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RESEARCH ARTICLE

GalNAc-T3 and MUC1, a combined predictor of prognosis and recurrence in solitary pulmonary adenocarcinoma initially diagnosed as malignant solitary pulmonary nodule (≤ 3 cm)

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Abstract

The significance of the polypeptide *N*-acetyl-galactosaminyl transferase-3 (GalNAc-T3) and mucin 1 (MUC1) in solitary pulmonary adenocarcinoma (SPA) initially diagnosed as malignant solitary pulmonary nodule (≤3 cm), especially as a combined predictor of prognosis and recurrence, was explored in this study. A retrospective analysis of 83 patients with SPA (≤3 cm), which revealed postoperative pathological diagnosis was lung adenocarcinoma after complete resection. Immunohistochemical staining was used to detect the expression of GalNAc-T3 and MUC1 in primary tumor specimens. The relationship between expression and various clinicopathological factors was analyzed, as well as the effects of patients' overall survival (OS) and disease-free survival (DFS). In all patients, GalNAc-T3 was highly expressed in 53 (63.9%) cases; MUC1 was highly expressed in 31 (37.3%) cases. The GalNAc-T3 expression was correlated with differentiation, pathological risk group, N stage, and TNM stage. The group with high GalNAc-T3 expression and low MUC1 expression (GalNAc-T3^{Hig}/MUC1^{Low}) is correlated to pathological differentiation and has a trend related to the TNM stage. The patients with better differentiation, lower pathological risk group, lower N stage, and GalNAc-T3 high expression had better overall survival, especially the GalNAc-T3^{Hig}/MUC1^{Low} group. Moreover, the moderate differentiation, N3 stage, and GalNAc-T3^{Hig}/MUC1^{Low} group were independent predictive factors for OS. Besides, patients with lower N stage, lower TNM stage, higher GalNAc-T3 expression got better disease-free survival (DFS), especially the GalNAc-T3^{Hig}/MUC1^{Low} group. The GalNAc-T3^{Hig}/MUC1^{Low} group was an independent predictive factor for DFS. In conclusion, GalNAc-T3 and MUC1 were combined predictors of prognosis and recurrence in SPA (≤3 cm).

Keywords GalNAc-T3 · MUC1 · Solitary pulmonary nodule · Pulmonary adenocarcinoma · Prognosis

Introduction

Lung cancer, one of the most severe human malignancies, has become the leading cause of cancer death in the world over the past decades [1]. With the increasing awareness of medical examination and the popularization of low-dose computer tomography (LDCT) technology, more and more ground-glass nodules (GGN) and solitary pulmonary nodules (SPN) have been found [2–4]. The SPN refers to a single lesion with a diameter of ≤3 cm, surrounded by air-containing lung tissue, without atelectasis, enlarged hilar, or pleural effusion [5]. In the National Lung Screening Trial, the detection rate of SPN in high-risk populations by low-dose CT was 23% [6]. The detection rate of SPN ranged from 8 to 51%, and the malignant rate was 1.1–12% in different studies [7].

The polypeptide *N*-acetyl-galactosaminyl transferase-3 (GalNAc-T3) is one of the members of the GalNAc-transferases family [8]. This family is the initiating enzyme that catalyzes mucin-type O-glycosylation of cell surface proteins and is widely expressed in human normal tissues and

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malignancies [9,10]. Its activity is closely related to the formation of mucins rich in O-sugar chains [11]. The O-glycan mucin abnormalities are a common phenomenon that occurs in a variety of epithelial-derived tumors and are closely related to malignant tumor adhesion, invasion, metastasis, and intracellular signal transduction [12–14]. On the other hand, the sugar chain of mucin 1 (MUC1), a high molecular weight glycoprotein, is mostly connected to the polypeptide backbone by O-glycosidic bonds [15]. It plays a vital role in cancer cell adhesion and metastasis, immune regulation, and cell signal transduction [16,17]. As a classical tumor marker, MUC1 can be used for diagnosis and recurrence monitoring of various cancers [18]. At the same time, a large of studies shown that MUC1 has necessary regulation on various malignant functions of lung cancer, and is correlated to the prognosis and recurrence of lung cancer [19–21].

In our previous study, the expression of GalNAc-T3 was closely correlated to the prognosis and recurrence of lung adenocarcinoma, as well as small (≤2 cm) peripheral lung adenocarcinoma [22,23]. We conjectured whether it is also related to solitary pulmonary adenocarcinoma (SPA) initially diagnosed as malignant solitary pulmonary nodule (≤3 cm). Based on that, we try to find a combined predictor different from the traditional single predictive factor. Therefore, we boldly speculate whether the expression of these two markers is related to the prognosis and recurrence of patients with SPA (≤3 cm). This is the first time that GalNAc-T3 and MUC1 are combined as a predictor to predict the prognosis and recurrence of SPA (≤3 cm).

Materials and methods

Patients and follow-up

Primary specimens from 83 patients (median age 66 years; range from 47 to 85 years) who underwent complete surgical resection of the solitary pulmonary adenocarcinoma (SPA) initially diagnosed as malignant solitary pulmonary nodule (≤3 cm) without any neoadjuvant therapy were consecutively obtained at the First Affiliated Hospital of Dalian Medical University between January 2009 and December 2010. Other than that, the inclusion criteria for the study were based on malignant solitary pulmonary nodule with a diameter of ≤3 cm, the postoperative pathological diagnosis of lung adenocarcinoma, identified by routine histopathologic examination. The seventh edition International Union Against Cancer/American Joint Committee on Cancer TNM classification was applied to all enrolled patients [24].

For the postoperative follow-up, patients were followed every 3 months within the first year and at approximately 5 to 6-month intervals thereafter. During the follow-up period, the data of physical examination, blood chemistry analysis, carcinoembryonic antigen determination, as well as chest, upper abdomen, brain CT scan were collected and analyzed to timely detect whether there were recurrence and metastasis. The terminal follow-up time was June 2018 (median follow-up: 49.4 months). The study was approved by the Medical Ethical Committee of the First Affiliated Hospital of Dalian Medical University. All patients provided written informed consent and agreed their tissue samples could be used for clinical research, but not commercial use.

Immunohistochemistry staining

All resected specimens were obtained from primary lesions, fixed with formalin, embedded with paraffin, serial 4 μm sections were prepared. Immunohistochemical staining (IHC), applied to specimens, was performed according to the instruction from 3,3′-diaminobenzidine (DAB) Kit (Zsbio, Beijing, China) strictly. Antibodies of GalNAc-T3 (Proteintech Group, Inc., Rosemont, Illinois, USA) and MUC1 (Proteintech Group, Inc., Rosemont, Illinois, USA) antibody diluted 1:100 in PBS containing 2% bovine goat serum (included in DAB Kit), respectively, according to the manufacturer's protocol. Then the slides were observed and photographed with an upright microscope.

Staining evaluation

The positive expression of GalNAc-T3 and MUC1 was indicated by pale brown cell cytoplasm, cytomembrane, and nucleus. Immunostaining was evaluated by two pulmonary pathologists using a blind protocol design. Histologic classification of each tumor was based on IASLC/ATS/ERS international multidisciplinary classification of lung adenocarcinoma [25]. The new classification included: (1) atypical adenomatous hyperplasia (AAH), (2) adenocarcinoma in situ (AIS), (3) minimally invasive adenocarcinoma (MIA), (4) lepidic (Lep), (5) acinar (Aci), (6) papillary (Pap), (7) micropapillary (Mp), (8) solid (Sol), and (9) mucinous (Mc). For each specimen, the total score of intensity expression (negative staining: 0 point; weak staining: 1point; moderate staining: 2 point; and strong staining: 3 point) multiplying stained cell numbers (positive cells as ≤25% of the cells: 1 point; 26–50% of the cells: 2 point; 51–75% of the cells: 3 point; >75% of the cells: 4 point). Subsequently, to accurately evaluate the overall expression of GalNAc-T3 and MUC1 in the primary lesions, three experienced pathologists, including a senior pathologist if necessary, carried out a receiver operating characteristic (ROC) curve based on the score of the primary lesions and adjacent tissues, as reported in previous articles [26]. The score closest to the upper left corner on the curve is selected as the cut-off score. As shown in Fig. 1, when the cut-off score of GalNAc-T3 expression is

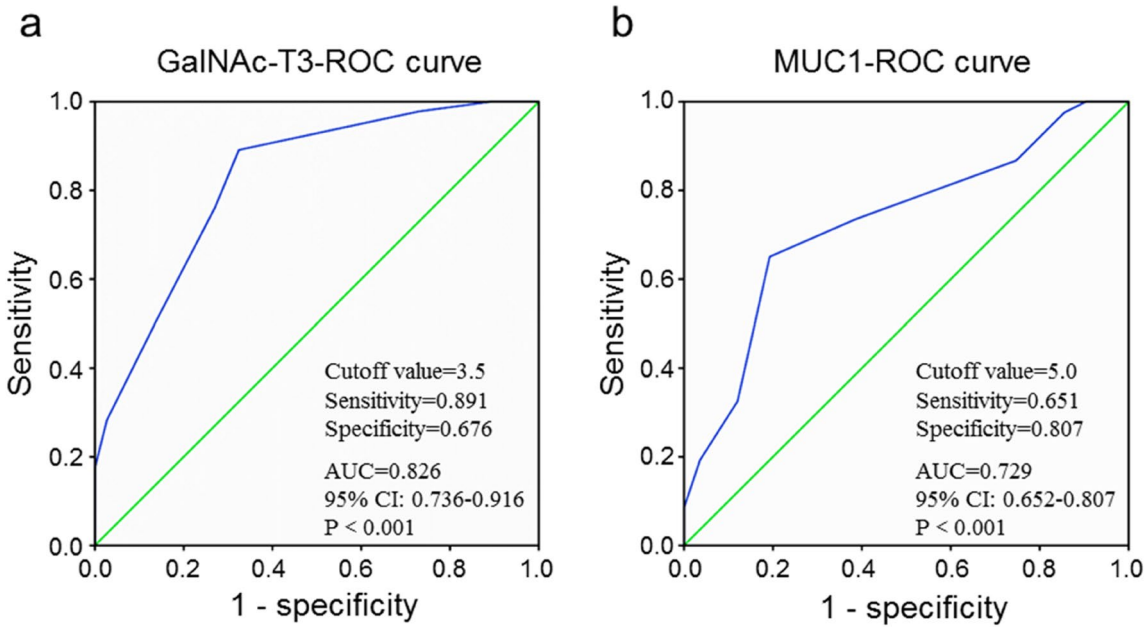

Fig. 1 ROC curve to analyze the diacritic expression of GalNAc-T3 and MUC1 expression. **a** The AUC of the GalNAc-T3-ROC curve was 0.826 (95% CI 0.736–0.916; $P < 0.001$) with a specificity of 67.6% and sensitivity of 89.1%. **b** The AUC of the MUC1-ROC curve was 0.729 (95% CI 0.651–0.807; $P < 0.001$) with a specificity of 80.7% and sensitivity of 65.1%

3.5 points, the sensitivity and specificity of GalNAc-T3 to distinguish lung adenocarcinoma from normal human lung tissue are 89.1% and 67.6%, (area under the ROC curve [AUC] = 0.826; 95% confidence interval [CI] 0.736–0.916; $P < 0.001$). Therefore, when the score is 3.5 points, GalNAc-T3 is considered to be highly expressed, otherwise it is considered to be less expressed. When the cut-off score of MUC1 expression is 5 points, the sensitivity and specificity are 80.7% and 65.1%, respectively (AUC = 0.729; 95% CI 0.651–0.807; $P < 0.001$). MUC1 was considered to be highly expressed when the score was 5 points.

Statistical analysis

ROC curve analyses were performed to assess the cut-off levels of GaINAC-T3 or MUC1 expression, and the comparison of areas under the ROC curve was performed by *Z* test. The associations between GaINAC-T3 or MUC1 expression and categorical variables were compared by the chi-square test. Survival curves were calculated using the Kaplan Meier method. The log-rank test was used to analyze overall survival (OS) and disease-free survival (DFS) time between different clinicopathological factors in lung adenocarcinoma. Multivariate analysis was performed using the Cox regression analysis. Data were analyzed by the SPSS 22 software (IBM Corporation, Armonk, NY, USA). Values of $P < 0.05$ were considered a statistically significant difference.

Results

Clinicopathologic characteristics

The basic clinicopathological characteristics of all patients are summarized in Table 1. Among the 83 patients who were initially diagnosed with malignant solitary pulmonary nodules (≤ 3 cm) and postoperatively diagnosed with lung adenocarcinoma, 45 (54.2%) cases were male, and 38 (44.8%) cases were female. 36 (48%) patients of all were over 64 years. From the postoperative pathological analysis, the number and percentage of well, moderate, and poor differentiation were 40 (48.2%), 37 (44.6%), and 6 (7.2%), respectively. From the point of postoperative pathology, IASLC/ATS/ERS classification risk grade of patients resulted in a low risk grade (9 [10.8%]; 1 AAH [1.2%], 1 AIS [1.2%], 2 MIA [2.4%], and 5 Lep [6.0%]), a moderate risk grade (60 [72.3]; 23 Pap [27.7%] and 37 Aci [44.6%]), and a high-risk grade (14 [16.9]; 33 Mp [3.6%], 9 Sol [10.8%], 2 Mc [2.4%]). All patients were in T1 and M0 stage. In the N stage, N0 accounted for the majority, 73 (88.0%), N1, N2 and N3 were 1 (1.2%), 7 (8.4%) and 2 (2.4%) patients respectively. Similar distribution can also

Table 1 Clinicopathologic characteristics in 83 patients with solitary pulmonary adenocarcinoma (≤3 cm)

Characteristics	Number (%)
Sex	
Male	45 (54.2)
Female	38 (44.8)
Age (years)	
≤66	42 (50.6)
>66	41 (49.4)
Differentiation	
Well	40 (48.2)
Moderate	37 (44.6)
Poor	6 (7.2)
IASLC/ATS/ERS classification risk group	
Low risk	9 (10.8)
AAH	1 (1.2)
AIS	1 (1.2)
MIA	2 (2.4)
Lepidic	5 (6.0)
Moderate risk	60 (72.3)
Papillary	23 (27.7)
Acinar	37 (44.6)
High risk	14 (16.9)
Micropapillary	3 (3.6)
Solid	9 (10.8)
Mucinous	2 (2.4)
T states	
T1	83 (100)
N states	
N0	73 (88.0)
N1	1 (1.2)
N2	7 (8.4)
N3	2 (2.4)
M states	
M0	83 (100)
TNM stage	
I	73 (88.0)
II	1 (1.2)
III	9 (10.8)
GalNAc-T3 expression	
High	53 (63.9)
Low	30 (36.1)
MUC1 expression	
High	31 (37.3)
Low	52 (62.7)

be seen in the TNM stage, 73 (88.0%) patients were TNM stage I, one patient with TNM stage II, and nine patients with TNM stage III.

Correlation between GalNAc-T3 or MUC1 expression and clinicopathologic factors

In IHC staining analysis, GalNAc-T3 was usually found in the cytoplasm, while MUC1 appeared more in the nucleus and cell membrane. The typical examples of IHC stains with different expression levels of GalNAc-T3 and MUC1 were shown in Fig. 2. In all 83 specimens, 53 (63.9%) cases were GalNAc-T3 high expression, while 31 (37.3%) cases were MUC1 high expression. Moreover, as shown in Table 2, the GalNAc-T3 expression level was significantly correlated with pathological differentiation, N stage, TNM stage, IASLC/ATS/ERS classification risk group ($P=0.001$, $P=0.017$, $P=0.008$, $P=0.048$, respectively). The correlation between various clinicopathological factors and MUC1 expression is temporarily not obvious. In Table 3, after combining GalNAc-T3 high expression and MUC1 low expression (GalNAc-T3^{Hig}/MUC1^{Low}), the GalNAc-T3^{Hig}/MUC1^{Low} group was significantly correlated with differentiation ($P=0.010$) and had a correlation trend with TNM stage ($P=0.052$). It was a pity that the GalNAc-T3^{Hig}/MUC1^{Low} group seem to weaken the correlation between GalNAc-T3 and clinicopathological factors, but, in clinical work, our focuses more on the impact on overall survival (OS) and disease-free survival (DFS).

Survival analyses

We analyzed the effects of GalNAc-T3 and MUC1 expression and clinicopathological factors on OS and DFS. In Table 4 and Fig. 3, among all the enrolled patients, those with better differentiation ($P=0.014$), N stage ($P=0.002$), IASLC/ATS/ERS classification risk group ($P=0.030$) and higher GalNAc-T3 expression ($P=0.035$) had better OS, especially GalNAc-T3 high expression Patients with MUC1 low expression ($P=0.005$). After combining the GalNAc-T3 high expression and MUC1 low expression cases, it's 5-year OS rate (90.0%) was significantly better than other groups (57.4%). In further multivariate analysis (Table 5), moderate differentiation (hazard ratio, [HR] = 3.818; 95% confidence interval [CI] 1.249–11.671; $P=0.019$), N3 stage (HR = 11.666; 95% [CI] 2.035–61.842; $P=0.006$) were independent risk factors for overall survival. Excitingly, the GalNAc-T3^{Hig}/MUC1^{Low} expression group (HR = 0.310; 95% CI 0.100–0.963; $P=0.043$) are independent protective factors for overall survival.

On the other hand, in Table 4 and Fig. 4, we found that the N stage ($P=0.040$), TNM stage ($P=0.049$), and GalNAc-T3 expression ($P=0.004$) were correlated with DFS. After the combination of MUC1 expression, the GalNAc-T3^{Hig}/MUC1^{Low} group ($P=0.004$) was closely tied to DFS, and

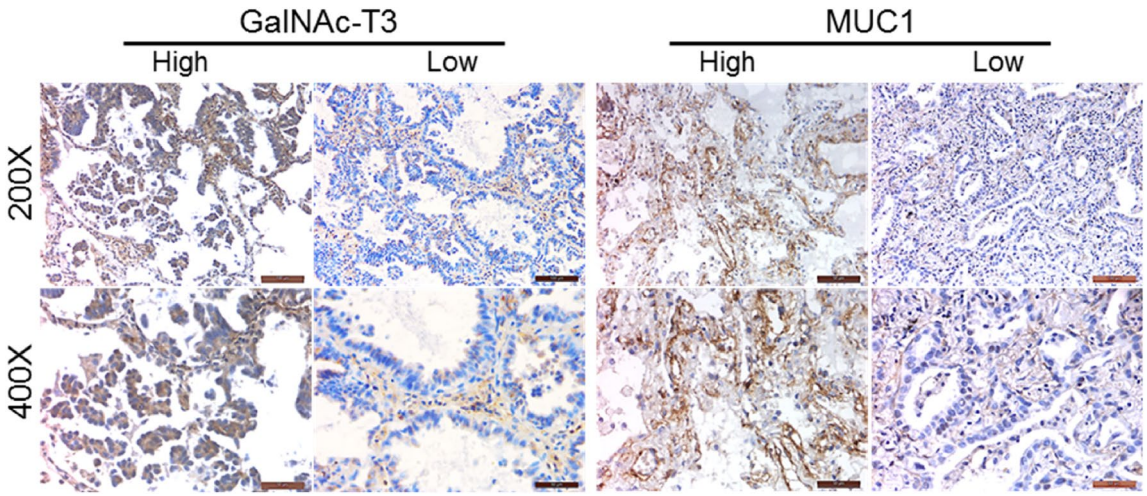

Fig. 2 Immunohistochemical analyses of GalNAc-T3 and MUC1 expression in solitary pulmonary adenocarcinoma (≤3 cm). The expression of GalNAc-T3 and MUC1 in SPA (≤3 cm) specimens ($n=83$) was detected by IHC assay. Original magnification ×200 and ×400 in the inset

Table 2 Relations between the level of GalNAc-T3 or MUC1 expression and clinicopathologic characteristics in solitary pulmonary adenocarcinoma (≤3 cm)

Characteristics	GalNAc-T3 expression		*P* value	MUC1 expression		*P* value
	High (%)	Low		High (%)	Low	
Over all	53 (63.9)	30		31 (37.3)	52	
Sex			0.210			0.318
Male	26 (57.8)	19		19 (42.2)	26	
Female	27 (71.1)	11		12 (31.6)	26	
Age (years)			0.198			0.094
≤66	24 (57.1)	18		12 (47.6)	30	
>66	29 (70.7)	12		19 (46.3)	22	
Differentiation			**0.001**			0.344
Well	31 (77.5)	9		12 (30.0)	28	
Moderate	22 (59.5)	15		17 (45.9)	20	
Poor	0 (0.0)	6		2 (33.3)	4	
Pathological risk group			**0.048**			0.244
Low risk grade (AAH + AIS + MIA + Lep)	7 (77.8)	2		3 (33.3)	6	
Moderate risk grade (Pap + Aci)	41 (68.3)	19		20 (33.3)	40	
High risk grade (Mp + Sol + Mc)	5 (35.7)	9		8 (57.1)	6	
T states						
T1	53 (63.9)	30		31 (37.3)	52	
N states			**0.017**			0.573
N0	51 (69.9)	22		26 (35.6)	47	
N1	0 (0.0)	1		0(0.0)	1	
N2	2 (28.6)	5		4(57.1)	3	
N3	0 (0.0)	2		1(50.0)	1	
M states						
M0	53 (63.9)	30		31 (37.3)	52	
TNM stage			**0.008**			0.374
I	51 (69.9)	22		26(35.6)	47	
II	0 (0.0)	1		0(0.0)	1	
III	2 (22.2)	7		5(55.6)	4	

Statistical significance was evaluated using the chi-square test. Differences were considered to be statistically significant for *P* values of < 0.05 which are shown in bold

Table 3 Relations between GalNAc-T3/MUC1 expression and clinicopathologic characteristics in solitary pulmonary adenocarcinoma (≤ 3 cm)

Characteristics	GalNAc-T3^{Hig}/ MUC1^{Low} (%)	Other	*P* value
Over all	38 (45.8)	45	
Sex			0.479
Male	19 (42.2)	26	
Female	19 (50.0)	19	
Age (years)			0.734
≤ 66	20 (47.6)	22	
> 66	18 (43.9)	23	
Differentiation			**0.010**
Well	24 (60.0)	16	
Moderate	14 (37.8)	23	
Poor	0 (0.0)	6	
Pathological risk group			0.626
Low risk grade (AAH + AIS + MIA + Lep)	5 (55.6)	4	
Moderate risk grade (Pap + Aci)	28 (46.7)	32	
High risk grade (Mp + Sol + Mc)	5 (35.7)	9	
T states			
T1	38 (45.8)	45	
N states			0.110
N0	37 (50.7)	36	
N1	0 (0.0)	1	
N2	1 (14.3)	6	
N3	0 (0.0)	2	
M states			
M0	38 (45.8)	45	
TNM stage			0.052
I	37 (50.7)	36	
II	0 (0.0)	1	
III	1 (11.1)	8	

Statistical significance was evaluated using the chi-square test. Differences were considered to be statistically significant for *P* value of < 0.05 which are shown in bold

GalNAc-T3^{Hig}/MUC1^{Low} high GalNAc-T3 and low MUC1 expression

5-year DFS rate reached 91.6% compared with other groups (66.2%). In the DFS multivariate analysis, the GalNAc-T3^{Hig}/MUC1^{Low} group (HR = 0.158; 95% CI 0.035–0.719; $P = 0.017$) was the independent protective factor of DFS (Table 6).

Discussion

Due to the poor prognosis and high recurrence, lung cancer has become one of the most severe malignant tumors [1]. Solitary lung nodules are one of the more and more early lung cancers detected in routine examination today [3]. In our study, we found that patients with SPA (≤ 3 cm) in the early N stage (N0, N1) and early TNM stage (I, II) had better overall and disease-free survival, which indicated that early diagnosis and early treatment are crucial in improving the prognosis of lung cancer. However, there are currently no specific clinical manifestations and diagnostic biomarkers to find the part with a poor prognosis for further adjuvant therapy, or the good part to avoid unnecessary overtreatment in lung cancer detected early.

GalNAc-T3 is one of the GalNAc-transferases families, which catalyzes the O-glycosylation of mucin-type on the cell surface [11]. On the other hand, the sugar chain of MUC1 is connected with the polypeptide skeleton by the O-glycoside bond mostly [8]. From this, we speculate whether there is a correlation between these two molecules in solitary pulmonary nodules. GalNAc-T3 and MUC1 are correlated to the occurrence and development of various human tumors, but there is no research on their expression and prognosis in solitary pulmonary nodules. We evaluated the expression of GalNAc-T3 and MUC1 in SPA (≤ 3 cm) by immunohistochemistry and analyzed

Table 4 Univariate analysis of five-year overall survival and five-year disease-free survival on different clinicopathological factors by Kaplan–Meier method

Variable	5-OS (%)	Log-rank test (*P* value)	5-DFS (%)	Log-rank test (*P* value)
Sex		0.824		0.717
Male	69.9		83.5	
Female	73.2		73.5	
Age (years)		0.691		0.907
≤66	66.6		76.9	
>66	76.3		79.6	
Differentiation		**0.014**		0.210
Poor	50.0		66.7	
Moderate	62.6		75.6	
Well	82.5		82.5	
Pathological risk group		**0.030**		0.752
Low risk grade (AAH + AIS + MIA + Lep)	87.5		80.0	
Moderate risk grade (Pap + Aci)	73.2		79.6	
High risk grade (Mp + Sol + Mc)	46.9		66.7	
N states		**0.002**		**0.040**
N0	76.0		80.3	
N1	0.0		0.0	
N2	68.6		83.3	
N3	50.0		0.0	
TNM stage		0.153		**0.049**
I	76.0		80.3	
II	0.0		0.0	
III	51.9		68.6	
GalNAc-T3 expression		**0.035**		**0.004**
Low	53.4		62.4	
High	82.3		87.2	
MUC1 expression		0.084		0.271
Low	78.7		80.5	
High	59.7		73.2	
GalNAc-T3/MUC1 expression		**0.005**		**0.004**
GalNAc-T3^{Hig}/MUC1^{Low}	90.0		91.6	
Other	57.4		66.2	

Statistical significance was evaluated using the log-rank test. Differences were considered to be statistically significant for *P* values of < 0.05 which are shown in bold

5-OS five-year overall survival, *5-DFS* five-year disease-free survival, *GalNAc-T3^{Hig}/MUC1^{Low}* high GalNAc-T3 and low MUC1 expression

their relationship with clinicopathological factors and survival. It was found that the expression of GalNAc-T3 and MUC1 was correlated with pathological differentiation, N stage, and TNM stage. Besides, compared with MUC1, GalNAc-T3 has a more significant impact on prognosis and recurrence. Still, after combining high expression of GalNAc-T3 with low expression of MUC1, the ability to predict prognosis and recurrence was more potent than that of single GalNAc-T3.

The O-glycosylation catalyzed by the GalNAc-transferases family is a key in tumor invasion and metastasis. The research of GalNAc-T3 in lung cancer is not many for the time being, by combining the existing research and our current results, we believed that GalNAc-T3 have a vital function in the occurrence and development of lung cancer. In our previous study, the expression of GalNAc-T3 in patients with small (< 2 cm) peripheral lung adenocarcinoma was related to the classification of International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS), and the low expression of GalNAc-T3 also means worse prognosis in patients with non-small cell lung cancer and small peripheral lung adenocarcinoma, which is consistent with our current results [22,23]. We not only found that

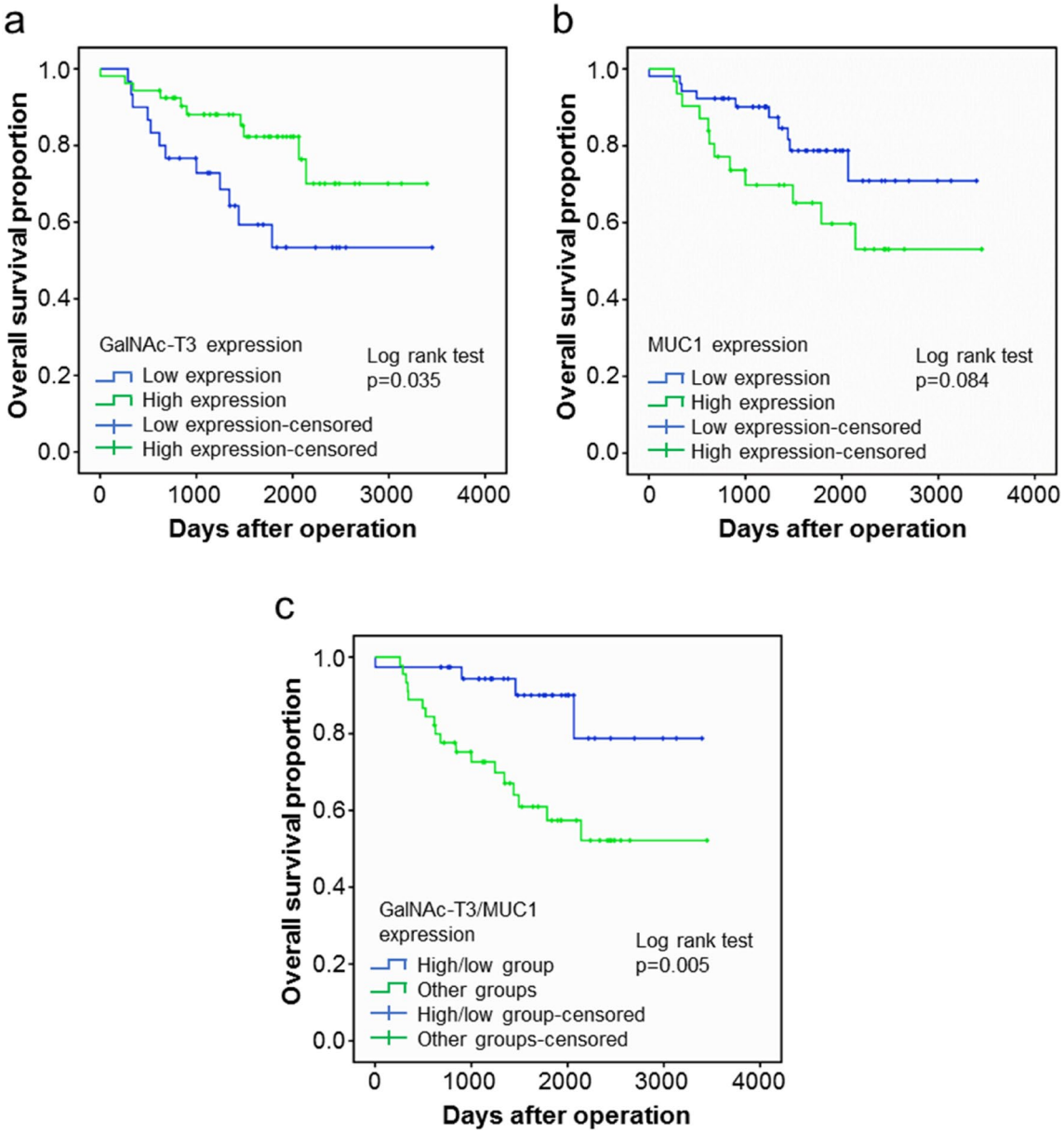

Fig. 3 Kaplan–Meier survival curves of overall survival in 83 patients with solitary pulmonary adenocarcinoma (≤3 cm). **a**, **b** GalNAc-T3 expression level has an effect on the overall survival of patients with SPA (≤3 cm), but MUC1 has no effect on overall survival. **c** The group with high GalNAc-T3 and low MUC1expression in SPA (≤3 cm) was associated with better overall survival ($P=0.005$) compared with other groups

GalNAc-T3 expression was closely correlated to differentiation, pathological risk group, N stage, TNM stage, and prognosis in patients with SPA (≤3 cm) but further proved that patients with low expression of GalNAc-T3 had a lower recurrence. But here's the difference, in early oral squamous cell carcinoma, high GalNAc-T3 expression meant lower DFS, which may be due to organ specificity [13]. Therefore, the effect of GalNAc-T3 on cancer development and progression depends on tissue specificity, possibly. Later, it turned out that the expression of GalNAc-T3 was different even in the same tissue. In earlier studies, the GalNAc-T3 expression in lung adenocarcinoma and matched adjacent tissues was explored [23]. The expression of GalNAc-T3 was low in poorly differentiated lung adenocarcinoma, high in high differentiation, and similar phenomena were found in pancreatic cancer [10]. This is also consistent with the information contained in our study. Of the 83 patients with high (48.2%) and moderate differentiation (44.6%), 63.9% of cases were highly expressed GalNAc-T3. Chances are, the dominant enzymes of the GalNAc-transferases family are

Table 5 Multivariate analysis of overall survival by Cox regression

Variable	Multivariate analysis		*P* value
	HR	95% CI	
Differentiation			
Poor	4.545	0.996–20.746	0.051
Moderate	3.818	1.249–11.671	**0.019**
Well	1		
N states			
N3	11.666	2.035–61.842	**0.006**
N2	0.581	0.127–2.667	0.485
N1	1.883	0.236–15.041	0.551
N0	1		
GalNAc-T3/MUC1 expression			
GalNAc-T3^{Hig}/MUC1^{Low}	0.310	0.100–0.963	**0.043**
Others	1		

Statistical significance was evaluated using the Cox regression test. Differences were considered to be statistically significant for *P* values of <0.05 which are shown in bold

GalNAc-T3^{Hig}/MUC1^{Low} high GalNAc-T3 and low MUC1 expression

different in different differentiation, and the high expression of dominant enzymes inhibited other glycosyltransferases in the family simultaneously [11,27,28]. The mechanism is not precise. This is why we insisted on exploring a more accurate combined predictor basis on the single GalNAc-T3, which can detect the prognosis and recurrence of SPA (≤ 3 cm) already.

As a classic tumor marker, MUC1 has a particular predictive effect on the prognosis and survival of various cancers [17,21]. In lung cancer, MUC1 is not only a marker of prognosis and recurrence, but also closely related to tumor histological subtypes, differentiation, smoking status, and growth pattern [19,20]. Moreover, the glycosylation of MUC1 is catalyzed by the GalNAc-Transfers family, so we chose MUC1 and GalNAc-T3 as a combined predictor. In our study, the relationship between MUC1 and clinicopathological factors was not visible. Perhaps it's because all patients were relatively early TNM stage (I, II: 89.2%), and the well and moderate differentiation reached 93.8%. MUC1 also showed a trend affecting OS, although no valuable *P* value was obtained in the survival analysis. But as shown in the Kaplan–Meier survival curve (Figs. 3b, 4b), the two curves were almost wholly separated in OS and DFS analysis. We cannot directly conclude that MUC1 is unrelated to prognosis and recurrence in SPA (≤ 3 cm), which will lose a lot of valuable information. A retrospective cohort study with more cases is underway, and we will analyze the significance of MUC1 in SPA (≤ 3 cm) in detail.

After combining the GalNAc-T3 high expression and the MUC1 low expression group, GalNAc-T3^{Hig}/MUC1^{Low} group was correlated to pathological differentiation closely and had a related trend with TNM stage. Although the relationship between the combined group and various clinicopathological factors has weakened, in actual clinical work, we pay more attention to the impact on the overall and disease-free survival of patients. In the survival analysis, the 5-year OS rate of the GalNAc-T3^{Hig}/MUC1^{Low} group was as high as 90.0%, and the 5-year DFS rate was 91.6%, which was better than the single marker. In the DFS univariate analysis, GalNAc-T3 expression and the combined group appeared to have the same predictive effect. Still, we found evidence that the combined group was superior in further multivariate analysis. When we established and tested the multivariate Cox stepwise regression model, we found that GalNAc-T3, MUC1, and IASLC/ATS/ERS classification risk group were eliminated in the stepwise regression. Only the GalNAc-T3^{Hig}/MUC1^{Low} group was one of the covariates of the optimal model compared with the single GalNAc-T3 or MUC1, especially in the DFS multivariate analysis. Therefore, we found that GalNAc-T3^{Hig}/MUC1^{Low} group was an independent predictor of OS and DFS, which can better predict the prognosis and recurrence of SPA (≤ 3 cm) patients. These results showed that the combination of GalNAc-T3 and MUC1 plays an essential role in the development of SPA (≤ 3 cm). It is hoped that our work can provide inspiration for actual clinical practice.

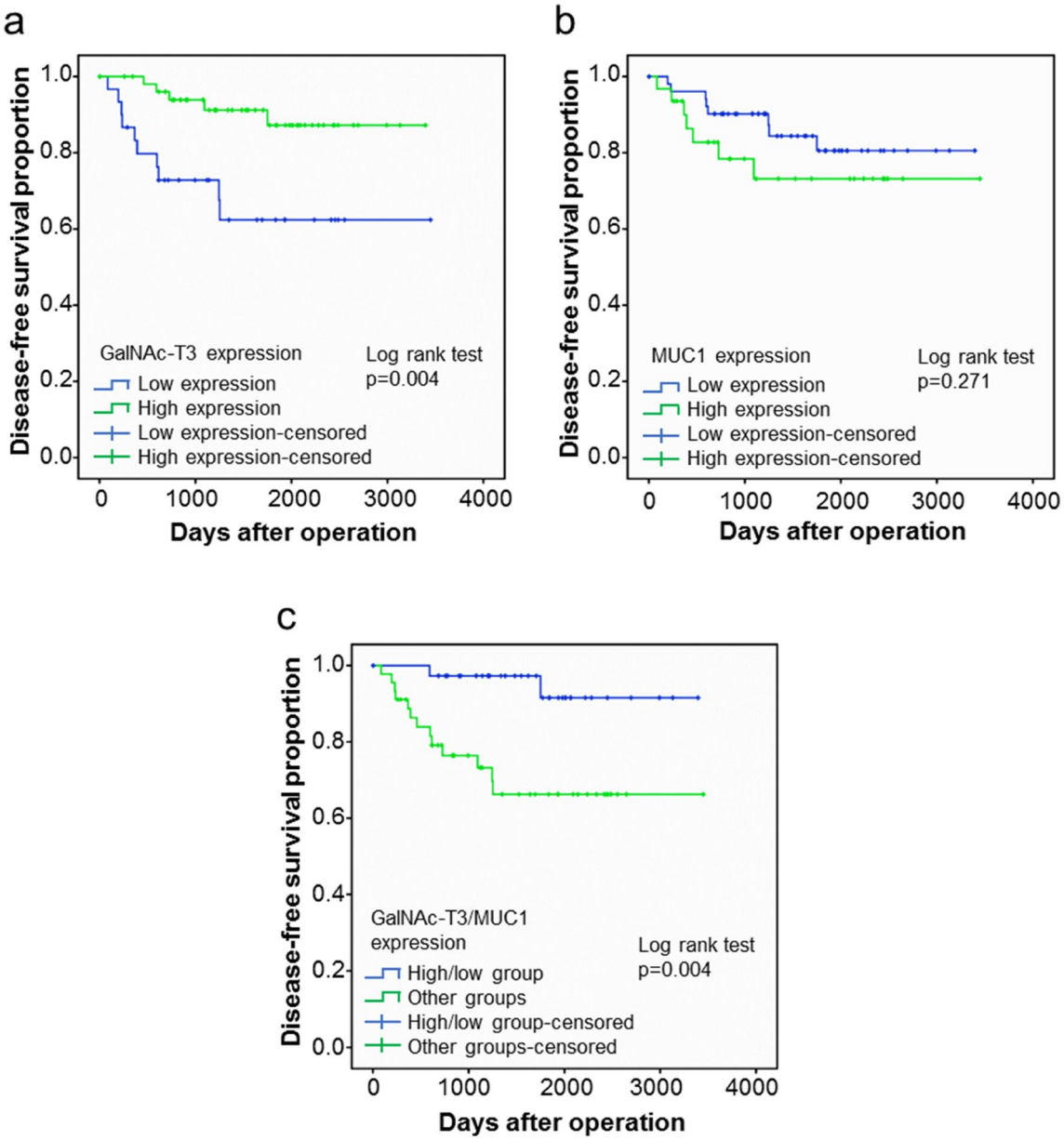

Fig. 4 Kaplan–Meier curves of disease-free survival in 83 patients with solitary pulmonary adenocarcinoma (≤3 cm). **a**, **b** GalNAc-T3 expression level has an effect on the disease-free survival of patients with SPA (≤3 cm), but MUC1 has no effect on disease-free survival. **c** The group with high GalNAc-T3 and low MUC1 expression in SPA (≤3 cm) was associated with better disease-free survival ($P=0.004$) compared with other groups

Table 6 Multivariate analysis of disease-free survival by Cox regression

Variable	Multivariate analysis		*P* value
	HR	95% CI	
N states			
N3	2.549	0.321–20.239	0.376
N2	0.513	0.066–4.004	0.524
N1	4.752	0.586–38.512	0.144
N0	1		
GalNAc-T3/MUC1 expression			
GalNAc-T3^{Hig}/MUC1^{Low}	0.158	0.035–0.719	**0.017**
Others	1		

Statistical significance was evaluated using the Cox regression test. Differences were considered to be statistically significant for *P* values of <0.05 which are shown in bold

GalNAc-T3^{Hig}/MUC1^{Low} high GalNAc-T3 and low MUC1 expression

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The study was approved by the Medical Ethical Committee of the First Affiliated Hospital of Dalian Medical University. No animal experiments were involved in this study. Works submitted for publication have no impact on public health or general welfare.

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