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Original Article

ELMO3 is a novel biomarker for diagnosis and prognosis of non-small cell lung cancer

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Abstract: Background: To investigated the diagnostic and prognostic value of engulfment and cell motility (*ELMO3*) in non small cell lung cancer (NSCLC). Methods: The expression of *ELMO3* at mRNA levels were detected using reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR) in 125 NSCLC patients' tissues and adjacent tissues, as well as in the serum of 125 NSCLC patients and 89 healthy controls. Then, receiver operating characteristic curve (ROC), Kaplan-Meier and Cox regression analysis were adopted to estimate the potential diagnostic and prognostic value of *ELMO3*, respectively. Results: *ELMO3* expression level was significantly up-regulated in NSCLC patients' tissues and serum compared with controls ($P<0.001$). Moreover, the expression of *ELMO3* was significantly associated with tumor size ($P=0.020$), TNM stage ($P=0.017$), lymph node metastasis ($P=0.045$) and distance metastasis ($P=0.033$). ROC showed the AUC was 0.917, and the optimal cutoff value was 0.735, providing a sensitivity of 92.8% and a specificity of 84.3%. Furthermore, Kaplan-Meier analysis indicated the high expression of *ELMO3* could lead to a shorter overall survival time. In multivariate analysis, *ELMO3* expression (HR=3.378, 95% CI=1.326-8.587, $P=0.011$) was proved to be linked with the prognosis of NSCLC and might act as an independent prognostic marker. Conclusion: The over-expression of *ELMO3* was a potential diagnostic and prognostic marker for NSCLC.

Keywords: Non small cell lung cancer, *ELMO3,* diagnosis, prognosis

Introduction

Lung cancer is a major cause of cancer-related death in worldwide, accounting for 18% (1.4 million) of cancer deaths in 2008 according to global cancer statistics [1]. It is traditionally classified into two major subtypes, small cell lung cancer and non-small cell lung cancer (NSCLC). Among them, NSCLC covers 80-85% [2]. Although the survival rate of patients with NSCLC has been improved due to the advances in surgical techniques and treatment strategies, the high rate of recurrence and metastasis were still lead to a short-term survival after surgical resection [3, 4]. NSCLC is a slow-developing cancer with a complex pathogenesis and its progression get involve in several stages as well as activation of many oncogenes and inactivation of tumor suppressor genes [5]. Because of the late-stage diagnosis and other complex factors, NSCLC has a poor prognosis and low cure rate. Therefore, the research for effective molecular markers for diagnosis and prognosis of lung cancer is an important issue at present.

The engulfment and cell motility (*ELMO*) proteins family which is consists of *ELMO1*, *ELMO2*, and *ELMO3*, widely distribute in mammals and participate in a various of life action including cell migration, cell chemotaxis transfer, cell polarity, apoptosis, dendritic development and so on [6-10]. Ras GTPase-binding domain (RBD) that only present in Elmo proteins and ElmoD protein (Elmo domain) is the most important characteristic of *ELMO* [11, 12]. In previous studies, most of the researches were about the function of *ELMO1* and *EMLO2,* the investigation about *ELMO3* was rarely. It was reported that *ELMO3* plays important roles in the cell

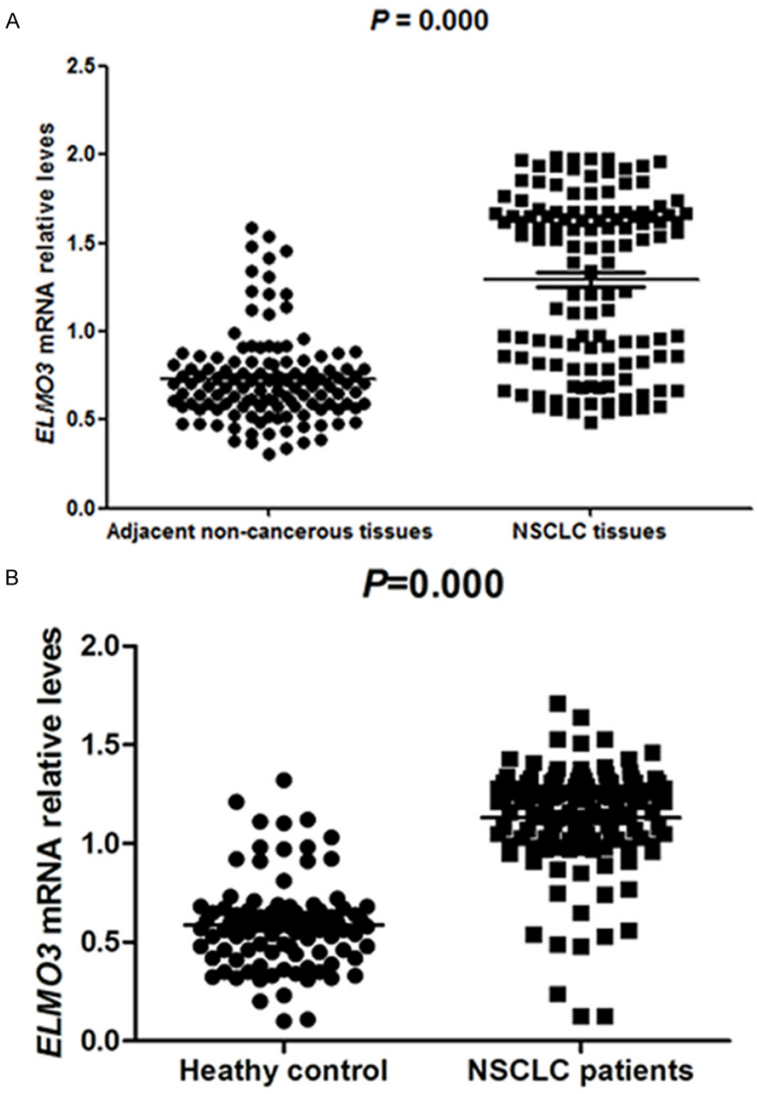

Figure 1. Expression level of *ELMO3* in NSCLC tissues and serum detected. A. The expression of *ELMO3* in NSCLC tissues was significantly higher than in adjacent tissues ($P<0.05$). B. The serum *ELMO3* expression were also markedly up-regulated in patients with NSCLC compared with healthy controls ($P<0.05$).

renewing and migration processes within the intestinal epithelia and in colorectal cancer [13]. Besides, *ELMO3* had been considered to be a promoter of metastatic dissemination of NSCLC in the study of Sφes et al. [14]. However, the diagnostic and prognostic of *ELMO3* in NSCLC remains unknown.

In this study, we investigated the expression level of *ELMO3* in NSCLC tissues and serum through quantitative real-time polymerase chain reaction (qRT-PCR), and analyzed the association of *ELMO3* with clinicopathological characteristics. Meanwhile, the diagnostic and prognostic values of *ELMO3* were also estimated.

Materials and methods

Patients and samples

A total of 125 patients with NSCLC were recruited. The study was permitted by the Ethics Committee of the hospital. None of them had received any adjuvant chemotherapy or radiotherapy. Besides, 89 healthy volunteers matching age and gender were obtained as healthy controls. Meanwhile, written informed consents had been signed by each participators in advance.

The tissues and adjacent tissues were collected from NSCLC patients and the serum was reserved. The serum from healthy controls were extracted, too. All the tissues were frozen by liquid nitrogen while the serum samples were put into blood collection tube of EDTA and stored at -80°C for RNA extraction. A 5-years' follow-up was performed to estimate the prognosis of NSCLC patients who had undergone curative surgical resection. The clinicopathologic characteristics including age, gender, tumor size, TNM stage, lymph node metastasis, and distant metastasis were recorded in a database. Patients who died from unexpected events or other diseases were excluded from our study.

RNA extraction and quantitative real-time polymerase chain reaction (QRT-PCR)

Total RNA was isolated from the tissues and adjacent tissues of 125 patients with NSCLC as well as the serum samples using the Trizol reagent (Invitrogen). The RNA was purified to the OD A260/A280 ratio reach to 2.0, the anal-

Table 1. Relationship between *ELMO3* expression and clinicopathological characteristics in NSCLC

Characteristic	Cases	*ELMO3* expression		x^2	*P*-values
		Low N=55	High N=70		
Gender					
Male	72	30	42	0.982	0.322
Female	53	25	28		
Age (years)					
≤60	83	40	43	1.762	0.184
>60	42	15	27		
Tumor size					
≤5 cm	89	45	44	5.400	0.020
>5 cm	36	10	26		
TNM stage					
Stage I	102	50	52	5.299	0.017
Stage II/III	23	5	18		
Lymph node metastasis					
Yes	34	10	24	4.034	0.045
No	91	45	46		
Distant metastasis					
Absent	98	48	50	4.566	0.033
Present	27	7	20		

ysis would be subsequently conducted. Reverse transcription was performed to synthesize the first chain of cDNA according to the TaqMan microRNA assay protocol (Applied Biosystems, Foster City, CA, USA). Then the RT-PCR reaction was carried out in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *ELMO3* primers were: forward; 5'-GGC CTT CTC AGA GCT CAT G-3' and reverse; 5'-TGA GGT TCA TGT TCA CGT AGC-3'. *β*-actin was taken as an internal control. Each sample was examined in triplicate, and the relative quantification of *ELMO3* expression was evaluated by the comparative cycle threshold (CT) method, normalized to *β*-actin.

Statistical analysis

All statistical analyses were performed using SPSS version 18.0 software. The data was stated as mean ± SD. The differences of *ELMO3* expression between NSCLC tissues and adjacent tissues as well between NSCLC serum and healthy serum were estimated via students' T-test, respectively. The correlations between *ELMO3* expression and clinicopathologic parameters were analyzed by chi-square test. The diagnostic value of serum *ELMO3* expression was evaluated through the establishment of receiver operating characteristic curve (ROC). Kaplan-Meier analysis was used to determine the overall survival time of patients with different expression of *ELMO3*. The log-rank test was used to analyze the significance of the result. Multivariate analyses were performed using Cox regression analysis to determine the factors affecting the prognosis of NSCLC. The difference was considered to be statistically significant when the *P* value was less than 0.05.

Results

Expression of ELMO3 was increased in NSCLC tissues and serum

To detect the expression of *ELMO3* in NSCLC patients, we analyzed the expression of *ELMO3* at mRNA level by qRT-PCR in NSCLC tissues and adjacent tissues, as well as in NSCLC patients' serum and healthy controls' serum. The expression of *ELMO3* at mRNA level in NSCLC tissues was significantly higher than that in adjacent tissues ($P<0.05$, **Figure 1A**). Similarly, the serum *ELMO3* expression was also markedly up-regulated in patients with NSCLC compared with healthy controls ($P<0.05$, **Figure 1B**). These results indicated that *ELMO3* could be an oncogene in NSCLC.

Correlation between ELMO3 expression and clinicopathological characteristics

To explore whether *EMLO3* expression was relevant to clinicopathological characteristics, we performed chi-square test. As indicated in **Table 1**, *ELMO3* expression was significantly correlated with tumor size ($P=0.020$), TNM stage ($P=0.017$), lymph node metastasis ($P=0.045$) and distant metastasis ($P=0.033$). Nevertheless, no significant correlation was observed among *ELMO3* expression with gender ($P=0.322>0.05$) and age ($P=0.184>0.05$).

Diagnostic value of ELMO3 for NSCLC

ROC curve was established to estimate the diagnostic value of ELMO3 with the serum

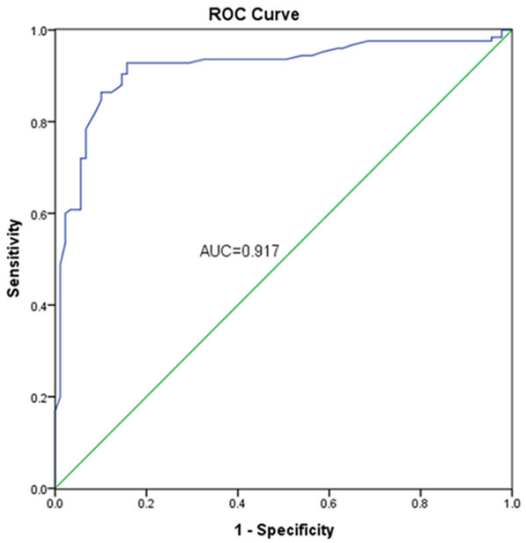

Figure 2. Diagnostic value of *ELMO3* via ROC which had a AUC of 0.917, combing with a sensitivity of 92.8% and a specificity of 84.3%.

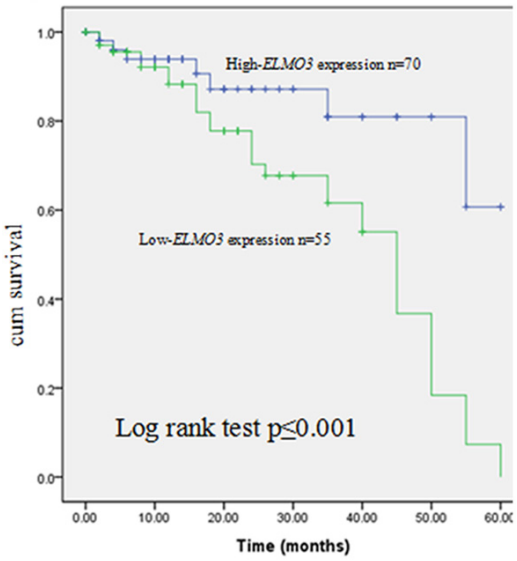

Figure 3. Association between *ELMO3* and overall survival time of NSCLC patients according to Kaplan-Meier analysis.

ELMO3 expression (**Figure 2**). The AUC value was 0.917 corresponding with a sensitivity of 92.8% and a specificity of 84.3% with a cutoff value was 0.735. This result might demonstrate that EMLO3 was a potential biomarker for differentiating NSCLC patients.

Association between EMLO3 expression and overall survival

The relationship between *ELMO3* expression and overall survival time of the patients with NSCLC was evaluated by Kaplan-Meier analysis. As determined by the log-rank test in **Figure 3**, the overall survival time of patients with high *ELMO3* expression was significantly lower than those with low *ELMO3* expression (*P*=0.001). Besides, whether *ELMO3* as well as clinicopathological characteristics could be used as prognostic markers for NSCLC patients were estimated via Cox regression analysis. The outcome indicated that no clinicopathological characteristics but *ELMO3* expression (*P*=0.011, HR=3.378, CI=1.326-8.587) alone was related to the prognosis of NSCLC. Moreover, it could be an independent prognostic markers in NSCLC (**Table 2**).

Table 2. Cox regression analysis of *ELMO3* and clinicopathological characteristics

Variables	HR	95% CI	*P* Value
ELMO3 gene	3.378	1.326-8.587	0.011

Discussion

NSCLC is the leading cause of lung cancer related deaths in world with a characteristic of a long asymptomatic latency and poor prognosis [15 16]. The 5-year survival rate was different along with the change of its stage and the rate could reach to 50% if it was early detected and received treatment [17]. Novel bio-markers with high sensitivity and specificity are therefore urgently needed to the early diagnosis of NSCLC and to promote the development of new treatments [6]. Wang et al. had reported that circulating *MACC1* was expressed higher in NSCLC patients than in benign disease patients or healthy volunteers, represents a potential noninvasive, diagnostic and prognostic marker for NSCLC [18]. Ulivi et al. found peripheral blood miR-328 expression could be as a potential biomarker for the early diagnosis of NSCLC according to relative analysis [19]. Although there are a lot of bio-markers such as mutations in the KRAS, epidermal growth factor receptor, TP53 genes, and changes in the expression levels of carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CK19), cancer antigen-125 (CA125), and neuron-specific enolase (NSE) have been confirmed in NSCLC now [20-22], the clinical value of them were still limited [23, 24].

ELMO3 has the most different protein sequences in *ELMO* family, but modular protein domains are similar with other members [25]. Previous reports have demonstrated that *ELMO* is upregulated in several cancer tissues, such as human glioma and lung cancer [14, 26, 27]. Molecular alterations between normal lung tissues and tumor tissues, are true drivers of the metastatic process, which are specific to the cohort of patients. *ELMO3* has been a negative impact on the survival of lung cancer patients with distant metastases causing additional morbidities. However, its diagnostic and prognostic role in NSCLC is still unclear.

In the study, we performed qRT-PCR to detect the *ELMO3* expression in NSCLC tissues and adjacent tissues as well as the serum *ELMO3* expression was tested at mRNA level. The result showed that *ELMO3* in NSCLC was significantly higher than that in adjacent tissues and the trend was similar with in serum, implying that *ELMO3* might serve as an oncogene in NSCLC. Besides, *ELMO3* was possible to be related to the occurrence and development of NSCLC through the analysis of the relationship between it and patients' clinicopathological characteristics including tumor size, TNM staging, lymph node metastasis and distance metastasis. The results also verified that *ELMO3* is essential for the progress and metastasis of NSCLC which was consistent with the outcome of previous study [28].

As its abnormal expression, we inferred *ELMO3* could be involved in the diagnosis or prognosis or both of them. Hence, we estimated the diagnostic and prognostic value of *ELMO3* furthermore. For a high AUC value, sensitivity and specificity on the basis of ROC, the diagnostic value of *ELMO3* was considered to be valuable. Meanwhile, the prognostic value was confirmed by Kaplan-Meier and Cox regression analysis. *ELMO3* might be a new potential bio-marker for the early detection and prognosis of NSCLC.

In conclusion, *ELMO3* is over-expressed in NSCLC patients and its expression is influenced by many clinicopathological characteristics including tumor size, TNM stage, lymph node metastasis and distant metastasis. Besides, *ELMO3* is identified to be an independent diagnostic and prognostic indicator in our NSCLC. To our knowledge, this is the first study to investigate the diagnostic and prognostic value of *ELMO3* in NSCLC. There are still several limitations in the study. On the one hand, the sample size of the study is small, and further studies with more patients are required to confirm the results. On the other hand, all of the patients in this study come from one hospital and the results may differ according to the techniques used. Hence, further studies are needed.

Disclosure of conflict of interest

None.

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