Increased expression levels of ppGalNAc-T13 in lung cancers: Significance in the prognostic diagnosis

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Abstract. ppGalNAc-T13 is upregulated along with reduced expression of GM1 in high metastatic sublines of the murine Lewis lung cancer cell line, but little is known about the implication of ppGalNAc-T13 expression in human cancers. Since lung cancer cell lines showed high expression levels of ppGalNAc-T13, we analyzed ppGalNAc-T13 expression in surgical lung cancer specimens to examine whether ppGalNAc-T13 can be used as a prognostic marker or a therapeutic target. We analyzed mRNA expression levels of *GALNT13* and its variant exon usages in surgical specimens by real-time RT-PCR, and the results were evaluated by correlating with clinical data. Ninety-one surgical specimens were analyzed. Consequently, recurrence-free survival was significantly shorter (P=0.045) in high expression group of *GALNT13* mRNA. In the analysis of tumor specific exon usage in *GALNT13* RNA sequence, one variant exon was significantly associated with worse prognosis. By contrast, in another variant exon, positive variant expression group showed better prognosis than negative group. We also tried to detect *GALNT13* mRNA in 63 serum samples from patients with lung cancers to examine whether *GALNT13* mRNA can be measured in body fluids, detecting significant levels in 4 samples. Finally, expression of GM1, ppGalNAc-T13 and trimeric Tn antigen was examined by immunohistochemistry in order to evaluate them as a prognostic factor. It was demonstrated that ppGalNAc-T13 and trimeric Tn antigen had a relationship with worse prognosis in 35 investigated lung cancer patients. In conclusion, our results suggest that ppGalNAc-T13 might be a useful prognostic factor of lung cancers.

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Abbreviations: PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; qPCR, quantitative polymerase chain reaction; PBS, phosphate-buffered saline; BSA, bovine serum albumin; FBS, fetal bovine serum; T13, ppGalNAc-T13

Key words: ppGalNAc-T13, lung cancer, tumor marker, prognosis, metastasis

Introduction

Lung cancer is the most frequent malignancy and the prognosis is still poor (1). One of the reasons for worse prognosis is that tumor metastasis is often seen at the diagnosis. In addition, despite development of some molecularly-targeted drugs that target for EGFR-mutation or ALK-rearrangement, there are certain patients who cannot get these benefits (2,3). Therefore, there is a strong need to define new therapeutic targets and biomarkers.

As mentioned above, metastasis is a major cause of death in lung cancer patients. In metastasis process, glycolipids and glycoproteins play important roles and are involved in cancer properties (4-7). Gangliosides, a kind of glycosphingolipid, are widely expressed in many tissues (8), but some gangliosides are very limitedly expressed in normal tissues, and their expression is enhanced in tumors. GD2 is highly expressed in small cell lung cancers, and is strongly associated with the malignant potential (9,10). Furthermore, anti-GD2 monoclonal antibodies induce apoptosis of small cell lung cancer cells (11). NeuGcGM3 is overexpressed in non-small cell lung cancer and an anti-idiotype vaccine targeting the NeuGcGM3 is effective in the treatment of patients with advanced non-small cell lung cancer (12). As for carbohydrates on glycoproteins, O-glycans also play important roles in the malignancy and survival of cancer cells (6). O-glycans are often highly sialylated in cancer cells (13,14). Among them, Tn antigen, which is

Table I. Sequences of primers used in nested and real-time RT-PCR.

Gene	Forward primer	Reverse primer
GALNT13 wild	5'-TGGCCAGTGATTTGATTGCC-3'	5'-AACGTGCATTCACAGTGTGC-3'
E5	5'-TGACTTTCCCTGCTTTCGTG-3'	5'-ATGACCTGCCCTTTTGAAGC-3'
E13'	5'-GTTACTTGCTCCCATGTTGGTC-3'	5'-CAGGTCCATTGAGTCTGATTTC-3'
E14	5'-TGGGAATATGATGCTGAGTCTTG-3'	5'-TTCATGTGCCCAAGGTCATG-3'
GALNT13 nested		
Outer	5'-GCATTGAGGGCTGTTATTTCAAG-3'	5'-AGAGTAGATAGTGTGGGGAACG-3'
Inner	5'-AAGAAGGGCCAGGAGAAATGG-3'	5'-AGAGTGCTCCAAGCTTCATTATG-3'

formed by polypeptide N-acetylgalactosaminyltransferases (ppGalNAc-T), and sialyl Tn antigen have been considered as tumor-associated antigens, and the expression levels of them are increased in many types of human cancers (15,16).

In the analysis of mechanisms for cancer metastasis, we previously demonstrated that reduced levels of ganglioside GM1 resulted in the increased invasion and metastatic potentials (17). We also found that ppGalNAc-T13 (T13), which is a family member of ppGalNAc transferases, was upregulated as a result of reduced GM1, leading to enhanced metastasis by formation of trimeric Tn antigen on Syndecan 1 in mouse Lewis lung cancer (18-20). In human, it has been reported that *GALNT13* mRNA is a strong predictor of poor clinical outcome in neuroblastoma patients (21). However, little is known about the relationship of the T13 expression and the phenotypes and prognosis in lung cancer patients. If the correlation between *GALNT13* gene expression levels in lung cancer tissues and patient prognosis is indicated, it can be expected as a new prognostic marker and a therapeutic target. In addition, if it is detected in blood, it can be a useful biomarker for early detection or minimal metastatic diseases.

In the present study, T13 expression levels in surgical lung cancer specimens were analyzed to examine whether T13 and its product can be used as a tumor prognostic marker.

Materials and methods

Sample collection. Ninety-one patients received pulmonary resection at Nagoya University Hospital (2008, January-2009, March). Before operation, each patient's serum sample was collected. The lung cancer tissues and sera were stored at -80˚C. The treatment policy was decided according to the standard protocol, and fully informed written consents were obtained from all patients. Our study protocol was approved by the Institutional Review Boards of Nagoya University Graduate School of Medicine.

Total RNA isolation and cDNA synthesis. Total RNA was isolated from tissue using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA). We added 1000 μl of TRIzol reagent to tissue, and the sample was homogenized by Psychotron™ (Niti-on Co., Ltd., Funabashi, Japan). Next, we incubated the homogenized sample for 5 min at room temperature prior to the addition of 0.2 ml of chloroform, and they were incubated for 3-min at room temperature. An upper aqueous phase was collected after centrifugation at 12,000 x g for 15 min at 4˚C, and mixed with 0.5 ml of 100% isopropanol. After incubation for 10 min at room temperature, the sample was centrifuged at 12,000 x g for 10 min at 4˚C and the supernatant was removed. After washing, the pellet with 1 ml of 75% ethanol, RNA pellets were dried for 30 min, and resuspended in 50 μl of RNase-free water and incubated at 55˚C for 10 min. For serum samples and cell lines, total RNA was extracted using Total RNA Purification™ kit (Norgen Biotek Corp., Thorold, Ontario, Canada) according to the manufacturer's instructions. We synthesized cDNA from total RNA and extracted genomic DNA using PrimeScript™ RT reagent kit with gDNA Eraser™ (Takara Bio, Kusatsu, Japan).

Quantitative real-time RT-PCR. Real-time quantitative PCR (qPCR) was performed with 8 ng of cDNA and SsoAdvanced™ Universal SYBR-Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) using CFX Connect™ Real-Time system (Bio-Rad Laboratories). Specific primers for *GALNT13*, its variant exon usages, and *GAPDH* were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Each primer sequence of wild-type and variant exons is shown in Table I. Cycling conditions were as follows: 30 sec at 95˚C, and then 40 cycles of 5 sec at 95˚C and 20 sec at 62˚C. Cq values were calculated with Bio-Rad CFX Manager™ version 2.1. *GALNT13* mRNA expression was normalized with an internal control, *GAPDH* mRNA.

Nested PCR. To detect expression of *GALNT13* mRNA in serum sample, nested PCR was performed. Each primer sequence of primary PCR and nested PCR is shown in Table I. First PCR was performed in 50 μl using 8 ng cDNA with 1 μl of KOD FX™ (Toyobo Co., Ltd., Osaka, Japan). Nested PCR were done using 1 μl of the first PCR product as a template. Both first and nested PCR were done under following conditions: 2 min at 94˚C, and then 40 cycles of 10 sec at 98˚C and 30 sec at 62˚C. PCR product was visualized on a 1.5% agarose gel.

Immunohistochemistry. Lung tumor samples were fixed in 4% paraformaldehyde for 24 h, followed by a solution of 30% sucrose/PBS until the tissues sank. After embedding in Tissue-Tek™ O.C.T Compound (Sakura Finetek Japan, Tokyo, Japan), 5 μm-thick frozen sections were made using Leica CM3050S™ cryostat (Leica Biosystems, Wetzlar, Germany). The sections treated with 4% paraformaldehyde for 10 min. Samples were then incubated with 3% H_2O_2 to inhibit endogenous peroxidase activity. In T13 immunostaining, sections were treated with Protein Block Serum-Free™ (Dako Japan,

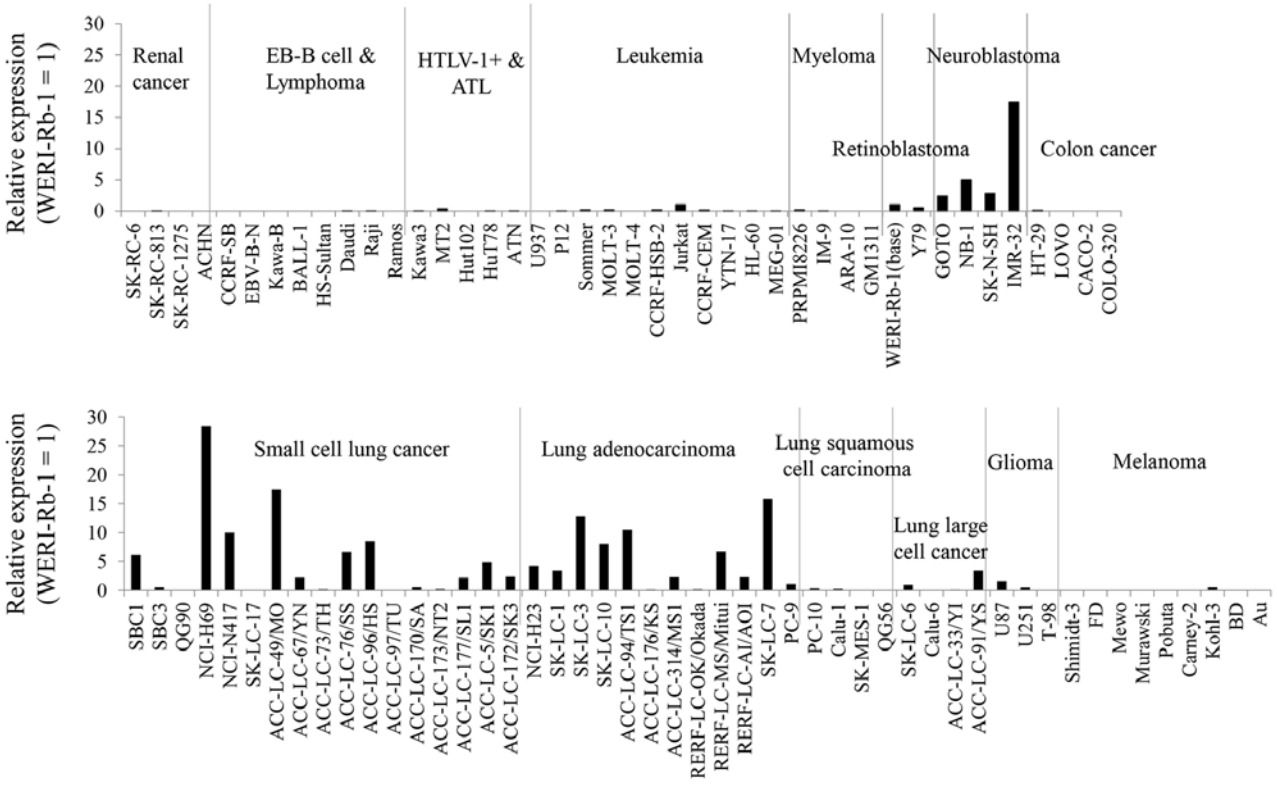


Figure 1. *GALNT13* mRNA expression levels in various human cancer cell lines. The expression levels were analyzed by qRT-PCR as described in Materials and methods, and normalized by *GAPDH*. Relative expression levels obtained by comparing with the value of a control cell line, WERI-Rb-1 were presented. *GALNT13* mRNA was found to be highly expressed in lung cancers and neuroblastomas. EB-B, Epstein-Barr virus transformed B cell. HTLV-1+, human T-lymphotropic virus type 1-positive. ATL, adult T-cell leukemia.

Tokyo, Japan) at room temperature for 30 min in order to block non-specific binding. The sections were incubated at room temperature for 60 min in 0.4 μg/ml goat polyclonal primary anti-ppGalNAc-T13 antibody (T18) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by applying a secondary biotinylated rabbit anti-goat IgG. In GM1 immunostaining, sections were treated with 3% BSA in PBS for 15 min for blocking. Sections were then incubated with cholera toxin B subunit-biotin conjugate (List Biological Laboratories, Campbell, CA, USA) diluted at 0.5 μg/ml with PBS plus 3% BSA, at room temperature for 60 min. In trimeric Tn antigen immunostaining, after treating with Protein Block Serum-Free™, the samples were incubated at room temperature for 60 min with mouse monoclonal primary anti-trimeric Tn antibody (MLS128) (1.6 μg/ml) (provided from Nakada, Kyoto Sangyo University, Kyoto, Japan). Then, a secondary biotinylated horse anti-mouse IgG was applied. The immunoreactivity of these sections was visualized using Vectastain™ Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) and Dako Liquid DAB+ Substrate Chromogen System™ (Dako Japan).

Cell culture. The cell line ACC-LC-94/TS1 was derived from human lung adenocarcinoma and cultured in DMEM with 7.5% fetal bovine serum (FBS; Sigma-Aldrich Japan).

Statistical analysis. The clinicopathological characteristics, overall survival and recurrence-free survival of the patients were obtained from medical records. The relationship was analyzed using the Wilcoxon-Mann-Whitney test. The Kaplan-Meier method was conducted to estimate survival differences and analyzed using log-rank test. The level of significance was set at $P<0.05$. These data were analyzed with IBM SPSS Statistics version 22.0 (IBM, Armonk, NY, USA).

Results

GALNT13 mRNA was highly expressed in human lung cancer cell lines and neuroblastoma cell lines. In order to examine *GALNT13* mRNA expression among human tumors, we determined mRNA expression levels in various human cancer cell lines (Fig. 1). From this result, *GALNT13* mRNA was found to be highly expressed in lung cancers and neuroblastomas. Among lung cancers, both adenocarcinoma cell lines and small cell lung cancer cell lines showed high expression levels.

Association of GALNT13 expression levels with clinical data of lung cancer patients. Then, to examine whether T13 affects the intensity and prognosis of the disease, we analyzed *GALNT13* mRNA expression levels in 91 surgical specimens by real-time RT-PCR, and the results were evaluated by correlating with pathological and clinical data. The *GALNT13* mRNA expression levels were normalized by *GAPDH* mRNA and then relative levels to that of a control cell line (ACC-LC-94/TS1, relative expression level=1) were calculated. We divided 91 cases into *GALNT13* mRNA high expression group (T13 high, n=16) and low expression group (T13 low, n=75) by value 0.001, cut-off point of sufficient expression levels.

Table II. Clinicopathological parameters of patients.

Characteristic	T13 high (N=16) no (%)	T13 low (N=75) no (%)	P-value[a]
Age (years)			0.831
Median	67	67	
Range	54-78	44-84	
Gender			
Female	4 (25)	17 (23)	0.841
Smoking status			0.343
Never smoked	3 (19)	15 (20)	
Former smoker	9 (56)	52 (69)	
Current smoker	4 (25)	8 (11)	
Comorbidity			
COPD	5 (31)	2 (37)	0.648
Diabetes mellitus	5 (31)	12 (16)	0.158
Hypertention	6 (38)	29 (39)	0.931
Tumor histologic type			
Adenocarcinoma	10 (63)	51 (68)	1.000
Non-adenocaricinoma	6 (37)	24 (32)	
UICC-7 stage			0.236
I	7 (44)	45 (60)	
II/III	9 (56)	30 (40)	
pT category			0.124
0-1	10 (63)	31 (41)	
2-4	6 (37)	44 (59)	
pN category			0.062
0	9 (56)	59 (79)	
1-2	7 (44)	16 (21)	

T13 high, *GALNT13* mRNA high expression group; T13 low, *GALNT13* mRNA low expression group. Cut-off point was value 0.001 of mRNA normalized expression levels. [a]Wilcoxon-Mann-Whitney test.

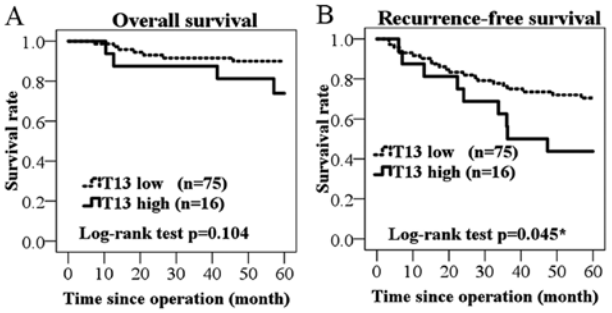


Figure 2. Kaplan-Meier analysis of lung cancer patients for overall survival (A) and recurrence-free survival (B). *GALNT13* mRNA high expression group (T13 high) showed a significantly shorter recurrence-free survival compared with the low expression group (T13 low). Patients numbers are shown in the figure. P=0.045. *P<0.05.

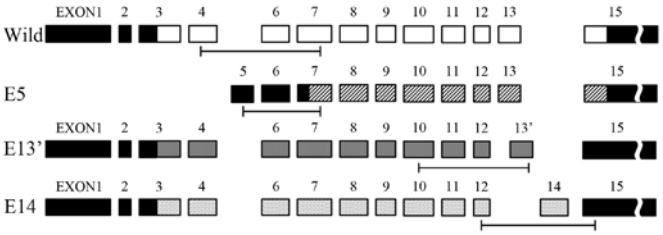


Figure 3. Exon structures of *GALNT13* mRNA and its exon usages. These data were obtained from GenBank and Ensembl. We selected three tumor specific variant exon usages named E5, E13' and E14. The black areas are non-cording regions. The lines under individual exon structures indicate PCR-amplified regions by designed primers for detection.

Clinicopathological parameters of 91 patients are shown in Table II. We found no differences in age, gender, smoking status and comorbidities. Lymph node metastasis tended to be higher in the T13 high group than in T13 low group, but this association was not significant (P=0.062, Wilcoxon-Mann-Whitney test).

Among the 91 cases, patients with high expression levels of *GALNT13* mRNA exhibited reduced overall survival (Fig. 2A). However, the difference in survival rates was not significant (P=0.104; log-rank test). On the other hand, T13 high group was significantly associated with worse prognosis as observed in recurrence-free survival (P=0.045; Fig. 2B).

It was reported that there were differential usages of exons in *GALNT13* RNA sequence, therefore, we chose three variant exons (named E5, E13' E14) and analyzed their expression levels. We obtained base sequence of *GALNT13* mRNA and variant exons from GenBank and Ensembl, and designed a specific PCR primer of each. Their exon structures and PCR targets are shown in Fig. 3.

Then, the survival terms of E5, E13' and E14-positive cases were examined by using 40 surgical specimens (Fig. 4). In E5, neither overall survival nor recurrence-free survival was significantly correlated between positive and negative groups (P=0.700, P=0.154, log-rank test; Fig. 4A). In E13'-positive group, significantly worse prognosis in overall survival, but not in recurrence-free survival was found (P=0.042, P=0.117, respectively; Fig. 4B). In turn, E14-positive group showed rather better prognosis than negative group, especially in the recurrence-free survival (P=0.019; Fig. 4C).

Detection of GALNT13 mRNA in sera. We examined whether T13 in sera can be used as a tumor marker. Some frozen serum samples from surgical patients were analyzed by PCR, but *GALNT13* mRNA could not be detected by current analytical condition. Since the expression levels in sera were considered to be less than in tumor tissues, we tried nested PCR, and were able to detect it as shown in Fig. 5A. Sixty-one frozen serum samples from surgical patients and two fresh serum samples from stage IV patients were examined. Among total of 63 serum samples examined by nested PCR, *GALNT13* mRNA could be detected in four of the specimens. The parameters of these four patients are shown in Fig. 5B.

Immunohistochemistry analysis revealed that staining of T13 and trimeric Tn antigen significantly correlated with worse prognosis. We evaluated the association between the prognosis and expression of T13, GM1 and trimeric Tn antigen by immunohistochemistry. In a mouse model, we revealed that

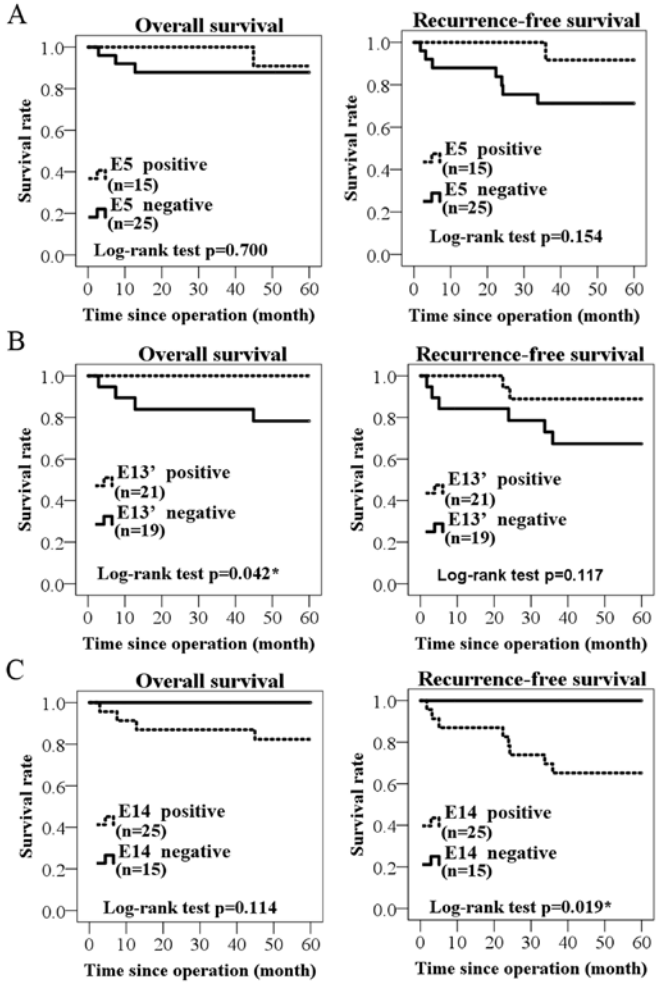
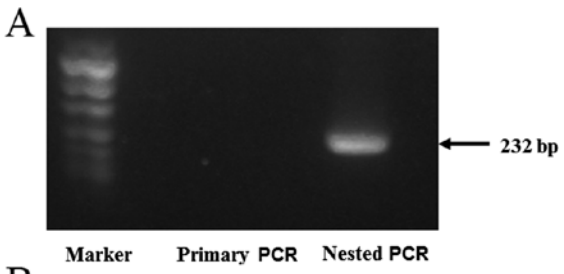


Figure 4. Kaplan-Meier analysis for overall and recurrence-free survival of patients having *GALNT13* RNA variant exon usages: E5 (A), E13' (B) and E14 (C). E13'-positive expression group was significantly associated with worse prognosis in overall survival. Conversely, E14-positive expression group showed significantly prolonged recurrence-free survival compared with the negative group. Number of patients in each group is shown in the figure.



B

Parameters of serum *T13* mRNA positive patients

Patient	Age	Sex	Histologic type	Stage	State after 5 years
1.†	74	male	ADC	ⅢA	dead
2.†	69	male	ADC	ⅠA	alive
3.†	70	male	SCC	ⅡA	dead
4.‡	75	male	ADC	Ⅳ	dead

Figure 5. Detection of *GALNT13* mRNA from patient serum samples by nested PCR. (A) Agarose gel electrophoresis of PCR products. Design of primers and qPCR are described in Table I. The 310 bp primary PCR product was negative in the central lane, but the 232 bp nested PCR product was positive on the right. (B) Sixty-one frozen serum samples from surgical patients and two fresh serum samples from stage 4 patients were evaluated by nested PCR. *GALNT13* mRNA could be detected in four of the specimen. The age, gender, histologic type, stage and state after five years of the four patients are shown. T13, *GALNT13*. †Surgical patient. ‡Stage 4 patient.

Table III. Correlation between T13, GM1 and trimeric Tn.

	T13 positive (N=13)	T13 negative (N=22)	P-value[a]
GM1			0.007[b]
Positive	0	9	
Negative	13	13	
Trimeric Tn			0.006[b]
Positive	10	6	
Negative	3	16	

T13, ppGalNAc-T13. [a]Fisher's exact test; [b]P<0.01.

reduced expression level of GM1 resulted in the upregulation of *GALNT13* gene, and T13 formed trimeric Tn antigen on Syndecan-1, leading to increased adhesion to extracellular matrix (20). Therefore, we also examined the correlation between T13, GM1 and trimeric Tn in lung cancer tissues. The tumor samples which showed positive staining in more than 10% of tumor cells were defined as positive expression. Images of the positive and negative staining of T13, GM1 and trimeric Tn are shown in Fig. 6. We analyzed 35 samples (patients received operation in May, 2008-December, 2008) and evaluated the correlation between the staining and the patients prognosis. We found that the T13 positive group tended to show worse overall survival than T13-negative group without significance (P=0.248, log-rank test; Fig. 7A). On the other hand, T13-positive group had strongly significant association with shortened recurrence-free survival (P=0.007). This result was similar to results of *GALNT13* mRNA expression analysis. As for GM1, GM1 positive group tended to show longer survival in overall and recurrence-free survival, but it was not significant (P=0.180, P=0.061; Fig. 7B). For trimeric Tn antigen, the positive group showed a trend of short survival, and there was a significant difference in the recurrence-free survival (P=0.007; Fig. 7C). The correlation between T13, GM1, and trimeric Tn expression is shown in Table III. There was a negative correlation between T13 and GM1 expression (P=0.007, Fisher's exact test). By contrast, positive correlation was observed in T13 and trimeric Tn expression (P=0.006).

Discussion

In the present study, we demonstrated that T13 expression was significantly associated with the worse prognosis of lung cancer patients. In pathological data, lymph node metastasis, which was a prognostic factor in resected non-small cell lung cancer (22), tended to be higher in the *GALNT13* mRNA high expression group. From these results, it can be considered that T13 plays an important role in the malignancy of cancer, particularly in metastasis process in lung cancers. This speculation is supported by our previous study, which showed that

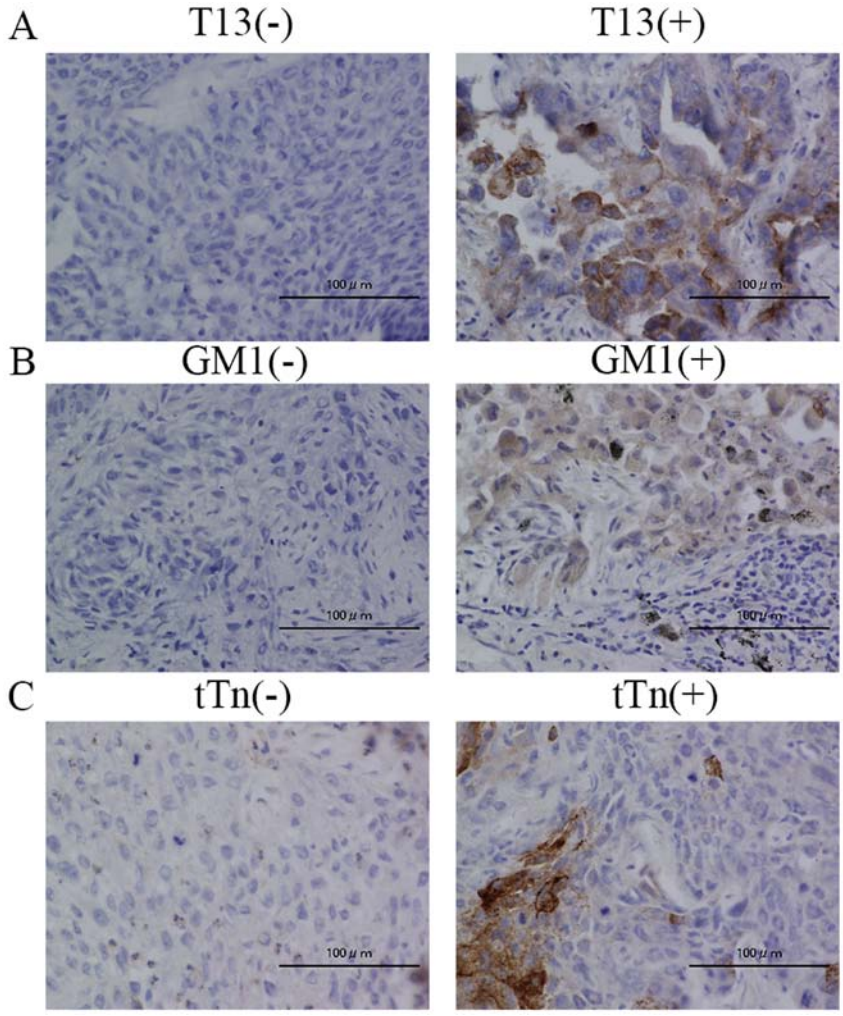


Figure 6. Expression of T13, GM1 and trimeric Tn antigen in lung cancer tissues as analyzed by immunohistochemistry. (A-C) Left panel is negative expression tissue and right panel is positive expression tissue of each staining by anti-ppGalNAc-T13 (T18) (A), cholera toxin B subunit (B), and anti-trimeric Tn antibody (MLS128) (C), respectively. Scale bars, 100 μm. T13, ppGalNAc-T13; tTn, trimeric Tn.

T13 induces high metastatic potential of murine Lewis lung cancer. In the study, we also revealed that T13 was upregulated as a result of reduced GM1, leading to enhanced metastasis by formation of trimeric Tn antigen on Syndecan 1 in mouse Lewis lung cancer (19,20). In this study, we found the negative correlation of GM1 and T13, and the positive correlation of trimeric Tn and T13 by immunohistochemistry. These results corresponded with our previous data in the experimental mouse metastasis model.

In a previous study, expression levels of *GALNT13* mRNA were analyzed in various adult and fetal human tissues. The expression level was highest in the fetal brain, followed by the adult brain. *GALNT13* mRNA was expressed at minimal or undetectable levels in the other tissues (18). It was also shown that T13 was able to form trimeric Tn antigen, and significant decrease in Tn antigen expression was found in the cerebellum of the T13 knockout mouse (18). However, roles of T13 have not been clarified. In the analysis of *GALNT13* mRNA expression levels among human tumor cell lines, we found that the expression levels were higher in only lung cancer and neuroblastoma lines (Fig. 1). Combined with the fact that *GALNT13* mRNA was scarcely expressed in normal lung tissues, T13 may be involved in the mechanism for evolution or malignant properties of lung cancers. We also demonstrated the association between T13 and metastatic potential and/or poor prognosis of lung cancer patients. Considering that T13 is mainly expressed in fetal brain, T13 and its product trimeric Tn may be involved in the cell growth or proliferation. In fact, it was reported that MLS128 monoclonal antibody, which binds an epitope consisting of three consecutive Tn-antigens, inhibited colon and breast cancer cell growth (23-25).

We also analyzed several variant exon usages in *GALNT13* mRNA sequence, and found that one variant exon expression had significant association with worse prognosis (Fig. 4). By contrast, another variant exon-positive group showed better prognosis than negative group. This was an interesting and surprising result. *GALNT13* mRNA differential usages of exons have different sequences of lectin like domain. Therefore, the reason for opposite result among variant exon usages may be attributed to the difference in the recognition of the substrate during the synthesis of O-glycans (26). Thus, tumor-specific and malignant property-associated variant exon usage may be important as targets for molecular therapy of cancers, although precise mechanisms remain to be investigated.

We demonstrated that T13 and trimeric Tn antigen had a relationship with worse prognosis of lung cancer patients by

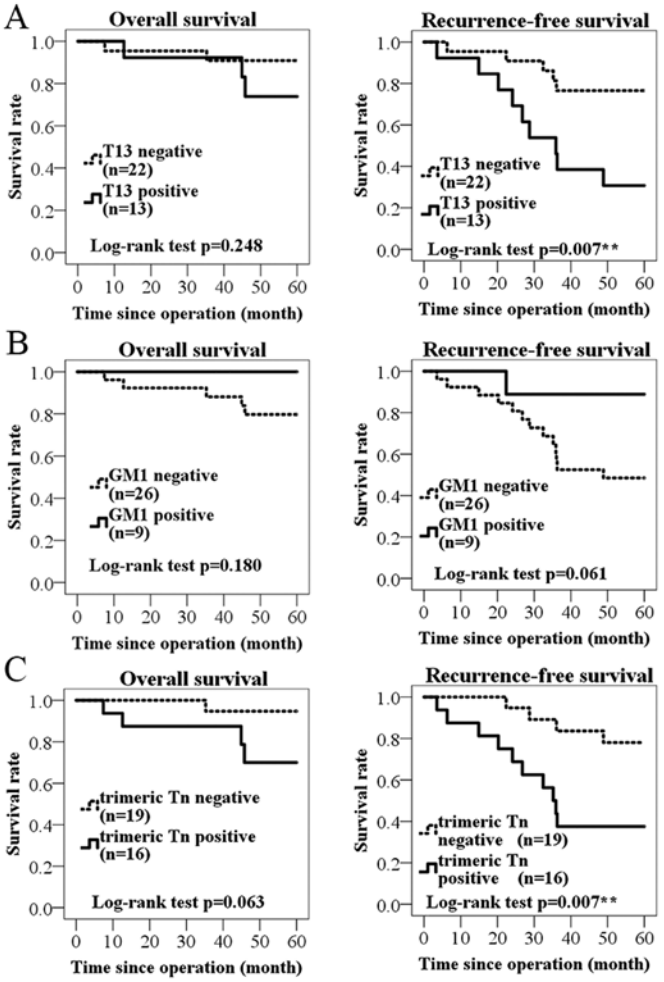


Figure 7. Kaplan-Meier analysis for overall and recurrence-free survival between positive and negative groups for T13 (A), GM1 (B), and trimeric Tn (C) in immunohistochemistry. (A) T13-positive group showed significantly worse prognosis in overall survival. (B) GM1-positive group showed longer survival, but it was not significant. (C) Trimeric Tn antigen-positive group showed significantly shorter survival in recurrence-free survival. T13, ppGalNAc-T13; tTn, trimeric Tn. **P<0.01.

immunohistochemistry. This result also suggests that T13 and trimeric Tn antigen can be used as a tumor marker. Although *GALNT13* mRNA could be detected in serum sample, the expression levels were too low to be stably quantified. If the carrier proteins of T13 or trimeric Tn are identified, we can more easily detect them in serum by using ELISA. Thus, they can be expected as a tumor marker, leading to early detection of lung cancers or minimal metastatic diseases.

The limitation of the present study is partly due to restricted selection of specimens. *GALNT13* mRNA detection and immunohistochemistry were conducted in primary tumors obtained in surgery. Because we did not get metastatic tumors and lymph nodes, we could not examine the difference in the expression levels between primary and metastatic tumors. Despite these study limitations, we demonstrated that higher expression of T13 in primary tumors were associated with the poor prognosis. Consequently, it can be an indicator for whether we should perform postoperative therapy and careful observation. That is because postoperative therapy largely affect patient survival, while a part of patients can obtain benefit (27,28). Therefore, by using specific antibodies, T13 and trimeric Tn antigen might be expected as a new target of molecular treatment.

In conclusion, the present study showed that high expression levels of *GALNT13* mRNA is associated with poor prognosis of lung cancer patients by using quantitative real-time RT-PCR. Furthermore, T13 and trimeric Tn antigen expression were strongly correlated with shortened survival in immunostaining. Our results suggest that T13 might be a useful prognostic factor and might be a new target for cancer treatment.

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