Article

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LINC00174 Accelerates the Progression of Non-Small Cell Lung Cancer via Modulating EP300/NFAT5 Axis

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Background: How long non-coding RNA (lncRNA) functions in cancer biology has attracted extensive attention recently. Various lncRNAs interfere with the advancement of non-small cell lung cancer (NSCLC). This work focused on how lncRNA LINC00174 functions in NSCLC.

Methods: The analysis of *LINC00174* expressions in NSCLC was performed with quantitative real-time PCR (qRT-PCR). The multiplication, migration, aggressiveness and apoptosis of NSCLC cells were evaluated by Cell Counting Kit-8 (CCK-8), Transwell assays and Flow cytometry. The binding relationship between LINC00174 and E1A-binding protein p300 (EP300) was verified by RNA immunoprecipitation experiment. The binding of EP300 to the promoter region of the nuclear factor of activated T cells 5 (*NFAT5*) was detected by dual luciferase reporter gene assay and immunocoprecipitation assay.

Results: The expression of LINC00174 was increased in NSCLC, which impaired the overall survival time in patients with NSCLC. Knocking down LINC00174 curbed the multiplication, migration and aggressiveness of cancer cells and promoted apoptosis. LINC00174 was directly combined with EP300. Knocking down LINC00174 inhibited the binding of EP300 and *NFAT5* promoter region, thereby curbing *NFAT5* transcription. In addition, NFAT5 overexpression facilitated cancer cell malignancy, which could be reversed by knockdown of LINC00174.

Conclusions: This study indicates that LINC00174 promotes *NFAT5* transcription by recruiting the EP300 to *NFAT5* promoter region, thereby accelerating the progression of NSCLC.

Keywords: LINC00174; EP300; NFAT5; NSCLC; proliferation

Introduction

The surgery and molecular targeted therapy have progressed in recent decades. However, the prognosis of non-small cell lung cancer (NSCLC) is still pessimistic due to the insidious early symptoms and easy metastasis [1,2]. Due to a limited understanding of the pathogenesis of NSCLC, the exploration of NSCLC tumorigenesis is a research hotspot [3].

Long non-coding RNAs (LncRNAs) are conceptually a category of RNA transcripts longer than 200 nucleotides, incapable of encoding proteins [4]. LncRNAs can modulate gene expressions through distinct mechanisms, including chromatin modification, transcription and post-transcription processing [5]. Furthermore, lncRNAs are important participants in cancer biology, which can lead to the imbalance of gene products, thus promoting or inhibiting the development of human cancer. LncRNA can also modulate tumorigenesis via multiple mechanisms, e.g., function as a molecular sponge to decoy miRNAs to reduce their availability or bind with proteins as a molecular guide to change their location [6,7]. *LINC00174* has been identified as an oncogenic factor in many human tumors [8–11]. For instance, LINC00174 facilitates the progression of breast cancer by negatively modulating miR-1827 expression [11]. However, the mechanism of how *LINC00174* works in NSCLC is still unclear.

E1A-binding protein p300 (EP300), mutated in some cancer cases, functions as a transcription co-factor and works with histone lysine acetyltransferase (KAT) [12–16]. The down-regulation of EP300 increases the apoptosis of NSCLC cells [17]. However, the mechanism of EP300 in NSCLC is also unclear. The nuclear factor of activated T cells 5 (NFAT5), part of the NFAT family, is a transcription factor that modulates the expressions of genes in osmotic stress [17]. *NFAT5* is highly expressed in pancreatic adenocarcinoma, adrenocortical carcinoma, and NSCLC and can promote cancer progression [18–20].

This research focused on deciphering the role of *LINC00174* in NSCLC. We aimed to understand the interaction of LINC00174 with the EP300/NFAT5 axis in NSCLC. The current results may offer new potential biomarkers or therapy targets for NSCLC.

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Materials and Methods

Patient Samples

Forty-eight patients with NSCLC in Yichang Central People's Hospital were included in this study. The patients did not receive any treatment prior to the surgery. NSCLC tissues and adjacent normal tissue specimens (>5 cm from the tumor margin) were collected and instantly frozen in liquid nitrogen (–196 °C). With written informed consent from the patients, this work was approved by the Ethics Committee of Yichang Central People's Hospital (Approval Number: YXLL_20210504). The collection and use of human samples followed the *Declaration of Helsinki*.

Cell Culture

The immortalized lung epithelial cell lines BEAS-2B (catalog number: AW-CNH004) and DMS114 (catalog number: BY-1144), Calu-1 (catalog number: SNL-278), DMS53 (catalog number: CL-0766), NCI-H1437 (catalog number: AW-CCH287), and NCI-H292 (catalog number: AW-CCH296) cells were available from ATCC (Manassas, VA, USA), and all of the cell lines were verified. All cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS; A5669401, Thermo Fisher Scientific, Waltham, MA, USA) and 100 U/mL penicillin and 100 µg/mL streptomycin (15140122, Invitrogen, Carlsbad, CA, USA) at 37 °C and 5% CO_2. The passage ratio was set at 1:3. Mycoplasma testing was performed before the experiments. STR profile was used to validate the purity of the cells before the experiments.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was reversely transcribed into complementary DNA (cDNA) by a cDNA synthesis kit (6111A, Takara, Dalian, China). qRT-PCR was accomplished by the SYBR® Premix Ex Taq™ kit (DRR041A, Takara, Dalian, China) on an ABI7300 real-time PCR (Applied Biosystems, Foster City, CA, USA) with a 20 µL system (containing cDNA, primers, and PCR kit). Amplification conditions were as follows: pre-denaturation (92 °C, 200 s); denaturation (97 °C, 20 s); annealing (62 °C, 1 min); a total of 45 cycles; and extension at 65 °C for 5 min. The primer sequences are detailed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) functioned as the internal control for LINC00174, EP300, and NFAT5.

Table 1. Primer sequences.

Name	Primer sequences
LINC00174	F: 5′-GTGGTTTGATCTTGGCTCAC-3′
	R: 5′-CCAGGGGGCTTCTTGTTGCAT-3′
EP300	F: 5′-GGCTGTATCAGAGCGTATTGTC-3′
	R: 5′-CCTCGAAATAAGGCAATTCC-3′
NFAT5	F: 5′-GAAGTGGACATTGAAGGCACT-3′
	R: 5′-CTGGCTTCGACATCAGCATT-3′
GAPDH	F: 5′-TGCACCACCAACTGCTTAGC-3′
	R: 5′-GGCATGGACTGTGGTCATGAG-3′
U6	F: 5′-CCGCCCGCCGCCAGGCCCC-3′
	R: 5′-ATATGGAACGCTTCACGAATT-3′

NFAT5, nuclear factor of activated T cells 5; *EP300*, E1A-binding protein p300; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Subcellular Isolation

The cytoplasmic and nuclear RNA purification kit (NGB-37400, Amyjet, Wuhan, China) was used for this assay. The subcellular fractions were obtained after centrifugation at 2500 g for 15 min. Next, LINC00174 expression in the cytoplasm and nucleus was then measured by qRT-PCR, with GAPDH (cytoplasm) and U6 (nuclei) as the controls of subcellular localization.

Transfection

Small interference RNAs (siRNAs) targeting the *LINC00174* gene (si-LINC00174#1: guide: 5′-UUAUCCAUCUUGAUAAAAGGU-3′, passenger: 5′-CUUUUAUCAAGAUGGAUAAAC-3′; and si-LINC00174#2: guide: 5′-UCAUUCAACACCAUUCACCUC-3′, passenger: 5′-GGUGAAUGGUGUUGAAUGAGG-3′), siRNA negative control (si-NC: guide: 5′-UCUACUGUCACUCAGUAGU-3′; passenger: 5′-ACUACUGAGUGACAGUAGA-3′), EP300 overexpression plasmid (EP300-OE), and *NFAT5* overexpression plasmid (NFAT5-OE), and their control plasmid (NC) were obtained from GenePharma Co., Ltd. (Shanghai, China). Calu-1 and NCI-H1437 cells were inoculated on a 6-well plate at 1×10^5 cells/well and cultured for 24 h. Then serum starvation was performed for 12 h, and subsequently, the cells were incubated with the oligonucleotides or plasmids [50 nM mixed with a transfection agent (Lipofectamine® 3000, Invitrogen, Carlsbad, CA, USA)]. The following designed experiments were performed after 24 h.

Cell Counting Kit-8 (CCK-8) Assay

Calu-1 and NCI-H1437 cells were prepared into cell suspension of 2×10^4/mL and inoculated onto 96-well plates (100 µL/well) before the culture started. At the indicated times, 10 µL of CCK-8 solution (C0037, Beyotime, Shanghai, China) was loaded into each well and incubated at 37 °C for 2 h. Then, the absorbance values were examined at 450 nm by a microplate reader.

Transwell Assay

Calu-1 and NCI-H1437 cells were made into single-cell suspension with serum-free RPMI-1640, with the density altered to 2×10^5 cells/mL. The suspension (200 µL) was loaded into the upper chamber of each Transwell insert (24-well insert; pore size 8 µm; Corning, New York,

NY, USA). The lower chamber was added with 500 µL of medium containing 10% FBS. These cells were cultured at 37 °C in 5% CO_2 for 48 h. After the chamber was taken out, the cells at the bottom of the membrane were instantly fixed with 4% paraformaldehyde, and 0.1% crystal violet solution was used to stain the cells. The remaining crystal violet solution was washed with phosphate-buffered solution (PBS). Ultimately, five fields of view were randomly chosen under an inverted microscope (CKX53, Olympus, Tokyo, Japan). The average value represented the migration ability of the cells. For the invasion experiments, the filter of Transwell insert was pre-coated with 50 µL of Matrigel (356234, BD Biosciences, San Jose, CA, USA), and the remaining steps followed the migration experiments.

Flow Cytometry Analysis

1×10^6 NSCLC cells were resuspended in 1 mL of PBS and followingly centrifuged (400 g, 2 min). The cells were then resuspended in 200 µL of PBS and instantly incubated with 10 µL of Annexin V-FITC and 10 µL of PI in darkness at 4 °C for 30 min. Then the cells were rinsed with PBS, and the apoptosis was measured with a FACSCount flow cytometer (FACSCount, BD Biosciences, San Jose, CA, USA).

Immunohistochemistry (IHC) Staining

Embedded tissue sections with a thickness of 4-µm were prepared for IHC analysis. The slices were dewaxed with xylene and rehydrated in ethanol. Then the antigen retrieval was performed with sodium citrate buffer solution (pH 6.0). The sections were incubated with 3% H_2O_2 for 10 min. The sections were washed in PBS, blocked in 10% goat serum for 30 min and incubated overnight with anti-NFAT5 antibodies (ab3446, 1:300, Abcam, Shanghai, China). They were then further incubated for 30 min at ambient temperature with a secondary antibody (ab6721, 1:2000, Abcam, Shanghai, China). The slices were stained with diaminobenzidine solution and then re-stained with 20% hematoxylin.

Staining was scored according to a previous study [21]. The ratio of positively stained tumor cells was meticulously scored at 0 (no tumor cell staining), 1 (<25%), 2 (25–50%), 3 (50–75%) and 4 (75–100%). The staining intensity was graded as 3 (brown, strong), 2 (yellowish-brown, medium), 1 (pale yellow, weak) and 0 (no staining). IHC score = the proportion of tumor cells with positive staining × staining intensity. NFAT5 expression was grouped into a high expression group (IHC staining score >6) and a low group (IHC score ≤6) as per the IHC staining score.

Western Blot Analysis

An equal amount of protein was denatured in boiling water and subsequently separated with SDS-PAGE (60V, 10 min; 120 V, 50 min) and electrotransferred (200 mA; 1 h) to a polyvinylidene fluoride membrane (IPVH00010, Millipore, Temecula, CA, USA), which was blocked with 5% skimmed milk at ambient temperature for 1 h. The membranes were incubated overnight at 4 °C with primary anti-EP300 antibody (ab275378, 1:1000), anti-NFAT5 antibody (ab3446, 1:1000) and anti-GAPDH antibody (ab9485, 1:1000) and then with the secondary antibody (ab6721, 1:4000) for 2 h. All of the antibodies were from Abcam (Cambridge, UK). The protein bands were visualized by the ECL system (P0018AFT, Beyotime, Shanghai, China).

RNA Binding Protein Immunoprecipitation (RIP) Assay

RIP analysis was conducted using the Magna RIP RNA binding protein immunoprecipitation kit (17-611, Millipore, Temecula, CA, USA). Calu-1 and NCI-H1437 cells were subsequently lysed in RIP buffer for 5 min, then centrifuged, and the supernatant was collected. RIP buffer containing magnetic beads coated with anti-EP300 antibody (ab275378, Abcam, Shanghai, China) or immunoglobulin G (IgG) antibody (ab172730, Abcam, Shanghai, China) was loaded and incubated at 4 °C overnight. The immunoprecipitated complex was obtained and then immersed in RIP buffer. The protein was removed using proteinase K, and the RNA was extracted. qRT-PCR was accomplished to probe the enrichment of LINC00174 in the complex.

Dual Luciferase Reporter Gene Assay

The target fragments of wild-type (WT) NFAT5 and the mutant type (MUT) NFAT5 were constructed and subsequently integrated into a PGL3 vector (E1771, Promega, Madison, WI, USA). NFAT5-WT reporter or NFAT5-MUT reporter (0.5 µg) was then co-transfected with the EP300 overexpression plasmid or its control plasmid (50 nM) into Calu-1 and NCI-H1437 cells. The luciferase activity was measured after 48 h.

Chromatin Immunoprecipitation (ChIP) Assay

A ChIP kit (17-371, Millipore, Temecula, CA, USA) was used in this assay. Calu-1 and NCI-H1437 cells transfected with si-LINC00174#1 or si-si-LINC00174#2 were crosslinked with 1% formaldehyde and rinsed with cold PBS containing protease inhibitors. The cells were then collected, resuspended in 200 µL lysis buffer, and incubated on ice for 10 min. The sample was centrifuged (2500 g, 15 min), and the supernatant was collected and diluted in a dilution buffer. The cross-linked chromatin was incubated with an anti-EP300 antibody (ab275378, Abcam, Shanghai, China) or control IgG (ab172730, Abcam, Shanghai, China) overnight at 4 °C. The antibody-protein-DNA complex was immunoprecipitated by salmon sperm DNA/protein A, with 5 M NaCl for reverse crosslinking. Samples were then purified by the QIAquick PCR purification kit (28104, QIAGEN, Chatsworth, CA, USA) and subjected to qRT-PCR detection.

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Statistical Analysis

The analysis was conducted with SPSS 20.0 (IBM, Chicago, IL, USA) and Graphpad Prism 9.3 (Graphpad, Boston, MA, USA). Data were presented as mean ± standard deviation value. Student's *t-test* was applicable for the detection of comparisons between two groups. For the data of multiple groups, one-way analysis of variance and Tukey post-hoc test were conducted for comparisons. The Kaplan-Meier analysis with a log-rank test was utilized to probe the overall survival rate. Pearson's correlation coefficient was applicable to examine the interrelation of gene expression. $p < 0.05$ reflects statistical significance.

Results

Clinical Significance of LINC00174 in NSCLC

StarBase database (https://starbase.sysu.edu.cn/) revealed that the expression of *LINC00174* was abnormally raised in both lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) (Fig. 1A,B). *LINC00174* expression in tumor tissues of 48 patients with NSCLC was significantly higher than in paracancerous tissues (Fig. 1C). High expression of *LINC00174* was pertinent to a shorter overall survival time (Fig. 1D). In addition, *LINC00174* expressions were markedly increased in the cancer cell lines (DMS 114, Calu-1, DMS 53, NCI-H1437, and NCI-H292) compared with that of BEAS-2B cells (Fig. 1E). qRT-PCR highlighted that *LINC00174* was predominantly present in the nuclei of Calu-1 and NCI-H1437 cells, suggesting that it could probably exert its biological function at the transcriptional level (Fig. 1F). Chi-square test suggested that high expression of *LINC00174* was relevant to positive lymph node metastasis, larger tumor size, and later TNM staging in patients with NSCLC (Table 2).

LINC00174 Depletion Curbs the Malignancy of NSCLC Cells

We selected the Calu-1 and NCI-H1437 cell lines with the most up-regulated *LINC00174* for the following experiments. Transfection of si-LINC00174#1 or si-LINC00174#2 significantly reduced expression levels of *LINC00174* (Fig. 2A). LINC00174 depletion dramatically

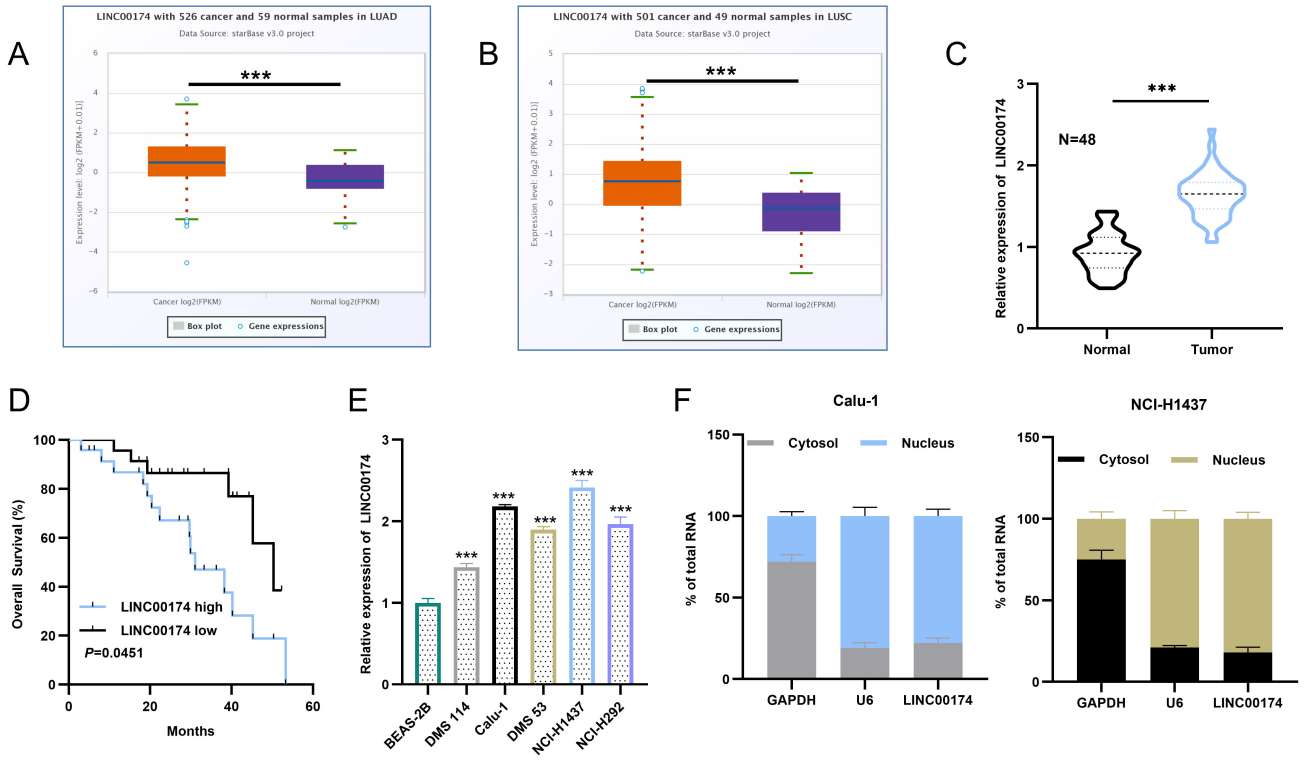

Fig. 1. Clinical significance of *LINC00174* in NSCLC. (A) *LINC00174* expression levels in lung adenocarcinoma (LUAD) were probed by the StarBase database. (B) *LINC00174* expression levels in lung squamous cell carcinoma (LUSC) were probed by the StarBase database. (C) *LINC00174* expressions in 48 pairs of non-small cell lung cancer (NSCLC) tissues and paracancerous tissues were determined by quantitative real-time PCR (qRT-PCR). (D) Kaplan-Meier curve showed the interplay between LINC00174 expressions and the overall survival (high expression group: n = 24; low expression group: n = 24). (E) The relative expressions of LINC00174 in different lung cancer cell lines (DMS 114, Calu-1, DMS 53, NCI-H1437 and NCI-H292) and BEAS-2B cells were examined by qRT-PCR. (F) Subcellular fraction assay showed the subcellular distribution of LINC00174. All of the experiments were performed in triplicate and repeated for 3 times. Note: ***$p < 0.001$.

Table 2. The correlation of *LINC00174* expression with clinicopathological variables.

Characteristics	All case (n = 48)	*LINC00174* expression		χ^2	*p* value
		Low	High		
Gender					
Male	28	13	15	0.343	0.558
Female	20	11	9		
Age (years)					
≤60	27	12	15	0.762	0.383
>60	21	12	9		
Lymphatic metastasis					
Negative	18	14	4	8.889	0.003**
Positive	30	10	20		
Histology					
Squamous cell carcinoma	19	9	10	0.087	0.768
Adenocarcinoma	29	15	14		
Tumor size					
<3 cm	17	12	5	4.463	0.035*
≥3 cm	31	12	19		
TNM stage					
I/II	16	12	4	6.000	0.014
III/IV	32	12	20		
Smoking history					
Yes	21	10	11	0.085	0.771
No	27	14	13		
Differentiation					
High and moderate	17	10	7	0.820	0.365
Poor	31	14	17		

*$p < 0.05$ and **$p < 0.01$.

restrained the cell viability (Fig. 2B). Transwell assay suggested that the migrative and invasive abilities of the cells were significantly inhibited after LINC00174 knockdown (Fig. 2C,D). In addition, knocking down LINC00174 led to the increased apoptosis rate of Calu-1 and NCI-H1437 cells (Fig. 2E).

LINC00174 Up-Regulates NFAT5 by Binding to EP300

Next, we focused on the interrelations between LINC00174 and EP300/NFAT5. First, NFAT5 expression in NSCLC was probed by IHC staining, which showed that NFAT5 expression levels in NSCLC tissues were remarkably higher (Fig. 3A,B). Consistently, high expression of NFAT5 mRNA was also observed (Fig. 3C). LINC00174 expression levels were positively correlated with NFAT5 mRNA expression levels in tumor tissues in the 48 selected NSCLC patients (Fig. 3D). Western blot showed that LINC00174 knockdown reduced NFAT5 expression in Calu-1 and NCI-H1437 cells (Fig. 3E). We performed a RIP experiment to verify the interaction of EP300 with LINC00174 in NSCLC. The findings showed that LINC00174 was significantly enriched in the anti-EP300 group of Calu-1 and NCI-H1437 cells (Fig. 3F), indicating an interaction between LINC00174 and EP300. Through an analysis of the Promo Database (http://alggen.lsi.upc.es), the binding sites for the promoter regions of EP300 and NFAT5 (three potential sites) were obtained (Fig. 3G). The dual luciferase reporter gene proved that the EP300 overexpression greatly enhanced the activity of NFAT5-WT in Calu-1 and NCI-H1437 cells, but that of NFAT5-MUT was not greatly affected (Fig. 3H). In addition, ChIP analysis results showed that the binding of EP300 to the NFAT5 promoter was blocked following the knockdown of LINC00174 (Fig. 3I). These results indicate that LINC00174 positively regulates NFAT5 expression by binding to EP300.

Knockdown of LINC00174 Rescues the Impacts of NFAT5 Overexpression on the Malignancy of NSCLC Cells

Western blot analysis highlighted that NFAT5 and EP300 expressions were markedly increased in Calu-1 and NCI-H1437 cells transfected with the NFAT5 overexpression plasmid while decreased after co-transfection with si-LINC00174#2 (Fig. 4A). Additionally, NFAT5 overexpression markedly strengthened the proliferative, migrative and invasive potential of Calu-1 and NCI-H1437 cells and in-

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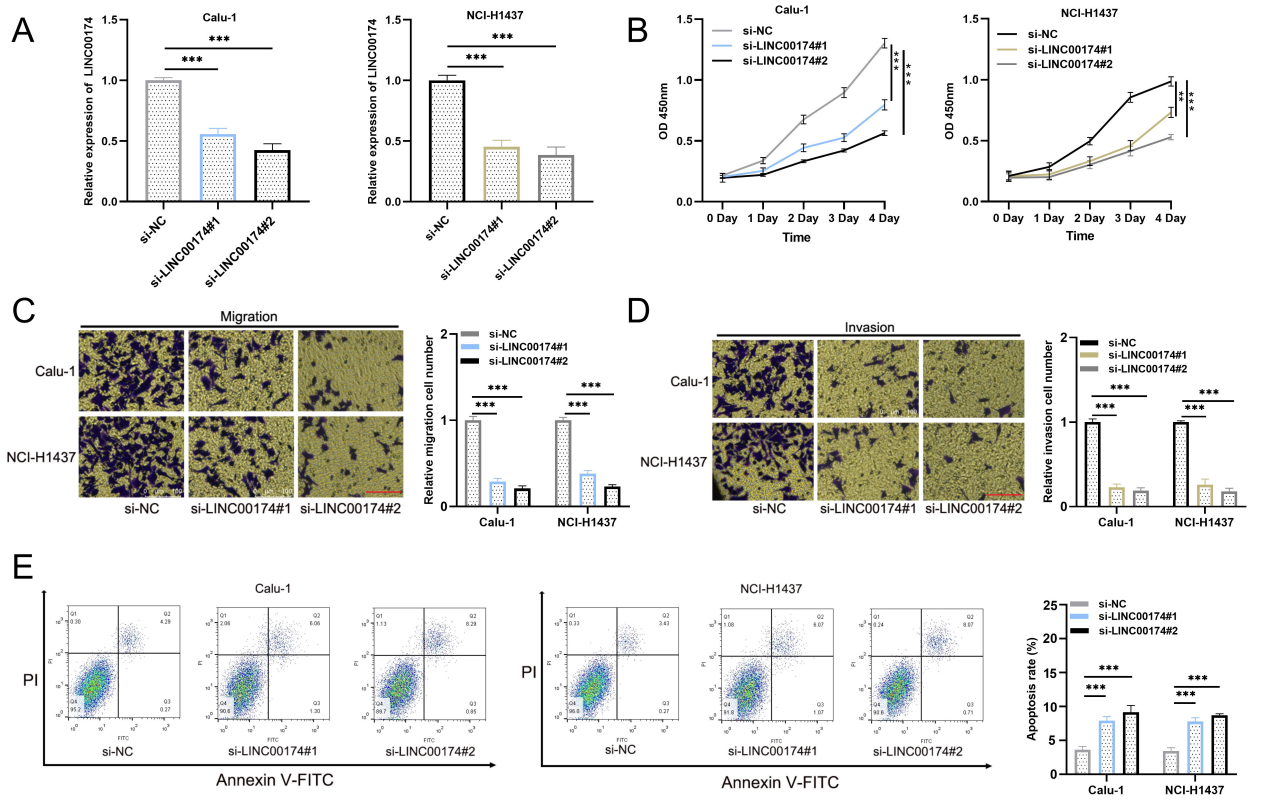

Fig. 2. Effects of LINC00174 depletion on the malignancy of NSCLC cells. (A) si-LINC00174#1 or si-LINC00174#2 was transfected into Calu-1 and NCI-H1437 cells, respectively, and the efficacy was rated by qRT-PCR. (B–E) The multiplication, migration, invasion and apoptosis of Calu-1 and NCI-H1437 cells after depleting LINC00174 were examined by Cell Counting Kit-8 (CCK-8) assay (B), Transwell assay (C,D) and flow cytometry (E). Scale bar = 100 μM. $^{**}p < 0.01$ and $^{***}p < 0.001$.

hibited apoptosis (Fig. 4B–E). Notably, LINC00174 knockdown rescued the impacts of NFAT5 overexpression on the malignant biological processes of Calu-1 and NCI-H1437 cells (Fig. 4B–E).

Discussions

LncRNA was previously categorized to be a by-product of the transcription process [22]. However, lncRNAs can be extensively implicated in the physiological and pathological processes by modulating gene expression, and they interfere with tumor progression by impairing multiple aspects of tumor cells [23,24]. Disorders of lncRNAs are vital in the carcinogenesis of NSCLC [25–27]. For instance, lncRNA TRERNA1 is increased in NSCLC and can accelerate disease progression by targeting FOXL1 [26]. LncRNA PCAT1 can interact with DKC1 to promote the growth, invasion and curb the apoptosis of NSCLC cells [27]. In this study, we discovered that the expression of *LINC00174* was increased in NSCLC, and this change indicates poor pathological features and poor prognosis of NSCLC. Functionally, LINC00174 knockdown reduced the malignancy of NSCLC cells and promoted apoptosis. This indicates that *LINC00174* may participate in NSCLC progression as an oncogenic lncRNA.

EP300 is a large multi-domain protein with histone acetyltransferase activity [28]. EP300 can bind to chromosomal proteins and regulate chromatin remodeling through histone acetylation, activating transcription of various genes [28,29]. Although *EP300* is a tumor suppressor that prevents carcinogenesis of normal epithelial cells [30,31], high expression of *EP300* in various categories of cancers is frequently observed and is pertinent to poor prognosis in patients [32,33]. EP300 knockdown can block the migration and aggressiveness of esophageal cancer cells [34]. In the current study, LINC00174 modulated the transcriptional activity of EP300, which partly explains the mechanism of *EP300* dysfunction in cancer biology.

NFAT5 is primarily identified as a transcription factor for interleukin-2 in T cells [35] and is also pivotal in tumor development [36]. *NFAT5* can restrain the aggressiveness of hepatocellular carcinoma cells and promote apoptosis via regulating PARP-1 expression [37]. Conversely, in pancreatic cancer, *NFAT5* was highly expressed and could predict the pessimistic prognosis of the patients, suggesting that it is a tumor promoter [18]. In NSCLC, *NFAT5* expression is elevated, which can strengthen the viability and migration of cancer cells [38]. In this study, we observed high expression of *NFAT5* in NSCLC tissues, which is coherent with the previous study [38], and discovered that *NFAT5* was

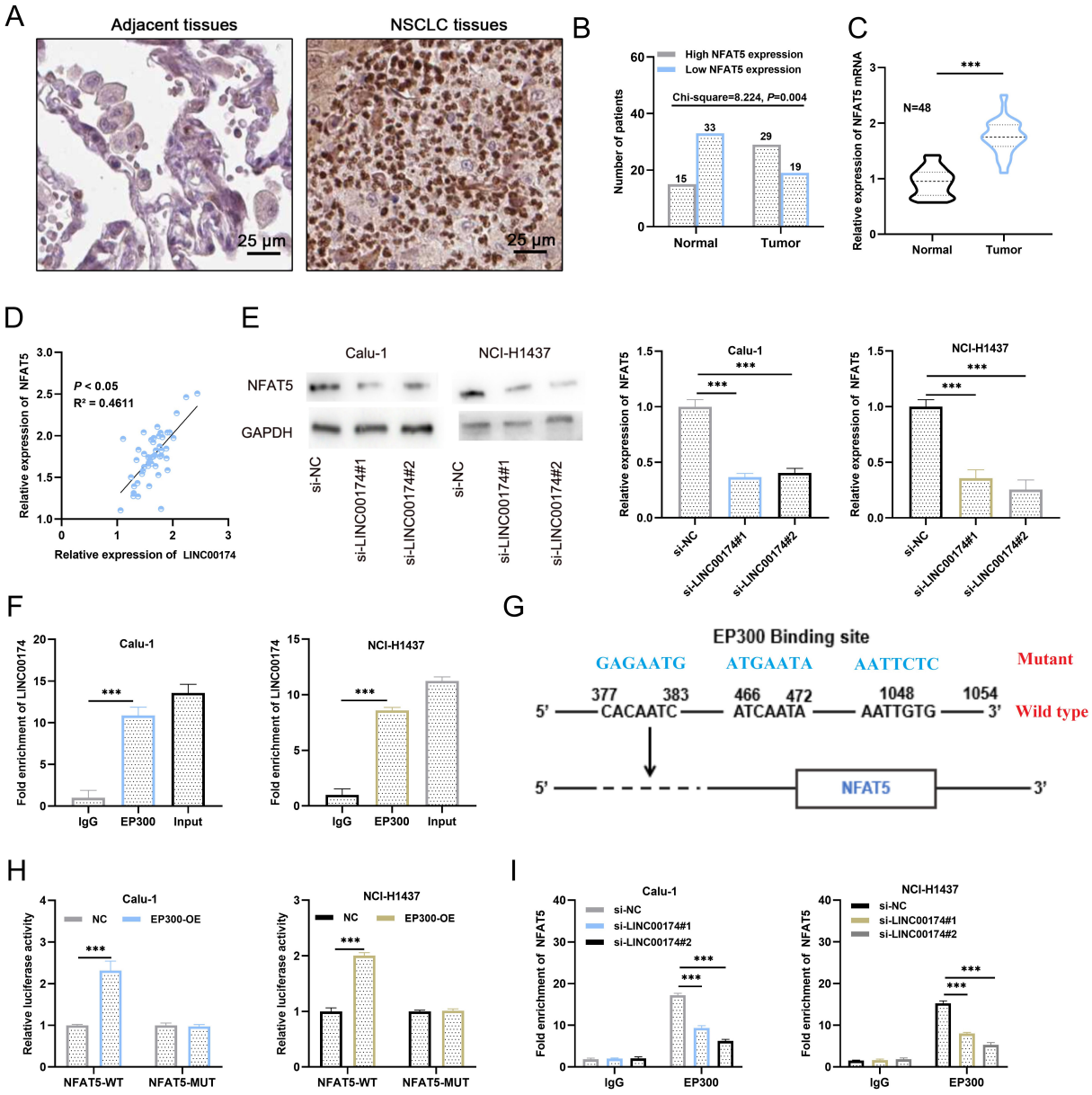

Fig. 3. LINC00174 induces *NFAT5* expression by binding to EP300. (A,B) NFAT5 expressions in NSCLC tissues and paracancerous tissues were estimated by IHC (n = 48). (C) qRT-PCR was applicable for the examination of NFAT5 mRNA expressions in NSCLC. (D) Pearson's correlation analysis showed the interrelation between NFAT5 and LINC00174 expressions in NSCLC tissues (n = 48). (E) The effect of LINC00174 depletion on NFAT5 expression was exposed by Western blot. (F) RIP experiment testified the direct binding relations between LINC00174 and EP300. (G) PROMO database predicted the binding sequence of EP300 to the *NFAT5* promoter region. (H) Dual-luciferase reporter gene experiment was used to detect the binding relations between EP300 and NFAT5 (WT, wild type; MUT, mutant type). (I) ChIP assay allowed the detection of the effect of LINC00174 knockdown on the binding of EP300 to the *NFAT5* promoter region. ***$p < 0.001$.

a downstream target of EP300. In addition, LINC00174 knockdown reduces the ability of EP300 to activate *NFAT5* transcription. It also reveals that LINC00174 depletion rescued the effect of NFAT5 on NSCLC cells. LINC00174 is a vital upstream modulator of *NFAT1*, and the biological function of LIN00174 is at least partially mediated by EP300.

Conclusions

LINC00174 is highly expressed in NSCLC, which impairs the prognosis in patients with NSCLC. Mechanistically, LINC00174 facilitates the transcription of *NFAT5* by recruiting EP300 to *NFAT5* promoter region, thereby promoting the proliferative, migrative and aggressive capabilities of NSCLC cells and inhibiting the apoptotic abilities.

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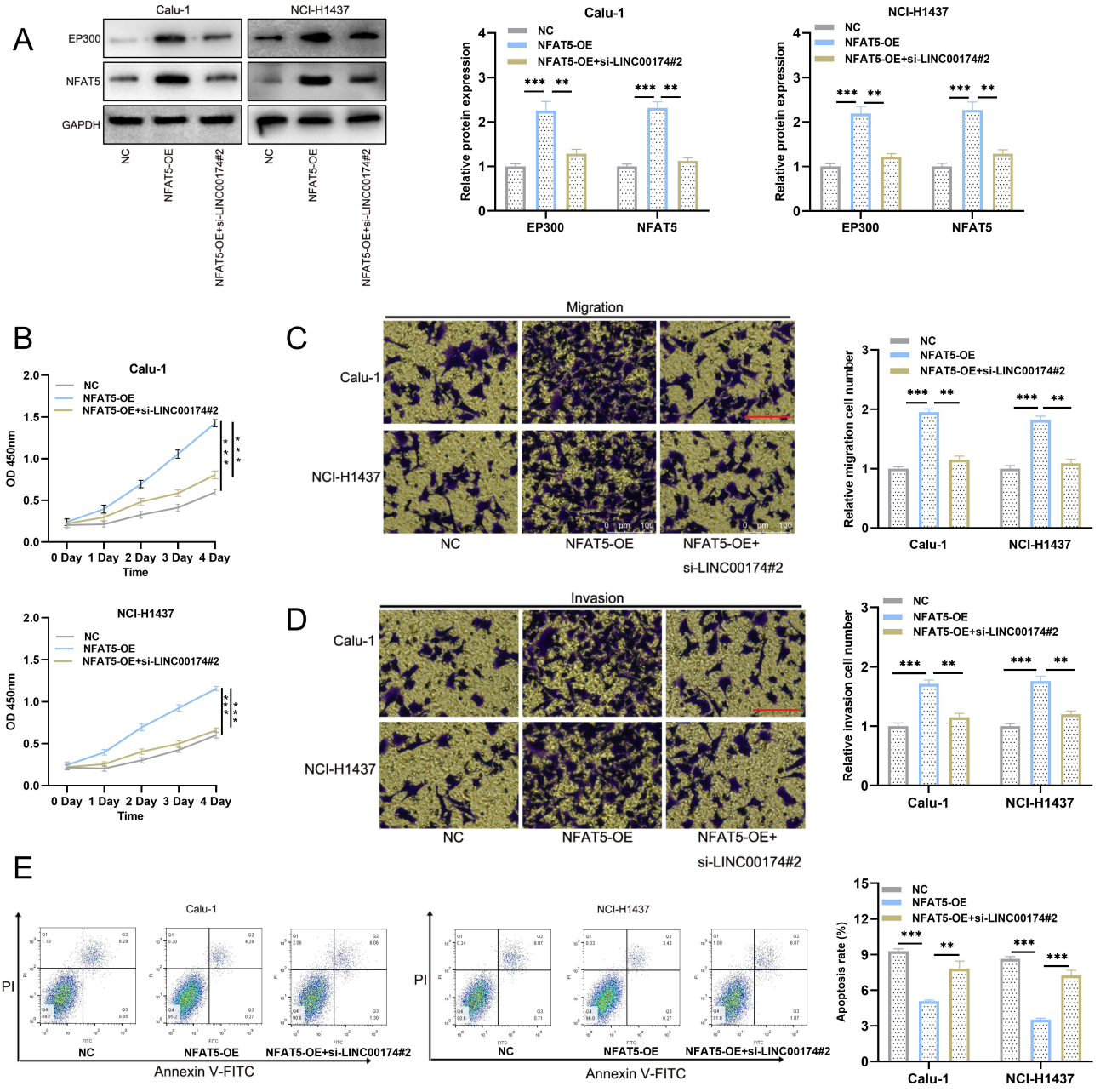

Fig. 4. LINC00174 knockdown reverses the impacts of NFAT5 overexpression on NSCLC cells. Calu-1 and NCI-H1437 cells were grouped into NC, NFAT5 overexpression, and NFAT5 overexpression + LINC00174 knockdown groups. (A) Western blot was applied for detecting EP300 and NFAT5 expressions. (B) The viability of NSCLC cells was measured by CCK-8 assay. (C–E) Transwell assay and Flow cytometry were applicable for detecting the migration, aggressiveness and apoptosis of NSCLC cells. Scale bar = 100 μM. $^{**}p < 0.01$ and $^{***}p < 0.001$.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

PX and GZ designed the research study. PX and ZC performed the research. PX, ZC and XG analyzed the data, PX drafted the manuscript. SG reviewed the manuscript and made necessary advice on the experiment design. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

With written informed consent from the patients, this work was approved by the Ethics Committee of Yichang Central People's Hospital (Approval Number: YXLL_20210504). The collection and use of human samples followed the *Declaration of Helsinki*.

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Conflict of Interest

The authors declare no conflict of interest.

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