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Exploring the Utility of Carbohydrate Associated Transferase Activities as Potential Tumor Markers for Human Gastric Cancer

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1. Introduction

Mucins are large, highly O-glycosylated proteins that are present at the surface of most epithelial cells and are involved in the protection and lubrication of the epithelium (Gendler and Spicer 1995). The expression of mucin genes is organ-and cell-type specific. The increased expression and altered glycosylation of mucins influence cellular growth, differentiation, transformation, adhesion and immune surveillance (Hollingworth and swanson 2004) and are associated with the development of cancer. Mucins have been implicated in the biologic behavior and progression of several cancers. During carcinogenesis, aberrant glycosylation leads to the development of tumor subpopulations with different adhesion properties (Taniguchi et al. 1996; Hiraiwa et al. 1996). Alterations of mucins during the pathogenesis of cancer have been well documented (Hakomori et al. 2002). The under glycosylation of mucins results in the creation of tumor associated cryptic carbohydrate core structures Tn, sialyl Tn and T (Taylor-Papadimitriou et al. 1999).

The Gram-negative bacterium Helicobacter pyroli infects over 50% of the world's population and causes various gastric diseases such as chronic gastritis, peptic ulcer and gastric cancer. Peripheral lymph node addressin (PNAd) containing 6-sulfo sialyl Lewis X-capped O-glycans on high endothelial venule (HEV)-like vessels are closely associated with pathogenesis of H pyroli-related diseases. The α1,4 GlcNAc-capped O-glycans expressed by gastric gland mucous cell-derived mucin prevents colonization of H pyroli. Thus, the differential expression of distinct O-glycans in stomach provides therapeutic potentialities based on specific carbohydrate modulation (Nakayama et al. 1999; Reis et al. 2000 and Kobayashi et al. 2009).

Werther et al. (1996) examined the frequency of sialyl Tn expression and its prognostic value in gastric cancer by immunohistochemical analysis of 340 gastric tumors and found that sialyl Tn expression is a marker of gastric cancer progression suggesting that cancer associated mucins play a role in the malignant behavior of the tumor. Santos-Silva et al. (2005) have reported that polymorphism in the MUC1 tandem repeat exerts influence on the expression of cryptic carbohydrate core structures in gastric cancer cells and the aggressive gastric tumors tend to express T antigen. Immunohistochemical study of Amado et al. (1998) for the expression of dimeric sialyl Lewis^x in 97 gastric carcinomas revealed a correlation

between high expression of dimeric sialyl Lewis^x and venous invasion and poor outcome in gastric cancer patients. Petretti *et al* (1999) studied RNA expression of several glycosyltransferases in surgical specimens of gastric carcinomas and found significant enhancement in the expression of ST3Gal IV and FucT IV.

A comprehensive study by Carvalho et al. (1999) on mucin expression in a panel of gastric carcinoma cell lines found no apparent relationship between the mucin core proteins and the simple mucin type or Lewis^x carbohydrate antigens that are expressed in each cell line. Thus there exists a complexity in glycosyltransferase activities which lead to several tumor-associated carbohydrate structures in gastric carcinoma.

A comparative study of gene-expression profiles of adenocarcinoma metastases and primary adenocarcinomas (Ramaswamy et al. 2003) indicated that a subset of primary tumors resembled metastatic tumors with respect to a gene-expression signature and that solid tumors carrying this gene-expression signature were most likely to be associated with metastasis and poor clinical outcome. These results further suggested that the metastatic potential of human tumors is encoded in the bulk of a primary tumor and thus challenged the notion that metastases arise from rare cells within a primary tumor that have the ability to metastasize (Poste et al. 1980). Thus, it became obvious that a very consistent change of some biological events in a primary tumor as opposed to the normal tissue has the possibility of being related to the malignant potential of the tumor. Recently, we examined the patterns of glycosyl- and glycan-sulfotransferase activities in several cancer cell lines and the resulting data strongly suggested an association of unique carbohydrate structures with signature potential in individual cancers (Chandrasekaran et al. 2006). The present study was aimed to identify the glycosyl- or sulfotransferase which exhibits the most marked and consistent change of activity in gastric tumorigenesis, by comparing the pattern of various glycosyl and glycan: sulfotransferase activities in tumorous and normal gastric tissues of each patient in ten gastric carcinoma cases.

2. Materials and methods

2.1 Tissue specimens

Human gastric tumor specimens were obtained during surgical procedures at Roswell Park Cancer Institute and stored frozen within 1h at -70°C. We studied the gastric tumor as well as non-tumor stomach tissue specimens from the same patient in ten gastric carcinoma cases and, in addition, two gastric tumor specimens in which case normal stomach tissue specimens were unavailable. The Pathology report on the tumors is presented in Table 1 and Table 4.

When the samples were collected from Pathology, a portion of the sample adjacent to that used for enzyme assay was fixed in formalin and embedded in paraffin. Slides were prepared from the paraffin blocks, and stained with hematoxylin and eosin by standard procedures. A board-certified pathologist studied the slides to determine the distribution of cell types within the tumor tissue compared to the control tissue from the same case (although in three cases non-tumor tissue was not available). Due to the invasiveness of the gastric cancer in these cases, both the tumor and the non-tumor tissue samples generally contained smooth muscle. The smooth muscle component existed at roughly equivalent percentage in the tumor sample and non-tumor control on a case by case basis sometimes being a little larger in either tumor or non-tumor sample. The major difference between the normal and tumor sample in this study was that the tumor contained malignant epithelial

cells, and the normal never did. With the exception of case 10, the amount of protein solubilized from the tumor and the corresponding non-tumor specimen by Triton X-100 did not vary much as evident from the values reported in **Table 1**. Thus, it becomes evident that a comparison of each glycosyltransferase and glycan:sulfotransferase activity per mg protein of the tissue extract between tumor and the corresponding non-tumor specimen is quite meaningful in understanding the quantitative change of each enzyme activity in tumorigenesis.

Gastric cancer cases	Tumor Site	STG	Smooth muscle (%)		Specimen Wt. (g)		Protein (mg) in Triton X-100 solubilized extract /g tissue	
			N	T	N	T	N	T
1	Overlapping lesion of stomach		30	40	3.5	1.9	52.8	67.4
2	Lesser curvature of stomach, NOS	3A	30	50	1.9	2.5	58.5	53.6
3	Lesser curvature of stomach, NOS	3A	20	30	4.2	1.9	54.6	42.2
4	Lesser curvature of stomach, NOS	2	N/A	70	6.0	1.0	60.0	41.0
5	Gastric antrum	3A	20	20	0.8	0.8	80.0	80.0
6	Gastric antrum	1B	20	30	0.8	0.6	60.4	67.5
7	Gastric antrum	2	10	0	1.7	0.5	52.4	82.8
8	Body of stomach	3A	10	10	0.6	2.1	68.9	80.2
9	Body of stomach	1B	20	10	0.6	2.0	52.9	86.9
10	Cardia, NOS	3A	40	30	1.9	2.3	112.0	23.5
11	Stomach, NOS	4	N/A	60	N/A	3.6		58.3
12	Lesser curvature of stomach, NOS		N/A	0	N/A	3.5		76.8

N; Non-tumor gastric tissue; T: Tumor gastric tissue N/A Not available

Table 1. The cancer site, stage and the details of the gastric tumor and non-tumor specimens from 12 patients

2.2 Acceptor compounds

The synthetic compounds used as acceptors in this study have already been used in our earlier studies (Chandrasekaran et al. 1995; 1996; 2001; 2004; 2005; 2006) and, thus, are well-documented acceptors for measuring the reported enzyme activities.

2.3 Processing of tissue specimens

The tissues were homogenized at 4°C with 4 volumes 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 1h using Speci-Mix (Thermolyne) and then centrifuged at 20,000g for 1h at 4°C. The clear fat-free supernatant was stored frozen at -20°C until use. Aliquots of 10µL from this extract were used in assays run in duplicates.

2.4 Enzyme assays

Glycosyltransferase activity in tumor extract was determined by mixing the tumor extracts with acceptor and radiolabeled monosaccharide donor 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 LS9000 scintillation counter. Controls for each assay contained the reaction mixture with everything except the acceptor. Radioactivity of control was subtracted from that of product 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 following are the conditions for individual enzymatic assays. Reaction temperature in all cases was 37°C.

2.5 Sialyltransferase

α2,3- and α2,6 sialyltransferase (ST) assay reactions proceeded for 2h in a mixture containing 100mM sodium cacodylate buffer (pH 6.0), 7.5mM acceptor, CMP-[9-^{3}H] NeuAc (typically 0.2μCi) and 10μl tumor extract in a total volume of 20μl (Chandrasekaran et al. 1995, 2005).

2.6 Gal/GalNAc transferases

βGlcNAc: β1,4Gal-T and αGalNAc:β1,3Gal-T assay mixtures in duplicate contained 0.1M Hepes-NaOH pH 7.0, 7mM ATP, 20mM Mn acetate, 1mM UDP-Gal. UDP [^{14}C] Gal (0.05μCi; 327mCi/mmol; Amersham), 0.5mM acceptor (unless otherwise stated) and 10μl tumor extract in a total volume of 20μL (Chandrasekaran et al. 2001). It was incubated for 4h; βGlcNAc:β1,4GalNAc-T assay mixtures in duplicate contained 0.1M Hepes-NaOH ph7.0, 7mM ATP, 20mM Mn acetate, UDP [^{3}H] GalNAc (0.20μCi; 7.8Ci/mmol: New England Nuclear Corp.) 7.5mM acceptor and incubated for 4h (Chandrasekaran et al. 2001).

2.7 Fucosyltransferases

α1,2, α1,3- , α1,4-fucosyltransferase and FT VI assay reactions were carried out for 2h in a reaction mixture containing 50mM Hepes buffer (pH 7.5), 5mM $MnC1_2$, 7mM ATP, 3mM NaN_3, 3mM synthetic acceptor, 0.05μCi GDP-[^{14}C]Fuc (290mCi/mmol) and 10μl tumor extract in a total volume of 20μl (Chandrasekaran et al. 1996).

2.8 Glycan: sulfotransferases

Sulfotransferase (Sulfo-T) assay reactions took 2h and required a mixture containing 100mM Tris-Maleate (pH 7.2), 5mM Mg Acetate, 5mM ATP, 10mM NaF, 10mM BAL, 7.5mM acceptor, 0.5μCi of [^{35}S]PAPS (specific activity 2.4Ci/mmol) and 10μl of tumor extract in a total volume of 30μl (Chandrasekaran et al. 2004).

2.9 Chromatographic methods

Dowex-1-Cl or Sep-Pak C18 cartridges were used to isolate radiolabeled product from the reaction mixture. For Gal-T, GalNAc-T and FT assays, the incubation mixture was diluted with 1ml water and passed through a 1ml bed volume of Dowex-1-Cl column (Chandrasekaran et al. 1996, 2001). The column was washed twice with 1ml water. The breakthrough and the water wash contained the [^{14}C]-galactosylated or [^{14}C]-fucosylated products formed with neutral acceptors. 3ml of 0.1M NaCl 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 Sep-Pak C18 cartridge (Water, Milford, MA), and elution of the product was done with 3ml methanol (Chandrasekaran et al. 2005; Palcic et al. 1998). For sulfotransferase assays, elution of the [35S]-sulfated compound from Dowex-1-Cl column could be achieved by 3ml of 0.2M NaCl (Chandrasekaran et al. 2001).

2.10 Effect of divalent cations on gastric tumor Gal: 3-O-sulfotransferase activity

For seeing the effect of divalent cations on Gal: 3-O-sulfotransferase activity the incubation mixture contained varying concentration (1-50mM) of Mg acetate, Mn acetate or Ca acetate under the standard incubation conditions. Gal3Sulfo-T4 activity was assayed using 3-O-Me Galβ1, 4 GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn as the acceptor. Gal3Sulfo-T2 activity was measured with the acceptor Galβ1, 4 GlcNAcβ1, 6(3-O-MeGalβ1, 3)GalNAcα-O-Bn.

2.11 N-Acetylglucosaminltransferase assay

The reaction mixture (30µl) contained 5mM acceptor, 70 mM Hepes-NaOH pH7.0, 7mM GlcNAc 1, 5 lactone, 14mM Mn acetate, 5mM ATP, 2mM NaN_3, 0.2µCi UDP-[6-^{3}H] GlcNAc (NEN-Dupont) and 15µl tumor extract and incubated for 4h at 37°C. The radioactive products from benzyl glycosides were separated by hydrophobic chromatography on Sep-Pak C18 cartridge and from allyl glycoside by Biogel P2 column chromatography as described above.

2.12 β N-acetylhexosaminidase treatment

10 µl of the [6-^{3}H] N-acetyl glucosamine containing products were incubated at 37°C for 20h with 0.1 unit of this enzyme as recommended by supplier (GLYKO) in a reaction volume of 20µl.

2.13 β1, 3Galactosidase (recombinant enzyme from calbiochem) treatment

10 µl of the [6-^{3}H] N-acetyl glucosamine containing product from Galβ1, 3 (GlcNAc β 1, 6) GalNAc α O-Al was mixed with 10µl of 0.1M citrate buffer-0.1% BSA pH 5.0 and 10µl (20 mU) recombinant enzyme and incubated at 37°C for 20h.

2.14 Lectin-agarose chromatography

PNA-agarose (Sigma), ConA-agarose (Sigma) and GSL II-agarose (Vector) columns of 5 ml bed volume were used under conditions as recommended by suppliers.

2.15 Thin layer chromatography

TLC was carried out on Silica gel GHLF (250 µm scored 20x20cm; Analtech, Newark DE). The solvent systems A [1 propanol/NH_4OH/H_2O (12/2/5 V/V)] and B [n-butanol/acetic acid/H_2O (3/2/1)] were used. The acceptor compounds were located on the plates by spraying with sulfuric acid in ethanol and heating at 100°C. The radioactive products were located by scraping 0.5cm width segments of silica gel and soaking them in 2 ml water in vials followed by liquid scintillation counting.

3. Results

3.1 Non-reducing terminal of complex carbohydrate chains

Several glycosyltransferases and glycan-sulfotransferases act on the non-reducing terminal of mucin Core2 tetra-and tri-saccharides and Globo backbone unit of gangliosides, core 1

disaccharide and Asparagine linked complex carbohydrate chains terminating in Gal residues, leading to a complexity of cancer-associated terminal glycan structures as shown in **Table 2.** Hence, we examined the levels of many of these enzyme activities in gastric tumor tissue as well as non-tumor gastric tissue of the same patient in ten gastric cancer cases for identifying any significant consistent change in any of these enzyme activities in gastric cancer. The specific acceptor compounds used in the present study for assaying the enzymes are reported in **Table 3.** The results on various enzyme levels are presented in **Tables 4** and **5.**

Carbohydrate chain terminal	Modifying glycosyl-and sulfo-transferases
1. *GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Ser/Thr (mucin core2 trisaccharide)	β1,3Gal/GalNAcTs; FTVI; β1,4Gal/GalNAcTs; GlcNAc6SulfoT
2. a) *Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Ser/Thr (mucin core2 tetrasaccharide) b) Asparagine linked complex carbohydrate chains terminating in *Gal residues	Gal3SulfoT2; Gal3SulfoT3 ST3Gal III; ST3Gal IV α1,2-FT; α1,4-GlcNAcT β1,3-GlcNAcT
3. a) Galβ1,4GlcNAcβ1,6(*Galβ1,3)GalNAcα-O-Ser/Thr b) GlcNAcβ1,6(*Galβ1,3)GalNAcα-O-Ser/Thr c) *Galβ1,3 GalNAcα-O-Ser/Thr (mucin core1 disaccharide) d) *Galβ1,3 GalNAcβ 1,3 Galα-O-R (Globo unit of gangliosides)	Gal3SulfoT4; ST3Gal I ST3Gal II; α1,2-FT α1,4-GlcNAcT β1,3-GlcNAcT

* Denotes the terminal unit utilized by the enzyme to transfer monosaccharide or sulfate

Table 2. Glycosyl-and sulfo-transferases involved in carbohydride chain terminal modification

3.2 Fucosyltransferase activities in gastric carcinoma

α1,3-FT contributed the major FT activity in both normal and tumor specimens in all cases. In nine cases, the tumor specimens as compared to normal (with the exception case 10) contained lower α1,3-FT activity (a decrease of 10-60%). There was a marked reduction (40-90%) of α1, 2-FT activity in seven cancer cases (2, 3, 4, 5, 6, 7 and 9). Thus, it is interesting to note that a decrease of α 1, 3- and α 1, 2- FT activities occurred in the same specimens. On the other hand, α1,4-FT activity and FTVI activity were present in increased level in the same five cancer cases (1, 3, 4, 7 and 10) and in decreased level in the same five cases (2, 5, 6, 8 and 9). Four tumor specimens (cases 7, 4, 1 and 10) which include two Signet ring cell CA and two mucin producing adenocarcinoma exhibited high elevation of FTVI activity (8.9, 10.5, 51.4 and 199.5 fold respectively) as compared to the corresponding normal specimen. Further, a highly noteworthy finding is that the gastrointestinal stromal tumor specimen (case 12) was distinct in having a low level of α1, 3-FT activity and negligible amounts of α1, 4-FT, α1,2-FT and FTVI activities as compared to other tumor specimens.

Glycosyl- or Sulfo-transferases	Acceptors
Fucosyltransferases: α 1, 2-FT α 1, 3-FT α 1, 4-FT FTVI	 Gal β-O-Bn 2-O-Me Gal β 1, 4 GlcNAc 2-O-Me Gal β 1, 3 GlcNAc GlcNAc β 1, 4 GlcNAc β-O-Bn
Sialyltransferases: ST3(O) ST3(N) ST6(N)	3-O-Me Gal β 1, 4 GlcNAc β 1, 6 (Gal β 1, 3) GalNAc α-O-Bn 2-O-Me Gal β 1, 3 GlcNAc β-O-Bn Gal β 1, 4 GlcNAc β 1, 6 (3-O-Me Gal β 1, 3) GalNAc α-O-Bn [corrected for ST3(N) activity]
β Gal/GalNAc transferase: β 1, 4 Gal-T β 1, 4 GalNAc-T α GalNAc: β 1, 3 Gal-T	 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Bn 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Bn 4-Fluoro GlcNAc β 1, 6 GalNAc α-O-Bn
Glycan: Sulfotransferases Gal 3 Sulfo-T_2 Gal 3 Sulfo-T_4 GlcNAc 6 Sulfo-T	Gal β 1, 4 GlcNAc β 1, 6 (3-O-Me Gal β 1, 3) GalNAc α-O-Bn 3-O-Me Gal β 1, 4 GlcNAc β 1, 6 (Gal β 1, 3) GalNAc α-O-Bn GlcNAc β 1, 3 Gal β 1, 4 Glc

Table 3. Specific acceptors for measuring Glycosyl- and Sulfo- transferase activities in gastric tumor and non-tumor specimens

3.3 Sialyltransferase activities in gastric carcinoma

ST3 (O) activity, which forms 3'-sialyl T hapten (NeuAcα2, 3Galβ1, 3GalNAcα) was the predominant sialyltransferase activity in all gastric tissue specimens. This activity increased (1.7-172.0 fold) in six (cases 2, 3, 4, 6, 9 and 10) and decreased in four (cases 1, 5, 7 and 8) gastric tissue specimens. ST6(N) activity synthesizing 6'-LacNAc type 2 unit (NeuAcα2,6Galβ1,4GlcNAcβ-) ranged 4% - 78% of the ST3(O) activity in the tumor tissue specimens studied and ST3(N) activity forming 3'-sialyl LacNAc was even far less than ST6(N) activity. Three out of four tumor specimens from Signet ring cell carcinoma (cases 2, 3 and 4) and three out of five tumor specimens from adenocarcinoma (cases 6, 9 and 10) showed an increased level of ST3 (O) activity. Two cases of Signet ring cell CA (1 and 3) and three cases of adinocarcinoma (7, 8 and 9) showed significant ST6 (N) activity in the range 8.2 – 78.0% of ST3 (O) activity.

3.4 β-GlcNAc : β1,4-Gal/GalNAc and α-GalNAc : β1,3-Gal transferase activities in gastric carcinoma

Three tumor specimens (cases 3, 4 and 10) while exhibiting an increase in β1, 4Gal-T activity, other tumor specimens contained lower level of this activity than the corresponding normal specimens. Six tumor specimens (cases 3, 4, 5, 6, 8 and 10) showed an increased level of β1, 4GalNAc-T activity. It is remarkable that two tumor specimens (cases 5 and 6) from adenocarcinoma contained only 30% and 40% level of α-GalNAc: β1, 3Gal-T activity as

compared to the normal specimens. On the contrary, the tumor specimen of case 10 as compared to other tumor specimens had a high level of this activity, which exceeded even the levels of its β1, 4Gal/GalNAc-T activities. The level of αGalNAc : β1,3Gal-T activity was also higher than β1,4Gal/GalNAc-T activity in Litinis Plastica specimen (case 11)

Enzyme Activity		Glycosyltransferase activities in Tissue specimens: Incorporation of [14C] or [3H] monosaccharides (CPMx10-3) into the enzyme specific acceptor catalyzed by 1mg protein of the tissue extract											
		1	2	3	4	5	6	7	8	9	10	11	12
Fucosyl-transferases													
α1,2-FT	N	127.2	146.8	76.8	62.9	197.2	109.4	70.9	102.7	108.9	19.9		
	T	111.2	83.3	49.0	35.0	16.5	22.0	132.1	34.4	62.5	131.9	88.5	2.5
		(0.9)	(0.6)	(0.6)	(0.6)	(0.1)	(0.2)	(1.9)	(0.3)	(0.6)	(6.6)		
α1,3-FT	N	449.6	494.1	533.2	422.2	448.8	428.4	526.3	367.8	483.5	166.4		
	T	390.5	382.6	448.3	184.0	180.2	198.2	352.8	278.9	328.7	655.0	452.2	107.0
		(0.9)	(0.8)	(0.8)	(0.4)	(0.4)	(0.5)	(0.7)	(0.8)	(0.7)	(3.9)		
α1,4-FT	N	68.3	9.1	268.3	71.5	12.3	405.2	189.5	173.6	417.0	18.4		
	T	193.9	2.3	322.6	201.3	0.0	211.3	325.6	91.2	294.5	671.6	193.4	0.9
		(2.8)	(0.3)	(1.2)	(2.8)	(0.0)	(0.5)	(1.7)	(0.5)	(0.7)	(36.5)		
FT-VI	N	3.1	138.6	42.7	1.5	16.7	137.9	13.6	5.4	227.0	0.8		
	T	159.2	60.6	51.7	15.8	1.2	2.7	121.0	3.8	181.7	159.6	90.5	0.8
		(51.4)	(0.4)	(1.2)	(10.5)	(0.1)	(0.0)	(8.9)	(0.7)	(0.8)	(199.5)		
Sialyl-transferases													
α2,3(O)ST	N	184.5	54.4	180.4	15.6	234.2	104.2	379.7	306.5	148.6	1.9		
	T	53.8	145.1	309.0	122.7	155.2	240.3	83.3	245.1	308.2	326.8	114.0	91.9
		(0.3)	(2.7)	(1.7)	(7.9)	(0.7)	(2.3)	(0.2)	(0.8)	(2.1)	(172.0)		
α2,3(N)ST	N	2.1	1.9	0.7	0.0	1.1	1.7	0.6	2.6	3.6	0.4		
	T	3.1	4.0	2.5	0.0	1.6	2.3	4.2	2.2	3.8	7.1	1.1	2.7
		(1.5)	(2.1)	(3.6)	(0.0)	(1.5)	(1.4)	(7.0)	(0.8)	(1.1)	(4.3)		
α2,6(N)ST	N	0.4	15.4	24.5	0.0	16.2	48.3	40.5	8.3	50.1	0.0		
	T	32.9	8.3	25.2	7.3	6.4	11.9	65.0	38.4	41.5	0.0	8.7	8.2
		(82.2)	(0.5)	(1.0)	(7.3)	(0.4)	(0.2)	(1.6)	(4.6)	(0.8)	(0.0)		
Gal/GalNAc transferases													
β1,3 Gal-T	N	36.6	34.8	67.2	25.6	70.2	102.0	88.1	98.9	110.6	39.7		
	T	43.3	44.1	65.9	31.6	19.4	36.3	138.1	83.0	166.4	244.9	103.3	23.8
		(1.2)	(1.3)	(1.0)	(1.2)	(0.3)	(0.4)	(1.6)	(0.8)	(1.5)	(6.2)		
β1,4 Gal-T	N	172.2	167.8	255.3	113.2	232.2	275.0	362.5	229.8	264.5	26.7		
	T	111.6	151.9	403.9	124.1	199.0	258.4	211.4	205.7	207.0	161.9	92.9	62.3
		(0.6)	(0.9)	(1.6)	(1.1)	(0.9)	(0.9)	(0.6)	(0.9)	(0.8)	(6.1)		
β1,4 GalNAc-T	N	51.7	44.3	36.0	12.2	36.8	38.5	39.7	37.9	74.8	15.3		
	T	27.2	38.1	50.5	25.8	44.0	43.9	32.4	40.3	28.7	61.0	28.9	10.1
		(0.5)	(0.9)	(1.4)	(2.1)	(1.2)	(1.1)	(0.8)	(1.1)	(0.4)	(4.0)		

N: Non-tumor gastric tissue; T: Tumor gastric tissueValues in parentheses indicate fold of enzyme activity in tumor with respect to the normal

Table 4. The levels of glycosyltransferase activities in human gastric non-tumor and tumor specimens

Gastric cancer cases	Tumor Histology	Differen-tiation		Sulfotransferase activities in tissue specimens: Incorporation of ^{35}S-sulfate (CPMx10^{-3}) into enzyme specific acceptors catalyzed by 1mg protein of the tissue extract		
				Gal3sulfo T2	Gal3sulfo T4	GlcNAc6 sulfo T
1	Signet ring cell CA Krukenberg Tumor	Poor	N T	0.8 60.7 (75.9)	3.4 49.1 (14.4)	33.8 43.8 (1.3)
2	Signet ring cell CA Krukenberg Tumor	Poor	N T	2.1 329.0 (156.7)	12.9 91.4 (7.1)	19.5 53.7 (2.8)
3	Signet ring cell CA, Krukenberg Tumor	Poor	N T	10.1 208.1 (20.6)	1.8 111.0 (61.7)	1.8 3.6 (2.0)
4	Signet ring cell CA, Krukenberg Tumor	Moderate	N T	0.1 2.6 (26.0)	0.1 0.5 (5.0)	0.1 0.7 (7.0)
5	Adenocarcinoma nos	Poor	N T	1.1 11.7 (10.6)	27.8 75.8 (2.7)	31.1 49.8 (1.6)
6	Adenocarcinoma nos	Poor	N T	1.4 2.6 (1.9)	6.7 34.3 (5.1)	6.0 3.3 (0.6)
7	Mucin producing adenocarcinoma	Poor	N T	0.3 2.1 (7.0)	0.4 59.6 (149.0)	1.9 5.2 (2.7)
8	Adenocarcinoma nos	Moderate	N T	1.3 2.7 (2.1)	0.7 2.3 (3.3)	2.9 4.1 (1.4)
9	Adenocarcinoma nos	Moderate	N T	0.4 3.3 (8.3)	7.1 19.8 (2.4)	1.9 15.3 (8.1)
10	Mucin producing adenocarcinoma	Poor	N T	0 1.4 (1.4)	0.2 12.3 (61.5)	2.3 86.3 (37.5)
11	Litinis Plastica	Poor	T	2.3	56.2	58.3
12	Gastrointestinal stromal sarcoma	Non-epithelial Poor	T	1.1	4.6	76.8

N: Non-tumor gasrteic tissue; T: Tumor gastric tissue Values in parentheses indicate fold of enzyme activity in tumor with respect to the normal

Table 5. The levels of sulfotransferase activities in human gastric non-tumor and tumor specimens

3.5 Glycan: Sulfotransferase activities in gastric carcinoma

Both Gal3Sulfo-T_4 activity specific for Galβ1, 3GalNAcα- and Gal3Sulfo-T_2 activity utilizing mainly Galβ1,4GlcNAcβ- were found, respectively, at 2.4→61.7 fold and 1.7→156.7 fold elevated level in all the gastric tumor specimens studied. On the other hand, an increased level of GlcNAc6-Sulfo-T activity was also evident but to a lesser extent in nine gastric tumor specimens. Five tumor specimens, all from adinocarcinoma (cases 5, 6 7, 9 and 10)

contained predominantly Gal3Sulfo-T_4 while Gal3Sulfo-T_2 dominated in three specimens of Signet ring cell CA(cases 1, 2 and 3).

3.6 The identity of Gal: 3-O-Sulfo transferases in human gastric tumor specimens

Acceptor specificities: A consistent marked elevated Gal: 3-O-Sulfotransferase activities in gastric tumors prompted us to establish further the identity of these enzymes with the known cloned Gal3Sulfotransferases (Chandrasekaran et. al. 2004). We studied four tumor specimens (cases 2, 3, 6 and 7) for acceptor specificities and the results are presented in **Table 6.**

Acceptor	Gal : 3-O-Sulfotransferase Activity % case 6	case 7	case 2	case 3
Galβ1,4GlcNAc	3.2	8.8	100.0 (2438)	100.0 (17550)
Galβ1,3GalNAcα-O-Al	100.0 (2679)	100.0 (3409)	22.7	16.5
3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn	230.2	326.8	48.4	42.9
Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn	11.1	16.7	80.1	73.2
Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Al	489.4	657.3	112.8	90.2
Galβ1,3GalNAcβ1,3Galα-O-Me	222.8	304.2	210.7	178.0
Fetuin triantennary asialo glycopeptide	21.3	15.9	44.0	29.7

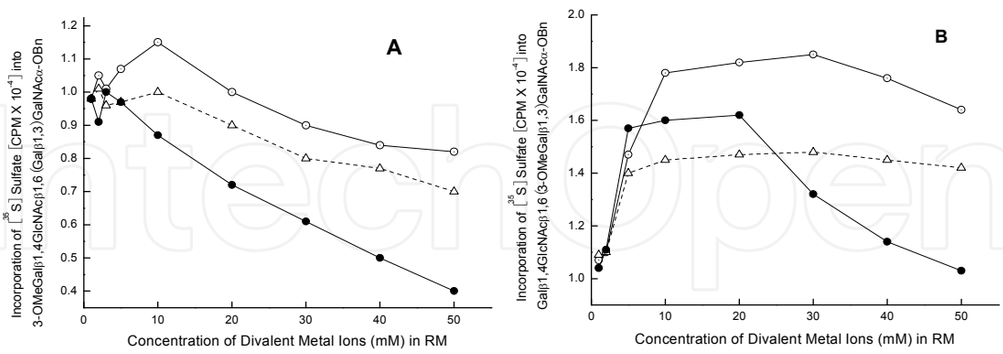
Table 6. Establishing the identity of Gal : 3-O-Sulfotransferases in Human Gastric Tumor Specimens

The values in parentheses are the actual CPM designated as 100% for the acceptor Galβ1, 3GalNAcα-O-Al in the cases 6 and 7 and for the acceptor Galβ1,4GlcNAc in the cases of 2 and 3

Gal3Sulfo-Ts of tumor specimens from cases 2 and 3 showed low activity towards Galβ1, 3GalNAcα-O-Al and that from cases 6 and 7 very low activity towards Galβ1, 4GlclNAc. Tumor specimens 6 and 7 contained mostly Gal3Sulfo-T_4 (Chandrasekaran et. al. 2004). Gal3Sulfo-Ts of tumor specimens 2 and 3 were found to be less active towards Fetuin triantennary asialo glycopeptides, which was established as the best acceptor in our earlier studies for Gal3Sulfo-T_3 (Chandrasekaran et. al. 2004). Further, the above enzymes were very active towards Galβ1, 3GalNAcβ1, 3Galα-O-Me whereas Gal3Sulfo-T_3 showed very low activity towards this acceptor. Gal3Sulfo-Ts of tumor specimens 2 and 3 were found to have the identity of cloned Gal3Sulfo-T_2 (Chandrasekaran et. al. 2004).

Influence of divalent metal ions (see **Fig 1**): None of the divalent metal ions Ca^{2+}, Mn^{2+} and Mg^{2+} had any significant stimulating effect on Gal3Sulfo-T of tumor specimen 7. In fact, a gradual decline in enzyme activity was noticed upon increasing the concentration of Mn^{2+} in the reaction mixture. Thus, this enzyme resembled Gal3Sulfotransferase from breast tumor (Chandrasekaran et. al. 1997) as well as the cloned Gal3Sulfo-T_4 (Chandrasekaran et. al. 2004). On the other hand, the metal ions Ca^{2+}, Mn^{2+} and Mg^{2+} stimulated the activity of tumor specimen 3. Mn^{2+} stimulated the activity between 5-20mM and then the activity decreased reaching the initial level at 50mM. This pattern of influence by Mn^{2+} on the activity was quite similar to that of Gal3Sulfotransferase from colon tissue (Chandrasekaran et. al. 1997) and cloned Gal3Sulfo-T_2 (Chandrasekaran et. al. 2004). On the contrary, the activity of cloned Gal3Sulfo-T_3 was stimulated by Mn^{2+} reaching the maximum at 40-50mM

(Chandrasekaran et. al. 2004). Thus, Gal3Sulfo-T of tumor specimen 7 was similar to GalSuflo-T_4 and that of tumor specimen 3 closely resembled Gal3Sulfo-T_2.



A. Case 7 tumor specimen; B. Case 3 tumor specimen ● Mn;△ Mg; ○ Ca

Fig. 1. Influence of divalent metal ions on gastric tumor Gal3 Sulfotransferase activities.

3.7 Identification of human gastric tissue α- GlcNAc transferase acting on terminal GlcNAc residue

The present investigation identified two N-acetylglucosaminyltransferase activities in gastric tissues using mucin core 2 tri- and tetra-saccharides as acceptors as reported in **Table 7**. An increased level (1.5-8.5 fold) of GlcNAc transferase activity on mucin core 2 tetrasaccharide was observed in three gastric tumor specimens as compared to that of the corresponding non-tumor specimens. Further, the level of this enzyme is about two-fold as compared to the corresponding activity of the other enzyme. The linkages of [6-^{3}H] GlcNAc residues attached to mucin core 2 tri- and tetra-saccharides were further examined by utilizing TLC and lectin-agarose chromatography techniques (see sections 3.8 and 3.9).

Gastric cancer cases		Incorporation [6-^{3}H] GlcNAc (CPM x 10^{-3}) catalyzed by 1 mg protein of the tissue extract	
		Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Al	Gal β 1, 3 (Gal β 1, 4 GlcNAc β 1, 6) GalNAc α-O-Bn
1	N	5.9	9.8
	T	5.8	14.4
2	N	8.4	18.4
	T	6.8	24.5
5	N	15.7	27.2
	T	5.2	26.7
10	N	2.1	2.7
	T	15.0	23.0
11	T	7.0	16.4

Table 7. The levels of human gastric tissue N-Acetylglucosaminyltransferase activities towards mucin core 2 tri-and tetra-saccharides

3.8 TLC studies

Fig 2 A (lanes A, B, C and D respectively) shows the mobilities (solvent system A) of the acceptor 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAcα-O-Bn, the [6-^{3}H] GlcNAc containing product, [6-^{3}H] GlcNAc containing product incubated with and without β-N-acetyl hexosaminidase (Jack bean). Lanes B, C and D show that the radioactive products have the same mobility. It is evident from these results that β-N-acetyl hexosaminidase (Jack bean) was not able to hydrolyze the [6-^{3}H] GlcNAc containing product. Fig 2 B (lanes A and B)shows the mobilities (solvent system B) of 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAcα-O-Bn and 3-O-Me Gal β 1, 3 (Gal β 1, 4 GlcNAc β 1, 6) GalNAcα-O-Bn. Lane c shows the mobilities of 3-O-Sulfo Gal β 1, 3 (Gal β 1, 4 GlcNAc β 1, 6) GalNAcα-O-Bn and Std. GlcNAc. Lanes D, E and F show respectively the mobilities of [6-^{3}H] GlcNAc containing product from 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAcα-O-Bn, 3-O-Me Gal β 1, 3 (Gal β 1, 4, GlcNAc β 1, 6) GalNAcα-O-Bn and 3-O-Sulfo Gal β 1, 3 (Gal β 1, 4, GlcNAc β 1, 6) GalNAcα-O-Bn after treatment with β-N-acetyl hexosaminidase (Jack bean). It is clear that [6-^{3}H] GlcNAc was released only from the radioactive products arising from the last two acceptors (Lanes E and F) indicating that [6-^{3}H] GlcNAc is β linked to mucin core 2 tetrasaccharide . **Fig 2 C** lane A shows the mobilities (Solvent system B) of acceptor Gal β 1, 3 (GlcNAc β 1, 6) GalNAcα-O-Al and Std. GlcNAc. Treatment with recombinant β 1, 3 Galactosidase resulted in the removal of Gal as evident from the faster mobility of the radioactive product (lane C). β-N-Acetylhexosaminidase (Jack bean) did not act on the radioactive product (compare lanes E and F).

3.9 Lectin-agarose chromatography

The primary sugar specificity of PNA is Gal linked β 1, 3to GalNAc. ConA binds Man and Glc and GSL II binds terminal GlcNAc but these lectins have additional structural requirements for binding. **Fig 3** shows PNA-agarose affinity chromatography of [6-^{3}H] GlcNAc containing product from Gal β 1, 3 (GlcNAc β 1, 6) GalNAcα-O-Al. More than 90% of the product binds to this column as shown in Fig 3 A indicating that [6-^{3}H] GlcNAc is not transferred to the Gal terminal. The radioactive product after recombinant β 1, 3 Galactosidase treatment was subjected to PNA-agarose affinity chromatography and it showed > 90% non-binding to this column. On the contrary to these results, Nakayama et al. (1999) without showing data just mentioned in their paper that their cloned human α 1, 4 N-acetylglucosaminyltransferase acted only on the Gal moiety of mucin core 2 trisaccharide. We find that [6-^{3}H] GlcNAc linkage to β 1, 6 linked GlcNAc in mucin core 2 is completely resistant to Jack bean β-N-acetyl hexosaminidase which has a broad specificity cleaving non-reducing terminal β 1- 2, 3, 4 or 6 linked GlcNAc and GalNAc residues (Li and Li 1970). This enzyme was also able to cleave very efficiently GlcNAc linked β 1, 4 to GlcNAc (N, N′-Diacetylchitobiose) (Li and Li 1970). Thus GlcNAc transferred to β 1, 6 linked GlcNAc has been found to be in α-linkage. Nakayama et al. (1999) reported that α 1, 4 GlcNAc-T was responsible for the formation of GlcNAc α 1, 4 Gal β-R which in turn was responsible for class III mucin ConA reactivity. In this connection, it is noteworthy that the present study found that the radioactive compound [6-^{3}H] GlcNAc α GlcNAc β 1, 6 (Gal β 1, 3) GalNAc α-O-Al did not bind to ConA-agarose as well as GSL II-agarose (**Fig 4 A and B)**. After the removal of Gal by recombinant β 1, 3 galactosidase treatment, the resulting radioactive compound still did not bind to GSL II-agarose (**Fig 4 C**).

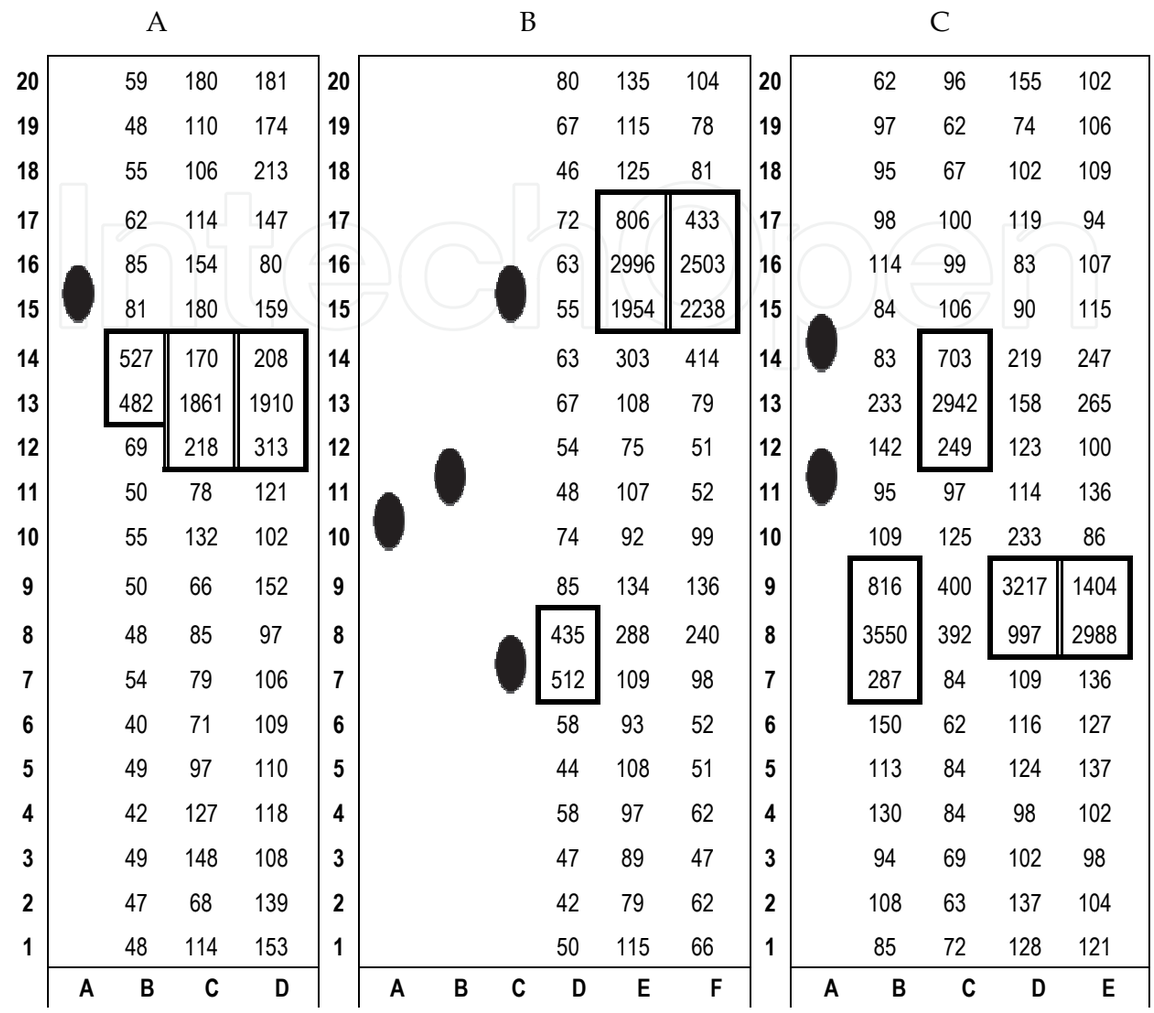
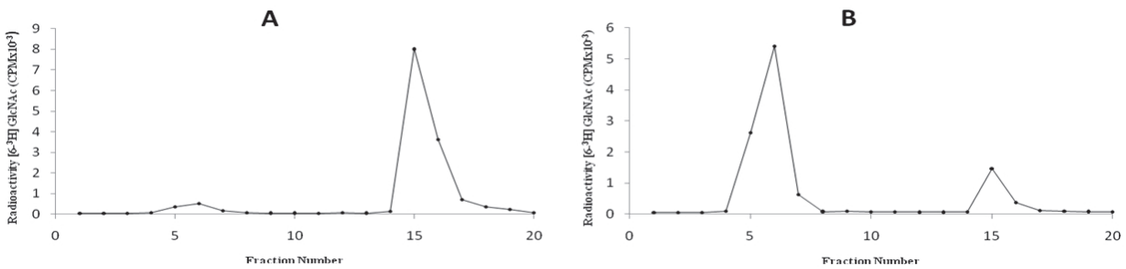


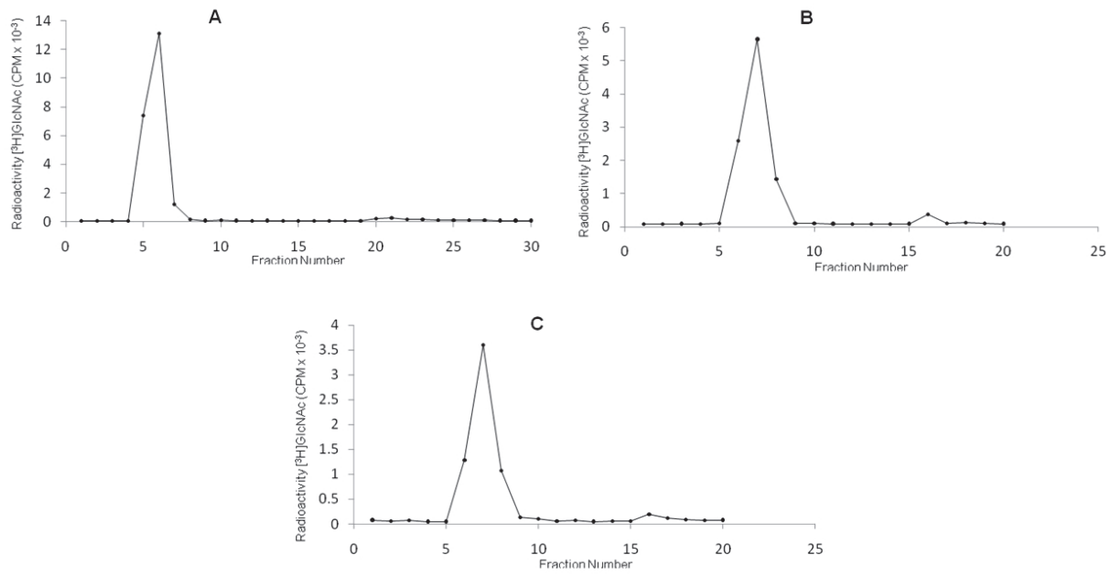
Fig. 2. TLC Identification of βGlcNAc: αGlcNAc transferase activity in human gastric tumor

Fig. 2 A (solvent system A).
A**:** Acceptor compound 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Bn
B**:** [6-^{3}H] GlcNAc containing product resulting from A
C and D**:** B incubated with and without β N-acetylhexosaminidase (Jack bean)
Fig. 2. B (solvent system B).
A**:** 3-O-Me Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Bn
B**:** 3-O-Me Gal β 1, 3 (Gal β 1, 4 GlcNAc β 1, 6) GalNAc α-O-Bn
C**:** 3-O-Sulfo Gal β 1, 3 (Gal β 1, 4 GlcNAc β 1, 6) GalNAc α-O-Bn and Std. GlcNAc
D, E and F**:** [6-^{3}H] GlcNAc containing products resulting from A, B and C respectively after β N- acetylhexosaminidase(Jack bean) treatment
Fig. 2. C (Solvent system B**).**
A**:** The acceptor Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Al and Std. GlcNAc
B**:** [6-^{3}H] GlcNAc containing product resulting from the acceptor in A
C**:** B after β 1, 3 galactosidase (recombinant) treatment
D and E**:** B incubated with and without β N- acetylhexosaminidase (Jack bean)



A: [6-^{3}H] GlcNAc containing product resulting from the acceptor Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Al
B: β 1, 3 galactosidase (recombinant) treated [6-^{3}H] GlcNAc containing product

Fig. 3. PNA-agarose affinity chromatography of [6-^{3}H] GlcNAc containing product



Affinity chromatography of [6-^{3}H] GlcNAc containing product resulting from the acceptor Gal β 1, 3 (GlcNAc β 1, 6) GalNAc α-O-Al on A: Con A-agarose; B: GSL II-agarose; C: GSL II-agarose after β 1, 3 galactosidase (recombinant) treatment

Fig. 4. Chromatography on ConA-agarose and GSL II-agarose

4. Discussion

Many risk factors have been associated with the development of gastric cancer, and the pathogenesis is most likely multifactorial. One postulation on the development of this disease involves a succession of histologic changes that commence with atrophic gastritis, advance to mucosal metaplasia, and eventually result in a malignancy (Layke et al. 2004). Tumor development and growth can be viewed as uncontrolled tissue growth. Tumor growth relies on blood supply by the blood vessels (Kobayashi et al. 2003). It has been known that a growing tumor secretes factors that induce blood vessel growth (i.e., neovascularization) to support its own growth and survival (Folkman et al. 1995). A primary cell type of the blood vessels, especially microvessels in a tumor, is an endothelial

cell. Therefore, focus has been to discover factors that specifically control endothelial cell proliferation (Davis et al. 2003). The roles of other factors are just beginning to be understood (Sogn et al. 2005). The tumor microenvironment needs to be completely characterized for understanding its role in tumor progression and metastasis (Sogn et al. 2005). Glycoproteins play a role in pathological processes due to immunological response to the altered oligosaccharides (Fukuda et al. 1996). They have roles in cell adhesion during inflammation and metastasis (Fukuda et al. 1996). Hence it is anticipated that glycoproteins may have a definite role in tumor microenvironment.

Recently an important role for galectins in cancer micrometastasis became evident from the report of Khaldoyandidi, et al. (2003) that interactions between T-antigen of breast cancer cells and galectin-3 mediate both homotypic and heterotypic intercellular adhesion of metastatic breast cancer cells under conditions of flow *in vitro* and *in vivo*. A substitution of sulfate at C-3 position of β-galactosyl residue in T-hapten enhanced its binding efficiency about 15 fold towards galectin-4 and 3 fold towards galectin-3 (Ideo et al. 2002). Similarly, the studies of our laboratory on galectin specificities showed that 3-O-Sulfo Galβ1, 4GlcNAc as compared to Galβ1, 4GlcNAc was 3 fold more efficient in binding to galectin-1 (Allen et al. 1998). Thus, the enhancement of the binding ability to galectins by 3-O-Sulfation of β-galactosyl residues appears to be a common characteristic of galectin family. In addition, 3'-sialylation Core1 in contrast to 3'-sulfated Core1 had very weak affinity for galectin-4 (Ideo et al. 2002). Hippo et al. (2001) analyzed six gastric cancer cell lines by Northern Blot and observed an up-regulation of galectin-4. Sulfatide was found as a major acidic glycolipid in human gastric mucosa (Natomi et al. 1993) and the expression of cerebroside sulfotransferase mRNA in endoscopic bioptic specimens of eleven gastric cancer cases was reported by Kobayashi et al. (1999). The present study finds several fold consistent increase in Gal3Sulfotransferase activity in gastric tumor. The resulting 3-O-Sulfogalactosyl residues in gastric tumor cell glycoproteins and glycolipids may facilitate the interaction of gastric tumor cells with galectin-4, resulting in intercellular homotypic and heterotypic adhesion of gastric cancer cells.

5. Conclusion

It becomes evident from the present study that among the various transferases, which can modify the terminal Gal residues in carbohydrate chains as shown in **Table 2**, only Gal3Sulfotransferases show a consistent several fold elevated activity in all gastric tumor specimens: Gal3-sulfo-T_4 (Gal3-sulfortransferase specific for Galβ1,3GalNAcα-O-Ser/Thr) and GlcNAc6-Sulfo-T are apparently associated with poorly differentiated gastric adenocarcinoma whereas poorly differentiated Signet ring cell gastric carcinoma expresses a high level of Gal3Sulfo-T_2, a Gal3Sulfotransferase acting on Galβ1,4GlcNAcβ- terminal unit. The most consistent change in glycosyltransferase activity could be found only with α1, 2-FT. A significant decrease in this activity was seen in the range 40 – 90% in seven gastric tumor specimens. Thus, the present study was able to show that down regulation of α1,2-FT activity accompanied by induction of Gal3-O-Sulfotransferase activities acting on Gal terminals could be involved in the facile sulfation of carbohydrate chains, which may contribute to the microenvironment suitable for interaction with galectin-4 in promoting tumor growth and metastasis. Furthermore, the sulfation of Gal terminal as well as GlcNAc residues would render the carbohydrate chains strong anionic charge, resistance to

degradation by sialidases, galactosidases and hexosaminidases and this would increase their half-life in receptor-mediated glycoprotein clearance.

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7. References

Allen HJ, Ahmed H, Matta KL (1998) Binding of synthetic sulfated ligands by human splenic galectin 1, a β-galactoside-binding lectin. Glycoconj J 15:691-695.

Amado M, Carneiro F, Seixas M, Clausen H, Sobrinho-Simoes M (1998) Dimeric sialyl-Le(x) expression in gastric carcinoma correlates with venous invasion and poor outcome. Gastroenterology 114:462-470.

Carvalho F, David L, Aubert JP, Lopez-Ferrer A, De Bolos C, Reis, CA, Gartner F, Peixoto A, Alves P, Sobrinho-Simoes M (1999) Mucins and mucin-associated carbohydrate antigens expression in gastric carcinoma cell lines. Virchows Arc 435:479-485.

Chandrasekaran EV, Jain RK, Larsen RD, Wlasichuk K, Matta KL (1995) Selectin-ligands and tumor associated carbohydrate structures: specificities of α2,3-sialyltransferases in the assembly of 3′-sialyl, 6-sulfo/sialyl Lewis a and x, 3′-sialyl, 6′-sulfo Lewis x and 3′-sialyl, 6-sialyl/sulfo blood group T-hapten. Biochemistry 34:2925-2936.

Chandrasekaran EV, Jain RK, Larsen RD, Wlasichuk K, DiCioccio RA, Matta KL (1996) Specificity analysis of three clonal and five non-clonal α1,3-L-fucosyltransferases with sulfated, sialylated, or fucosylated synthetic carbohydrates as acceptors in relation to the assembly of 3′-sialyl-6′-sulfo Lewis x (the L-selectin ligand) and related complex structures. Biochemistry 35:8925-8933.

Chandrasekaran EV, Jain RK, Vig R, Matta KL (1997) The enzymatic sulfation of glycoprotein carbohydrate units: blood group T-hapten specific and two other distinct Gal:3-O-sulfotransferases as evident from specificities and kinetics and the influence of sulfate and fucose residues occurring in the carbohydrate chain on C-3 sulfation of terminal Gal. Glycobiology 7:753-768.

Chandrasekaran EV, Chawda R, Piskorz C, Locke RD, Ta A, Sharad G, Odunsi K, Lele S, Matta KL (2001) Human ovarian cancer, lymphoma spleen, and bovine milk GlcNAc:β1,4Gal/GalNAc transferases: two molecular species in ovarian tumor and induction of GalNAcβ1,4Glc synthesis by α-lactalbumin. Carbohydr Res 334:105-118.

Chandrasekaran EV, Lakhaman SS, Chawda R, Piskorz CF, Neelamegham S, Matta KL (2004) Identification of physiologically relevant substrates for cloned Gal:3-O-sulfotransferases (Gal3STs): distinct high affinity of Gal3ST-2 and LS180 sulfotransferase for the Globo H backbone, Gal3ST-3 for N-glycan multiterminal Galβ1, 4GlcNAcβ- units and 6-sulfoGalβ1, 4GlcNAcβ-, and Gal3ST-4 for the mucin core-2 trisaccharide. J Biol Chem 279:10032-10041.

Chandrasekaran EV, Xue J, Xia J, Chawda R, Piskorz C, Locke RD, Neelamegham S, Matta KL (2005) Analysis of the specificity of sialyltransferases toward mucin Core2, Globo, and related structures. Identification of the sialylation sequence and the effects of sulfate, fucose, methyl and fluoro substituents of the carbohydrate chain

in the biosynthesis of selectin and siglec ligands and novel sialylation by cloned α2,3(O)sialyltransferase. Biochemistry 44:15619-15635.

Chandrasekaran EV, Xue J, Neelamegham S, Matta KL (2006) 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. Carbohydr Res 341:983-994.

Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1:27-31.

Gendler S J, Spicer A P (1995) Epithelial mucin genes. Annu Rev. Physiol. 57:607-634

Hakomori S (2002) Glycosylation defining cancer malignancy: new wine in an old bottle. Proc Natl Acad Sci USA 99:10231-10233.

Hippo Y, Yashiro M, Ishii M, Taniguchi H, Tsutsumi S, Hirakawa K, Kodama T, Aburatani H (2001) Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res 61:889-895.

Hiraiwa N, Dohi T, KawakamiKimura N, Yumen M, Ohmori K, Maeda M, Kannagi R (1996) Suppression of sialyl Lewis X expression and E-selectin-mediated cell adhesion in cultured human lymphoid cells by transfection of antisense cDNA of an alpha1→3 fucosyltransferase (Fuc-T VII). J Biol Chem 271:31556-31561.

Hollingsworth M A, Swanson B J (2004) Mucins in cancer: Protection and control of the cell surface. Nat. Rev. Cancer 4:45-60

Ideo H, Seko A, Ohkura T, Matta KL, Yamashita K (2002) High-affinity binding of recombinant human galectin-4 to SO_3-→3Galβ1→3GalNAc pyranoside. Glycobiology 12:199-208.

Khaldoyanidi SK, Glinsky VV, Sikora L, Glinskii AB, Mossine VV, Quinn TP, Glinsky GV, Sriramarao P (2003) MDA-MB-435 human breast carcinoma cell homo- and heterotypic adhesion under flow conditions is mediated in part by Thomsen-Friedenreich antigen -galecin-3 interactions. J Biol Chem 278:27-4134.

Kobayashi T, Honke K, Tsunematsu I, Kagaya H, Nishikawa S, Hokari K, Kato M, Takeda H, Sugiyama T, Higuchi A, Asaka M (1999) Detection of cerebroside sulfotransferase mRNA in human gastric mucosa and adenocarcinoma. Cancer Lett 138:45-51.

Kobayashi M, Lee H, Nakayama J, Fukuda M (2009) Roles of gastric mucin-type O-glycans in the pathogenesis of Helicobacter pylori infection. Glycobiol. 19:453-461

Layke JC, Lopez PP (2004) Gastric Cancer: Diagnosis and treatment options. Am Family Physician 69:1133-1140.

Li S-C, Li Y-T (1970) Studies on the glycosidases of Jack bean meal. III Crystallization and properties of β N-acetylhexosaminidase. J. Biol. Chem. 245:5153-5160

Nakayama J, Yeh J C, Misra A K, Ito S, Katsuyama T, Fukuda M (1999) Expression cloning of a human α1,4- N acetyl glucosaminyltransferase that forms GlcNAcα1, 4 Galβ-R, a glycan specifically expressed in the gastric gland mucous cell-type mucin. Proc. Natl. Acad. Sci. USA 96:8991-8996.

Natomi H, Saitoh T, Sugano K, Iwamori M, Fukayama M, Nagai Y (1993) Systemic analysis of glycosphingolipids in the human gastrointestinal tract: enrichment of sulfatides with hydroxylated longer-chain fatty acids in the gastric and duodenal mucosa. Lipids 28:737-742.

Palcic MM, Heerze LD, Pierce M, Hindsgaul O (1988) The use of hydrophobic synthetic glycosides as acceptors in glycosyltransferase assays. Glycoconj J 5:49-63.

Petretti T, Schulze B, Schlag PM, Kemmner W (1999) Altered mRNA expression of glycosyltransferases in human gastric carcinomas. Biochim Biophys Acta 1428:209-218.

Poste G, Fidler IJ (1980) The pathogenesis of cancer metastasis. Nature 283:139-146.

Ramaswamy S, Ross KN, Lander ES, Golub TR (2003) A molecular signature of metastasis in primary solid tumors. Nat Genet 33:49-54.

Reis CA, David L, Carvalho F, Mandel U, de Bolos C, Mirgorodskaya E, Clausen H, Sobrinho-Simoes M (2000) Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreated mucins (MUC5AC and MUC2) in human gastric carcinomas. J. Histochem. Cytochem. 48:377-388

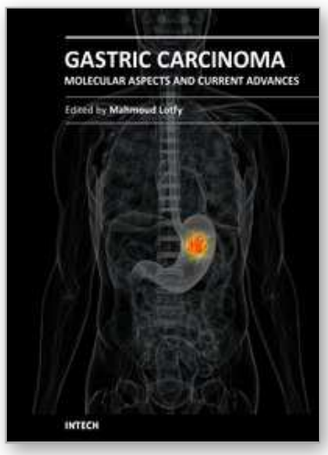
Santos-Silva F, Fonseca A, Caffrey T, Carvalho F, Mesquita P, Reis C, Almeida R, David L, Hollingsworth MA (2005) Thomsen-Friedenreich antigen expression in gastric carcinomas is associated with MUC1 mucin VNTR polymorphism. Glycobiology 15:511-517.

Sogn JA, Anton-Culver H, Singer DS (2005) Meeting Reports: NCI Think Tanks in Cancer Biology. Cancer Res 65:9117-9120.

Taniguchi N, Yoshimura M, Miyoshi E, Ihara Y, Nishikawa A, Fuji S. (1996) Remodeling of cell surface glycoproteins by N-acetylglucosaminyltransferase III gene transfection: modulation of metastatic potentials and down regulation of hepatitis B virus replication. Glycobiology 6:691-694.

Taylor-Papadimitriou J, Burchell J, Miles DW, Dalziel M (1999) MUC1 and cancer. Biochim Biophys Acta 1455:301-313.

Werther JL, Tatematsu M, Klein R, Kurihara M, Kumagai K, Llorens P, Neto JG, Bodian C, Pertsemlidis D, Yamachika T, Kitou T, Zkowitz S (1996) Sialosyl-Tn antigen as a marker of gastric cancer progression: an international study. Int J Cancer 69:193-199.



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Gastric cancer is one of the most common tumors worldwide. It has a heterogeneous milieu, where the genetic background, tumor immunology, oxidative stress, and microbial infections are key players in the multiple stages of tumorigenesis. These diverse factors are linked to the prognosis of the gastric cancer and the survival of gastric cancer patients. This book is appropriate for scientists and students in the field of oncology, gastroenterology, molecular biology, immunology, cell biology, biology, biochemistry, and pathology. This authoritative text carefully explains the fundamentals, providing a general overview of the principles followed by more detailed explanations of these recent topics efficiently. The topics presented herein contain the most recent knowledge in gastric cancer concerning the oncogenic signaling, genetic instability, the epigenetic aspect, molecular features and their clinical implications, miRNAs, integrin and E-cadherin, carbohydrate-associated-transferases, free radicals, immune cell responses, mucins, Helicobacter-pylori, neoadjuvant and adjuvant therapy, prophylactic strategy for peritoneal recurrence, and hepatic metastasis.

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