INTRODUCTION

Lung cancer is the leading cause of neoplasm-associated death worldwide, accounting for approximately one million deaths per year around the world. 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%. The poor clinical outcome has been attributed to neoplasm invasion, distant metastasis, and recurrence. Thus, it is still urgent to explore
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 lncRNAASPRY4-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 and purity were determined with the use of the Nanodrop® 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−ΔΔCt) 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 1 Correlation between LINC00691 expression and clinic pathological factors of NSCLC patients |
|---|---|---|---|
| Characteristics | Numbers | LINC00691 expression | P value |
| 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 cm | 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 | |
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.²⁻¹⁸ 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 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
the clinical prognosis of NSCLC patients is essential for individualized treatments, and several methods have been used to determine the clinical progression of NSCLC.\textsuperscript{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.\textsuperscript{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\textsuperscript{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\textsuperscript{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\(\beta\) (GSK3\(\beta\)) activity. Ke et al\textsuperscript{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.\textsuperscript{29} However, the prevalence of obesity in primary students and adults population is high.\textsuperscript{30,31} In our subsequent study, we plan to study the potential mechanism by which LINC00691 influences the clinical prognosis.

### 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.

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### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: Cancer J Clin. 2015;65(1):5-29.
2. Hirsch FR, Scagliotti GV, Mulshine JL, et al. Lung cancer: current therapies and new targeted treatments. Lancet (London, England). 2017;389(10066):299-311.
3. Dutkowska AE, Antczak A. Comorbidities in lung cancer. Pneumonol Alergol Pol. 2016;84(3):186-192.
4. Chivima B. Lung cancer. Nursing Stand (Royal College of Nursing (Great Britain)). 1987;29(22):61.
5. Liang Y, He L, Yuan H, Jin Y, Yao Y. Association between RUNX3 promoter methylation and non-small cell lung cancer: a meta-analysis. J Thorac Dis. 2014;6(6):694-705.
6. Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (Review). Oncol Rep. 2017;37(1):3-9.
7. Carpenter S. Long noncoding RNA: Novel links between gene expression and innate immunity. Virus Res. 2016;212:137-145.
8. Lagarde J, Uszczynska-Ratajczak B, Carbonell S, et al. High-throughput annotation of full-length long noncoding RNAs with capture long-read sequencing. Nat Genet. 2017;49(12):1731-1740.
9. Ferre F, Colanenti A, Helmer-Citterich M. Revealing protein-RNA interaction. Brief Bioinform. 2016;17(1):106-116.
10. Tani H, Imamachi N, Mizutani R, et al. Genome-wide analysis of long noncoding RNA turnover. Methods Mol Biol (Clifton, NJ). 2015;1262:305-320.
11. Luo ML. Methods to Study Long Noncoding RNA Biology in Cancer. Adv Exp Med Biol. 2016;927:69-107.
12. Wang P, Chen D, Ma H, Li Y. LncRNA SNHG12 contributes to multidrug resistance through activating the MAPK/Slug pathway by sponging miR-181a in non-small cell lung cancer. Oncoarget. 2017;8(48):84086-84101.
13. Yang BF, Cai W, Chen B. LncRNA SNHG12 regulated the proliferation and apoptosis by regulating miR-21. Cancer Biol Ther. 2016;17(1):104-113.
14. Wang T, Huang W, Lu H, et al. Identification and validation of a TGF-beta-associated long non-coding RNA of head and neck squamous cell carcinoma by bioinformatics method. J Transl Med. 2018;16(1):46.
15. Chen B, Wang C, Zhang J, Zhou Y, Hu W, Guo T. New insights into long noncoding RNAs and pseudogenes in prognosis of renal cell carcinoma. Cancer Cell Int. 2018;18:157.
16. She J, Yang P, Hong Q, Bai C. Lung cancer in China: challenges and interventions. Chest. 2013;143(4):1117-1126.
17. Crawford J, Wheatley-Price P, Feliciano JL. Treatment of lung cancer in medically compromised patients. Am Soc Clin Oncol Educ Book Am Soc Clin Oncol Annual Meeting. 2016:35:e484-491.
18. Ke L, Xu SB, Wang J, Jiang XL, Xu MQ. High expression of long non-coding RNA ATB indicates a poor prognosis and regulates cell proliferation and metastasis in non-small cell lung cancer. Cancer Cell Int. 2016;16:927-937.
19. Zhang H, Wang Y, Lu J, Zhao Y. Long non-coding RNA LINC00222 regulates GSK3beta activity and promotes cell apoptosis in lung adenocarcinoma. Biomed Pharmacother = Biomedecine & pharmacotherapie. 2018;106:755-762.
20. Sun C, Li S, Zhang F, et al. Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-E2F3 pathway. Oncotarget. 2016;7(32):51784-51814.
21. Gao J, Lin X, He Y, et al. The Comparison of Different Obesity Indexes and the Risk of Lung Cancer: A Meta-Analysis of Prospective Cohort Studies. Nutr Cancer. 2019;71(6):908-921.
22. He L, Ren X, Chen Y, et al. Prevalence of overweight and obesity among primary school children aged 5 to 14 years in Wannan area. China. Nutr Hosp. 2014;30(4):776-781.
23. She J, Cao B, Wang X, et al. Clinical characteristics and interventions. J Transl Med. 2016;15(1):599-605.
24. Gao J, Lin X, He Y, et al. The Comparison of Different Obesity Indexes and the Risk of Lung Cancer: A Meta-Analysis of Prospective Cohort Studies. Nutr Cancer. 2019;71(6):908-921.
25. He L, Ren X, Chen Y, et al. Prevalence of overweight and obesity among primary school children aged 5 to 14 years in Wannan area. China. Nutr Hosp. 2014;30(4):776-781.
26. He L, Ren X, Qian Y et al Prevalence of overweight and obesity among a university faculty and staffs from 2004 to 2010 in Wuhu, China. Nutr Hosp. 2014;29(5):1033-1037.

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