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Research Article

A High-level Over-Expression of N-and O-Glycan Glycosyltransferases in Pancreatic Tumors and Diabetic Neutrophils: An Unique Pathological Situation in Pancreatic Cancer and Diabetic Retinopathy

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ABSTRACT

Diabetes is a widely existing disease in patients at risk of pancreatic cancer. It originates from pancreatic endocrine gland whereas pancreatic cancer develops from exocrine glands. Leukocyte cell surface glycans are involved in leukocyte-endothelial cell adherence and retinal endothelial cell death in diabetic retinopathy. A stimulation of hexosamine biosynthesis pathway occurs in diabetes and in pancreatic cancer controlled by oncogene KRAS variant. We examined 5 pancreatic non-tumor and 14 pancreatic tumor tissue specimens for quantitative changes in glycosyltransferse (GTs) activities in pancreatic tumorigenesis by following the incorporation of ^{14}C or ^{3}H monosaccharide (CPM) into specific acceptor catalyzed by 1 mg protein of Triton X-100 solubilized tissue extract. As compared to pancreatic non-tumor tissue specimens with a very low level of GTs activities, pancreatic tumor specimens on average contained 26.0, 42.9, 331.7, 121.0 and 62.8-fold of α1-2, α1-3, α1-4, α1-6 FTs and FTVI activities respectively. The major sialyltransferase α2-3 (O)ST and sialomucin glycoproteins increased 95.4 and 4.0-fold; N-glycan αMan: β1-2GlcNAc-T, chain elongating βGal: β1-3 GlcNAc-T and N-glycan GalNAc capping β1-3/1-4 GalNAc-T were respectively 95.0, 2.7 and 14.8-fold and the mRNAs of FUT-4, β1-3 and β1-4 GalNAc-Ts were 8.3, 12.0 and 2.4-fold respectively.

The increase in activity in neutrophils of retinopathy vs. normal was: β1-2-GlcNAc-T (9.0 fold), β1-3-GlcNAc-T (2.5), α1-3- FT (3.5), α1-6-FT (3.3), FTVII (1.9), αGalNAc: β1-3-GalT (1.4), βGlcNAc: β1-4-Gal-T (2.1), α2-3-(O)ST (2.1), α2-3-(N)ST (4.5), α2-6-(N)ST (8.1). GalNAc replacing Gal in LacNAc terminals results in changes of glycosyltransferase specificities and the modified GalNAc β1-4GlcNAc by FTs and STs bind to lectins such as WGA. In contrast to a low-level expression-difference of glycosyltransferases between tumor and non-tumor specimens from stomach, prostate and colon, a multifold increase in GTs in pancreatic tumor would indicate their significant role in invasion and intractability of pancreatic cancer.

Keywords: Pancreatic cancer; Tumor specimens; Diabetic retinopathy neutrophils; N-and O- glycans, over-expression; Glycosyltransferase activities

INTRODUCTION

Diabetes is a metabolic disease associated with the endocrine glands of pancreas and is a widely existing disease in patients at risk of pancreatic cancer. Diabetic retinopathy is a progressive vision threatening complication of diabetes [1]. Increased leukocyte-endothelial cell adhesion appears to be a mechanism for capillary occlusion in diabetic retinopathy [2]. O-linked oligosaccharides on the surface of leukocytes play a crucial role in leukocyte-endothelial cell adherence through adhesion molecules such as selectins and integrins [3,4].

High glucose driven metabolic dysfunction such as increased involvement of the hexosamine pathway, accumulation of advanced glycation end-products and the induction of chronic low grade inflammatory signaling in the retina play an important pathological role [5]. Two types of glycosylation reaction namely O-GlcNAc [6] and β1-4GlcNAc branch from Man in complex N-glycans [7,8] were shown to be involved in type 2 diabetes.

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Pancreas is sandwiched adjacent to stomach, gall bladder and small intestine and it lies in proximity to nearby blood vessels. Hence pancreatic tumor can spread quickly into other organs. Further many pancreatic cancers are encased in main blood vessels passing through pancreas and so surgical intervention is rather difficult. Pancreatic ductal adenocarcinoma (PDA) which develops from exocrine glands of pancreas is a very lethal cancer with a 5-year survival rate of ~5% [9].

Malignant progression proceeds with the early acquisition of activating mutations in the KRAS oncogene, which occurs in >90% of cases, and subsequent loss of tumor suppressors including INK4A/ARF, TP53, and SMAD4 [10]. $Kras^{G12D}$ serves a vital role in controlling tumor metabolism through stimulation of glucose uptake and channeling of glucose intermediates into the hexosamine biosynthesis (HBP) and pentose phosphate pathways [11]. A transgenic model of PDA showed that the inhibition of autophagy in presence of Kras and Trp53 mutations promotes cancer formation [12]. Several studies indicate that glycans and glycosylation of cellular proteins participate in the process of cancer cell adhesion, dissemination and metastasis [13-26].

As $Kras^{G12D}$ mediates pancreatic tumor maintenance by stimulating hexosamine biosynthesis pathway (HBP) and associated protein glycosylation [11], it is pertinent to ascertain whether the stimulation of HBP leads to overexpression of any specific or several glycosyltransferase activities and glycans in pancreatic tumor tissues. The present study examined the levels of several glycosyltransferase activities, their mRNA in some cases and sialylated O-glycans in tumor and non-tumor pancreatic tissue specimens and found their multifold overexpression in pancreatic tumor. Several glycosyltransferase activities were also found at higher level in neutrophils of diabetic retinopathy patients. The unprecedented exorbitant increase in the capacity of glycosylation could lead to detrimental biological consequences.

MATERIALS AND METHODS

Tissue specimens

Human pancreatic and prostate tumor and non-tumor tissue specimens were obtained from pathology after surgical procedures at Roswell Park Cancer Institute and stored frozen within 1 h at 70°C. It is to be noted that in pancreatic cancer unlike many other cancers, there is a paucity of pancreatic tumor and non-tumor specimens from the same patients available for study. We studied the pancreatic tumor as well as non-tumor tissue specimens from the same patient in three pancreatic carcinoma cases (1T, 1N; 2T, 2N; 3T, 3N) and 11 pancreatic tumor specimens in which case normal pancreatic tissue specimens (6T-16T) were unavailable.

Two non-tumor pancreatic tissue specimens, for which the corresponding tumor specimens (4N, 5N) were unavailable, were also used. The prostate tumor and non-tumor tissue specimen from the same patient in nine prostate cancer cases (1T, 1N-9T, 9N) and three other available prostate tumor tissues (10T-12T) were used in this study for the purpose of comparison with pancreatic tumor data. When the tissue samples were collected from Pathology, a portion of the sample used for enzyme assay was fixed in formalin and embedded in paraffin. Slides were prepared from the paraffin block and stained with hematoxylin-eosin by standard procedures. A board-certified pathologist studied the slides to determine the distribution of cell types within the tumor and non-tumor tissues. The tumor tissue contained malignant epithelial cells while the non-tumor did not.

The pathology report containing pancreatic cancer diagnostic details and the amount of tissue specimens available for our studies is presented in Table 1. A comparison of each glycosyltransferase activity per mg protein of the Triton X-100 solubilized extract of tumor specimens with that of non-tumor specimens was made as it is relevant for understanding the quantitative change of each enzyme activity in tumorigenesis.

Table 1: Pancreatic cancer diagnostic details of the patients and tumor and non-tumor tissue specimens.

Case	Site	Histology	Grade	Tissue (mg)
14490	Tail of Pancreas	Mucinous cystadenocarcinoma	Well differentiated	1T: 960 1N: 560
14596	Tail of Pancreas	Infiltrating duct CA	Moderately differentiated	2T: 715 2N: 2290
15667	Head of Pancreas	Infiltrating duct CA	Moderately differentiated	3T: 740 3N: 860
15770	Ampulla of Vater	Ampullary adenocarcinoma	Poorly differentiated	4N: 215
15788	Ampulla of Vater	Ampullary adenocarcinoma	Moderately differentiated	5N: 715
15291	Head of Pancreas	Infiltrating duct CA	Poorly differentiated	6T: 150
15647	Head of Pancreas	Infiltrating duct CA	Moderately differentiated	7T: 410
15655	Other specified parts of Pancreas	Infiltrating duct CA	Poorly differentiated	8T: 350
11911	Ampulla of Vater	Ampullary adenocarcinoma	Moderately differentiated	9T: 220
12459	Head of Pancreas	Infiltrating duct CA	Poorly differentiated	10T: 160
12461	Distal Pancreas	Mucinous adenocarcinoma	Moderately differentiated	11T: 770
12876	Head of Pancreas	Infiltrating duct CA	Moderately differentiated	12T: 620
12956	Ampulla of Vater	Ampullary adenocarcinoma	Moderately differentiated	13T: 350
13767	Junction of Ampulla and Duodenal mucosa	Intestinal type adenocarcinoma	Moderately differentiated	14T: 290
12573	Head of Pancreas	Adenocarcinoma	Poorly differentiated	15T: 310
12584	Head of Pancreas	Adenocarcinoma	Poorly differentiated	16T: 280

N: Non-tumor; T: Tumor.

Acceptor compounds

The synthetic compounds and modified glycopeptides used as acceptors in this study as shown in Table 1 have already been reported in our earlier studies [27-32] and, thus, are well-documented acceptors for measuring the reported enzyme activities.

Processing of tissue specimens

The tissues were homogenized at 4°C with four volumes (4 mL/g tissue) of 0.1 M Tris Maleate pH 7.2, 0.1% NaN_3 using kinematica. After adjusting the concentration of Triton X-100 to 2% by adding 20% Triton X-100, 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. Protein in the clear fat-free supernatant was measured by micro BCA method and then stored frozen at -20°C until use. Aliquots of 10 µL from this extract were used in most assays run in duplicates.

Glycosyltransferase activity in tumor lysate was determined by mixing the lysates 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, USA) and a Beckman LS9000 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 Figures and Tables. All assays were run in duplicate. We have taken care to ensure that the 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. 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-[^{14}C] NeuAc (0.05 µCi; 293 mCi/mmol Perkin-Elmer) and 10 µL cell extract in a total volume of 20 µL [30]. GlcNAc:1-4Gal-T and GalNAc:1-3Gal-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 10 µL tissue extract in a total volume of 20µ L [27].

It was incubated for 4 h GlcNAc:1-3/4GalNAc-T assay mixtures in duplicate contained 0.1 M Hepes–NaOH pH7.0, 7 mM ATP, 20 mM Mn acetate. UDP [6-^{3}H] GalNAc (0.20 µCi; 7.8 Ci/mmol: NEN-Dupont) 7.5 mM acceptor, 10µL tissue extract and incubated for 4 h [27]. α1-2-, α1-6-, α1-3- and α1-4-FTs assay reactions were carried out for 2 h in a reaction mixture containing 50 mM Hepes buffer (pH 7.5), 5 mM MnC12, 7 mM ATP, 3 mM NaN3, 3 mM synthetic acceptor or 40 µg of IgG glycopeptide acceptor, 0.05 µCi GDP-[U-^{14}C] Fuc (290 mCi/ mmol; NEN-Dupont) and 10 µL tissue extract in a total volume of 20 µl [26,28,29]. GlcNAc-T assay mixture (20µL) in duplicate contained 70 mM Hepes-NaOH pH 7.0, 7mM GlcNAc 1,5 lactone, 14mM Mn acetate, 5mM ATP, 0.05µCi UDP [^{14}C] GlcNAc (200 mCi/mmol; Amersham), 5 mM synthetic acceptor or 40 µg Fetuin glycopeptide acceptor and 10 µL tissue extract and incubated for 4 h at 37°C [32].

Dowex-1-Cl or Sep-Pak C18 cartridges were used to isolate radiolabeled product from the reaction mixture. For α1-6 FT and α Man: β1-2 GlcNAc-T assays, the incubation mixture was diluted with 1 ml water and passed through a 1 ml bed volume of Dowex-1-Cl column [26,27]. The column was washed twice with 1 ml water. The breakthrough and the water wash contained the [^{14}C] fucosyl or [^{14}C] GlcNAc-yl products formed from glycopeptide acceptors. About 3 ml of 0.1 M NaCl was used to obtain [^{14}C]-fucosylated products from sialylated acceptors after water elution. For other glycosyl transferase assays, the radioactive products from benzylglycosides were separated by hydrophobic chromatography on Sep-Pak C18 cartridge (Water, Milford, MA, USA), and elution of the product was done with 3 ml methanol [30].

Reverse-exchange sialylation of sialomucin glycoproteins in pancreatic tissue specimens

We have well documented reverse sialylation activity of ST3 Gal II [α2-3(O)ST] which converts CMP to CMP-NeuAc using specific donor NeuAc α2-3 Gal β1-3 GalNAcα units [33, 34]. Radiolabeling of sialic acid in NeuAc α2-3 Gal β1-3 GalNAc units of several glycoconjugates were shown by using ST3 Gal II and CMP [9-^{3}H or ^{14}C] NeuAc utilizing the slow natural breakdown of CMP NeuAc into CMP and NeuAc at 37° C. The Triton X-100 solubilized tissue extracts (1N, 1T, 2N, 2T, 3N, 3T, 4N, 5N, 8T, 11T, 12T and 13T; 0.1mL each) were incubated separately at 37°C for 20 h in0.1M Na cacodylate pH 6.0, 0.2 µCi CMP-[^{14}C] NeuAc, and 25mU ST3Gal II (reaction volume 0.16ml). After incubation the reaction mixtures were diluted with 1.0ml water and dialyzed in the cold room against 2L of deionized distilled water with four changes for 72 h, lyophilized to dryness and then picked up in 1.0 ml water containing 0.2% Triton X-100. Incorporation of [^{14}C] NeuAc per mg protein of these extracts was determined.

Isolation of neutrophils from control and diabetic retinopathy patients

Human polymorphonuclear leukocytes were isolated from freshly collected blood obtained by venipuncture in 10 U/ml heparin (Elkins-Sinn, Cherry Hill, NJ, USA). After gradient separation, erythrocytes were removed by hypotonic lysis. Polymorphonuclear leukocytes were then stored in Ca^{2+}-free HEPES buffer at 4°C. Cell viability was >99% and >90% of the isolated leukocytes were neutrophils. ~50×10^6 neutrophils were further purified by sorting, using a FACS-Vantage instrument [35, 36]. Such samples were obtained from normal/control individual and diabetic retinopathy patients using protocols approved by the University at Buffalo Health Sciences IRB.

Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA isolation from tissue specimens, quantitative RT-PCR and quantification of mRNA levels of glycosyltransferase genes FUT 4, β1-3 GalNAc-T, and β1-4 GalNAc-T were performed as described before [37].

RESULTS

Evolution has selected the most diverse molecules such as glycoproteins containing complex glycan structures as the communication interface between cells and extracellular environment [38]. The early studies indicated the apparent role of glycans in cancer by showing that altered glycosylation on the surfaces or secreted proteins of tumor cells is common in pancreatic cancer and is thought to promote cancer progression [39,40]. Antibody-

glycan microarray method found pro-inflammatory stimuli to alter the expression and glycosylation of mucins MUC 1, MUC 5AC and MUC 16 in multiple pancreatic cancer cell lines [41]. Since pancreatic tumor cells are usually part of an inflammatory environment, they are exposed to a variety of cytokines and growth factors [41]. It appears that the emergence of particular glycan structures on these cells may be functionally important in cancer progression [41]. Haptoglobin, leukemia-inhibitory factor receptor, centrosome-associated protein and vacuolar protein sorting-associated protein in pancreatic cancer sera were shown to express N-glycans containing core α1-6 Fuc, terminal GalNAc, α2-6 sialyl GalNAc and α1-2 Fuc as well as hybrid complex structures [42].

The present study used specific acceptors for assaying glycosyltransferase activities in tumor and non-tumor tissue specimens from pancreas and prostate cancer patients and also in normal and diabetic neutrophils as listed in Table 2.

Fucosyltransferases in pancreatic cancer

The results presented in Figure 1 indicate that α1-2-, α1-3-, α1-4-FT and FT VI and α1-6-FT activities are either absent or very low in pancreatic non-tumor tissue specimens (Figure 1 A-E specimens 1-5). But all these FTs showed highly increased level of activities in all pancreatic tumor tissue specimens (Figure 1 A-E specimens 6-19) except for the tissue13T which expressed only α1-6FT at high level (Figure 1 E specimen 16) It is interesting to note that the increase in α1-3- and α1-4- FT activities are very high and multifold in all pancreatic tumor specimens except for 13T. FUT-4 mRNA (Table 3.) was very low in pancreatic non-tumor specimens in the range 0.04→0.15 whereas the pancreatic tumor specimens contained FUT-4 mRNA in the range 0.60→0.99.

Table 2: Acceptors used for assaying glycosyltransferases in tumor and non-tumor tissue specimens.

	Acceptors
Fucosyltransferases	
α1-2- FT[a]	D-Fuc β1-3 GalNAcα-O-Benzyl (Bn)
α1-3- FT	2-O-MeGalβ1-4GlcNAcβ-O-Bn
α1-4- FT	2-O-MeGalβ1-3GlcNAcβ-O-Bn
FT VI[b]	GlcNAcβ1-4GlcNAcβ-O-Bn
α1-6- FT	Bovine IgG diantennary agalacto defucosyl glycopeptide
FT VII	NeuAc α2-3 Galβ1-4GlcNAcβ-O-Bn
Sialyltransferases	
α2-3 (O)ST	Galβ1-3 GalNAcα-O-Bn
α2-3 (N)ST	4-O-MeGalβ1-4GlcNAcβ-O-Bn
α2-6 (N)ST	GalNAcβ1-4GlcNAcβ-O-Bn
α2-6 (O)ST	3-O-MeGalβ1-3GalNAcα-O-Bn
βGal/GalNAc Transferases	
β1-4Gal-T	3-O-MeGalβ1-3(GlcNAcβ1-6) GalNAcα-O-Bn
αGalNAc: β1-3Gal-T	4-FluoroGlcNAcβ1-6 GalNAcα-O-Bn
β1-3/4GalNAc-T	GlcNAcβ-O-Bn
GlcNAc Transferases	
Man: β1-2GlcNAc-T	Fetuin triantennary asialo aglacto glycopeptide devoid of terminal GlcNAc residues
Gal: β1-3GlcNAc-T	Galβ1-4GlcNAcβ-O-Bn

[a]α1-2 fucosyltransferase acts on terminal βGal as well as on 6-deoxy βGal (D-fucose) as determined by using chemically synthesized compounds [28]; [b]Our earlier studies established that FT VI which is an α1-3-FT can also utilize GlcNAcβ1-4GlcNAc whereas α1-6- FT acts only on Asn-linked chitobiose attached to trimannosyl core [29,31].

Table 3: m-RNA expression of FUT-4, β-1-3GalNAc-T and β-1-4GalNAcT in pancreatic tumor and non-tumor tissue specimens.

Tissue Specimens		m-RNA FUT-4	m-RNA β1-4 GalNAc-T	m-RNA β1-3GalNAc-T
Pancreatic Normal	1N	0.04	1.95	0.20
	2N	0.15	2.91	0.49
	3N	0.06	1.85	0.22
	5N	0.04	0.80	0.05
Pancreatic Tumor	1T	0.60	5.34	0.75
	9T	0.99	10.10	11.03
	10T	0.30	2.57	0.88
	11T	0.40	5.31	2.05
	12T	0.72	3.63	1.59
	13T	0.47	2.02	0.96
	14T	0.60	3.92	3.58

N: Non-tumor; T: Tumor

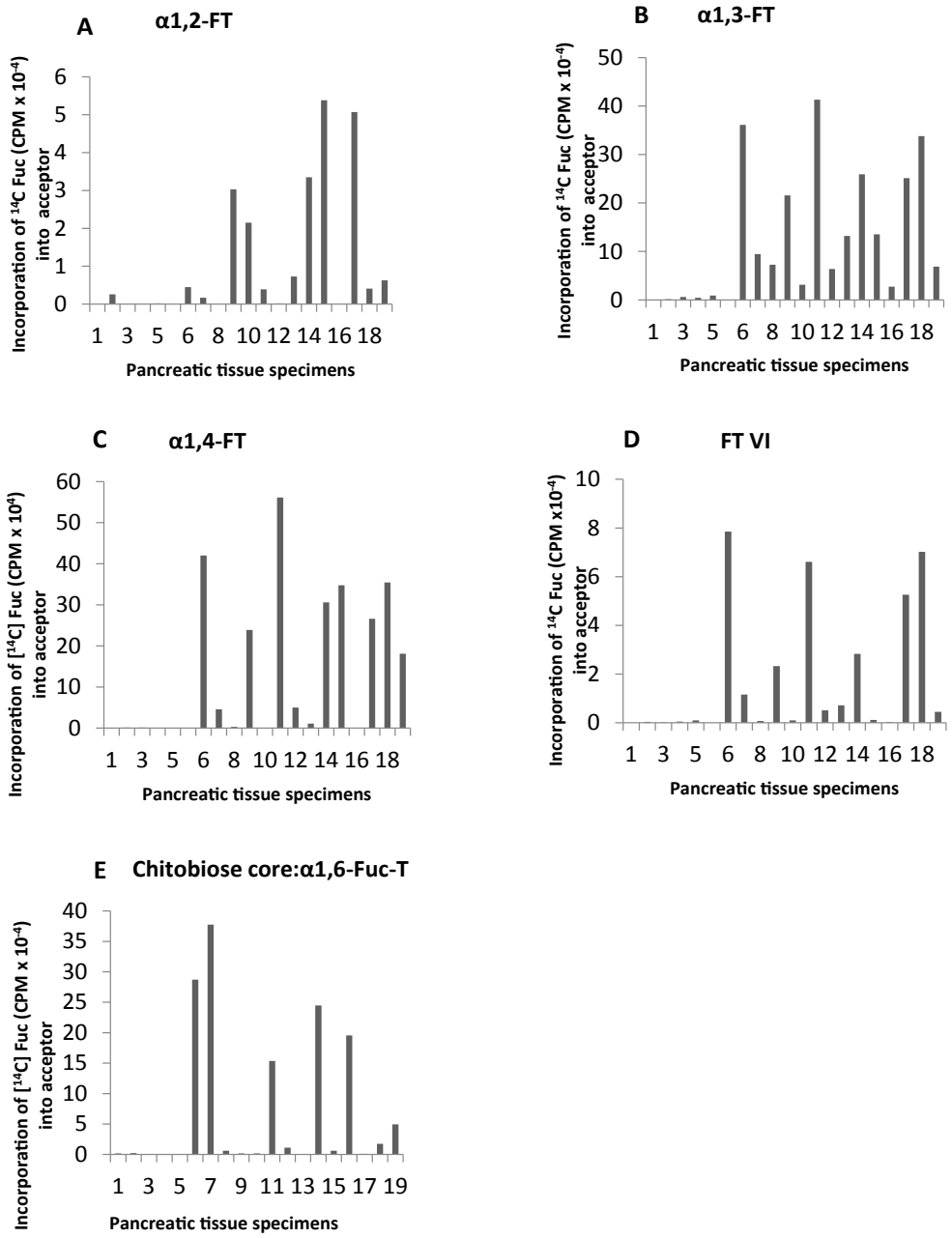

Figure 1: Fucosyltransferase activities in pancreatic tumor and non-tumor tissue specimens. Fucosyltransferase activity: Incorporation of ^{14}C Fuc (CPM x 10^{-4}) into specific acceptor catalyzed by 1mg protein. A) α1-2FT; B) α1-3FT; C) α1-4FT; D) FT VI; E) Chitobios core: α1-6FT Pancreatic tissue specimen: 1-5: Non-tumor specimens 1N-5N; 6-19: Tumor specimens 1T-3T; 6T-16T.

Sialyltranferases and sialomucin glycoproteins in pancreatic cancer

The data presented in Figure 2 indicate that all sialyltransferase activities are either absent or too low in pancreatic non-tumor specimens. The major sialyltransferase activity in pancreatic tumor is α2-3 (O)ST which forms NeuAc α2-3 Galβ1-3 GalNAc α-O-Ser/Thr. Except for pancreatic tumor specimen 6T, 7T, 9T,10T and 14T (Figure 2 Specimens 9-11, 13 and 17) others exhibited multifold increase in α2-3 (O) ST activity. Pancreatic tumor α2-3 (N)ST activity was much lower and α2-6 (N) ST activity was minor as compared to that of α2-3 (O)ST.

An investigation on the level of sialomucin type glycoprotein in five non-tumor (1N-5N) (Figure 2 Specimens 1-5) and seven tumor (1T-3T, 8T, 11T-13T) pancreatic tissue specimens (Figure 2 Specimens 6-8, 11, 14-16) using the technique of exchange sialylation showed a significant increase in sialomucin type glycoproteins in pancreatic tumor specimens as compared to non-tumor specimens examined. It is evident from the above results that pancreatic tumor contains an elevated level of sialomucin type glycoproteins and increased activities of α1-3- and α1-4-FTs and α2-3 (N) ST which favor the formation of Lewis blood group structures.

N-glycan associated glycosyltransferases in pancreatic cancer

As shown in Figure 3, N-linked complex-type chain initiating enzyme αMan: β1-2 GlcNAc-T, N-glycan chitobiose inner core α1-6-FT (Figure 1 E), N-glycan chain elongating βGal: β1-3GlcNAc-T

(Figure 3 D) and N-glycan GalNAc capping β GlcNAc: β1-3/β1-4 GalNAc-T (Figure 3 C) were found either absent or very low in non-tumor pancreatic tissue specimens (Figure 3 specimens 1-5). αMan: β1-2 GlcNAc-T in pancreatic tumor specimens except for

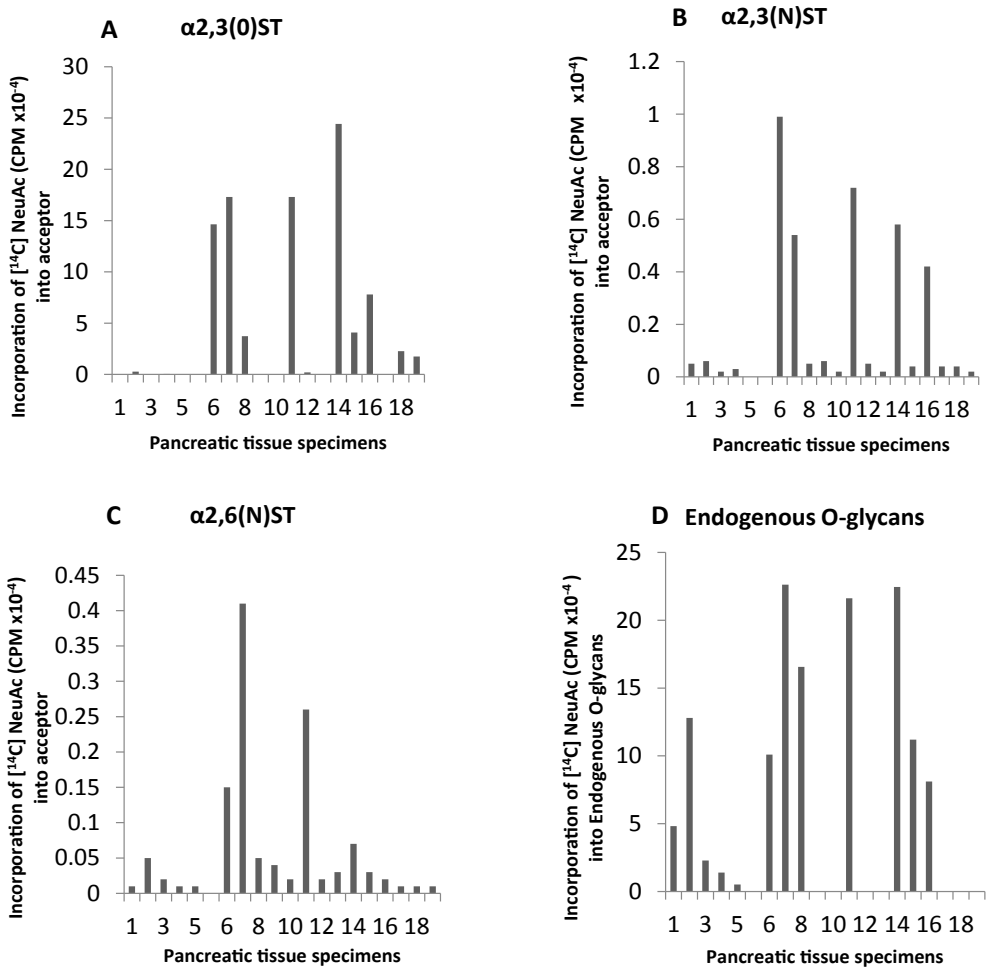

Figure 2: Sialyltransferase activities and sialomucin glycoprotein expression levels in pancreatic tumor and non-tumor tissue specimens. Sialyltransferase activity: Incorporation of ^{14}C NeuAc (CPM x 10-4) into specific acceptor catalyzed by 1mg protein. A) α2-3(O)ST; B) α2-3(N)ST; C) α2-6(N)ST; D) endogenous sialomucin glycoproteins Pancreatic tissue specimen: 1-5: Non-tumor specimens 1N-5N; 6-19: Tumor specimens 1T-3T; 6T-16T.

6T, 7T, 9T, 10T and 14T (Figure 3 E specimens 9, 10, 12, 13 and 17) showed increased activity, the increase being several fold in 1T, 2T, 8T, 11T and 13T (Figure 3 E specimens 6,7, 11, 14 and 16). Several fold α1-6-FT activity was found in tumor specimens 1T, 2T, 3T, 8T, 9T, 11T, 13T and 16T (Figure 1E specimens 6-8, 11, 12, 14, 16 and 19). It is noteworthy there is an inverse relationship between α1-2-FT and α1-6-FT; most of the tumors showing overexpression of α1-6-FT had low levels α1-2-FT (Figure 1A and 1E).

Significant increase in β1-3 GlcNAc-T was seen with 1T, 2T, 3T, 8T, 11T and 13T (Figure 3 D specimens 6-8, 11, 14 and 16). β1-3/β1-4 GalNAc-T activity exhibited an increase of severalfold in pancreatic tumor specimens 1T, 2T, 3T, 8T, 11T, 12T, 13T and 16T (Figure 3 C specimens 6-8, 11, 14-16 and 19). These findings indicate an over-expression of N-glycan associated glycotransferases in pancreatic tumors. The levels of β1-4 GalNAc-T and β1-3 GalNAc-T mRNAs (Table 3) in pancreatic tumor specimens were much higher than that in non-tumor specimens. Range: β1-4 GalNAc-T N: 0.80→2.91 and T: 2.02/10.10; β1-3 GalNAc-T N: 0.05→0.49 and T: 0.75→11.03.

A comparison of pancreatic, gastric, and prostate tumors for mucin core 1 initiating enzyme αGalNAc: β1-3Gal-T and mucin core 2 elongating enzyme GlcNAc: β1-4Gal-T.

As shown in Table 4, core2 β1-4 Gal-T activity is much lower in pancreatic non-tumor specimens as compared to the level of these enzymes in the non-tumor specimens from stomach [43] and prostate. Activity range: pancreas 0.1→0.3; stomach 2.7→36.3 and prostate 6.3→40.2. The β1-4 Gal-T activities range in tumor specimens were pancreas 0.2→38.5; stomach 6.2→40.4 [43] and prostate 6.4→40.2 (except 7T 73.5).

Hence the increase in β1-4 Gal-T activity level with respect to non-tumor tissue is multifold in pancreatic tumor as compared to that of gastric and prostate tumors. Core1 β1-3 Gal-T activity in both pancreas and prostate non-tumor specimens is much lower than stomach non-tumor specimens [43]. The β1-3 Gal-T activities range in non-tumor and tumor specimens are as follows: Pancreas N 0.1→0.6; T 0.2→19.8; stomach N 2.6→11.1; T 1.9→24.5 [43] and prostate N 0.8→1.6; T 0.6/1.4. It is clear from this data, the increase in β1-3 Gal-T activity is multifold in pancreatic tumor whereas there was no increase in prostate tumor specimens and less significant increase in gastric tumor specimens [43]. Thus, it becomes evident that both mucin β1-3 and β1-4 Gal-T activities which were quite low in normal pancreas were highly elevated in pancreatic tumorigenesis and this happening of a stimulation in the synthesis of mucin-type structures appears to be unique to pancreatic cancers due to over-expression of αGalNAc: β1-3Gal-T and core2 GlcNAc: β1-4 Gal-T.

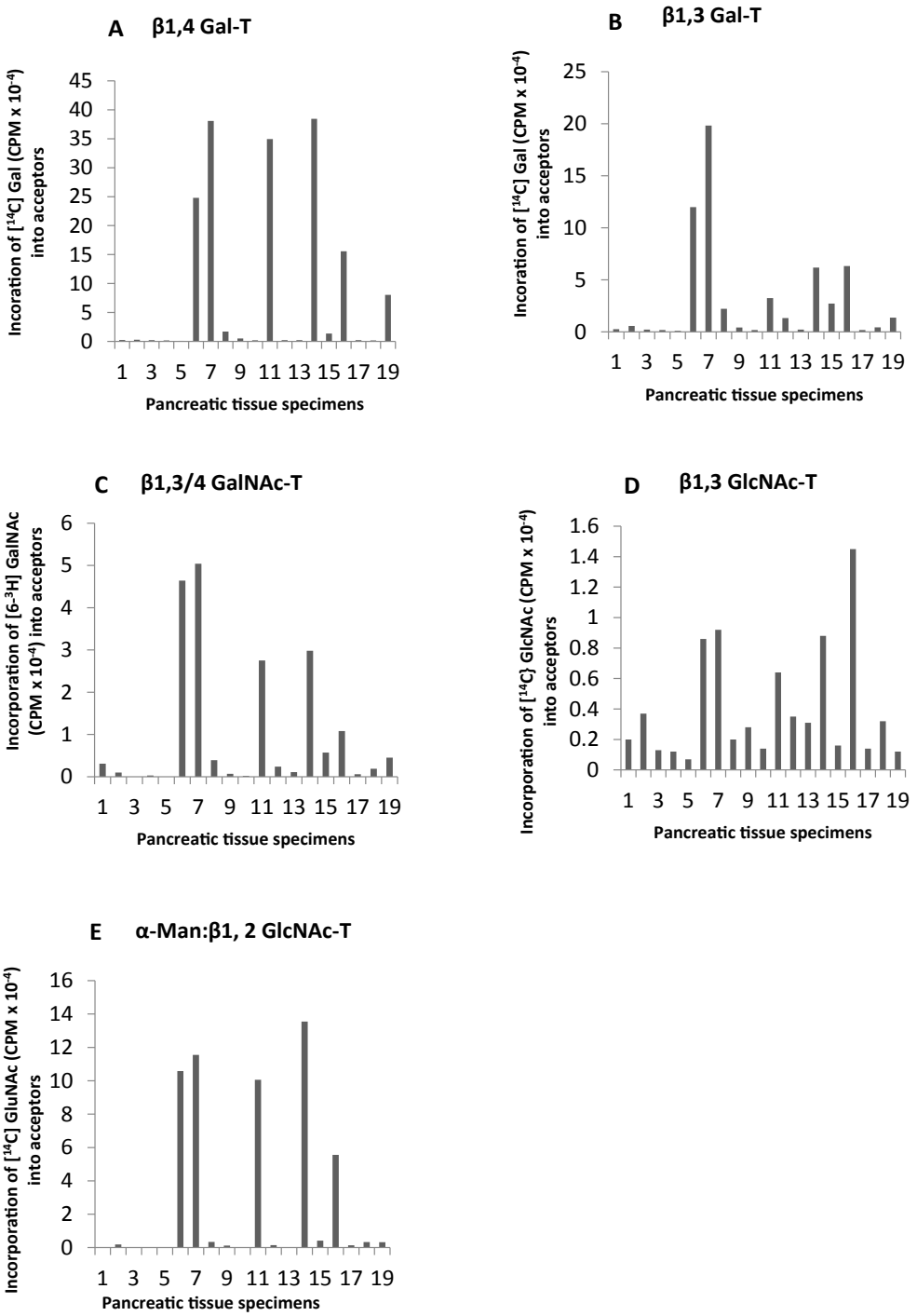

Figure 3: B-Galactosyl, β-N-acetylglucosaminyl and β-N-acetylgalactosaminyl transferase activities in pancreatic tumor and non-tumor tissue specimens Galactosyl, N-Acetylglucosaminyl and N-Acetylgalactosaminyl transferase activities: incorporation of ^{14}C Gal, ^{14}C GlcNAc or 6-^{3}H GalNAc (CPM x 10^{-4}) into specific acceptor catalyzed by 1mg protein.A) GlcNAc: β1-4 Gal-T; B) α GalNAc: β1-3 Gal-T; C) GlcNAc: β1-3/4 GalNAc-T; D) Gal: β1-3 GlcNAc-T; E) αMan: β1-2GlcNAc-T Pancreatic tissue specimen: 1-5: Non-tumor specimens 1N-5N;6-19: Tumor specimens 1T-3T; 6T-16T.

Altering terminals in carbohydrate chains and its effects on gycosyltransferases activities and the binding of plant lectins

Table 5 presents the results from replacing Gal with GalNAc in LacNAc type I and type 2 acceptors. Remarkably, FT IV which is known to be strictly capable of carrying out α1-3 fucosylation only is shown here to be considerably active (25.1%) with GalNAc β1-3GlcNAc β-O-Bn as compared to Gal β1-3 GlcNAc β-O-Bn (1.7%). The α1-4-FT from human lung [44] utilized both Galβ1-3 GlcNAc β-O-Bn (100.0%) and GalNAc β1-3GlcNAc β-O-Bn (96.9%) to the same extent whereas it acted more on GalNAc β1-4GlcNAc β-O-Bn (51.2%) as compared to Gal β1-4 GlcNAc β-O-Bn (12.0%).

Cloned FT V acted almost equally on type 1 and type 2 containing either Gal or GalNAc whereas FT VI utilized poorly Galβ1-3 GlcNAc β-O-Bn (5.0%) but acted on GalNAc β1-3GlcNAc equally well (93.8%) as on type 2 LacNAc containing either Gal or GalNAc (100.0% and 97.4%). Cloned ST3Gal III acted poorly on LacNAc type 1 and type 2 when Gal was replaced by GalNAc (3.0% and 0.7%). Further, it is noteworthy that Cloned ST6Gal I in contrast to ST3 Gal III acted fairly well on LacNAc type 2 containing GalNAc (LacdiNAc) (65.2%) whereas LacNAc type 1 containing Gal or GalNAc was a poor acceptor (3.0% and 8.1%).

The plant lectin SNA-1[45] bound comfortably well to α2-6

Table 4: A Comparison of the levels of αGalNAc: β1-3Gal-T and GlcNAc: β1-4 Gal-T activities in stomach, prostate and pancreatic normal and tumor specimens.

Pancreas			Stomach[a]			Prostate		
Tissue specimens	β1-3 Gal-T	β1-4 Gal-T	Tissue specimens	β1-3 Gal-T	β1-4 Gal-T	Tissue specimens	β1-3 Gal-T	β1-4 Gal-T
Incorporation of [^{14}C] Gal (CPMx10^{-4}) into the acceptor catalyzed by 1mg protein								
1N	0.3	0.2	1N	4.0	2.7	1N	0.8	32.1
1T	12.0	24.8	1T	24.5	16.2	1T	1.2	24.8
2N	0.6	0.3	2N	3.7	17.2	2N	0.7	6.3
2T	19.8	38.1	2T	4.3	11.2	2T	0.7	6.4
3N	0.2	0.2	3N	3.5	16.8	3N	1.2	23.8
3T	2.2	1.7	3T	4.4	15.2	3T	1.1	36.2
4N	0.2	0.2	4N	7.0	23.2	4N	0.5	26.3
			4T	1.9	19.9	4T	0.6	26.6
5N	0.1	0.1	5N	10.2	27.5	5N	1.6	40.2
			5T	3.6	25.8	5T	1.3	37.9
6T	0.4	0.5	6N	9.9	23.0	6N	0.7	12.2
			6T	6.3	20.6	6T	1.1	11.7
7T	0.2	0.2	7N	6.7	25.5	7N	1.6	16.9
			7T	6.6	40.4	7T	1.4	73.5
8T	3.2	35.0	8N	2.6	11.3	8N	0.9	36.3
			8T	3.2	12.4	8T	0.9	34.0
9T	1.3	0.2	9N	8.8	36.3	9N	0.8	34.4
			9T	13.8	21.1	9T	0.7	13.7
10T	0.2	0.2	10N	11.1	26.5	10T	0.8	23.8
			10T	16.6	20.7			
11T	6.2	38.5	11T	10.3	9.3	11T	1.0	42.2
12T	2.7	1.4	12T	2.4	6.2	12T	0.7	30.7
13T	6.3	15.6						
14T	0.2	0.2						
15T	0.4	0.2						
16T	1.4	8.0						
Mean Value								
N	0.3	0.2	N	6.8	21.0	N	1.0	25.4
T	4.0	11.7	T	8.2	18.3	T	1.0	26.7
Fold	13.3	58.5		1.2	0.9		1.0	1.1

N: Non-tumor; T: Tumor; [a] In order to compare the values for gastric tissue specimens were used from an earlier report [43].

sialylated LacNAc type 2 containing either Gal or LacdiNAc GalNAc. In contrast, WGA did not bind to α2-6 sialylated LacNAc type 2 containing Gal but bound very tightly to α2-6 sialylated type 2 containing GalNAc β1-4GlcNAcβ sequence. It also exhibited non-binding to Lewis X structure but tight binding to GalNAc containing Lewis X structure. The above findings using some examples illustrate that GalNAC replacing Gal has distinct biological consequences and this kind of alteration may have an important role in pancreatic cancer.

Thus, the increased acceptor activity of FT III, FT IV and FT VI on GalNAc terminal LacNAc type 2 LacdiNAc (25.1%), GalNAc β1-3GlcNAcβ type 1 (51.2%) and GalNAcβ1-3GlcNAcβ type 1 (93.8%) respectively and almost equal activity of FT V on GalNAc terminal GalNAcβ1-3GlcNAcβ (88.0%), negligible activity of ST3 Gal III on GalNAc terminal Galβ1-3GlcNACβ and GalNAcβ1-4GlcNAc (3.0% and 0.7% respectively) and moderate activity of ST6 Gal I on GalNAc terminal in GalNAcβ1-4GlcNAcβ (65.2%) would indicate that GalNAc replacing Gal in LacNAc terminals may play a significant role in pancreatic cancer invasion. Non-binding of WGA to 6 sialyl Galβ1-4 GlcNAc and Gal β1-4 (Fuc α1-3) GlcNAc and tight-binding of WGA to 6 sialyl GalNAc β1-4 GlcNAc and GalNAc β1-4 (Fuc α1-3) GlcNAc [45] would suggest the utility of WGA as a tool for detecting pancreatic cancer.

A comparison of several glycosyltranferase activities in tumor and non-tumor tissue specimens from pancreas, gastric, prostate and colon cancers

The glycosyltransferase activities of tumor and non-tumor specimens from the same patient in 9 prostate cancer cases and 3 other prostate tumor specimens are presented in Table 6. The mean values of the folds of activities for tumor with respect to non-tumor specimens from pancreas, gastric, prostate and colon cancers are presented in Table 7.

Mean values (fold of activity) for fucosyltransferases in the case of

Table 5: Biological consequence of sugar-alteration by showing the effect of GalNAc replacing Gal in LacNAc type 1 and type 2 carbohydrate Chain terminals on glycosylation and plant lectin-binding.

Glycosyltransferases	**Fucosyl and sialyl transferase activities: Incorporation of [^{14}C] Fuc or [9-^{3}H] NeuAc (CPMx10^{-4}) into acceptor compounds**			
Fucosyltransferases	Galβ1-4GlcNAc β-O-Bn	GalNAcβ1-4 GlcNAc β-O-Bn	Galβ1-3GlcNAc β-O-Bn	GalNAcβ1-3GlcNAc β-O-Bn
FT IV (HL60 cell lysate)	100.0% (4.69)[a]	101.9%	1.7%	25.1%
α-1,4FT(FTIII) (purified from human lung tumor)	12.0%	51.2%	100.0% (6.59)	96.9%
Cloned FT V	100.0% (2.53)	109.4%	90.1%	88.0%
Cloned FTVI	100.0% (5.27)	97.4%	5.0%	93.8%
Sialyltransferases				
Cloned ST3Gal III	100.0% (2.42)	0.7%	123.1%	3.0%
Cloned ST6Gal I	100% (4.02)	65.2%	3.0%	8.1%
Plant Lectins	**Binding characteristics of synthetic compounds**			
	NeuAc α2-6 Gal β1-4GlcNAc	NeuAc α2-6 Gal NAc β1-4GlcNAc	NeuAc α2-6 Gal β1-4(6-O-sulfo) GlcNAc	
SNA-1	Regular binding	Regular binding	Tight binding	
WGA	Non-binding	Tight binding		
	Galβ1-4 (Fucα1-3) GlcNAc	GalNAcβ1-4 (Fucα1-3) GlcNAc	Gal/GalNAc β1-3 (Fucα1-4) GlcNAc	
WGA	Non-binding	Tight binding	Non-binding	

[a]The CPM for 100% activity is given in parenthesis for each enzyme; Note: α1-2 Fucosyltransferase, α1-3 Galactosyltransferase and 3-O-sulfotransferases act on Gal terminal but cannot act on GalNAc terminal.

Table 6: Fucosyltransferases, sialyltransferases, GlcNAc: β1-3/4GalNAc transferases and αMan: β1-2 GlcNAc transferase activities in human prostate tumor and non-tumor tissue specimens.

Tissue specimen	**Incorporation of [^{14}C] Fuc or [9-^{3}H] NeuAc or [^{3}H] GalNAc or [^{3}H] GlcNAc (CPMx10^{-4}) into the acceptor catalyzed by 1mg protein of TritonX-100 solubilized tissue extract**							
	α1-2 FT	α1-3 FT	α1-6 FT	α2-3 (O)ST	α2-3 (N)ST	α2-6 (N)ST	βGlcNAc: β 1-3/4 GalNAc-T	αMan: β1-2 GlcNAc-T
1N	2.49	5.38	16.19	7.11	0.36	0.23	1.20	1.26
1T	1.58	7.17	13.96	10.31	0.98	0.60	1.84	0.94
2N	ND	ND	11.96	4.79	0.08	0.10	0.36	0.53
2T	ND	ND	12.92	6.87	0.11	0.10	0.51	0.63
3N	3.8	3.14	17.02	8.46	0.47	0.21	1.49	2.64
3T	4.65	3.82	9.50	5.90	0.71	0.41	2.25	1.54
4N	ND	ND	7.39	4.90	0.31	0.18	1.24	1.11
4T	ND	ND	1.46	9.30	0.48	0.35	1.13	2.33
5N	3.81	0.79	27.50	4.88	1.87	0.89	3.34	6.04
5T	4.46	1.94	22.02	3.11	0.72	0.22	3.60	1.00
6N	1.73	3.84	6.99	10.18	0.42	0.23	1.26	1.28
6T	3.94	2.73	7.20	6.74	0.36	0.30	1.48	0.48
7N	2.91	2.00	18.27	8.08	1.04	0.55	2.21	4.20
7T	6.87	5.73	9.79	13.41	4.54	3.50	5.74	2.37
8N	5.53	1.93	19.69	4.10	1.16	0.57	3.17	1.67
8T	4.83	1.69	6.04	2.48	0.70	0.37	2.54	2.00
9N	4.15	1.77	14.47	4.60	0.85	0.34	1.21	1.30
9T	1.48	1.72	8.36	3.61	0.65	0.24	3.05	2.10
10T	ND	ND	10.29	2.96	0.26	0.15	1.03	1.36
11T	ND	ND	21.26	20.27	1.18	0.71	1.68	3.17
12T	ND	ND	8.08	9.52	0.65	0.21	1.49	1.86
MV: N	3.49	2.69	15.50	6.34	0.73	0.37	1.72	2.23
T	3.97	3.54	10.91	7.87	0.95	0.60	2.20	1.65
Fold	1.3	1.5	0.7	0.7	1.6	1.9	1.3	0.7

N: Non-tumor; T: Tumor; MV: Mean Value; Fold: Activity in tumor *vs* non-tumor

Table 7: A comparison of the mean values of the fold of glycosyltransferase activities in tumor and non-tumor tissue specimens from pancreatic, gastric, prostate and colon cancers.

	Mean value of the fold of activities in tumor vs non-tumor tissue specimens			
Glycosyltransferases	**Pancreatic Cancer[a]**	**Gastric Cancer [b]**	**Prostate Cancer[c]**	**Colon Cancer[d]**
Fucosyltransferases				
α1-2FT	26.0	1.2	1.3	9.0
α1-3FT	42.9	1.0	1.5	1.1
α1-4FT	331.7	4.7	ND	1.7
FTVI	62.8	27.4	ND	1.2
α1-6FT	121.0	ND	0.7	ND
Sialyltransferases				
α2-3 (O)ST	95.4	19.1	0.7	20.7
α2-3 (N)ST	9.3	2.3	1.6	8.7
α2-6(N)ST	4.0	9.9	1.9	19.5
Gal/GalNAc-transferases				
GlcNAc: β1-4 Gal-T	58.5	1.4	1.3	3.2
αGalNAc: β1-3 Gal-T	13.3	1.6	1.1	6.1
GlcNAC: β1-3/4 GalNAc-T	14.8	2.2	1.3	
GlcNAc-transferases				
Gal: β1-3 GlcNAc-T	2.7	ND	ND	
αMan: β1-2 GlcNAc-T	95.0	ND	0.9	

ND: Not determined; [a] for the values of pancreas, refer Figures 1-3; [b]for comparison purpose, the mean value of glycosyltransferase activity in gastric tumor and non-tumor specimens from the same patient (10 patients) were calculated from the data of an earlier report [43]; [c] for the values of prostate, refer Tables 4 and 6; [d] the fold of glycosyltransferase activity in colon tumor *vs* normal colon tissue was calculated from our previous data [31].

pancreatic tumor specimens are multifold higher when compared to gastric and prostate tumor specimens. In the case of FT VI only two gastric tumor specimens showed very high activities (51.4 and 199.5) that reflected in the high mean value (27.4). In the case of sialyltransferases, the mean values for pancreatic tumors are very much higher than that of prostate tumors. As only two gastric specimens contained high α2-3 (O)ST (7.9 and 172.0) and α2-6 (N) ST (7.3 and 82.2) activities, the mean values for α2-3 (O)ST (19.1) and α2-6 (N)ST (9.9) appear to be high reflecting these activities. Otherwise increase in fold of activity in pancreatic tumors is truly high as compared to gastric tumors. The activities of other enzymes Gal/GalNAc transferases and GlcNAc transferase have increased multifold in pancreatic tumor as evident from the mean values. All glycosyltransferase activities did not increase to a multiple level in gastric and prostate tumor specimens.

Neutrophil glycosyltransferase activities in diabetic retinopathy

The neutrophils were isolated separately from blood of five donors, one being a control individual and the other four diabetic retinopathy patients. The glycosyltransferase activities of these neutrophil samples were determined, and the results are presented in Table 8. Fucosyltranseferases α1-2 and α1-4 activities are absent in control neutrophils and either absent or in trace amount in diabetic neutrophils. α1-3-FT which forms Lewis X and FT VII which forms sialyl Lewis X from 3 sialyl Galβ1-4 GlcNAc are the major fucosyltransferase activities in neutrophils. α1-3-FT activity was almost 4-5-fold in diabetic neutrophils except for donor D (1.6 fold). FT VII activity was about 2-3-fold in diabetic neutrophil except for donor D (0.9 fold).

The major sialyltransferase activity is α2-3 (O)ST which is about 2-3-fold in diabetic neutrophils. The control neutrophils contained minor activities of other sialyltransferases α2-6 (O)ST, α2-3 (N)ST and α2-6 (N)ST but the diabetic neutrophils had about 2-3-fold, 3-8-fold and 7-13-fold activities of α2-6 (O)ST, α2-3 (N)ST and α2-6 (N)ST respectively. The core2 β1-4 Gal-T forming LacNAc type 2 which had high activity in neutrophils also exhibited an increase of activity (1.7/2.9-fold) in diabetic neutrophils. There was an appreciable increase in αGalNAc: β1-3 Gal-T activity in diabetic neutrophils (1.1→1.7-fold).

Among the glycosyltransferase activities associated with N-glycan biosynthesis, in the case of α1-6 FT which is also a major fucosyltransferase activity in neutrophils, an increase of 1.3→6.5-fold activity was found in diabetic neutrophils. The key enzyme in complex N-glycan biosynthesis namely αMan: β1-2 GlcNAc-T, there was multifold increase activity in diabetic neutrophils (4.6/12.2-fold). The complex N-glycan chain elongating enzyme Gal: β1-3 GlcNAc-T which is a major activity in neutrophils exhibited 1.9→3.1-fold increase in activity in diabetic neutrophils.

DISCUSSION

The findings of the present study validate the role of specific sets of enzymes that contribute towards the assembly of abnormally glycosylated molecules in pancreatic cancer progression. The overall profiling of glycosyltransferases (GTs) has been useful in identifying potential glycan structures altered in cancer [31]. A functional glycomics study pointed out that gene expression data would be more powerful when used in conjunction with biochemical data such as GTs activities [46]. A recent study found that changes in glycan structures, generally but not uniformly, correlate with alterations in transcript abundant for the corresponding biosynthetic enzymes [47].

Further, it is evident that a greater degree of regulation is necessary for the synthesis of distal branching and capping modifications of

Table 8: Glycosyltransferase activities of neutrophils isolated from the blood of control and diabetic retinopathy patients.

Glycosyltransferase	Glycosyltransferase activities: Incorporation of [^{14}C] - Fuc, Gal or GlcNAc or [9-^{3}H] NeuAc [CPMx10^{-3}] into the acceptor catalyzed by 1 mg protein of the neutrophil extract					
	Neutrophil Donors					
	Control	Patients				
		A	B	C	D	Mean Value
A. Fucosyltransferases						
α1-2FT	0	0.2	0.3	0	0	
α1-3FT	7.1	25.4	33.8	29.3	11.1	
		[3.6]	[1.6]	[4.1]	[1.6]	3.5
α1-4FT	0	0.6	1.1	0.1	0	
FTVII	20.3	40.8	48.2	55.2	18.7	
		[2.0]	[0.9]	[2.7]	[0.9]	1.9
α1-6FT	20.8	56.7	59	134.7	26.3	
		[2.7]	[1.3]	[6.5]	[1.3]	3.3
B. Sialyltransferases						
α2-3 (O)ST	13.8	28.4	23.8	38.9	26.3	
		[2.1]	[1.9]	[2.8]	[1.9]	2.1
α2-6 (O)ST	0.8	1.9	1.5	2.1	1.6	
		[2.4]	[1.9]	[2.6]	[2.0]	2.2
α2-3 (N)ST	1.7	5.8	5.5	13.1	5.9	
		[3.4]	[3.5]	[7.7]	[3.5]	4.5
α2-6 (N)ST	0.7	4.6	4.1	9.1	4.8	
		[6.6]	[6.9]	[13.0]	[6.9]	8.1
C. Galactosyltransferases						
β1-4 Gal-T	173.9	328.8	335.1	506.4	288.8	
		[1.9]	[1.7]	[2.9]	[1.7]	2.1
αGalNAc: β1-3 Gal-T	23.8	24.9	40	39.6	29.3	
		[1.1]	[1.2]	[1.7]	[1.2]	1.4
D. N-Acetylglucosaminyl-transferases						
β1-3 GlcNAc-T	83.6	156.8	197	261.7	197.7	
		[1.9]	[2.4]	[3.1]	[2.4]	2.5
αMan: β1-2 GlcNAc-T	15.1	138.9	147.05	183.9	69.7	
		[9.2]	[4.6]	[12.2]	[4.6]	9

The fold of enzyme activity in patients with respect to that in control is shown in brackets. The mean value of enzyme activities in fold is shown in the last column

glycan chains as they are more accessible for mediating biological interactions in order to play regulation and functional roles in biological systems [47]. Cancer specific glycan expressions are better measured by following the GTs activities rather than the underlying transcription profile. Thus, it is apparently highly meaningful if cancer studies focus on GTs activities and related biochemistry rather than relying entirely on gene expression.

Fucosylation in pancreatic cancer

The present study found highly elevated level of fucosyltransferase activities in pancreatic tumor and this finding is quite consistent with the following earlier reports that fucosyltransferases regulate the synthesis of tumor associated carbohydrate determinants Lewis X, Lewis Y, sialyl Lewis X and sialyl Lewis a in pancreatic cancer [20]. A study on fucosyltransferase activities in human pancreatic tumor tissues and pancreatic tumor cell lines showed that FUT6 transcript was detected only in pancreatic cancer tissues but is not expressed in normal pancreas [21].

Increased branching of N-linked oligosaccharides and increased fucosylation and sialylation were observed in pancreatic cancer serum proteins [22]. Concentration of fucosylated haptoglobin increased in the sera of patients with pancreatic cancer compared to those of other types of cancer and normal controls [23]. Triantennary N-glycans containing a Lewis X –type fucose markedly increased at the Asn 211 site of haptoglobin N-glycan. Difucosylated tetra antennary N-glycans were observed only at this site in pancreatic cancer patients [23]. RNase 1 from healthy pancreatic cells contains neutral complex biantennary structures. In contrast, RNase1 glycans from tumor cells (Capan-1) were fucosylated hybrid and complex biantennary glycans with GalNAc-GlcNAc antennae [24].

Sialylation in pancreatic cancer

Cancer associated alteras largely occur from glycan branching of lactosamine chain in both N- and O- glycans [26]. One aspect of lactosamine extension is associated with formation of Lewis blood

group structures such as CA 19-9 antigen which is a commonly used serum-based marker of pancreatic cancer [48]. A recently identified carbohydrate antigen sialyl-TRA in addition to CA 19-9 was shown to be an accurate serological biomarker of pancreatic cancer [49].

The attachment between PCI pancreatic carcinoma cells and activated endothelial cells is mediated by sialyl Lewis a in pancreatic carcinoma and E-selectin in endothelial cells [50,51]. The level of surface sialyl Lewis a expression of PCI cells correlates with a number of metastatic colonies in the liver [52]. Capan-1 and MDA Panc-3 cells contained RNase1 glycans with sialylated structures completely absent in the healthy pancreas [24].

SialylLea and integrin mediate the process from adhesion to implantation of human pancreatic cancer SW1990 cells to endothelial cells [13]. CD44 and integrin play important roles in the initial attachment of SW1990 cells to mesothelial cells [13]. SW1990 cell adhesion to E selectin is mediated by ligands on mucinous glycoproteins [14].

MUC4 mucin is aberrantly expressed in pancreatic tumors with no detectable expression in the normal pancreas [15]. A progressive increase of MUC4 expression in pancreatic intraepithelial neoplasia suggests its association with disease development [15]. Over- expression of MUC1 by tumor cells simultaneously mediates and blocks specific molecular interactions between exogenous cell ligands and cell surface receptors [16]. Muc1 is heavily glycosylated in normal epithelial but is overexpressed and differentially glycosylated in pancreatic cancer.

This altered glycosylation includes the shortened core-1 O-glycans for monosialyl and disialyl T-antigens [17]. In consistent with the findings of these earlier studies, the present study found that the major sialyltransferase activity in pancreatic tumor as α2-3 (O)ST implying predominant expression of sialyl T-antigen. Further a moderate level of α2-3 (N)ST activity and multifold overexpression α1-3 and α1-4 FT activities would favor the expression of sialyl Lewis X and Sialyl Lewis a in pancreatic cancer. In support of the high level α2-3(O)ST activity we have also shown in the present study a significant increase in the level of sialomucin glycoproteins containing sialyl T-hapten units in pancreatic tumor.

Asn-linked glycans in pancreatic cancer

Our present findings that the increase in the activities N-glycan associated glycosyltransferases are several folds in pancreatic tumor are supported by earlier studies showing the occurrence of Lewis X bearing triantennary and tetraantennary N-glycans in haptoglobin and triantennary glycans with GalNAc-GlcNAc antennae in RNase in pancreatic cancer [23,24].

There was a remarkable increase (40%) in core fucosylated biantennary glycans in the pancreatic cancer serum RNase 1, suggesting that there is a subset of tumor associated glycoforms of RNase1 [25]. Lectin antibody microarrays were utilized to detect unique glycosylation patterns of proteins in serum [53]. α1-β glycoprotein response to SNA resulted in specific detection of pancreatic cancer with high sensitivity and specificity [53]. The resulting scatterplots also showed the ability to clearly distinguish pancreatic cancer from chronic pancreatitis, diabetics or normal samples [53]. The response of protein amyloid to SNA also indicated its acceptable ability to detect pancreatic cancer [53].

Glycosyltransferases in neutrophils of diabetic retinopathy

The present study showed that the neutrophils from diabetic retinopathy patients contained a significant increase in glycosyltransferase activities of O-glycans biosynthesis and multifold increase in N-glycan biosynthesis associated glycosyltransferase activities. The glycosyltransferase activities of diabetic neutrophils indicate that α1-3-FT and FT VII forming respectively Lewis X and sialyl Lewis X, with the capability of producing N-glycans containing core α1-6 and elongated α1-3 fucosylated lactosamine chain are dominant in diabetic neutrophils whereas core1. GalNAc: β1-3 Gal-T and α2-3 (O)ST act together giving rise to sialyl T antigen.

Diabetic retinopathy is characterized by capillary occlusion, formation of microvascular lesions and retinal neovascularization adjacent to ischemic areas of retina [54, 55]. Studies using human tissue demonstrate a strong relationship between leucocyte endothelial cell adhesion and retinal capillary damage in diabetes [2]. The changes in the expression of O-linked oligosaccharides on the surface of leucocytes appear to be involved in the increased adhesion to endothelial cells [56]. Further carbohydrate composition changes of glycoconjugates constituting the glycocalyx of microvascular cells could be involved in the alterations of cell-cell interactions observed in diabetic retinopathy [57]. The metabolic dysfunction in diabetes includes increased involvement of the hexoamine pathway and accumulation advanced glycation end- products [5]. An increase in the level of O-GlcNAc in diabetes was found to lead to insulin resistance [6]. The MGAT4A gene that encodes the major Gn T-IV enzyme Gn T-IVa in pancreas is down-regulated in diabetic patients [7,8]. Cell surface residency of the glucose transporter GLU T2 was impaired in Gn T-IVa deficient βcells [7,8].

P-selectin binds its main ligand PSGL-1 with high affinity and mechanical stability. E-selectin and L-selectin recognize many ligands with lower intrinsic affinity [58,59]. To compensate for their intrinsically low association rates, the E- and L- selectin determinants are thought to be presented in closely spaced clusters. N-glycan linked 6-Sulfo sialyl Lewis X has the critical function of L-selectin-dependent lymphocyte homing and recruitment [60]. A synthetic mucin core 2 compound GalNAcβ1-4 (Fucα1-3) GlcNAcβ1-6 (NeuAc α2-3 Galβ1-3) GalNAc α-OMe was ~ 6-fold better than sialyl Lewis X as inhibitor of L-and P- selectin binding [61].

PSGL-1 expressed on leucocytes is a high affinity ligand for E-, P-, and L- selectins. This glycoprotein has 71 Ser/Thr sites for O-glycans and 3 Asn sites for N-glycans. Sialyl Lewis X expressed on PSGL-1 represents the prototype oligosaccharides that bind selectins [36]. Using a CRISPR-Cas 9 tool kit to selectively truncate O-glycans, N-glycans and glycosphingolipids, it was shown that leucocytes rolling on P- and L- selectins is ablated in cells lacking O-glycans and an increased cell rolling velocity was found in cells with N-glycan truncation[62-71].The present study showed a multifold increase in N-glycan associated glycosyltransferase activities and a significant increase in O-glycan associated glycosyltransferase activities in diabetic retinopathy neutrophils. Thus, it is apparent that diabetic neutrophils can bring adverse changes to leucocyte-endothelial cell adhesion process due to their increased capacity of N- and O-glycans associated glycosylations.

CONCLUSION

It is evident from the present study that an exorbitant increase in the activities of the entire spectrum of glycosyltransferases we have examined is happening in pancreatic tumorigenesis. It is apparent that altered glycosylations on the surfaces and secreted proteins of pancreatic tumor cells may be the outcome of multifold increase in the level of N- and O-glycans associated glycosyltransferase activities in pancreatic tumor. The use of chemically synthesized well defined acceptors of enzymes leads to the discovery of new activities. α1-4 GlcNAc–capped O-glycans frequently expressed in pancreatic cancer cells indicated the use of α1-4 GlcNAc-T mRNA expressed in the mononuclear cell fraction of peripheral blood for the detection of pancreatic cancer.

We identified subsequently in gastric tumor αGlcNAc-T acting on terminal βGlcNAc in mucin core 2 trisaccharide GlcNAc β1-6 (Galβ1-3) GalNAcα- by showing the product to bind completely to PNA-agarose, its complete resistance to Jack bean β-N-acetylhexosaminidase and non-binding to PNA-agarose after recombinant β1-3-galactosidase treatment . If such α GlcNAc-T occurs in pancreatic tumor, it will be additionally useful for early detection and tracking of pancreatic cancer. Further, as shown in Table 8, pancreatic tumors express high levels of GlcNAc: β1-3/4GalNAc-T. The appearance of β1-4GalNAc-T combined with high expression of α1-3-FT can generate GalNAcβ1-4 (Fucα1-3) GlcNAcβ located on N-glycans as well as O-glycans. These glycans can bind to E-selctins which can lead to pancreatic cancer progression.

In contrast to normal colon, gastric and prostate tissues, normal pancreatic tissue is unique due to the fact that both N-glycan and O- glycan glycosyltransferase activities are very low, and this situation is akin to normal breast tissue. Pancreatic tumor cells are part of inflammatory microenvironment being exposed to a variety of cytokines and growth factors. Multiple mucin domains interact differently and regulate different components of the tumor micro environment. Several over-expressed mucins impede drug delivery to pancreatic tumors, suggesting that targeting mucin biosynthesis through mucin core 2 β1-6 GlcNAc-T (GCNT3) may improve drug responsiveness. Talniflumate alone and in combination with low dose gefitinib reduced GCNT3 expression leading to the disrupted production of mucins in vivo and invitro. Apparently certain glycan structures play a role in cancer progression by affecting tumor cell invasiveness, ability to disseminate through the blood circulation and to metastasize in distant organs.

During metastasis tumor cell-derived glycans enable binding to cells in their microenvironment including endothelium and blood constituents through glycan-binding receptors-lectins. Our earlier studies found a multifold elevation of Gal:3-O-sulfotransferases (Gal3-sulfo-T2 and Gal3-sulfo-T4) in contrast to glycan:glycosyltransferases in breast, colon and gastric tumors indicating an apparent role of sulfated glycans and sufation process in the progression of these cancers. PAP (3′phospho adenosine 5′ phosphate) is a known potent inhibitor of enzymatic sulfation. PAP exhibited Ki of 10μM with Gal3sulfo-T2 purified from colon cancer LS180 cells . Apparently, PAP may have use as metabolic inhibitor of these cancers.

The present study showed that α2-3 (O)ST (ST3Gal II) increased almost 100-fold in pancreatic tumorogenesis. We found earlier that this enzyme has reversible sialylation activity by converting 5′ CMP and also 5′ UMP to 5′ CMP-and 5′ UMP-NeuAc by utilizing the donor NeuAcα2-3Galβ1-3GalNAcα-units. Further, it was shown that 5′ UMP-NeuAc is an inactive sialyl donor for the sialylation of glycans by ST3Gal II, ST3Gal III and ST6Gal I. 5′ UMP is apparently an efficient inhibitor of glycan sialylation process and has a potential of inhibiting pancreatic cancer progression.

We found in the present study neutrophils from diabetic retinopathy patients contained a high-level of glycosyltransferases involved in N- and O-glycan biosynthesis. In support of our finding it has been shown by others that increased involvement of the hexosamine pathway is a detrimental consequence of cell and tissue exposure to high glucose in diabetes . A transketolase inhibitor Benfotiamine was able to divert hexose metabolism to the pentose pathway and inhibit the development of diabetic retinopathy in animal models. KRAS variant controlled increase in hexosamine pathway for the growth of pancreatic tumor may as well be inhibited by specifically targeting pancreas with such metabolic inhibitors of hexosamine pathway for preventing pancreatic cancer invasion.

A recent report suggests that oral administration of mannose may be a safe and selective therapy in the treatment of cancer. It is apparent that oral mannose would affect the glycan patterns in pancreatic and other cancers. Some glycosyltransferases are shed in serum and β1-4 Gal-T attracted much attention as a biomarker for ovarian cancer. Thus, GlcNAc: β1-3/4GalNAc-T may have the potential to serve as pancreatic cancer serum biomarker that can be used for follow-up of cancer patients in oral mannose treatment.

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REFERENCES

1. Klein R, Klein EKB, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy: Four-year incidence and progression of diabetic retinopathy when age at diagnosis is 30 years or more. Arch Ophthalmol. 1989;107:244-249.
2. McLeod DS, Lefer DJ, Merges C, Lutty GA. Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid. Am J Pathol. 1995;147:642-653.
3. Lasky LA. Selectins Interpreters of cell-specific carbohydrate information during inflammation. Science. 1992;258:964-969.
4. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76:301-314.
5. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414:813-820.
6. Vaidyanathan K, Wells L. Multiple tissue-specific roles for the O-GlcNAc post-translational modification in the induction of and complications arising from type II diabetes. J Biol Chem. 2004; 289:34466-34471.

7. Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell. 2005;122:337-349.

8. Ohtsubo K, Chen MZ, Olefsky JM, Marth JD. Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. Nat Med. 2001;17:1067-1075.

9. Hidalgo M. Pancreatic Cancer. N Engl J Med. 2010;362:1605-1617.

10. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA (2006) Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2006;20:1218-1249.

11. Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell. 2012;149:656-670.

12. Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, MacKay G. p53 status determines the role of autophagy in pancreatic tumour development. Nature. 2013;504:296-300.

13. Hosono J, Narita T, Kimura N, Sato M, Nakashio T. Involvement of adhesion molecules in metastasis of SW1990, human pancreatic cancer cells. J Surg Oncol. 1968;67:77-84.

14. Sawada T, Ho JJL, Chung YS, Sowa M, Kim YS. E-selectin binding by pancreatic tumor cells is inhibited by cancer sera. Int J Cancer. 2004;57:901-907.

15. Singh AP, Moniaux N, Chauhan SC, Meza JL, Batra SK. Inhibition of MUC4 Expression Suppresses Pancreatic Tumor Cell Growth and Metastasis. Cancer Research. 2004;64:622-630.

16. McDermott KM, Crocker PR, Harris A, Burdick MD, Hinoda Y. Overexpression of MUC1 reconfigures the binding properties of tumor cells. Int J Cancer. 2001;94:783-791.

17. Swanson BJ, McDermott KM, Singh PK, Eggers JP, Crocker PR. MUC1 Is a Counter-Receptor for Myelin-Associated Glycoprotein (Siglec-4a) and Their Interaction Contributes to Adhesion in Pancreatic Cancer Perineural Invasion. Cancer Res. 2007;67:10222-10229.

18. Ishizone S, Yamauchi K, Kawa S, Suzuki T, Shimizu F. Clinical utility of quantitative RT-PCR targeted to α1,4-N-acetylglucosaminyltransferase mRNA for detection of pancreatic cancer. Cancer Sci. 2006;97:119-126.

19. Kim YS, Itzkowitz SH, Yuan M, Chung YS, Satake K. Lex and Ley Antigen Expression in Human Pancreatic Cancer. Cancer Res. 1988;48:475-482.

20. Mas E, Pasqualini E, Caillol N, Battari AE, Crotte C. Fucosyltransferase activities in human pancreatic tissue: comparative study between cancer tissues and established tumoral cell lines. Glycobiol. 1998;8:605-613.

21. Nakano M, Nakagawa T, Ito T, Kitada T, Hijioka T. Site-specific analysis of N-glycans on haptoglobin in sera of patients with pancreatic cancer: A novel approach for the development of tumor markers. Int J Cancer. 2008;122:2301-2309.

22. Li C, Simeone DM, Brenner DE, Anderson MA, Shedden KA. Pancreatic Cancer Serum Detection Using a Lectin/Glyco-Antibody Array Method. J Proteome Res. 2007;6:1126-1138.

23. Peracaula R, Royle L, Tabarés T, Mallorquí-Fernández G, Barrabés S.Glycosylation of human pancreatic ribonuclease: differences between normal and tumor states. Glycobiol. 2003;13:227-244.

24. Barrabés S, Pagès-Pons L, Radcliffe CM, Tabarés G, Fort E. Glycosylation of serum ribonuclease 1 indicates a major endothelial origin and reveals an increase in core fucosylation in pancreatic cancer. Glycobiol. 2007;17:388-400.

25. Wu YM, Nowack DD, Omenn GS, Haab BS. Mucin Glycosylation Is Altered by Pro-Inflammatory Signaling in Pancreatic-Cancer Cells. J Proteome Res. 2009;8:1876-1886.

26. Chandrasekaran EV, Jain RK, Larsen RD, Wlasichuk K, DiCioccio RA. 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. 1996;35:8925-8933.

27. Chandrasekaran EV, Chawda R, Piskorz C, Locke RD, Ta A.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. 2001;334:105-118.

28. Chandrasekaran EV, Chawda R, Locke RD, Piskorz CF, Matta KL. Biosynthesis of the carbohydrate antigenic determinants, Globo H, blood group H, and Lewis b: a role for prostate cancer cell α1,2-L-fucosyltransferase. Glycobiology. 2002;12:153-162.

29. Chandrasekaran EV, Chawda R, Rhodes JM, Locke RD, Piskorz CF. The binding characteristics and utilization of Aleuria aurantia, Lens culinaris and few other lectins in the elucidation of fucosyltransferase activities resembling cloned FT VI and apparently unique to colon cancer cells. Carbohydr Res. 2003;338:887-901.

30. Chandrasekaran EV, Xue J, Xia J, Chawda R, Piskorz C. Analysis of the Specificity of Sialyltransferases toward Mucin Core 2, 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. 2005;44:15619-15635.

31. Chandrasekaran EV, Xue J, Neelamegham S, Matta KL. 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. 2006;341:983-994.

32. Chandrasekaran EV, Matta KL.Gastric Carcinoma-Molecular Aspects and Current Advances: Exploring the utility of carbohydrate associated transferase activity as potential tumor markers for human gastric cancer (Intertech Publishers). 2011;123-140.

33. Chandrasekaran EV, Xue J, Xia J, Locke RD, Patil SA. Mammalian Sialyltransferase ST3Gal-II: Its Exchange Sialylation Catalytic Properties Allow Labeling of Sialyl Residues in Mucin-Type Sialylated Glycoproteins and Specific Gangliosides. Biochemistry. 2011;50:9475-9487.

34. Chandrasekaran EV, Xue J, Xia J, Locke RD, Patil SA. Characterization of Cancer Associated Mucin Type O-Glycans Using the Exchange Sialylation Properties of Mammalian Sialyltransferase ST3Gal-II. J Proteomic Res. 2012;11:2609-2618.

35. Beauharnois ME, Lindquist KC, Marathe D, Vanderslice P, Xia J. ffinity and Kinetics of Sialyl Lewis-X and Core-2 Based Oligosaccharides Binding to L- and P-Selectin. Biochemistry. 2005;44:9507-9519.

36. Marathe D, Chandrasekaran EV, Lau JTY, Matta KL, Neelamegham S. Systems-level studies of glycosyltransferase gene expression and enzyme activity that are associated with the selectin binding function of human leukocytes. FASEB J. 2008;22:4154-4167.

37. Nasirikenari M, Segal BH, Ostberg JR, Urbasic A, Lau JTY. Altered granulopoietic profile and exaggerated acute neutrophilic inflammation in mice with targeted deficiency in the sialyltransferase ST6Gal I. Blood. 2006;108:3397-3405.

38. Dalziel M, Crispin M, Scanlan CN, Zitzmann N, Dwek RA. Emerging Principles for the Therapeutic Exploitation of Glycosylation. Science. 2014;343:1235681.

39. Dube DH, Bertozzi CR.Glycans in cancer and inflammation--potential for therapeutics and diagnostics. Nat Rev Drug Discov. 2005;4:477-488.

40. Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta. 1999;1473:21-34.

41. Wu YM, Nowack DD, Gilbert S Omenn GS, Haab BB. Mucin Glycosylation Is Altered by Pro-Inflammatory Signaling in Pancreatic-Cancer Cells. J Proteome Res. 2009;8:1876-1886.

42. Iwai K, Ishikura H, Kaji M, Sugiura H, Ishizu A. Importance of E-selectin (ELAM-1) and sialyl lewisa in the adhesion of pancreatic carcinoma cells to activated endothelium. Intl J Cancer. 1993;54:972-977.

43. Chandrasekaran EV, Xue J, Piskorz C, Locke RD, Tóth K. Potential tumor markers for human gastric cancer: an elevation of glycan: sulfotransferases and a concomitant loss of α1,2-fucosyltransferase activities. J Cancer Res Clin Oncol. 2007;133:599-611.

44. Chandrasekaran EV, Chawda R, Rhodes JM, Xia J, Piskorz C. Human lung adenocarcinoma α1,3/4-L-fucosyltransferase displays two molecular forms, high substrate affinity for clustered sialyl LacNAc type 1 units as well as mucin core 2 sialyl LacNAc type 2 unit and novel α1,2-L-fucosylating activity. Glycobiology. 2001;11:353–363.

45. Chandrasekaran EV, Xue J, Xia J, Khaja SD, Piskorz CF. Novel interactions of complex carbohydrates with peanut (PNA), Ricinus communis (RCA-I), Sambucus nigra (SNA-I) and wheat germ (WGA) agglutinins as revealed by the binding specificities of these lectins towards mucin core-2 O-linked and N-linked glycans and related structures. Glycoconj J. 2016;33:819-836.

46. Comelli EM, Head SR, Gilmartin T, Whisenant T, Haslam SM. A focused microarray approach to functional glycomics: transcriptional regulation of the glycome. Glycobiology. 2006;16:117-131.

47. Nairn AV, Aoki K, dela Rosa M, Porterfield M, Lim JM. Regulation of glycan structures in murine embryonic stem cells: Combined Transcript profiling of glycan related genes and structural analysis. J Biol Chem. 2012;287:37835-37856.

48. Court CM, Ankeny JS, Hou S, Tseng HR, Tomlinson JS. Improving pancreatic cancer diagnosis using circulating tumor cells: prospects for staging and single-cell analysis. Expert Rev Mol Diagn. 2015;15:1491-1504.

49. Barnett D, Liu Y, Partyka K, Huang Y, Tang H. The CA19-9 and sialyl-TRA antigens define separate subpopulations of pancreatic cancer cells. Sci Rep. 2017;7:4020.

50. Kaji M, Ishikura H, Kishimoto T, Omi M, Ishizu A. E-selectin expression induced by pancreas-carcinoma-derived interleukin-1α results in enhanced adhesion of pancreas-carcinoma cells to endothelial cells. Intl J Cancer. 1995;60:712-717.

51. Drabik A, Bodzon-Kulakowska A, Suder P, Silberring J, Kulig J. Glycosylation Changes in Serum Proteins Identify Patients with Pancreatic Cancer. J Proteome Res. 16:1436–1444.

52. Kishimoto T, Ishikura H, Kimura C, Takahashi T, Kato H. Phenotypes correlating to metastatic properties of pancreas adenocarcinoma in vivo: The importance of surface sialyl Lewis antigen. Intl J Cancer. 1996;69:290-294.

53. Li C, Simeone DM, Brenner DE, Anderson MA, Shedden KA. Pancreatic Cancer Serum Detection Using a Lectin/Glyco-Antibody Array Method. J Proteome Res. 2009;8:483-492.

54. Davis MD. Diabetic Retinopathy: A clinical overview Diabetes Care. 1992;15:1844-1874.

55. Kohner EM, Chibber R.Diabetic retinopathy. In Diabetic Angiopathy. Oxford Univ Press .1992;233-247.

56. Chibber R, Ben-Mahmud BM, Coppini D, Christ E, Kohner EM. Activity of the glycosylating enzyme, core 2 GlcNAc (β1,6) transferase, is higher in polymorphonuclear leukocytes from diabetic patients compared with age-matched control subjects: Relevance to capillary occlusion in diabetic retinopathy. Diabetes. 2000;49:1724-1730.

57. Rellier N, Ruggiero-Lopez D, Lecomte M, Lagarde M, Wiernsperger N. In vitro and in vivo alterations of enzymatic glycosylation in diabetes. Life Sciences. 1999;64:1571-1583.

58. McEver RP. Selectins: lectins that initiate cell adhesion under flow. Curr Opin Cell Biol. 2002;14:581-586.

59. Ley K, Kansas GS.Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. Nat Rev Immunol. 2004;4:325-35.

60. Mitoma J, Bao X, Petryanik B, Schaerli P, Gauguet JM.Critical functions of N-glycans in L-selectin-mediated lymphocyte homing and recruitment. Nat Immunol. 2007;8:409-418.

61. Jain RK, Piskorz CF, Huang BG, Locke RD, Han HL. Inhibition of L- and P-selectin by a rationally synthesized novel core 2-like branched structure containing GalNAc-Lewisx and Neu5Acα2-3Galβ1-3GalNAc sequences. Glycobiol. 1998;8:707-717.

62. Stolfa G, Mondal N, Zhu Y, Yu X, Buffone Jr A.Using CRISPR-Cas9 to quantify the contributions of O-glycans, N-glycans and Glycosphingolipids to human leukocyte-endothelium adhesion. Sci Rep. 2016;30392.

63. Kaur S, Kumar S, Momi N, Sasson AR, Batra SK. Mucins in pancreatic cancer and its microenvironment. Nat Rev Gastroenterol Hepatol. 2013;10:607-620.

64. Rao CV, Janakiram NB, Madka V, Kumar G, Scott EJ. Small-Molecule Inhibition of GCNT3 Disrupts Mucin Biosynthesis and Malignant Cellular Behaviors in Pancreatic Cancer. Cancer Res. 2016;76:1965-1974.

65. Häuselmann I, Borsig L. Altered tumor-cell glycosylation promotes metastasis. Front Oncol. 2014;4:1-15.

66. Chandrasekaran EV, Jain RK, Vig R, Matta KL.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. Glycobiol. 1997;7:753-767.

67. Chandrasekaran EV, Lakhaman SS, Chawda R, Piskorz CF, Neelamegham S. 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. 2004;279:10032-10041.

68. Chandrasekaran EV, Xue J, Xia J, Locke RD, Matta KL. Reversible Sialylation: Synthesis of Cytidine 5'-Monophospho-N-acetylneuraminic Acid from Cytidine 5'-Monophosphate with α2,3-Sialyl O-Glycan-, Glycolipid-, and Macromolecule-Based Donors Yields Diverse Sialylated Products. Biochemistry. 2008;47:320-330.

69. Berrone E, Beltramo E, Solimine C, Ape AU, Porta M. Regulation of Intracellular Glucose and Polyol Pathway by Thiamine and Benfotiamine in Vascular Cells Cultured in High Glucose. J Biol Chem. 2006;281:9307-9313.

70. Gonzalez PS, O'Prey J, Cardaci S, Barthet VJA, Sakamaki J. Mannose impairs tumour growth and enhances chemotherapy. Nature. 2018;563:719-723.

71. Odunsi K, Ghamande S, Chandrasekaran EV, Ta A, Moysich KB. Evaluation of β1,4-galactosyltransferase as a potential biomarker for the detection of subclinical disease after the completion of primary therapy for ovarian cancer. Am J Obstet Gynecol. 2002;187:575-580.