

The clinical significance of transforming acidic coiled-coil protein 3 expression in non-small cell lung cancer

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Received August 3, 2015; Accepted September 23, 2015

DOI: 10.3892/or.2015.4373

Abstract. The relationship between TACC3, a member of the transforming acidic coiled-coil proteins (TACCs) family, and lung carcinoma remains unclear. The present study was designed to explore the prognostic and clinical significance of TACC3 in non-small cell lung cancer (NSCLC). An immunohistochemistry (IHC) assay was performed to analyze the expression of TACC3 in 195 lung cancer cases. The mRNA and protein levels of TACC3 were examined by quantitative reverse transcription-PCR or western blotting. The correlation between TACC3 expression and clinicopathological factors was analyzed by χ^2 analysis and Fisher's exact test. Kaplan-Meier analysis and the Cox proportional hazards model were used to examine the correlation of prognostic outcomes with TACC3. The results showed that the levels of TACC3 mRNA and total protein were higher in lung cancer lesions than paired non-cancerous tissues. IHC analysis revealed that TACC3 was highly expressed in 94 (48.2%) cases. The expression of TACC3 was strongly correlated with smoking status, histological classification, differentiation, cytokeratin 19 fragment levels, T stage and the clinical stage of NSCLC patients. Univariate and multivariate analyses demonstrated that TACC3 is a useful biomarker for NSCLC prognosis. The low TACC3 expression group exhibited better progression-free survival (PFS) among patients who received anti-microtubule chemotherapy. In conclusion, the results showed that a high level of TACC3 expression was correlated with advanced clinicopathological classifications, poor overall survival (OS) and poor recurrence-free survival (RFS) in NSCLC patients.

Our findings indicate that TACC3 is a potential prognostic marker and therapeutic target for NSCLC.

Introduction

Lung cancer is the most common cause of cancer mortality globally and the second most common type of cancer (1). The high mortality associated with lung cancer is primarily attributable to late diagnosis and metastasis at the time of diagnosis. Identifying effective tumor biomarkers of this malignancy in the early stages and preventing metastasis may therefore be the best options for improving the generally dismal survival rate of lung cancer patients.

Structural and numerical abnormalities of the centrosome are significantly associated with chromosome instability, aneuploidy and tumorigenesis (2,3,4-8). The transforming acidic coiled-coil proteins (TACCs) family is characterized by a highly conserved carboxyl-terminus coiled-coil 'TACC domain', which is important for the interaction with spindle/centrosome dynamics (2,4,9). The TACC family contains TACC1, TACC2 and TACC3 in mammals (2-4,10). Although increasing evidence indicates that abnormalities in human TACCs (TACC1-3) may correlate with the development of some human malignancies, the functions of these three proteins in mammals are largely unknown (11-13).

The human TACC1-3 genes are located on chromosome 8p11, 10q26 and 4p16.3, respectively (2,4,11-17). TACC3 is mainly expressed in the developing embryo and in adult testis, thymus, peripheral blood leukocytes, spleen and intestinal tissues (4,17). Several studies had shown that TACC3 may be associated with some human tumor, but the expression status of TACC3 in cancer remains controversial and unclear. For instance, a microarray analysis suggested that TACC3 is upregulated during the transition of ductal carcinoma *in situ* to invasive ductal carcinoma, while an immunohistochemical analysis demonstrated that TACC3 is downregulated in resected breast tumors (18,19). A similar discrepancy was found in the expression of TACC3 in ovarian tumor and thyroid cancer (20-22). In addition, TACC3 has been identified as a potential glioblastoma multiforme oncogene and may be overexpressed in multiple myeloma cases and lung cancer (23-25). Therefore, it is necessary to explore the expression status of TACC3 in lung cancer and the relationship between TACC3

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Key words: non-small cell lung cancer, immunohistochemistry, TACC3, prognosis, chemotherapy sensitivity

expression and clinicopathological characteristic of lung cancer.

In the present study, we focused on the expression patterns of TACC3 in primary non-small cell lung cancer (NSCLC) lesions and metastatic lymph nodes. Statistical analysis indicated a relationship between high TACC3 expression levels and the clinicopathological features of NSCLC. Multivariate Cox regression analyses suggested that higher TACC3 expression in NSCLC predicts poorer survival independent of other factors.

Materials and methods

Patients and tumor specimen. The present study is reported according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) (26). A total of 207 consecutive cases of primary NSCLC with surgical resection were collected from Sun Yat-sen University Cancer Center (SYSUCC; Guangzhou, China) between January 2003 and June 2004. Fig. 1 shows the inclusion and exclusion criteria for the present study. Ultimately, a total of 195 patients were enrolled.

We evaluated conventional clinical characteristics as shown in Table I. All 195 cases were reevaluated with respect to histological subtype, differentiation and tumor stage. The follow-up interval ranged from 0.9 to 139.4 months (median 27.3 months, mean 49.5 months). The 5-year cumulative survival rate for all patients was 30%.

A total of 195 paraffin-embedded NSCLC tissue samples and 11 matched adjacent paraffin-embedded non-cancerous human lung tissues were obtained from the archives of the Department of Sample Resources, SYSUCC. These samples had been histologically and clinically diagnosed. Fifty metastatic lymph nodes were also obtained from the above-mentioned patients. Eleven paired freshly frozen lung carcinoma and non-cancerous tissues adjacent to cancer lesions were obtained from the same patients. The present study was conducted with the consent of the patients and the approval of the Institute Research Ethics Committee of SYSUCC.

Immunohistochemistry. Immunohistochemistry was performed as previously described (27). The relevant sections were incubated with an anti-TACC3 monoclonal antibody (1:800; Abcam, Cambridge, UK) at 4°C overnight. Immunohistochemical kit (SP-9001 rabbit SP kit, lot: 50581654) was obtained from Zhongshan Golden Bridge Biotechnology Co. Ltd. (Beijing, China). As a negative control, the primary antibody was replaced by IgG.

The degree of immunostaining was examined and evaluated independently by two pathologists without knowledge of the clinical characteristics of the cases. The proportion of positively stained tumor cells varied from 0 to 100%, and the intensity of staining varied from weak to strong. The proportion of stained tumor cells was graded according to the following criteria: 0 (no positive tumor cells), 1 ($\leq 10\%$ positive tumor cells), 2 (11–25% positive tumor cells), 3 (26–40% positive tumor cells) and 4 ($\geq 41\%$ positive tumor cells). The intensity of staining was quantified using the following score system: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown) and 3 (intense staining, brown). The TACC3

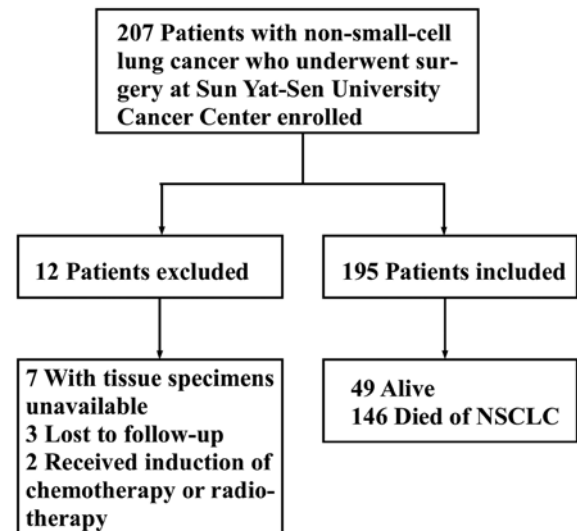


Figure 1. Inclusion and exclusion criteria for the present study.

expression index was calculated by multiplying the two scores to obtain a final score of 0, 1, 2, 3, 4, 6, 9 or 12. The cut-off value for high and low levels of expression was based on a measurement of heterogeneity using a log-rank test statistical analysis with respect to the overall survival (OS) rate. The carcinomas were finally classified as low expression (score <5) or high expression (score ≥ 6).

Western blotting. Western blot analysis was performed as previously described (27). The membrane was incubated with primary antibodies (1:1,000) at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG secondary antibody (1:3,000) (both from Abcam) at room temperature for 1 h. A mouse β -actin monoclonal antibody (1:4,000; Cell Signaling Technology, Danvers, MA, USA) was used as an internal loading control. The membrane was detected using an enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, UK) detection system (Amersham Biosciences Europe, Freiburg, Germany) according to the manufacturer's instructions.

RNA extraction and quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from 11 paired freshly frozen lung cancer and non-cancerous tissues using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was treated with RNA-free DNase, and 2.0 μ g of total RNA was used to generate cDNA using the RETROscript kit (Promega, Madison, WI, USA). The full-length open reading frame of TACC3 was PCR amplified from cDNA samples. RT-PCR was performed using iQ SYBR-Green Supermix on a MYiQ qPCR machine (both from Bio-Rad, Hercules, CA, USA). The following primers were used to amplify TACC3: forward, 5'-CCTCTTCAAGCGTTTGTAGAAAC-3' and reverse, 5'-GCCCTCCTGGGTGATCCTT-3'. The following primers were used to amplify β -actin: forward, 5'-GACTCATGACCACAGTCCATGC-3' and reverse, 5'-AGAGGCAGGGATGATGTTCTG-3'. The

Table I. Correlation between TACC3 expression and clinicopathological characteristics.

Characteristics	TACC3 expression: no. of patients (%)		P-value ^b
	Low ^a (101)	High ^a (94)	
Gender			
Male	76 (49.7)	77 (50.3)	0.258
Female	25 (59.5)	17 (40.5)	
Age (years)			
≤60	54 (50.5)	53 (49.5)	0.682
>60	47 (53.4)	41 (46.6)	
Smoking status ^c			
No	40 (63.5)	23 (36.5)	0.027
Yes	61 (46.6)	70 (53.4)	
Differentiation			
Well-moderate	53 (63.9)	30 (36.1)	0.004
Poor	48 (42.9)	64 (57.1)	
T stage ^d			
T1	23 (69.7)	10 (30.3)	0.024
T2-4	78 (48.1)	84 (51.9)	
N stage ^d			
N0	54 (56.8)	41 (43.2)	0.168
N1-3	45 (46.9)	51 (53.1)	
M stage ^d			
M0	82 (49.7)	83 (50.3)	0.169
M1	19 (63.3)	11 (36.7)	
Clinical stage ^d			
I	36 (66.7)	18 (33.3)	0.001
II	18 (32.1)	38 (67.9)	
III	28 (50.0)	28 (50.0)	
IV	19 (65.5)	10 (34.5)	
Histological type			
Adenocarcinoma	67 (59.3)	46 (40.7)	0.004
SCC	34 (41.5)	48 (58.5)	
SCCA ^e			
(-)	49 (53.8)	42 (46.2)	0.320
(+)	6 (40.0)	9 (60.0)	
CEA ^f			
(-)	40 (55.6)	32 (44.4)	0.741
(+)	30 (52.6)	27 (47.4)	
CYFRA 21-1 ^g			
(-)	11 (73.3)	4 (26.7)	0.025
(+)	7 (35.0)	13 (65.0)	

TACC3, transforming acidic coiled-coil protein 3; NSCLC, non-small-cell lung cancer; SCCA, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment; ^aTACC3 low expression, score <5; high expression, score ≥6; ^btwo-sided P-values were calculated using Pearson's χ^2 test or continuity correction to evaluate the significance of the correlations. Bold print indicates statistical significance. ^cSmoking status is unknown for 1 patient. ^dTumor size, lymph node involvement, distant metastasis and clinical stage were classified or reclassified according to the seventh edition of the International Union Against Cancer (UICC) staging system for Lung Cancer. N stage is unknown for 4 patients. ^eSCCA: -, normal (0-1.50 ng/ml); +, elevated (>1.50 ng/ml); SCCA unknown for 89 patients. ^fCEA: -, normal (0-5 ng/ml); +, elevated (>5 ng/ml); CEA unknown for 66 patients. ^gCYFRA 21-1: -, normal (0.1-3.30 ng/ml); +, elevated (>3.30 ng/ml); CYFRA 21-1 unknown for 160 patients.

primer sets were designed and produced by PrimerDesign Ltd. (Southampton, UK). Cycles were as follows: 95°C for 3 min

(1 cycle), 94°C for 30 sec followed by 60°C for 10 sec and 72°C for 15 sec (40 cycles), and 95°C for 30 sec (1 cycle). Melt curve

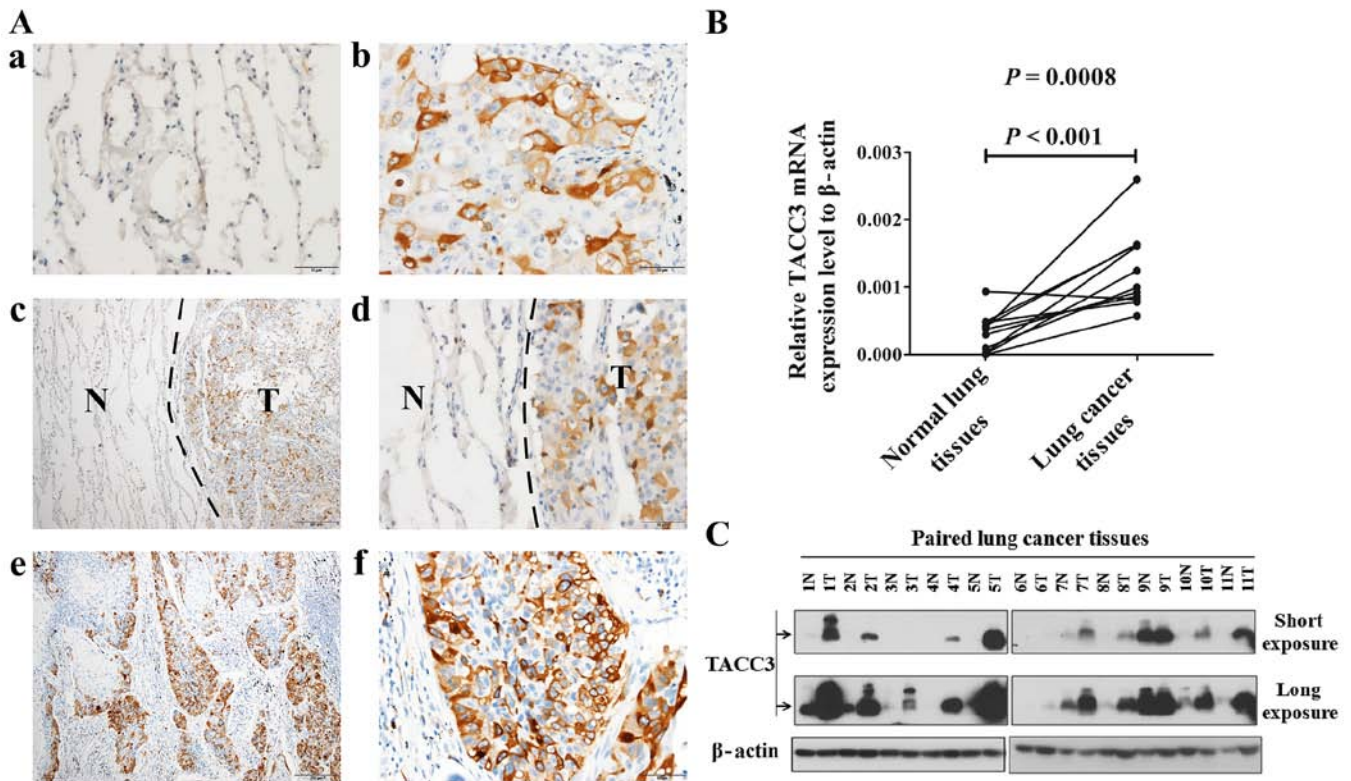


Figure 2. The expression of TACC3 is elevated in fresh frozen primary NSCLC tissues compared with non-cancerous tissues adjacent to the cancer lesions. (A) Expression of TACC3 in different tissues as determined by immunohistochemical staining. a, Lack of TACC3 staining in the alveolar epithelium of a normal lung (magnification, x400). b, Cytoplasmic TACC3 staining in primary lung cancer lesions (magnification, x400). c and d, Higher expression of the TACC3 protein in primary lung cancer lesions compared with adjacent non-cancerous tissues (c, magnification, x100; d, magnification, x400). e and f, Strong cytoplasmic TACC3 staining in the matched metastatic lymph nodes of adenocarcinomas (e, magnification, x100; f, magnification, x400). (B) Expression analysis of TACC3 mRNA in paired and fresh frozen primary NSCLC (T) and non-cancerous tissues (N) from the same patient by real-time RT-PCR. β -actin was used as an internal control. The reactions were performed in triplicate in three independent experiments. (C) Expression analysis of TACC3 protein in paired and fresh frozen primary NSCLC tissues (T) and non-cancerous tissues (N) from the same patient by western blotting. β -actin was used as an internal reference.

analysis was performed for each reaction to ensure amplification specificity.

Statistical analysis. All statistical analyses were performed using the SPSS version 16.0 statistical software package (SPSS, Inc., Chicago, IL, USA).

The data are presented as the mean \pm SEM unless otherwise indicated. Differences among variables were identified by χ^2 analysis or two-tailed Student's t-tests. Correlations between TACC3 expression and clinicopathological factors were assessed by χ^2 analysis and Fisher's exact test. Survival curves were plotted using Kaplan-Meier survival analysis and compared using a log-rank test. Further analyses of survival curves were calculated based on stratifying age, TNM classifications, histological type and clinical stages. Univariate and multivariate Cox proportional hazards models were used to evaluate the relative risks (RRs) of death associated with TACC3 expression and other variables. In our analyses, we defined the RR of 1.000 as baseline for factors including gender (male), age (≤ 60 years), smoking status (non-smoker), differentiation (well-moderate), SCC (0-15.0 ng/ml), T1, N0, M0, clinical stage (I-II), and low level of TACC3 expression. In all cases, $P < 0.05$ was considered to indicate a statistically significant result.

Results

Immunohistochemistry reveals high TACC3 expression in NSCLC tissue samples. As shown in Fig. 2A, normal lung alveolar epithelium did not express TACC3 protein (Fig. 2A-a, -c and -d). TACC3 immunoreactivity was generally confined to the cytoplasm of tumor cells in NSCLC lesions and metastatic lymph nodes (Fig. 2A-b, -c, -d, -e and -f). In all NSCLC tissue samples, TACC3 protein was observed in 180 of 195 (92.3%) human NSCLC samples, and strong cytoplasmic staining of TACC3 protein was detected in 64 (35.5%) tumors. Among the corresponding metastatic lymph nodes, 47 of 50 (94%) cases displayed positive staining (Fig. 2A-e and -f), and 48.9% (23 of 47) of cases exhibited strong staining.

TACC3 expression is upregulated in NSCLC tissue samples as determined by real-time RT-PCR and western blotting. To investigate the mRNA and protein expression levels of TACC3 in NSCLC lesions, real-time RT-PCR and western blot analyses were performed in paired freshly frozen NSCLC tissues and adjacent non-cancerous tissues from the same patients. As determined by real-time RT-PCR analysis, all 11 human NSCLC samples exhibited higher TACC3 mRNA expression compared to paired non-cancerous tissues (Fig. 2B). Western

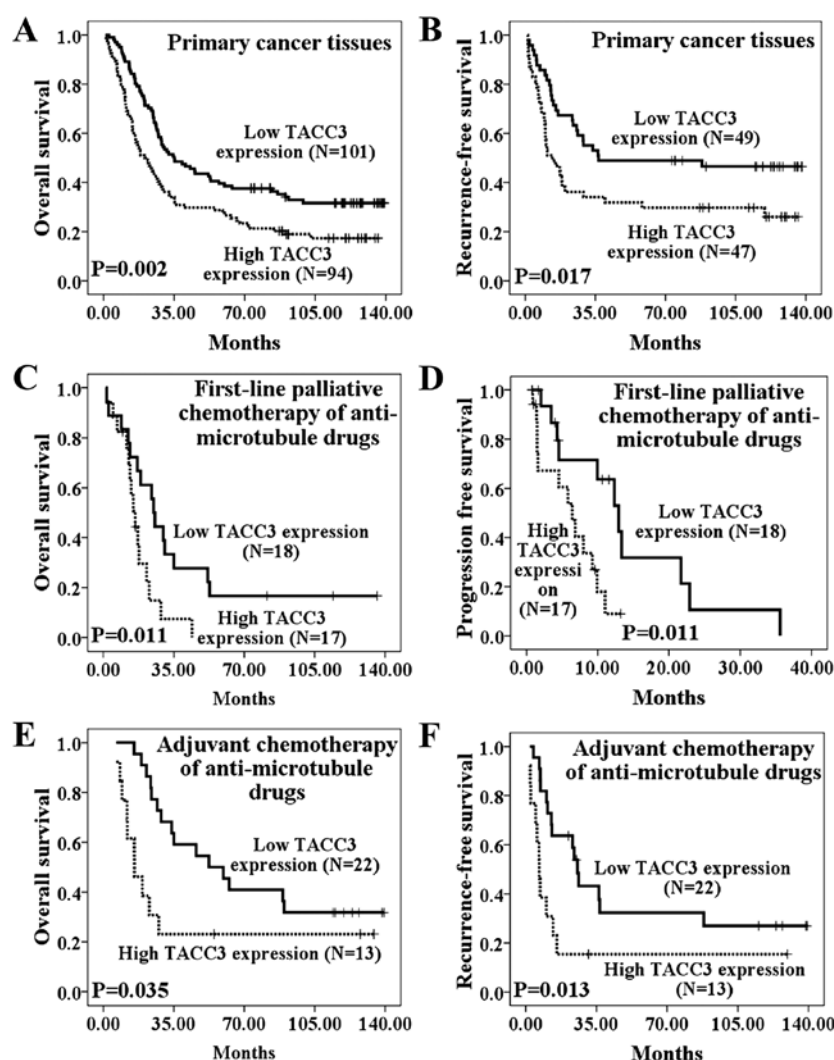


Figure 3. Influence of TACC3 expression on NSCLC patient survival and TACC3 expression levels are correlated with sensitivity to anti-microtubule drugs. (A) OS curves and analysis of 195 primary NSCLC tissues demonstrated that patients with high TACC3 expression have short survival times (P=0.002). (B) The duration of RFS differed significantly between patients with high and low TACC3 expression (P=0.017), with better survival in the low TACC3 expression group. TACC3 low expression, score <5; high expression, score ≥ 6 . (C and D) The low TACC3 expression group exhibited better overall survival (P=0.011) and progression-free survival (P=0.011) among patients who received anti-microtubule chemotherapy as a first-line palliative therapy. (E and F) Among patients who received anti-microtubule chemotherapies as adjuvant chemotherapy, low TACC3 expression was also correlated with longer overall survival time (P=0.035) and longer recurrence-free survival time (P=0.013) compared with patients expressing high TACC3 levels.

blot analysis demonstrated that TACC3 protein was markedly overexpressed in the NSCLC lesions but weakly detected in adjacent paired non-cancerous tissues (Fig. 2C).

Correlation between the clinicopathological features and TACC3 expression levels. The immunoreactivity results of TACC3 expression levels were statistically analyzed to determine their relationships with the clinical characteristics of the NSCLC patients. As presented in Table I, the expression of TACC3 was strongly correlated with smoking status, histological classification, differentiation, CYFRA21-1 levels, T stage and the clinical stage of NSCLC patients. However, there was no significant correlation between the expression level of TACC3 protein and gender, age, squamous cell carcinoma antigen (SCCA), carcinoembryonic antigen (CEA), tumor suppressor group of factors (TSGF), N classification or distant metastasis of NSCLC patients.

TACC3 expression is correlated with poor overall and recurrence-free survival. After demonstrating the correlation of TACC3 expression with clinicopathological characteristics, we proceeded to examine the relationship between TACC3 expression level and the patients survival. The patients OS time was defined as the time from the day of surgery to (i) the point of death due to recurrence or metastasis or (ii) to the end point of the most recent follow-up if the patient was alive. Recurrence-free survival (RFS) was calculated from the date of curative surgery to the day of disease recurrence, disease progression, death or the most recent follow-up. Patients with metastases were excluded from the RFS analysis.

The relationship between TACC3 expression levels and OS time (P=0.002) is presented in Fig. 3A. In primary NSCLC lesions, the low TACC3 expression group had better OS (median, 35.10 months), whereas the high TACC3 expression group had poor OS (median, 20.33 months).

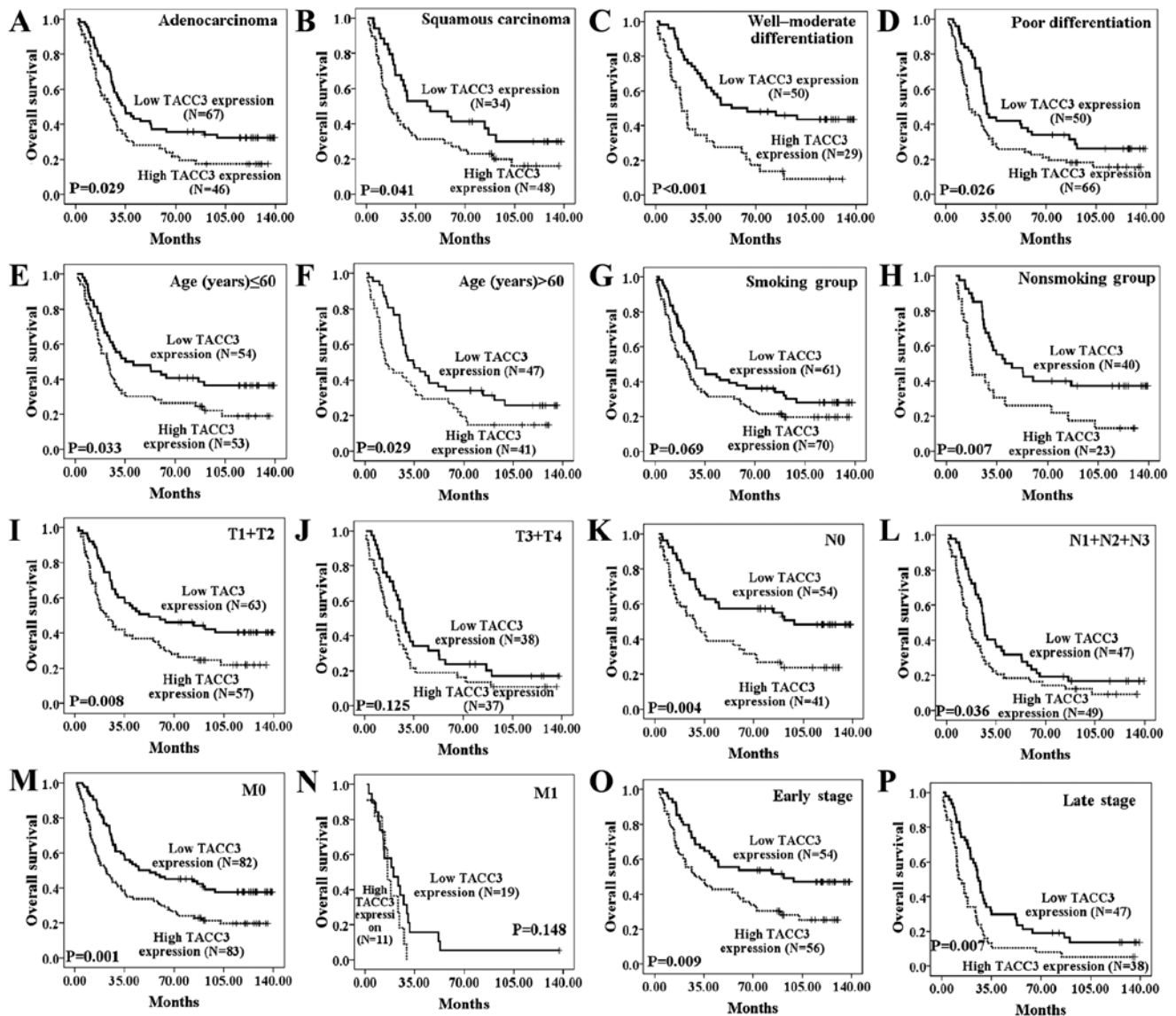


Figure 4. The influence of TACC3 expression on overall survival of NSCLC patients stratified by clinicopathological characteristics. Kaplan-Meier curves were stratified by TACC3 expression level according to histological type, tumor differentiation, age, smoking status, as well as T, N, M and clinical stage (A-P). Patients with high TACC3 expression had poor OS regardless of histological type (A and B), tumor differentiation (C and D), age (E and F), N stage (K and L) and clinical stage (O and P). Patients with high TACC3 expression have poorer OS in the non-smoking group (H), early T stage (T1+T2) (K), and M0 stage (M) subgroups. TACC3 low expression, score <5; high expression, score ≥ 6 . Smoking status is unknown for 1 patient. N stage is unknown for 4 patients.

The cumulative 5-year survival rate was 38.6% in the low TACC3 expression group and 25.5% in the high TACC3 expression group. Furthermore, patients with a low TACC3 expression had better OS regardless of the patients age, histological type, tumor differentiation, N stage and clinical stage. Moreover, the OS showed a statistically significant difference between the high and low TACC3 expression groups in the non-smoking group, early T stage (T1+T2) and M0 stage subgroups (Fig. 4A-P).

As shown in Fig. 3B, RFS also differed significantly between patients with high and low TACC3 expression levels ($P=0.017$). RFS also significantly differed between the high and low TACC3 expression groups in the younger age group (≤ 60 years), among cases with well-moderated differentiation, and in the early T stage (T1+T2), N0 and M0 stages, and early clinical stage (I-II) subgroups (Fig. 5A-O).

Univariate and multivariate analyses indicate an independent role of TACC3 in the prognosis of NSCLC. As shown in Tables II and III, univariate Cox regression analyses revealed that a higher level of TACC3 was associated with a significantly increased risk of cancer-related death ($P=0.002$, $RR=1.663$; $P=0.019$, $RR=1.849$) in NSCLC patients. The RRs indicated that the level of SCCA, differentiation and clinical staging were also effective predictors. Multivariate Cox regression analyses suggested that higher TACC3 expression in NSCLC predicted worse survival independent of other factors ($P=0.005$, $RR=1.999$; $P=0.022$, $RR=1.825$). As expected, clinical staging was also recognized as independent prognostic factors.

Taken together, the univariate and multivariate analyses suggested that TACC3 may be a useful biomarker for the prognosis of NSCLC patients.

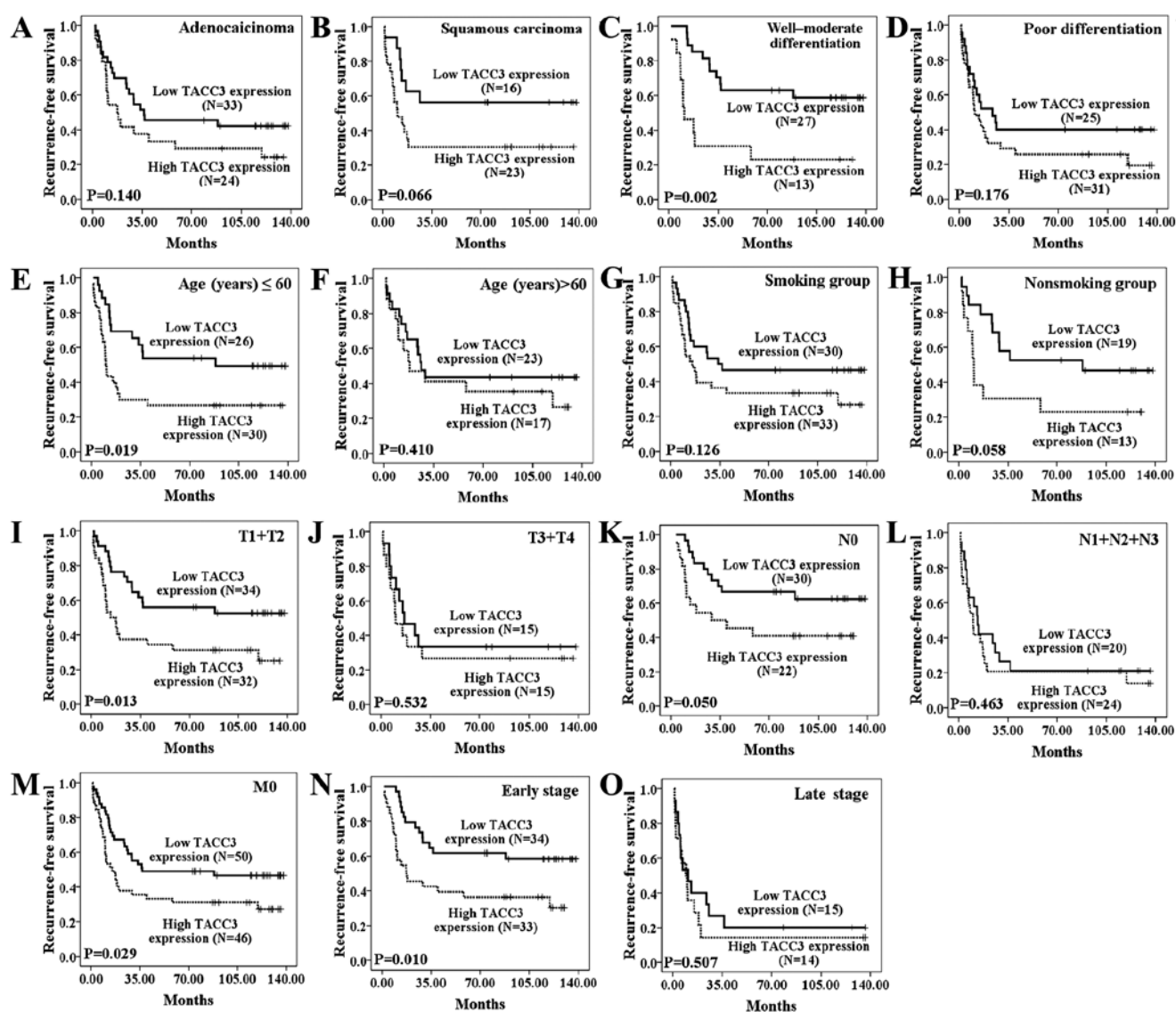


Figure 5. The influence of TACC3 expression on recurrence-free survival of NSCLC patients stratified by clinicopathological characteristics. (A and B) The duration of RFS did not differ significantly between the two groups among the histological type subgroups ($P=0.140$ and $P=0.066$). In addition, the duration of RFS differed significantly between the high and low TACC3 expression groups among the well-moderate differentiation (C), younger age (≤ 60 years) (E), early T stage (T1+T2) (I), N0 stage (K), M0 stage (M), and early clinical stage (I+II) (N) subgroups. The duration of RFS did not differ significantly between the high and low TACC3 expression groups among the poor differentiation (D), older age (>60 years) (F), smoking (G) or non-smoking groups (H), or the late T stage (T3+T4) (J), advanced N stage (N1+N2+N3) (L) and late clinical stage (III+IV) (O) subgroups. TACC3 low expression, score <5 ; high expression, score ≥ 6 .

TACC3 expression levels are correlated with sensitivity to anti-microtubule drugs. As shown in Fig. 6, 35 patients received adjuvant chemotherapy with platinum drugs (cisplatin or carboplatin) and anti-microtubule drugs, including paclitaxel (16 patients), docetaxel (7 patients) and vinorelbine (12 patients). Among the patients who progressed after adjuvant chemotherapy and those who received palliative therapy, 35 received first-line palliative therapy with platinum and anti-microtubule drugs, including paclitaxel (20 patients), docetaxel (6 patients) and vinorelbine (9 patients).

OS of patients who received chemotherapy was defined as the time from the day of the first chemotherapy treatment to (i) the point of death due to recurrence or metastasis or (ii) to the end point of the most recent follow-up if the patient was alive. Progression-free survival (PFS) of patients who received

palliative chemotherapy was defined as the interval between the first chemotherapy treatment and disease progression or last contact. RFS of patients who received adjuvant chemotherapy was calculated from the date of the first chemotherapy treatment to the day of disease recurrence, disease progression, death or the most recent follow-up.

To explore whether expression of TACC3 was correlated with sensitivity to anti-microtubule drugs, we used Kaplan-Meier analysis and log-rank tests to examine the relationship between the TACC3 expression level and survival in patients who received anti-microtubule chemotherapy. As shown in Fig. 3C and D, the low TACC3 expression group exhibited better OS ($P=0.011$) and better PFS ($P=0.011$) among patients who received anti-microtubule chemotherapy as a first-line palliative therapy. Among patients who received anti-micro-

Table II. Univariate and multivariate Cox regression analysis of potential prognostic parameters for NSCLC patients regarding overall survival.

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	P-value	RR (95% CI)	P-value
Gender				
Male	1.000	0.108		
Female	0.716 (0.477-1.077)			
Age, years				
≤60	1.000	0.441		
>60	1.137 (0.820-1.575)			
Smoking status				
No	1.000	0.186		
Yes	1.268 (0.891-1.804)			
Histology				
Adenocarcinoma	1.000	0.475		
Squamous carcinoma	1.132 (0.806-1.589)			
Differentiation				
Well-moderate	1.000	0.077		
Poor	1.376 (0.966-1.961)			
SCCA ^a				
(-)	1.000	0.006	1.000	0.009
(+)	2.336 (1.280-4.264)		2.304 (1.233-4.304)	
CEA ^b				
(-)	1.000	0.082		
(+)	1.433 (0.955-2.150)			
CYFRA 21-1 ^c				
(-)	1.000	0.753		
(+)	1.142 (0.499-2.611)			
Clinical stage ^d				
I-II	1.000	<0.001	1.000	<0.001
III-IV	2.269 (1.629-3.161)		3.255 (1.990-5.323)	
TACC3 expression ^e				
Low	1.000	0.002	1.000	0.005
High	1.663 (1.199-2.306)		1.999 (1.231-3.248)	

TACC3, transforming acidic coiled-coil protein 3; NSCLC, non-small cell lung cancer; SCCA, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment; TSGF, tumor-specific growth factor; CI, confidence interval; ^aSCCA: -, normal (0-1.50 ng/ml); +, elevated (>1.50 ng/ml); SCC unknown for 89 patients. ^bCEA: -, normal (0-5 ng/ml); +, elevated (>5 ng/ml); CEA unknown for 66 patients. ^cCYFRA 21-1: -, normal (0.1-3.30 ng/ml); +, elevated (>3.30 ng/ml); CYFRA 21-1 unknown for 110 patients. ^dTumor staging was classified or reclassified according to the seventh edition of the International Union Against Cancer (UICC) staging system for Lung Cancer. ^eTACC3 low expression: score <5; high expression, score ≥6.

tubule chemotherapies as adjuvant chemotherapy, low TACC3 expression was also correlated with longer OS time (Fig. 3E, P=0.035) and longer RFS time (Fig. 3F, P=0.013) compared with patients expressing high TACC3 levels.

Discussion

Members of the TACC family play important roles in cell growth, differentiation and gene regulation by affecting centrosome/spindle dynamics (10,17,28-29). In addition, the deregulation of human TACCs (TACC1-3) may be involved in

the development and progression of some human malignancies (3,10,13,15,25,30,31).

A previous immunohistochemical analysis of lung cancer showed TACC3 expression is higher in primary lung cancer tissues than in normal lung tissues and high level of TACC3 is associated with a poor prognosis (25). However, that study did not use western blot analysis and real-time RT-PCR to verify the conclusion and did not analysis the expression of TACC3 in metastatic lymph node tissues. In the present study, we performed western blot analysis and real-time RT-PCR as well as immunohistochemical analysis to determine whether

Table III. Univariate and multivariate Cox regression analysis of potential prognostic parameters for NSCLC patients regarding recurrence-free survival.

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	P-value	RR (95% CI)	P-value
Gender				
Male	1.000	0.361		
Female	0.751 (0.406-1.389)			
Age, years				
≤60	1.000	0.802		
>60	0.973 (0.560-1.565)			
Smoking status				
No	1.000	0.849		
Yes	1.054 (0.651-1.807)			
Histology				
Adenocarcinoma	1.000	0.575		
Squamous carcinoma	1.127 (0.669-1.896)			
Differentiation				
Well-moderate	1.000	0.016	1.000	0.297
Poor	1.911 (1.128-3.235)		1.336 (0.760-2.456)	
SCCA ^a				
(-)	1.000	0.122		
(+)	2.136 (0.737-6.270)			
CEA ^b				
(-)	1.000	0.302		
(+)	1.329 (0.743-2.611)			
CYFRA 21-1 ^c				
(-)	1.000	0.706		
(+)	1.709 (0.167-3.336)			
Clinical stage ^d				
I-II	1.000	<0.001	1.000	<0.001
III-IV	2.640 (1.566-4.451)		2.614 (1.560-4.408)	
TACC3 expression ^e				
Low	1.000	0.019	1.000	0.022
High	1.849 (1.107-3.086)		1.825 (1.093-3.047)	

TACC3, transforming acidic coiled-coil protein 3; NSCLC, non-small cell lung cancer; SCCA, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment; TSGF, tumor-specific growth factor; CI, confidence interval; ^aSCCA: -, normal (0-1.50 ng/ml); +, elevated (>1.50 ng/ml); SCC unknown for 89 patients. ^bCEA: -, normal (0-5 ng/ml); +, elevated (>5 ng/ml); CEA unknown for 66 patients. ^cCYFRA 21-1: -, normal (0.1-3.30 ng/ml); +, elevated (>3.30 ng/ml); CYFRA 21-1 unknown for 110 patients. ^dTumor staging was classified or reclassified according to the seventh edition of the International Union Against Cancer (UICC) staging system for Lung Cancer. ^eTACC3 low expression, score <5; high expression, score ≥6.

TACC3 expression is upregulated in primary NSCLC lesions compared with normal lung tissues. The present study indicated that high TACC3 expression in primary NSCLC lesions is correlated with poorer OS (25). We present that high TACC3 expression in primary NSCLC lesions is also associated with poorer RFS, which has not been reported. These data provide strong evidence for a relationship between high TACC3 expression levels and poor prognosis in NSCLC patients and indicate that the TACC3 expression level is an independent prognostic factor for NSCLC patients. In addition,

we compared the expression of TACC3 in metastatic lymph nodes tissues with normal lung tissues and demonstrated that expression of TACC3 is also upregulated in metastatic lymph nodes tissues. These results indicate that alterations in TACC3 protein levels may be involved in both tumor development and progression.

We observed a strong correlation between more advanced clinical staging and higher T classification and higher TACC3 expression. These results may indicate a correlation between TACC3 overexpression and clinical progression in NSCLC.

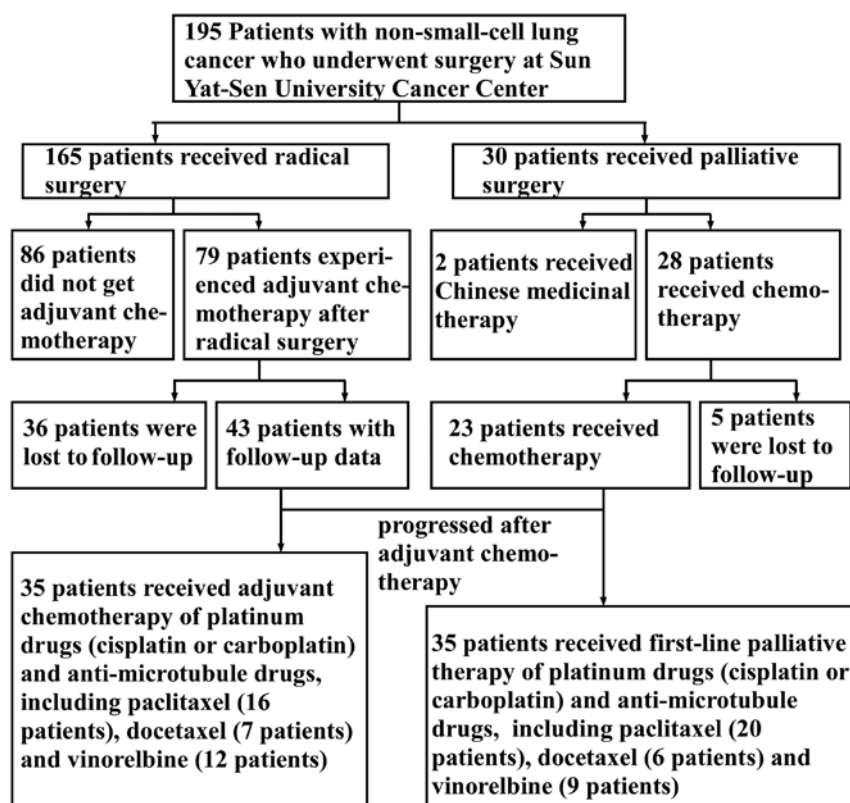


Figure 6. Treatments received by patients enrolled in the present study.

Recent studies in cervical cancer have suggested that TACC3 overexpression promotes epithelial-mesenchymal transition (EMT) by activating the phosphatidylinositol 3-kinase (PI3K)/Akt and extracellular signal-regulated protein kinases (ERKs) signal transduction pathways (32,33). As EMT plays a major role during cancer invasion and metastases, it is worthwhile to further investigate the role of TACC3 in the progression of NSCLC.

TACC3 expression has been observed in various cell lines and tissues, particularly those undergoing rapid growth and differentiation. Sadek *et al* found that TACC3 is upregulated during the early differentiation of many varied cell types (17). Another study showed TACC3 plays an important role in murine embryonic development and may be involved in differentiation (34,35). These observations showed the potential relationship between TACC3 and differentiation stage in normal tissues. However, the correlation between TACC3 and the differentiation status in tumor tissues has not been studied to date. The present study demonstrated that high levels of TACC3 expression were significantly correlated with poor differentiation in lung cancer, which had not been reported. Further experiments are needed to explore the molecular mechanisms of relationship between TACC3 expression and differentiation in tumor tissues.

Furthermore, the present study revealed that patients who smoked, squamous cell carcinoma patients and those with elevated CYFRA21-1 levels tend to have high expression levels of TACC3. More analysis is required to investigate the mechanisms underlying changes in TACC3 expression in NSCLC patients.

The phosphorylation and localization of TACC3 are novel pharmacodynamic indicators of Aurora A activity (36). Yim *et al* identified TACC3 as a possible direct target of anti-microtubule drugs such as paclitaxel in cervical carcinoma cells (37). Subsequent studies have demonstrated that paclitaxel induces strong polyploidy and apoptosis in TACC3-depleted murine fibroblast cells and that the downregulation of TACC3 enhances paclitaxel chemosensitivity in breast carcinoma cells (38,39). However, if the expression of TACC3 in tumor tissue by immunohistochemical analysis correlates with the sensitivity to anti-microtubule drugs has not been reported yet. Our results indicate that low TACC3 expression may enhance chemosensitivity of anti-microtubule drugs in patients of lung cancer. However, larger samples of patients and further experiments *in vitro* are needed to verify the correlation between TACC3 expression status and chemosensitivity to anti-microtubule drugs.

In conclusion, we demonstrated that TACC3 expression is associated with the clinicopathological classification of NSCLC and that TACC3 is an independent prognostic factor for patient survival. In addition, TACC3 overexpression may be associated with chemosensitivity to anti-microtubule drugs. Our findings suggest that the TACC3 expression level may be a potential prognostic factor and a new therapeutic target for NSCLC.

Acknowledgements

The present study was supported by the National Scientific Foundation of China (no. 81372887), the National Basic

Research Program of China (grant no. 2013CB910500), and the Guangdong Science and Technology Project (no. 2013B021800167).

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