring peaks and/or other influences on these signals. These effects suggest that sulfate and Fuc are attached to the
TABLE II
Composition analysis and mass spectrometry of purified fractions

| Fraction | m/z | HNa<sup>a</sup> | HS<sub>O</sub><sup>b</sup> | NeuAe | Fuc | Gal | GlcNaC | GalNaC-o
|----------|-----|----------------|----------------|-------|-----|-----|-------|-------|
| FN-1*    | 408 | +Na           | —                | 1     | 1   | 1   | —     | —     |
| FN-2*    | 554 | +Na           | —                | —     | —   | 1   | 1     | —     |
| FN-3     | 757 | —Na           | —                | —     | 1   | 1   | 1     | —     |
| FI-3*    | 537 | +Na           | —                | —     | —   | —   | —     | —     |
| FI-4*    | 699 | +Na           | —                | 1     | 1   | 1   | 1     | —     |
| FI-5<sup>a</sup> | 902 | +Na           | 1                | 1     | 1   | 1   | —     | —     |
| FI-9<sup>b</sup> | 762 | +Na           | 1                | —     | 1   | 1   | 1     | —     |
| FI-10*   | 999 | +Na           | 1                | —     | 1   | 1   | 1     | —     |
| FI-12    | 1,121 | -H         | —                | 1     | 2   | 2   | 1     | —     |
| FI-14    | 1,486 | -H      | 1                | —     | 2   | 3   | 2     | —     |
| FI-15    | 2,590 | +Na         | 2                | —     | 2   | 3   | 2     | —     |
| FIIL-2*  | 988 | -2H+Na      | 2                | —     | 1   | 1   | —     | —     |
| FIIL-5*  | 1,288 | -2H+Na | 1                | 1     | 2   | 1   | 1     | —     |
| FIIL-6*  | 2,099 | -2H+Na | 2                | —     | 3   | 4   | 3     | 1     |
| FIIL-7*  | 2,099 | -2H+Na | 2                | —     | 3   | 4   | 3     | 1     |
| FIIL-11* | 1,953 | -2H+Na | 2                | 2     | 4   | 3   | 1     | —     |
| FIIL-12* | 1,588 | -2H+Na | 2                | —     | 2   | 3   | 2     | 1     |
| FIIL-12* | 1,791 | -2H+Na | 2                | —     | 2   | 3   | 3     | 1     |
| FIIL-8*  | 2,201 | -3H+2Na | 3                | —     | 3   | 4   | 3     | 1     |
| FIIL-10* | 1,893 | -3H+2Na | 3                | —     | 2   | 3   | 3     | 1     |
| FIIL-10* | 2,055 | -3H+2Na | 3                | —     | 2   | 4   | 3     | 1     |

<sup>a</sup> Indicates the fractions that were also analyzed by NMR spectroscopy and for which complete structures were proposed (Table VI). The samples were analyzed for carbohydrate composition by hydrosylation and HPLC, and by MALDI-TOF mass spectrometry. —, not available.

<sup>b</sup> Fractions were analyzed in positive or negative ion mode, and the number of H or Na subtracted and/or added in the pseudomolecular ions are indicated. The nominal value of m/z is given excluding the fractional mass increment of each atom, e.g. H is counted as 1 instead of as 1.008. The error in mass assignment was about ±1/1000. The composition shown for each compound was deduced based on the ion mass, allowing for calibration errors, and the monosaccharide composition analysis.

<sup>c</sup> FN was analyzed by MALDI-TOF mass spectrometry in positive ion mode. The indicated mass and composition revealed one sulfate, one Fuc, two Gal, and two GlcNAc. This confirms the presence of sulfated Lewis X determinants in the original fractions. No 4,6-dimethyl-GlcNAc was detected, indicating the relative lack of Galβ1-3GlcNAc and, hence, lack of Lewis a determinants in the original fractions.

TABLE III
Mass spectrometry of neutral saccharide mixture (FN)

The total neutral saccharide fraction FN was analyzed by MALDI-TOF mass spectrometry in positive ion mode.

| m/z    | Fuc | Gal | HexNAc<sup>ab</sup> | GalNAc-o | Also found in<sup>c</sup> |
|--------|-----|-----|--------------------|----------|------------------------|
| 570    | 1   | 1   | 1                  | —        | FN-2                   |
| 627    | 1   | 1   | —                  | —        | FN-3                   |
| 789    | 1   | 1   | 1                  | —        | FI-10                  |
| 935    | 2   | 2   | 1                  | —        | FI-12                  |
| 1,081  | 2   | 2   | —                  | —        | FIIL-11                |
| 1,154  | 3   | 2   | 1                  | —        | FIIL-12                |
| 1,300  | 1   | 3   | 2                  | —        | FIIL-12                |

<sup>a</sup> All m/z values correspond to pseudomolecular ions due to loss of one H. The nominal value of m/z is given excluding the fractional mass increment of each atom; e.g. H is counted as 1 instead of as 1.008. The error in mass assignment was about ±1/1000. The composition shown for each compound was deduced based on the ion mass, allowing for calibration errors, and the monosaccharide composition analysis.

<sup>b</sup> HexNAc can be either GlcNAc or GalNAc.

<sup>c</sup> The components also found in further purified fractions are indicated.

TABLE IV
Mass spectrometry of monoacidic saccharide mixture (FI)
The total monoacidic fraction FI was analyzed by MALDI-TOF mass spectrometry in negative ion mode. —, not available.

| m/z    | HS<sub>O</sub> | Fuc | Gal | GlcNaC | GalNaC-o | Also found in<sup>b</sup> |
|--------|----------------|-----|-----|--------|----------|------------------------|
| 667    | 1              | 1   | 1   | 1      | —        | FI-9                   |
| 738    | 1              | 1   | 1   | 1      | —        | FI-9                   |
| 813    | 1              | 1   | 1   | 1      | —        | FI-9                   |
| 929    | 1              | 1   | 1   | —      | —        | FI-9                   |
| 975    | 1              | 1   | 1   | 2      | 1        | FI-9                   |
| 1,121  | 1              | 2   | 2   | 1      | —        | FI-9                   |
| 1,178  | 1              | 1   | 1   | 2      | 1        | FI-9                   |
| 1,381  | 1              | 1   | 2   | 3      | 1        | FI-9                   |
| 1,486  | 1              | 2   | 3   | 2      | 1        | FI-9                   |

<sup>a</sup> All m/z values correspond to pseudomolecular ions due to loss of one H. The nominal value of m/z is given excluding the fractional mass increment of each atom; e.g. H is counted as 1 instead of as 1.008. The error in mass assignment was about ±1/1000. The composition shown for each compound was deduced based on the ion mass, allowing for calibration errors, and the monosaccharide composition analysis.

<sup>b</sup> The components also found in further purified fractions are indicated.

<sup>c</sup> Instead of GalNAc-o, the m/z value indicated the presence of an unknown group (R in Fig. 5, and Table IV) of mass 131.
and one GlcNAc per GalNAc-ol residue. The NMR spectrum (Table V) revealed the signals from GalNAc-ol typical of a core 2 \(\text{Gal}_b^1\_3(\text{GlcNAc}_b^1\_6)\text{GalNAc-ol}\) (33), the signals characteristic for an sulfated Lewis X determinant as described above, and a signal at 4.46 ppm for H-1 of the Gal linked to the 3-position of GalNAc-ol, Gal3. Hence, the proposed structure is as shown for structure 9 in Table VII. The alternative structure, in which sulfate is on Gal3 whereas Gal4,6 of the Lewis X determinant is nonsulfated, is not consistent with the data. For such a structure, one would have expected a signal from Gal4,6 H-1 at 4.44 ppm (instead of 4.46 ppm for H-1 of the unsubstituted Gal3) and signal from Gal4,6 \(\text{H-3 eq}\) at 3.49 ppm, typically clearly visible outside the bulk signals between 3.5 and 4.0 ppm for compounds having an unsubstituted Lewis X determinant (see Fig. 8, described below).

A superscript to the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. Two or three superscripts map out the pathway from the residue toward the GalNAc-ol residue. In the column headings, the structures are represented by a short-hand symbolic notation: \(\ominus = \text{GalNAc-ol}; \blacksquare = \beta-\text{Gal}; \diamond = \beta-\text{GlcNAc}; \square = \alpha-\text{Fuc}; \triangle = \text{NeuAc}(\alpha2–3)\); \(\bigcirc = \text{NeuAc}(\alpha2–6)\). The linkage position is specified by the direction of the connecting bars as follows:

\[\begin{array}{c}
\text{Gal}^3 \\
\text{GlcNAc}^6 \\
\text{Fuc}^3,6 \\
\text{NeuAc}^6 \\
\text{Fuc}^3,3,3,1,4,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3,3,4,5,6,6,3,6,2,3
Sulfated Lewis X on Colon Cancer Mucin

TABLE VI

1H chemical shifts of structural reporter group protons of constituent monosaccharides for di- and triacidic oligosaccharide-alditols isolated from human colonic cancer cell line mucin

n.d., not determined; —, absent in the structure.

| Chemical shift in compound | GalNAc-ol | H-2 | 4.379 | 4.388 | 4.390 | 4.392 | 4.387 | 4.392 | 4.401 |
|---------------------------|----------|-----|-------|-------|-------|-------|-------|-------|-------|
|                           | H-3      | 4.067 | 4.067 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
|                           | H-4      | 3.523 | 3.428 | 3.454 | 3.456 | 3.499 | 3.451 | 3.465 | 3.465 |
|                           | H-5      | 4.233 | 4.255 | 4.207 | 4.227 | 4.148 | 4.229 | 4.229 | 4.229 |
|                           | Gal      | 2.042 | 2.065 | 2.040b | 2.041b | 2.041b | 2.044 | 2.040b | 2.039b |
|                           | H-1      | 4.541 | 4.528 | 4.446 | 4.448 | 4.459 | 4.445 | 4.445 | 4.447 |
|                           | H-3      | 4.117 | 4.113 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
|                           | H-4      | 3.927 | n.d.  | 4.108 | 4.109 | 4.107 | 4.104 | 4.104 | 4.105 |
|                           | Fuc      | 1.800 | 1.800 | -     | -     | -     | -     | -     | -     |
|                           | 2.774    | -     | -     | -     | -     | -     | -     | -     | -     |
|                           | 2.031    | -     | -     | -     | -     | -     | -     | -     | -     |
|                           | 1.691    | -     | -     | -     | -     | -     | -     | -     | -     |
|                           | 2.724    | -     | -     | -     | -     | -     | -     | -     | -     |
|                           | 2.031    | -     | -     | -     | -     | -     | -     | -     | -     |
|                           | GlcNac   | 4.699 | 4.699 | 4.699 | 4.688 | 4.688 | 4.688 | 4.688 | 4.688 |
|                           | 5.115    | 5.115 | -     | -     | -     | 5.116 | -     | -     | -     |
|                           | 4.809    | 4.813b | -     | -     | -     | 4.813 | -     | -     | -     |
|                           | CH₃      | 1.175 | 1.175 | -     | -     | -     | 1.177 | -     | -     |
|                           | Fuc      | 5.136 | 5.139 | 5.140 | 5.140 | 5.140 | 5.140 | 5.140 | 5.140 |
|                           | 4.833    | 4.813 | 4.810 | 4.813 | 4.813 | 4.813 | 4.813 | 4.813 | 4.813 |
|                           | CH₃      | 1.175 | 1.176 | 1.176b | 1.176b | 1.176b | 1.177 | 1.177 | 1.176 |
|                           | Fuc      | 5.096 | 5.097 | 5.103 | 5.096 | 5.097 | 5.097 | 5.097 | 5.097 |
|                           | 4.807b   | 4.813 | 4.810 | 4.813 | 4.813 | 4.813 | 4.813 | 4.813 | 4.813 |
|                           | CH₃      | 1.175 | 1.176 | 1.176b | 1.176b | 1.176b | 1.177 | 1.177 | 1.176 |

* A superscript at a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. Two or three superscripts map out the pathway from the residue toward the GalNAc-ol residue. In the column headings, the structures are represented by a short-hand symbolic notation: ○ = GalNAc-ol; ■ = β-Gal; ● = β-GlcNAc; □ = α-Fuc; △ = NeuAcα2–3; ○ = NeuAcα2–6). The linkage position is specified by the direction of the connecting bars as follows:

\[ \text{GalNAc-ol} \quad \text{H-2} \quad \text{Gal} \quad \text{Fuc} \quad \text{Nac} \]

**b** Assignments may have to be interchanged.

**fated Lewis X Determinants**—Fraction FII-12 was heterogeneous, but a main component (FII-12A) could clearly be identified. The mass and composition of this main component was the same as for fraction FI-10 with addition of one more sulfate, Gal, Fuc, and GlcNAc (Table II), indicating the presence of an additional sulfated Lewis X determinant. Accordingly the NMR spectrum (Fig. 6, Table VI) showed the presence of two Fucα1–3GlcNAc moieties and two 3-HSO₃⁻-Gal moieties. The signals from GalNAc-ol was characteristic of core 1 with H-2 at 4.39 ppm as found for core 1 and core 2 but not other cores, and H-5 at 4.15 ppm, as found for core 1 but not core 2. Additional information was provided by the signal from Galβ H-4 (33, 34)
found at 4.106 ppm. This signal is shifted downfield to about 4.13 ppm in GlcNAcβ1–3Gal containing structures, compared with its position at about 3.9 ppm in structures with an unsub-

| No. | Relative amount | Fraction | Structure |
|-----|----------------|----------|-----------|
| 1<sup>a</sup> | 70 | FI-3 | NeuAc2–6 / Galβ1–3 / GlcNAc-ol / Galβ1 |
| 2<sup>a</sup> | 5 | FI-4 | GlcNAc-ol / Galβ1–3 / NeuAc2–3 |
| 3<sup>a</sup> | 35 | FI-4 | NeuAc2–6 / Galβ1–3 / GlcNAc-ol / Galβ1 |
| 4<sup>a</sup> | 10 | FII-2 | NeuAc2–6 / Galβ1–3 / GlcNAc-ol / Galβ1 |
| 5<sup>a</sup> | 40 | FI-5 | GlcNAcβ1–6 / Galβ1–3 / NeuAc2–3 |
| 6<sup>a</sup> | 60 | FI-9 | Galβ1–4GlcNAcβ1–6 / HSO<sub>3</sub>-3 Fucol–3 / R |
| 7<sup>a</sup> | 230 | FII-10 | Galβ1–4GlcNAcβ1–6 / HSO<sub>3</sub>-3 Fucol–3 / Galβ1–3 |
| 8<sup>a</sup> | 100 | FII-12 | Galβ1–4GlcNAcβ1–6 / HSO<sub>3</sub>-3 Fucol–3 / Galβ1–3 |
| 9<sup>a</sup> | 180 | FIII-8 | Galβ1–4GlcNAcβ1–6 / HSO<sub>3</sub>-3 Fucol–3 / Galβ1–3 |

<sup>a</sup> Previously published structures. For structures 1 and 2 the NMR data were reported by van Halbeek <i>et al.</i> (33), for structures 3–5 by van Halbeek <i>et al.</i> (61), for structure 6 by Vliegenthart <i>et al.</i> (62), for structure 7 by Strecker <i>et al.</i> (26), and for structure 10 by Lo-Guidice <i>et al.</i> (22).

<sup>b</sup> Structures not reported before, but strongly supported by composition, mass spectrometry, and NMR analysis.

<sup>c</sup> Structures not reported before. Overall features strongly supported by composition, mass spectrometry, and NMR analysis, but assignments of partial terminal determinants to particular antennae are tentative.

A Triantennary Core 2-based Saccharide with Three Sulfated...
Lewis X Determinants—The most complex structure of the mucin sample was found in fraction FIII-8. This was also the major triacidic fraction (Fig. 3c). Mass spectrometry and composition analysis revealed three sulfate groups, three Fuc, three GlcNAc, and four Gal per GalNAc-ol, indicating a multiantennary highly sulfated compound (Table II). The NMR spectrum (Fig. 7, Table VI) suggested that all sulfate groups, Fuc and GlcNAc residues, and three Gal residues were forming three sulfated Lewis X determinants based on the same arguments as given above. Only one Gal was not sulfated, as indicated by H-1 at 4.46 ppm, but instead was substituted with GlcNAc at position 3 and 6, as indicated by H-4 at 4.106 ppm. The identification of this branching Gal as Gal3 was based on the expected presence of -4GlcNAc also described above. Moreover, permethylation analysis of the desulfated and defucosylated fractions FII-6 and FII-7 revealed the expected presence of -GlcNAc also described above.

Triantennary “Partial” Structures—The remaining structures can be regarded as not fully elaborated parts of structure 12. Their masses were consistent with the same composition as structure 12 minus sulfate, Fuc, and/or Gal, suggesting that one or more of their antennae lacked one of these residues compared with structure 12.

The presence of Galβ1–4GlcNAc rather than Galβ1–3GlcNAc as the backbone in the antennae was based on the observation that the same NMR reporter groups as described for sulfated Lewis X determinants above. Moreover, permethylation analysis of the above structures indicated by the same NMR signals as described for fraction FIII-8 above.

In addition, the permethylation analysis of FII-7 and FII-11 revealed 3,6-disubstituted GalNAc-ol (detected as 1,4,5-tri-methyl-3,6-diacetyl-GalNAc-ol by gas chromatography-mass spectroscopy) but no 3-substituted Gal, as expected for this core (data not shown).

Previous data on NMR of numerous O-linked glycans (25) show slight differences between the reporter groups from each of the three antennae. For example, the signal from Fuc H-1 is at about 5.09, 5.11, or 5.13 ppm, depending on whether it is attached to GlcNAc6,3, GlcNAc6, or GlcNAc3,3, respectively (compare Figs. 6 and 7, and Table V and VI). Similarly, H-1 of Gal are slightly different for each antenna. The published data indicate that these differences are due to the local environment within each antenna, e.g. whether GlcNAc is linked β1–3 or β1–6, rather than interactions between the antennae or conformational shifts of the overall structure. Tentative structural interpretations based on this assumption is presented for fraction FII-6, FII-7, FII-11, FII-12, and FII-10 below.

The saccharide alditols in FII-6 and FII-7 had the same mass and composition: identical to FIII-8 except one sulfate group less (Table II). As expected from this composition, the NMR spectra of FII-6 and FII-7 had a signal at 3.49 ppm (Fig. 8) typical of Gal H-2 from a nonsubstituted Lewis X determinant; Gal H-2 from sulfated or sialylated Lewis X determinants as well as most other structural motifs is found at >3.53 ppm and is usually obscured by the many other signals between 3.50 and 4.0 ppm. The only other significant difference between the NMR spectra of FII-6 and FII-7 compared with FIII-8 concerned the signals assigned to Gal H-1. In each case, a signal due to a sulfated Gal had been lost and a new signal due to a nonsulfated Gal had appeared. For FII-7 (Fig. 8) the latter was found at 4.47 ppm, which is the same as that found for H-1 of Gal linked to a fucosylated GlcNAcβ1–3, and the signal assigned to the sulfated Gal1,3,3 in FII-8 (Fig. 7) had disappeared. For FII-6, the new signal from the nonsulfated Gal was instead found at 4.45 ppm, previously found for H-1 of Gal linked to Fucα1–3GlcNAcβ1–6, and the peak due to the overlapping signals from sulfated Gal4,6,3 and Gal4,6 had decreased to half its size. From these data, it is clear that the main structures in FII-6 and FII-7 differ. The most likely difference is that in FII-7 the nonsulfated Gal is on the bottom antenna (structure 14, Table VII), whereas for FII-6 it is on neither of the top two antennae (structure 13, Table VII).

The major peak of the MALDI-TOF spectrum of FII-11 (Table II) indicated a component with one sulfate and one Fuc less than FIII-8. The sulfate and Fuc are probably missing from
the same antenna because, if they were missing from different antennae, there should have been a signal at 3.49 ppm from Gal H-2 of the nonsulfated Lewis X determinant (described above), but this signal was lacking in the NMR spectrum of FII-11. Of the three Fuc H-1 signals the one at 5.11 ppm, typical of Fuc\textsubscript{3,6}, was deficient compared with the NMR of FIII-8. Hence, the most likely structure of the major component of FII-11 is structure 15 in Table VII.

Fraction FII-12 contained a second less abundant component (FII-12B) in addition to the major biantennary saccharide (structure 11) described above. This compound had the same composition as the latter with the addition of one GlcNAc (Table II), suggesting the presence of a third incomplete antenna. Most of the NMR signals from this compound overlapped with the more intense signals from the major compound (Fig. 6), but three signals in the NMR were clearly resolved from those of the major component and were weaker, indicating that they came from single protons of the less abundant component. They indicated the attachment of the additional GlcNAc to position 6 of GalNAc-ol. The signal at 4.532 ppm for GlcNAc\textsubscript{6} H-1, instead of at 4.532 as in the minor component of FII-12 (Fig. 6, structure 16 in Table VII), suggests that this GlcNAc is sulfated at the 6-position (35); possible signals from the two H-6 of the sulfated GlcNAc at 4.38 ppm and 4.29 ppm were observed partially resolved from the GalNAc H-2 (4.39 ppm) and the Gal H-4 (4.27 ppm) signals, respectively (data not shown).

Other Fractions—The quantity and/or heterogeneity of many fractions precluded NMR analysis, but the compositional analysis combined with mass spectrometry still provided the relative ratios of sulfate and sugar residues for many component saccharides (Tables II–IV). In most cases, these ratios are consistent with structures that are incompletely elaborated parts of the fully characterized structures shown in Table VII. Thus, the number of Fuc or sulfate groups was always equal or less than the number of GlcNAc and the number of Gal minus one. A saccharide in fraction FI-12 was an exception to this, having the same compositions as FI-10 but with one extra Fuc. Since there is no place for this Fuc on the 6-linked antenna in the structure of FI-10 (structure 9 in Table VII), this Fuc is probably linked to Gal\textsubscript{3} as in a structure described by Lo-
Guidice et al. (22). Notably, there was no evidence for additional NeuAc-containing compounds. Fraction FN-4 differed from all others in containing significant amounts of Man and GalNAc; the ratio of Man:GalNAc:Fuc:Gal:GlcNAc:GalNAc-ol was 3.0:1.0:1.0:3.9:2.3:1.0. The presence of contaminating N-linked saccharides, suggested by Man, was also indicated in the mass spectrum of this fraction by, e.g., a peak at \( m/z \) 1645 for \((M+Na)^+\) of a biantennary complex structure. The presence of Man in the intact mucin indicates the presence of N-linked saccharides as minor components.

The yield of released saccharides (calculated based on amount of hexose in the intact mucin compared with in the oligosaccharide alditols, Table I) was about 40%, which is slightly lower than usual for this type of experiment (about 50%). It is also clear that the released saccharides contained relatively more Gal, GalNAc, and Fuc compared with the intact mucin, suggesting that complex saccharides were more efficiently recovered during \( \beta \)-elimination and subsequent purification. However, it is unlikely that any particular type of saccharide has been selectively lost since the recovered saccharides had both simple and complex structures and included neutral, sialylated, and sulfated representatives.

**DISCUSSION**

Here we provide strong evidence that the mucin from LS174T-HM7 colon cancer nude mouse xenografts carry sulfated Lewis X determinants as a major structural motif, most of which is found on di- and triantennary saccharides not reported previously (Table VII). Structural analysis by NMR spectroscopy and mass spectrometry was necessary to demonstrate these structures, which would not have been detected by any of the known lectins or monoclonal antibodies frequently used as probes for cancer saccharides.

Since the evidence for sulfated Lewis X determinants is
based mainly on a combined interpretation of composition analysis and NMR spectra, the question may be raised: is an alternative structural interpretation possible? The only other known position of Fuc that gives NMR signals similar to those of Fucα1–3GlcNAc is those of Fucα1–4GlcNAc as in Lewis a determinant. The signal that most clearly distinguishes these two alternatives comes from Fuc H-1. Numerous NMR spectra have been reported (25)\(^2\) for saccharides containing Galβ1–4(Fucα1–3)GlcNAc within a wide variety of structural contexts. In all cases, the chemical shift of Fuc H-1 was between 5.09 and 5.14 ppm, as found also for the structures reported here. Many structures containing Galβ1–3(Fucα1–4)GlcNAc have been reported, and in all but one δ Fuc H-1 was between 5.00 and 5.05. Both trisaccharide determinants are regarded as rigid, and the presence of NeuAc or sulfate on the 3-position of the Gal has little effect on their conformation and the NMR signals from the Fuc (less than 0.01 ppm shifts for the Fuc H-1) (36–38). The range of shifts for Fuc H-1 is instead mainly caused by structural features outside the trisaccharide determinants, i.e. mainly the nature of the linkage of GlcNAc to next sugar residue as described under “Results.” It is highly unlikely that the structural environment in the present compounds would cause Fuc H-1 of a Lewis a determinant to shift into the range of the Lewis X determinant; when found in O-linked glycans with core structures similar to those found here, its chemical shift is about 5.03. Only in one case has the Fuc H-1 signal of the Lewis A determinant been found above 5.05 ppm (5.088 ppm); this was when Fuc was directly linked to a sialylated core 3 (Galβ1–3[Galβ1–4]GlcNAcβ1–3GalNAc)[α1–2 NeuAc]; Ref. 39), a type of structure not detected in the LS174T-HM7 mucin saccharides. Hence, the data are consistent with the presence of sulfated Lewis X determinants in the major components, but are not consistent with similar amounts of any other known fucosylated motif. The permethylation analysis of the defucosylated, desulfated fractions FII-7 and FII-11 support this conclusion, by providing evidence for Galβ1–4GlcNAc, but not Galβ1–3GlcNAc, as a major structural moiety in the glycans of these fractions. Other fucosylated motifs, such as Lewis A, may, however, be present in minor components and may also be functionally significant. Recently we found that a monoclonal antibody specific for sulfated Lewis a but nonreactive with sulfated Lewis X (40) does indeed bind the mucin of LS174T-HM7 cells. The HM7 mucin saccharide structures raise two principal questions: 1) what is the significance of their difference from the saccharide structures of other mucins, and 2) what are their significance for cancer? Regarding the first question, the HM7 mucin saccharides are unusually homogeneous; most mucins isolated from tissue that have been analyzed in detail have a wider variety of terminal determinants and core structures (22, 41, 42). In contrast, a much more homogeneous saccharide pattern was observed in the few cases of structurally characterized purified mucins (43), or mucins from a uniform cell source (16). Hence, the relative homogeneity of the saccharides structures in LS174T- HM7 cells may be due to the homogeneity of these cells, i.e. their clonal origin. A caveat in the interpretation is that since the LS174T-HM7 cells were grown as xenografts in nude mice, it is to be expected that some mucin-like glycoproteins from the admixed mouse tissues, such as vasculature and infiltrating mouse immune cells, could contribute some saccharide components. However, these would be likely to be among the small neutral or sialylated saccharides that are commonly found in many glycoproteins with O-linked saccharides, and not the complex structures with sulfated Lewis X determinants. The particular saccharide structures of the LS174T-HM7 mucin is, as mentioned above, unique among mucins characterized so far in having a large number of 3-sulfated Lewis X determinants, known to be efficient selectin ligands (37, 44). This determinant have only been found previously in quantitatively minor saccharide components of a few other mucins (22, 32, 45) and human milk (44). The glycans of the L-selectin ligand GlyCAM-1 are similar in coming from one to three Lewis X determinants and large amounts of sulfate; however, in contrast to the HM7 mucin saccharides, they are sialylated instead of sulfated on the 3-position of Gal, and the sulfate is bound to the 6-positions of Gal and GlcNAc (43). The saccharide composition of the LS174T-HM7 differ from the parent LS174T cells, from which it has been selected as a clone. The latter mucin has not been structurally characterized, but its carbohydrate composition (Table I) clearly shows about 3-fold less sulfate, and 3 times more NeuAc. Moreover, the relative amounts of Gal and GlcNAc compared with GalNAc suggest that the saccharides in the LS174T mucin are simpler. Mucin from a different colon carcinoma cell line (CL.16E, a clone of HT29 cells) also has very different structures (16) compared with LS174T-HM7. These saccharide were mainly sialylated, but had no Fuc, and the small amount of sulfate was linked to the 6-position, not 3-position, of Gal; the core structures included type 1 and 2 as in the LS174T-HM7 mucin but in addition included core 4 with the GlcNAcβ1–3GalNAc linkage that is not found in the LS174T-HM7 mucin. These differences suggest that each clonal cell line may express a different program for O-linked oligosaccharide synthesis. Are these programs induced and/or selected in cancer cells, as proposed (46, 47), or do they reflect a differentiation program of some normal cell type, fetal or adult, of the intestinal mucosa? The detection of multiple subpopulations of human goblet cells by lectin histochemistry (48) supports the idea of normal subtypes and/or clones with different expression of saccharides and/or mucin subtypes such as sulfomucin (49). Although sulfated Lewis X determinants are not prominent among the structures reported for O-linked saccharides from human meconium (32) or adult large intestinal mucins of healthy individuals (41, 42), they might be prominent in the saccharides from a particular, possibly minor, goblet cell subtype. Regarding the second question, many lines of evidence suggest that mucin expression contributes to cancer metastasis. There is a relationship between cancer metastasis and bulk quantity of mucin produced by some cancer cells. Among cell lines, those producing more mucins often produce more metastasis, including the LS174T-HM7 cells analyzed in this study (10, 17). In one proposed mechanism, the bulky mucin may prevent cell adhesion molecules such as integrins and E-cadherin from mediating cell-substratum and cell-cell interaction (50), in turn resulting in dedifferentiation and increased invasiveness of the tumor cell (51). Recent studies in which mucin saccharide synthesis was inhibited support this idea for the LS174T-HM7 cells (52, 53). In a later step, specific selectin-binding saccharide epitopes carried by the mucin may help circulating tumor cells to initiate the attachment and extravasation necessary for metastasis (4, 46, 54, 55), in a way analogous to the normal attachment-extravasation pathway for leukocytes (3). Hence, the same mucin may prevent cell adhesion at one site and promote it at another depending on its saccharide structures and what ligands are involved. The mucin from LS174T-HM7 cells is likely to bind selectins very well because the sulfated Lewis X determinant binds selectins well (37, 44, 56). If the mucin remains attached to the cell surface, it could enhance metastatic activity according to the mechanism mentioned above. However, if the mucin is
released from the cell surface, it could also act as an inhibitor of immune cell targeting, another mechanism for increased malignancy.

In a possible third mechanism, cell-bound or released mucin could inhibit tumor cell lysis by natural killer (NK) cells (57). Cell surface lectins of NK cells bind sulfated saccharides, resulting in signals inhibiting target cell killing (37, 58).

Mucin from HM7 and other colon cancer cells have also been shown to interact with galectin-3 (59), a β-galactoside-binding lectin expressed by many cancer cells themselves as well as normal intestinal epithelium and various immune cells (60), but the function of this binding is unknown.

In conclusion, the present results underscore the fact that the relationship between colon cancer malignancy and mucin saccharide structures is complex and probably has to be understood in terms of detailed structures rather than overall quantity or composition. The saccharide structure of the LS174T-HM7 mucin not only differs dramatically from the saccharides of the few other colon carcinoma mucins that have been analyzed. In addition, the LS174T-HM7-mucin does have saccharides that may very well contribute strongly to malignancy, despite the fact that these cells do not express sialyl-Lewis X or sialyl-Lewis A determinants, and do not have a saccharide profile previously regarded as typical of malignant cells. Hence, for further elucidation of the role of saccharides in colon cancer, full structural analysis of a larger collection of colon cancer mucins, and the development of reagents detecting a larger range of specific saccharide determinants, such as the sulfated Lewis X, is necessary. With the development of improved methods, exemplified by MALDI-TOF mass spectrometry of the sulfated saccharides in this report, such analysis should now be possible despite the small amounts of sample obtainable.

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