Tripartite motif-containing 29 as a novel biomarker in non-small cell lung cancer

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Abstract. Tripartite motif-containing 29 (TRIM29) is a member of the tripartite motif (TRIM) protein family. TRIM29 has been reported to be deregulated in a number of cancer types, suggesting the oncogenic function of TRIM29. However, its clinical significance in non-small cell lung cancer (NSCLC) has not been fully elucidated. In the present study, the TRIM29 expression status was investigated by immunohistochemical analysis in paraffin-embedded specimens obtained from 320 patients with surgically resected NSCLC, treated between 2000 and 2007. High TRIM29 expression was significantly associated with smoking (P=0.012), T stage (P=0.015) and M stage (P=0.003). Furthermore, elevated TRIM29 expression level was correlated with reduced overall (OS) and disease-free survival. In addition, high TRIM29 expression was an independent prognostic factor for OS [P=0.003, hazard ratio (HR)=2.102, 95% confidence interval (CI), 1.069-3.193]. In conclusion, these results suggest that TRIM29 may be a useful prognostic marker in NSCLC patients and a potential molecular target for NSCLC treatment.

Introduction

Non-small cell lung cancer (NSCLC) accounts for ~80% of all lung cancer cases (1) and is the most common cause of cancer-associated mortality worldwide (2). Globally, the annual diagnosis rate of new NSCLC cases is ~1.6 million, with the rate increasing over the last decades (3). A previous study demonstrated the importance of molecular characterization of these tumors and the identification of potential molecular targets for treatment (3). However, the prognosis in these patients remains poor as the overall 5-year survival is <15% (4). These unsatisfactory clinical outcomes indicate the requirement for more reliable predictors of survival and novel therapeutic targets (3).

The tripartite motif (TRIM) family members are involved in numerous biological processes and, when altered, are implicated in a number of pathological conditions (5). TRIM29, which is also known as ataxia-telangiectasia group D complementing protein (ATDC), is a member of the TRIM family proteins (6). TRIM29 is highly expressed in gastric cancer and is involved in the differentiation, proliferation and progression of gastric cancer cells (7). In addition, TRIM29 was found to be overexpressed in bladder cancer, ovarian serous papillary tumors and endometrial neoplasms (8,9). However, the clinical significance and prognostic value of TRIM29 expression in NSCLC patients remain unclear. The present study aimed to investigate the association between TRIM29 expression and the clinicopathological features of NSCLC, as well as examine the potential role of TRIM29 as a prognostic factor in NSCLC patients.

Materials and methods

Patients and histological evaluation. NSCLC patients who were diagnosed, treated and followed-up at the Department of Thoracic Surgery (Shandong Qianfoshan Hospital, Jinan, China) between January 2000 and January 2007 were recruited in this study. The inclusion criteria were as follows: Patients with surgically resected and pathologically confirmed primary NSCLC, complete medical records and available paraffin-embedded specimens were included. In total, 320 patients with NSCLC were enrolled, including 221 patients treated with lobectomy and 99 patients treated with pneumonectomy. Clinical variables were retrieved from the patients' medical records, including age, gender, and survival or disease progression (Table I). Tumor diagnosis and histological classification were based on a new multidisciplinary classification of lung cancer proposed by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society (10). Tumors were staged according to the 7th edition of the TNM Classification of Malignant Tumors (11,12). Normal bronchial epithelium obtained from noncancerous

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Table I. Association between TRIM29 expression and clinicopathological features.

Characteristic	TRIM29 expression		P-value
	High	Low	
Patients, n (%)	79 (24.7)	241 (75.3)	
Gender, n (%)			0.092
Male	65 (82.3)	214 (88.8)	
Female	14 (17.7)	27 (11.2)	
Mean age ± SD, years	57.32±11.8	59.26±9.77	0.113
Smoking status, n (%)			0.012
Smoker	50 (63.3)	200 (83)	
Non-smoker	29 (36.7)	41 (17)	
Histology, n (%)			0.821
SCC	31 (39.2)	184 (76.3)	
ADC	48 (60.8)	57 (23.7)	
Pathological stage, n (%)			0.210
Stage I	28 (35.4)	110 (45.6)	
Stage II	26 (32.9)	72 (29.9)	
Stage III	21 (26.6)	50 (20.7)	
Stage IV	4 (5.1)	9 (3.7)	
T stage, n (%)			0.015
T1	23 (29.1)	30 (12.4)	
T2	40 (50.6)	163 (67.6)	
T3	12 (15.2)	20 (8.3)	
T4	4 (5.1)	28 (11.7)	
N stage, n (%)			0.920
N0	48 (60.8)	147 (61)	
N1	15 (19)	51 (21.2)	
N2	16 (20.2)	43 (17.8)	
M stage, n (%)			0.003
M0	29 (36.7)	205 (85.1)	
M1	50 (63.3)	36 (14.9)	

TRIM29, tripartite motif-containing 29; SD, standard deviation; SCC, squamous cell carcinoma; ADC, adenocarcinoma; T stage, tumor size; N stage, lymph nodes; M stage, distant metastases.

lung tissue of the NSCLC patients was used as the control. The study was approved by the Institutional Review Board at the Shandong Qianfoshan Hospital. Written informed consent was obtained from all patients.

Follow-up. Standardized follow-up was conducted every 3 months for the first 2 years after surgery, every 6 months for the 3rd year and yearly thereafter. Follow-up included physical examination, complete blood count, chest computed tomography scans, brain magnetic resonance imaging scans and abdominal ultrasound. The median follow-up period for surviving patients was 31.5 months (6-72 months). Disease-free survival (DFS) was defined as the time from the initial surgery until a documented relapse, including locoregional recurrence and distant metastasis. The overall survival (OS) was defined from the date of the initial surgery until mortality or the end of follow-up.

Immunohistochemical analysis. Tissue sections (6 μm) were deparaffinized in xylene, rehydrated and heated at 100˚C in citrate buffer (pH 6.0) for 5 min for nonenzymatic antigen retrieval. The sections were then incubated with monoclonal mouse anti-human TRIM29 antibody (cat. no. H00023650-B01; dilution, 1:100; Novus Biologicals, LLC, Littleton, CO, USA) for 60 min at room temperature, followed by incubation with a goat anti-mouse immunoglobulin G antibody (cat. no. AI-9200; dilution, 1:1,000; Vector Laboratories, Inc., Burlingame, CA, USA) for 1 h at room temperature. Staining was performed with 3,3'-diaminobenzidine chromogen for 5 min, followed by counterstaining using hematoxylin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 5 min, as previously described (13). Rabbit IgG (cat. no. NP-001; Epitomics Inc., Burlingame, CA, USA) was used as a negative control. TRIM29 expression was scored as follows: 0, no staining;

Table II. Univariate analyses of overall survival and disease-free survival in 320 patients with non-small cell lung cancer.

	Overall survival		Disease-free survival	
Characteristic	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
TRIM29 expression		0.007		0.014
Low vs. high	1.52 (1.120-2.061)		1.51 (1.089-2.119)	
Gender		0.134		0.982
Female vs. male	0.56 (0.267-1.049)		0.97 (0.670-1.427)	
Smoking status		0.025		0.190
No vs. yes	1.48 (1.049-2.138)		0.80 (0.570-1.121)	
Age, years		0.079		0.447
≤60 vs. >60	1.43 (1.036-1.901)		0.82 (0.642-1.213)	
Histology		0.814		0.625
SCC vs. ADC	1.07 (0.766-1.403)		1.34 (0.979-1.858)	
Stage		<0.001		<0.001
I/II vs. III/IV	2.69 (1.933-3.550)		1.86 (1.314-2.648)	
T stage		<0.001		<0.001
T1/T2 vs. T3/T4	2.84 (2.082-3.995)		2.35 (1.621-3.429)	
N stage		<0.001		<0.001
N0 vs. N1/N2	2.30 (1.725-3.120)		1.96 (1.419-2.695)	
M stage		<0.001		<0.001
M0 vs. M1	2.54 (1.894-3.725)		2.02 (1.711-3.502)	

CI, confidence interval; SCC, squamous cell carcinoma; ADC, adenocarcinoma; T stage, tumor size; N stage, lymph nodes; M stage, distant metastases.

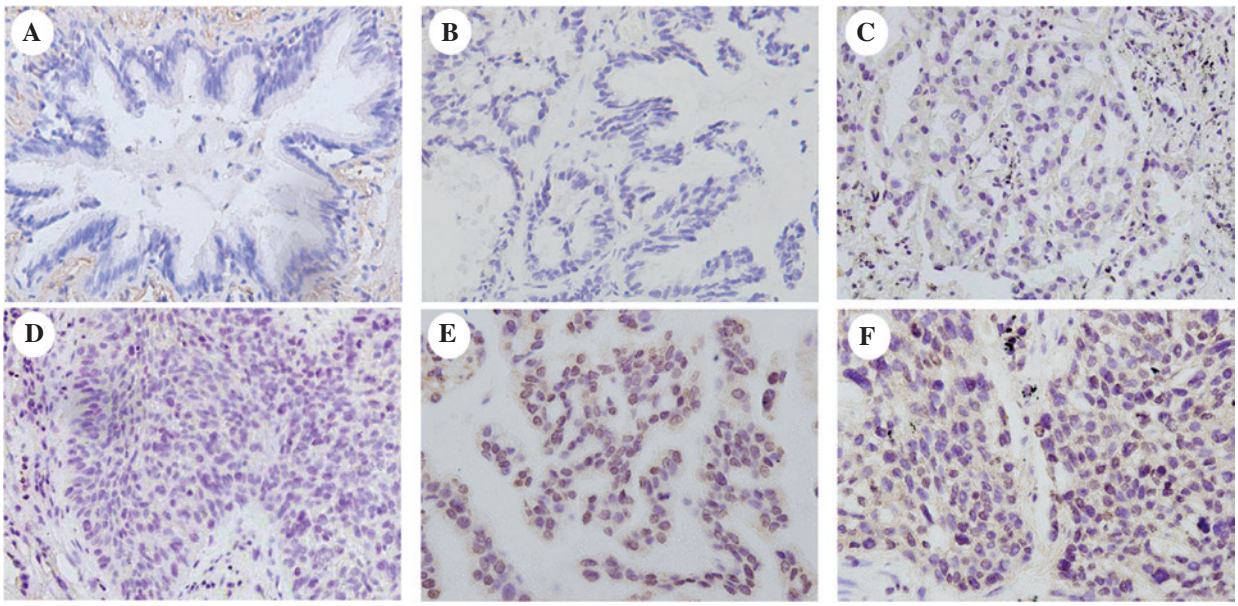

Figure 1. Immunohistochemical staining of TRIM29 in lung cancer tissue specimens. (A) Negative staining in normal bronchial epithelium of noncancerous lung tissue (control). (B) Negative control using rabbit immunoglobulin. (C) Weak TRIM29 staining in lung adenocarcinoma. (D) Weak TRIM29 staining in a case of squamous cell carcinoma. (E) Positive TRIM29 staining in a case of lung adenocarcinoma. (F) Positive TRIM29 staining in a case of squamous cell carcinoma. TRIM29, tripartite motif-containing 29.

1+, <10% of tumor cells expressing TRIM29; 2+, 10-50% of tumor cells expressing TRIM29; and 3+, >50% of tumor cells expressing TRIM29. Scores of 0 and 1+ were classified as low TRIM29 expression, whereas scores of 2+ and 3+ were classified as high TRIM29 expression. The scoring is a modification of a previously described classification (14). Two

Table III. Multivariate analyses of overall survival and disease-free survival in 320 patients with non-small cell lung cancer.

	Overall survival		Disease-free survival	
Characteristic	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
TRIM29 expression		0.003		0.064
Low vs. high	2.102 (1.069-3.193)		1.384 (0.982-1.952)	
Gender		0.036		0.331
Female vs. male	1.921 (1.044-3.535)		1.327 (0.750-2.347)	
Smoking status		0.312		0.406
No vs. yes	1.277 (0.795-2.052)		0.813 (0.499-1.325)	
Age, years		0.001		0.692
≤60 vs. >60	1.752 (1.274-2.410)		1.070 (0.766-1.495)	
Histology		0.007		0.040
SCC vs. ADC	1.611 (1.141-2.275)		1.506 (1.020-2.225)	
Stage		0.415		0.649
I/II vs. III/IV	1.196 (0.778-1.838)		0.891 (0.543-1.463)	
T stage		<0.001		<0.001
T1/T2 vs. T3/T4	2.050 (1.371-3.065)		2.246 (1.427-3.537)	
N stage		<0.001		<0.001
N0 vs. N1/N2	2.053 (1.592-3.413)		1.817 (1.100-2.713)	
M stage		<0.001		0.001
M0 vs. M1	2.316 (1.618-3.313)		1.952 (1.320-2.888)	

CI, confidence interval; SCC, squamous cell carcinoma; ADC, adenocarcinoma; T stage, tumor size; N stage, lymph nodes; M stage, distant metastases.

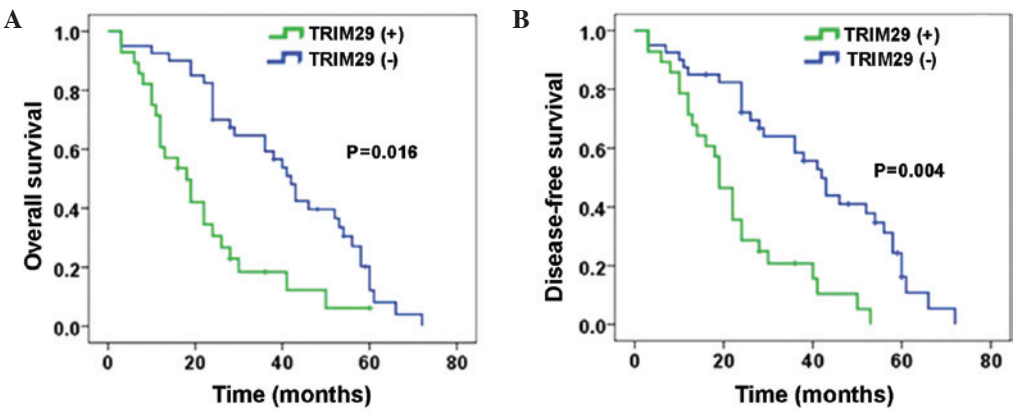

Figure 2. Kaplan-Meier curves for (A) overall survival and (B) disease-free survival in all non-small-cell lung cancer patients. TRIM29, tripartite motif-containing 29; (+), positive expression; (-), negative expression.

pathologists blinded to the patients' clinical data interpreted all the slides and agreed on the classification of the sections into the low or high TRIM29 expression groups.

Statistical analysis. In order to determine any possible associations between qualitative clinicopathological variables and TRIM29 expression, the χ^2 test or two-tailed Fisher's exact test was applied. Survival differences among the high- and low-expression groups were calculated using the Kaplan-Meier method along with the log-rank test. The Cox proportional hazards model was used for multivariate analyses of OS and DFS. SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. P<0.05 was considered to indicate a statistically significant difference.

Results

Association between TRIM29 expression and clinicopathological features of NSCLC patients. Various patient characteristics and their correlation with TRIM29 expression are listed in Table I. The mean age at diagnosis was

58.9±10.7 years (range, 34-82 years). A total of 138 patients (43.1%) were diagnosed with stage I, 98 (30.6%) with stage II, 71 (22.2%) with stage III and 13 (4.1%) with stage IV disease. TRIM29 expression was predominantly localized in the nuclear compartments of the tumor cells, while the normal bronchial epithelia exhibited negative or low staining (Fig. 1).

The association between total TRIM29 expression and various clinical parameters was investigated. As shown in Table I, a high TRIM29 expression was significantly associated with smoking (P=0.012), a higher T stage (P=0.015) and a higher M stage (P=0.003). However, no statistically significant correlation was observed between TRIM29 expression and other clinical features.

Association between TRIM29 expression and OS or DFS. The prognostic relevance of TRIM29 expression and other clinicopathological parameters with regard to OS and DFS in patients with NSCLC was investigated (Table II; Fig. 2A and B). Patients with high TRIM29 expression presented shorter OS and DFS rates compared with patients with low TRIM29 expression (P=0.007 and P=0.014, respectively). In addition, pathological stage (I/II vs. III/IV) and TNM stage were demonstrated to be independent prognostic factors affecting OS and DFS (P<0.001; Table II). Furhtermore, high TRIM29 expression was demonstrated to be an independent prognostic factor affecting OS (P=0.003; hazard ratio, 2.102; 95% confidence interval, 1.069-3.193) using multivariate analyses (Table III).

Discussion

The aim of the present study was to determine the prognostic significance of TRIM29 protein expression in NSCLC. Based on the findings of the current study, TRIM29 overexpression appears to be associated with aggressive tumor behavior and ultimately influences patients' clinical outcomes. In addition, a high TRIM29 expression was prevalent in smokers and was found to be an unfavorable clinical factor in NSCLC.

Recent evidence has suggested that TRIM29 promotes cancer cell proliferation via inhibiting the nuclear activities of *p53*, which is a major tumor suppressor gene involved in the determination of proliferation or growth arrest at the cellular level (15,16). TRIM29 binds p53 and represses the expression of p53-regulated genes, including *p21* and *NOXA* (15,17). In addition, TRIM29 is selectively expressed in basal cells of the normal prostate gland. In a previous study, immunohistochemical staining with anti-TRIM29 antibody revealed the same expression pattern as that observed for staining with 34βE12 in prostate cancer and its benign mimics, indicating that TRIM29 may be useful for distinguishing prostate cancer from benign tissues (18). Furthermore, younger females with early-stage breast cancer who were not administered adjuvant systemic therapy had a significantly lower risk of relapse when their tumor exhibited a high TRIM29 expression (19). This finding suggests that loss of TRIM29 expression in normal breast luminal cells can contribute to malignant transformation and lead to progression of breast cancer in premenopausal women (19). The expression of TRIM29 was also significantly associated with progression to muscle-invasive bladder cancer and was identified as an independent prognostic marker (20). In addition, TRIM29 has been reported to upregulate matrix metalloproteinase 9 to promote lung cancer cell invasion by activating the extracellular signal-regulated kinase and c-Jun N-terminal kinase signaling pathways (21). Previous results have identified a DNA repair pathway leading from MAPK-activated protein kinase-2 and ataxia telangiectasia mutated to TRIM29 (22). Therefore, as TRIM29 has been used as a therapeutic target to radiosensitize pancreatic ductal adenocarcinoma and improve the efficacy of DNA-damaging treatment in pancreatic cancer (22), the use of TRIM29 as a potential therapeutic target for the treatment of NSCLC was investigated in the present study.

In conclusion, in the present study, TRIM29 expression was investigated by immunohistochemical analysis in paraffin-embedded specimens obtained from 320 patients with surgically resected NSCLC, treated between 2000 and 2007. High TRIM29 expression was significantly associated with smoking, T stage and M stage. Furthermore, elevated TRIM29 expression level was correlated with reduced OS and DFS and was an independent prognostic factor for OS in NSCLC. Therefore, TRIM29 may be a useful prognostic marker in NSCLC patients and a potential molecular target for NSCLC treatment.

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