RESEARCH ARTICLE

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Increased levels of long noncoding RNA LINC00691 correlate with poor prognosis in non–small-cell lung cancer patients

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Abstract

Background: Although long intergenic non-protein coding RNA 691 (LINC00691) has been functionally identified in several tumors, the association between LINC00691 and non–small-cell lung cancer (NSCLC) has not been reported. The objective of our study was to explore the clinical significance of LINC00691 in NSCLC.

Methods: RT-PCR was performed to detect LINC00691 levels in 177 pairs of human NSCLC tissues and matched normal lung specimens. A chi-squared test was used to analyze the possible influence of LINC00691 on the clinical progress of NSCLC. Kaplan-Meier methods were used to determine differences in patient survival. The prognostic value of survival variables was evaluated using univariate and multivariate analyses.

Results: We found that LINC00691 levels were increased in tumor specimens compared with matched normal lung tissues ($P < .01$). Increased LINC00691 levels correlated with lymph node metastasis ($P = .025$) and advanced TNM stage ($P = .002$) in NSCLC patients. Moreover, clinical investigations revealed that NSCLC patients with high LINC00691 expression had a shorter overall survival than those with low LINC00691 expression ($P = .0042$). Finally, Cox regression assays confirmed LINC00691 as an independent prognostic factor for NSCLC patients.

Conclusions: The aberrant expression of LINC00691 may function as a reliable marker for the progression and prognosis of NSCLC in patients.

KEYWORDS
LINC00691, long noncoding RNA, NSCLC, prognosis

1 | INTRODUCTION

Lung cancer is the leading cause of neoplasm-associated death worldwide, accounting for approximately one million deaths per year around the world.[1] About 85% of all lung cancer is estimated to be non–small-cell lung cancer (NSCLC). Although several diagnostic techniques and treatments for lung cancer have been developed, the overall five-year survival rate of advanced NSCLC is still < 15%.[2,3] The poor clinical outcome has been attributed to neoplasm invasion, distant metastasis, and recurrence.[4] Thus, it is still urgent to explore

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precise and unique markers for developing effective therapies. Some study found there is a strong association between methylation of the RUNX3 promoter and NSCLC.[5]

Noncoding RNAs have recently emerged as decisive modulators of gene expression.[6] Long noncoding RNAs (lncRNAs) that are composed of >200 nucleotides usually display a limited capacity for encoding protein.[7] They have been detected with the progression of whole-genome sequencing technology.[8] Increasing evidence has indicated that lncRNAs act as important modulators in various cellular processes, including cell growth, differentiation, invasion, and apoptosis.[9,10] Furthermore, several studies have revealed that many lncRNAs are abnormally expressed in various tumors and involved in the development and progression of tumors.[11] For instance, studies have shown the involvement of lncRNA SNHG12 in NSCLC and gastric carcinoma,[12,13] lncRNASPRY4-IT1 in hepatocellular carcinoma and ovarian cancer,[14,15] and lncRNA MEG3 in gastric cancer and cervical cancer.[16,17] Thus, lncRNAs have the potential to be prognostic and diagnostic molecular markers, as well as therapeutic targets for NSCLC in the clinic.

Long intergenic non-protein coding RNA 691 (LINC00691) is a novel lncRNA that was newly identified as being unregulated in NSCLC by amazing microarray data.[18] Recently, the high expression of LINC00691 has been reported in several tumors, including renal cell carcinoma and head and neck squamous cell carcinoma.[19,20] However, the expression profiles and functions of LINC00691 in NSCLC patients remain largely unknown, and its effects in other tumors have not been reported. In this study, we aimed to further confirm whether LINC00691 is deregulated in NSCLC. We also aimed to study the prognostic value of LINC00691 in NSCLC patients.

2 | PATIENTS AND METHODS

2.1 | Patients and tissue samples

In total, NSCLC tissues and normal lung specimens were collected between July 2011 and June 2015 from 177 patients diagnosed with NSCLC. All patients were treated at The First Affiliated Hospital of Shandong First Medical University. Patients had not received chemotherapy or anti-tumor therapy and were aged between 41.6 and 70 years (average age, 54.7 years). NSCLC and normal lung specimens were demonstrated histological. All samples were collected and stored at −80°C for further RNA experiments. Clinical and biological information was available for all patients. The clinic pathological data are shown in Table 1. We calculated overall survival time based on the time elapsed between the surgical procedure and the patient's death. Written informed consent was obtained from all participants.

2.2 | RNA extraction and RT-PCR assays

Total RNA was extracted from NSCLC tissues and matched normal lung tissues using TRIZOL reagent (Invitrogen). RNA concentration

TABLE 1 Correlation between LINC00691 expression and clinic pathological factors of NSCLC patients

Characteristics	Numbers	LINC00691 expression		P value
		Low	High	
Sex				
Male	110	51	59	NS
Female	67	34	33	
Age				
≤60	82	40	42	NS
>60	95	45	50	
Histological grade				
Middle or low	126	64	62	NS
High	51	21	30	
Histological classification				
SCC	87	46	41	NS
AD	90	39	51	
Tumor size				
≤3 cm	108	56	52	NS
>3 m	69	29	40	
TNM stage				
I-II	115	65	50	.002
III-IV	62	20	42	
Lymph node metastasis				
Negative	130	69	61	.025
Positive	47	16	31	
History of smoking				
Ever	100	49	51	NS
Never	77	36	41	

and purity were determined with the use of the Nanodrop[#01] spectrophotometer (Thermo Fisher Scientific). The Reverse Transcription System Kit (TaKaRa) was used for the reverse transcription of total RNA into cDNA. RT-PCR was performed on the Bio-Rad CFX99 real-time PCR System (Biosystems) using KAPA-PROBEE FAST[#] qPCR Kits (Promega) and TaqMan probes (Invitrogen). GAPDH was used as an internal control. The detailed primer sequences included in this study are shown in Table 2. The fold changes between the two groups were calculated using relative quantification ($2^{-\Delta\Delta Ct}$) methods as described previously.

2.3 | Statistical analysis

All statistical analyses were carried out using SPSS 19.0 (IBM SPSS Statistics for Windows). The differences between groups were analyzed using the Student's t test or chi-squared test. The Kaplan-Meier method was used to analyze the possible association between

TABLE 2 Primer sets used in the present study

Gene names	Sequences (5'-3')
LINC00691: Forward	AGGGATTCTGGGCTACGAGT
LINC00691: Reverse	ATGTGCTCTTCTTGCCTTGG
GAPDH: Forward	CTTTGGTATCGTGGAAGGACTC
GAPDH: Reverse	GTAGAGGCAGGGATGATGTTCT

the upregulation of LINC00691 and clinical factors. The prognostic value of LINC00691 was further verified using the Cox proportional-hazards regression model. A value of $P < .05$ was considered to be statistically significant.

3 | RESULTS

3.1 | LINC00691 expression was upregulated in NSCLC specimens

To study the effects of LINC00691 expression on the progression of NSCLC, we detected its expression in 177 NSCLC patients. As shown in Figure 1, we found that LINC00691 was distinctly unregulated in NSCLC tissues compared with the matched normal tissues ($P < .01$). Our observations were consistent with previous microarray data analyzed by Zhao et al[18] Thus, our results, together with previous results, confirmed that LINC00691 expression is increased in NSCLC and could play a functional role.

3.2 | LINC00691 expression was not associated with the clinic pathological parameters of NSCLC

We explored the clinical relationship of LINC00691 expression in NSCLC patients. All NSCLC tissues expressing LINC00691 at levels less than the median expression level were assigned to the low-expression group. The others were assigned to the high-expression group. As presented in Table 2, the patients with advanced TNM stages ($P = .002$) and positive lymph node metastasis ($P = .025$) exhibited higher levels of LINC00691. However, no association between the levels of LINC00691 and additional clinical factors, such as gender and histological grade, was observed.

3.3 | Increased LINC00691 correlated with unfavorable clinical outcomes

Next, we performed survival analysis with clinical follow-up information to evaluate whether the deregulation of LINC00691 levels has prognostic potential for the overall survival of NSCLC patients. As shown in Figure 2, the data of the Kaplan-Meier assays revealed that the 5-year overall survival rate was 23.06% in the group with a low level of expression of LINC00691, and 44.15% in the group with high LINC00691 tissue expression. Moreover, the log-rank
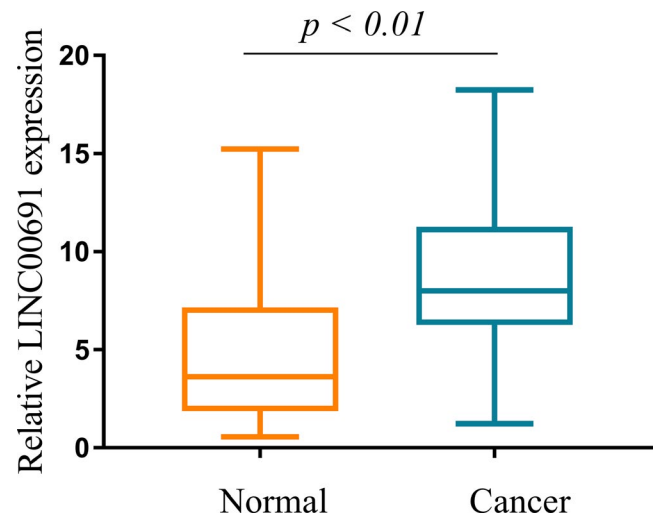

FIGURE 1 Expression of LINC00691 determined by quantitative real-time PCR in 177 paired human NSCLC and adjacent normal tissues

test confirmed that patients with higher LINC00691 expression had a shorter overall survival time than those with lower LINC00691 expression ($P = .0042$). More importantly, univariate Cox regression assays revealed that the TNM stage and LINC00691 expression were possible prognosis-related factors. In addition, we further performed multivariate analyses, confirming increased LINC00691 expression to be a poor independent prognostic factor for NSCLC patients (HR = 3.016, 95% CI: 1.217-3.889, $P = .006$, Table 3).

4 | DISCUSSION

NSCLC is the most common neoplasm type and has the highest mortality rate in China.[21] To date, the potential mechanisms underlying tumor genesis and the progression of tumors remain largely unclear, resulting in ineffective treatment for NSCLC patients. Predicating
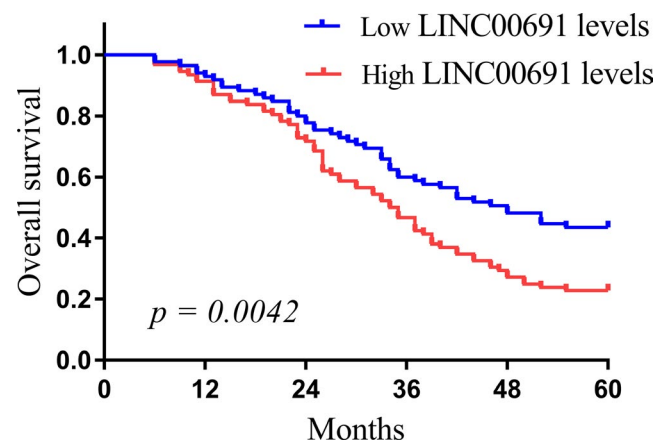

FIGURE 2 LINC00691 expression levels divided into high-expression and low-expression groups. High expression of LINC00691 indicated poor prognosis in NSCLC patients

Risk factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
TNM stage	3.327	1.325-4.652	.001	2.785	1.126-4.023	.004
Lymph node metastasis	2.988	1.462-3.955	.006	2.654	1.215-3.766	.018
LINC00691 expression	3.561	1.562-4.346	.002	3.016	1.217-3.889	.006
Age	1.325	0.658-1.784	.236	-	-	-
Histological grade	1.289	0.781-1.654	.128	-	-	-
Histological classification	1.389	0.845-2.126	.117	-	-	-
Tumor size	1.235	0.733-2.126	.239	-	-	-
History of smoking	1.171	0.568-1.774	.472	-	-	-

TABLE 3 Univariate and multivariate analysis of clinic pathologic factors for overall survival in 177 patients with NSCLC

the clinical prognosis of NSCLC patients is essential for individualized treatments, and several methods have been used to determine the clinical progression of NSCLC.[22,23] However, effective methods are limited. The identification of new targets involved in NSCLC tumor genesis may result in the development of novel diagnostic and prognostic strategies. Recently, with the application of high-throughput sequencing and bioinformatics, more and more dysregulated lncRNAs have been identified in various tumor tissues, including NSCLC.[24,25] In addition, a growing number of studies have shed light on the biogenesis and function of lncRNAs. In the future, lncRNAs may become novel diagnostic and prognostic biomarkers for tumors.

Since the first lncRNA was identified, more than 100 lncRNAs have been reported to be abnormally expressed in various tumors, including NSCLC. Sun et al[26] reported that lncRNA NEAT1, a functional lncRNA reported in various tumors, is also highly expressed in NSCLC, where its overexpression distinctly results in the acceleration of NSCLC cell proliferation and metastasis via modulation of the miRNA-377-5p-E2F3 axis. Zhang et al[27] showed that lncRNA LINC00222 exerts oncogenic activity in NSCLC because its overexpression inhibits the proliferation and metastasis of tumor cells by regulating glycogen synthase kinase 3 β (GSK3β) activity. Ke et al[28] indicated that high expression of lncRNA ATB correlates with an advanced clinical stage and an unfavorable prognosis for NSCLC patients. In addition, its overexpression promotes NSCLC cell proliferation and metastasis. These results imply that lncRNAs have an important role in NSCLC. Recently, a newly identified lncRNA LINC00691 attracted our attention because of its high levels in microarray data. However, its role in tumors remains largely unstudied, and whether LINC00691 is involved in the clinical progression has not been investigated.

In this study, by detecting the level of LINC00691 in 177 NSCLC patients, we further confirmed that LINC00691 is an unregulated lncRNA in NSCLC, which is in line with previous results. First, we analyzed the association between LINC00691 and clinical factors, finding that patients with advanced TNM stage and positive metastasis exhibited high levels of LINC00691. Then, we performed Kaplan-Meier assays to explore whether LINC00691 could be involved in

the clinical prognosis of NSCLC patients, and the observations revealed that the overall survival of patients with high LINC00691 expressions was distinctly reduced. Finally, using the multivariate Cox proportional-hazard model, we confirmed that high LINC00691 expression is recognized as an independent prognostic factor for NSCLC patients. Overall, our present study has provided clinical evidence that LINC00691 may be a potential and useful tumor marker for the prognosis of NSCLC.

Some limitations of this study should be noted. First, because of the small sample size in the current study, further studies using a larger number of patients are required to confirm our findings. Second, neither functional assays nor an investigation on mechanisms was performed. Additionally, a meta-analysis found that central obesity is associated with lung cancer development.[29] However, the prevalence of obesity in primary students and adults population is high.[30,31] In our subsequent study, we plan to study the potential mechanism by which LINC00691 influences the clinical prognosis.

5 | CONCLUSIONS

Our present study, for the first time, has provided important clinical evidence that LINC00691 is highly expressed in NSCLC and is associated with poor clinical prognosis for NSCLC patients. The identification of lncRNAs as a potential prognostic marker will help build multiparametric models for prognostic purposes in NSCLC.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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