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LINC01117 promotes the malignant proliferation of lung adenocarcinoma by increasing HOXD8 mRNA stability

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Abstract. The present study aimed to investigate the functional role and molecular mechanisms of long intergenic non-protein coding RNA 1117 (LINC01117) in lung adenocarcinoma (LUAD). Bioinformatics analysis was performed to assess the expression level and prognostic value of LINC01117. Gene expression in LUAD and lung squamous cell carcinoma tissues was determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and gene and protein expression levels in LUAD cells were detected using RT-qPCR and western blotting, respectively. Cell Counting Kit-8, 5-ethynyl-2'-deoxyuridine, live cell imaging and colony formation assays were used to evaluate the proliferation of LUAD cells. Actinomycin D was used to inhibit RNA transcription. The results indicated that LINC01117 was significantly upregulated in both LUAD and lung squamous cell carcinoma, and its elevated expression was positively associated with poor prognosis in LUAD. Knockdown of LINC01117 inhibited cell proliferation, whereas its overexpression promoted proliferation. Furthermore, LINC01117 exhibited positive co-expression with the transcription factor homeobox D8 (HOXD8); knockdown or overexpression of LINC01117 reduced or increased HOXD8 expression at both the RNA and protein levels, respectively. Conversely, HOXD8 knockdown did not significantly alter LINC01117 expression; however, HOXD8 knockdown inhibited cell proliferation and reversed the pro-proliferative effects induced by LINC01117 overexpression. Mechanistically, LINC01117 was associated with increased HOXD8 mRNA stability. In conclusion, LINC01117 may serve as a notable prognostic biomarker that promotes cell proliferation in LUAD. HOXD8 may function as a downstream target of LINC01117, with LINC01117 exerting its pro-proliferative effects by increasing HOXD8 mRNA stability.

Introduction

Among all cancer types, lung cancer has the highest incidence and is the most common cause of cancer-related deaths worldwide (1). Non-small cell lung cancer (NSCLC) constitutes >85% of lung cancer cases, with lung adenocarcinoma (LUAD) representing the most prevalent pathological subtype (2). Despite notable advances in targeted therapy and immunotherapy in recent years, the survival rate for patients with LUAD remains <20% (3). Furthermore, LUAD is a highly heterogeneous tumor (4) and different patients with the same subtype may exhibit marked variations in response to the same treatment (5,6). Therefore, exploring the molecular mechanisms underlying LUAD progression and identifying reliable biomarkers are essential for developing individualized treatment and improving patient survival outcomes.

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts >200 nucleotides in length that lack protein-coding capacity (7). Accumulating evidence has confirmed that lncRNAs participate in diverse biological processes through mechanisms such as transcriptional regulation, post-transcriptional modulation and epigenetic modification (8-12). Extensive research has also shown that lncRNAs modulate critical processes such as cell proliferation, metastasis and drug resistance, thereby either promoting or suppressing tumor development (13-16). Notably, certain lncRNAs have shown promise as diagnostic or prognostic biomarkers, and have the potential to serve as therapeutic targets (17-19). Therefore, investigating the role of lncRNAs in tumors is critical for elucidating pathogenesis and advancing personalized therapeutic strategies.

Long intergenic non-protein coding RNA 1117 (LINC01117), located at chromosome 2q31.1, is a relatively novel lncRNA that has only been investigated in a limited number of studies thus far. One study has suggested that LINC01117 may be included in a prognostic gene signature for breast cancer (20). A recent study reported that, in postmenopausal patients with uterine fibroids, LINC01117 was markedly

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downregulated in fibroid tissues compared with in the adjacent myometrium, whereas no notable change was observed between these tissues in premenopausal patients with uterine fibroids (21). Another study has demonstrated that LINC01117 may promote the migration of LUAD cells (22). However, the molecular mechanisms by which LINC01117 may promote malignant proliferation in LUAD remains unclear.

lncRNAs are closely associated with protein-coding genes (PCGs) and regulate various biological processes through these interactions (23). Homeobox D8 (HOXD8), a transcription factor located on chromosome 2q31.1, is positioned upstream of LINC01117. The function of HOXD8 in tumorigenesis remains controversial; while some studies have proposed that it acts as a tumor suppressor and inhibits cancer progression (24-26), others have indicated that it may function as an oncogene. For example, HOXD8 hypermethylation has been identified as a highly sensitive and specific biomarker for biliary tract cancer (27) and one study has demonstrated its elevated expression in NSCLC tissues, where it may promote proliferation, cancer stemness and migratory abilities (28). Given their genomic proximity, further investigation into the functional relationship between LINC01117 and HOXD8 is warranted. Therefore, the present study aimed to investigate the functional roles of LINC01117 in LUAD and explore its regulatory mechanism involving HOXD8.

Materials and methods

Data acquisition from The Cancer Genome Atlas (TCGA) database. In the present study, RNA sequencing (RNA-seq) data and the related clinical characteristics for 535 LUAD and 502 lung squamous cell carcinoma (LUSC) tumor tissues, along with 59 non-tumor control samples from patients in TCGA-LUAD cohort and 49 non-tumor control samples from patients in TCGA-LUSC cohort, were acquired from TCGA database (https://portal.gdc.cancer.gov/). Part of the bioinformatics analyses was performed using R software (version 4.4.2; https://www.r-project.org/) (29). The R package 'edgeR' (version 4.4.2) was used to perform differential expression analysis on these RNA-seq data (30). Differential expression was assessed based on log2 fold change and false discovery rate. Patients with an overall survival (OS) time of <60 days were excluded to minimize bias from non-cancer-related deaths or perioperative mortality, which may not accurately represent the true disease prognosis. After excluding patients with an OS of <60 days or incomplete clinical characteristics, 448 patients with LUAD and 444 patients with LUSC were retained for subsequent analysis. The evaluated clinical parameters included survival status, sex, age, tumor size (T) stage, lymph node metastasis (N) stage and tumor-node-metastasis (TNM) stage. The clinical characteristics of patients from TCGA cohort are listed in Table I.

Survival analyses. Survival analyses were performed using the Kaplan-Meier (KM) method and Cox proportional hazards regression. Patients with LUAD from TCGA were stratified into high- and low-expression groups using the median LINC01117 expression as the cutoff. The median expression level was 3.44 in LUAD and 12.59 in LUSC. OS differences were assessed using the two-sided log-rank test. KM curves were fitted using the 'survival' package (version 3.7.0; https://CRAN.R-project.org/package=survival) (31) and visualized using 'survminer' (version 0.5.1; https://CRAN.R-project.org/package=survminer) (32) in R. Univariate Cox regression was conducted to evaluate the association between LINC01117 expression and OS using the 'survival' package (version 3.7.0). Multivariate Cox regression was further performed to determine whether LINC01117 was an independent prognostic factor, adjusting for age, sex, T stage, N stage and TNM stage. Hazard ratios and 95% confidence intervals were calculated.

Correlation analysis. Raw RNA-seq count data from TCGA-LUAD cohort were normalized for library size and composition bias using the trimmed mean of M-values method in the edgeR package. Based on the edgeR-normalized RNA-seq expression matrix of patients with LUAD, the correlations between LINC01117 and 17,109 PCGs from TCGA-LUAD cohort were evaluated in R (version 4.4.2). Gene expression values were log2-transformed [log2(x + 1)] prior to correlation analysis. The 'limma' package (version 3.62.2; https://bioconductor.org/packages/limma) (33) was used for preprocessing of the expression matrix, and Pearson correlation coefficients and corresponding P-values were calculated using the cor.test function from the stats package (version 4.4.2), which is included in the base R distribution. Genes with r>0.4 and P<0.05 were considered significantly correlated, and scatter plots with fitted regression lines were generated for visualization.

Correlation analysis using gene expression profiling interactive analysis (GEPIA). To further investigate the correlation between LINC01117 and HOXD8, the GEPIA online database (http://gepia.cancer-pku.cn/) was used. The TCGA-LUAD tumor cohort was selected, and the correlation between the two genes was assessed based on GEPIA-provided log2TPM expression values. Pearson's correlation coefficient was calculated and the corresponding scatter plot was downloaded.

Tissue collection. Fresh-frozen paired tumor and adjacent non-tumor tissues (21 LUAD and 9 LUSC cases) were used to verify the expression of LINC01117. The median age of patients with LUAD was 60 years (range, 40-76 years), including 11 women and 10 men. The median age of patients with LUSC was 61 years (range, 45-73 years), including 3 women and 6 men. These tissue specimens were collected from The Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) between May 10, 2016 and July 25, 2019. All patients were newly diagnosed with tumors, and had not undergone radiotherapy or chemotherapy prior to sample collection. The patient tissue samples used in the current study were originally collected with approval from the Ethics Committee of The Second Affiliated Hospital of Xi'an Jiaotong University (approval no. 2016036). The present study and all experimental procedures involving these samples were separately approved by the same committee (approval no. 2022185). The present study adhered to The Declaration of Helsinki and all specimens were obtained only after securing written informed consent from each patient prior to tissue collection.

Table I. Clinical characteristics of patients in The Cancer Genome Atlas cohort.

	Patients with LUAD (n=448)		Patients with LUSC (n=444)	
Clinical factor	L-group (n=224)	H-group (n=224)	L-group (n=222)	H-group (n=222)
Vital status				
Alive	165 (73.7)	134 (59.8)	138 (62.2)	133 (59.9)
Dead	59 (26.3)	90 (40.2)	84 (37.8)	89 (40.1)
Age, years				
≤65	104 (46.4)	109 (48.7)	92 (41.4)	81 (36.5)
>65	120 (53.6)	115 (51.3)	130 (58.5)	141 (63.5)
Sex				
Female	116 (51.8)	124 (55.4)	58 (26.1)	57 (25.7)
Male	108 (48.2)	100 (44.6)	164 (73.9)	165 (74.3)
Stage				
1	124 (55.4)	117 (52.2)	106 (47.7)	106 (47.7)
2	52 (23.2)	59 (26.3)	79 (35.6)	70 (31.5)
3/4	48 (21.4)	48 (21.4)	37 (16.7)	46 (20.7)
T stage				
1	80 (35.7)	71 (31.7)	47 (21.2)	53 (23.9)
2	121 (54.0)	120 (53.6)	134 (60.4)	126 (56.7)
3/4	23 (10.3)	33 (14.7)	41 (18.5)	43 (19.4)
N stage				
0	151 (67.4)	143 (63.8)	142 (64.0)	141 (63.5)
1	38 (17.0)	49 (21.9)	62 (27.9)	58 (26.1)
2	35 (15.6)	32 (14.3)	18 (8.1)	23 (10.4)

Data were shown as n (%), L-group represents low-expression group, H-group represents high-expression group. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

Cell line culture and transfection. In total, four LUAD cell lines (A549, H1299, PC-9 and H1975) and one normal bronchial epithelial cell line (BEAS-2B) were used in the present study. All cell lines were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences and were cultured in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Biological Industries; Sartorius AG) at 37˚C in a 5% CO_2 humidified atmosphere. The full-length LINC01117 sequence was cloned into the pEX-3 (pGCMV/MCS/Neo) vector to generate the LINC01117 overexpression plasmid; the corresponding empty pEX-3 (pGCMV/MCS/Neo) vector was used as the negative control (NC) for overexpression experiments. Vectors were purchased from Shanghai GenePharma Co., Ltd. For knockdown experiments, gene-specific small interfering RNAs (siRNAs) were designed and a non-targeting scrambled siRNA was used as the NC (siNC). All plasmids and siRNAs were synthesized by Shanghai GenePharma Co., Ltd. PC-9 cells at 70-80% confluence were transfected with the LINC01117 overexpression plasmid or the corresponding NC plasmid, whereas A549, PC-9 and NCI-H1299 cells at 60-70% confluence were transfected with siRNAs or the corresponding siNC. Transient transfection was performed using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, and all procedures were carried out at room temperature. siRNAs were transfected at a final concentration of 50 nM, and plasmids were transfected at 2 μg/well. Cells were transfected with siRNAs or plasmids for 6 h in a humidified atmosphere at 37˚C with 5% CO_2, after which the transfection medium was replaced with fresh complete medium. Total RNA was isolated 24 h post-transfection to confirm overexpression efficiency or evaluate knockdown efficiency. For subsequent experiments, transfected cells were reseeded at 24 h post-transfection. Cells were harvested 48 h post-transfection for protein extraction, and total RNA was also isolated at 48 h post-transfection to assess the expression of other genes. Following transfection, the cells were further incubated in a humidified atmosphere at 37˚C with 5% CO_2. The siRNA sequences targeting the relevant genes are listed in Table II.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Cytoplasmic and nuclear RNA was isolated from A549, NCI-H1299, PC-9 and H1975 cells using the Cytoplasmic & Nuclear RNA Purification Kit (Norgen Biotek Corp.). Total tissue RNA and cellular RNA were extracted using the FAST1000 and FAST200 kits (Shaanxi Xianfeng Biotechnology Co., Ltd.), respectively. Total RNA was reverse transcribed into cDNA using the PrimeScript™ RT Reagent Kit (Takara Bio, Inc.) under the following conditions: 37˚C

Table II. siRNA sequences for target genes.

siRNA	Sequence, 5'-3'
LINC01117-siRNA1	GCGAUACGUGAUCAUUUAATT
LINC01117-siRNA2	GGUUAAUUGCUUGCCCAAATT
LINC01117-siRNA3	GCAAACGAAGAGAUGGUAATT
HOXD8-siRNA1	GCCUGACAAAUUAACUUCUTT
HOXD8-siRNA2	GGUUCCAGAACAGGAGAAUTT
HOXD8-siRNA3	GUCGCUUCCAAACUCUAGATT
siNC	UUCUCCGAACGUGUCACGUTT

HOXD8, homeobox D8; LINC01117, long intergenic non-protein coding RNA 1117; NC, negative control; si, small interfering.

Table III. Primer sequences.

Gene	Sequence, 5'-3'
GAPDH	F: CTCCTCCACCTTTGACGCTG R: TCCTCTTGTGCTCTTGCTGG
LINC01117	F: ACCTCCTGACCCTGAAAGCA R: TGTTGGGGTCTGTCCCATCT
U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT
HOXD8	F: AGCAGCTCCTGGTAGACGAAGAG R: AGGGCTAGGGCGTGGGAAAC

F, forward; HOXD8, homeobox D8; LINC01117, long intergenic non-protein coding RNA 1117; R, reverse.

for 15 min and 85˚C for 5 sec, followed by maintenance at 4˚C. Quantitative amplification was then performed with TB Green® Premix Ex Taq™ II (Takara Bio, Inc.) under the following cycling conditions: 95˚C for 30 sec for initial denaturation, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. For nucleo-cytoplasmic fractionation, U6 was used as a nuclear fractionation control to verify enrichment of the nuclear RNA fraction, whereas GAPDH was used as a cytoplasmic fractionation control (34,35). The target gene expression levels were quantified via the $2^{-\Delta\Delta Cq}$ method (36). All primers were synthesized by Sangon Biotech Co., Ltd., the sequences of which are listed in Table III.

Colony formation assays. Following transfection, cells were seeded into 6-well plates (200 cells/well) and incubated in a humidified incubator with 5% CO_2 at 37˚C for 7 days. Subsequently, at room temperature, colonies were fixed with 4% formaldehyde for 20 min, stained with 0.5% crystal violet for 20 min and images were captured. Colonies containing >50 cells were manually counted for analysis.

Live cell imaging to evaluate cell proliferation. Transfected cells were seeded into 24-well culture plates at a density of 2×10^4 cells/well. After adhesion, the plates were placed into the live cell imaging system (Cytation5; BioTek; Agilent Technologies, Inc.) for real-time monitoring, with images captured every 2 h. Cell proliferation was then evaluated by quantifying the cells; the cell proliferation rate (%) was calculated as the relative increase in cell number per unit area compared that at 0 h using the formula: $[(Nt/A)-(N_0/A)]/(N0/A)$ x100, where Nt is the cell number at the indicated time point, N_0 is the cell number at 0 h, and A is the imaging area.

Cell Counting Kit-8 (CCK-8) assay. Transfected cells were seeded into 96-well plates at a density of 2×10^3 cells/well. After 24, 48 and 72 h of incubation, 10 µl CCK-8 reagent (Shanghai Qihai Futai Biotechnology Co., Ltd.) was added to the medium and the plates were incubated for 40 min. The absorbance at 450 nm was then measured using a microplate reader (BioTek; Agilent Technologies, Inc.).

5-ethynyl-2'-deoxyuridine (EdU) assay. The EdU kit was purchased from Guangzhou RiboBio Co., Ltd. Post-transfection, 8×10^3 cells/well were seeded into 96-well plates and incubated at 37˚C for 48 h in a cell incubator. The medium was then replaced with 50 µM EdU-containing medium for an additional 2-h incubation. At room temperature, following fixation with 4% paraformaldehyde for 30 min, the cells were stained with Apollo solution for 30 min on a shaker in the dark, and nuclear DNA was counterstained with Hoechst reagent for 30 min on a shaker in the dark, according to the manufacturer's protocol. Fluorescence images were captured using a Nikon Ti-S inverted fluorescence microscope (Nikon Corporation). The EdU-positive rate (%) was determined as: (Apollo-stained cells/Hoechst-stained nuclei) x100.

Western blotting. Proteins were extracted from NCI-H1299 and PC-9 cells using RIPA lysis buffer (Beyotime Biotechnology). Protein concentration was determined using a BCA Protein Assay Kit (cat. no. P0012S; Beyotime Biotechnology). Equal amounts of protein (20 µg/lane) were separated by 10% Tris-glycine/SDS-PAGE. Following electrophoretic transfer onto PVDF membranes, the membranes were blocked with 5% non-fat milk at 37˚C for 1 h. The membranes were then incubated overnight at 4˚C with primary antibodies against HOXD8 (cat. no. sc-515357; dilution 1:200; Santa Cruz Biotechnology, Inc.) and GAPDH (cat. no. 2118; dilution 1:1,000; Cell Signaling Technology, Inc.), with the latter serving as the internal control. The membranes probed for GAPDH and HOXD8 were incubated with HRP-conjugated goat anti-rabbit IgG secondary antibody (cat. no. 7074; dilution, 1:1,000; Cell Signaling Technology, Inc.) and HRP-conjugated goat anti-mouse IgG secondary antibody (cat. no. 7076; dilution, 1:1,000; Cell Signaling Technology, Inc.), respectively, at 37˚C for 1 h. The signals were visualized using an HRP chemiluminescent detection kit (cat. no. WBKLS0050; MilliporeSigma) and captured with an imaging system (model no. 5200; Tanon Science and Technology Co., Ltd.). Band intensities were semi-quantified by densitometric analysis using ImageJ software (version 1.54g; National Institutes of Health).

Actinomycin D (ActD) treatment. ActD was purchased from TargetMol (cat. no. TN9827) and dissolved in dimethyl sulfoxide. In PC-9 cells, ActD was added to the LINC01117

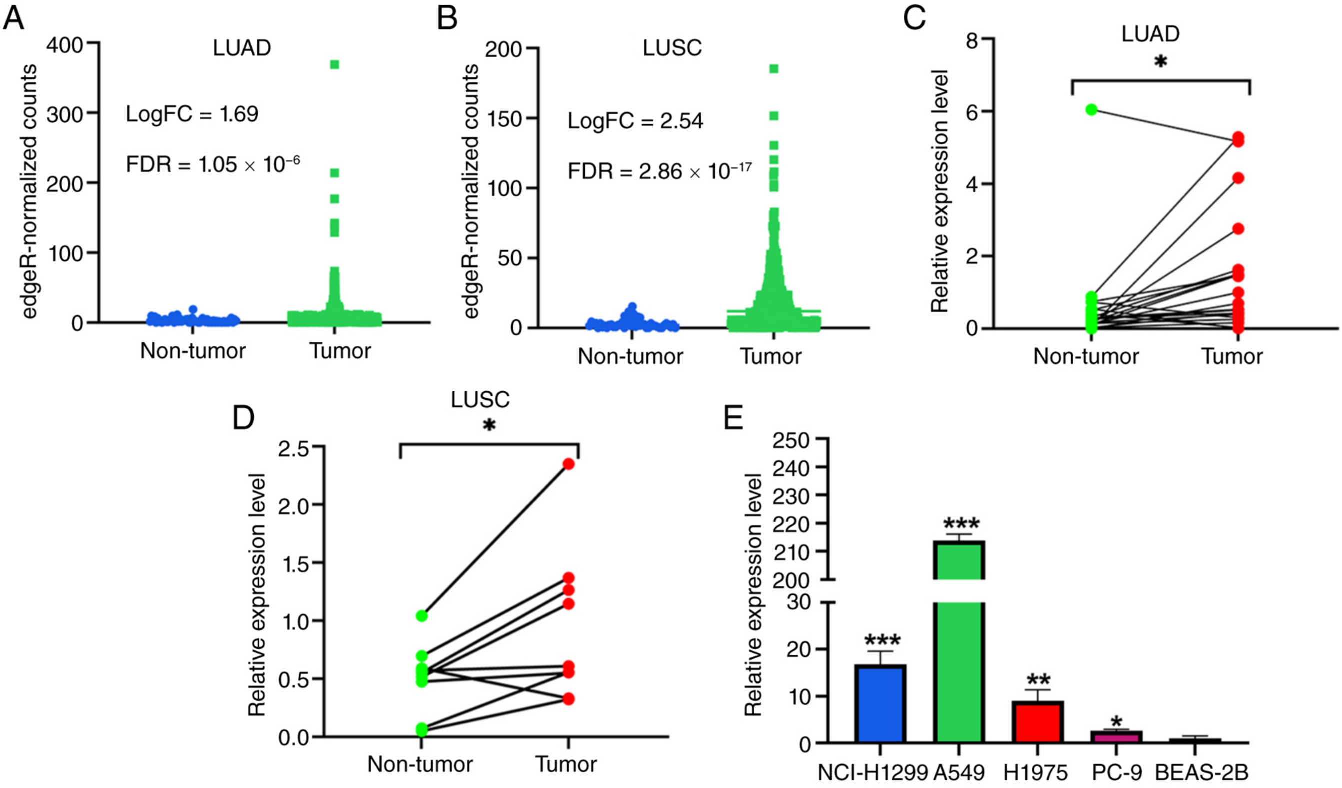

Figure 1. LINC01117 is highly expressed in LUAD and LUSC. LINC01117 expression in TCGA (A) LUAD and (B) LUSC datasets. LINC01117 expression in human (C) LUAD and (D) LUSC tissues. (E) LINC01117 expression in LUAD cell lines compared with in a normal bronchial epithelial cell line. *P<0.05, **P<0.01, ***P<0.001 vs. BEAS-2B or as indicated. FC, fold change; FDR, false discovery rate; LINC01117, long intergenic non-protein coding RNA 1117; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

overexpression and NC groups to achieve a final concentration of 10 μg/ml, and the cells were incubated at 37˚C for 0, 2, 4, 6 and 8 h after treatment. Cells were then harvested at room temperature for total RNA extraction. HOXD8 mRNA levels at each time point were quantified by RT-qPCR as aforementioned and normalized to the 0-h time point to assess degradation kinetics.

Statistical analysis. All statistical analyses were performed using GraphPad Prism 9.0 (Dotmatics). The normality of data distribution was assessed using the Shapiro-Wilk test. For normally distributed data, comparisons between two groups were performed using the Student's t-test, with paired t-tests used for tissue samples and unpaired t-tests used for other two-group comparisons, whereas the Mann-Whitney U test was used for non-normally distributed data. For comparisons involving multiple groups, one-way ANOVA was applied, followed by Tukey's post hoc test. Data following a normal distribution are presented as the mean ± standard deviation, whereas non-normally distributed data are presented as the median (interquartile range). Pearson correlation analysis was used to assess the correlation between gene expression levels. For tissue samples, each sample represented an independent biological replicate. RNA expression levels were measured with at least three technical replicates per sample, and the mean value was used for statistical analysis. All other *in vitro* experiments were performed with at least three independent biological replicates. For bioinformatics analyses based on TCGA data, the sample size was determined by all eligible patients meeting the inclusion criteria in the public database. Due to the retrospective nature of the present study, no *a priori* power calculation was performed. Two-sided P<0.05 was considered to indicate a statistically significant difference.

Results

LINC01117 is upregulated in LUAD and LUSC. Differential expression analysis of TCGA data revealed that LINC01117 was upregulated in LUAD (Fig. 1A) and LUSC (Fig. 1B) tumor tissues compared with in non-tumor tissues. Consistent with this, analysis of the collected clinical tissue samples showed that LINC01117 was significantly upregulated in the tumor tissues from 21 LUAD (Fig. 1C) and nine LUSC (Fig. 1D) samples relative to the matched adjacent non-tumor tissues. Furthermore, LINC01117 expression was elevated in LUAD cell lines compared with in the normal bronchial epithelial BEAS-2B cell line (Fig. 1E). Based on these findings, the A549 and H1299 cell lines were selected for subsequent LINC01117 knockdown experiments, whereas the PC-9 cell line was used for overexpression experiments.

LINC01117 is positively associated with poor prognosis in patients with LUAD. KM analysis of TCGA data revealed that elevated LINC01117 expression was associated with poorer outcomes in LUAD (Fig. 2A) but not in LUSC (Fig. 2B). Among the patients with LUAD, the median survival time was significantly shorter in the high-expression group (3.14 years) than the low-expression group (4.93 years). The 3- and 5-year

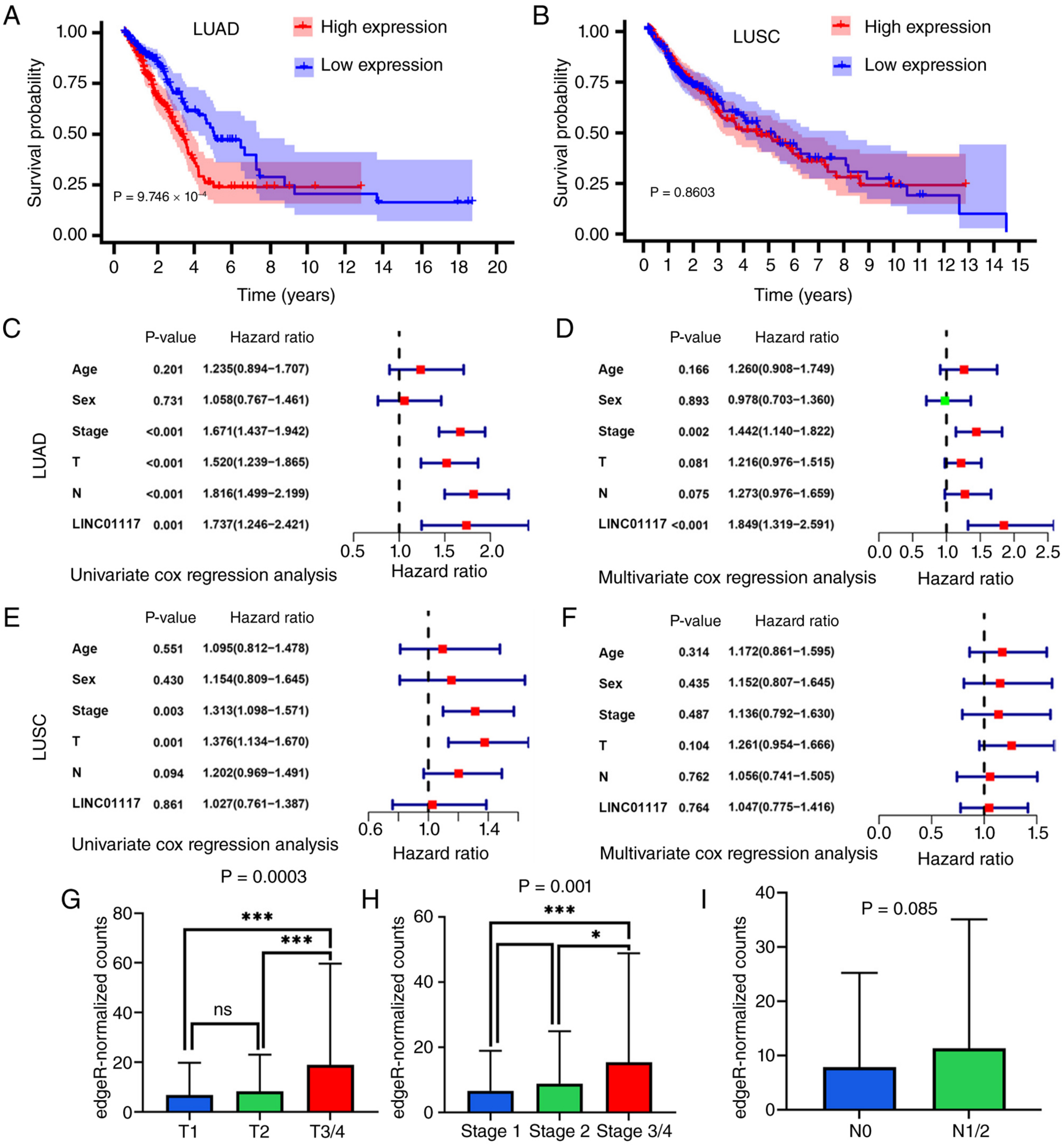

Figure 2. LINC01117 is associated with a poor prognosis in LUAD. Kaplan-Meier survival analyses of patients with (A) LUAD and (B) LUSC based on LINC01117 expression. (C) Univariate and (D) multivariate Cox regression analyses of LINC01117 in LUAD. (E) Univariate and (F) multivariate Cox regression analyses of LINC01117 in LUSC. Expression of LINC01117 in (G) different T stages, (H) different TNM stages and (I) different N stages. *P<0.05 and ***P<0.001. LINC01117, long intergenic non-protein coding RNA 1117; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; N, lymph node metastasis; ns, not significant; T, tumor size; TNM, tumor-node-metastasis.

survival rates were 50.5 and 24.0% in the high-expression group vs. 68.3 and 47.0% in the low-expression group, respectively. Univariate Cox regression analysis confirmed a significant association between LINC01117 and OS (Fig. 2C). Multivariate Cox regression analysis further demonstrated that LINC01117 remained independently associated with LUAD prognosis after adjusting for clinical characteristics (Fig. 2D). These results suggested that LINC01117 may be an independent prognostic biomarker for LUAD. By contrast, neither the univariate nor multivariate Cox regression analyses showed a significant prognostic association between LINC01117 and LUSC (Fig. 2E and F). Therefore, the present study focused primarily on investigating the functional mechanisms of LINC01117 in LUAD.

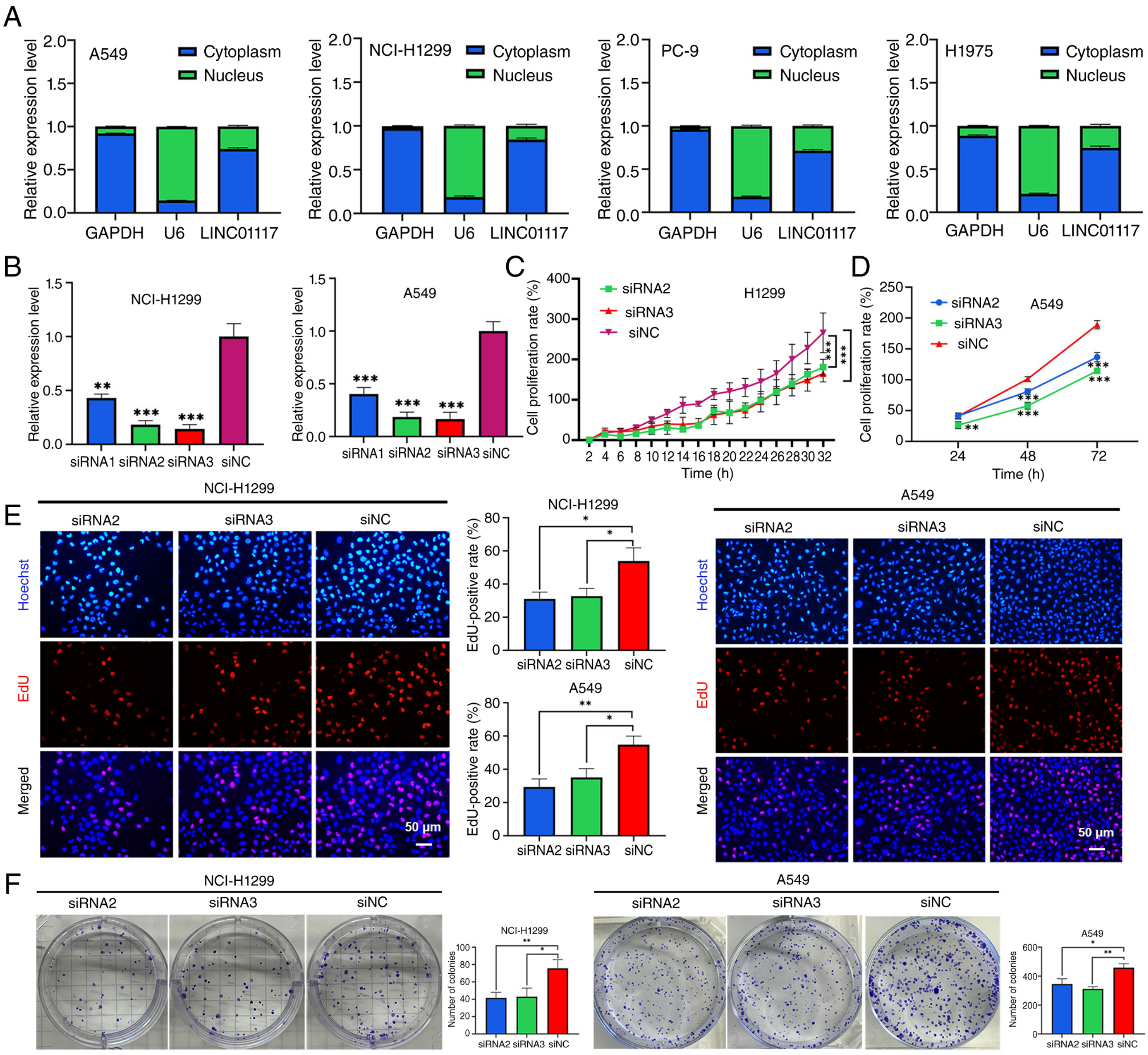

Figure 3. Knockdown of LINC01117 inhibits LUAD cell proliferation. (A) Subcellular distribution of LINC01117 in LUAD cells. (B) Knockdown efficiency of LINC01117 using siRNAs. (C) In NCI-H1299 cells, live-cell imaging assay measured cell proliferation following LINC01117 knockdown. (D) In A549 cells, Cell Counting Kit-8 assay measured cell proliferation following LINC01117 knockdown. In NCI-H1299 and A549 cells, (E) EdU and (F) colony formation assays assessed cell proliferation following LINC01117 knockdown. *P<0.05, **P<0.01, ***P<0.001 vs. siNC or as indicated. EdU, 5-ethynyl-2'-deoxyuridine; LINC01117, long intergenic non-protein coding RNA 1117; LUAD, lung adenocarcinoma; NC, negative control; si, small interfering.

To further evaluate the prognostic value of LINC01117 in LUAD, its association with clinical stage was analyzed. One-way ANOVA revealed a significant difference in expression among the T1, T2 and T3/4 groups; subsequent Tukey's multiple-comparisons test showed that expression levels in both the T1 and T2 groups were significantly lower than those in the T3/4 group, whereas no significant difference was observed between the T1 and T2 groups (Fig. 2G). One-way ANOVA revealed a significant difference in expression among the stage 1, stage 2 and stage 3/4 groups; subsequent Tukey's multiple-comparisons test showed that expression levels in both the stage 1 and stage 2 groups were significantly lower than those in the stage 3/4 group, whereas no significant difference was observed between the stage 1 and stage 2 groups (Fig. 2H). Although no significant differences were observed across N stages, the LINC01117 expression levels showed an increasing trend with advancing N stage (Fig. 2I). These findings collectively suggested that elevated LINC01117 expression may predict poor prognosis in patients with LUAD.

Knockdown of LINC01117 inhibits LUAD cell proliferation. To further understand the role of LINC01117 in LUAD, cytoplasmic and nuclear RNA were extracted from A549, NCI-H1299, PC-9 and H1975cells, and RT-qPCR analysis of the fractionated RNA showed enrichment of U6 in the nuclear fraction and GAPDH in the cytoplasmic fraction, supporting successful nucleo-cytoplasmic separation. LINC01117 expression was then detected using RT-qPCR, and the results

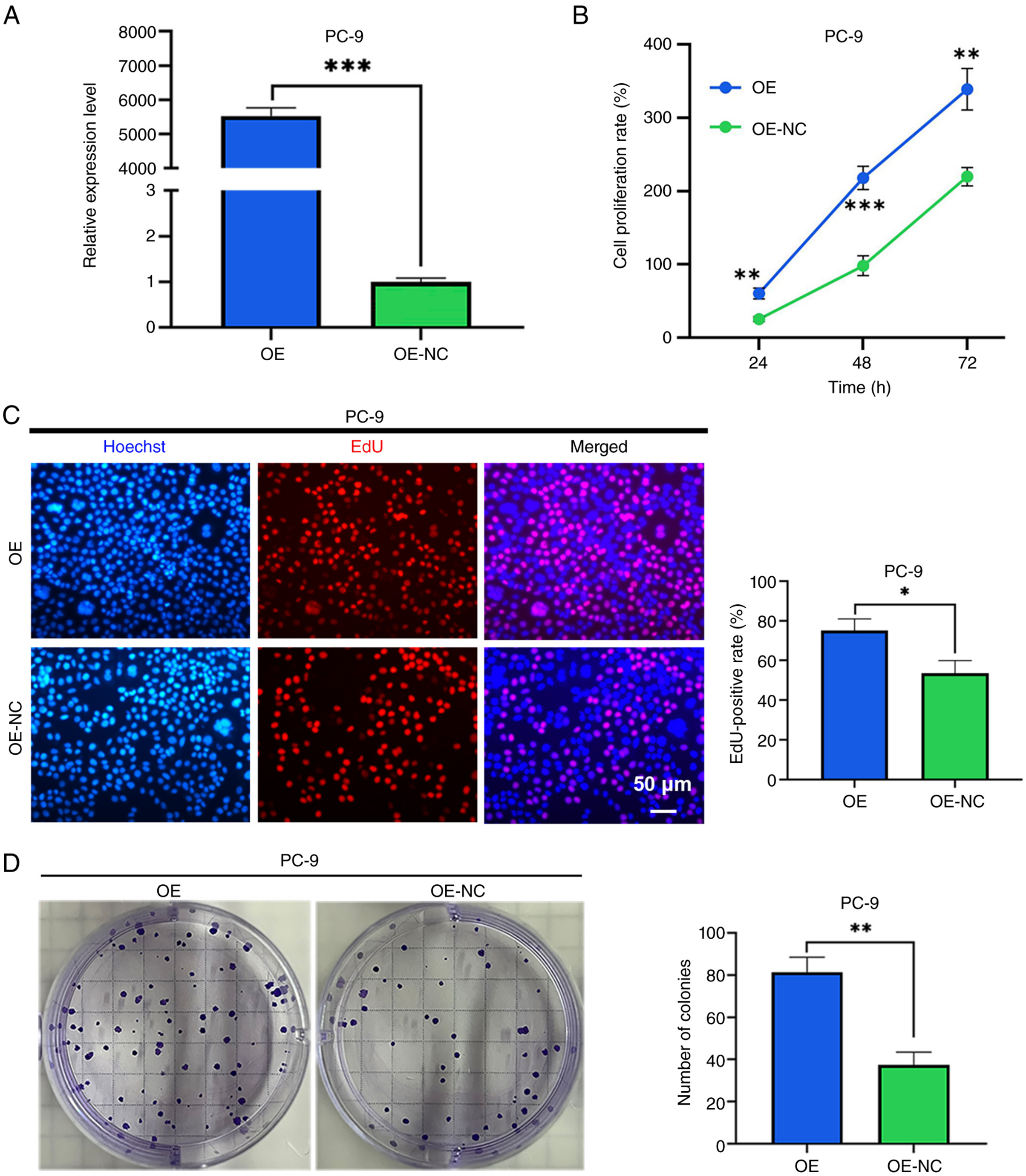

Figure 4. OE of LINC01117 promotes lung adenocarcinoma cell proliferation. (A) OE efficiency of LINC01117 in PC-9 cells. (B) Cell Counting Kit-8, (C) EdU and (D) colony formation assays assessed the proliferation of PC-9 cells following LINC01117 OE. *P<0.05, **P<0.01, ***P<0.001 vs. OE-NC or as indicated. EdU, 5-ethynyl-2'-deoxyuridine; LINC01117, long intergenic non-protein coding RNA 1117; NC, negative control; OE, overexpression.

indicated that LINC01117 was mainly localized in the cytoplasm (Fig. 3A).

Subsequently, A549 and NCI-H1299cells were transfected with siRNAs targeting LINC01117 to achieve knockdown of expression. RT-qPCR analysis revealed that siRNA2 and siRNA3 exhibited higher knockdown efficiencies compared with siRNA1 (Fig. 3B) and were therefore selected for further experiments. Live cell imaging demonstrated significantly lower NCI-H1299 cell survival rates in the siRNA2 and siRNA3 groups compared with those in the siNC group (Fig. 3C). To improve the reliability of the findings, the CCK-8 assay was used to assess cell proliferation in the A549 cell line,

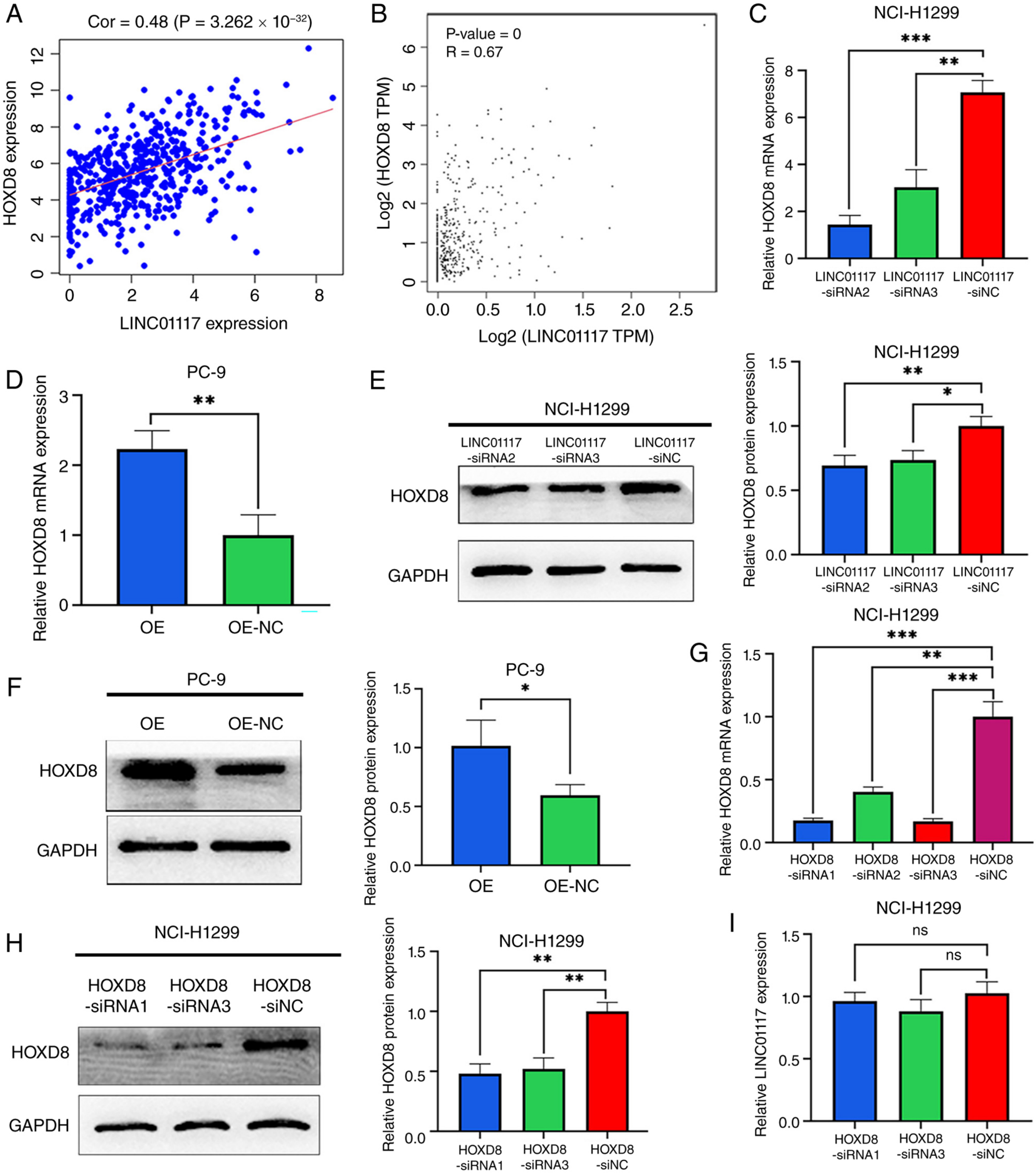

Figure 5. HOXD8 is a downstream target gene of LINC01117. (A) Co-expression of LINC01117 and HOXD8 in The Cancer Genome Atlas-lung adenocarcinoma dataset. (B) Co-expression of LINC01117 and HOXD8 in the Gene Expression Profiling Interactive Analysis online database. HOXD8 mRNA expression following LINC01117 (C) knockdown and (D) OE. HOXD8 protein expression following (E) LINC01117 knockdown and (F) OE. Validation of HOXD8 knockdown efficiency at the (G) mRNA and (H) protein levels. (I) LINC01117 expression after HOXD8 knockdown. *P<0.05, **P<0.01, ***P<0.001. HOXD8, homeobox D8; LINC01117, long intergenic non-protein coding RNA 1117; NC, negative control; ns, not significant; OE, overexpression; si, small interfering.

and the results showed that LINC01117 knockdown inhibited cell proliferation compared with in the siNC group (Fig. 3D). In NCI-H1299 and A549 cells, the EdU assay revealed markedly lower EdU-positive rates in the LINC01117 knockdown groups than in the NC group (Fig. 3E). Similarly, the colony formation assay showed a significant reduction in colony numbers following LINC01117 knockdown compared with the NC in NCI-H1299 and A549 cells (Fig. 3F). Collectively, these results demonstrated that LINC01117 knockdown inhibited LUAD cell proliferation.

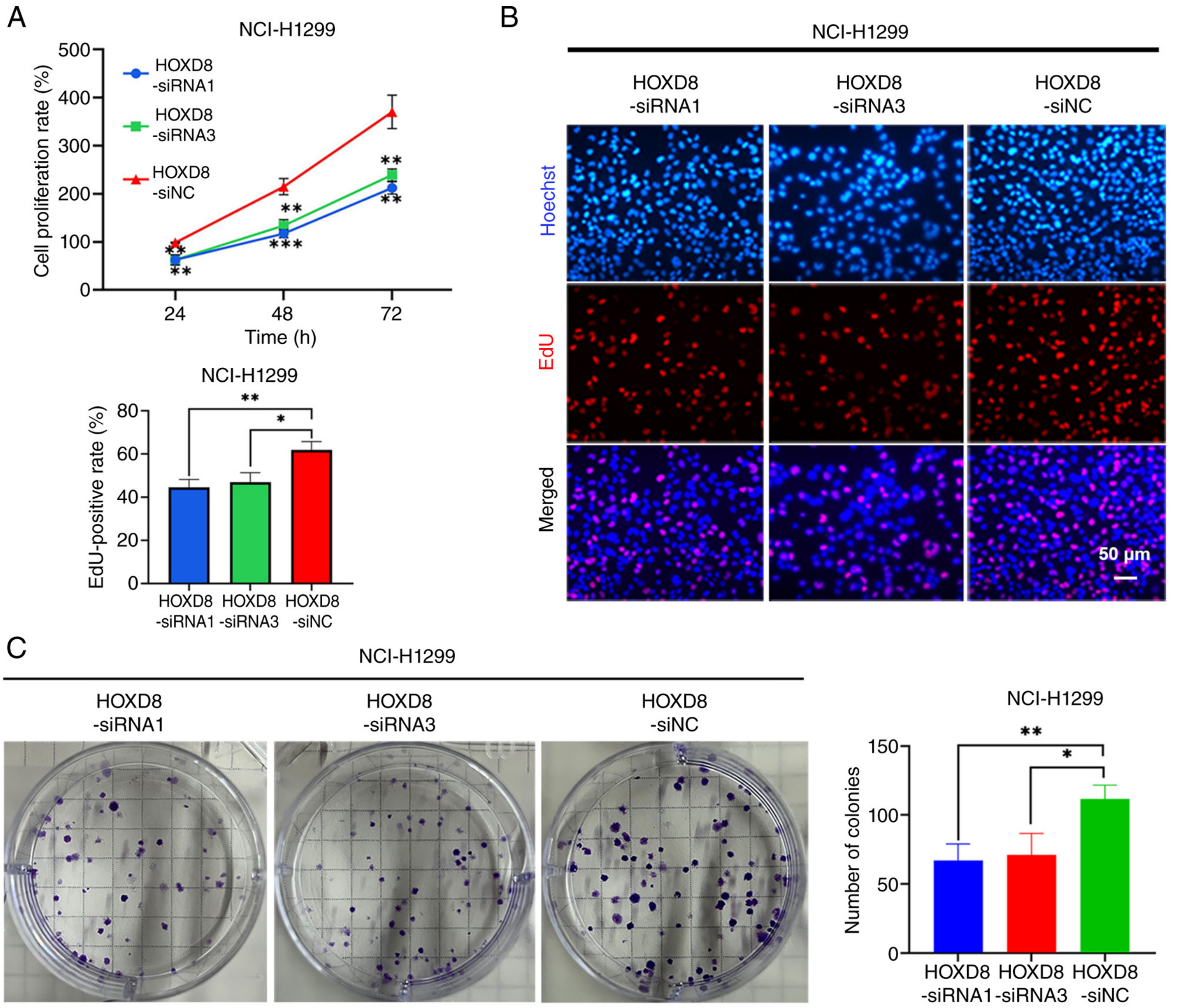

Figure 6. Knockdown of HOXD8 inhibits lung adenocarcinoma cell proliferation. In NCI-H1299 cells, (A) Cell Counting Kit-8, (B) EdU and (C) colony formation assays measured cell proliferation following HOXD8 knockdown. *P<0.05, **P<0.01, ***P<0.001 vs. HOXD8-siNC or as indicated. EdU, 5-ethynyl-2'-deoxyuridine; HOXD8, homeobox D8; NC, negative control; si, small interfering.

Overexpression of LINC01117 promotes LUAD cell proliferation. LINC01117 was successfully overexpressed in PC-9 cells using a plasmid vector (Fig. 4A). The CCK-8 assay results demonstrated that the LINC01117 overexpression group exhibited a significantly higher cell proliferation rate compared with that in the control group (Fig. 4B). Similarly, the EdU assay revealed a markedly higher EdU-positive rate in the LINC01117 overexpression group than in the NC group (Fig. 4C). Furthermore, the colony formation assay showed a significantly increased number of colonies in the LINC01117 overexpression group relative to the control group (Fig. 4D). Collectively, these results indicated that LINC01117 overexpression promoted LUAD cell proliferation.

HOXD8 is a downstream target gene of LINC01117. To investigate the mechanisms by which LINC01117 may promote LUAD progression, Pearson correlation analyses between LINC01117 expression and the expression levels of all PCGs from TCGA-LUAD cohort were performed. The results revealed that LINC01117 was most significantly co-expressed with HOXD8 (Fig. 5A). This co-expression pattern was validated using data from the GEPIA database (Fig. 5B).

The regulatory relationship between LINC01117 and HOXD8 was then examined. Knockdown of LINC01117 significantly reduced the mRNA and protein levels of HOXD8 (Fig. 5C and E), whereas LINC01117 overexpression increased the mRNA and protein levels of HOXD8 (Fig. 5D and F). To determine whether HOXD8 reciprocally regulated LINC01117, NCI-H1299 cells were transfected with siRNAs to knockdown HOXD8 expression. RT-qPCR analysis revealed that, among the three HOXD8 siRNAs, siRNA1 and siRNA3 exhibited higher knockdown efficiencies than siRNA2 and were therefore selected for further experiments (Fig. 5G). The knockdown efficiencies were also successfully validated at the protein level (Fig. 5H). Notably, HOXD8 knockdown did not alter LINC01117 expression (Fig. 5I). These results

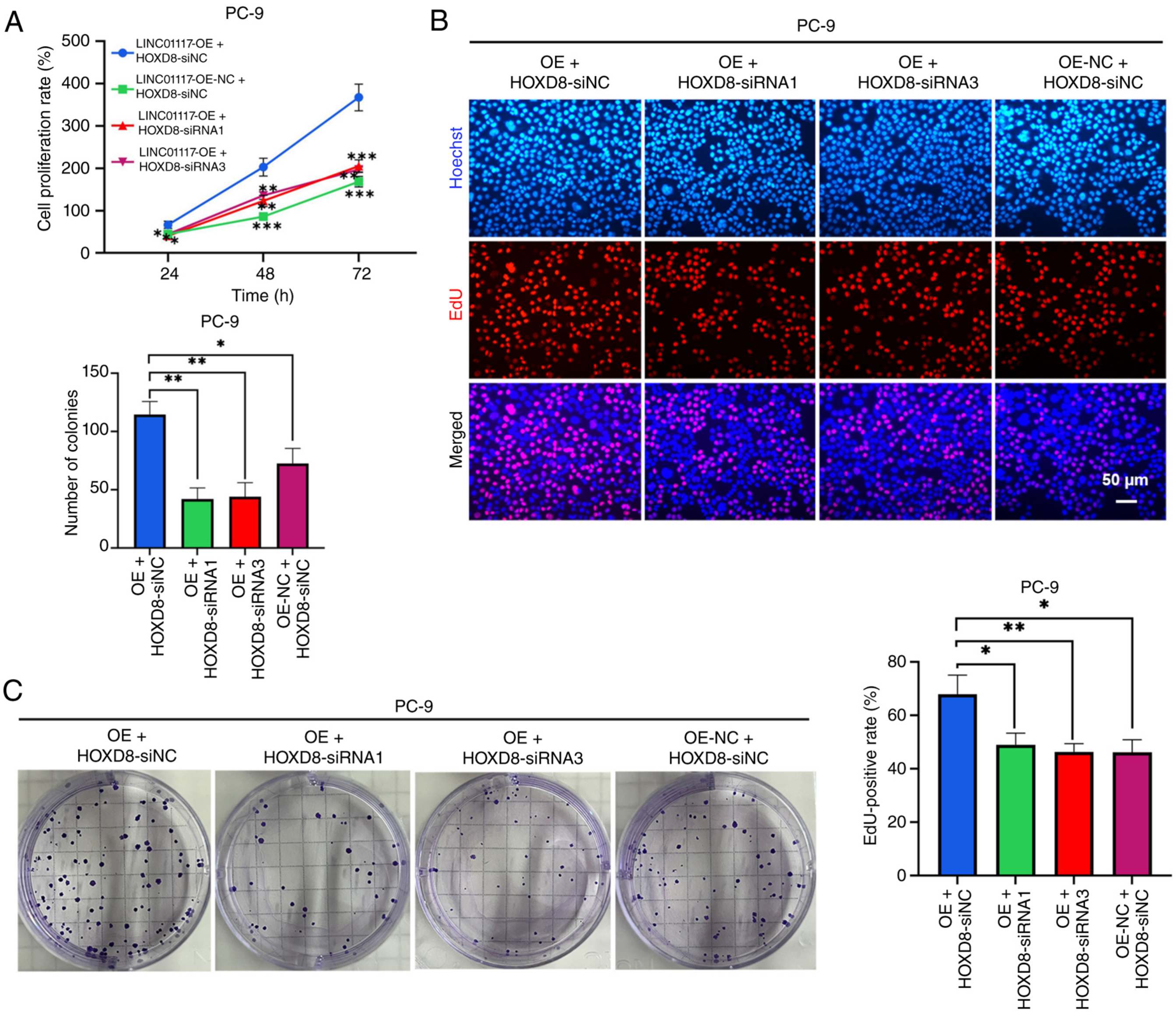

Figure 7. LINC01117 promotes lung adenocarcinoma cell proliferation by targeting HOXD8. (A) CCK-8, (B) EdU and (C) colony formation assays measured the proliferation of LINC01117-overexpressing cells following HOXD8 knockdown. *P<0.05, **P<0.01, ***P<0.001 vs. LINC01117-OE + HOXD8-siNC or as indicated. EdU, 5-ethynyl-2'-deoxyuridine; HOXD8, homeobox D8; LINC01117, long intergenic non-protein coding RNA 1117; NC, negative control; OE, overexpression; si, small interfering.

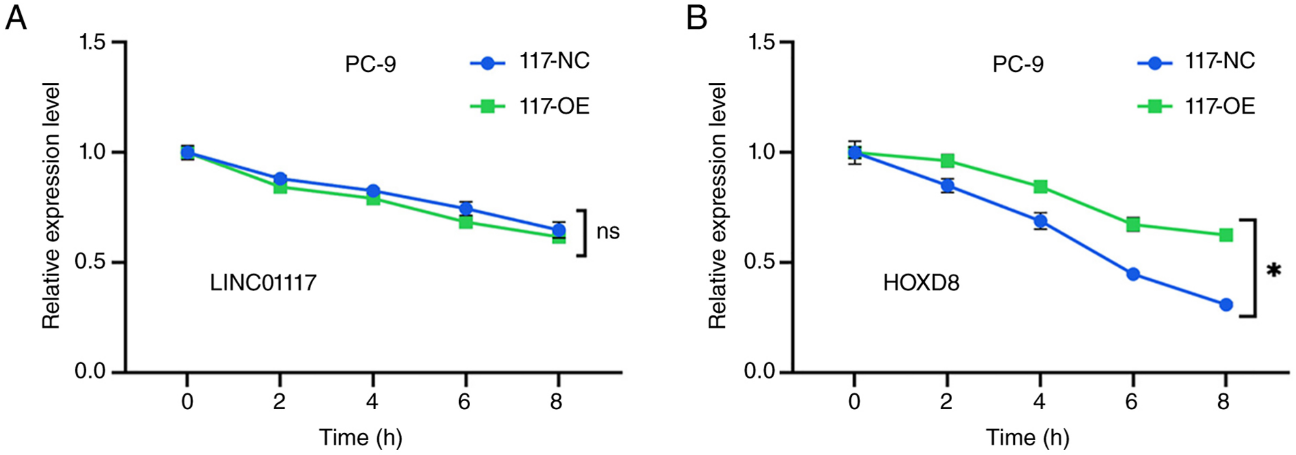

Figure 8. LINC01117 maintains HOXD8 mRNA stability. (A) Degradation rate of LINC01117 in LINC01117 OE group and NC group. (B) Degradation rate of HOXD8 mRNA in LINC01117 OE group and NC group. *P<0.05. HOXD8, homeobox D8; LINC01117, long intergenic non-protein coding RNA 1117; NC, negative control; ns, not significant; OE, overexpression.

demonstrated that HOXD8 may be a downstream target gene of LINC01117.

LINC01117 promotes LUAD cell proliferation by targeting HOXD8. Having established that LINC01117 promotes LUAD cell proliferation and that HOXD8 may be a downstream target gene of LINC01117, the present study aimed to determine whether HOXD8 itself is involved in NCI-H1299 cell proliferation. CCK-8 assays showed that knockdown of HOXD8 significantly suppressed the cell proliferation rate (Fig. 6A). EdU assays showed that the EdU-positive rate in the HOXD8 knockdown groups was significantly lower than that in the NC group (Fig. 6B). Additionally, colony formation assays showed that the number of colonies in the HOXD8 knockdown groups was significantly lower than that in the NC group (Fig. 6C). These results indicated that HOXD8 promoted LUAD cell proliferation.

It was thus considered that LINC01117 may promote LUAD cell proliferation by targeting HOXD8. To test this, NCI-H1299 cells were transfected with a LINC01117 overexpression plasmid, followed by transfection with HOXD8 siRNAs to reduce HOXD8 expression, and cell proliferation was subsequently examined. The results of the CCK-8 assay showed that knockdown of HOXD8 in LINC01117-overexpressing cells significantly inhibited cell proliferation compared with the LINC01117-overexpression + HOXD8-siNC group (Fig. 7A). In addition, HOXD8 knockdown significantly reduced the EdU-positive rate in LINC01117-overexpressing groups compared with in the LINC01117-overexpression + HOXD8-siNC group (Fig. 7B). Furthermore, the colony formation assay demonstrated that the number of colonies was significantly reduced in HOXD8-knockdown LINC01117-overexpressing groups compared with that in the LINC01117-overexpression + HOXD8-siNC group (Fig. 7C). Thus, HOXD8 knockdown reversed the pro-proliferative effect of LINC01117 overexpression. Collectively, these results indicated that LINC01117 may promote cell proliferation through HOXD8.

LINC01117 is associated with increased HOXD8 mRNA stability. As aforementioned, changes in LINC01117 expression lead to corresponding changes in HOXD8 expression at both the mRNA and protein levels, suggesting that LINC01117 may stabilize HOXD8 mRNA. To assess this hypothesis, ActD was used to inhibit *de novo* RNA transcription. In PC-9 cells, the degradation rate of LINC01117 in the LINC01117 overexpression group did not differ significantly from that in the NC group (Fig. 8A). However, the degradation rate of HOXD8 mRNA was significantly slower in the LINC01117 overexpression group compared with the control (Fig. 8B). These results indicated that LINC01117 was associated with increased HOXD8 mRNA stability.

Discussion

Lung cancer accounts for the highest number of cancer-related deaths (1); therefore, an improved understanding of the molecular mechanisms underlying LUAD is crucial for improving patient survival rates. Notably, a number of studies have focused on identifying prognostic lncRNA biomarkers, some of which have demonstrated clinical value (37,38). LINC01117 is a relatively new lncRNA, the functions and mechanisms of which in lung cancer remain largely unclear. The present study demonstrated that LINC01117 was upregulated in LUSC tumor tissues, and in LUAD tumor tissues and cell lines. Furthermore, high LINC01117 expression was significantly associated with a poor prognosis in patients with LUAD, but showed no significant association with prognosis in patients with LUSC. Thus, the present study focused on elucidating the functions and mechanisms of LINC01117 in LUAD.

Notably, the survival curves of the high- and low-expression groups tended to converge after ~10 years in patients with LUAD; this suggests that the prognostic impact of LINC01117 may be more pronounced in the early stages of disease progression. This pattern may be influenced by several factors, including tumor heterogeneity, subsequent treatment interventions and dynamic changes in the tumor microenvironment, such as cancer-associated inflammation and immune modulation. Further studies are warranted to elucidate the underlying mechanisms.

In the present study, bioinformatics analysis revealed a positive association between LINC01117 expression and the T and TNM stages of LUAD, whereas no significant association was observed with N stage. Although an increasing trend in LINC01117 expression was observed with higher N stage, this difference was not statistically significant. Therefore, this finding should be interpreted with caution and requires further validation in larger cohorts. It remains unclear whether this result stems from limitations in TCGA sample size or indicates a true absence of association with metastasis. To elucidate the function and mechanism of LINC01117 in LUAD, knockdown and overexpression experiments were performed. Collectively, these results suggested that LINC01117 promoted LUAD cell proliferation.

Most ncRNAs regulate biological processes by interacting with PCGs (39). Therefore, to identify potential targets of LINC01117, correlation coefficients between LINC01117 and all PCGs were calculated in the present study. Among them, HOXD8 exhibited the strongest positive correlation coefficient with LINC01117. Notably, both LINC01117 and HOXD8 are located at the 2q31.1 chromosomal locus, with HOXD8 positioned upstream of LINC01117. In the present study, knockdown of LINC01117 significantly reduced HOXD8 expression, whereas its overexpression significantly increased HOXD8 expression at both the mRNA and protein levels. By contrast, knockdown of HOXD8 did not significantly alter the LINC01117 expression levels. Collectively, these findings indicated that HOXD8 may be a downstream target of LINC01117.

A previous study reported that lncRNA LINC01116 post-transcriptionally regulates HOXD8 to promote bladder cancer progression (40), which aligns with the lung cancer findings in the present study. HOXD8 is a transcription factor implicated in various cancer types (27). However, the role of HOXD8 appears to be context-dependent. In some tumors, HOXD8 acts as an oncogene; for example, it is upregulated in glioma, and promotes glioma cell proliferation and migration (41). By contrast, it may function as a tumor suppressor in certain cancer types, such as gastric cancer, where its expression is downregulated and negatively associated with tumor malignancy (42). Studies have indicated that HOXD8 contributes to chemoresistance, promotes proliferation and metastasis, and frequently serves as a target for ncRNAs (24-26,43). In the present study, HOXD8 knockdown reversed the LINC01117-induced increase in cell proliferation. These results suggested that LINC01117 may promote LUAD cell proliferation by targeting HOXD8. Moreover, the present study demonstrated that LINC01117 was associated with increased HOXD8 mRNA stability.

The present study has several limitations that should be acknowledged. First, the expression of LINC01117 was only assessed in 21 LUAD and nine LUSC tumor tissues and adjacent non-tumor tissues. In future studies, the sample size should be increased and additional clinical data should be collected to further analyze the association between LINC01117 expression and clinicopathological factors. Second, while the present study focused on the role of LINC01117 in cell proliferation, its potential involvement in metastasis was not investigated. Third, the results suggested that LINC01117 may regulate HOXD8 mRNA stability, but the precise molecular mechanism underlying this regulation remains to be fully elucidated. Finally, the mechanism by which LINC01117 may promote the malignant proliferation of LUAD was not verified *in vivo*, and further *in vivo* validation is required to confirm the biological role of LINC01117 in LUAD development. Despite these limitations, the current study provides novel insights into the potential mechanism by which LINC01117 contributes to LUAD progression. It offers a theoretical basis for the development of LINC01117 as a potential therapeutic target in LUAD.

Another limitation of the present study should be acknowledged. Although U6 was used as a nuclear fractionation control to verify the quality of nucleo-cytoplasmic separation (34,35), U6 is a small nuclear RNA rather than a lncRNA, and therefore may not represent an ideal universal endogenous reference for lncRNA RT-qPCR normalization. Thus, although the present data support the predominantly cytoplasmic localization of LINC01117, this conclusion would be further strengthened by additional approaches, such as validation with multiple reference transcripts, absolute quantification or RNA-fluorescence *in situ* hybridization analysis in future studies.

In conclusion, the results of the present study demonstrated that LINC01117 was upregulated in LUAD and may serve as an independent prognostic biomarker. Mechanistically, LINC01117 promoted LUAD cell proliferation by increasing HOXD8 mRNA stability. Overall, the present study identified LINC01117 as an oncogene that may drive LUAD progression and predict poor patient outcomes.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LiZ collected human tissue samples, validated the expression of LINC01117 in tissues and cells, performed the bioinformatics and subcellular localization analyses of LINC01117, and drafted the manuscript. JS assessed the effects of LINC01117 knockdown on cell proliferation. CZ assessed the effects of LINC01117 overexpression on cell proliferation. YD assessed HOXD8 expression following LINC01117 knockdown and overexpression, and evaluated LINC01117 expression after HOXD8 knockdown. YC assessed the effects of HOXD8 knockdown on cell proliferation, as well as the effects of HOXD8 knockdown on cell proliferation in the LINC01117-overexpression group. QZ examined the effect of LINC01117 overexpression on the mRNA stability of HOXD8. LoZ and SY supervised the project, contributed to the study design and revised the manuscript. LiZ, LoZ and SY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committees of The Second Affiliated Hospital of Xi'an Jiaotong University (approval nos. 2016036 and 2022185). All subjects provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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