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The pattern of glycosyl- and sulfotransferase activities in cancer cell lines: a predictor of individual cancer-associated distinct carbohydrate structures for the structural identification of signature glycans

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Abstract—Carbohydrate chains of cancer glycoprotein antigens contain major outer changes dictated by tissue-specific regulation of glycosyltransferase genes, the availability of sugar nucleotides, and competition between enzymes for acceptor intermediates during glycan elongation. However, it is evident from recent studies with recombinant mucin probes that the final glycosylation profiles of mucin glycoproteins are mainly determined by the cellular repertoire of glycosyltransferases. Hence, we examined various cancer cell lines for the levels of fucosyl-, β-galactosyl, β-*N*-acetylgalactosaminyl-, sialyl-, and sulfotransferase activities that generate the outer ends of the oligosaccharide chains. We have identified glycosyltransferases activities at the levels that would give rise to *O*-glycan chains as reported by others in breast cancer cell lines, T47D, ZR75-1, MCF-7, and MDA-MB-231. Most breast cancer cells express Gal-3-*O*-sulfotransferase specific for T-hapten Galβ1→3GalNAcα-, whereas the enzyme from colon cancer cells exhibits a vast preference for the Galβ1,4GlcNAc terminal unit in *O*-glycans. We also studied ovarian cancer cells SW626 and PA-1 and hepatic cancer cells $HepG_2$. Our studies show that α1,2-L-fucosyl-T, α(2,3) sialyl-T, and 3-*O*-Sulfo-T capable of acting on the mucin core 2 tetrasaccharide, Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-, can also act on the Globo H antigen backbone, Galβ1,3GalNAcβ1,3Galα-, suggesting the existence of unique carbohydrate moieties in certain cancer-associated glycolipids. Briefly, our study indicates the following: (i) 3′-Sulfo-T-hapten has an apparent relationship to the tumorigenic potential of breast cancer cells; (ii) the 3′-sulfo Lewis^x, the 3-*O*-sulfo-Globo unit, and the 3-fucosylchitobiose core could be uniquely associated with colon cancer cells; (iii) synthesis of a polylactosamine chain and T-hapten are favorable in ovarian cancer cells due to negligible sialyltransferase activities; and (iv) a 6′-sialyl LacNAc unit and 3′-sialyl T-hapten appear to be prevalent structures in hepatic cancer cell glycans. Thus, it is apparent that different cancer cells are expressing unique glycan epitopes, which could be novel targets for cancer diagnosis and treatment.

Keywords: Cancer; Glycosyltransferase; Signature carbohydrate; Sulfotransferase; Tumor

1. Introduction

An important area of cancer research is to search for biomarkers for a very early detection of the disease, thus avoiding the cancer spread and metastasis. Prostate specific antigen (PSA), the biomarker of prostate cancer,[1–3] performs poorly when used to differentiate prostate cancer from benign prostatic hyperplasia.[4] Pancreatic cancer is another leading cause of cancer-related deaths

Abbreviations: Al, allyl; BAL, British anti-Lewisite; Bn, benzyl; CPM, counts per minute; FetTA, fetuin triantennary; FT, fucosyltransferase; Globo backbone, Galβ1,3GalNAcβ1,3Galα-; GP, glycopeptide; LacNAc type 1, Galβ1,3GlcNAc; LacNAc type 2, Galβ1,4GlcNAc; Lewis^a, Galβ1,3(Fucα1,4)GlcNAc; Lewis^b, Fucα1,2Galβ1,3(Fucα1,4)-GlcNAc; Lewis^x, Galβ1,4(Fucα1,3)GlcNAc; Lewis^y, Fucα1,2Galβ1,4-(Fucα1,3)GlcNAc; Me, methyl; MeOH, methanol; PAPS, 3′-phospho adenosine, 5′-phosphosulfate; T-hapten, Galβ1,3GalNAcα-.

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in Western countries.[5–7] Monoclonal antibodies for CA19-9, a mucin-associated sialyl Lewis^a, along with other serum tumor markers have been proposed for diagnosis and follow up of these diseases.[5] CA 125 is used world wide as a marker for detection and followup of ovarian cancer with some limitations.[8] At present, biomarkers unique for cancers of breast, colon, stomach, lung, and other organs are practically non-existent.

Changes in glycosylation are commonly observed in human carcinomas[9,10] and may contribute to the malignant phenotype downstream of certain oncogenic events. Carbohydrate antigens associated with cancer can be divided into three major classes: (i) Glycosphingolipids of the ganglio and globo series. (ii) Lacto series type 1 (Galβ1,3GlcNAc) and type 2 (Galβ1,4GlcNAc) chains carrying sialyl Le^a and sialyl Le^x determinants that can occur in both tumor-associated glycolipids and glycoproteins. (iii) Tumor-associated glycoprotein antigens may be either *N*- or *O*-glycans. Recent studies emphasize the importance of understanding the structure of carbohydrates present in tumor tissue for two clinical needs. In one aspect, antibodies against unique carbohydrates in tumor-associated antigens, especially those secreted into blood, may provide new and better biomarkers. Secondly, glycans uniquely expressed on cancer cells provide targets for novel cancer therapy. The levels of glycosyltransferase activities are altered in cancer cells and hence target structures can be selected on an enzymatic basis considering the specificity of the particular enzymes involved in the assembly of these complex carbohydrate antigens. Detailed structural determination of carbohydrate chains of already known tumor associated antigens has shown the existence of both *N*- and *O*-glycan chains in CA 125,[11] a glycosylation difference between prostate cancer cell PSA and normal PSA,[12–15] and a difference in *N*-glycan chains of human pancreatic ribonuclease isolated from healthy pancreas versus pancreatic cancer.[16] It has become apparent from these structural analyses of glycans that major changes are at the outer end of carbohydrate chains in cancer-associated antigens. This has prompted the use of lectins to detect the outer-end glycosylation changes of tumor-associated glycoconjugates. The changes in the activities of glycosyltransferases reflect the structures of glycans expressed by cancer cells. Thus, a knowledge of glycosyltransferase activities in cancer cells can reveal valuable information about the structures likely to be expressed by cancer cells and allows the identification and selection of better carbohydrate epitopes for immunohistochemical detection of cancer. Tumor-associated mucins from breast, pancreas, and colon carcinomas are known to contain mucin core 2 branched structures.[17,18] The target epitopes that can occur as part of core 2 O-linked glycoproteins associated with cancers can be identified by measuring the activities of various glycosyltransferases on defined acceptors. Mucin core 2 structure Galβ1,3(GlcNAcβ1,6)GalNAcα-Ser/Thr serves as a carrier of selectin ligands.[19] The malignant potential of a tumor cell is yet to be defined in terms of the relative amounts of sialyl Lewis^x and sialyl Lewis^a, which serves as the selectin ligands to that of sialyl Tn and Tn epitopes expressed by the same tumor cell.[20,21] As cancer-associated specific changes in the cellular repertoire of glycosyltransferases define the final glycosylation profiles of glycoconjugates,[22] the present study was undertaken to examine the patterns of various glycosyltransferase activities in several cancer cells and then verify these patterns in two tumor tissues. The results of the present study strongly suggest an association of unique carbohydrate structures with signature potential in individual cancers.

2. Experimental

2.1. Origin of various cell lines

2.1.1. Breast cancer cell lines. BT20 was established by cultivation of spilling cells from thin breast tumor slices from a 74-year-old female patient. DU4475 was derived from metastatic cutaneous nodule of a 70-year-old female patient with advanced breast cancer. MCF-7, MDA-MB-231, T47D, and ZR-75-1 were established by cultivation of cells from the pleural effusion of female breast cancer patients. MDA-MB-435S was evolved from an estrogen receptor-negative, invasive, and metastatic MDA-MB-435S cell line isolated from the pleural effusion of a 31-year-old female with metastatic ductal adenocarcinoma of the breast and were not tumorigenic in athymic nude mice. MDA-435/LCC6 was derived from the spontaneous ascites of MDA-MB-435 in nude mice. They grow as both malignant ascites and solid tumors in vivo in nude mice and nude rats. MDA-435/LCC6^{MDR1} (the classical MDR1 resistance pattern) was isolated by transduction of rapidly proliferating MDA-435/LCC6 cells in vitro with an amphotropic retrovirus containing the coding sequence of the full-length MDR1 cDNA. Recently, both homotypic and heterotypic intercellular adhesions of MDA-MB-435 cells under conditions of flow in vivo and in vitro were shown to be mediated by the interactions between T-antigen and galectin-3 expressed by MDA-MB-435 cells.[24]

2.1.2. Colon cancer cell line. Colo205 was isolated from ascitic fluid of a 70-year-old male with colon carcinoma. LS180 was derived from the colon tumor of a 58-year-old female with Duke's type B colon adenocarcinoma. SW1116 was derived from a Grade II colon adenocarcinoma of a 73-year-old male.

2.1.3. Ovarian cancer cell lines. SW626 was initiated from a poorly differentiated cystadenocarcinoma of the

ovary taken from a 46-year-old female. PA-1 was obtained by culturing ascitic fluid cells from a 12-year-old female with teratocarcinoma of the ovary.

2.1.4. Hepatic carcinoma cell line. HepG2 was derived from hepatocellular carcinoma tissue of a 15-year-old male.

2.2. Culturing of cells

The cells were cultivated in tissue culture as described earlier.[23] The colon carcinoma cell line, Colo205, the hepatic carcinoma cell line, $HepG_2$, the breast carcinoma cell lines, BT20 and MCF-7, and the ovarian teratocarcinoma cell line, PA-1, were grown in minimal essential medium; the colon carcinoma cell line, LS180, and the breast carcinoma cell lines, DU4475, T4TD, and ZR-75-1 were grown in RPMI 1640; the colon carcinoma cell line, SW1116, the ovarian carcinoma cell line, SW626, the breast carcinoma cell lines, MDA-MB-231, MDA-MB-435S, MDA-435/LCC6, and MDA-435/LCC6^{MDR1} were grown in Leibovitz's L-15 medium. All media were supplemented with 10% fetal bovine serum and the antibiotics per milliliter, penicillin (10 U), streptomycin (10 μg), and amphotericin B (0.25 μg) in 250 mL T-flasks under conditions as recommended by American Type Culture Collection, except for DU4475, which was grown as a suspension. MDA-435/LCC6 and MDA-435/LCC6^{MDR1} were kindly provided by Dr. Ralph Bernacki of this Institute.

2.3. Extraction of cells

The cells were homogenized with 0.1 M Tris–Maleate pH 7.2 containing 2% Triton X-100 using a Dounce all-glass, hand-operated homogenizer. The homogenate was centrifuged at 16,000*g* for 1 h at 4 °C. Protein was measured on the supernatants by the BCA micromethod (Pierce Chemical Co.) with BSA as the standard. The supernatants were adjusted to 5 mg protein/mL by adding the necessary amount of extraction buffer and then stored frozen at −20 °C until use.[23] Aliquots (10 μL) of the extracts were used in assays run in duplicate.

2.4. Acceptor compounds

The chemical synthesis of acceptors in the present study have already been published.[25,26] GlcNAcβ1,4GlcNAcβ-O-Bn was obtained from Toronto Research Chemicals. Fetuin triantennary glycopeptides were available from our earlier studies.[27,28] Highly purified ancrod, 35 kDa, a glycoprotein containing exclusively NeuAcα2,3Galβ1,3GlcNAcβ-terminating *N*-glycan chains, was a generous gift from Dr. Chris Nolan of Abbott Laboratories (Chicago, IL).[29]

2.5. Tumor tissues

Colon tumor (CC9338) and normal colon (14,891) tissues and breast tumor (BC9400) and normal breast (14,445) tissues were obtained during surgical procedures at Roswell Park Cancer Institute and stored frozen within 1 h at −70 °C. The tissues were homogenized at 4 °C with 4 vols of 0.1 M Tris–Maleate pH 7.2 0.1% NaN_3 using kinematica. After adjusting the concentration of TritonX-100 to 2%, these homogenates were mixed in the cold room for 1 h using Speci-Mix (Thermolyne) and then centrifuged at 20,000*g* for 1 h at 4 °C. The clear, fat-free supernatant was stored frozen at −20 °C until use. Aliquots of 10 μL from this extract were used in assays run in duplicates. Glycosyltransferase activity in cell lysate was determined by mixing the lysates with acceptor and radiolabeled donor (monosaccharide) under the reaction conditions detailed below, followed by separation of unreacted donor from the radioactive product using anionic or hydrophobic chromatography. In all cases, the radioactive content of isolated products was determined by using 3a70 scintillation cocktail (Research Products International, Mount Prospect, IL) and a Beckman LS6500 scintillation counter. Controls for each assay contained the reaction mixture with everything except the acceptor. Radioactivity of product was subtracted from that of control to obtain the results presented in the tables. All assays were run in duplicate. Results from duplicate runs did not vary by more than 5%.

The acceptors used for measuring the glycosyltransferase and sulfotransferase activities are given in Table 1. The following are the conditions for individual enzymatic assays. Reaction temperature in all cases was 37 °C. α2,3- and α2,6 sialyltransferase (ST) assay reactions proceeded for 2 h in a mixture containing 100 mM sodium cacodylate buffer (pH 6.0), 7.5 mM acceptor, CMP-[9-^{3}H]NeuAc (typically 0.2 μCi), and 10 μL of cell extract in a total volume of 20 μL.[30] βGlcNAc:β1,4Gal-T and αGalNAc:β1,3Gal-T assay mixtures in duplicate contained 0.1 M Hepes–NaOH pH 7.0, 7 mM ATP, 20 mM Mn acetate, 1 mM UDP-Gal, UDP [^{14}C]Gal (0.05 μCi; 327 mCi/mmol; Amersham), 0.5 mM acceptor (unless otherwise stated) and the enzyme in a total volume of 20 μL. It was incubated for 4 h.[30] βGlcNAc:β1,4GalNAc-T assay mixtures in duplicate contained 0.1 M Hepes–NaOH pH 7.0, 7 mM ATP, 20 mM Mn acetate. UDP [^{3}H] GalNAc (0.20 μCi; 7.8 Ci/mmol: New England Nuclear Corp.) 7.5 mM acceptor (unless otherwise stated) and the enzyme in a total volume of 20 μL and incubated for 4 h.[31]

α1,2, α1,6-, α1,3-, and α1,4-fucosyltransferase (FT) assay reactions were carried out for 2 h in a reaction mixture containing 50 mM Hepes buffer (pH 7.5), 5 mM $MnCl_2$, 7 mM ATP, 3 mM NaN_3, 3 mM

Table 1. Acceptors used for assaying the enzymes in cancer cell lines

Enzyme	Acceptor used for measuring the enzyme activity
Sialyltransferase	
α2,3-Sia-TI or TII (ST3:Galβ1,3GalNAc)	Gal3Meβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn
α2,3-Sia-TIV (ST3:Galβ1,4GlcNAc)	Galβ1,4GlcNAcβ1,6(Gal3Meβ1,3)GalNAcα-O-Bn Gal2Meβ1,3GlcNAcβ-O-Bn
α2,6-Sia-TI (ST6:Galβ1,4GlcNAc)	Galβ1,4GlcNAcβ1,6(Gal3Meβ1,3)GalNAcα-O-Bn
Gal-/GalNAc-transferase	
β-GlcNAc:β1,3/4-Gal-T	Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn
β-GlcNAc:β1,3/4-GalNAc-T	Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn
α-GalNAc:β1,3-Gal-T	GlcNAc4Fβ1,6GalNAcα-O-Bn
Fucosyltransferase	
α1,2-Fuc-T	Galβ-O-Bn
α1,3-Fuc-T	Gal2Meβ1,4GlcNAc
α1,4-Fuc-T	Gal2Meβ1,3GlcNAc Fetuin triantennary asialo glycopeptide Ancrod
Fuc-TVI	GlcNAcβ1,4GlcNAcβ-O-Bn
Fuc-TVII	Fetuin triantennary sialo glycopeptide
α1,6-Fuc-T	Fetuin triantennary asialo agalacto glycopeptide
Sulfotransferase	
Gal3-*O*-Sulfo-T_2 or T_3	Galβ1,4GlcNAcβ1,6(Gal3Meβ1,3)GalNAcα-O-Bn
Gal3-*O*-Sulfo-T_4	Gal3Meβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn
GlcNAc6-*O*-Sulfo-T	Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn

synthetic acceptor or 40 μg of fetuin-based acceptor, 0.05 μCi GDP-[14C]Fuc (290 mCi/mmol) and 10 μL cell extract in a total volume of 20 μL.[29] Sulfotransferase (Sulfo T) assay reactions took 2 h and required a mixture containing 100 mM Tris–Maleate (pH 7.2), 5 mM Mg Acetate, 5 mM ATP, 10 mM NaF, 10 mM BAL, 7.5 mM acceptor, 0.5 μCi of [^{35}S]PAPS (specific activity 2.4 Ci/mmol), and 10 μL of cell extract in a total volume of 30 μL.[32]

Dowex-1 [Cl^-] or Sep-Pak C_{18} cartridges were used to isolate radiolabeled product from the reaction mixture. For GalT, GalNAc-T, and FT assays, the incubation mixture was diluted with 1 mL of water and passed through a 1-mL Dowex-1 [Cl^-] column.[29,33] The column was washed twice with 1 mL of water. The breakthrough and the water wash contained the [^{14}C]-galactosylated or [^{14}C]-fucosylated products formed with neutral acceptors. NaCl (3 mL of 0.1 M) was used to obtain [^{14}C]-fucosylated products from sialylated acceptors after water elution. For sialyltransferase assays, the radioactive products from benzylglycosides were separated by hydrophobic chromatography on a Sep-Pak C_{18} cartridge (Waters, Milford, MA), and elution of the product was done with 3 mL of MeOH.[34] For sulfotransferase assays, elution of the [^{35}S]-sulfated compound from the Dowex-1 [Cl^-] column was achieved by 3 mL of 0.2 M NaCl.[32]

3. Results and discussion

3.1. *O*-Glycans in breast cancer cell lines

Several glycosyltransferases and Gal3-*O*-sulfotransferases are capable of elongating the mucin core 2 tetrasaccharide as well as the Globo backbone unit. Such action of these enzymes could lead to a complexity of cancer associated terminal glycan structures, as illustrated in Figure 1.

Müller and Hanisch[22] expressed a MUC1 fusion protein MFP6 in the breast cancer cell lines ZR-75-1, MDA-MB-231, MCF-7, and T47D and studied the *O*-glycans of the fusion proteins after releasing by hydrazinolysis and then labeling with 2-aminobenzamide. In order to test the hypothesis of predicting glycan signatures from the pattern of glycosyltransferase activities, we first examined the levels of activities of various enzymes in these four cell lines, followed by other cell lines as reported in Table 2. The levels of the carbohydrate structures likely to arise from these enzyme activities are presented in Table 3. Müller and Hanisch[22] found that NeuAcα2,3Galβ1,3GalNAcα- units constituted the major structure of MFP6 proteins expressed in T47D (68.6%), MDA-MB-231 (65.5%), and ZR-75-1 (86.1%). Our present study also showed that α2,3-Sia-TI or TII was the most dominant enzyme as compared to α2,3-

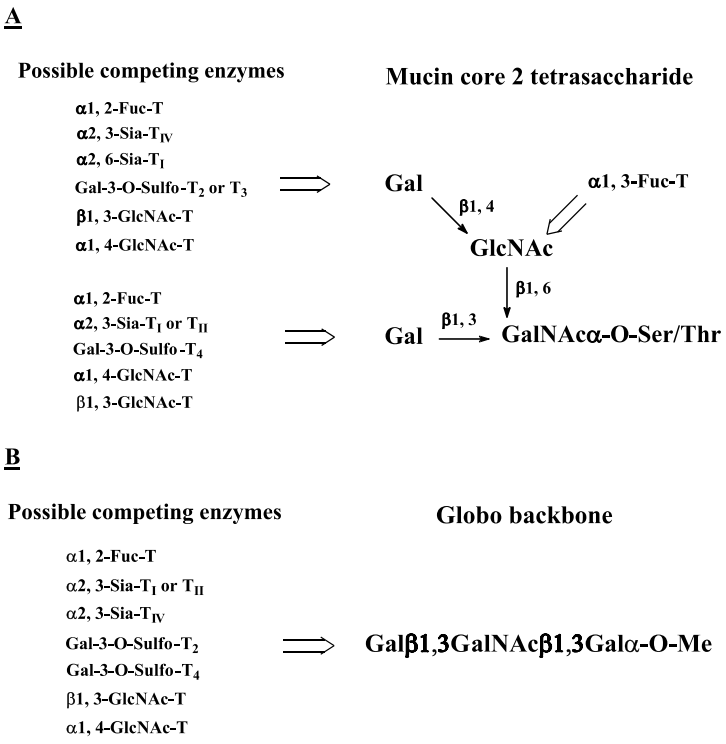

Figure 1. Glycosyl- and sulfotransferases acting on the mucin core 2 tetrasaccharide and the Globo backbone.

Table 2. Sialyl-, fucosyl-, β-galactosyl-, β-*N*-acetylgalactosaminyl-, and glycan-3-*O*-sulfotransferase activities in breast cancer cell lines as determined by the incorporation of radioactive sugar or [^{35}S] sulfate [CPM × 10^{-3}] catalyzed by 1 mg of protein cell extract

ENZYME	MDA-MB-231	MCF-7	T47D	ZR-75-1	DU4475	BT20	MDA-MB435S	MDA-435/LCC6	MDA-435/LCC6 MDR1
Sialyltransferases									
α2,3(O)ST	126.5	105.2	86.8	368.1	8.7	197.8	257.4	171.6	159.1
α2,3(N)ST	10.6	9.4	4.2	12.3	2.4	18.3	26.3	18.4	17.7
α2,6(N)ST	0	3.4	8.6	74.1	0	0	86.5	56.0	64.1
Fucosyltransferases									
α1,2-L-FT	0.4	2.9	1.2	0.3	46.9	0.4	0	0	0.1
α1,3-L-FT	30.5	48.1	40.6	105.3	60.4	20.8	0	0	0
α1,4-L-FT	0.5	1.1	0.5	156.5	255.1	241.5	0	0	0
FTVI	0	0.4	0.3	0	1.9	0	2.5	0	0
α1,6-L-FT	631.2	362.0	127.3	612.4	611.4	205.6	496.6	444.9	447.8
FetTA-AsialoGP	200.1	195.1	ND	ND	221.6	105.4	96.1	99.9	103.8
FetTA-SialoGP	0	0	ND	ND	31.7	9.6	0	0	0
Ancrod	0	0	ND	ND	33.8	41.3	0	0	0
βGal/GalNAc-Transferases									
GlcNAc:β1,3/4Gal-T	494.4	284.9	358.1	552.7	155.5	260.7	80.1	400.1	295.8
GlcNAc:β1,3/4GalNAc-T	152.1	13.4	ND	ND	103.3	33.1	34.7	86.2	65.7
αGalNAc:β1,3Gal-T	28.8	23.3	30.6	36.3	47.4	35.6	11.1	45.3	43.6
Glycan:sulfotransferases									
Galβ1,3GalNAcα-:3-*O*-Sulfo-T	1.2	3.4	0.3	2.1	51.8	<0.1	7.2	62.6	28.1
Galβ1,4GlcNAcβ:3-*O*-Sulfo-T	0.2	19.4	0.3	19.1	361.2	0.3	0.1	0.5	0.3
GlcNAc:6-*O*-Sulfo-T	0.3	0	0	0.2	0.2	0.1	0	0.2	0.2

ND: Not determined.

sia-TIV in all these cell lines. They also observed in MCF-7 that sialylated carbohydrate chains comprise only 5.2% of the *O*-glycan chains. The present study finds that except for ZR-75-1, which exhibits high sialyltransferase activities, T47D, MDA-MB-231, and MCF-7 expressed almost the same level of α2,3-sia-TI or TII.

Table 3. A comparison among several cancer cell lines for the potential signature carbohydrate structure predicted solely based on the enzyme activity data

The predicted carbohydrate structural unit	Breast cancer cell lines									Colon cancer cell lines			Ovarian cancer cell lines		Hepatic cancer cell line
	MDA-MB-231	MCF-7	T47D	ZR-75-1	DU4475	BT20	MDA-MB-435S	MDA-435/LCC6	MDA-435/LCC6MDR1	Colo205	LS180	SW1116	SW626	PA-1	HepG2
(i) 6-Fucosyl chitobiose core GlcNacβ1,4(Fucα1,6)GlcNAc	5X	3X	X	5X	5X	2X	4X	4X	4X	4X	4X	4X	4X	4X	3X
(ii) 3-Fucosyl chitobiose core GlcNacβ1,4(Fucα1,3)GlcNAc	O	T	T	O	T	O	T	O	O	9X	16X	X	O	O	O
(iii) Lewis[a] Galβ1,3(Fucα1,4)GlcNAc	O	O	O	6X	10X	10X	O	O	O	13X	9X	4X	X	O	O
(iv) Lewis[x] Galβ1,4(Fucα1,3)GlcNAc	X[a]	2X	2X	4X	3X	X	O	O	O	9X	9X	8X	3X	3X	T
(v) 3′-Sialyl T-hapten NeuAcα2,3Galβ1,3GalNAcα	4X	3X	3X	12X	T	6X	9X	6X	6X	X	3X	3X	T	T	6X
(vi) 3′-Sulfo Lewis[x] 3-*O*-SulfoGalβ1,4(Fucα1,3)-GlcNAc	O	X	O	X	18X	O	O	O	O	T	10X	5X	X	O	ND[c]
(vii) 3′-Sialyl Lewis[x] NeuAcα2,3Galβ1,4(Fucα1,3)-GlcNAc	T[b]	T	T	T	O	T	O	O	O	T	T	T	O	O	T
(viii) 3′-Sialyl Lewis[a] NeuAcα2,3Galβ1,3(Fucα1,4)-GlcNAc	O	O	O	T	T	T	O	O	O	T	T	T	O	O	O
(ix) T-hapten Galβ1,3GalNAcα	T	T	T	T	T	T	T	T	T	O	O	O	X	X	T
(x) 3′-Sulfo T-hapten 3-*O*-SulfoGalβ1,3GalNAcα	O	O	O	O	7X	O	X	9X	4X	T	5X	3X	T	O	ND
(xi) 3′-Sialyl LacNAc NeuAcα2,3Galβ1,4GlcNAc	O	O	O	O	O	O	X	X	X	T	T	T	O	O	X
(xii) 6′-Sialyl LacNAc NeuAcα2,6Galβ1,4GlcNAc	O	O	O	X	O	O	X	X	X	T	O	O	O	O	5X
(xiii) Lewis[b] Fucα1,2Galβ1,3(Fucα1,4)-GlcNAc	O	O	O	O	X	O	O	O	O	T	T	T	T	T	O
(xiv) Lewis[y] Fucα1,2Galβ1,4(Fucα1,3)-GlcNAc	O	O	O	O	X	O	O	O	O	T	T	T	T	T	O

[a] X denotes the lowest concentration that was considered as 1-fold when a comparison of a particular structure was made among all the cancer cell lines studied here.
[b] T refers to trace amount.
[c] ND—Not determined.

MDA-MB231 and ZR75-1 cell extracts contain α2,3-Sia-T IV enzyme that constructs the NeuAc2,3Galβ1,4GlcNAc and Müller and Hanisch[22] reported this sequence in the oligosaccharide chains of *O*-glycans of cell lines MDA-MB231 and ZR75-1 but not in MCF-7 cell line. Our enzymatic studies have demonstrated that MCF-7 contains a low level of α2,3-Sia-T IV but expresses α1,2-L-FT and α1,3-L-FT capable of generating the Fucα1,2Gal and Fucα1,3GlcNAc linkages, respectively. Thus, the presence of these fucosyltransferases suggested the existence of Fucα1,2Galβ1,3GalNAc and Galβ1,4(Fucα,3)GlcNAc, the structures that were reported to be part of *O*-glycans in MCF-7.[22] A low level of α2,3-sialyl-T IV activity suggests the absence of sialyl Lewis^x structure in MCF-7, which agrees with another report.[35] However, the Lewis^x structure moiety linked at C-6 position of GalNAc is reported to be part of *O*-glycans in MCF-7. Overall the expression profiles of α(2→3)-sialyl-Ts and fucosyltransferases in our studies agree with the structures reported by Müller and Hanisch[22] for these breast cancer cell lines.

3.2. Sulfated *O*-glycans of cancer cells

We extended our study of determining the levels of these enzymes to some other breast cancer cell lines (see Table 2). As sulfotransferases and sialyltransferases can compete for the same site of acceptors (see Fig. 1), it became important to study sulfotransferases. Among the breast cell lines, DU4475 is unique as it has negligible sialyltransferase activities but a high level of 3-*O*-sulfotransferase specific for Galβ1,4GlcNAc. MCF-7 and ZR-75-1 also can express this enzyme at a low level. The presence of 1,3-FT would generate sulfo Lewis^x in these cell lines. MCF-7 contains both α1,2-L-FT and α1,3-L-FT activities that can generate the Lewis^y moiety, but it is not reported in *O*-glycans.[22] Gal3-*O*-sulfo-T seems to prevent the action of α1,2-L-FT on Galβ1,4GlcNAc. The cell lines MDA-435/LCC6, MDA-435/LCC6^{MDR1} and even DU4475 have sulfotransferase activity that incorporate sulfate at the C-3 position of galactose in the T antigen to give 3′-sulfo-T-hapten. We were the first to report that a core 2 structure distinguishes the sulfotransferase activities in breast and colon cell lines.[36,37] Two distinct types of Gal-3-*O*-sulfotransferases were revealed. One that is specific for the Galβ1,3GalNAcα-moiety is expressed by breast cancer cell lines, and the other that shows preference for the Galβ1,4GlcNAc branch in mucin core 2 is present in colon cancer cell lines and in some breast cell lines.[37] In this context, the availability of modified analogs of core 2 tetrasaccharide has proven to be important in determining the uniqueness of these enzymes. The specificity of sulfotransferase in colon cancer cell lines and DU4475 breast cell line predicts the presence of 3-*O*-sulfo Galβ1,4GlcNAcβ1,6-(Galβ1,3)GalNAcα-. On the other hand, Gal3Sulfo T-4 cloned by Seko et al.[38] is present in breast cancer cell lines, synthesizing the Galβ1,4GlcNAcβ1,6(3-O-SulfoGalβ1,3)GalNAcα sequence. These core 2 structures would serve as acceptors for α(2→3)-sialyltransferase and α(1→3)L-fucosyltransferase to generate more complex carbohydrate structures. The levels of glycosyltransferase and sulfotransferase activities in colon and, as a comparison that of ovarian cancer cell lines are reported in Table 4. A comparison of the levels of the predicted carbohydrate structures arising from these enzyme activities is presented in Table 3. Three colon cell lines we have tested had very low α1,2-FT activity but a high level of both α1,3- and α,14-FT activities, the α1,4-FT activity being highest in Colo205. They all expressed FTVI activity, the level being highest in LS180. Colo205 showed a very weak expression of 3-*O*-sulfotransferase activity toward Galβ1,4GlcNAcβ. On the contrary, high levels of this enzyme activity are present in LS180 and SW1116. Since the levels of both α2,3-sia-TIV and α1,2-FT activities appear to be low, in all cases, this would suggest that the Galβ1,4GlcNAcβ arm in the core 2 tetrasaccharide is prone to the action of Gal-3-*O*-sulfotransferase to give 3-*O*-sulfoGalβ1,4GlcNAcβ. The dominancy of the 3′-sulfo Lewis^x determinant in LS180 and SW1116 can be expected as these cells contain a high level of α1,3-L-fucosyltransferase activity. The enzyme α2,3-sia-TI or TII activity is dominated in LS180 and SW1116. The occurrence of the 3′-sulfo Lewis^x determinant has also been demonstrated in mucin glycoprotein expressed by LS174T-HM7, a highly metastatic subline of colon carcinoma LS174T.[39] The expression of a di-*O*-sulfo mucin core 2 tetrasaccharide seems to be favorable with DU4475, since it lacks all sialyltransferase activities (see Fig. 1 for biosynthesis).

Our present findings demonstrate the power of well-defined acceptors for studying these enzymes. Brown et al.[40] analyzed mRNA from LS180 cells using GlycoV1 Genechip microarrays and detected only α2,3-Sia-TIV. On the contrary, the present study identified α2,3-Sia-TI or TII as the overwhelming sialyltransferase activity, not only in LS180, but also in other colon cell lines Colo205 and SW1116. In agreement with our present data on fucosyltransferase activities, they also detected FTIII and FTVI in LS180 cells. But the presence of FTVII in colon cancer cells could not be ruled out, when considering that Fetuin triant sialylglycopeptide acted as a good acceptor for the FTs of colon cancer cell lines. Glycosyltransferase expression in human colonic tissues was examined by Kemmner et al.[41] using oligonucleotide microarrays. Their restricted analysis of 39 glycosyltransferases present on the Genechip U95A indicated that sialyltransferases α2,6-Sia-TI, α2,3-Sia-TIV, fucosyltransferases FTIII, FTVI, and FTVIII(α1,6-L-FT) and also GalNAcT-1(polypeptide:GalNAc-T) and β1,4GalT-2 may be responsible for the aberrant biosynthesis of carbohydrates in colonic

Table 4. Sialyl-, fucosyl-, β-galactosyl-, β-*N*-acetylgalactosaminyl-, and glycan:3-*O*-sulfotransferase activities of colon cancer, ovarian cancer, leukemia, and hepatic cancer cell lines as determined by the incorporation of radioactive sugar or [^{35}S] sulfate [CPM × 10^{-3}] catalyzed by 1mg protein of the cell extract

Enzyme	Colon cancer			Ovarian cancer		Hepatic cancer
	Colo205	LS180	SW1116	SW626	PA-1	HepG2
Sialyltransferases						
α2,3(O)ST	34.0	86.7	95.9	9.0	5.0	185.7
α2,3(N)ST	3.5	7.4	7.5	1.3	1.3	22.6
α2,6(N)ST	4.2	0	0	0	0	362.8
Fucosyltransferases						
α1,2-L-FT	3.8	5.5	3.4	2.7	0.9	0
α1,3-L-FT	267.7	257.8	223.7	88.7	86.7	11.8
α1,4-L-FT	319.0	210.8	89.4	24.0	0.8	0
FTVI	101.3	180.3	11.8	2.3	0.8	0
α,6-L-FT	413.6	350.9	343.9	438.9	443.5	292.0
FetTA-AsialoGP	391.7	399.7	199.0	165.4	148.9	39.6
FetTA-SialoGP	227.2	251.2	61.1	0	0	43.6
Ancrod	77.5	75.0	16.5	0	0	0
β-Gal/GalNAc-Transferases						
GlcNAc:β1,3/4Gal-T	297.5	226.6	503.4	478.2	187.0	478.3
GlcNAc:β1,3/4GalNAc-T	27.6	73.0	171.5	142.8	75.5	148.1
α-GalNAc:β1,3Gal-T	31.5	23.7	62.2	65.4	29.5	146.3
Glycan:sulfotransferases						
Galβ1,3GalNAcα-:3-*O*-Sulfo-T	1.3	33.4	17.3	2.5	0.8	ND
Galβ1,4GlcNAcβ-:3-*O*-Sulfo-T	6.4	216.0	92.5	15.7	0	ND
GlcNAc:6-*O*-Sulfo-T	0.1	0.2	0.4	0	ND	ND

carcinogenesis and metastasis. Their findings seem to fit with the present data on the pattern of most of the glycosyltransferase activities obtained for colon cancer cell lines.

3.3. Unique specificity of fucosyl-, sialyl-, and sulfotransferases in shaping the terminal end of oligosaccharide chains in glycolipids, *N*- and *O*-glycans

We investigated the specificity of α(2,3)-Sia-T, Gal-3-*O*-sulfo-T, and α1,2-Fuc-T using the core 2 branched structure and the Galβ1,3GalNAcβ1,3Galα- sequence that occurs in glycolipids as a Globo H precursor (see Fig. 1). The Globo H antigen was originally characterized by Hakomori and co-workers in a human breast cancer cell line.[42] Immunostaining using murine MoAb MBr1 demonstrated this antigen in other human tissues including prostate cancer and small-cell lung carcinoma. We reported that α1,2-Fuc-T in LNCaP cells acts more efficiently (4-fold) on the mucin core 2 Galβ1,4GlcNAcβ unit as well as on the Globo H precursor trisaccharide as compared to its activity toward Galβ1,3GalNAcβ- arm in mucin core-2.[43] In another study, we examined the specificity of three cloned Gal-3-sulfotransferases.[32] Gal3SulfoT-2, which is known to incorporate sulfate at C-3 position of galactose in Galβ1,4GlcNAcβ, behaves similarly to α1,2-Fuc-T transferase and α2,3-Sia-TIV toward these substrates; it acts with the same efficiency on both the Globo trisaccharide and core-2 tetrasaccharide. Gal-3-sulfoT4 can also utilize the Globo H precursor to the same extent as Galβ1,3GalNAcα-. α2,3-Sia-TI or TII generates NeuAcα2,3Galβ1,3GalNAc- in mucin and also acts on the Globo H precursor to give the NeuAcα2,3Galβ1,3GalNAcβ1, 3Galα→sequence. α2,3-Sia-TII has been reported to be involved in the biosynthesis of NeuAcα2,3Galβ1,3GalNAcβ1,3Galα,3Galβ1, 4GlcβCer.[44,45] There is some controversy on the specificity of α2,3-Sia-TIV.[46] Some groups report that this enzyme is involved in the synthesis of NeuAc2, 3Galβ1,3GalNAcβ in glycolipids, while other groups suggest that it can generate NeuAc2,3Galβ1,4GlcNAcβ followed by fucosylation to give SLe^x.[47,48] Our studies[49] demonstrate clearly the specificity of α2,3-Sia-TIV toward the mucin core 2 Galβ1,4GlcNAc unit as well as the Galβ1,3GalNAcβ1,3Galα- sequence.

Our study indicates three types of sulfated carbohydrate epitopes, 3-*O*-sulfoGalβ1,3GalNAcβ1,3Galα-, 3-*O*-sulfoGalβ1,3GalNAcα- and 3-*O*-sulfoGalβ1,4GlcNAcβ as the signatures of cancer cells. The 3-*O*-sulfo-T activity toward Galβ1, 4GlcNAcβ- in DU4475 is 18-fold that of MCF-7 and ZR-75-1, which are the only other two breast cancer cell lines expressing this activity. These results would indicate the signature carbohydrate structure expressed by DU4475 as 3′-Sulfo Lewis^x. It is interesting to note that the tumorigenic breast cell lines MDA-MB-435/LCC6 and MDA-MB-435/LCC6^{MDR1} exhibit about 9- and 4-fold Gal-3-*O*-sulfotransferase activity specific for Galβ1,3 GalNAcα- unit as compared to its level in the non-tumorigenic parent cell line MDA-MB-435S. Further, the former cell lines contain 4–5-fold

GlcNAc:β1,4Gal-T activity and less sialyltransferase activities as compared to the latter. Thus it appears that an increase in Gal-3-*O*-sulfotransferase with a concomitant decrease in Gal-3-*O*-sialyltransferase acting on Galβ1,3GalNAcα- has a relationship to the tumorigenic potential of breast cancer cell lines. The absence of FT activities in MDA-MB-435 series would suggest that 3-*O*-sulfoGalβ1,3GalNAcα- as well as 3-*O*-sulfoGalβ1,4GlcNAcβ could be signature structures of tumorigenic breast cancer cells.

For comparison purposes, the enzyme activity levels of two ovarian cancer cell lines SW626 and PA-1 (Table 4) were examined, and the predicted carbohydrate structures are presented in Table 3. These cell lines contain only a small amount of sialyltransferase and glycan sulfotransferase activities and considerably a low level of α1,3-L-fucosyltransferase activity as compared to the colon cancer cell lines, indicating that the biosynthesis of polylactosamine chain is quite favored in these cell lines. The hepatic cancer cell line HepG2 appears to be capable of synthesizing α2,6-sialylated *N*-glycans and α2,3-sialyl T-hapten. It is interesting to note that, except for the occurrence of a significant level of α1,6-L-FT activity, other FT activities are either negligible or absent in HepG2 thus indicating the existence of a reciprocal relationship in the expression of α2,6(*N*)sialyltransferase and α1,2- and α1,3/4-L-fucosyltransferases. From the level of α1,6-L-FT activity it is apparent that, as compared to T47D cell line, the other breast cell lines BT20, MCF-7, and MDA-MB-435 series, respectively, express about 2-, 3-, and 4-fold and the rest, namely MDA-MB-231, DU4475, and ZR-75-1, express about 5-fold α1,6-fucosylated *N*-glycans. The colon cancer cells Colo205, LS180, and SW1116 contained almost the same level of α1,6-FT activity, indicating the presence of same amount of α1,6-fucosylated *N*-glycans. Both sialo and asialo fetuin triantennary glycopeptides served as good acceptors for the fucosyltransferases of the colon cell lines, indicating the facile synthesis of sialyl Lewis^x and sialyl Lewis^a. Ancrod was also utilized as an acceptor that would further indicate the formation of sialyl Lewis^a. Hence, Colo205, LS180, and SW1116 could express sialyl Lewis^a, sialyl Lewis^x and sialyl T-hapten.

3.4. Signatures of glycans

3.4.1. The patterns of glycosyltransferase activities in the two tumor tissues examined closely resemble that of the parent cancer cells (see Table 5). When compared to a colon tumor specimen, normal colon tissue examined contained significantly very low levels of sialyltransferase as well as α1,2-FT activities. Further, lower levels of α1,3-FT, α1,4-FT, and FTVI as well as GlcNAc:β1,4Gal-T and αGalNAc:β1,3Gal-T activities in the normal colon tissue were also noticed. In comparison to breast tumor, all the glycosyltransferase activities examined in the normal breast tissue specimen were found to be extremely low. The colon tumor in comparison to the breast tumor had 18-fold α1,3-FT activity. The α1,4-FT activity in breast tumor was comparatively negligible; but there was a significant level of α1,2-FT activity. These FT activities thus mirrored the *O*-glycan chains from MUC1 fusion protein of MCF-7 reported by Müller and Hanisch. In contrast to the presence of Galβ1,4GlcNAc utilizing Gal-3-*O*-sulfotransferase in MCF-7, ZR-75-1, and DU4475, the other sulfo-T specific for Galβ1,3GalNAc was found in this breast tumor. We identified the latter enzyme in MDA-MB-435 series and DU4475 and in a number of breast tumor specimens in an earlier study.[37]

It is interesting to note that the tumorigenic cell lines MDA-435/LCC6 and MDA-435/LCC6^{MDR1} as compared to the non-tumorigenic parent cell line MDA-MB-435S express 4–5-fold GlcNAc:β1,3/4Gal-T activity; DU4475 expressing negligible amount of sialyltransferase activities, as well as a low level of GlcNAcβ1,3/4Gal-T activity as compared to other cell lines, contains a high level of GlcNAc:β1,3/4GalNAc-T activity as well as α-GalNAc:β1,3Gal-T activity. In this context, it makes sense in comparing this data to the situation in breast tumor. As compared to the colon tumor specimen, the breast tumor specimen showed a high level of GlcNAc:β1,3/4GalNAc-T activity and a low level of α2,3(O)ST and α-GalNAc:β1,3Gal-T activities.

Our analysis of glycosyltransferase activities in a colon tumor tissue (see Table 4) indicated a pattern of fucosyltransferase activities akin to that of colon cancer cells being present in this tissue. From the levels of α1,2-FT activity in tumor specimens as measured with four different acceptors (see Table 5), it became evident that Globo-based acceptors, namely Galβ1,3GalNAcβ1,3Galα-O-Me and D-Fucβ1,3GalNAcβ1,3Galα-O-Me are better acceptors than the Core 1 acceptor Galβ1,3GalNAcα-O-Al and Galβ-*O*-Bn for tumor α1,2-FT. Further, the dominance of Gal-3-*O*-sulfotransferase activity specific for Galβ1,4GlcNAc over α2,3(*N*)sialyltransferase activity in colon cancer cell lines and colon tumor tissue would suggest that 3-sulfo Lewis^x terminal carbohydrate would be a signature carbohydrate structure associated with colon cancer.

Further, it is quite interesting to note that while the breast tumor tissue akin to breast cancer cell lines was almost devoid of FTVI activity, the colon tumor tissue contained a significant level of this activity thus resembling the colon cancer cell lines. It appears that *N*-glycans with a 3-fucosylchitobiose core could be a signature carbohydrate structure associated with colon cancer.

3.4.2. Sialyl and sulfo groups as modulators of glycan function in cancer. Seko et al.[50] reported that Gal3-Sulfo-T_2 is the dominant Gal3Sulfo-T in normal colon and

Table 5. Sialyl-, fucosyl-, β-galactosyl-, β-*N*-acetylgalactosaminyl-, and glycan-sulfotransferase activities in colon tumor versus normal colon tissue and breast tumor versus normal breast tissue as determined by incorporation of radioactive sugar or [^{35}S] sulfate [CPM × 10^{-3}] catalyzed by 1mg protein of the tissue extract

Enzyme	Colon tumor CC9338	Normal colon 14,891	Breast tumor BC9400	Normal breast 14,445
Sialyltransferases				
α2,3(O)ST	721.4	35.5	283.0	4.9
α2,3(N)ST	7.8	0.9	2.3	0.3
α2,6(N)ST	148.0	7.6	11.6	1.8
Fucosyltransferases				
α1,2-L-FT				
(i) Galβ-O-Bn	20.6	3.9	9.8	0
(ii) Galβ1,3GalNAcα-O-Al	36.8	4.1	5.5	
(iii) Galβ1,3GalNAcβ1,3Galα-O-Me	49.1	7.2	13.7	0.4
(iv) D-Fucβ1,3GalNAcβ1,3Galα-O-Me	89.8	7.3	16.2	0
α1,3-L-FT:Gal2Meβ1,4GlcNAc	540.9	503.9	30.5	0
α1,2-L-FT plus α1,4-L-FT:Galβ1, 3GlcNAcβ1,3Galβ-O-Me	578.9	328.6	16.8	
α1,4-L-FT:Gal2Meβ1,3GlcNAc	727.5	425.9	4.6	0
FTVI:GlcNAcβ1,4GlcNAcβ-O-Bn	139.1	117.8	0.3	0
β-Gal/GalNAc-Transferases				
(a) β-GlcNAc:β1,3/4Gal-T	421.0	131.0	403.4	0.5
(b) β-GlcNAc:β1,3/4GalNAc-T	85.3		153.2	
α-GalNAc:β1,3Gal-T	102.2	16.7	38.6	0.9
a and b by using other acceptors				
(i) GlcNAcβ1,6Manα-O-Al	a. 493.4	241.6	443.6	3.2
	b. 54.5		99.2	
(ii) GlcNAcβ1,2Manβ1,6Glcβ-O-Al	a. 277.7	99.2	370.5	0.3
	b. 25.6		57.9	
(iii) GlcNAcβ1,3Galβ-O-Me	a. 247.9		338.5	
	b. 20.9		60.9	
(iv) GlcNAcβ1,4GlcNAcβ-O-Bn	a. 307.6	106.5	436.7	1.1
	b. 18.9		57.8	
Glycan:sulfotransferases[a]				
Galβ1,3GalNAcα-:3-*O*-Sulfo-T	40.1		60.2	
Galβ1,4GlcNAcβ-:3-*O*-Sulfo-T	181.2		5.0	
GlcNAc:6-*O*-Sulfo-T	52.8		69.8	

[a] Our earlier study[36] found an elevation of Gal-3-*O*-Sulfo-T activities in colon and breast cancer by showing that three normal breast tissue specimens and six breast tumor specimens had Galβ1,3GalNAcα-3-*O*-Sulfo-T activities in the range of 0.2→18.9 and 36.1→301.7, respectively; seven normal colon tissue specimens and five colon tumor specimens contained Galβ1,4GlcNAcβ-3-*O*-Sulfo-T activities in the range of 2.6→35.8 and 129.0→346.9, respectively.

colon cancer. They also noticed a significantly lower level of this enzyme in non-mucinous adenocarcinoma.[50] We identified Gal3Sulfo-T_2 in colon carcinoma LS180 cells and also showed that it is more than 2-fold active with the Globo backbone Galβ1,3GalNAcβ1,3Galα- when compared to its activity toward Galβ1,4GlcNAcβ- unit in the mucin core 2 structure.[32] Immunohistochemical investigation of colon specific sulfomucins and sialyl Tn antigen in colorectal tumors at different stages of progression by Yamachika et al.[51] indicated that sulfomucins decrease and sialyl Tn antigen increases during tumor progression. Izawa et al.[52] found preferential expression of the major L-selectin ligand, sialyl 6-sulfo Lewis^x in non-malignant colonic epithelia and sialyl Lewis^x in cancer tissues. Their transfection experiments[52] showed that the determinant 6-sulfo Lewis^x was synthesized mainly by Fuc-TVI in colonic epithelia. Consistent with this data, we found that colon cancer cells LS180, SW1116, and Colo205 were unique in expressing Fuc-TVI, which is either absent or present in trace amounts in other cancer cell lines examined in this study.[23]

Capon et al.[39] identified a sulfated Lewis^x determinant as the major structural motif in mucins isolated from a nude mice xenograft tumor produced by LS174T-HMT, a highly metastatic subline of human colon carcinoma LS174T. Varki and his group[53,54] found that carcinoma growth and metastasis formation is attenuated in P-selectin-deficient mice, and all three selectins can bind to colon carcinoma cell lines in a calcium-dependent fashion. They also obtained evidence for the involvement of distinct selectin ligands on colon carcinoma mucins in mediating pathological interactions among platelets, leukocytes and endothelium.[55] On the contrary, Velcich et al.[56] found that mice that are genetically deficient in Muc2 apomucin, the most abundant

gastrointestinal mucin, develop colorectal cancer, indicating the involvement of Muc2 in the suppression of colorectal cancer.

Cancer cells are known to exhibit a metabolic shift from oxidative to elevated anaerobic glycolysis (the Warburg effect) to cope with hypoxic environments. Koike et al.[57] found that hypoxic culturing induced significantly the transcription of genes for Fuc-TVII and α2,3-Sia-TI in cancer cells and also induced a marked increase in the expression of the selectin ligands sialyl Lewis^x and sialyl Lewis^a determinants on the cell surface of colon cancer cells. Kannagi[58] suggested that the impairment of GlcNAc 6-sulfation and α2,6-sialylation upon malignant transformation facilitates the synthesis of sialyl Lewis$^{x/a}$ in colon cancer cells. It has long been known that an increase of sialylation and a decrease of sulfation of carbohydrate determinants are associated with malignant transformation of colonic epithelial cells. Thus, glycan sulfotransferases and sialyltransferases seem to play a vital role in the modulation of glycan function in cancer.

The present study has well documented the fact that α2,3(O)ST activity is the most predominant sialyltransferase activity that accounts for 70–90% of the total sialylating activity in breast and colon cancer cell lines as well as in the two tumor tissues that were examined. We have also found that cloned α2,3(O)ST (ST3 Gal II) can sialylate very efficiently not only T-hapten (Galβ1, 3GalNAcα-) and the Globo backbone (Galβ1,3GalNAcβ1,3Galα-) but also LacNac type 1 (Galβ1,3GlcNAcβ-).[49] We have shown previously[36,37] that Gal-3-*O*-sulfotransferases of human breast and colon tumor tissues, which act on Galβ1,3GalNAcα- and Galβ1, 4GlcNAcβ-, respectively, act poorly on Galβ1,3GlcNAcβ-. Thus, it appears that the formation of 3′-sialyl Lewis^a and 3′-sulfo Lewis^x could be the favorable events in colon cancer. Further the predominancy of α2,3(O)ST activity in breast and colon cancer cells precludes the possibility of mucin core 1 chain (Galβ1,3GalNAcα-O-Ser/Thr) elongation in these cells. But such a possibility seems to exist in ovarian cancer cells, as they express almost negligible amount of sialyltransferase activities.

A knowledge of glycoconjugate biosynthesis and the existing structures of glycans is helpful in interpreting the glycosyltransferase activity patterns. For example, α2,6-Sia-TI prefers LacNAc type II glycan branches located at the α(1,3)-linked mannose branch of *N*-glycans.[59] A lack of core 2 β1,6GlcNAc-T activity can lead to *O*-glycans having extended core 1.[60] Le^a, Le^b, and sialyl Le^a are known to be located at the C-3 branch from GalNAcα. A monoclonal antibody specific for 3-*O*-sulfo Lewis^a tetrasaccharide is capable of binding to sulfomucin, a high molecular weight glycoprotein in colon mucosa, suggesting the existence of 3-*O*-SulfoGalβ1,3(Fucα,4)GlcNAcβ1,3, Galβ1,3 as part of sulfomucin.[51] A decrease of this epitope in colon cancer is reported, whereas the expression of sulfo Lewis^x is not altered.[51] Thus, it becomes obvious that activities and specificity of the chain terminating enzymes such as fucosyl-, *N*-acetylgalactosaminyl, sialyl-, and sulfotransferases from cancer cells and tissues, along with a knowledge of oligosaccharide biosynthesis, would help to determine and select the carbohydrate epitopes present at the outer ends of known as well as unidentified cancer-associated antigens for immunological targeting. Thus several factors such as hydrolases, sugar nucleotide and PAPS synthases, sugar nucleotide and PAPS transporters to Golgi, etc. also determine glycosyl structures of cancer cells. Harvey,[61] in his article on matrix-assisted laser desorption/ionization (MALDI) mass spectrometry of carbohydrates and glycoconjugates, points out that a knowledge of glycotransferase activities plays an important role in structural determination by MS analysis. Thus a determination of the glycan structures of different cancer cells using systematic mass spectrometry becomes the obvious choice to conclude on the signature status of a glycan structure. Hence, work along this line becomes the focus of this laboratory.

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