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LINC00511 promotes the progression of non-small cell lung cancer through downregulating LATS2 and KLF2 by binding to EZH2 and LSD1

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Abstract. – OBJECTIVE: Lung cancer is a malignant tumor with extremely high morbidity and mortality. Recent studies have identified the vital role of LINC00511 (lncRNAs) in the development and progression of non-small cell lung cancer (NSCLC). In this research, we aim to explore the biological function of LINC00511 in the development and metastasis of NSCLC.

PATIENTS AND METHODS: LINC00511 expression in 57 paired NSCLC patients' tissues and matched normal tissues were detected by Real-time quantitative polymerase chain reaction (RT-qPCR). Cell proliferation assay, colony formation assay and transwell assay were conducted to observe the biological behavior changes of NSCLC cells through the influence of LINC00511. In addition, dual-luciferase reporter gene assay, RNA immunoprecipitation assay (RIP) and, chromatin immunoprecipitation (ChIP) were performed to discover the potential targets of LINC00511 in NSCLC cells.

RESULTS: LINC00511 was highly expressed in NSCLC tissues and cell lines compared with controls. LINC00511 expression was positively correlated with tumor size, tumor stage, lymph node metastasis and distant metastasis, but negatively correlated with overall survival (OS) of NSCLC patients. Receiver Operating Characteristic (ROC) curves suggested that LINC00511 could be an effective indicator to distinguish NSCLC patients from normal people. Cell counting kit-8 (CCK-8), flow cytometry and transwell assay showed that knockdown of LINC00511 in A549 cells decreased viability, accelerated apoptosis and inhibited invasive and migratory abilities. Overexpression of LINC00511 in PC9 cells obtained the opposite biological effects. Chromatin fractionation predicted that LINC00511 was mainly distributed in the nucleus. RIP and ChIP assay showed that LINC00551 directly bound to lysine-specific demethylase 1 (LSD1) and enhancer of zeste homolog 2 (EZH2). It inhibited expressions of LATS2 and KLF2 by binding to their promoter regions.

CONCLUSIONS: LINC00511 is upregulated in NSCLC tissues and cell lines. It is closely related to tumor size, tumor stage, lymph node metastasis and, distant metastasis of NSCLC patients. Knockdown of LINC00511 attenuates proliferative, migratory and invasive capacities, but induces apoptosis of NSCLC cells. LATS2 and KLF2 are target genes of LINC00511, which are regulated by LINC00511 through binding to EZH2 and LSD1, thus influencing the progression of NSCLC.

Key Words:
NSCLC, LINC00511, EZH2, LSD1, LATS2, KLF2.

Introduction

Lung cancer is a malignant tumor with extremely high morbidity and mortality, which has been well concerned in recent years. There are millions of new cases of lung cancer per year throughout the world. In China, the mortality of lung cancer increases at a rate of 4.5% per year. It is estimated that by 2030, 1.7 million middle-aged people in China will die of lung cancer each year[1,2]. More than 80% of lung cancer cases pathologically belong to non-small cell lung cancer (NSCLC). Although progresses have been made in the treatment of NSCLC, the 5-year survival rate remains low. The pathogenesis of NSCLC is a complex process involving various factors, such as heredity, environment and living habits. The exact

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molecular mechanism of NSCLC pathogenesis remains unclear.

Long noncoding RNAs (lncRNAs) are a class of transcripts without an open reading frame and distributed in the nucleus or cytoplasm. They have a length greater than 200 nucleotides, and are structurally similar to that of mRNA[3]. In 2012, Stanford Medicine conducted the first large-scale analysis of lncRNA expression in cancer was conducted in Stanford Medicine. 1065 tumor-related lncRNAs have been identified through RNA sequencing of 64-tumor samples[4]. LncRNA mainly exerts its biological functions by regulating expressions of functional genes at epigenetic, transcriptional and post-transcriptional levels[5,6]. Many studies have reported the important regulatory role of lncRNA in tumors. Highly expressed LINC00473 regulated by cAMP/CREB axis in LKB1-inactivated lung cancer promotes tumor growth[7]. UCA1 serves as a ceRNA and promotes the proliferative and metastatic rates of NSCLC cells, through alleviating the inhibitory effect of miR-193a-3p on its target gene ERBB4[8]. By analyzing the TCGA database, Zhang et al[9] found that highly expressed SPRY4-IT1 in lung adenocarcinoma promotes tumor metastasis. LUCAT1 is closely related to the clinical prognosis of NSCLC. It promotes the proliferative rate of NSCLC cells by inhibiting expressions of p21 and p57 through binding to PRC2[10].

Studies have shown that LINC00511 is involved in the development of multiple tumors, such as tongue squamous cell carcinoma[11], bladder cancer[12], and pancreatic ductal adenocarcinoma[13]. LINC00511 is highly expressed in NSCLC tissues and cell lines and it indicates a poor prognosis in NSCLC patients. LINC00511 knockdown inhibits proliferative, migratory and invasive capacities of A549 and SPCA1 cells, and inhibits tumorigenesis *in vivo*, but promotes cell apoptosis *in vitro*. In addition, LINC00511 exerts its carcinogenesis effect by partially inhibiting p57 expression through direct binding to enhancer zeste homolog 2 (EZH2). LINC00511 may be a useful hallmark and potential therapeutic target in NSCLC[14]. However, other potential targets and mechanisms regulated by LINC00511 are still needed to be further explored.

Patients and Methods

Microarray Data and Data Preprocessing

The lncRNA expression profile of GSE30219 was downloaded from the Gene Expression Omnibus database. The downloaded dataset included 293 lung tumor samples and 14 non-tumoral lung samples. The *p*-values obtained for all genes were adjusted using the Limma R package. An adjusted *p*-value < 0.01 was designated as the cut-off value.

Sample Collection

A total of 57 cases of NSCLC and non-tumoral adjacent tissues were harvested from the radical or palliative resection in Danyang People's Hospital of Jiangsu Province from January 2007 to December 2017. Non-tumoral adjacent tissues located 5 cm away from the tumor tissues were resected. Personal information and detailed clinical information of enrolled patients were collected. All tissues were pathologically diagnosed as NSCLC. Fresh specimens that were surgically resected were immediately frozen in liquid nitrogen. None of the patients were treated with preoperative radiotherapy. The study was approved by the Ethics Committee of the Danyang People's Hospital of Jiangsu Province and all patients signed informed consent.

Cell Culture and Transfection

Normal human bronchial epithelial cell line (BEAS-2B) and lung cancer cell lines (A549, PC9 and H460) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA, USA) containing 10% FBS (fetal bovine serum) (Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were incubated in a 5% CO_2 incubator at 37°C.

One day prior to cell transfection, cells in good growth condition were seeded into 6-well plates with serum-free medium at a density of 5×10^5 cells per well. Transfection was performed when the confluence was up to 70-80% following the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Plasmids or Lipofectamine 2000 were diluted in Opti-MEM and then mixed together at room temperature for 15-20 min maintenance. Relative transfection plasmids, including si-LINC00511, pcDNA-LINC00511, si-LSD1, si-EZH2, pcDNA-LATS2 and negative control were purchased from Ribobio (Guangzhou, China).

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA in treated cells was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga,

Japan). RNA concentration was detected using a spectrometer. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq TM (TaKaRa, Otsu, Shiga, Japan). The relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method. Primers used in the study were as follows: LINC00511: F: TCTCCTTGTGCAAACCTCC, R: CCCACATTGAGCGAATGCC; P21: F: GTCGCTGTCTTGCACTCTGG, R: CCAATCTGCGCTTGGAGTGATA; LATS1: F: CCACCCTACCCAAAACATCTG, R: CGCTGCTGATGAGATTTGAGTAC; LATS2: F: TAGAGCAGAGGGCGCGGAAG, R: CCAACACTCCACCAGTCACAGA; PTEN: F: TGGCGGAACTTGCAATCCTCAGT, R: TCCCGTCGTGTGGGTCCTGA; KLF2: F: GCCTGTGGGTTCGCTATAAA, R: AAGGAATGGTCAGCCACATC; EZH2: F: GTGGAGAGATTATTTCTCAAGATG, R: CCGACATACTTCAGGGCATCAGCC; CoREST: F: GCCGCACCTCAGCTTATTATG, R: CCGGCATCAGTTCTGCCAT; LSD1: F: CCTGAAGAACCATCGGGTGT, R: CCTTCTGGGTCTGTTGTGGT; GAPDH: F: GAAGATGGTGATGGGATTTC, R: GAAGGTGAAGGTCGGAGTC.

Cell Proliferation Assay

Transfected cells were prepared for suspension with a density of 1×10^4/ mL. 200 μL of suspension was added in each well of 96-well plate. Each group set 10 replicates. 10 μL of 5 mg/mL cell counting kit-8 (CCK-8) solution (Dojindo Laboratories, Kumamoto, Japan) was added in each well after cell culture for 6, 24, 48, 72 and 96 h, respectively. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow Cytometry

Apoptosis was observed 48 h after transfection in each group by Annexin V-fluorescein isothiocyanate (FITC)/Propidium Iodide (PI) double staining flow cytometry. Cell density was adjusted to 5×10^5 cells/ml. Subsequently, cells were washed with phosphate-buffered saline (PBS), resuspended in 500 μL of binding buffer, and incubated with 5 μL of Annexin V-FITC and 10 μL of PI at 18-28°C in dark. The mixture was centrifuged for 15 min and analyzed using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The experiment was repeated 3 times.

Transwell Migration and Invasion Assays

50 mg/L Matrigel was melted at 4°C and diluted in serum-free medium at a ratio of 1:5.5. Matrigel was coated on the upper transwell chamber with 50 μL per well and incubated for 60 min at 37°C. Cells were resuspended in medium containing 1% FBS and adjusted to 1×10^5 cells/mL. 400 μL of suspension and 600 μL of medium containing 10% FBS were added in the upper and bottom chamber, respectively. After cell culture for 24 h, cells were fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 15 min. Inner cells were carefully cleaned. Penetrating cells were captured and calculated in 10 randomly selected fields of each sample. Procedures of cell invasion assay were the same as the above except for Matrigel pre-coating (BD Pharmingen, San Diego, CA, USA).

Western Blot

Cells were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer in the presence of a protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA) to harvest total cellular protein. The protein concentration of each cell lysate was quantified using the BCA (bicinchoninic acid) protein assay kit (Pierce, Rockford, IL, USA). An equal amount of protein sample was loaded onto a 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gel and then transferred to a PVDF (polyvinylidene difluoride) membrane (Roche, Basel, Switzerland) after separation. After blocking with skim milk, membranes were incubated with primary antibody (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C and then incubated with HRP (horseradish peroxidase) conjugated secondary antibody for 2-3 h at room temperature. Finally, an image of the protein band was captured by the Tanon detection system using enhanced chemiluminescence (ECL) reagent (Thermo-Fisher Scientific, Waltham, MA, USA).

Chromatin Fractionation

Until cell growth to 1×10^6/mL, 200 μl of Lysis Buffer J was added to fully lyse the cells. After centrifugation, the supernatant contained cytoplasmic RNA, and the remaining liquid contained nuclear RNA. The supernatant was transferred to new tubes. Buffer SK was added in the supernatant containing cytoplasmic RNA, and absolute ethanol was added in the liquid containing nuclear RNA. Column centrifugation was performed to elute cytoplasmic RNA and nuclear RNA, and finally subjected to qRT-PCR for determination.

RNA Immunoprecipitation

The Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA,

USA) was used for RIP assay. 100 µl of cell lysis was incubated with RIP buffer containing magnetic beads conjugated with antibodies of LSD1, EZH2 and CoREST or control IgG (Millipore, Billerica, MA, USA) for 6 h at 4°C. The beads were then washed with washing buffer, followed by incubation with 0.1% SDS/0.5 mg/ml Proteinase K at 55°C for 30 min. RNA concentration was measured using a NanoDrop (Thermo-Fisher Scientific, Waltham, MA, USA), followed by quality assessment using a bioanalyzer (Agilent, Santa Clara, CA, USA). QRT-PCR was finally conducted after isolation and purification of immunoprecipitated RNA.

Chromatin Immunoprecipitation (ChIP)

A549 and PC9 cells were fixed in 1% formaldehyde for 10 min. Anti-RNA polymerase, normal mouse IgG and corresponding antibodies were then immunoprecipitated overnight at 4°C. Protein G agarose (Thermo Fisher Scientific, Waltham, MA, USA) was added to collect immune complexes. The beads were resuspended in elution buffer and incubated overnight at 65°C before DNA extraction. DNA purification was performed using a spin column and quantified using qRT-PCR.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$). Measurement data and classification data were compared using the *t*-test. Analysis of survival data was performed using Kaplan-Meier method. Receiver Operating Characteristic (ROC) curves were utilized for analyzing the diagnostic value of LINC00511 in NSCLC. $p<0.05$ considered the difference was statistically significant.

Results

High Expression of LINC00511 in NSCLC

First, the expression profile of lncRNAs in the NSCLC dataset GSE30219 extracted from the GEO database was analyzed (Figure 1A). LINC00511 expression was remarkably upregulated in NSCLC tissues (Figure 1B), and negatively correlated with the OS of NSCLC patients (Figure 1C).

Further, we determined LINC00511 expression in NSCLC tissues collected from our hospital. Identically, LINC00511 was highly expressed in NSCLC tissues than non-tumoral tissues (Figure 2A). Based on the median expression of LINC00511, 57 NSCLC patients were divided into high-expression group and low-expression group (Figure 2B). LINC00511 expression was positively correlated with tumor size, tumor stage, lymph node metastasis and distant metastasis of NSCLC patients. However, LINC00511 expression was not correlated to sex and age of NSCLC patients (Table I). Based on the tumor size of NSCLC tissues, higher expression of LINC00511 was found in NSCLC patients with tumor tissues larger than 3 cm in diameter compared with those smaller than 3 cm (Figure 2C). Survival curves suggested the prognostic value of LINC00511 in NSCLC (Figure 2D). ROC curves indicated a certain diagnostic significance of LINC00511 in NSCLC, which can distinguish NSCLC tissues from normal lung tissues (AUC=0.9821, $p<0.05$) (Figure 2E).

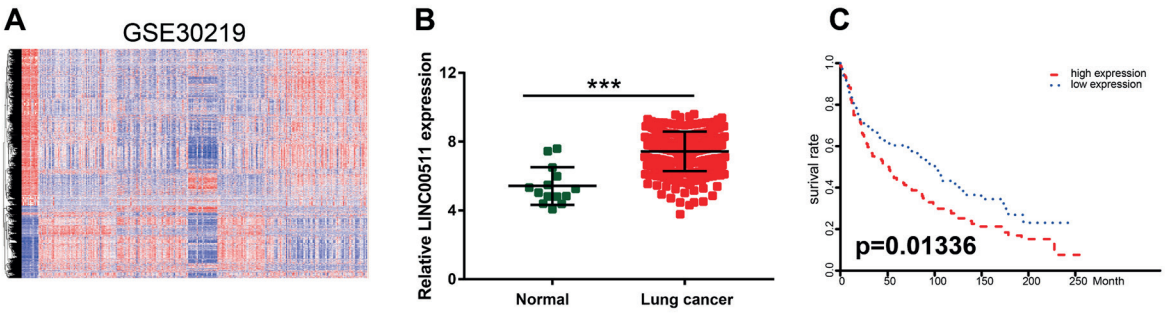

Figure 1. LINC00511 was highly expressed in the NSCLC dataset GSE30219. **A,** Differentially expressed lncRNAs in GSE30219. **B,** LINC00511 expression was significantly upregulated in GSE30219. **C,** Expression level of LINC00511 was negatively correlated to OS of NSCLC patients.

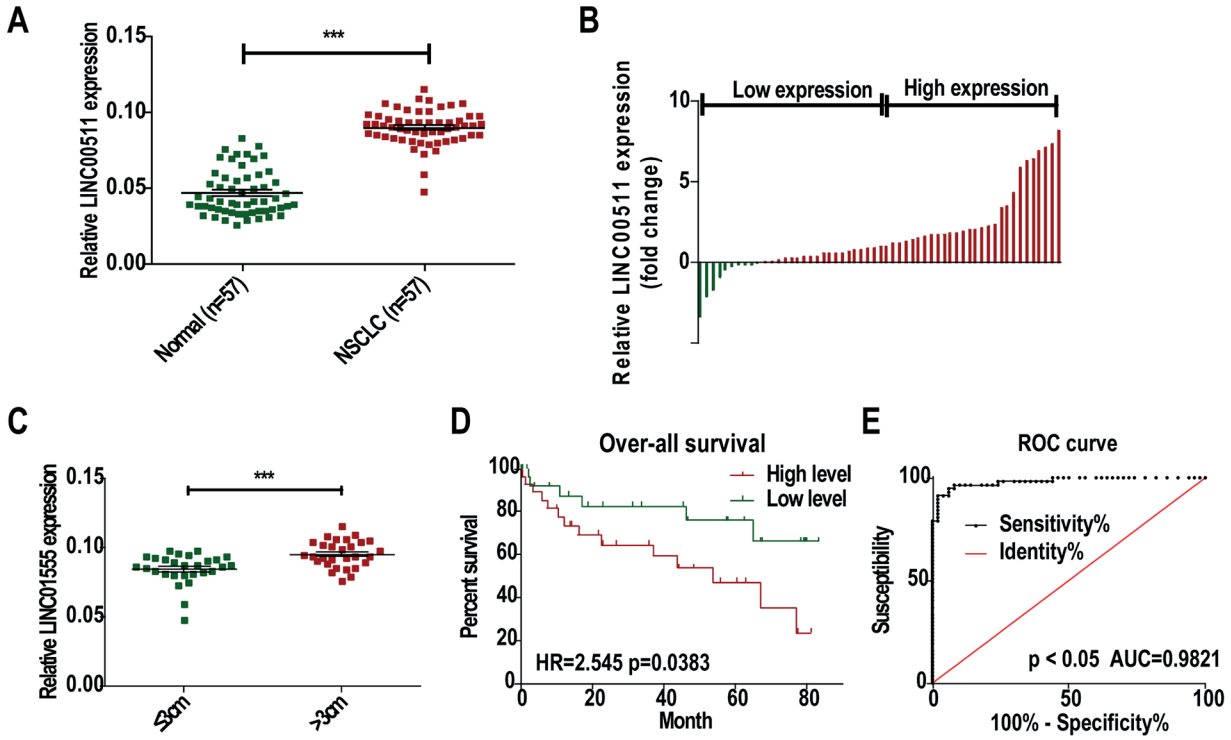

Figure 2. LINC00511 was highly expressed in NSCLC. **A-B,** LINC00511 expression in 57 NSCLC tissues was significantly higher than non-tumoral adjacent tissues detected by qRT-PCR. **C,** LINC00511 expression in NSCLC patients with >3 cm tumors was significantly higher than those with tumor size ≤ 3 cm detected by qRT-PCR. **D,** The OS of NSCLC patients with higher expression of LINC00511 was significantly lower than those with lower expression**. E.** ROC curves showed diagnostic sensitivity of LINC00511 in NSCLC.

Overexpression of LINC00511 in NSCLC Cells Promoted Proliferative Rate

QRT-PCR was used to detect the expression level of LINC00511 in normal lung cell line (BEAS2B) and lung cancer cell lines (A549, PC9 and H460). Expression level of LINC00511 was markedly higher in lung cancer cells compared with controls. Among three-lung cancer cell lines,

Table I. Correlation between LINC00511 expression and clinicopathological features in NSCLC patients (n = 57).

Clinicopathologic features	Number of cases	LINC00511expression		*p*-value
		Low (n=28)	High (n=29)	
Age (years)				0.5136
≤ 56	26	14	12	
> 56	31	14	17	
Gender				0.5092
Male	28	15	13	
Female	29	13	16	
Tumor size				0.0237
≤ 3 CM	30	19	11	
> 3 CM	27	9	18	
TNM stage				
I-II	24	16	8	0.0239
III-IV	33	12	21	
Lymph node metastasis				
Absent	27	18	9	0.0120
Present	30	10	20	

A549 and PC9 cells showed a relatively high and low expression of LINC00511, respectively, and were selected for the subsequent experiments (Figure 3A). Transfection of si-LINC00511 in A549 cells or LINC00511 overexpression plasmid in PC9 cells remarkably downregulated or upregulated LINC00511 expression, respectively (Figure 3B and 3C). By conducting CCK-8 assay and flow cytometry, LINC00511 knockdown in A549 cells was found to inhibit cell viability (Figure 3D) but accelerated cell apoptosis (Figure 3E). On the contrary, LINC00511 overexpression in PC9 cells increased viability (Figure 3F), but inhibited apoptosis (Figure 3G). Transwell assay was then performed for determining invasive and migratory abilities of NSCLC cells influenced by LINC00511. LINC00511 knockdown greatly inhibited invasive and migratory abilities of A549 cells (Figure 4A). Contrarily, LINC00511 overexpression in PC9 cells promoted invasive and migratory abilities (Figure 4B).

LINC00511 Directly Bound to EZH2 and LSD1 and Inhibited Expressions of LATS2 and KLF2

To further explore the mechanism of LINC00511 in regulating NSCLC, chromatin fractionation assay was conducted to locate the intracellular expression of LINC00511. LINC00511 was mainly expressed in the nucleus of A549 and PC9 cells (Figure 5A). It is reported that nuclear lncRNA exerts a regulatory function by binding to an RNA-binding protein. Previous studies revealed that AGAP2-AS1 specifically binds to LSD1 and EZH2, resulting in the occupancy of H3K27me3 on the promoter regions of LATS2 and KLF2. Expressions of LATS2 and KLF2 are inhibited, thereafter participating in the development of lung cancer[15]. CoREST forms a ternary complex with LSD1 and HDAC1/2, which further regulates the methylation and demethylation of certain histones[16-18]. PTEN[19], p21[20] and LATS1[21] are also greatly involved in the development of lung cancer. In this experiment, RIP assay showed that LINC00511 in A549 and PC9 cells could directly bind to LSD1 and EZH2, and had no binding relationship with CoREST (Figure 5B). LINC00511 knockdown remarkably upregulated mRNA levels of LATS2 and KLF2 in A549 and PC9 cells, while mRNA levels of p21, LATS1, and PTEN did not obviously change (Figure 5C). Western blot indicated the upregulated LACT2 and KLF2 in A549 and PC9 cells after LINC00511 knockdown (Figure 5D). The above data suggested that LINC00511 could bind to EZH2 and LSD1 and regulate expressions of LATS2 and KLF2.

We then explored the regulatory effects of EZH2 and LSD1 on LATS2 and KLF2. The mRNA levels of LATS2 and KLF2 in A549 and PC9 cells markedly increased after knockdown of LSD1 and EZH2 (Figure 6A), suggesting that LSD1 and EZH2 can regulate the transcriptional levels of LATS2 and KLF2. ChIP assay elucidated that EZH2, LSD1, H3K27me3 and H3K4me2 could bind to the promoter regions of LATS2 and KLF2 in A549 and PC9 cells (Figure 6B). After knockdown of LINC00511, levels of EZH2, LSD1, H3K27me3 and H3K4me2 that bound to LATS2 and KLF2 in A549 and PC9 cells were downregulated (Figure 6C and 6D). These results indicated that LINC00511 could inhibit expressions of LATS2 and KLF2 by promoting the binding of EZH2 and LSD1 to the promoter regions of LATS2 and KLF2.

LATS2 Promoted Apoptosis of NSCLC Cells

To verify the biological function of LATS2, transfection efficacy of pcDNA-LATS2 was first verified in A549 and PC9 cells (Figure 7A). Decreased viability (Figure 7B) and accelerated apoptosis (Figure 7C) were observed after LATS2 overexpression in A549 and PC9 cells.

Gain-of-function experiment was carried out to verify the involvement of LATS2 in LINC00511-mediated NSCLC progression. Co-transfection of si-LINC00511 and si-LATS2 could partially reverse the decreased viability (Figure 8A), accelerated apoptosis (Figure 8B) and inhibited migratory abilities (Figure 8C) induced by LINC00511 knockdown.

Discussion

Strong abilities of proliferation, migration and invasion are distinguishing features of tumor cells, which are closely associated with tumor occupying, lymph node metastasis, and distant metastasis[22,23]. LncRNA is involved in the pathological and physiological processes of many human diseases. Dysregulated lncRNA in tumors is correlated with tumor metastasis, progression, and prognosis[23-27]. Epigenetic regulation exerts a key part in promoting the occurrence and progression of tumors[28]. As a common event in tumor development, chromosomal instability may be involved in tumor invasion and metastasis[29].

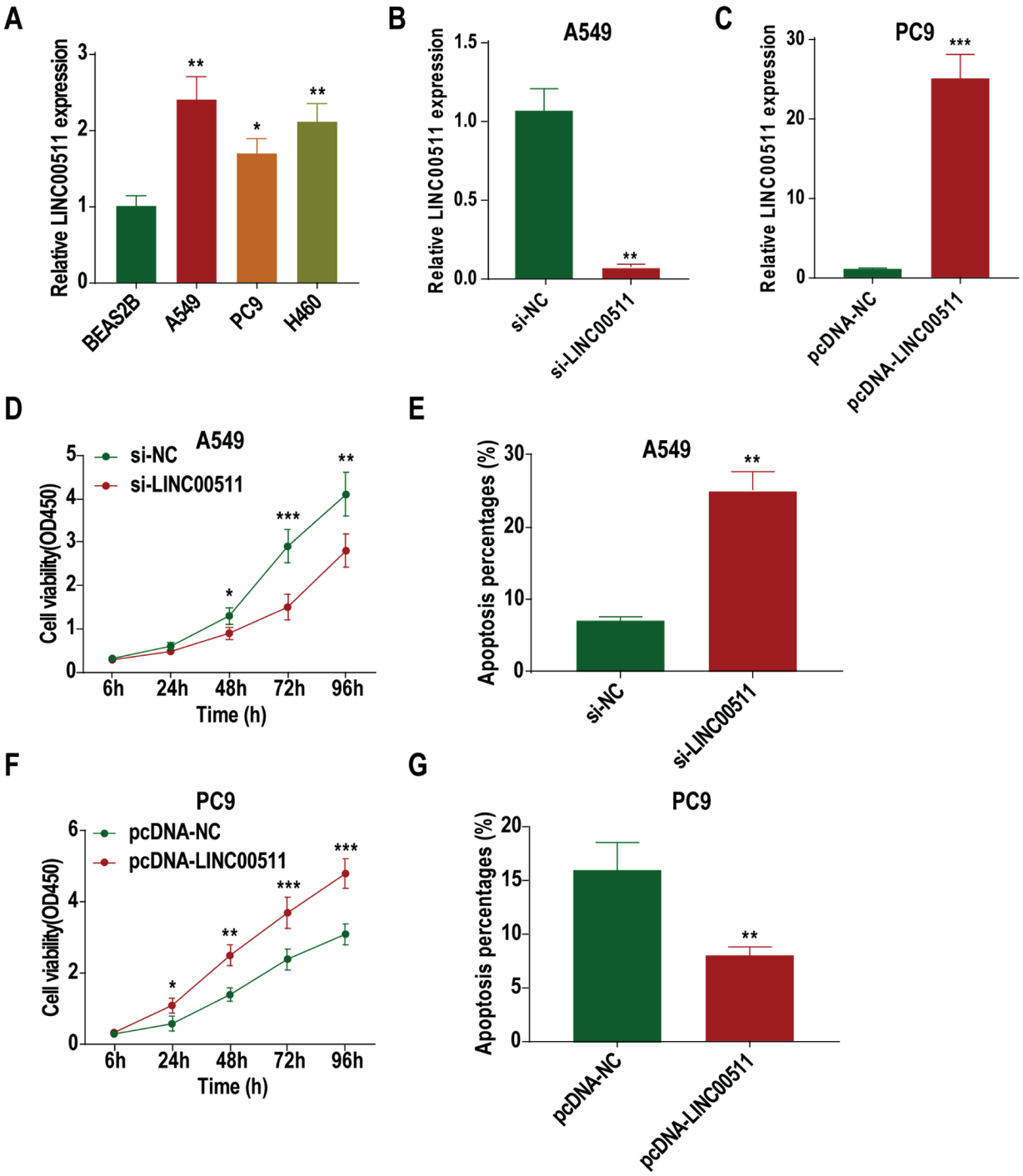

Figure 3. LINC00511 was highly expressed in NSCLC cells and promoted cell viability. **A,** Expression level of LINC00511 in normal lung cell line (BEAS2B) and lung cancer cell lines (A549, PC9 and H460) detected by qRT-PCR. **B,** QRT-PCR result showed a significant decrease in the expression level of LINC00511 after knockdown of LINC00511 in A549 cells. **C,** QRT-PCR result showed a significant increase in the expression level of LINC00511 after overexpression of LINC00511 in PC9 cells. **D,** CCK-8 showed decreased viability after knockdown of LINC00511 in A549 cells. **E,** Flow cytometry showed accelerated apoptosis after knockdown of LINC00511 in A549 cells. **F,** CCK-8 showed increased viability after overexpression of LINC00511 in PC9 cells. **G,** Flow cytometry showed inhibited apoptosis after overexpression of LINC00511 in PC9 cells.

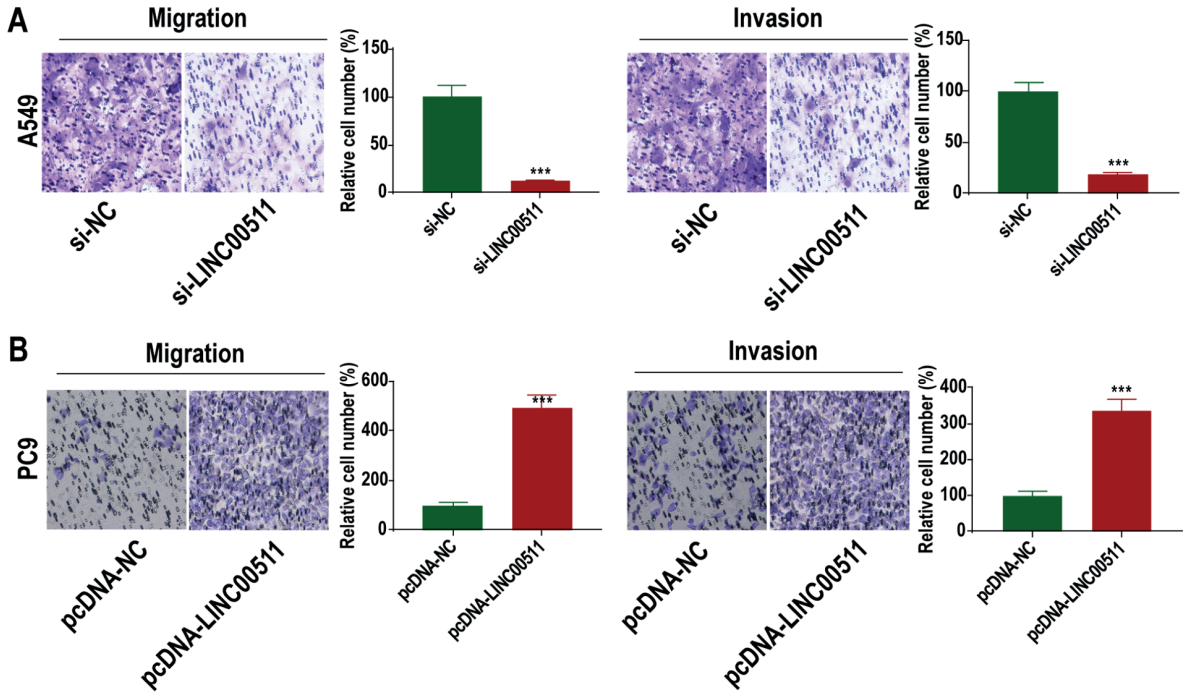

Figure 4. LINC00511 overexpression promoted invasion and migration of NSCLC cells. **A,** Transwell assay showed decreased invasion and migration after knockdown of LINC00511 in A549 cells (magnification 40×). **B,** Transwell assay showed increased invasion and migration after overexpression of LINC00511 in PC9 cells (magnification 40×).

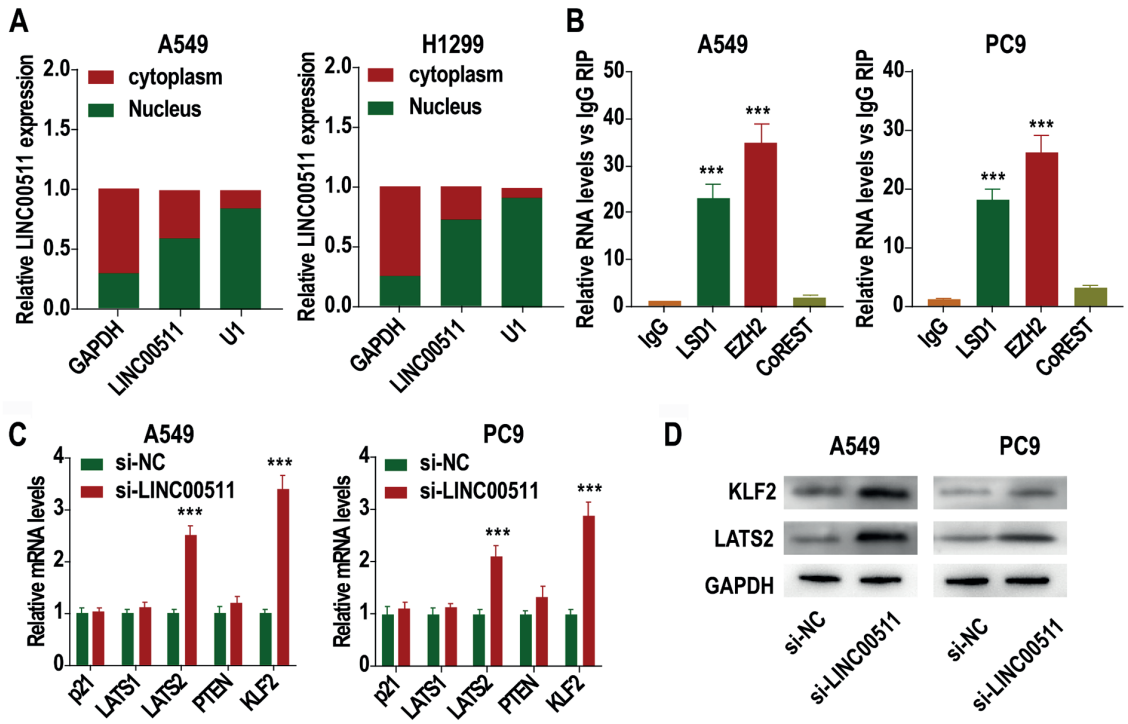

Figure 5. LINC00511 directly targeted on EZH2 and LSD1. **A,** LINC00511 mainly distributed in nucleus of A549 and PC9 cells through qRT-PCR detection after chromatin fractionation. GAPDH or U6 was the loading control of cell nucleus or cytoplasm, respectively. **B,** RIP assay showed that LINC00511 in A549 and PC9 cells could directly bind to LSD1 and EZH2, but had no binding relationship with CoREST. **C,** The mRNA levels of p21, LATS1, LATS2, PTEN and KLF2 after LINC00511 knockdown detected by qRT-PCR. **D,** Protein levels of p21, LATS1, LATS2, PTEN and KLF2 after LINC00511 knockdown detected by Western blot.

LncRNA is capable of regulating epigenetic alteration by recruiting chromatin-remodeling complexes to specific genomic loci. Functionally, lncRNAs could be served as oncogenes or tumor-suppressor genes[30,31].

Epigenetic disorder is a major cause leading to the development of many tumor diseases, including NSCLC[22,23]. Histone methylation is the main content of epigenetic regulation[32]. Wagner et al[33] have found that histone methylation modification is key to the epigenetic mechanism involved in transcriptional regulation in NSCLC. As a crucial epigenetic factor, lncRNA may participate in the epigenetic regulation in tumors. For example, upregulated lncRNA AGAP2-AS1 represses expressions of LATS2 and KLF2 through interacting with EZH2 and LSD1 in NSCLC cells, thus promoting the proliferative, migratory, invasive and tumorigenic abilities, but inhibiting apoptosis of NSCLC cells[15]. LATS2 is a member of the LATS family located on 13q11-q12, encoding a silk/suru amino acid protein kinase. It is distributed in the centrosome and responsible for the accumulation of gamma-tubulin and spindle formation, exerting a crucial role in centrosome mitosis and maintenance of genomic stability[34,35]. LATS2 is able to inhibit the occurrence and development of tumor by regulating cell cycle progression[36]. As a member of Hippo signaling pathway, LATS2 participates in the dynamic balance of tumor cell growth, proliferation and apoptosis, as well as regulates cell contact inhibition. LATS2 deficiency would destruct Hippo signaling pathway, finally losing its function of tumorigenesis inhibition[37]. Histone methylation is an essential approach to epigenetic regulation. PRC2 mediates the methylation regulation of H3K27me3, which is critical for Polycomb (Pc) gene silencing, a classical epigenetic phenomenon that maintains transcriptional silencing through cell division[38-40]. By binding to PRC2, lncRNAs are capable of regulating the transcription of target genes[14]. EZH2 is a key catalytic part of PRC2, which catalyzes H3K27me3, and in turn induces chromatin compaction and transcription-

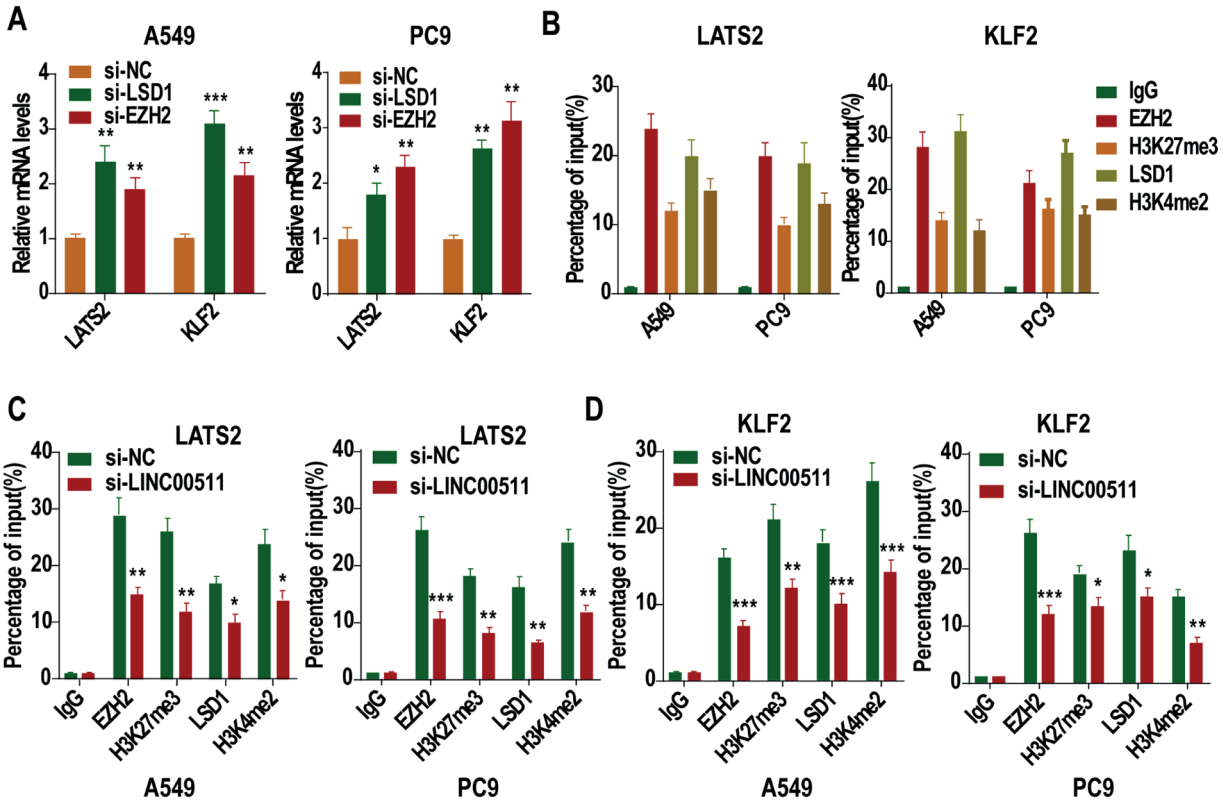

Figure 6. LINC00511 downregulated expressions of LATS2 and KLF2 through EZH2 and LSD1. **A,** The mRNA levels of LATS2 and KLF2 in A549 and PC9 cells markedly increased after knockdown of LSD1 and EZH2. **B,** ChIP assay elucidated that EZH2, LSD1, H3K27me3 and H3K4me2 could bind to the promoter regions of LATS2 and KLF2 in A549 and PC9 cells. IgG was served as negative control. **C, D,** Levels of EZH2, LSD1, H3K27me3 and H3K4me2 bound to LATS2 and KLF2 in A549 and PC9 cells were downregulated after knockdown of LINC00511.

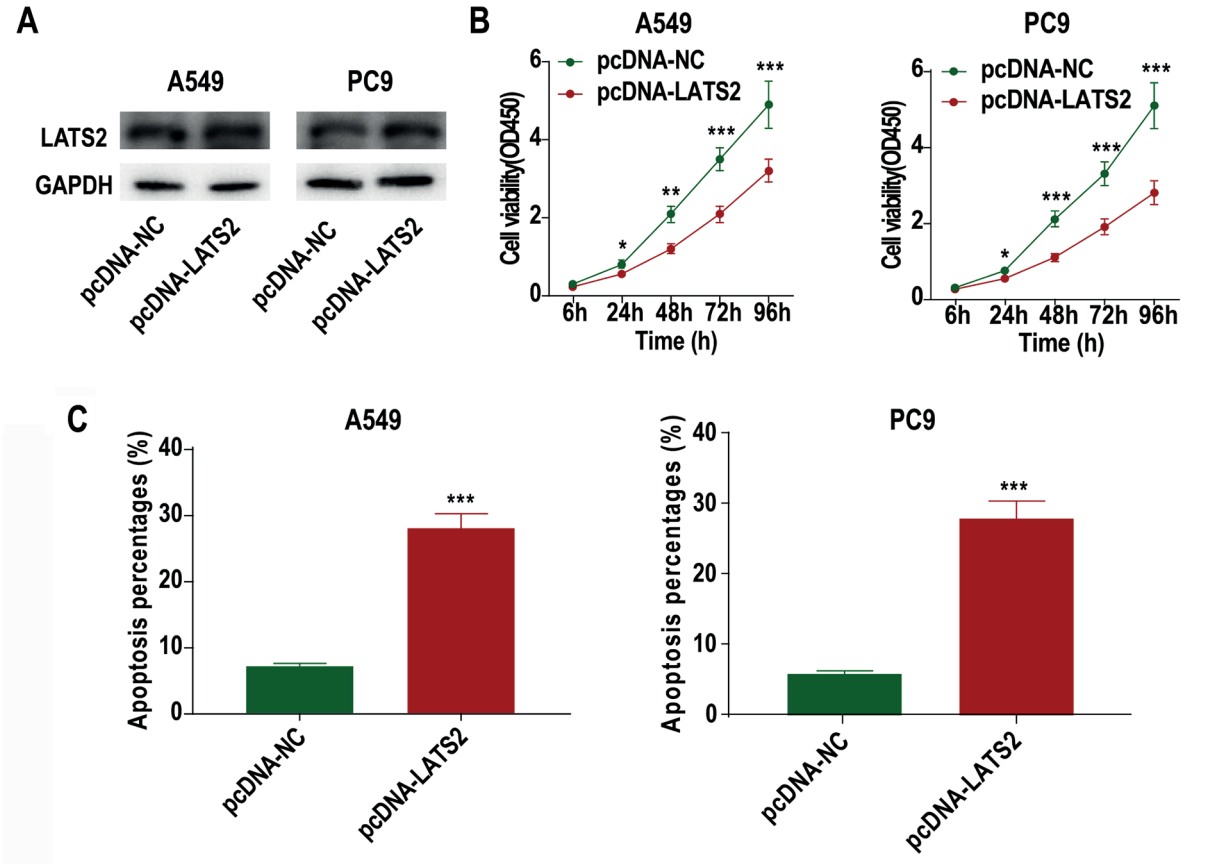

Figure 7. LATS2 promoted apoptosis of NSCLC cells. LATS2 was overexpressed in A549 and PC9 cells. **A,** Protein level of LATS2 was upregulated detected by Western blot. **B,** Cell viability decreased detected by CCK-8. **C,** Apoptosis was accelerated detected by flow cytometry.

al repression[41]. EZH2 shows great functions in the development of various tumors through its target gene promoter histone methylation (H3K27me3). Previous studies have highlighted the significant role of histone methylation in the development of NSCLC[42,43]. LSD1 is an important member of the amine oxidase family, also known as KI-AA0601, AOF2, and KDM1. Its role in tumorigenesis, development, invasion, and metastasis has been well recognized[44-46]. For example, over-activation of epidermal growth factor (EGF) signaling promotes LSD1 expression. Conversely, inhibition of LSD1 expression blocks EGF-induced proliferative and migratory abilities of ovarian cancer cells[47]. LSD1 affects tumor progression and invasion with the deacetylase complex (NuRD) by interacting with the tumor-suppressor gene p53 and activating TGF-β signaling pathway, finally regulating human telomerase reverse transcriptase (hTERT)[48-50]. In this work, LINC00511 was highly expressed in NSCLC tissues through analyzing online database. LINC00511 was correlated to the overall survival of NSCLCL patients. LINC00511 expression in the collected 57 NSCLC tissues and non-tumoral tissues was identical to that in the downloaded dataset. Meanwhile, we analyzed the correlation between LINC00511 expression and pathological characteristics of NSCLC patients. The data showed a positive correlation between LINC00511 expression with tumor size, tumor stage, lymph node metastasis and distant metastasis of NSCLC patients. ROC curves suggested the diagnostic potential of LINC00511 in NSCLC. Subsequent *in vitro* experiments demonstrated the promotive effects of LINC00511 on proliferative, migratory and invasive capacities of A549 and PC9 cells. Apoptosis of NSCLC cells, however, was inhibited by LINC00511. RIP assay suggested that LINC00511 directly combined with EZH2 and LSD1, and exerted regulatory effects on both of them. Furthermore, ChIP assay confirmed that EZH2 and LSD1 could directly bind to

LATS2, KLF2, H3K27me3 and H3K4me4. EZH2 and LSD1 were capable of regulating LATS2 and KLF2. LINC00511 could regulate LATS2, KLF2, H3K27me3 and H3K4me4 as well. LATS2 exerted regulatory effects on proliferative and apoptotic rates of A549 and PC9 cells that were similar to LINC00511. Therefore, we proved that LINC00511 regulated EZH2 and LSD1, further regulating methylation of LATS2 and KLF2d promoters H3K27me3 and H3K4me4, respectively. Gain-of-function experiments also confirmed our speculation. Nevertheless, some shortcomings in this experiment should be noteworthy. First of all, we were unable to design *in vivo* experiments to verify our results. Secondly, the potential mechanism of KLF2 in NSCLC pathogenesis is still needed to be further elucidated.

Conclusions

LINC00511 is upregulated in NSCLC tissues and cell lines. It is closely related to tumor size, tumor stage, lymph node metastasis and distant

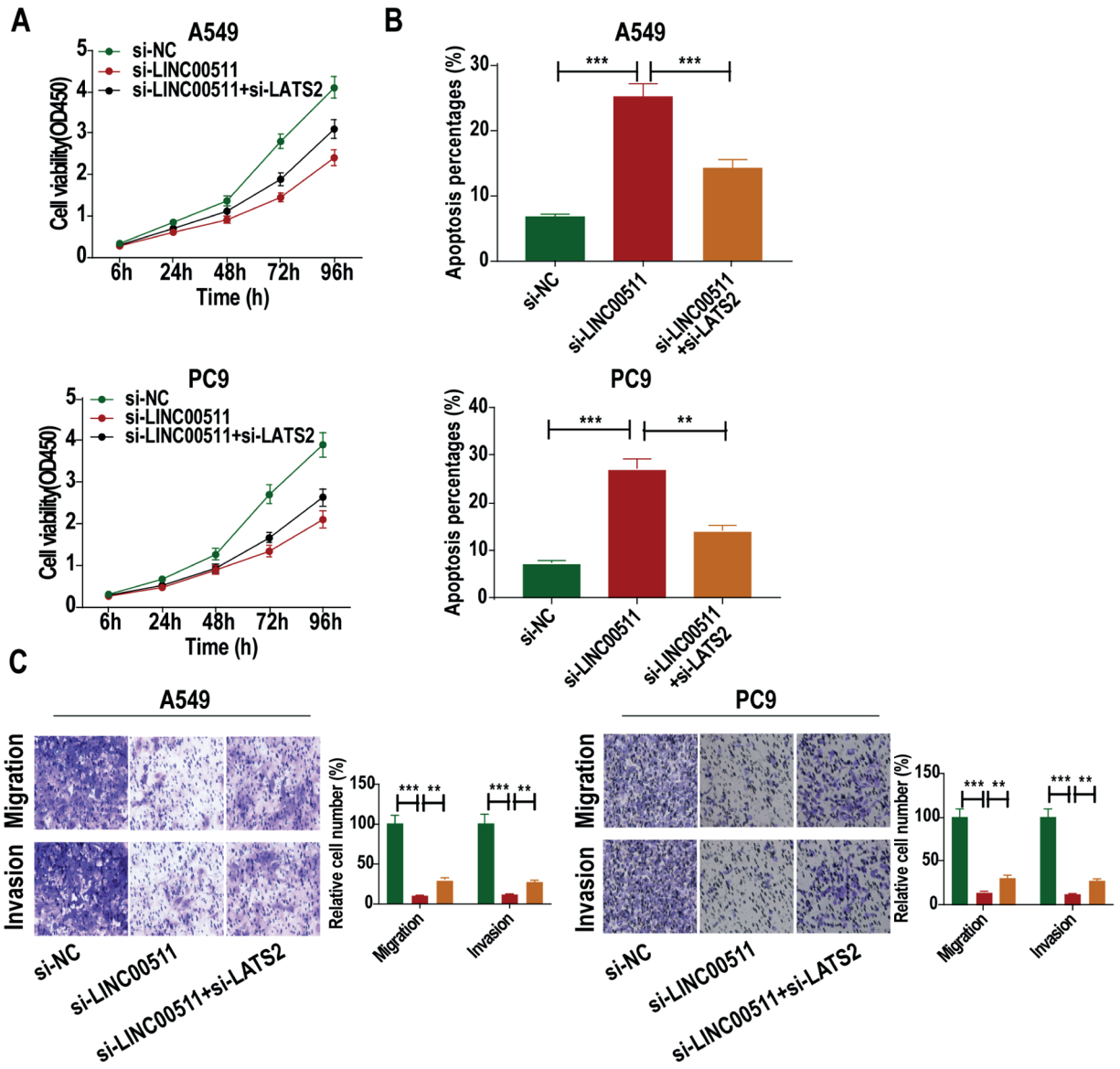

Figure 8. LINC00511 exerted biological function in NSCLC through regulating LATS2. Co-transfection of si-LINC00511 and si-LATS2 could partially reverse the decreased viability **(A)**, accelerated apoptosis **(B)** and inhibited migratory abilities **(C)** induced by LINC00511 knockdown (magnification: 40×).

metastasis of NSCLC patients. Knockdown of LINC00511 attenuates proliferative, migratory and invasive capacities, but induces apoptosis of NSCLC cells. LATS2 and KLF2 are target genes of LINC00511, which are regulated by LINC00511 through binding to EZH2 and LSD1, thus influencing the progression of NSCLC.

Conflicts of interest

The authors declare no conflicts of interest.

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