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Decreased expression of long non-coding RNA LINC00261 is a prognostic marker for patients with non-small cell lung cancer: a preliminary study

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Abstract. – OBJECTIVE: The previous study found that long non-coding RNA LINC00261 (LINC00261) was significantly down-regulated in non-small cell lung cancer (NSCLC). However, the function of LINC00261 in the progression of NSCLC has not been reported. The present work aimed to explore the prognostic value of LINC00261 in patients with NSCLC.

PATIENTS AND METHODS: The expression of LINC00261 was determined in NSCLC tissues and matched normal lung tissues by quantitative Real-time PCR (qRT-PCR). Furthermore, we evaluated the relationship of LINC00261 and clinicopathological features with survival of patients with NSCLC. Finally, univariate and multivariate Cox regression analyses were used to explore whether LINC00261 was an independent predictor of survival.

RESULTS: We found that the LINC00261 expression level in NSCLC tissues was suppressed compared with that in adjacent normal lung tissues ($p < 0.01$). Low expression of LINC00261 was found to significantly correlate with TNM stage ($p = 0.005$), lymph node status ($p = 0.020$), and distant metastasis ($p = 0.004$). Then, Kaplan-Meier analysis demonstrated that low LINC00261 expression level was associated with poorer overall survival ($p = 0.0013$). Furthermore, multivariate analysis showed that low expression of LINC00261 was an independent adverse prognostic factor of NSCLC ($p = 0.004$).

CONCLUSIONS: We firstly provided evidence that LINC00261 expression was associated with poor prognosis of NSCLC patients and may serve as an independent prognostic indicator.

Key Words:
LINC00261, NSCLC, Prognosis.

Introduction

Lung cancer remains the most frequent cause of cancer-related deaths worldwide, accounting for 23% of cancer deaths in 2014 according to global tumor statistics[1]. Non-small cell lung cancer (NSCLC) accounted for 75-80% of all lung cancer and was comprised mainly of adenocarcinoma and squamous cell carcinoma[2]. Despite advanced of current therapies include surgery, chemotherapy, radiation therapy, and targeted therapy, the outcome of NSCLC is still poor with the 5-year survival rate about 15%[3]. High rates of distant metastasis are the important reason responsible for the poor outcome[4]. Thus, identification of novel molecular biomarkers predicting the clinical prognosis in NSCLC patients is particularly urgent.

Long non-coding RNAs (lncRNAs) are a class of newly discovered non-coding RNA molecules, which are greater than 200 nt and have no protein-coding ability[5]. Previous studies[6,7] revealed that lncRNAs are involved in many biological processes, including imprinting, epigenetic regulation, drug resistance, and tumor metastasis. Given the critical role of lncRNAs in progression and development of tumors, several researchers reveal potential mechanism by which lncRNAs exert their oncogenes or tumor suppressor. For instance, lncRNAs could influence the expression of tumor-related genes[8]. Recently, it was reported that lncRNAs are able to competitively inhibit miRNAs by acting as a molecular sponge[9]. Indeed, growing studies have demonstrated that lncRNAs can serve as prognostic markers in various tumors' types. However, only a few lncRNAs are studied.

In the present investigation, we focused on a new lncRNA, lncRNA LINC00261, which was located in 20p11.21. Although previous studies showed the role of LINC00261 in progression of several tumors[10,11], the function of LINC00261 in NSCLC has not been investigated. This is the first

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Table I. Sequence of the primers used in this study.

Genes	Primer sequences
LINC00261	Forward primer: GTCAGAAGGAAAGGCCGTGA Reverse primer: TGAGCCGAGATGAACAGGTG
GADPH	Forward primer: GCTCTCTGCTCCTCCTGTTC Reverse primer: ACGACCAAATCCGTTGACTC

report on the clinical significance of LINC00261 in patients with NSCLC.

Patients and Methods

Patients and Specimens

From 2007 to 2011, 150 NSCLC patients who underwent complete resection of the tumor in Beijing Chest Hospital, Capital Medical University, were subsequently enrolled in our study. The patients did not undergo radical surgery treatment, radiotherapy, and chemotherapy before surgery. All tissues were immediately frozen in liquid nitrogen after excision and, then, were stored at −80°C. The diagnosis of all samples was histopathologically confirmed by two pathologists. The overall survival was calculated from the day of primary surgery to death or last follow-up. The clinical features of all enrolled patients were shown in Table II. This examination was approved by the Research Ethics Committee of Beijing Chest Hospital, Capital Medical University. Written informed consent was obtained from all of the patients.

Quantitative Real-time PCR

Total RNA from tissues was extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA concentration and purity were determined using NanoDrop ND–1000. LINC00261 expression level was determined by qRT-PCR which was performed using SYBR Premix Ex TaqTM II (TaKaRa, Otsu, Shiga, Japan) on a Light Cycler. GAPDH was used to normalize mRNA expression levels. Primers for LINC00261 and GAPDH are listed in Table I. Relative quantification ($2^{-\Delta\Delta Ct}$) method was used for calculating fold changes. All reactions were run in triplicate.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism v5.0 (Graphpad Software Inc., La Jolla, CA, USA). The differences between two groups were analyzed using the two-sided Student's *t*-test. Relationships between LINC00261 expression level and the clinicopathological characteristics were studied using the chi-square test. Survival curves were obtained by the Kaplan-Meier method and compared by the log-rank test. Univariate and multivariate analysis were performed to identify the independent risk factors for NSCLC. The values were considered to be statistically significant at $p < 0.05$.

Results

Levels of LINC00261 in NSCLC Tissues and Matched Normal Tissues

Firstly, we analyzed the expression level of LINC00261 by qRT-PCR in NSCLC tissues and matched normal lung tissues. As shown in Figure 1, our results showed that LINC00261 was significantly downregulated in NSCLC tissues when compared with the paired adjacent normal tissues ($p < 0.01$). The data indicated that LINC00261 may play an anti-oncogene role in progression of NSCLC.

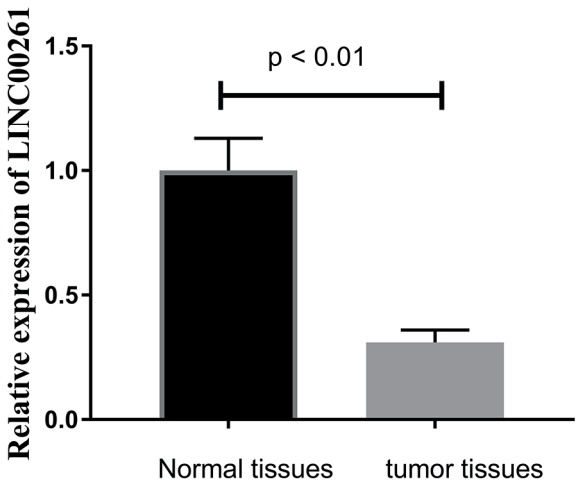

Figure 1. Comparison of LINC00261 expression levels between NSCLC tissues and adjacent normal lung tissues.

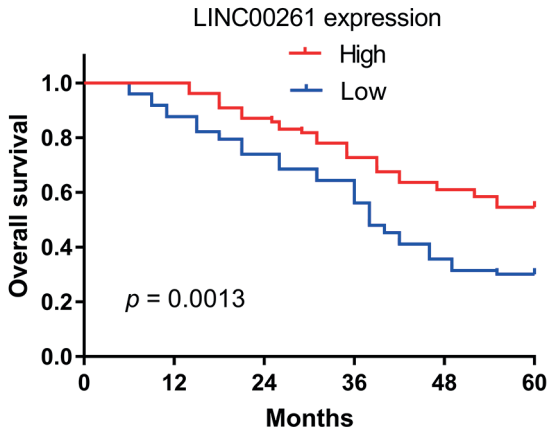

Figure 2. The expression of LINC00261 in relation to overall survival in the patients with NSCLC.

Association of LINC00261 Expression with Clinicopathological Characteristics of NSCLC Patients

To better understand the potential roles of LINC00261 in NSCLC development, the patients were divided into high and low expression groups by the median expression level of LINC00261 (4.17-fold). Then, we analyzed the association between LINC00261 expression and clinicopathological characteristics of NSCLC. As shown in Table II, we found that low expression of LINC00261 significantly correlated with TNM stage ($p = 0.005$), lymph node status ($p = 0.020$), and distant metastasis ($p = 0.004$). However, there was no association between LINC00261 expression and other clinical factors, such as age, gender, and tumor size, and histologic type ($p > 0.05$).

The Prognostic Significance of LINC00261 in NSCLC

Moreover, we performed Kaplan-Meier method to explore the prognostic value of LINC00261 in NSCLC patients. As shown in Figure 2, the overall survival of patients with low LINC00261 expression was significantly worse than that of LINC00261-high patients ($p = 0.0013$). In univariate analysis, TNM stage, lymph node status, distant metastasis, and low expression of LINC00261 were associated with poor survival. Further multivariate analysis indicated that LINC00261 expression was an independent prognostic factor in NSCLC (HR = 2.231, 95% CI: 1.448-4.138, $p = 0.004$) (Table III).

Discussion

Even though plenty of effort has been made to explore the diagnostic and prognostic biomarkers

Table II. Correlation of LINC00261 expression with clinicopathological features of NSCLC.

		LINC00261 expression		
Clinicopathological features	**Number of cases**	**High n (%)**	**Low n (%)**	***p*-value**
Age (years)				NS
< 60	60	31 (51.7)	29 (48.3)	
≥ 60	90	46 (51.1)	44 (48.9)	
Gender				NS
Male	95	47 (49.5)	48 (50.5)	
Female	55	30 (54.5)	25 (45.5)	
Tumor size (cm)				NS
< 3	52	24 (46.2)	28 (53.8)	
≥ 3	98	53 (54.1)	45 (45.9)	
Histologic type				NS
SCC	88	41 (46.6)	47 (53.4)	
AD	62	36 (58.1)	26 (41.9)	
TNM stage				0.005
I+II	93	56 (60.2)	37 (39.8)	
III+IV	57	21 (36.8)	36 (63.2)	
Lymph node status				0.020
Yes	48	18 (37.5)	30 (62.5)	
No	102	59 (57.8)	43 (42.2)	
Distant metastasis				0.004
Yes	47	16 (34)	31 (66)	
No	103	61 (62.1)	42 (37.9)	

Table III. Cox regression analysis of factors associated with overall survival in 150 NSCLC patients.

	Univariate analysis		Multivariate analysis	
Variables	**HR (95%CI)**	***p*-value**	**HR (95%CI)**	***p*-value**
Age (years), ≥60 vs. <60	1.381 (0.772-1.933)	0.371	-	-
Gender, Male vs. female	1.893 (0.482-2.684)	0.419	-	-
Tumor size (cm), <3 vs. ≥3	2.381 (0.894-3.131)	0.113	-	-
Histologic type, SCC vs. AD	1.669 (0.789-2.321)	0.193	-	-
TNM stage, III+IV vs. I+II	2.983 (1.783-5.993)	0.001	2.673 (1.483-5.213)	0.001
Lymph node status, Yes vs. No	3.213 (1.583-6.321)	0.008	2.783 (1.231-4.582)	0.013
Distant metastasis, Yes vs. No	2.563 (1.663-5.326)	0.001	2.103 (1.339-4.263)	0.003
Low LINC00261 vs. High LINC00261	2.731 (1.731-4.993)	0.002	2.231 (1.448-4.138)	0.004

for NSCLC, the sensitivities and specificities of current biomarkers are not satisfactory[12]. It has been confirmed that lncRNAs were involved in the tumorigenesis and progression of various tumors. The abnormal expression of lncRNAs are believed to serve as novel prognostic and diagnostic biomarkers in tumors[13,14]. Importantly, some lncRNAs have been shown to be significantly associated with prognosis of patients with NSCLC[15,16]. However, most of the lncRNAs have not been investigated.

Recently, several studies have revealed that the expression levels of lncRNAs are dysregulated in some different tumors, including NSCLC. For instance, Muller et al[10] reported that the expression levels of LINC00261 in pancreatic cancer tissues were significantly down-regulated compared with those in normal pancreatic tissues. Fan et al[17] found that over-expression of LINC00261 inhibited gastric cancer metastasis by affecting EMT. Further clinical research indicated that low LINC00261 expression was associated with poor prognosis of gastric cancer patients. Wang et al[18] reported that LINC00261 was lowly expressed in choriocarcinoma tissues, and its over-expression suppressed cell proliferation and invasion. Those findings indicated that LINC00261 functioned as a tumor suppressor in tumors. Recently, Yu et al[19] confirmed that LINC00261 was downregulated in NSCLC tissues compared with adjacent non-cancerous tissues. Given their findings, we further explored the biological function of LINC00261 in NSCLC.

In the present study, in line with the previous work, our results also showed that LINC00261 expression levels were significantly decreased in NSCLC tissues compared with matched non-cancerous tissues. Also, we found that low expression of LINC00261 was significantly correlated with TNM stage, lymph node status, and distant metastasis, which strongly suggested that LINC00261 was involved in metastasis of NSCLC. Further survival assay indicated that low LINC00261 expression level was associated with poorer overall survival. Finally, multivariate Cox hazard regression analysis identified low LINC00261 expression as an independent indicator of unfavorable prognosis.

To be honest, there are some limitations in our study. Firstly, we only explore the clinical significance of LINC00261 in patients with NSCLC. The tumor cell experiments have not been performed. Secondly, the results might be inaccurate to indicate the majority of NSCLC patients because of relatively small sample capacity. Thus, further *in vitro* and *in vivo* assay and a large sample size were needed to confirm our assessment.

Conclusions

We firstly explored the prognostic value of LINC00261 in NSCLC patients. Our findings suggested that LINC00261 may serve as a novel prognostic marker and therapeutic target in NSCLC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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