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Altered expression of sialylated carbohydrate antigens in HT29 colonic carcinoma cells

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***Abbreviations:* Le^a - Lewis A, S Le^c - sialyl Lewis C (NeuAca2,3Gal-β1,3-GlcNAc), Le^x - Lewis X, S Le^x - sialyl Lewis X, S Le^a - sialyl Lewis A, Le^y - Lewis Y, S Le^y - sialyl Lewis Y, STn - sialyl Tn, HPLC - high pressure liquid chromatography, PBS - phosphate buffered saline**

To determine whether cell growth conditions impacted carbohydrate expression, HT29 cells were gradually transferred from a conventional glucose-containing media to a glucose-free galactose containing media. Indirect immunofluorescence on acetone fixed cells showed increased expression of sialyl Lewis A antigen (CA19-9), sialyl Lewis C (DUPAN2) and Tn/sialyl-Tn on the surface of HT29 cells grown in the glucose-free galactose containing media compared to those grown in the glucose containing media. Sialyltransferases responsible for the synthesis for these sialylated epitopes were increased in the galactose-fed HT29 cells. Media overlying the cells was subjected to isopycnic ultracentrifugation in cesium chloride and the fractions derived from both glucose and galactose media with equivalent buoyant densities of 1.56 g/L, which are predicted to contain mucin glycoforms, were further separated by HPLC using a Mono-Q anion exchange column. The chromatograph of eluent from the sample derived from the cells growing in the galactose containing media showed an increased peak that reacted with the anti-sialyl Lewis A antibody, CA19-9. These results show that alteration of *in vitro* culture conditions may cause HT29 colonic carcinoma cells to alter the expression of sialylated carbohydrates.

Introduction

Alteration of cell surface carbohydrates during malignant transformation is a recognized phenomenon in a variety of tumors [1,2]. There has been interest in the specific role that carbohydrate antigens might play in tumor invasion and metastatic potential. For instance, a poor prognosis in patients with colorectal carcinoma was found to correlate with expression of the carbohydrate antigen sialosyl-Tn on this tumor and this correlation was independent of tumor grade and stage [3]. Differences in metastatic potential are attributable to various cellular properties [4,5] including sialylated complex carbohydrates [6,7]. The biological factors that cause increases in sialylated carbohydrate antigens in the metastases remain unknown. It has been postulated that biosynthesis of sialylated mucin core and peripheral carbohydrate structures might be affected by factors at the site of metastases or selective metastases of subpopulations of cancer cells producing these antigens [7–9]. Thus, the microenvironment of the primary tumor may be responsible for enhanced expression of sialylated antigens with metastases in part resulting from preferential colonization of differentially glycosylated tumor cells.

In the present study, we examined immunoreactivity of antibodies to core and peripheral mucin carbohydrate structures and glycosyltransferases responsible for the synthesis of these carbohydrate structures on HT29 colonic carcinoma cell lines that were grown in glucose- and galactose containing media. These different culture conditions have previously been associated with alterations in growth rate, morphological appearance, disaccharidase activity and mucin gene expression [10,11]. Differences in the antibody reactivity with cell surface and secreted products from the cells and sialyltransferase activities were observed when the growth conditions in which the cells were grown were altered.

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Methods and materials

Cell growth conditions

HT 29 colonic adenocarcinoma cells [American Type Tissue Collection, USA] were grown in McCoy's 5a media (modified) [Life Technologies, USA]. Cells were progressively transferred from this glucose-containing media to a glucose-free, galactose-containing media over a five day period as previously described [10]. Media was supplemented with 10% fetal bovine serum [Life Technologies] and antibiotics [100 units/mL penicillin G, 100 mg/mL streptomycin sulfate and amphotericin B 0.25 mg/mL; Life Technologies]. The cells were grown at 37°C in humidified atmosphere with 5% CO_2. Prior to reaching confluence, cells were passaged using 1% trypsin. Culture media overlying cells grown in the conventional glucose-containing McCoy's 5a (modified) media and overlying the cells grown in the glucose-free, galactose containing media were collected, lyophilized to 33% volume and stored at −70°C. Cells grown in glucose medium grew at a faster rate, requiring passage every 2–3 days, as compared to cells grown fed the galactose medium which required passage only every 4–5 days. The cells cultured under these distinct conditions exhibited morphological alterations that we have seen previously [10] and were previously described in detail [11].

Immunofluorescence

HT29 cells under different growth conditions were evaluated for reactivity with antibodies to carbohydrate antigens using a modification of the indirect immunofluorescence method described by Godfrey et al [12]. HT29 cells were plated onto 8-well chamber slides (Nunc Inc., USA), incubated at 37°C in humidified atmosphere with 5% CO_2 and allowed to grow to 80% confluence in either the glucose-containing or galactose containing McCoy's 5a media supplemented as described above. Monolayers were washed with PBS (pH 7.4) at room temperature prior to fixation with pre-chilled (−20°C) acetone for 10 minutes, and washed × 3 (PBS, pH 7.4). Cells were incubated with a primary antibody for 3 hours at room temperature and then washed with PBS × 3 to remove unbound antibody. Cells were then incubated with a 1:20 dilution of FITC conjugated goat anti-mouse polyvalent immunoglobulin (Sigma, St. Louis, MO) as a secondary antibody for 30 minutes at 37°C. Excess secondary antibody was washed away with PBS. The slides were mounted and examined under fluorescence microscopy. Experiments were repeated at least eight times for cells grown in both carbohydrate containing media. Photographs were taken following equivalent exposure times. Monoclonal antibodies were obtained from the following sources: CO514 [anti-Lewis A], CO431 [anti-Lewis B], B93.1 [anti-Lewis X], CSLEX1 [anti-sialyl Lewis X] [13], and B32.21 [anti-Lewis Y] were the gift of Mark Reddish of Biomera Corp.; CA19-9 [anti-sialyl Lewis A] was a gift from Hiliary Koprowski, Thomas Jefferson University, Philadelphia, PA [14]; DUPAN-2 [anti-sialyl Lewis C] [15]; B72.3 [anti-sialyl Tn] was a gift from David Colcher, University of Nebraska Medical Center, Omaha, NE [16]; 3C9 [anti-T] [17], HH5 [anti-A type 3 chain] [18], HH8 [anti-T] [17], HH14 [anti-H type 3 chain] [19], and PMH1 [anti-MUC2 GalNAc] [20] were the gift of Henrik Clausen, University of Copenhagen, Copenhagen, Denmark.

Preparation of mucins

Isolation techniques as previously described for purification of intestinal goblet-cell derived mucins were applied to spent cell culture media [21]. To minimize proteolytic degradation [22], 5 mM *N* -ethylmaleimide (Sigma Chemical Co., USA), 2 mM phenylmethylsulfonyl fluoride (Sigma) and 0.01% sodium azide (Sigma) were added along with 5 mM EDTA to harvested cell culture media. The media was centrifuged at 30,000 × g for 30 minutes at 4°C to remove cellular and particulate debris. Components of the soluble supernatant were subdivided by buoyant density using isopycnic ultracentrifugation in cesium chloride (Fisher Scientific, USA) with a starting density of 1.46 g/mL. The suspension was placed into polyallomer centrifuge tubes (Du Pont Co. Inc., USA) and centrifuged in a Sorvall 50.2 Ti rotor (Du Pont) at 105,000 × g for 48h at 4°C. Eight fractions of equal volume were collected and analyzed for buoyant density by a refractometer (Abbe 3L, Milton Roy Co., USA). Fractions were then placed into wetted cellulose dialysis tubing (50 kDa exclusion; Spectrum Medical Industries Inc., USA) and dialyzed against deionized water for 48h at 4°C. Total protein content of each fraction was determined by the method of Lowry [23] with a known concentration of albumin (Fraction V, Sigma) used as standard. Glycoprotein concentrations were determined by the periodic-acid Schiff assay [24] using crude porcine mucin (Sigma) as the reference standard.

Components of Fraction 2 that were expected to contain mucin glycoforms were further separated by ion exchange chromatography using a modification of the technique described by Raouf et al [25]. Dialyzed fractions were combined, lyophilized and resuspended to a concentration of 10 mg protein/mL and 10 mg protein was applied to a 5 mm × 50 mm Mono-Q anion exchange column (Q8HR, Waters Scientific, USA). Samples were eluted at a flow rate of 1 mL/min using 50 mM Tris-HCl (Sigma), pH 8.0 with a continuous salt gradient of 0–2.5 M NaCl (Sigma). Continuous optical density monitoring (280 nm) was used during the collection of sixty 1-mL fractions. Samples were stored at −20°C until subsequent use in assays.

Immunoblotting

Reactivity of antibodies to HPLC fractions was determined by dot blot immunoassay, as described by Towbin and Gor-

don [26]. Briefly, 2.5 μg of fraction protein was applied as a spot onto nitrocellulose paper and allowed to air dry for 1 hour. The paper was then incubated with 3% bovine serum albumin (Sigma, Fraction V) in 10 mM Trizma base (Sigma) with 0.9% saline at pH 7.4 for 1h at 37°C. After being washed with tris-saline, antibodies to carbohydrate structures were applied in a dilution of 1:500 and incubated with the paper overnight at 4°C. After washing with tris-saline at room temperature to remove unbound antibody, a 1:2,500 dilution of goat antimouse polyvalent immunoglobulin peroxidase conjugated (Sigma) was added to the nitrocellulose strips and incubated for 1h at room temperature. Horseradish peroxidase color development was performed with a reagent containing 4-chloro-1-naphthol (Bio-Rad, USA) in methanol and 30% hydrogen peroxide (Sigma) was added to develop reactions.

Assay of sialyltransferase activities

HT29 cells grown to confluence in glucose- or galactose-containing media in two T175 flasks were harvested by trypsinization. After neutralization of trypsin with serum-containing medium and washing 2× with PBS, the cells were homogenized in about 0.7 mL of 0.25M sucrose by succesive passing through 22, 25, and 26 guage needles. Sialyltransferase activities in the cell homogenate was measured in a 50 μl assay mixture which contained the following ingredients: 50 mM N-morpholinoethane sulfonic acid, pH 6.5 (Sigma); 1% Triton X-100 (Sigma); 5 mM $MnCl_2$ (Fisher Scientific); 1 mg/mL bovine serum albumin (Sigma); 20 mM CMP-[^{3}H]NeuAC (American Radioloabeled Inc., St. Louis, MO), 4,300 dpm/nmol; 8 mM Galβ1,3GlcNAc (Toronto Research Chemical Inc.) or 2 mg/mL asialo ovine submaxillary mucin [27], and 0.6–1.0 mg of cell homogenate. The endogenous enzyme activity was measured without the acceptor present.

After incubation at 37°C for 2h, the radiolabeled product was isolated from the supernatant (13,000 × g, 2 min) of the reaction mixture by Dowex 1-phosphate when Galβ1, 3GlcNAc was used as an acceptor [28] and on Bio-Gel P4 column when asialo ovine submaxillary mucin was the acceptor [27]. Enzyme activity was linear with time for up to 2 hours and for protein amounts used. Enzyme activity was expressed as nmole sialic acid transferred/h/mg protein. Protein was measured with the Bradford reagent (Bio-Rad) using bovine serum albumin as the standard. The net glycosyltransferase activity was estimated by subtracting the activity without acceptor from that with exogenously added acceptor.

Characterization of the product generated by CMP-NeuAc:Galβ1-3GlcNAc sialyltransferase

To prepare the product generated by CMP-NeuAc: Galβ1,3GlcNAc sialyltransferase for structural characterization, the reaction mixture was scaled up 10-fold and the reaction time was increased to 6h. The sialylated product was isolated on a 1 × 6 cm Dowex 1-phosphate column [28], concentrated, and then subjected to chromatography on a Bio-Gel P-4 column (100-200 mesh, 1.5 × 110 cm). The column was developed with 0.1 M Tris HCl, pH 7.5 at a flow rate of about 0.36 mL per min. The fractions which contained the radiolabeled product were combined, concentrated, and subjected to desalting on a Bio-Gel P-4 column (1 × 25 cm). Then, an aliquot (200 μl) of the radiolabeled product was adjusted to 50 mM sodium phosphate, pH 6.8 and treated with 5 units of α2,3 neuraminidase (V-Labs, Inc., Covington, LA) at 37°C for 2h [29]. The neuraminidase-treated sample was added to 2–3 mg each of glucose, lactose, raffinose, and stachyose, which were used for calibration of the column, prior to chromatography on a Bio-Gel P-4 (100–200 mesh) column (1.5 × 110 cm). The column was developed with 0.1 M Tris HCl, pH 7.5 as described above. Another aliquot (100 μl) of the product was treated and analyzed the same way but in the absence of α2,3 neuraminidase.

Results

Cell culture immunofluorescence studies

Expression of carbohydrate structures sialyl-Lewis A, sialyl Tn and sialyl-Lewis C were increased on cells grown in galactose-containing media compared to HT29 cells that were grown in a glucose-containing media (Figure 1). The Lewis A and Lewis Y antigens were expressed equally well by HT29 cells grown in either the glucose-containing media or galactose-containing media (Figure 2). Antibodies that did not react with HT29 cells grown under either culture conditions were Lewis B, Lewis X, sialyl Lewis X, T, MUC2-GalNAc, or those to blood groups A_3 or H_3.

Secreted glycoform separations

Cell culture supernatant and secreted cell products were separated by a density gradient cesium chloride ultracentrifugation (Figure 3). The buoyant densities of equivolume fractions of cells grown in glucose containing media ranged from 1.61 g/mL in the most dense fraction to 1.36 g/mL in the least dense fraction. The cesium chloride buoyant density fractions of supernatant pooled from cells grown in galactose containing media were similar among corresponding fractions and ranged from 1.64 g/ml to 1.37 g/mL. As we have previously shown in isolation techniques of mucins from intestinal mucosa, the most dense cesium chloride density gradient (Fraction #1) had relatively high nucleic acid content whereas the middle density fractions contain mucin glycoforms [21,30]. Buoyant density fractions #2 with densities of 1.56 g/mL for cells grown in both glucose- and galactose-containing media and a glycoprotein-to-protein ratio of about 0.2 were collected for further

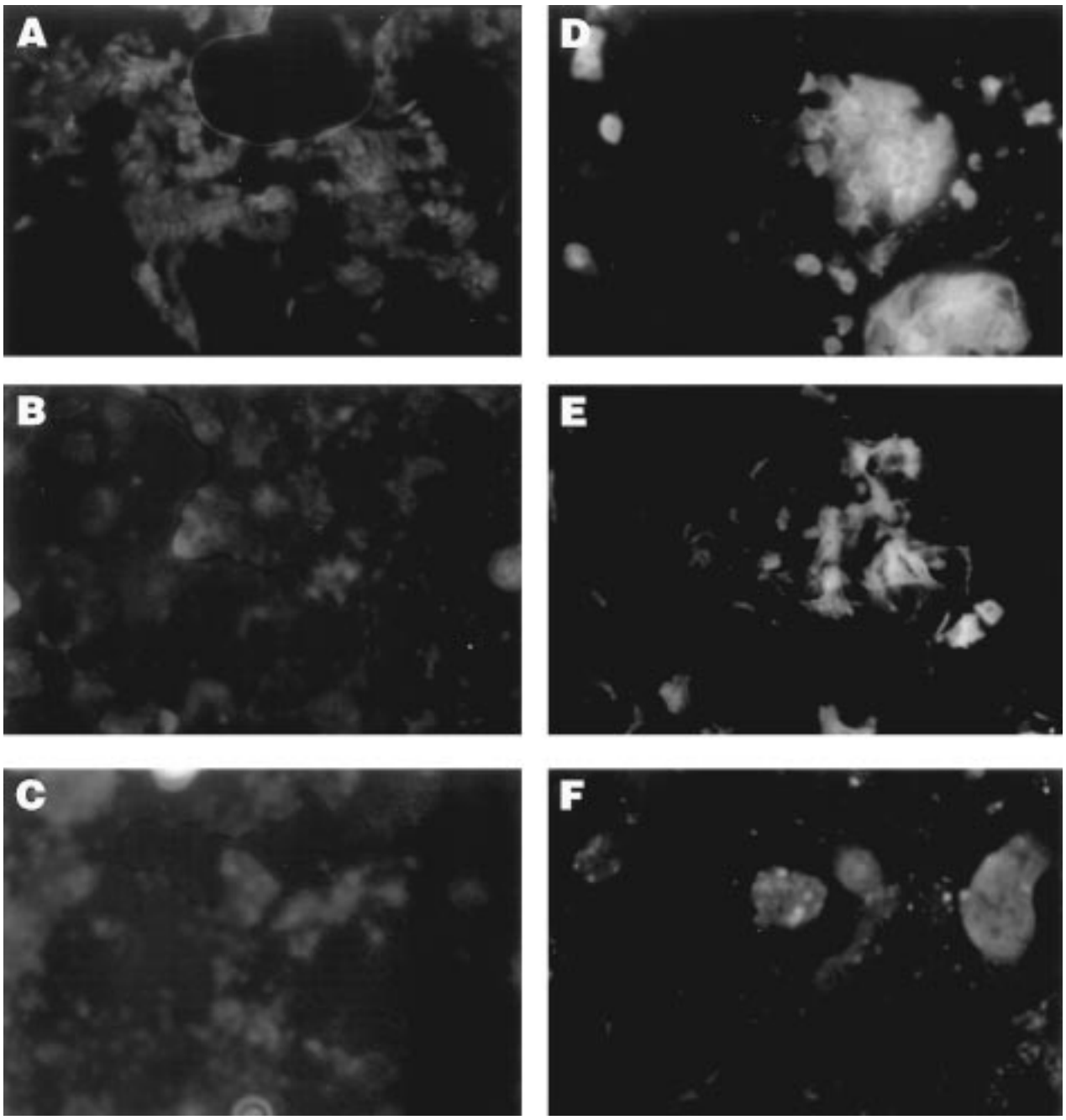

Figure 1. Expression of sialylated Lewis A (A and D), sialosyl Tn antigen (B and E) and sialylated Lewis C (C and F) as detected using immunocytochemistry of acetone fixed HT29 cells grown in McCoy's 5a (modified) medium with glucose (A, B and C) or containing galactose (D, E and F) as the carbohydrate source. Photomicrographs were taken using fluorescent microscopy.

separation of the components by ion exchange chromatography.

HPLC fraction immunoreactivity studies

HPLC chromatographs of mucin fractions in supernatants derived from HT29 cells grown in glucose- and galactose-containing media are shown in Figure 4. The chromatographs show general similarity throughout the salt gradient; fraction region F derived from HT29 cells grown in galactose-containing media shows greater peaks.

The results of dot-blot immunoassays with anti-carbohydrate antibodies that showed reactivity in cell fluorescence studies are shown in Figure 4. Lewis A and Lewis Y antigens, which had shown equal expression in cell immunofluorescence studies, were not detected in similar

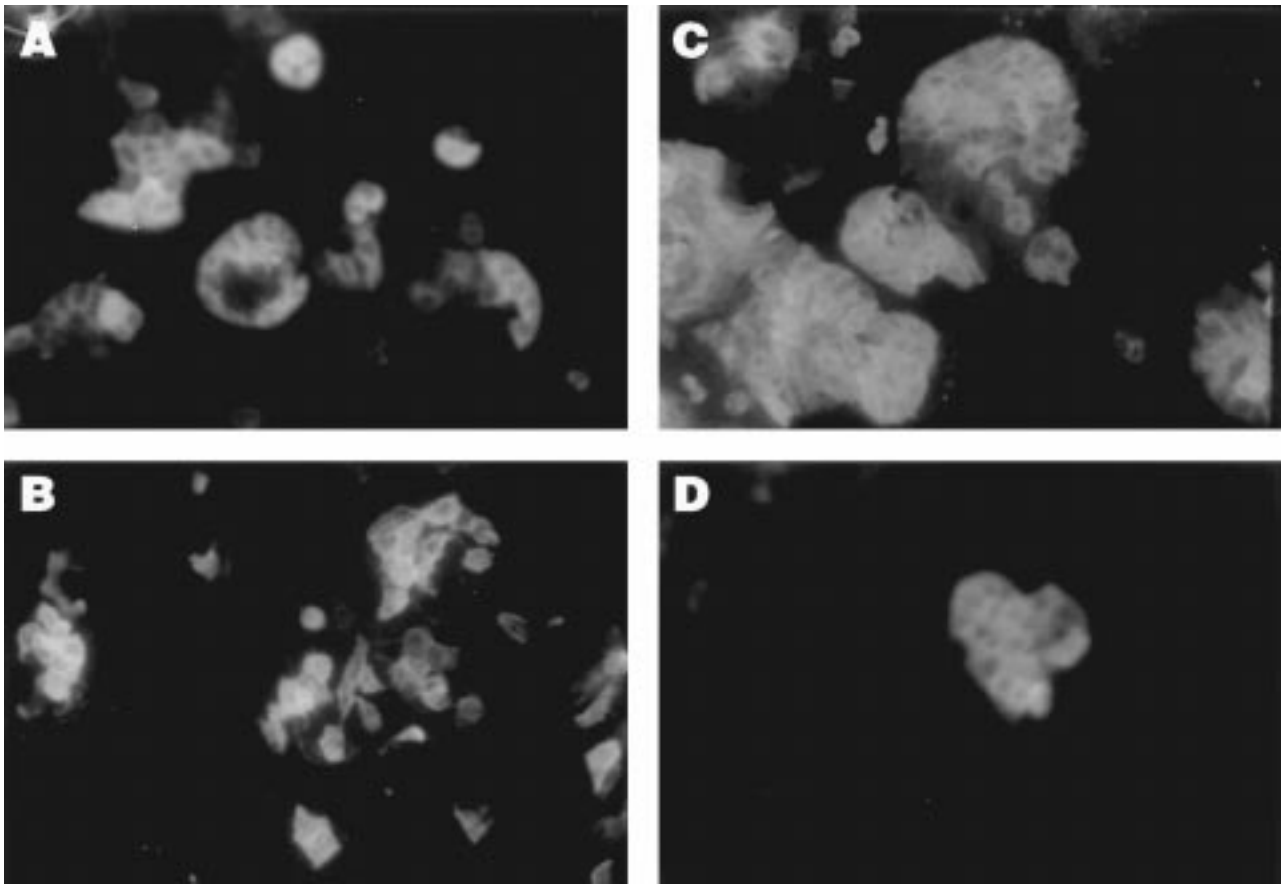

Figure 2. Expression of Lewis A (A and C) and Lewis Y (B and D) antigens on acetone fixed HT29 cells grown in McCoy's 5a (modified) media with either glucose (A and B) or galactose (C and D) as the carbohydrate source. Photomicrographs taken using fluorescent microscopy.

HPLC ion exchange chromatography fraction regions of the glucose and galactose grown cells. Sialyl Lewis C structures were detected in multiple fraction regions from supernatants of galactose-grown cells, while reactivity was not detected in any eluent fraction regions of glucose grown cells. Sialyl Tn antigen was found in different fractions of supernatants derived from cells cultured in glucose or galactose. In contrast, sialyl Lewis A antigens were seen in similar HPLC fractions from both cell types. However, measurements of optical density (280 nm) showed that greater amounts of material were present in the galactose grown cells.

Sialyltransferase activities in glucose- and galactose-fed HT29 cells

As shown in Table 1, after HT29 cells were switched from glucose-containing to galactose-containing medium, sialyltransferase activities measured with both Galβ1,3GlcNAc and asialo ovine submaxillary mucin acceptors were increased. A higher degree of enhancement of sialyltransferase activity was observed with Galβ1,3GlcNAc acceptor than that with asialo ovine submaxillary mucin acceptor.

Characterization of the reaction product generated by CMP-NeuAC:Galβ1,3GlcNAc sialyltransferase

When assay of a glycosyltransferase activity is performed using a crude homogenate, several different products may be generated from an acceptor. In the case when asialo ovine submaxillary mucin was used as the acceptor, the sialylated product was shown to be sialic acidα2,6 GalNAcαser/thr [31]. Therefore, the activity measured in the crude homogenate of HT29 cells with this acceptor is most likely the CMP-NeuAc:GalNAcα Ser/Thr α2,6 sialyltransferase. However, when Galβ1,3GlcNAc is used as an acceptor, more than one sialylated product may be generated. Therefore, the structure of the sialylated product(s) derived from this disaccharide acceptor needs to be characterized in order to know which sialyltransferase activity is measured. Using this disaccharide as the acceptor to generate the sialylated product on a preparative scale, we obtained about 39 nmoles of the radiolabeled product. When the sialylated product was treated with α2,3 neuraminidase, the radiolabeled sialic acid was totally cleaved (Figure 5). This result indicated that the product was primarily sialic acidα2,3Galβ1,3GlcNAc and the activity measured was

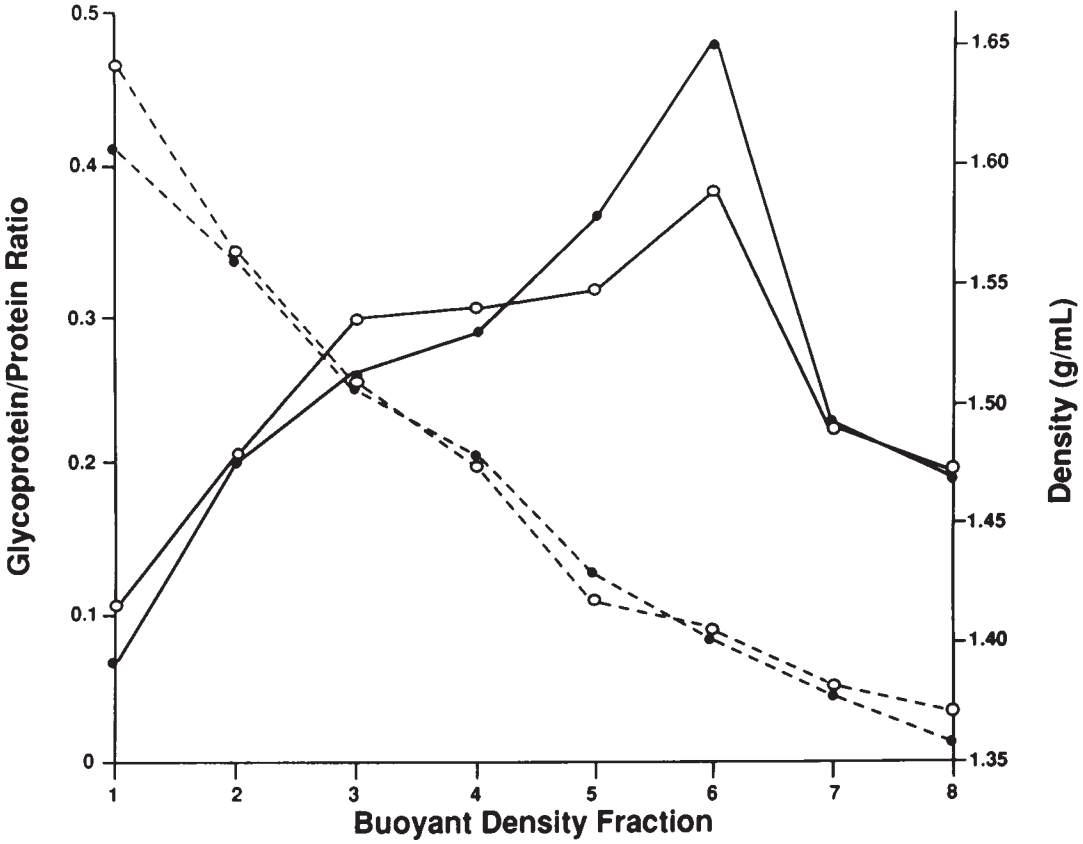

Figure 3. Characterization of cesium chloride buoyant density fractions of cell growth media from HT29 cells grown in media containing either glucose (closed circles) or galactose (open circles). Similar cesium chloride buoyant densities (dashed lines) were found in equivalent fractions following ultracentrifugation. Following dialysis against deionized water, glycoprotein (mg/mL) and protein (mg/mL) determinations (see Methods) were made and the glycoprotein-to-protein ratio (solid lines) for each of the eight fractions showed similarities for cells grown using either of the two carbohydrate sources.

CMP-NeuAc:Galβ1,3GlcNAc (NeuAc-Gal) α2,3 sialyltransferase. There were insignificant amounts of α2,6 sialyltransferase activity in the cells using Galβ1,3GlcNAc as the acceptor.

Discussion

A change in the growth environment of a colonic carcinoma cell line can lead to profound changes in the phenotype function of cellular processes. Replacing glucose with a glucose-free carbohydrate (galactose) source in the media of HT29 cells which induces an enterocyte-like differentiation in the cells, led to an alteration of the surface and secreted carbohydrate antigens. Increases in the sialylated carbohydrate antigens were observed in both mucin core antigens (i.e. STn) and peripheral carbohydrate antigens (i.e., SLe^a, SLe^c).

In the present study, cells grown in both glucose and galactose containing media had strong expression of Le^a and Le^y. In the normal adult intestinal tract, Lewis and ABH antigens are expressed in the goblet cell mucins of only the proximal portion of the colon without expression in the distal colonic mucosa [32]. In distal colonic tumors, re-expression of ABH antigens has been a common finding [33,34]. The type 2 chain (Galβ1,4GlcNAc) Le^y expression has been demonstrated in the majority of colonic adenocarcinomas [35–38]. In some instances, Lewis antigen expression has been found to be restricted to cell surfaces, whereas secreted products such as mucus glycoproteins were not found to express the same antigen [39,40]. In the present set of experiments, SLe^a expression was found on HT29 cells grown in the galactose media and secreted products of the same cells that had a high buoyant density fraction and was contained in one HPLC peak.

Sialic acid concentrations of intestinal glycoproteins tend to be higher in the fetal and newborn period and decrease with aging [41]; however, increased sialylation is one of the common changes of tumor mucins [42]. Carbohydrate moieties of colonic mucins are heterogeneous and consist of both core and peripheral antigens. Sialylated oligosaccharide structures are prominent in mucin-type glycoproteins from human colorectal cancers and are re-

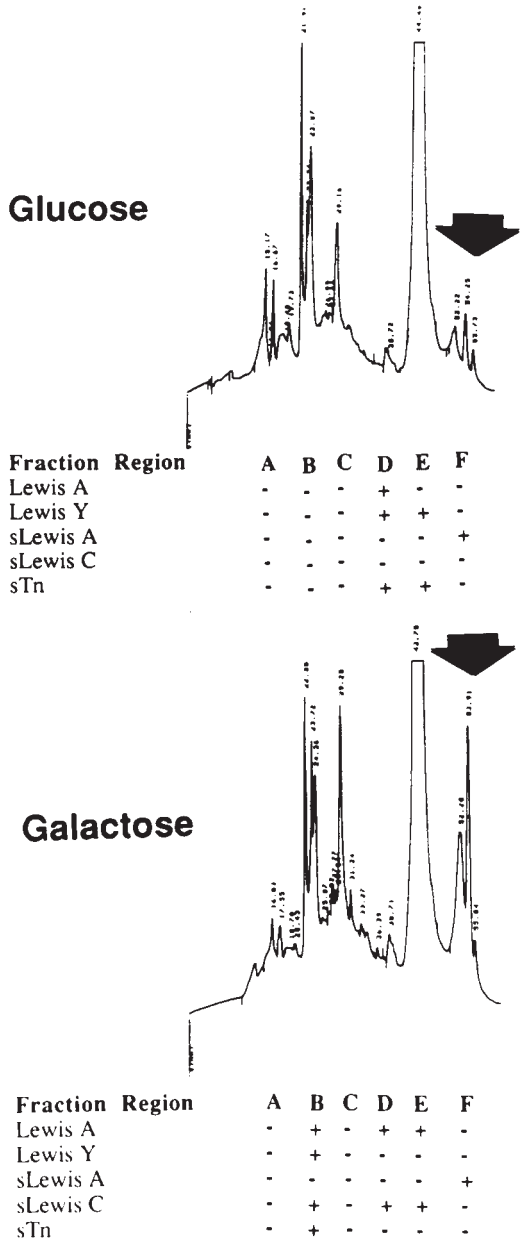

Figure 4. High pressure liquid chromatography profiles of cesium chloride buoyant density fraction 2 (1.56 g/mL) of cell growth media from HT29 cells grown in media containing either glucose (Panel A) or galactose (Panel B). Reactivity of antibodies to carbohydrate structures are shown for the various ion exchange chromatography peak regions. The arrow defines the peak differences between material derived from glucose- and galactose-grown cells, which reacted with sialylated Lewis A antibody.

sponsible for tumor-associated antigenicity. Partial removal of cell surface sialic acids has been shown to increase adhesion to substratum and cellular aggregation, and sialic acids on oligosaccharides O-linked to surface glycoproteins inhibit cellular aggregation and adhesion to substratum [43]. In the present study we found that the cells grown in the glucose environment and their secreted products had reduced sialylated mucin associated oligosaccharide structures. Thus, cellular microenvironments in the primary tumor are probably important factors dictating cellular events.

Mucin-type glycans are synthesized by sequential addition of single sugars to the acceptor as catalyzed by a series of glycosyltransferases [44]. STn is synthesized by the transfer of sialic acid from CMP-NeuAc to peptide-linked GalNAc, a reaction catalyzed by GalNAc-peptide: α2,6 sialyltransferase [31,44]. SLe^c is the precursor of SLe^a and Galβ1,3GlcNAc:α2,3 sialyltransferase is responsible for the synthesis of this sialylated structure. In this report we show that by switching HT29 cells from the glucose-containing to galactose-containing medium, the activities of these two sialyltransferases were increased in these cells. These results provide the explanation for increased expression of STn, SLe^c, and SLe^a tumor-associated blood group antigens in the galactose-fed HT29 cells as compared to glucose-fed HT29 cells.

An increase in glycosyltransferase activities has also been explored in the CaCo-2 human colonic adenocarcinoma cell line. These cells have also been found to develop an enterocyte-like differentiation with acquisition of brush border and hydrolytic enzymes spontaneously with prolonged culturing [45] with the state of enterocytic differentiation of HT29 cells correlated with *N*-glycan processing [46], in contrast to HT29 cells that require an alteration of the carbohydrate in the growth media to invoke differentiation. For CaCo-2 cells, the *N*-acetylglucosaminyltransferases II through V were less in cells that were harvested after a short period in culture compared with CaCo-2 cells that were harvested after longer periods of time in culture [47]. Similarly, UDP-GalNAc:NeuACα2,3Galβ-R (GalNAc to Gal) β-1,4-*N*-acetylgalactosaminyltransferase activity was increased in CaCo-2 cells that were maintained in culture for 20–21 days to induce differentiation as compared to cells harvested immediately after reaching confluence [48]. Interestingly, the activity of the α2,6 sialyltransferase that adds NeuAc in α2,6 linkage to *N*-acetyllactosaminyl sequences of glycoprotein *N*-linked chains but not the corresponding α2,3 sialyltransferase increases during *in vitro* maturation by prolonged incubation of confluent CaCo-2 cells [49].

Alterations in the growth conditions of HT29 cells other than those described here, such as the addition of sodium butyrate or methotrexate to culture media, leads to development of a permanently differentiated clonal derivative of HT29 cells [39] but lack the STn structures [50,51]. However, homogenous mucin secreting populations of HT29 cells obtained by adaptation in methotrexate containing media have a high concentration of the CMP-NeuAc: Galβ1,3GalNAc α2,3 sialyltransferase. Addition of benzyl-*N*-acetyl-α-D-galactosaminide, a potential inhibitor of the β1,3-galactosyltransferase resulted in decreased sialic acid content and decreased secretion of mucins [51]. Thus, this

Table 1. *Sialyltransferase Activities in HT29 cells Grown in Glucose- and Galactose-Containing Media.* Sialyltransferase activities were measured in the crude homogenate in the presence and absence of the acceptors, Galβ1,3GlcNAc and asialo ovine submaxillary mucin. The net sialyltransferase activity was determined by subtracting the endogenous activity (−) from the activity measured in the presence of the acceptor (+). Results are the mean values from two separate assays.

	Sialyltransferase Activities (nmole sialic acid transferred/h/mg protein)					
	Galβ1,3GlcNAc Acceptor			*Asialo Ovine Submaxillary Mucin Acceptor*		
HT29 Cells	(−)	(+)	Net	(−)	(+)	Net
Glucose-Fed	0.98	1.05	0.07	0.50	1.71	1.21
Galactose-Fed	0.35	2.31	1.96	0.45	2.83	2.38

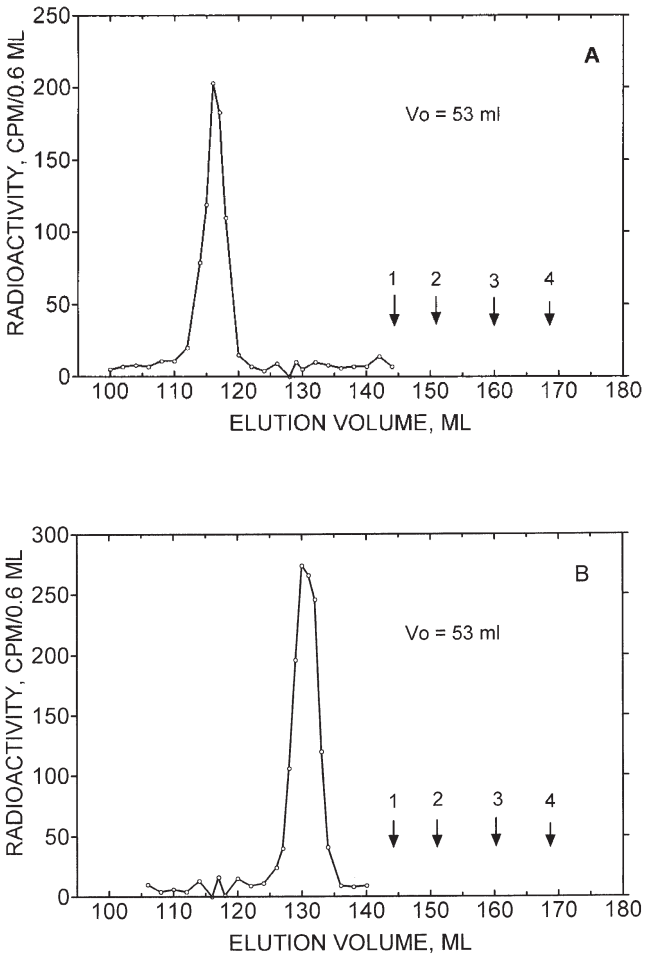

Figure 5. Bio-Gel P-4 column (1.5 × 110 cm) chromatograms of the reaction product of Galβ1,3GlcNAc: sialyltransferase: (A) without and (B) with treatment with α2,3 neuraminidase. The column was developed with 0.1 M Tris HCl, pH 7.5 at a flow rate of 0.36 mL per minute. The eluent was collected 1 mL per fraction. The column void volume was 53 mL. The column was calibrated with stachyose (1), raffinose (2), lactose (3), and glucose (4). In frame A, 100 μl aliquot of the isolated product was applied to the column after incubation in 50 mM sodium phosphate buffer, pH 6.8 without α2,3 neuraminidase at 37 °C for 2 hours. In frame B, 200 μl of the isolated product was applied to the column after treatment with α2,3 neuraminidase for 2 hours.

HT29 culture system could also serve as an *in vitro* experimental model for studying the regulation of the expression of glycosyltransferase genes involved in the synthesis of the mucin glycans associated with tumor progression and metastasis.

We previously showed that HT29 cells progressively transferred to a glucose-free galactose containing medium resulted in a reversible alteration of mucin gene expression, with increased expression of MUC3 mRNA and decreased expression of MUC2 mRNA compared to H29 cells grown in glucose-containing media [10]. Thus, it is likely that the component increased in the HPLC chromatograph shown in Figure 4 is MUC3 core protein, that is glycosylated with sialyl Lewis A. This remains to be confirmed when appropriate reagents to detect MUC3 protein become available. The possible association between increased sialylated carbohydrate antigens and MUC3 (and other mucin gene expression) is of biological significance and requires further investigation.

Acknowledgments

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