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Upregulation of LINC00504 is associated with aggressive progression and poor prognosis in non-small cell lung cancer

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Abstract. – OBJECTIVE: Long noncoding RNA LINC00504 (LINC00504) has been demonstrated to be dysregulated in several tumors. However, its function in tumor progression remains largely unclear. The goal of our present study was to determine the expressions of LINC00504 in non-small cell lung cancer (NSCLC) and its clinical associations.

PATIENTS AND METHODS: The expressions of LINC00504 were detected in 181 pairs of NSCLC specimens and adjacent normal tissues using qRT-PCR assays. Chi-square tests were applied to analyze the associations of LINC00504 expressions with various clinicopathological parameters of NSCLC specimens. The possible associations between LINC00504 expressions and overall survival were investigated using Kaplan-Meier assays. Univariate and multivariate assays were performed to further determine the prognostic value of LINC00504 levels in NSCLC patients.

RESULTS: We showed that LINC00504 expressions were distinctly decreased in clinical NSCLC specimens compared to adjacent non-tumor specimens ($p < 0.01$). High LINC00504 expressions were strongly associated with TNM stage ($p = 0.038$) and lymph node status ($p = 0.010$). In addition, Kaplan-Meier analysis indicated that NSCLC patients with high LINC00504 expression tend to have shorter overall survival ($p = 0.0036$). More importantly, the results of multivariate analysis indicated LINC00504 as an independent prognostic marker (p = 0.0028).

CONCLUSIONS: These findings revealed that LINC00504 might act as a prognostic biomarker for NSCLC patients.

Key Words:
LncRNA LINC00504, Non-small cell lung cancer, Prognosis.

Introduction

Lung cancer is the most commonly malignant neoplasm and one of the leading causes of cancer-associated deaths worldwide, responsible for > 620,000 deaths annually in China alone[1]. Non-small cell lung cancer (NSCLC) accounts for roughly 75% of lung cancer cases[2]. Despite the development in the current systematic treatments, including scientific surgical section with multiple beneficial techniques and adjuvant therapies over the last 20 years, the long-term survivals of patients with NSCLC remain poor[3,4]. Up to date, the recurrence and metastasis rate remains high, which contributes to the poor clinical outcome of NSCLC patients[5]. The detailed mechanisms underlying the etiology of this cancer are largely unclear. Therefore, it is of great significance to identify biomarkers for early detection and targeted treatments of NSCLC.

In recent years, several studies[6] have identified a group of long non-coding RNAs (lncRNAs) which was over 200 base pairs in length with no protein-coding capacity. LncRNAs have functional roles in regulating the expression of the encoded proteins by influencing their upstream promoters, which highlights the importance of lncRNAs in various physiological and pathological processes[7,8]. Growing dysregulated lncRNAs in various diseases were identified and their roles as new players in tumor progression by serving as tumor promoters or anti-oncogenes were also functionally explored[9,10]. Recently, a novel molecular mechanism has been discovered in which crosstalk between lncRNAs and mRNAs occurs by competing for shared miRNAs response elements[11,12]. Growing studies[13,14] about tumor-related projects provide

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the conclusive evidence which confirms that some functional lncRNAs may serve as miRNA sponges to suppress the expressions of miRNAs, eventually resulting in the increased expressions of the targeting genes at posttranscriptional level. These findings suggest that lncRNAs have great potential used as novel diagnostic and prognostic biomarkers[15]. However, many lncRNAs remain to be studied for their clinical significance.

Recently, Feng et al[16] identified a novel colon cancer-related lncRNA, LINC00504 whose high expression and tumor-promotive roles were functionally identified in colon cancer. Besides, previous studies also reported that LINC00504 was abnormally expressed in several tumors, such as head and neck squamous cell carcinoma and acute myeloid leukemia[17,18]. However, little was known about the expression and functions of LINC00504 in NSCLC. In this study, we aimed to preliminarily explore its prognostic value in this tumor.

Patients and Methods

Patients and Tissue Samples

We collected 181 paired primary NSCLC specimens and normal non-tumor specimens from NSCLC patients who had undergone surgical removal. All collected samples were frozen immediately in liquid nitrogen and stored at –80°C for RT-PCR assays. The diagnosis of NSCLC was demonstrated by two pathologists via the frozen section and a microscope. All patients were confirmed to receive treatments without chemotherapy or radiotherapy prior to excision. The clinical data from all patients were obtained using the observation of medical graphs and pathology information. The five-year survival rate of all NSCLC patients was retrieved from the follow-up center of our hospital. Informed consent was obtained from all patients. Our present research was approved by the Research Ethics Committee of Jining No.1 People's Hospital of Jining Medical University.

Quantitative Real-Time PCR

TRIzol which was purchased from TaKaRa Technology (Hangzhou, Zhejiang, China) was used for the preparation of total RNAs from NSCLC and normal lung specimens. The purity and concentration of total RNAs were determined by a spectrophotometer (NanoDrop, Zhezjiang, Hangzhou, China). For the reverse transcription of collected RNAs into cDNAs, the Primer-Script RT-PCR kit (Zhongxuan, Kunming, Yunnan, China) was used. Real-time PCR analyses were used for the determination of lncRNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which was used as internal control via SYBR Premix Ex Taq (TaKaRa, Pudong, Shanghai, China). The reaction conditions were: pre-denaturation at 95°C for 10 min for 1 cycle, denaturation at 95°C for 30 s, annealing at 58°C for 1 min, extension at 72°C for 30 s for a total of 40 cycles. All experiments were run in triplicate and the comparative cycle threshold (CT) methods were applied for the quantification of the relative levels of LINC00504. The primer used in this study were synthetized by Kesinai Technology (Pudong, Shanghai, Chian) and the primer sequence was as follows: primer sequence for LINC00504: sense: 5'-GCTATTTGCCATCGTGCTT-3'; antisense: 5'-AGTTTTGCCGAGAATGT-3'. For GAPDH: sense: CAATGACCCCTTCATTGAC: antisense: GACAAGCTTCCCGTTCTCAGT.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Student's *t*-tests were used to analyze the differences between the groups. The Chi-square test was applied for the examination of the associations between LINC00504 expressions and the clinicopathological factors. analysis. Survival curves were constructed by the use of the Kaplan-Meier methods. Cox regression assays were applied to examine factors related to survivals. A statistically significant difference was demonstrated using a value of $p < 0.05$.

Results

LINC00504 Is Markedly Upregulated in NSCLC Specimens

To study whether the levels of LINC00504 were altered in NSCLC progression, the expressions of LINC00504 in 181 pairs of NSCLC specimens and adjacent non-tumor specimens were examined applying qRT-PCR. As shown in Figure 1, the higher expressions of LINC00504 were observed in NSCLC samples compared with normal lung tissues ($p < 0.01$). Our findings suggested this new lncRNA as a functional factor in NSCLC progression.

Relationships Between LINC00504 Expressions and Clinicopathologic Features in NSCLC

To explore the clinical values of LINC00504 in NSCLC patients, 181 patients were divided in two

Table I. Correlation of LINC00504 expression with clinicopathological features of NSCLC.

Clinicopathological features	No. of cases	LINC00504 expression		*p*-value
		High	Low	
Age (years)				0.207
< 60	95	43	52	
≥ 60	86	47	39	
Gender				0.500
Male	115	55	60	
Female	66	35	31	
Tumor size (cm)				0.149
< 3	114	52	62	
≥ 3	67	38	29	
Histologic type				0.415
SCC	100	47	53	
AD	81	43	38	
TNM stage				0.038
I+II	116	51	65	
III+IV	65	39	26	
Lymph node status				0.010
Yes	134	59	75	
No	47	31	16	
Distant metastasis				0.039
Yes	133	60	73	
No	48	30	18	

groups (High group and Low Group) using the relative expression of LINC00504 in all NSCLC specimens. Then, the Chi-square test was applied for the statistics assays and the results were summarized in Table I. High LINC00504 expressions were strongly associated with distant metastasis (p = 0.039), TNM stage (p = 0.038), and lymph node status (p = 0.010). However, there were no significant correlations of LINC00504 expression with other clinical features (all $p > 0.05$). Thus, these findings provided preliminary evidence that increased LINC00504 levels may contribute to clinical progression of NSCLC.

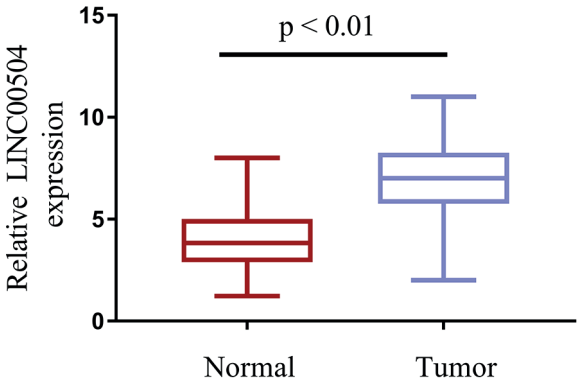

Figure 1. The relative levels of LINC00504 between NSCLC tissues and noncancerous lung tissues.

Prognostic Values of LINC00504 Expression in NSCLC Patients

For the exploration of the possible effects of high LINC00504 levels in clinical prognosis of NSCLC patients, we checked five-year survival data from follow-up department and performed the Kaplan-Meier methods to analyze these data. As shown in Figure 2, the results of log-rank tests revealed that the overall survival of patients with high levels of LINC00504 was distinctly shorter than those with low levels of LINC00504 (p = 0.0036). In addition, univariate analysis provided evidence that TNM stage, distant metastasis, lymph node status, and LINC00504 expression were associated with survival rate of NSCLC patients (All $p < 0.05$, Table II). Moreover, we performed multivariate analysis for further determination of prognostic value of LINC00504 expression in NSCLC patients. As shown in Table II, high LINC00504 expression was an independent prognostic marker for the overall survival of NSCLC patients (HR=2.895, 95% CI: 1.184-4.218, p =0.028).

Discussion

Nowadays, the number of new cases of NSCLC is rising rapidly and this cancer has become the

extreme obstacle in the improvement of life expectancy all over the world[19]. The poor prognosis of NSCLC patients is due to metastasis and recurrence. Facilitating the earlier screening and optimized treatments of NSCLC is critical for a favorable long-term survival[20]. Thus, the identification of sensitive biomarkers is necessary because of the small number of effective cancer biomarkers. In recent years, the detection of lncRNAs was considered to be ideal biomarker due to their regulatory effects in tumor development and the advancements of sequencing technology which made it possible to examine the levels of dysregulated lncRNAs in quantities[21,22]. Thus, the discovery of lncRNAs possessing diagnostic and prognostic values is of great significance.

Several lncRNAs have been reported as oncogenes or cancer promoters in NSCLC. For instance, lncRNA NKILA, a lowly expressed lncRNA in lung cancer which was demonstrated by RT-PCR, was clinically demonstrated to be associated with advanced clinical stages and a poorer overall survival, and its tumor-suppressive function in inhibiting metastasis of lung cancer cells was also confirmed i*n vitro* via the NF-κB/Snail signal[23]. LncRNA GAS5, a newly identified lncRNA whose downregulation was demonstrated in both lung cancer tissues and cells, was shown to suppress the proliferation and metastasis and to be involved in the modulation of radiosensitivity by sponging miRNA-135b[24]. Recently, LINC00504 as a novel lncRNA, attracted our attention and their roles in tumors remained largely unclear. Feng et al[16] firstly reported that LINC00504 was highly expressed in colon cancer, indicating that it may act as an oncogene in this tumor. Functional investigations indicated that knockdown of LINC00504 inhibited the proliferation and metastasis of colon cancer cells by interacting with c-Myc in both *in vitro* and *in vivo*. However, the roles of LINC00504 in other tumors have not been investigated.

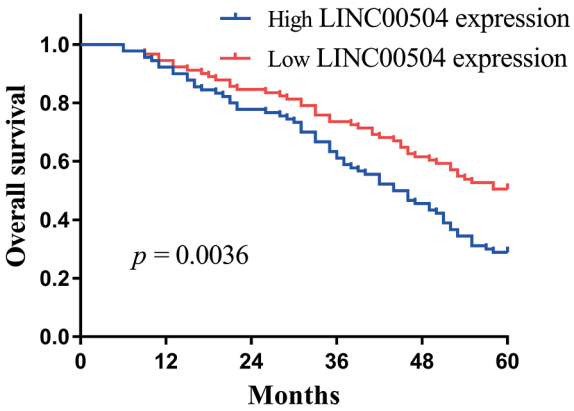

Figure 2. Correlation between LINC00504 expressions and survival in NSCLC patients using Kaplan-Meier survival analysis.

In this study, by examining the levels of LINC00504 in NSCLC specimens and matched normal tissues from 181 patients from our hospital, we preliminarily confirmed that LINC00504 expression was upregulated in NSCLC specimens compared to matched non-tumor tissues. Then, we explored the clinical significance of LINC00504 expressions in NSCLC patients using Chi-square tests, finding that high LINC00504 levels were associated with distant metastasis, TNM stage, and lymph node status. Moreover, the prognostic value of LINC00504 was further explored using Kaplan-Meier methods, which provided evidence that high LINC00504 levels predicted a shorter five-year overall survival of NSCLC patients. Finally, we carried out multivariate analysis to determine the value of clinical application of LINC00504 as a novel biomarker, finding that

Table II. Cox regression analysis of factors associated with overall survival in 181 NSCLC patients.

	Univariate analysis		Multivariate analysis	
Variables	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Age (years)	0.896 (0.432-1.775)	0.216	-	-
Gender	0.985 (0.572-1.928)	0.185	-	-
Tumor size (cm)	1.336 (0.672-2.327)	0.157	-	-
Histologic type	1.285 (0.785-2.428)	0.118	-	-
TNM stage	2.986 (1.327-4.762)	0.013	2.675 (1.146-4.426)	0.018
Lymph node status	2.776 (1.472-4.452)	0.019	2.554 (1.261-4.028)	0.031
Distant metastasis	3.018 (1.387-4.882)	0.009	2.875 (1.174-4.462)	0.014
LINC00504 expression	3.261 (1.366-4.364)	0.014	2.895 (1.184-4.218)	0.028

LINC00504 might be a potential molecular biomarker for predicting the clinical outcome of NSCLC patients.

Conclusions

We first provided evidence that LINC00504 expressions were distinctly upregulated in NSCLC and correlated with unfavorable patients' prognosis, suggesting that it may have potential use as a novel prognostic biomarker for NSCLC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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