Expression pattern of polypeptide N-acetylgalactosaminyltransferase-10 in gastric carcinoma

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Abstract. The aim of this study was to detect the expression of the pp GalNac-T10 protein in gastric carcinoma and to investigate the role of pp GalNac-T10 in the occurrence and development of gastric cancer tissues. pp GalNac-T10 protein expression was immunohistochemically analyzed in 96 gastric adenocarcinoma tissue samples, and in 88 5-cm adjacent non-tumor gastric mucosa samples, used as controls. pp GalNAc-T10 expression in gastric cancer tissues was higher compared with that in the adjacent non-tumor gastric tissue. pp GalNAc-T10 protein expression had a significant positive correlation with histological type and degree of differentiation of gastric cancer ($P<0.05$). Expression in diffuse type gastric cancer was notably higher compared with that in intestinal type gastric cancer. Expression in poorly differentiated tumors was markedly higher compared with that in high and mid degree differentiated tumors. pp GalNAc-T10 protein expression was not significantly positively correlated with clinical stage, depth of invasion or lymph node metastasis ($P>0.05$). pp GalNAc-T10 expression is a useful indicator of tumor differentiation in gastric cancer.

Introduction

Recently, sugar chains have drawn extensive attention from sugar biologists due to their increasing role in tumor growth and invasion and metastasis of cancer. Each step of the sugar chain synthesis requires a specific glycosyltransferase to catalyze the transfer of a monosaccharide moiety from a glycosyl donor to an acceptor substrate; therefore, the abnormal sugar chain is controlled by the transfer of enzymes from sugar chain synthesis catalyzed glycosylation in the final analysis. Polypeptide N-acetylgalactosaminyltransferase (pp GalNAc-T, EC2.4.1.4.1) is an O-sugar chain synthesis-starting glycosyltransferase, which catalyzes the transfer of the GalNAc group of UDP-GalNAc to the polypeptide chain Thr or Ser hydroxyl in a specific sequence in order to synthesize GalNAcα-O-Ser/Thr peptide; the peptide chain gradually extends under the action of other glycosyltransferases (1). A growing number of studies have demonstrated that pp GalNAc-Ts is closely connected with tumor occurrence and development. Ishikawa *et al* (2) identified that pp GalNAc-T3 expression is a useful indicator of tumor differentiation in early gastric cancer and its expression is positively correlated with the depth of tumor invasion and lymph node metastasis. Wu *et al* (3) revealed that the expression of pp GalNAc-T14 is associated with clinicopathological characteristics of breast carcinoma. Berois *et al* (4) reported that pp GalNAc-T6 is expressed in the majority of human breast carcinomas, but not in normal breast epithelium and is sporadically found in non-malignant breast diseases. Gu *et al* (5) hypothesised that low expression of pp GalNAc-T3 may be a useful indicator of early tumor recurrence in stage I lung adenocarcinoma. pp GalNAc-T10 is a member of the pp GalNAc family; however, limited study has been conducted. In this study, an immunohistochemical method was applied to detect pp GalNAc-T10 protein expression in different gastric mucosal lesions. Expression of pp GalNAc-T10 in gastric cancer tissues was identified to be higher than that in adjacent non-tumor gastric tissue. pp GalNAc-T10 protein expression in gastric cancer was significantly positively correlated with histological type and degree of differentiation of the gastric cancer. Therefore, pp GalNAc-T10 may be a specific biomarker for gastric cancer.

Materials and methods

Collection of tissue samples and histological classification. In this study, 96 gastric adenocarcinoma surgical samples and 88 5-cm adjacent cancer non-tumor gastric mucosa control samples were used. All specimens were collected from the two Affiliated Hospitals of Suzhou University. The study was approved by the ethics committee of Southern Medical University, Guangzhou, China. The patients, ranging in age from 38 to 83 years (median age, 62 years; mean age, 63.4 years), had received no other treatment prior to surgery. All patients provided informed consent to participate in this

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study. The clinicopathological parameters included gender, age, TNM stage, Laurén's type, histological grade, depth of tumor invasion and lymph node metastasis (Table II) in which TNM staging system is according to the 2004 AJCC (American Joint Committee on Cancer)/UICC (Union Internationale Contre le Cancer) gastric cancer staging.

Tissue microarray production. Cores (diameter 1 mm) were taken from the marked region of individual formalin-fixed paraffin-embedded gastric adenocarcinoma specimens and the 5-cm adjacent cancer non-tumor gastric mucosa control samples. In 8 cases, a non-tumor gastric mucosa control sample was missing. Two cores from each tissue were assembled adjacently into a single recipient TMA block (Outdo Biotech, Shanghai, China).

Immunohistochemistry. For pp GalNAc-T10 immunostaining, quenching of endogenous peroxidase activity was performed with 3% H_2O_2 in PBS for 20 min, which was then blocked with normal goat serum (Gibco, Carlsbad, CA, USA) for 20 min. Anti-pp GalNAc-T10 (Abcam, Cambridge, MA, USA) was incubated overnight at 4˚C and peroxidase-conjugated goat anti-mouse polyvalent antibody (Maixin-Bio, Jinan, China) was incubated for 60 min at room temperature. Reactions were revealed with diaminobenzidine, washed in water, counter-stained in Mayer's hematoxylin and dehydrated in ethanol and xylene and were then mounted. Between each step, sections were washed in PBS. For every assay, negative controls using PBS without primary antibody were included.

Immunocytochemical analysis. The immunostaining frequency for each tumor was scored as follows: 0 for negative samples or ≤5% stained tumor tissue; 1 for staining between 6 and 25% of the tumor tissue; 2 for staining between 26 and 50% of the tumor tissue; 3 for staining between 51 and 75% of the tumor tissue; and 4 for staining >75% of the tumor tissue. Signal intensity was scored as strong (3), moderate (2), weak (1) and null (0). Total immunostaining score resulted from the addition of both parameters. Scores were established jointly by four observers under a multi-head microscope. Clinicopathological information was masked to the observers.

Statistical analysis. SPSS 13.0 statistical software was used for statistical analysis. Gastric cancer tissues and adjacent non-tumor gastric tissue protein expression differing in intensity were tested with the two sample rate of the matched pair, designed to compare the Chi-square. The Chi-square test was applied to analyze the correlation between intensity and clinicopathological parameters of the protein expression. $P<0.05$ was considered to indicate a statistically significant difference.

Results

pp GalNAcT10 expression in gastric cancer tissues and adjacent non-tumor gastric tissues. To determine whether there are differences between the expression of pp GalNAc-T10 in cancerous gastric cancer tissues and adjacent non-cancerous gastric mucosa, we designed paired experiments of gastric cancer tissues and adjacent non-tumor gastric mucosa. Under observation using an optical microscope, we identified that

Table I. pp GalNAc-T10 expression in 88 cases of gastric cancer and adjacent non-tumor gastric tissue.

Tissue	Strong positive	Weakly positive	Negative	P-value
Gastric cancer	56	16	16	0.007
Adjacent non-tumor gastric	48	33	7	

pp GalNAc-T10, polypeptide N-acetylgalactosaminyltransferase 10.

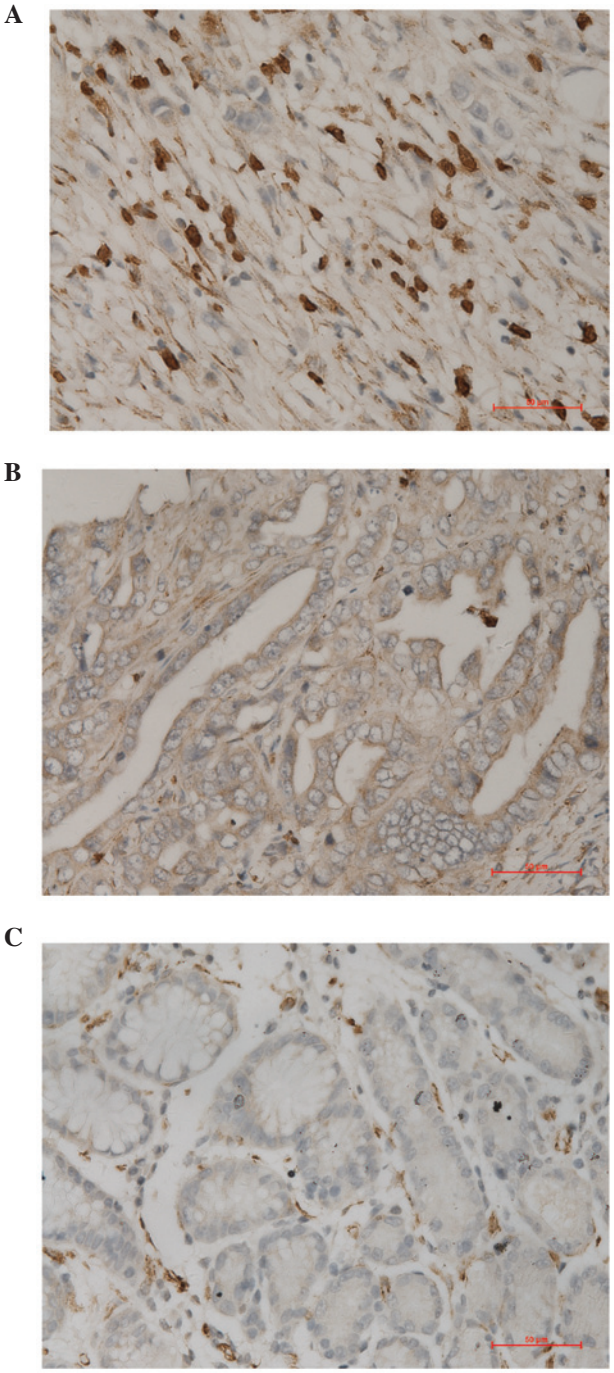

Figure 1. pp GalNAc-T10 expression in (A) diffuse-type gastric cancer, (B) intestinal type gastric cancer and (C) normal gastric mucosa. (Eli Vision; magnification, x400). pp GalNAc-T10, polypeptide N-acetylgalactosaminyl-transferase 10.

Table II. pp GalNAc-T10 expression and clinicopathological parameters in 96 patients with gastric carcinoma.

Clinicopathological parameters	n	pp GalNAc-T10 expression			P-value
		Strong positive	Weakly positive	Negative	
Age (years)					0.753
≥60	62	39	13	10	
<60	34	23	5	6	
Gender					0.699
Female	29	17	6	6	
Male	67	45	12	10	
TNM stage (AJCC)					0.588
I to II	32	20	5	7	
III to IV	64	42	13	9	
Depth of tumor invasion					0.416
T1 to T2	22	16	2	4	
T3 to T4	74	46	16	12	
Lymph node metastasis					0.564
Yes	70	47	13	10	
No	26	15	5	6	
Histological grade					0.016
High, mid	28	12	8	8	
Low	68	50	10	8	
Laurén's type					0.022
Intestinal	62	34	14	14	
Diffuse	34	28	4	2	

pp GalNAc-T10, polypeptide N-acetylgalactosaminyltransferase 10; AJCC, American Joint Committee on Cancer.

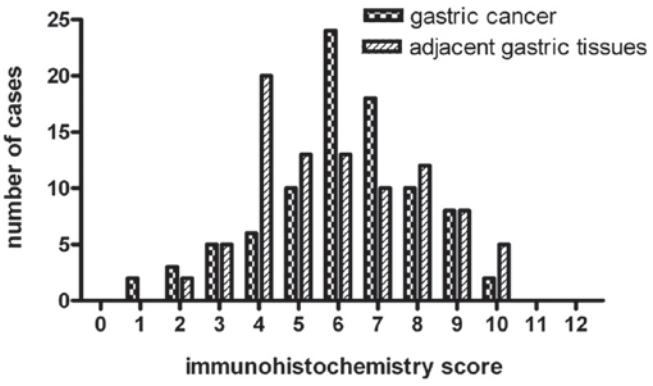

Figure 2. pp GalNAc-T10 immune response scores in 96 cases of gastric cancer and 88 cases of adjacent non-tumor gastric tissue. pp GalNAc-T10, polypeptide N-acetylgalactosaminyltransferase 10.

pp GalNAc-T10 protein is expressed in gastric cancer cells and normal gastric epithelial cell cytoplasm and cell membrane, which was observed as brown granules (Fig. 1). Statistical analysis results demonstrated (Fig. 2) that the expression of pp GalNAc-T10 in gastric cancer tissues was higher than that in adjacent non-tumor gastric cancer organizations, and that the difference was significant (P<0.01; Table I).

Correlation between pp GalNAc-T10 expression in gastric cancer tissues and clinicopathological parameters. In order to further determine the correlation between pp GalNAc-T10 expression and gastric cancer clinicopathological parameters, correlation analysis between the pp GalNAc-T10 expression strength and gastric cancer clinicopathological parameters was conducted. The results revealed that pp GalNAc-T10 protein expression had a significant positive correlation with gastric cancer histological type (differentiation degree; P<0.05) and expression in diffuse-type gastric cancer was significantly higher than that in the intestinal-type gastric cancer. The intensity of expression in poorly differentiated gastric carcinoma was significantly higher than that in moderate and highly differentiated cases. There was no significant positive correlation with age, gender, clinical stage, depth of invasion or lymph node metastasis (P<0.05; Table II).

Discussion

It is widely acknowledged that glycosyltransferases affect the development of tumors by altering the structure of sugar chains. The changes in cell surface sugar chains have an impact on the interaction between cancer cells, and cancer cells and the extracellular matrix. This in turn has a major impact on the behavior of cancer cells, including tumor growth, invasion and metastasis. The T-antigen O-glycosylation originates in

the peptide: the enzyme family of pp GalNAc-Ts. Certain members of the pp GalNAc-T family have reported abnormal expression in squamous, colon, stomach and lung cancer, leukemia and other types of cancer. pp GalNAc-T10 is a member of the pp GalNAc-T family, which is highly expressed in human gastrointestinal tissues (6), but at present there is no study relating pp GalNAc-T10 expression to cancer. In this study, pp GalNac-T10 protein expression was immunohistochemically analyzed, in 96 gastric adenocarcinoma samples and 88 5-cm adjacent cancer non-tumor gastric mucosa control samples. Results demonstrated that pp GalNAc-T10 protein expression in gastric cancer tissues was higher than that in adjacent non-tumor gastric tissues, and the expression had a significant positive correlation with the degree of tumor differentiation ($P<0.05$). Expression in poorly differentiated gastric carcinoma was significantly higher than that in cases of high and mid degree in differentiation, and the expression intensity in the diffuse-type gastric cancer was significantly higher than in the intestinal-type gastric cancer. pp GalNAc-T10 protein expression was not significantly positively correlated with clinical stage, depth of invasion or lymph node metastasis ($P>0.05$). Therefore, abnormal expression of pp GalNAc-T10 may be a significant event which determines whether normal cells tranform into malignant poorly differentiated gastric cancer cells. This may also be used as a diagnostic indicator of malignant gastric cancer cells. Through analysis of the protein expression in gastric cancer cells, we may be able to provide suggestions for the diagnosis of gastric cancer development and treatment.

We speculate that the impact of pp GalNAc-T10 on the proliferation and differentiation of gastric cancer results from its action on certain cell signaling pathways, particularly the transforming growth factor-β (TGF-β) signal-regulated pathway. The TGF-β superfamily is a newly discovered family of proteins that regulate cell growth and differentiation. Studies have reported that disruption of TGF-β signaling in diffuse-type gastric carcinoma models may accelerate tumor growth (7) and TGF-β decreases cancer-initiating cell proliferation within diffuse-type gastric carcinoma cells (8). Otherwise, N-acetyl aminotransferase is likely to play an important role in diffuse-type gastric cancer cells (9) in a TGF-β signal-regulated pathway. TGF-β1 mRNA level is significantly decreased in the overexpression of pp GalNAc-T2 (10). Therefore, we hypothesize that the pp GalNAc-T family may be correlated with the TGF-β family. Further studies are required to study the impact of pp GalNAc-T10 on the proliferation and differentiation of gastric cancer, and its correlation with TGF-β signaling pathway.

In the occurrence and development of gastric cancer, changes in sugar chains originate from the transferring of the glycosyltransferase. Through in-depth study of the pp GalNAc-T family we may be able to obtain a deeper knowledge of the pathogenesis of gastric cancer and provide a more effective reference for existing diagnosis and treatment options.

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