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Membrane-associated, Fucose-containing Glycoproteins and Glycolipids of Cultured Epithelial Cells from Human Colonic Adenocarcinoma and Fetal Intestine*

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Abstract—*Fucose-containing glycoproteins and glycolipids were compared in three human colon cancer cell lines and five human fetal intestinal epithelial cell lines. Cells were labeled by culturing cells in the presence of* L-[^{3}H]-*fucose. Fucose was incorporated into both the membrane and cytoplasmic fractions of all three colon cancer cell lines to a much lesser extent than into fetal cells. When the relative fucose labeling of glycolipids and glycoproteins were examined, a much greater proportion of fucose labeling in the membrane was associated with lipid in colon cancer cells* (11.6–16.7%) *compared to fetal intestinal cells* (1.3–2.5%). *Fluorographic analysis of SDS-polyacrylamide gel electrophoresis of fucose-labeled glycoproteins revealed a rather uniform labeling pattern of fetal intestinal cells which was distinct from those of colon cancer cells. Thin-layer chromatographic analysis of fucose labeled glycolipids of all three colon cancer cell lines indicated the presence of fucose-containing glycolipids with carbohydrate chain lengths greater than five sugars. Glycolipids of the SKCO-1 cells in particular appear to consist predominantly of complex fucose-containing glycolipids. These results indicate that significant qualitative differences in the fucose-containing glycoproteins and glycolipids exist between the membranes of human colon cancer cells and fetal intestinal cells and that complex fuco-glycolipids with long carbohydrate side chains are present in the three human colon cancer cell lines.*

INTRODUCTION

FUCOSE-CONTAINING glycoproteins and glycolipids are present in the membranes of many mammalian cells, particularly in the intestinal epithelial cells [1–5]. Fucose-containing glycoconjugates have been observed to be more enriched in the surface membranes compared to other monosaccharides constituents [6–8], except for *N*-acetylneuraminic acid. Although the precise role of fucose in the biological properties of mammalian cells is not yet clear, fucose-containing glycoconjugates are antigenically active and appear to play an important role in the determination of blood group activity [9].

Studies by various investigators indicate that considerable alteration in fucose-containing glycoconjugates occur with malignant transformation [9–11]. Virally transformed cells have been reported to express in their surface membranes fucose-containing glycopeptides with more highly branched oligosaccharide side chains than those found in the control cells [10]. Alterations in the antigenic expression of blood group-active fucose-containing glycolipids and glycoproteins have also been observed to occur in human gastrointestinal adenocarcinoma [4, 5, 12, 13]. Recently, we compared a human colon cancer and a fetal intestinal epithelial cell line and observed that colonic cancer cell membranes contain fucose-labeled, trypsin-labile, high mole-

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cular weight glycopeptides not present in fetal cells [14]. In the present study we compared in more detail the fucose-containing glycoproteins and glycolipids in the membranes of three human colon cancer cell lines and five human fetal intestinal epithelial cell lines.

MATERIALS AND METHODS

Cell cultures

Human colon cancer cell lines, SKCO-1 and HT-29 were kindly supplied to us by Dr. J. Fogh, Sloan-Kettering Cancer Institute, Rye, NY and SW-480 was provided by Col. A. Leibovitz from Scott-White Clinic, Temple, TX. Five human fetal intestinal cell lines were established in our laboratory, three from fetal small intestine (HFS) and two from colon (HFC). The fetal cell lines showed epithelioid morphology and density-dependent inhibition of growth, and did not produce tumors when injected subcutaneously into nude mice. All cultures were free of mycoplasma. All cell lines were maintained on Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). The cells were grown in 75 cm^2 growth area tissue culture flasks (Falcon Plastics, Oxnard, CA) and the medium was changed twice a week. All of the studies were carried out on cells at 60% confluency.

Fucose labeling of membrane glycoconjugates

Cells grown in T-75 flasks at 60% confluency were washed three times with Dulbecco's phosphate-buffered Ca^{2+} and Mg^{2+}-free saline (DBPS). Twenty-four milliliters of fresh medium containing L-[^{3}H]-fucose (13 Ci/mmol, New England Nuclear, Boston, MA) were added to the cells. After 92 hr incubation the medium was removed and the cells were washed three times in phosphate-buffered saline and centrifuged at 2000 rev/min for 10 min. The washed cells were sonicated and the homogenate was centrifuged at 105,000 ***g*** for 60 min to obtain the membrane and cytoplasmic fractions. The membrane pellet did not contain unbroken cells or cell debris when examined by phase microscopy. Although this membrane pellet is crude and probably consists of various subcellular membranes, it is called membrane fraction for convenience.

SDS-Gel electrophoresis

SDS-Polyacrylamide gel electrophoresis in a discontinuous buffer system was carried out according to the method of Laemmli [15] with a vertical slab gel apparatus (Bio-Rad Laboratories, Richmond, CA). Samples were dissolved in 0.021 M Tris buffer, pH 6.8, containing 2% sodium dodecylsulphate, 5% 2-mercaptoethanol, 0.001% bromophenol blue and 10% glycerol. Just before applying the gel, samples were heated at 100°C for 3 min. Gel thickness was 1.5 mm and the current applied was 50 mA/gel. An average run finished within 2 hr. Gels were stained with Coomassie brilliant blue for proteins according to the method of Fairbanks *et al.* [16]. Ten-percent gels with 4% stacking gels were used in all experiments. Approximately equal counts of 50,000 counts/min were applied to each lane.

Autoradiography

Autoradiography was performed according to the method of Bonner and Laskey [17]. Gels were incubated with dimethylsulfoxide to remove water, then impregnated with PPO (2.5-diphenyl-oxazole), dried and exposed to X-ray film (Kodak X-O mat R film).

Protein measurement

Protein was measured by the method of Lowry *et al.* [18] using crystalline bovine serum albumin as standard.

Isolation of L-[^{3}H]-*fucose labeled glycolipids*

Total lipids were extracted from the membrane fraction of three human colon cancer cell lines with 20 vols of chloroform:methanol (2:1) by sonication, filtered and the residue re-extracted with 10 vols of chloroform:methanol:water (1:2:0.15). Extracts were combined and concentrated at 40°C under vacuum and dialyzed against distilled water for two days at 4°C. The dialyzate was dried and applied on a 1 × 10 cm DEAE-Sephadex column. Labeled neutral glycolipids along with other lipids were eluted with 50 ml chloroform:methanol:water (30:60:8) and the ganglioside fraction, also containing sulfoglycolipids, was eluted with chloroform:methanol: 0.8 M sodium acetate (30:60:8).

Thin-layer chromatography and fluorography

Neutral, labeled, fucose-containing glycolipid fractions of the membrane preparations from three colon cancer cell lines were applied on plates coated with silica gel 1-B (Baker) and were developed in chloroform:methanol:water (60:35:65). Thin-layer chromatographic plates were dried at 50°C for 10–15 min. The plates were either used for fluorography or scraped for radioactivity counting. The plates were impregnated with the scintillation fluid by dipping them into 20% 2,5-diphenyloxazole (PPO) in toluene, dried and exposed to X-ray film (Kodak, X-Omat R XR_2) for several days at −70°C and developed. Another set of plates were scraped at every 0.5-cm distance from the origin up to 9 cm from the origin and the

radioactivity was counted in a Packard Tri-Card liquid scintillation spectrometer.

RESULTS

Table 1 summarizes the data on the incorporation of L-[^{3}H]-fucose into glycoconjugates of cellular homogenate, membrane and cytoplasmic fractions of human colon cancer cells and fetal intestinal cells. Although the total amount of L-[^{3}H]-fucose incorporation into the cancer cells per T-75 flask was much higher than that into the fetal cells, when the values were expressed per mg protein in each fraction the opposite results were obtained. This is consistent with the observation that more cancer cells are recovered than fetal cells per flask (5 to 10-fold) and indicate that on an individual cell basis, fetal cells incorporate about 2 to 4-fold more L-[^{3}H]-fucose into both the membrane and cytoplasmic fractions. The total proteins in the membrane and the cytoplasmic fractions added up to the homogenate protein values in all cells. Mean cell numbers per T-75 flasks were: HT-29, 39×10^6; SKCO-1, 95×10^6; SW-480, 16×10^6; HFC-172, 0.96×10^6; HFC-188, 1.1×10^6; HFS-188, 1.2×10^6; HFS-185, 1.0×10^6; and HFS-176, 0.95×10^6. Mean protein content per 10^6 cells were as follows: HT-29, 0.31 mg; SKCO-1, 0.40 mg; SW-480, 0.27 mg; HFC-172, 0.26 mg; HFC-188, 0.28 mg; HFS-188, 0.26 mg; HFS-185, 0.30 mg; and HFS-176, 0.28 mg. However, when the percentage of the total membrane-associated radioactivity associated with the lipid fraction was examined, colon cancer cells exhibited much greater incorporation of L-[^{3}H]-fucose into glycolipids fraction compared to fetal intestinal cells (Table 2).

Figure 1 shows fluorograms of SDS-polyacrylamide gels of fucose-labeled membrane glycoproteins. The fucose-labeled glycoprotein profiles were different between the colon cancer and fetal intestinal cells. Although some bands such as the 80,000 dalton band may be shared by fetal intestinal and some cancer cells, further studies using two-dimensional gel electrophoretic methods or immunological studies are necessary to determine this point. All five fetal intestinal epithelial cell lines showed nearly identical labeling patterns regardless of whether they were established from fetal colon or small intestine. In contrast, the labeling patterns of the three colon cancer cells were different from each other. All three cancer cell lines seemed to share the 110,000 dalton band, but the 200,000 dalton band was present only in SKCO-1 cells. An 80,000 dalton band was present in both HT-29 and SW-480 cells, but was not detectable in SKCO-1 cells. Radioactive bands were observed at the dye front of all three colon cancer cell lines, but were only faintly visible with fetal cell lines.

Table 2. Percentage L-[^{3}H]-*fucose radioactivity associated with the lipid fraction of the membrane pellet*

Cells	Lipid fraction (%)*
Colon cancer cells	
HT-29	16.7†
SKCO-1	11.6
SW-480	16.0
Fetal intestinal cells	
HFC-172	1.8
HFC-188	2.5
HFS-188	2.1
HFS-185	1.6
HFS-176	1.3

*Percentage of the total radioactivity in the membrane pellet (1 mg protein).
†Values are mean of 3 experiments.

Table 1. Incorporation of L-[^{3}H]-*fucose into glycoconjugates in the homogenate, membrane and cytoplasmic fractions*

Cells	Homogenate		Cytoplasmic fraction		Membrane fraction	
	Total radioactivity*	Specific radioactivity†	Total radioactivity*	Specific radioactivity†	Total radioactivity*	Specific radioactivity†
Colon cancer cells						
HT-29	3.31 ± 0.78‡	0.27 ± 0.08	0.90 ± 0.28	0.19 ± 0.03	1.61 ± 0.30	0.39 ± 0.14
SKCO-1	1.09 ± 0.27	0.28 ± 0.06	0.39 ± 0.08	0.23 ± 0.05	0.62 ± 0.21	0.48 ± 0.11
SW-480	2.72 ± 0.19	0.62 ± 0.20	0.65 ± 0.21	0.30 ± 0.05	1.55 ± 0.25	0.76 ± 0.23
Fetal intestinal cells						
HFC-172	0.29 ± 0.08	1.15 ± 0.16	0.05 ± 0.02	0.68 ± 0.14	0.12 ± 0.04	1.51 ± 0.39
HFC-188	0.38 ± 0.13	1.21 ± 0.10	0.09 ± 0.04	0.89 ± 0.08	0.30 ± 0.08	1.78 ± 0.24
HFS-188	0.36 ± 0.11	1.15 ± 0.24	0.08 ± 0.01	0.68 ± 0.10	0.22 ± 0.08	1.57 ± 0.15
HFS-185	0.32 ± 0.06	1.05 ± 0.18	0.06 ± 0.02	0.58 ± 0.18	0.23 ± 0.06	1.51 ± 0.31
HFS-176	0.30 ± 0.04	1.19 ± 0.30	0.05 ± 0.01	0.72 ± 0.16	0.22 ± 0.05	1.94 ± 0.45

*Count/min $\times 10^{-6}$ per 75 cm^2 flask.
†Count/min/mg protein $\times 10^{-6}$.
‡Values are mean ± S.E. of three experiments.

Figure 2 shows the fucose labeling pattern of the two colon cancer cell lines, HT-29 and SW-480, shown after SDS-gel electrophoresis on a 15% gel. A faint fast-migrating band is observed slightly behind the GL-4a standard. The pooled chloroform:methanol extract of labeled HT-29 and SW-480 cells containing neutral glycolipids isolated by DEA-Sephadex chromatography showed an identical band, while the non-extractable fraction showed markedly retarded mobility. This result suggests that the radioactive band seen at the dye front of the 10% gel and seen as a fast-migrating band on the 15% gel probably represents fucose-containing glycolipids with sugar side chains of greater than five saccharide subunits. This is supported by the data presented in Fig. 3, which show that fucose-containing glycolipids have carbohydrate side chains of greater than five subunits and that SKCO-1 cells appear to have predominantly fucose-labeled glycolipids with much longer or more complex carbohydrate side chains than HT-29 or SW-480 cells.

Figure 4 shows the fluorographic patterns of a thin-layer chromatogram of fucose-labeled glycolipids. As was suggested by the data in Fig. 3, all three cancer cell lines contained fucose-labeled glycolipids with carbohydrate side chains longer than five sugars. SKCO-1 cells in particular appear to contain mainly fuco-glycolipids with longer carbohydrate chains.

DISCUSSION

Considerable alterations in quantity and quality of glycoconjugates have been observed with malignant transformation [19, 20]. However, despite the relative abundance of fucose-containing glycoconjugates in the gastrointestinal mucosal cells, compared to other cells only limited data are available on this group of glycoconjugates. A marked reduction in protein-associated fucose content in both the membrane and cytoplasmic fractions of human cancerous tissues with a concomitant reduction in fucosyltransferase activities compared with the normal colonic tissues has been reported from this laboratory [4, 5]. Fucose-containing, blood group-active glycoconjugates appear during embryogenesis as early as the seventh week of gestation in the epithelial wall of the gut [21, 22]. Our previous preliminary study with a human colon cancer cell line and a human fetal intestinal epithelial cell line suggested that a qualitative difference in fucose-containing glycoproteins may be present between the two types of cell lines [14]. The present experiments further extend this study to fucose-containing glycoproteins and glycolipids of three human colon cancer cell lines and five human fetal intestinal epithelioid cell lines. A significant reduction in incorporation of fucose into the membrane fractions of individual human colon cancer cells when compared with the human fetal intestinal epithelioid cells is consistent with the reduced levels of fucose-containing glycoproteins observed in the colon cancer tissues reported previously [23]. Although in the present study most of the fucose label was associated with protein fractions in both cell types, a much greater proportion (12%) of the label was incorporated into the lipid fractions of the cancer cells than into those of fetal cells (1–2%). In our previous study on human colonic tissues the quantitative analysis of fucose-containing glycolipids in the normal and cancerous colonic mucosa had not been carried out due to the limited amount of tissue available, although the contents of certain glycolipids such as sulfoglycolipids, gangliosides and galactosyl and lactosyl-ceramide were observed to be markedly different in the tumor tissues [13].

The electrophoretic analysis of fucose-labeled

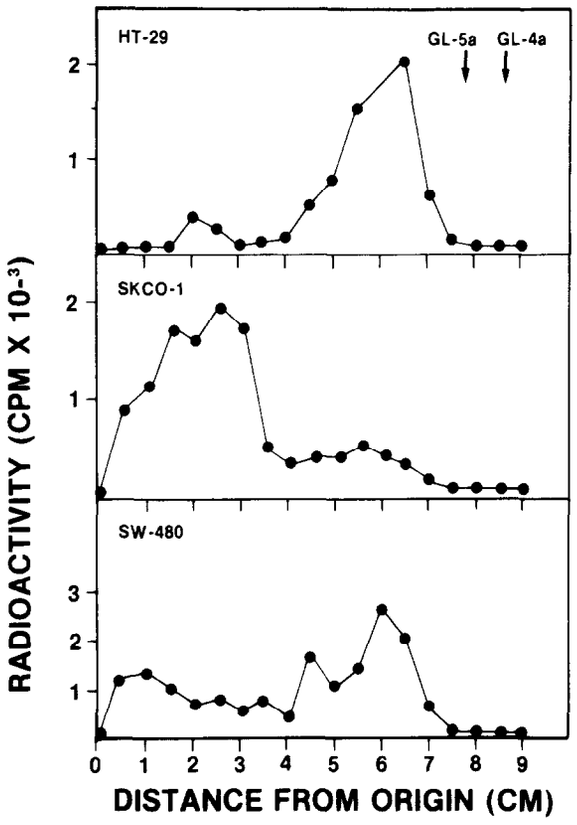

Fig. 3. Thin-layer chromatogram of L-[^{3}H]*-fucose-labeled glycolipids. Neutral* L-[^{3}H]*-fucolipids were applied on thin-layer chromatographic plates and developed in solvent as described in Materials and Methods. Silica gel was scraped from the origin every* 0.5 cm *and the radioactivity counted. GL-4a, GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 'Cer; GL-5a, GalNAcα1 → 3GalNAcβ1 → 3Galα1-4 Galβ1 → 4Glcβ1 → 1'Cer.*

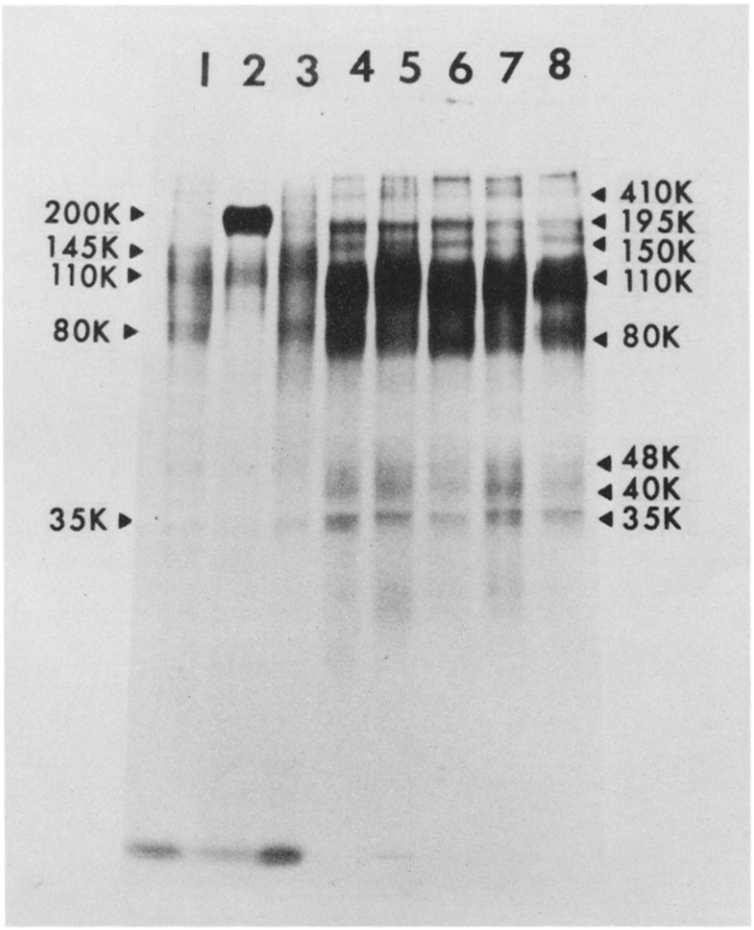

Fig. 1. Fluorography of SDS-polyacrylamide gel electrophoresis of L-[^{3}H]-*fucose-labeled membrane glycoconjugates. Cell lines examined are:* (1) *HT*-29; (2) *SKCO*-1; (3) *SW*-480; (4) *HFC*-172; (5) *HFC*-188; (6) *HSF*-188; (7) *HFS*-183; *and* (8) *HFS*-176.

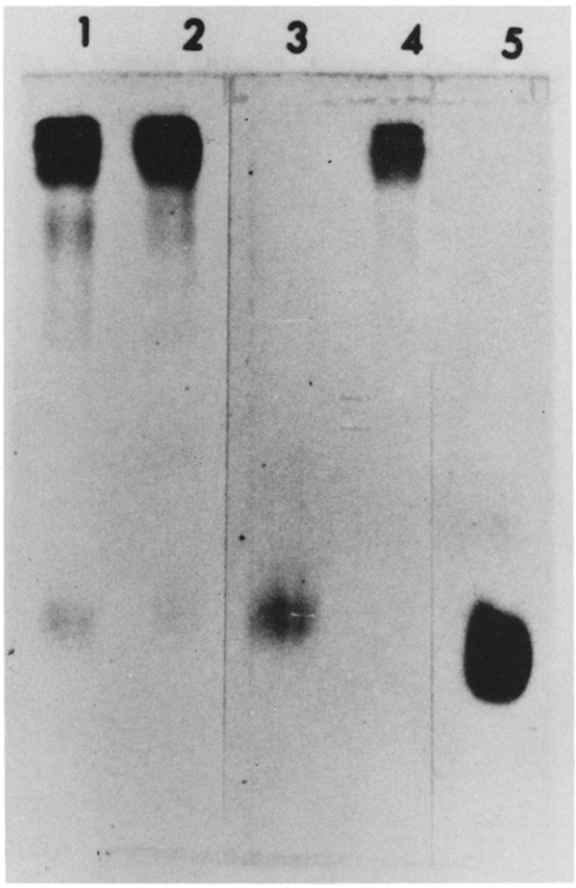

Fig. 2. Fluorography of SDS-polyacrylamide gel electrophoresis /15% gel) of L-[^{3}H]-*fucose-labeled membrane glycoconjugates.* (1) *Membrane pellet of HT-29 cells;* (2) *membrane pellet of SW-480 cells;* (3) *chloroform:methanol extract of membrane pellet of HT-29 and SW-480 cells eluted from DEAE-Sephadex column with chloroform:methanol:water* (30:60:8); (4) *chloroform:methanol:insoluble fraction of membrane pellet of HT-29 and SW-480 cells;* (5) *a glycolipid standard, GL-4a.*

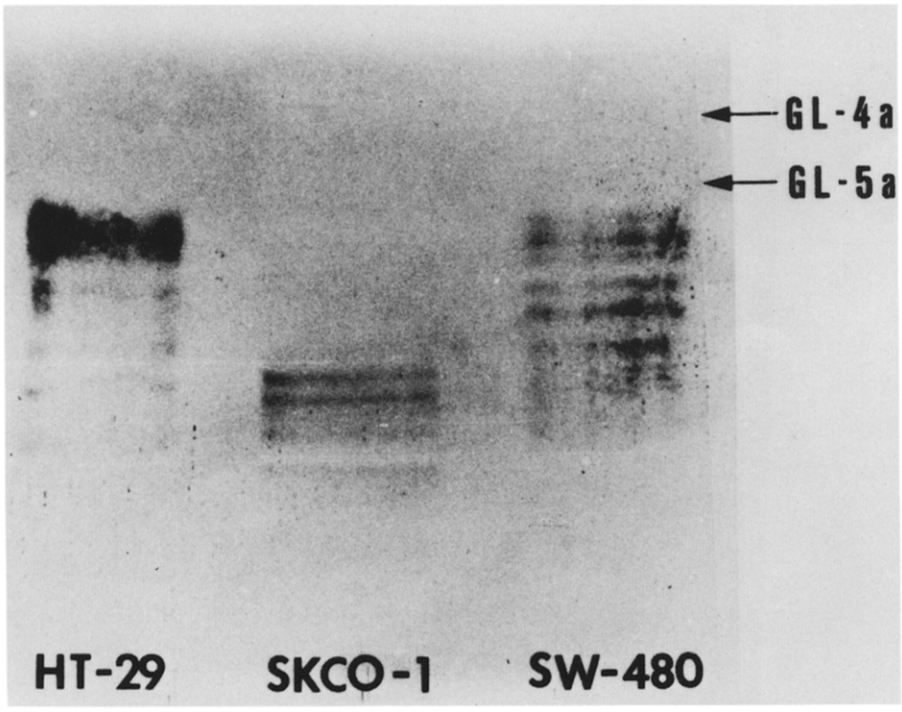

Fig. 4. Fluorography of thin-layer chromatogram of L-[^{3}H]-*fucose-labeled membrane glycolipids.*

membrane glycoproteins of the cancer cells showed much simpler patterns than those of human fetal intestinal cells. The latter showed a remarkably similar labeling pattern regardless of whether they were small intestinal or colonic in origin. The three colon cancer cell lines showed different glycoprotein labeling patterns. In particular, in SKCO-1 cells most of the labeled fucose was associated with a 200,000 dalton protein. Although not shown, nearly all the fucose radioactivity in this molecule could be precipitated with treatment by anti-CEA antisera, confirming our previous observation that CEA is a fucose-containing glycoprotein [23]. Whether the labeled glycoproteins in the colon cancer cells are biochemically and/or immunological distinct from those in the human fetal intestinal cells remains to be determined.

A very low labeling of fuco-glycolipid in the fetal intestinal cells may be due either to the low cellular content or to low level of turnover of this type of glycolipids. Due to the small amount of total glycolipids in these cells, we have not been able to measure the fucolipid content. However, our preliminary study indicates that fetal intestinal epithelial cells incorporate a considerable amount of [^{3}H]-galactose into gangliosides and non-fucosylated neutral lipids. Since the fetal intestinal cells contained only a trace amount of fucose-labeled glycolipids, the fuco-lipid study was limited to human cancer cell lines.

Thin-layer chromatographic studies indicate that three cancer cell lines contained rather heterogeneous fucose-containing glycolipids of carbohydrate side chains greater than five subunits. Interestingly, SKCO-1 cells seem to consist predominantly of fucolipids with longer carbohydrate side chains than HT-29 or SW-480 cells. SKCO-1 cells has been shown to contain 1000-fold carcinoembryonic antigen than the other two cell types, which have only trace amounts of CEA. It is also interesting to note that SKCO-1 cells are much less tumorigenic than HT-29 or SW-480 cells when injected into nude mice. Whether the presence of CEA and/or fucolipids with longer carbohydrate chains represents a more differentiated phenotype in the human colon cancer cells or not will only be decided once further studies are carried out. Marked reduction or deletion in human blood group A or B-active fuco-glycolipids in human gastrointestinal adenocarcinoma when compared to adjacent normal mucosa has been reported by Hakomori and others [12, 13]. These results suggest incomplete synthesis of blood group fuco-glycolipids since activities of glycosyltransferases responsible for the blood group antigenicity are markedly reduced in the tumor tissues [12, 13]. Yang and Hakomori also reported the accumulation of a novel type of fucose-containing glycolipid, lacto-*N*-fucopentaosyl III-ceramide, in some human adenocarcinomas [24]. However, they did not examine the presence or absence of this fuco-glycolipid in the normal mucosal tissues. The complex fuco-glycolipids with longer carbohydrate side chains detected in human colon cancer cell lines in the present study need to be characterized with regard to their structure and antigenicity as well as their presence or absence in human colon cancer tissues and normal colonic mucosa.

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