European Review for Medical and Pharmacological Sciences 2017; 21: 3808-3812

Long non-coding RNA Loc344887 is a potential prognostic biomarker in non-small cell lung cancer

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Abstract. – OBJECTIVE: Long non-coding RNA NmrA-like family domain containing 1 pseudogene (Loc344887) has been shown to be aberrantly expressed in non-small cell lung cancer (NSCLC). However, the biological function of Loc344887 in NSCLC remains unknown. The present study aimed to explore the prognosis value of Loc344887 in NSCLC patients.

PATIENTS AND METHODS: Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to detect the expression level of Loc344887 in NSCLC tissues and adjacent non-tumor lung tissues. Correlations between Loc344887 expression and the clinicopathological features of NSCLC patients were then evaluated. Overall survival was examined using Kaplan-Meier curves. Cox regression analyses were performed to judge whether plasma Loc344887 was an independent predictor of survival.

RESULTS: The expression levels of Loc344887 were increased in NSCLC tissues compared with those in normal lung tissues ($p < 0.05$). High Loc344887 expression level was significantly associated with lymph node metastasis ($p = 0.005$), advance stage ($p = 0.001$), and poorer differentiation ($p = 0.014$). Furthermore, patients with high expression of Loc344887 had a significantly shorter overall survival time compared with those with low Loc344887 expression in NSCLC ($p < 0.001$). Further analysis by Cox regression showed that high expression of Loc344887 in NSCLC was an independent predictor of poor prognosis.

CONCLUSIONS: These findings, for the first time, suggested the potential roles of Loc344887as a prognostic factor and as a therapeutic target for NSCLC.

Key Words:
Loc344887, NSCLC, Prognosis, Overall survival.

Introduction

Lung cancer accounts for 29% of male and 26% of female cancer estimated deaths worldwide and ranks first in terms of cancer-related mortality in the China[1,2]. The main types of lung cancer are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC)[3]. NSCLC is the most common type of lung cancer, and approximately 30% of patients have locally advanced disease at diagnosis[4]. Although there has been a great advancement on traditional and novel treatments, the overall survival of NSCLC patients remains poor because most patients are diagnosed after the tumor has metastasized[5,6]. Recent research indicated that using biomarkers to guide targeted therapy could be a benefit for the treatment of this malignancy[7]. Thus, much is needed to identify an efficient biomarker for early diagnosis and prognosis of NSCLC.

Long non-coding RNAs (lncRNAs) are important new members of the ncRNA family, which are longer than 200 nucleotides[8]. It has been confirmed that lncRNAs are involved in various biological functions and pathological processes, such as stem cell pluripotency, cellular development, and differentiation[9,10]. An increasing number of lncRNAs have been reported to play critical roles in cancer progression and metastasis[11,12]. In addition, recent studies[13-15] demonstrated that dysregulation of lncRNAs is correlated with diagnostic and prognostic markers for various tumors such as gastric cancer, glioma, and NSCLC. However, the detailed biological role of most lncRNAs remains unknown.

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Recently, a novel lncRNA, NmrA-like family domain containing 1 pseudogene (Loc344887), was reported to be up-regulated in NSCLC[16]. However, its clinical significance in NSCLC remains unknown. In the present work, we aimed to explore the expression pattern of Loc344887 and its prognostic value in patients with NSCLC.

Patients and Methods

Patient Data and Tissue Samples

Formalin-fixed and paraffin-embedded sections of paired NSCLC tissues and adjacent normal lung tissues were obtained from patients who underwent radical resection for NSCLC. All tissues were collected from July 2010 to December 2011 in the Shouguang People's Hospital and Linyi People's Hospital. None of the patients received chemotherapy, radiotherapy or immunotherapy before surgery. Among these 173 NSCLC cases, there were 74 males and 99 females, and their age ranged from 28 to 77 years. Patients were staged according to the 7th edition of the International System of Staging for Lung Cancer. Clinicopathologic information about the patient samples used in this research is presented in Table II. Overall survival is defined as the time elapsed from the surgery to the death of the patients with NSCLC. Survival data for patients that are listed as <1 month were omitted from the survival analysis. This study was approved by the Ethics Committee of The Shouguang People's Hospital and Linyi People's Hospital, and an informed consent form was signed by each patient.

RNA Extraction and Quantitative Real-time PCR

Total RNA was extracted from NSCLC tissues utilizing the Trizol reagent (Invitrogen, Carlsbad, CA, USA). The concentration and purity of the total RNA were detected with UV spectrophotometer analysis at 260 nm. The cDNA template was amplified by real-time PCR using the SYBR Premix Dimmer Eraser kit (TaKaRa Biotechnology, Dalian, China). Real-time PCR was performed using the SYBR Green PCR Kit purchased from TaKaRa Biotechnology. The levels of tissue Loc344887 were normalized using U6. Primer sequence was shown in Table I. Relative quantification of RNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate.

Table I. The primer sequence of Loc344887 and U6.

Gene	Sequence
Loc344887	F: 5'-GAGTGGTGGAAGTGGCTCTC-3'
	R: 5'-AGTTTGGCTGAGGATGTTCG-3'
U6	F: 5'-CTCGCTTCGGCAGCACA-3'
	R: 5'-AACGCTTCACGAATTTGCGT-3'

Statistical Analysis

All statistical analysis was performed using 5.0 (GraphPad Software, La Jolla, CA, USA). The results of two independent groups were analyzed using a *t*-test, and the association analysis was analyzed by Chi-square test. Survival curves were determined using the Kaplan-Meier method, and differences between them were evaluated by the log-rank test. The survival data were evaluated by univariate and multivariate Cox regression analyses. A *p*-value of 0.05 was used to determine statistical significance.

Results

The Expression of Loc344887 was Upregulated in NSCLC Tissues

To determine whether Loc344887 was up-regulated in NSCLC, we measured the Loc344887 expression level in 173 human NSCLC and pair-matched normal lung normal tissues using qRT-PCR. As shown in Figure 1, it was observed that the expression levels of Loc344887 in NSCLC tissues were significantly higher than those in pair-matched lung tissues ($p < 0.01$). These results were in line with the previous stu-

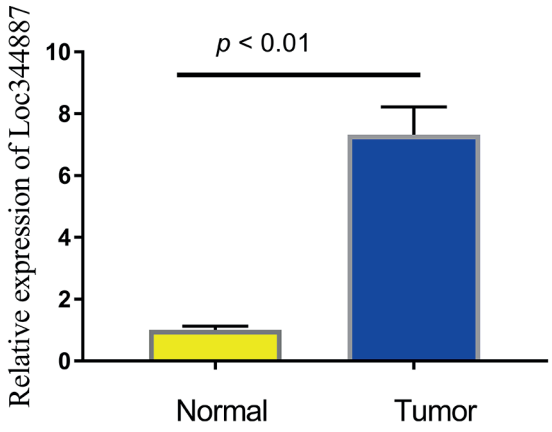

Figure 1. Loc344887 expression level in NSCLC tissues and normal lung tissues.

Table II. Correlation of Loc344887 expression with clinicopathological characteristics in NSCLC.

Variables	Case (n)	Loc344887 expression level High (n=87)	Low (n=86)	p
Age (years)				NS
≤65	78	37	41	
>65	95	50	45	
Gender				NS
Male	74	40	34	
Female	99	47	52	
Smoking				NS
Yes	118	61	57	
No	55	26	29	
Tumor size				NS
≤3cm	76	34	42	
>3cm	97	53	44	
Lymph node metastasis				0.005
Yes	60	39	21	
No	113	48	65	
Stage				0.001
I/II	113	46	67	
III/IV	60	41	19	
Differentiation				0.014
Well/Moderate	122	54	68	
Poor	51	33	18	

dy by Yu et al[16]. They suggested up-regulation of Loc344887 in NSCLC tissues by microarray gene expression analysis.

Association Between Loc344887 Expression and Clinical Characteristics in NSCLC

The associations of Loc344887 expression with various clinicopathological parameters of NSCLC tissues were analyzed by the chi-square test. The patients with NSCLC were divided into two groups according to the median value of Loc344887 expression level, including high-expression group (n = 87) and low-expression group (n = 86), respectively. The correlation between high Loc344887 expression with clinicopathological variables of 173 NSCLC patients was illustrated in Table II. We found that high Loc344887 was positively associated with lymph node metastasis ($p = 0.005$), stage ($p = 0.001$) and differentiation ($p = 0.014$) in NSCLC patients. However, no significant association was observed between Loc344887 expression and age, gender, smoking and tumor size ($p > 0.05$, respectively).

Association Between Loc344887 Expression and Prognosis in NSCLC Patients After Curative Surgery

Kaplan-Meier analysis was performed to analyze the correlation between Loc344887 expression and 173 patients' survival. As shown in Figure 2, the results showed that patients with higher Loc344887 expression had shorter overall survival time than that with lower Loc344887 expression ($p = 0.0011$). The 5-year survival rate was 67% in the low Loc344887 group compared with 87% in the high Loc344887 group.

Furthermore, we examined whether Loc344887 expression was an independent prognostic factor for NSCLC. Cox proportional hazards model analysis was performed. In univariate analysis,

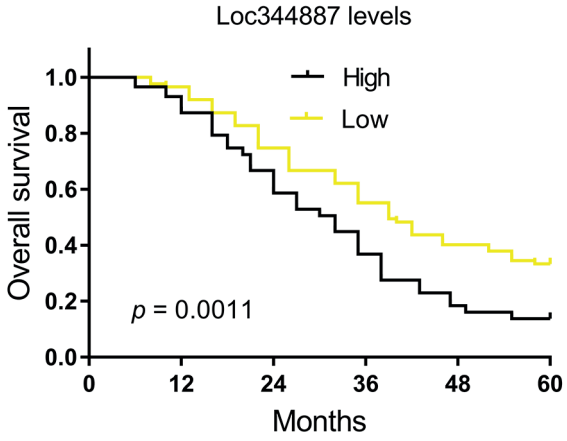

Figure 2. Survival analysis of the Loc344887 expression levels with overall survival of patients with NSCLC after surgery.

Table III. Prognostic factors for overall survival by univariate and multivariate analysis.

Clinicopathological features		Univariate analysis		Multivariate analysis	
		p-value	HR (95% CI)	*p*-value	HR (95% CI)
Age (years)	≤65 *vs*. >65	0.341	0.863 (0.683-1.441)	-	-
Gender	Male *vs*. Female	0.418	0.933 (0.731-1.642)	-	-
Smoking	Yes *vs*. No	0.558	0.641 (0.422-1.381)	-	-
Tumor size	≤3 cm *vs*. >3 cm	0.138	0.691 (0.413-1.327)	-	-
Lymph node metastasis	No *vs*. Yes	0.003	3.923 (1.523-6.523)	0.007	3.213 (1.231-5.239)
Stage	I/II *vs*. III/IV	0.006	3.131 (1.532-5.623)	0.009	2.783 (1.314-4.772)
Differentiation	Well/Moderate *vs*. Poor	0.011	3.312 (1.238-4.321)	0.015	2.893 (1.117-3.641)
Loc344887 levels	Low *vs*. High	0.003	4.317 (1.523-7.532)	0.005	3.783 (1.349-6.362)

we found that status of Loc344887 expression was significantly correlated with overall survival of NSCLC patients (p = 0.003, Table III). Moreover, in multivariate analysis, that Loc344887 level was also an independent prognostic factor for overall survival in NSCLC patients (p = 0.005, Table III).

Discussion

NSCLC remains the first tumor in morbidity and mortality among males in spite of significant improvements in its therapeutic strategy[17]. Patients are usually diagnosed with advanced stage NSCLC, which has limited therapy options[18]. Thus, there is an urgent need to detect NSCLC at an early stage to improve survivability with a better chance of cure. Many researchers believed that lncRNAs were ideal biomarkers for the diagnosis and prognosis of NSCLC, because they are simple to detect and toughly correlated with clinical progression[19].

Increasing evidences have demonstrated that the abnormally expressed lncRNAs have multiple functions in the regulation of NSCLC progression. For instance, Sun et al[20] reported that lncRNA NEAT1 was significantly increased in NSCLC and contributes to the development of NSCLC by regulation of miR-377-3p-E2F3 pathway. Li et al[21] found that lncRNA RGMB-AS1 promoted NSCLC cells proliferation, migration, and invasion, and predicted poor prognosis in patients with NSCLC. The findings by Nie et al[22] revealed that the upregulation of lncRNA ANRIL promoted proliferation of NSCLC cells by silencing KLF2 and P21 expression. Recently, a new lncRNA Loc344887 grasped our attention. It was found that Loc344887 expression was significantly up-regulated in NSCLC tissues by microarray gene expression analysis. Then, another study by Wu et al[23] reported that the expression of Loc344887 was significantly elevated in gallbladder cancer tissues and cell lines. *In vitro* assays showed that downregulation of Loc344887 suppressed migration, invasion, and proliferation by regulating induces EMT in gallbladder cancer. To our best knowledge, this is the first and only paper on the role of Loc344887 in tumors. Whether Loc344887 was involved in the progression of NSCLC remained unknown.

In the present investigation, we performed RT-PCR to detect the expression levels of Loc344887 in NSCLC tissues. We found that Loc344887 expression was significantly up-regulated in NSCLC tissues and these results were consistent with the results of microarray gene expression analysis. Then, we observed that Loc344887 expression correlated with lymph node metastasis, advanced stage, and poorer differentiation, which roughly indicated that Loc344887 may play a positive oncogene in the progression of NSCLC. Furthermore, Kaplan-Meier analysis revealed that patients with low level of Loc344887 had favorable trends of overall survival. Also, both the univariate and multivariate analyses confirmed that Loc344887 expression is an independent prognostic factor for overall survival.

Conclusions

Our present work firstly reports the prognostic value of Loc344887 in NSCLC patients. Low expression of Loc344887 is a good prognosis indicator in patients with NSCLC. Further studies are needed to investigate the mechanism underlying the oncogenetic function of Loc344887 for this malignancy.

Conflict of interest

The authors declare no conflicts of interest.

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