Original Article
Elevated RABEX-5 expression predicts poor prognosis in non-small-cell lung cancer

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Abstract: RABEX-5 has been studied in various solid tumors, but its role in non-small-cell lung cancer (NSCLC) remains unknown. This study is aimed to investigate the expression, the potential relevance to clinicopathological characters and prognostic significance of RABEX-5 in patients with NSCLC. A total of 120 NSCLC patients who underwent radical surgery between 2005 and 2010 were enrolled in the study. The clinicopathological data and survival time were reviewed. The mRNA and protein expression of RABEX-5 from the paired tumor specimens and adjacent normal tissues were determined, and its relationship with clinicopathological variables and prognosis was analyzed. Univariate and multivariate analyses were performed to investigate the prognostic significance of RABEX-5 for NSCLC. We found the mRNA and protein expression levels of RABEX-5 were significantly elevated in NSCLC tissues. The increased RABEX-5 expression was correlated strongly with tumor recurrence (P=0.005). The 5-year median OS and DFS were significantly shorter in the higher RABEX-5 expression group compared to that in the lower RABEX-5 expression group. Multivariate Cox analysis indicated that high RABEX-5 expression was an independent prognostic factor for OS and DFS (P<0.001). This data suggests that RABEX-5 is a potentially useful indicator for a poor prognosis for NSCLC.

Keywords: Non-small-cell lung cancer, RABEX-5, prognosis

Introduction

Although the incidence of lung cancer incidence rates began declining in the 1990s as a result of reductions in smoking prevalence, however, Lung cancer is still the first cause of tumor-related death in both developing and developed countries [1, 2]. As a subgroup of lung cancer, the Non-small cell lung cancer (N-SCLC) accounts for approximately 85% of all lung cancer cases [3]. For the treatment, the NCCN guidelines recommend the surgery, radiotherapy, and platinum-based combination chemotherapy for N-SCLC [4]. Although the diagnosis and treatment for the N-SCLC have made a good progress, and basic and clinical study have been conducted in recent years, the long-term outcome remains poor [5]. Exploring novel and special promising predictive factors is still urgent needed to improve the prognosis of N-SCLC.

RABEX-5 is a guanine nucleotide exchange factor (GEF) for RAB5, a small GTPase regulating endocytosis and vesicle transport, including the regulation of cell growth, signal transduction, and many other aspects of cellular processes [6]. Several studies found that RABEX-5 expression is significantly higher in several solid tumors, such as breast cancer [7], gastric cancer [8], prostate cancer and colorectal cancer [9, 10]. The data of these studies indicated that RABEX-5 may play an oncogenic role in tumorigenesis and progression. What's more, in prostate cancer, the study showed that high RABEX-5 mRNA expression predicted poor prognosis.

However, the expression, clinical value and prognostic implications of RABEX-5 in NSCLC have not yet been studied.

In the present study, we first detected the expression of RABEX-5 in NSCLC paired tumor specimens and adjacent normal tissues. Secondly, we examined the relationship between RABEX-5 and clinicopathological variables. Lastly, we investigated the prognosis value of RABEX-5 in NSCLC patients.

Table 1. Clinicopathological Characteristics by RABEX-5 protein expression

| Variables | Number | RABEX-5 protein expression | | P value |
		Low (n=60)	High (n=60)	
Age (years)				
≥ge	66 (55.0%)	32 (53.3%)	34 (56.7%)	0.121
<65	54 (45.0%)	28 (46.7%)	26 (43.3%)	
Gender (%)				
Male	100 (83.3%)	50 (83.3%)	50 (83.3%)	0.208
Female	20 (16.7%)	10 (16.7%)	10 (16.7%)	
Smoking history				
No	8 (6.7%)	5 (8.3%)	3 (5.0%)	0.367
Yes	112 (93.3%)	55 (91.7%)	57 (95.0%)	
Performance status				
ECOG 0-1	110 (91.7%)	62 (91.2%)	63 (92.6%)	0.211
ECOG 2	10 (8.3%)	6 (8.8%)	5 (7.4%)	
NSE				
Normal	51 (42.5%)	24 (40.0%)	27 (45.0%)	0.154
Elevated	69 (57.5%)	36 (60.0%)	33 (55.0%)	
Tumor location				
Left	59 (49.2%)	30 (50.0%)	29 (48.3%)	0.379
Right	61 (50.8%)	30 (50.0%)	31 (51.7%)	
Lymph node metastasis				
No	57 (47.5%)	28 (46.7%)	29 (48.3%)	0.801
Yes	63 (52.5%)	32 (53.3%)	31 (51.7%)	
Clinical stage				
I	29 (24.2%)	20 (33.3%)	9 (15.0%)	0.018
II	66 (55.0%)	34 (56.7%)	32 (53.3%)	
III	25 (20.8%)	6 (10.0%)	19 (31.7%)	
Chemotherapy				
Etoposide + platinum	90 (75.0%)	46 (76.7%)	44 (73.3%)	0.544
Irinotecan + platinum	30 (25.0%)	14 (23.3%)	16 (26.7%)	
Thoracic irradiation				
No	51 (42.5%)	25 (41.7%)	26 (43.3%)	0.332
Yes	69 (57.5%)	35 (58.3%)	34 (56.7%)	
LDH				
Normal (<240 U/L)	74 (61.7%)	38 (63.3%)	36 (60.0%)	0.298
Elevated (≥240 U/L)	46 (38.3%)	22 (36.7%)	24 (40.0%)	
Tumor recurrence				
No	73 (60.8%)	48 (80.0%)	25 (41.7%)	0.005
Yes	47 (39.2%)	12 (20.0%)	35 (58.3%)	

Materials and methods

Ethics statement

The protocol of this study was approved by the Research Ethics Committee of Tianjin Nankai Hospital, China. Written informed consent was obtained from all participants.

Study population

Patients who had histologically confirmed NSCLC from 2005 to 2010 were reviewed from our hospital tumor registry. The inclusion criteria for this study included: (1) patients who underwent radical surgery; (2) patients who pathologically confirmed patients with NSCLC; (3) patients who had matched fresh surgical specimens and adjacent normal tissues; (4) the clinical data and the follow-up information were complete. The exclusion criteria included: (1) patients who underwent palliative surgery; (2) patients who had distant metastasis or peritoneal dissemination that was found during the operation; (3) patients who received treatment, such as chemotherapy, or radiation therapy prior to radical surgery. Based on these inclusion and exclusion criteria, a total of 120 NSCLC patients were enrolled this study. Median follow-up time for all patients was 65.0 months.

Data collection

The detailed clinical characteristics including smoking history, age, gender, ECOG performance status, disease extent, therapeutic strategies as well as survival were obtained. The stage was determined according to the American Joint Committee on Cancer (AJCC) lung cancer TNM staging system. Histological typing was performed by at least two expert pathologists, working independently in a double-blinded fashion. Total follow-up information was collected from clinic visit or from family contact. The OS was defined as time from the date of diagnosis to the date of death or last visit.

RNA preparation, quantitative real-time PCR

Total RNA was isolated from the 120 matched surgical specimens and adjacent normal tissues using Trizol reagent (Invitrogen) according
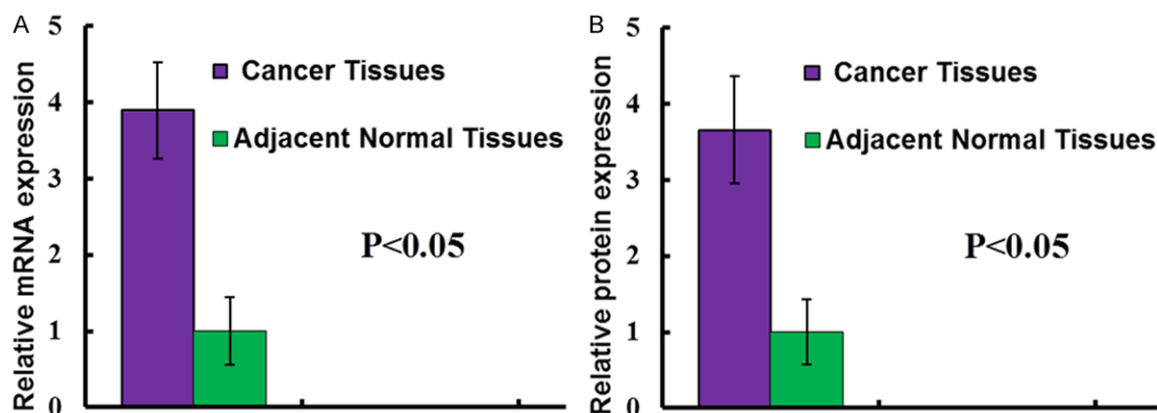
Figure 1. The expression level of RABEX-5. A. Real-time qRT-PCR of RABEX-5 mRNA expression in NSCLC tissues and adjacent normal tissues. The results of the real-time qRT-PCR were analyzed by using the $2^{-\Delta\Delta Ct}$ method. B. The relative levels of protein expression.
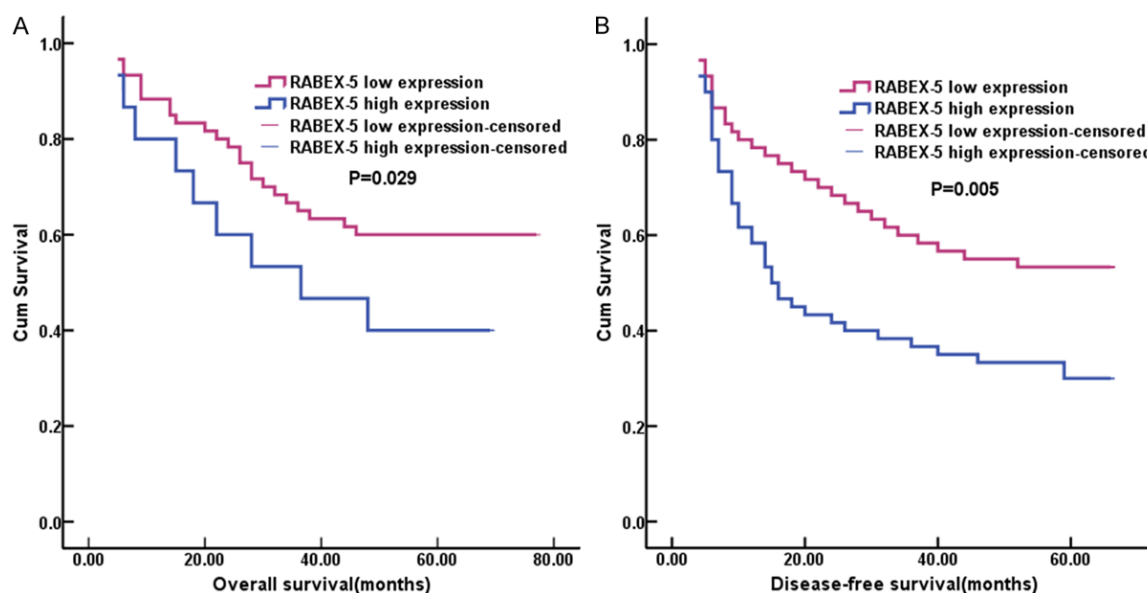
Figure 2. Overall and disease-free survival curves of NSCLC patients with RABEX-5 protein expression. A. The overall survival was significantly worse in the high RABEX-5 expression group compared to low expression group (P=0.029). B. The patients with high RABEX-5 expression had shorter disease-free survival (DFS) than patients with low RABEX-5 expression (P=0.005).

to the manufacturer's instruction. QRT-PCR was performed using SYBR Green polymerase chain reaction master mix according to the manufacturer's instructions (Applied Biosystem). The primers were as follows: RABEX-5: forward 5'-ATGTGGATCAATCGGATCTCCT-3', reverse 5'-GCTTTGTGGTACTCTTCCCTCC-3'; D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward 5'-CCATCAATGACCCCTTCA-TTG-3', reverse 5'-GACGGTGCCATGGAATTT-3'. The results of qRT-PCR were analyzed by using the $2^{-\Delta\Delta Ct}$ method. Relative mRNA expression of

RABEX-5 gene (R) was calculated following the formula: R=densitometric units of RABEX-5/ densitometric units of GAPDH. Experiments were repeated three times.

Western blot

Total of 120 paired fresh tumor tissues and adjacent normal tissues were homogenized in NP40 lysis buffer. Equal amounts of protein were separated by SDS-PAGE, then the proteins were transferred to polyvinylidene fluoride

Table 2. Univariate and Multivariate Analyses With Regard to DFS (n=120)

Variable	Univariate analysis	Multivariate Cox regression	
	p-Value	HR (95% CI)	p-Value
Age (years)			
<65			
≥65	0.391		-
NSE			
Normal (<15.2 µg/L)			
Elevated (≥15.2 µg/L)	0.667		-
LDH			
Normal (<240 U/L)			
Elevated (40 U/L)	0.470		-
Lymph node metastasis			
No			
Yes	0.148		-
Clinical stage			
I			
II			
III	0.011	2.775 (1.575-4.699)	0.005
Performance status			
ECOG 0-1			
ECOG 2	0.012	1.674 (1.101-3.157)	0.033
RABEX-5 expression			
Low			
High	0.005	3.366 (1.904-7.221)	<0.001

(PVDF) membranes, blocked and incubated with the appropriate primary antibodies and then probed with secondary antibody. Signals were detected using enhanced chemiluminescence substrate (Perkin-Elmer Life Sciences). Quantification of the Western blot data were performed by measuring the intensity of the hybridization signals using the Image analysis program. Relative protein expression of RABEX-5 was calculated by the formula: R=densitometric units of RABEX-5/densitometric units of GAPDH. Accordingly, the RABEX-5 protein expression levels were further divided into high and low groups using median expression level as the cut-off point for further analysis.

Statistical analysis

Continuous variables were described using mean ± SD, the categorical variables were analyzed by a chi-squared test. Paired t test was used to compare the RABEX-5 expression level between tumor and normal specimens. χ^2 test was employed to investigate the correlation between the RABEX-5 expression and clinicopathologic variables. The Kaplan-Meier method and log-rank tests were performed to compare survival curves. Furthermore, the Multivariate analyses were conducted to identify significant independent prognostic factors for the prognosis. Hazard ratios (HR) and 95% confidence interval (CI) were generated. Significance was defined as p-Values (two sides) <0.05. The statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, United States).

Results

Patient characteristics

A total of 100 (83.3%) male and 20 (16.7%) female with NSCLC treated with radical surgery were included in this study. **Table 1** summarizes the clinicopathological characteristics. Among these patients, Most of the patients (n=110; 91.7%) had better performance status (ECOG 0-1). Among all the NSCLC patients, 45 lymph node negative patients received platinum-based adjuvant chemotherapy alone, while 55 lymph node positive patients received concurrent or sequential chemoradiotherapy. Median follow-up time for all patients was 65.0 months.

RABEX-5 expression was elevated in NSCLC tissues

RABEX-5 mRNA expression level of 120 matched NSCLC surgical specimens and adjacent normal tissues was detected by qRT-PCR. Our data showed that RABEX-5 mRNA expression was elevated in NSCLC tissues compared to that of adjacent normal tissues (**Figure 1A**). Consistent with the mRNA expression, the RABEX-5 protein expression was also significantly higher in cancer tissues (**Figure 1B**).

Relationship between RABEX-5 expression and clinicopathological variables in NSCLC patients

As the **Table 1** shows, the elevated RABEX-5 protein expression correlated with clinical stage

Table 3. Univariate and Multivariate Analyses for OS (n=120)

Variable	Univariate analysis	Multivariate Cox regression	
	p-Value	HR (95% CI)	p-Value
Age (years)			
<65			
≥65	0.221		-
NSE			
Normal (<15.2 µg/L)			
Elevated (≥15.2 µg/L)	0.369		-
LDH			
Normal (<240 U/L)			
Elevated (40 U/L)	0.901		-
Lymph node metastasis			
No			
Yes	0.063		-
Clinical stage			
I			
II			
III	0.012	2.877 (1.665-6.335)	0.001
Performance status			
ECOG 0-1			
ECOG 2	0.088		-
RABEX-5 expression			
Low			
High	0.029	3.858 (1.944-8.259)	<0.001

(P=0.018). Most importantly, our study first found the high RABEX-5 protein level correlated with C-SCLC recurrence (P=0.005). However, the RABEX-5 protein expression was not correlated with age, gender, Performance status and NSE.

Relationship between RABEX-5 protein expression and survival outcomes in NSCLC patients

Our data showed that the median OS of all NSCLC patients in present study was 48.0 months. As the **Figure 2A** showed, the median OS was statistically worse in the high RABEX-5 expression group compared to low RABEX-5 expression group (P=0.029; **Figure 2A**), and also the patients with high RABEX-5 expression had shorter median DFS than patients with low RABEX-5 expression (P=0.005; **Figure 2B**).

The univariate analysis found that the clinical stage (P=0.011), performance status (P=0.012), and RABEX-5 expression (P=0.005) were prognostic factors for DFS (**Table 2**), while

the clinical stage (P=0.012), RABEX-5 expression (P=0.029) were associated with OS (**Table 3**). The further multivariate analysis identified that, for the NSCLC patients, the clinical stage (HR=2.775, 95% CI, 1.575-4.699; P=0.005), performance status (HR= 1.674, 95% CI, 1.101-3.157; P= 0.033), and elevated RABEX-5 level (HR=3.366, 95% CI, 1.904-7.221; P<0.001) were independent factors associated with DFS (**Table 2**). While the statically significant independent prognosis factors for OS were clinical stage (HR=2.877, 95% CI, 1.665-6.335; P=0.001) and the elevated RABEX-5 level (HR=3.858, 95% CI, 1.944-8.259; P<0.001) (**Table 3**).

Discussion

To date, our present study is the first report to explore the association of RABEX-5 expression with clinicopathological variables and survival outcome in patients with NSCLC. Our data confirmed the RABEX-5 expression was elevated in NSCLC, and the elevated RABEX-5 protein expression was associated with tumor recurrence. The further multivariate analysis identified that high RABEX-5 protein expression predicted a poor long-term prognosis.

Lung cancer is the most common cause of tumor death in the United States. Over 200,000 new cases of lung and bronchial cancer will be diagnosed in 2015, and 150,000 deaths are estimated to occur because of the cancer [1]. According to the tumor biology, therapy, and prognosis, the lung cancer was divided into NSCLC and small cell lung cancer (SCLC) [11]. NSCLC accounts for more than 85% of all lung cancer cases, and it includes non-squamous carcinoma and squamous cell carcinoma [5, 12]. Many studies and systematic empirical research specific on NSCLC have identified certain prognostic factors and several biomarkers for NSCLC [13-16]. These findings improved the outcome of the NSCLC, however, the 5-year survival rate for the NSCLC is only 16% [17]. It is important to identify the novel and reliable prognostic markers to improve the prognosis of NSCLC.

The RABEX-5 abnormal expression has been reported in several types of solid tumors [18, 19]. However, there is not much data about the expression and clinical value of RABEX-5 in NSCLC. Therefore, we first detected the expression using paired NSCLC tissue and adjacent normal tissues, collected and analyzed the relationship between the RABEX-5 expression and clinicopathological data. Our data identified that the RABEX-5 expression was elevated in NSCLC compared to the normal tissues (**Figure 1**). This result was consistently with Zhang's study, they found the RABEX-5 mRNA expression was also elevated in in patients with prostate cancer [9]. As the recent study found that the RABEX-5 expression was correlated with the clinicopathological characters in solid tumors, we further investigated the associations of elevated RABEX-5 mRNA expression and clinicopathological varibels in NSCLC. As the **Table 1** showed that the higher RABEX-5 protein expression correlated with tumor recurrence (P=0.005) significantly. In contrast, there was no correlation between the RABEX-5 expression and age, gender, performance status, lymph node metastasis and NSE.

Recently, as the novel oncogene, the potential prognostic value of RABEX-5 has been identified in prostate cancer. In the present study, our data showed that the 5-year OS and DFS was significantly shorter in the high RABEX-5 expression group compared to low RABEX-5 expression group (OS: P=0.029; **Figure 2A**; DFS: P=0.005; **Figure 2B**) in NSCLC patients (**Figure 2**).

Further multivariate Cox analysis confirmed that high RABEX-5 expression was an independent poor prognostic factor for long-term outcome in NSCLC patients (**Tables 2** and **3**). In addition, clinical stage were also identified as independent prognostic factors for overall survival (**Table 3**), which was consistent with the other reports [20]. These data indicates that RABEX-5 protein expression, if validated in further study, has the potential to be used as a valuable prognostic factor in NSCLC.

This study has several potential limitations. Although the mRNA and protein measurement was performed accurately from the fresh paired surgical specimens, and the survival data collection was conducted rigorously, we have relative small size patients, which limited the level of evidence. Accordingly, further large size study and protein level detection needs to be conducted to validate our result. Also, based on our data and the previous studies, the RABEX-5 might be involved in the carcinogenesis and progression of tumors [7-9, 21]. However, we have not too much data to elucidate the molecular mechanism involved in these processes.

Conclusions

These findings of our study indicate that the RABEX-5, as a newly identified oncoprotein, is highly elevated in NSCLC, predicts poor long-term survival. It might serve as a novel potential prognostic marker and potential therapeutic target.

Disclosure of conflict of interest

None.

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