Prognostic significance of UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase-3 (GalNAc-T3) expression in patients with gastric carcinoma

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Aberrant glycosylation occurs during development of gastric carcinomas. The initiation of mucin-type *O*-glycosylation is regulated by GalNAc-T3 (UDP-*N*-acetylgalactosamine:polypeptide *N*-acetylgalactosaminyltransferase-3). However, the clinical significance of GalNAc-T3 expression in human gastric carcinoma has not yet been demonstrated. In the present study, we investigated the relationship between immunohistochemical GalNAc-T3 expression and various clinicopathologic factors, including prognosis, in 117 gastric carcinoma patients. Of 117 gastric carcinomas examined, 59 (50.4%) showed strong expression of GalNAc-T3. Strong expression was detected in 38 of 59 (64.4%) differentiated type and in 21 of 58 (36.2%) undifferentiated gastric carcinomas, indicating that the expression of GalNAc-T3 correlated significantly with tumor differentiation (P=0.0023, χ^2 test). Overall 5-year survival rate in patients with strong GalNAc-T3 expression (71.0%) was significantly better than that of patients with weak expression (49.3%) (P=0.0197, log-rank test). Multivariate analysis identified GalNAc-T3 expression as an independent prognostic factor (P=0.0158, Cox proportional hazards model). Our data suggest that GalNAc-T3 expression may be a useful marker for prognosis and differentiation of gastric carcinomas. (Cancer Sci 2003; 94: 32–36)

Although the mortality rate of gastric carcinoma has improved with advances in treatment, it remains the major cause of cancer death in Japan.[1)] The major treatment for gastric carcinoma is surgery. However, the prognosis after surgery is related to the extent of metastasis and the possible recurrence of peritoneal dissemination. Therefore, it is important to predict prognosis after initial surgery in order to provide additional treatment when necessary, since prognosis in such patients is related to the extent of metastasis, recurrence and peritoneal dissemination.

Alterations of mucin-type (*O*-linked) glycoproteins on the surface of the cancer cell may contribute to changes in cancer cell growth regulation, immune recognition, and cell adhesion, all of which may in turn influence the invasive and metastatic characteristics and capabilities of the cancer.[2–6)] Generally, tumor-associated carbohydrate antigens are produced through incomplete synthesis of carbohydrate chains resulting in their accumulation as precursor forms or through neosynthesis of carbohydrate chains via the activation of certain glycosyltransferases, such as GalNAc-T (UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase).[7, 8)] Thus, GalNAc-T, which regulates the initiation of *O*-glycosylation of mucins, may be important for a better understanding of tumor-associated aberrant *O*-glycosylation. To date, nine distinct human *GalNAc-T* genes have been cloned,[9)] and it is known that multiple GalNAc-Ts are expressed in various tissues.[10)] In the GalNAc-T family, we previously reported that GalNAc-T3 was detected only in glands and adenocarcinoma.[11)] We further showed that patients with colorectal carcinoma that overexpressed GalNAc-T3 had a significantly better prognosis than those with weak expression.[12)] GalNAc-T3 may be one of the most important transferases to regulate *O*-glycosylation in adenocarcinomas. However, little is known regarding the function and the role of GalNAc-T3 in human gastric carcinoma tissues, and there is no information as to the relationship between GalNAc-T3 expression and clinicopathologic factors, including prognosis, of patients with gastric carcinoma.

In the present study, we demonstrate that GalNAc-T3 expression in gastric tumor tissue correlated strongly with good prognosis in patients with gastric carcinoma.

Materials and Methods

Patients. The subjects were 117 patients (79 males, 38 females) with primary gastric carcinoma who underwent surgical resection at the Department of Surgery I, University Hospital of Occupational and Environmental Health, Japan, between 1980 and 1982. Table 1 shows the histological classifications, stages of the carcinomas and other clinicopathologic factors. Clinicopathologic findings were determined according to the International Union Against Cancer (UICC) TNM Classification of Malignant Tumors[13)] and the Japanese Classification of Gastric Carcinoma (tumor location, lymphatic invasion, venous invasion, liver metastasis, and histopathological type).[14)]

Anti-GalNAc-T3 antibody. Polyclonal antibodies against human GalNAc-T3 were generated by multiple immunizations of a New Zealand white rabbit using synthetic peptides as described previously.[11)] A dilution of 1:2000 in phosphate-buffered saline (PBS) containing 2% bovine serum albumin was used to immunostain paraffin-embedded sections.

Immunohistochemistry. Tumor samples resected from patients with gastric carcinoma were fixed in formalin and embedded in paraffin. Immunohistochemistry was performed using a streptavidin-biotin-peroxidase complex method.[15)] Tissue sections (2 μm thick) were pretreated twice for 5 min per treatment with citrate buffer (0.01 mol/liter; pH 6.0) at 100°C in a microwave oven. Endogenous peroxidase activity was blocked by preincubating the slides in 3% H_2O_2 in absolute methanol for 5 min. Each slide was preincubated in normal goat serum for 10 min and then incubated with polyclonal GalNAc-T3 antibody for 60 min. The antibody-treated slides were then washed thoroughly, incubated in goat anti-rabbit immunoglobulin for 10 min, washed, and incubated with streptavidin-biotinylated horserad-

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ish peroxidase complex for 5 min. Finally, diaminobenzidine was used as a chromogen and the sections were lightly counterstained with hematoxylin. Substitution of PBS for the primary antibody was used as the negative control. A section of normal colorectal mucosa was used as the positive control.[11]

Histopathology. The sections were examined by two independent observers who were blinded as to the clinical status of the patients. In each section, the staining was evaluated by counting the frequency of labeled cells in 5 high power fields containing 100 tumor cells each. This count was classified using the following criteria: 0, negative; 1+, <25% positive; 2+, 25–50% positive; 3+, 50–75% positive; and 4+, 75–100% positive. 0, 1+ and 2+ were considered weak GalNAc-T3 expression. 3+ and 4+ were considered strong GalNAc-T3 expression.[12]

Statistical analysis. Statistical analysis was performed using the χ^2 and Mann-Whitney *U* tests. For survival analysis, the Kaplan-Meier method was applied and statistical significance was calculated using the log-rank test. Univariate and multivariate analyses were performed using a Cox proportional hazards model. Differences were considered statistically significant if the *P* value was <0.05. All analyses were performed using the StatView statistical package (version 5.0, SAS Institute, Inc., Cary, NC).

Table 1. Clinicopathologic variables

Variable	
Age (yrs)	
Median (range)	62 (24–82)
Gender	
Male	79
Female	38
Tumor location	
Upper third of the stomach	23
Middle third of the stomach	47
Lower third of the stomach	47
Size (cm)	
Median (range)	5.0 (0.7–16.5)
Lymphatic invasion	
Negative	26
Positive	91
Venous invasion	
Negative	50
Positive	67
Lymph node metastasis	
Negative	50
Positive	66
Liver metastasis	
Negative	109
Positive	8
Depth of invasion	
Tis	0
T1	32
T2	55
T3	27
T4	3
TNM stage	
0	0
I	51
II	13
III	27
IV	26
Histopathological type	
Differentiated	59
Undifferentiated	58

Results

Immunohistochemical staining of GalNAc-T3 expression in gastric carcinoma tissues. GalNAc-T3 immunoreactivity was detected in all cases, and predominantly localized to the cytoplasm. Strong GalNAc-T3 expression was always noted in glandular epithelial cells of the normal gastric mucosa (Fig. 1A). Fig. 1 shows representative immunohistochemical staining in carcinomas with strong GalNAc-T3 expression (B, D) and in carcinomas with weak GalNAc-T3 expression (C, E). Of 117 gastric carcinoma tissues, 59 (50.4%) were defined as having strong GalNAc-T3 expression, and 58 (49.6%) as having weak expression.

Relationship between GalNAc-T3 expression and clinicopathologic factors. We next examined the relationship between GalNAc-T3 expression and various clinicopathologic factors (Table 2). Strong GalNAc-T3 expression in the tumor was seen in 38 of 59 differentiated-type gastric carcinomas (papillary adenocarcinoma, well-differentiated and moderately differentiated tubular adenocarcinoma) (64.4%) and in 21 of 58 undifferentiated-type gastric carcinoma (poorly differentiated adenocarcinoma, signet-ring cell carcinoma, mucinous adenocarcinoma) (36.2%). Thus, GalNAc-T3 expression correlated significantly with histopathological type (P=0.0023, χ^2 test). However, there was no significant relationship between GalNAc-T3 expression and other clinicopathologic factors (Table 2).

Prognosis. Fig. 2A shows the Kaplan-Meier survival curve for all patients. The 5-year and 10-year overall survival rates of pa-

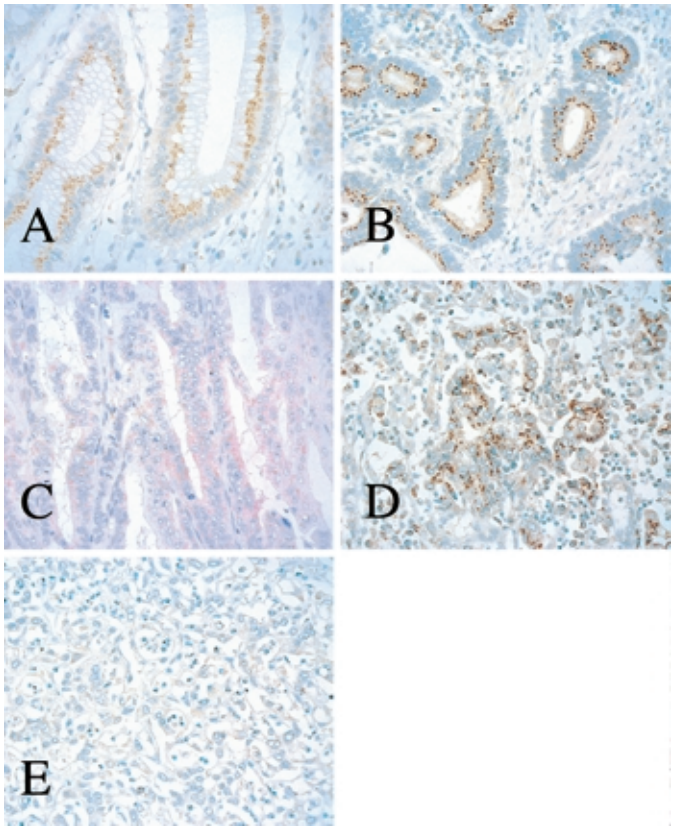

Fig. 1. Immunohistochemical expression of GalNAc-T3 in normal gastric mucosa and gastric carcinoma. Note the intense granular immunoreactivity for GalNAc-T3 appearing as a brown reaction product. A) Strong GalNAc-T3 expression in normal mucosa. B) Strong GalNAc-T3 expression in differentiated type. C) Weak GalNAc-T3 expression in differentiated type. D) Strong GalNAc-T3 expression in undifferentiated type. E) Weak GalNAc-T3 expression in undifferentiated type. Original magnification, ×400.

Table 2. Relation between clinicopathologic variables and GalNAc-T3 expression

Variable	No. of patients (%)		*P* value
	Strong GalNAc-T3 expression *n*=59 (50.4)	Weak GalNAc-T3 expression *n*=58 (49.6)	
Age			0.9244
>62 years	29 (49.2)	28 (48.3)	
≤62 years	30 (50.8)	30 (51.7)	
Gender			0.2118
Male	43 (72.9)	36 (62.1)	
Female	16 (27.1)	22 (37.9)	
Tumor location			0.1328
Upper third of the stomach	15 (25.4)	8 (13.8)	
Middle third of the stomach	25 (42.4)	22 (37.9)	
Lower third of the stomach	19 (32.2)	28 (48.3)	
Size			0.4039
>5 cm	31 (52.5)	26 (44.8)	
≤5 cm	28 (47.5)	32 (55.2)	
Lymphatic invasion			0.9606
Negative	13 (22.0)	13 (22.4)	
Positive	46 (78.0)	45 (77.6)	
Venous invasion			0.408
Negative	23 (39.0)	27 (46.6)	
Positive	36 (61.0)	31 (53.4)	
Lymph node metastasis			0.7077
Negative	24 (41.4)	26 (44.8)	
Positive	34 (58.6)	32 (55.2)	
Liver metastasis			0.4903
Negative	56 (94.9)	53 (91.4)	
Positive	3 (5.1)	5 (8.6)	
Depth of invasion			0.853
T1	16 (27.1)	16 (27.6)	
T2	27 (45.8)	28 (48.3)	
T3	15 (25.4)	12 (20.7)	
T4	1 (1.7)	2 (3.4)	
TNM stage			0.6867
I	25 (42.4)	26 (44.8)	
II	8 (13.6)	5 (8.6)	
III	16 (27.1)	11 (19.0)	
IV	10 (16.9)	16 (27.6)	
Histopathological type			0.0023
Differentiated	38 (64.4)	21 (36.2)	
Undifferentiated	21 (35.6)	37 (63.8)	

tients with strong GalNAc-T3 expression were 71.0% and 66.4%, respectively. In contrast, the 5-year and 10-year survival rates in patients with weak GalNAc-T3 expression were 49.3% and 41.1%, respectively. The survival rate for the strong GalNAc-T3 expression group was significantly higher than that for the weak GalNAc-T3 expression group (P=0.0197, log-rank test).

Because GalNAc-T3 expression correlated with histopathological type, we analyzed the survival rate in each histopathological subgroup in order to exclude the influence of histopathology. Patients with strong GalNAc-T3 expression tended to have higher survival rates than those with weak GalNAc-T3 expression in both the differentiated-type gastric carcinoma subgroup (5-year survival rate, 71.9% vs. 48.9%; 10-year survival rate, 68.2% vs. 37.2%; Fig. 2B) and the undifferentiated-type subgroup (5-year survival rate, 69.2% vs. 49.5%; 10-year survival rate, 62.9% vs. 43.1%; Fig. 2C). However, these differences were not statistically significant.

In univariate analysis of the factors affecting survival, TNM stage, lymphatic invasion, venous invasion, lymph node metastasis, liver metastasis, depth of invasion, and GalNAc-T3 immunoreactivity were found to be significant (Table 3). In contrast, histopathological type did not correlate significantly with survival (P=0.4728). Multivariate analysis using the Cox proportional hazards model showed that only GalNAc-T3 expression (P=0.0158) and TNM stage (P=0.0313) were significant independent prognostic factors among the eight variables (Table 4).

Discussion

In this study, we found that the survival rate for patients with gastric carcinoma that strongly expresses GalNAc-T3 is significantly better than that of patients with tumors having weak GalNAc-T3 expression (Fig. 2A), and multivariate Cox analysis identified GalNAc-T3 expression as an independent prognostic factor in patients with gastric carcinoma (Table 4). Our results add further support to a previous report indicating that GalNAc-T3 expression is a useful marker for prognosis in patients with colorectal carcinoma.[12] Further investigation will be necessary to determine whether GalNAc-T3 expression correlates with the prognosis of patients with other types of adenocarcinoma. Since GalNAc-T3 expression correlated with tumor differentiation (Table 2), it was necessary to exclude the influence of differentiation on the survival rate in patients with gastric carcinoma. Thus, we analyzed the relationship between Gal-

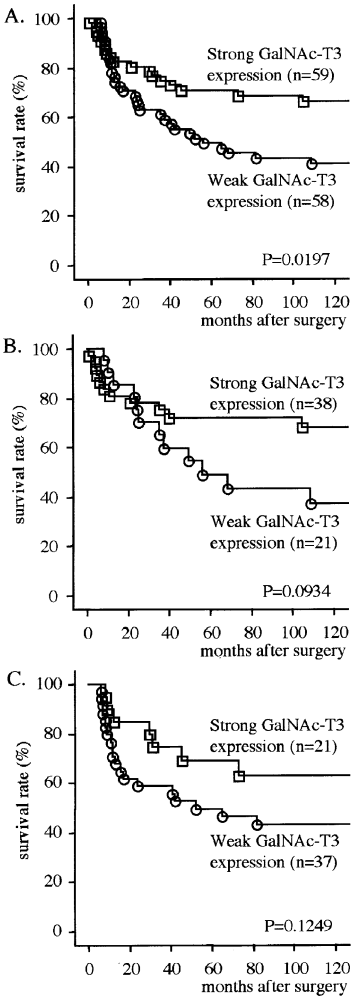

Fig. 2. Kaplan-Meier survival curves of patients with gastric carcinoma. A) Overall. □ strong GalNAc-T3 expression (n=59), ○ weak GalNAc-T3 expression (n=58), P=0.0197. B) Patients with differentiated-type adenocarcinoma. □ strong GalNAc-T3 expression (n=38), ○ weak GalNAc-T3 expression (n=21), P=0.0934. C) Patients with undifferentiated-type adenocarcinoma. □ strong GalNAc-T3 expression (n=21), ○ weak GalNAc-T3 expression (n=37), P=0.1249.

NAc-T3 expression and survival rate in each histopathological subgroup. Regardless of the type of differentiation (Fig. 2, B and C), patients with strong GalNAc-T3 expression had higher survival rates than those with weak GalNAc-T3 expression. However, the differences were not statistically significant, probably due to the small sample size.

In order to examine the relationship between GalNAc-T3 expression and prognosis in 117 gastric carcinoma patients, we had to decide the cut-off value of GalNAc-T3 expression. We classified 117 gastric carcinomas into either 0–25%, 25–50%, 50–75% or 75–100% GalNAc-T3 expression, and examined three different cut-off values: 25%, 50% and 75%.[12] With 25% or 75% as the cut-off value, GalNAc-T3 no longer predicted 5-year survival in patients with gastric carcinoma. Thus, GalNAc-T3 was a useful and independent prognostic marker in gastric carcinoma patients only if the cut-off value of GalNAc-T3 expression was set at 50% in our study. Furthermore, we divided the patients into three categories (weak GalNAc-T3 expression, 0–25%; moderate expression, 25–50%; and strong expression, 50–100%) and investigated the relationship between expression level of GalNAc-T3 and prognosis. The 5-year survival rate

Table 4. Multivariate analysis of prognostic factors for overall survival by the Cox hazards model

Variable	RH	χ^2	95% CI	P
GalNAc-T3 expression				
Strong vs. weak	2.208	5.830	1.161–4.199	0.0158
TNM stage				
I, II vs. III, IV	2.802	4.639	1.097–7.155	0.0313
Size				
≤5 cm vs. >5 cm	1.163	0.211	0.610–2.219	0.6464
Lymphatic invasion				
Positive vs. negative	0.905	0.018	0.212–3.860	0.8926
Venous invasion				
Positive vs. negative	0.672	1.151	0.325–1.389	0.2833
Lymph node metastasis				
Positive vs. negative	0.313	3.371	0.091–1.082	0.0663
Liver metastasis				
Positive vs. negative	0.487	2.605	0.203–1.167	0.1066
Depth of invasion				
T1, T2 vs. T3, T4	0.862	0.171	0.427–1.741	0.6794

RH, relative hazard; 95% CI, 95% confidence interval.

Table 3. Univariate analysis of the relationship between clinicopathologic variables and survival in 117 patients with gastric carcinoma

Variable	RH	χ^2	95% CI	P
GalNAc-T3 expression				
Strong vs. weak	1.972	5.229	1.102–3.529	0.0222
TNM stage				
I, II vs. III, IV	6.460	30.590	3.335–12.514	<0.0001
Size				
≤5 cm vs. >5 cm	2.029	5.812	1.141–3.608	0.0159
Lymphatic invasion				
Positive vs. negative	0.175	8.512	0.054–0.565	0.0035
Venous invasion				
Positive vs. negative	0.354	10.223	0.188–0.669	0.0014
Lymph node metastasis				
Positive vs. negative	0.124	22.549	0.053–0.294	<0.0001
Liver metastasis				
Positive vs. negative	0.148	22.548	0.067–0.325	<0.0001
Depth of invasion				
T1, T2 vs. T3, T4	2.309	7.897	1.288–4.138	0.0050
Histopathological type				
Undifferentiated vs. differentiated	0.814	0.516	0.464–1.427	0.4728

RH, relative hazard; 95% CI, 95% confidence interval.

was 37.5% in the weak GalNAc-T3 expression group, 55.0% in the moderate expression group and 71.0% in the strong expression group. However, there was no statistically significant difference (P=0.0543, log-rank test), probably due to the small sample size.

With regard to the relationship between GalNAc-T3 expression and differentiation in adenocarcinoma, we found that GalNAc-T3 expression was also related to differentiation in colorectal carcinoma tissues[12] and that expression of the *GalNAc-T3* gene was induced in two colorectal carcinoma cell lines by a differentiation-promoting agent, sodium butyrate (data not shown). Our results show that GalNAc-T3 expression is significantly correlated with the histopathological type of gastric carcinoma. These findings also support the hypothesis that *GalNAc-T3* gene expression may serve as a marker for differentiation in adenocarcinomas. In this regard, it has been reported that GalNAc-T3 is differentially expressed by pancreatic adenocarcinoma cell lines, with a tendency for higher expression in more differentiated cell lines.[16] These data indicate that the expression of GalNAc-T3 might correlate with the differentiation of some adenocarcinomas.

Malignant transformation of glandular epithelial cells is accompanied by changes in the biochemical characteristics of mucins. These changes include both an altered expression of *mucin* genes and an aberrant glycosylation of mucin core peptides.[17, 18] Alterations in cell surface and secreted glycoproteins are associated with carcinogenesis, and may play a significant role in determining the metastatic behavior of tumor cells.[19, 20] We observed variable GalNAc-T3 expression in gastric carcinoma tissues. Because GalNAc-T3 regulates *O*-glycosylation of mucins, aberrant glycosylation of mucins may be related to cancer-associated changes of GalNAc-T3 expression and the change of GalNAcT-3 activity may play an important role in tumor behavior.

We investigated the association between GalNAc-T3 expression and recurrence as peritoneal dissemination. Three of 7 cases (42.9%) with metachronous peritoneal dissemination showed strong GalNAc-T3 expression (P=0.7168, χ^2 test). We also investigated the association between GalNAc-T3 expression and synchronous peritoneal dissemination. Seven of 15 cases (46.7%) with synchronous peritoneal dissemination showed strong GalNAc-T3 expression (P=0.7550, χ^2 test). In this study, there were a few cases currently diagnosed as synchronous or metachronous peritoneal dissemination, and this might be the reason why no significant association was found between GalNAc-T3 expression and peritoneal dissemination.

In conclusion, our results indicate that the expression of GalNAc-T3 is strongly correlated with a better prognosis of patients with gastric carcinoma. Our results suggest that GalNAc-T3 expression could be a useful marker for prognosis of patients with gastric carcinoma and could help to identify those patients in need of closer follow-up and more aggressive treatment.

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1. Statistics and Information Department, Minister's Secretariat, Ministry of Health. Vital Statistics 1998 Japan. Vol. 1 (in Japanese). Tokyo: Health and Statistics Association; 2000. p. 284–90.
2. Kuan SF, Byrd JC, Basbaum CB, Kim YS. Characterization of quantitative mucin variants from a human colon cancer cell line. *Cancer Res* 1987; **47**: 5715–24.
3. Bresalier RS, Niv Y, Byrd JC, Duh QY, Toribara NW, Rockwell RW, Dahiya R, Kim YS. Mucin production by human colonic carcinoma cells correlates with their metastatic potential in animal models of colon cancer metastasis. *J Clin Invest* 1991; **87**: 1037–45.
4. Ligtenberg MJ, Buijs F, Vos HL, Hilkens J. Suppression of cellular aggregation by high levels of episialin. *Cancer Res* 1992; **52**: 2318–24.
5. Majuri ML, Mattila P, Renkonen, R. Recombinant E-selectin-protein mediates tumor cell adhesion via sialyl-Le(a) and sialyl-Le(x). *Biochem Biophys Res Commun* 1992; **182**: 1376–82.
6. Kojima N, Handa K, Newman W, Hakomori S. Inhibition of selectin-dependent tumor cell adhesion to endothelial cells and platelets by blocking O-glycosylation of these cells. *Biochem Biophys Res Commun* 1992; **182**: 1288–95.
7. Hull SR, Bright A, Carraway KL, Abe M, Hayes DF, Kufe DW. Oligosaccharide differences in the DF3 sialomucin antigen from normal human milk and the BT-20 human breast carcinoma cell line. *Cancer Commun* 1989; **1**: 261–7.
8. Ho SB, Kim YS. Carbohydrate antigens on cancer-associated mucin-like molecules. *Semin Cancer Biol* 1991; **2**: 389–400.
9. Hagen KG, Bedi GS, Tetaert D, Kingsley PD, Hagen FK, Balys MM, Beres TM, Degand P, Tabak LA. Cloning and characterization of a ninth member of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase family, ppGaNTase-T9. *J Biol Chem* 2001; **276**: 17395–404.
10. Clausen H, Bennett EP. A family of UDP-GalNAc: polypeptide N-acetylgalactosaminyl-transferases control the initiation of mucin-type O-linked glycosylation. *Glycobiology* 1996; **6**: 635–46.
11. Nomoto M, Izumi H, Ise T, Kato K, Takano H, Nagatani G, Shibao K, Ohta R, Imamura T, Kuwano M, Matsuo K, Yamada Y, Itoh H, Kohno K. Structural basis for the regulation of UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl transferase-3 gene expression in adenocarcinoma cells. *Cancer Res* 1999; **59**: 6214–22.
12. Shibao K, Izumi H, Nakayama Y, Ohta R, Nagata N, Nomoto M, Matsuo K, Yamada Y, Kitazato K, Itoh H, Kohno K. Expression of UDP-N-acetyl-alpha-D-galactosamine-polypeptide GalNAc N-acetylgalactosaminyl transferase-3 in relation to differentiation and prognosis in patients with colorectal carcinoma. *Cancer* 2002; **94**: 1939–46.
13. Sobin LH, Wittekind CH. International Union Against Cancer. TNM classification of malignant tumors. 5th ed. New York: John Wiley & Sons, Inc; 1997.
14. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma. 13th ed. Tokyo: Kanehara & Co Ltd; 1999.
15. Hsu SM, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. *Am J Clin Pathol* 1981; **75**: 816–21.
16. Sutherlin ME, Nishimori I, Caffrey T, Bennett EP, Hassan H, Mandel U, Mack D, Iwamura T, Clausen H, Hollingsworth MA. Expression of three UDP-N-acetyl-alpha-D-galactosamine:polypeptide GalNAc N-acetylgalactosaminyltransferases in adenocarcinoma cell lines. *Cancer Res* 1997; **57**: 4744–8.
17. Kim YS, Gum J Jr, Brockhausen I. Mucin glycoproteins in neoplasia. *Glycoconj J* 1996; **13**: 693–707.
18. Lesuffleur T, Zweibaum A, Real FX. Mucins in normal and neoplastic human gastrointestinal tissues. *Crit Rev Oncol Hematol* 1994; **17**: 153–80.
19. Hakomori S. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. *Adv Cancer Res* 1989; **52**: 257–331.
20. Schirrmacher V. Cancer metastasis: experimental approaches, theoretical concepts, and impacts for treatment strategies. *Adv Cancer Res* 1985; **43**: 1–73.