Iran J Public Health, Vol. 53, No.9, Sep 2024, pp.2049-2058

Original Article

LUCAT1 Activates the Malignant Phenotypes of Lung Cancer Cells via Regulating *P53* Ubiquitination

Junfeng Zhang, Weiyi Gong, Xinle Wang, Longbo Yang

Department of Thoracic Surgery, Zhongkegengjiu Hospital of Anhui/Gengjiu Clinical Medical College, Anhui Medical University, Hefei, 230001, China

***Corresponding Author:** Email: 18056080928@163.com

(Received 14 Dec 2023; accepted 10 Feb 2024)

Abstract

Background: Long non-coding RN (lncRNAs) have been implicated in lung cancer, but the mechanisms stay unclear. We investigated the theatrical role and mechanism of lncRNA Lung cancer associated transcript 1 *LUCAT1* in the malignant progress of lung cancer.

Methods: From May 2022 to March 2023, a total of thirty normal and cancerous tissues were collected from patients diagnosed with non-small cell lung cancer at Zhongke Gengjiu Hospital in Anhui Province, China. The human SPC-A1 and A549 cell lines were chosen as the subjects for the relevant cellular experiments in this study. LncRNAs were expressed in a different manner identified by bioinformatics methods, and the expression levels in lung cancer tissues as well as cells were verified by the qRT-PCR assay. The biological role of *LUCAT1* in NSCLC was determined by CCK-8, EdU, and transwell assay.

Results: The regulation of ubiquitin of *P53* by *LUCAT1* was studied, which showed that *LUCAT1* was significantly elevated in NSCLC cell lines and patients' tissues ($P<0.05$). High levels of *LUCAT1* promoted the proliferation, invasion, and migration of NSCLC cells. Mechanism studies showed that *LUCAT1* was mainly located in the nucleus, which bound to *P53* and mediated the ubiquitinated degradation of *P53*. Meanwhile, *LUCAT1* knockdown attenuated the ubiquitination process of *P53*. In addition, rescue experiments illustrated that *LUCAT1* induced the proliferation and invasion of NSCLC cells, and played a key role in the survival and tumorigenicity of NSCLC cells by mediating the ubiquitination of *P53*.

Conclusion: Collectively, *LUCAT1* activated the malignant phenotypes of NSCLC cells via regulating *P53* ubiquitination, which provided a new idea for the diagnosis and treatment of NSCLC.

Keywords: Lung cancer associated transcript 1; *P53*; Ubiquitination; Non-small-cell lung cancer

Introduction

Long non-coding RNAs (lncRNAs) are particularly verbalized in differentiated cancer tissues that are with respect to a function defined as transcripts of at least 200 nucleotides in length with no protein-coding potential (1, 2). Many lncRNAs are known to control major carcinogenic factors in various cancers (3). It is reported that lncRNAs interact with oncogenes (3-5). Growing proof connotates that lncRNAs play a considerable role in reconstituting cell viability and genomic stability by regulating gene expression (6, 7). lncRNAs modulate tumor-genesis and

tumor-immunity through direct interactions with endogenous biomolecules like mRNA, miRNA, and protein (8, 9).

P53, an important tumor suppressor, encoded by the *TP53* gene, known as the "gene guardian", is critical to cell cycle progress, apoptosis, and DNA repair through its transcriptional activity (10-12). Although *TP53* is widely recognized as an oncogene, carcinogenic outcomes of the mutant *P53* protein, such as dysregulation of metabolic pathways, enhanced tumor aggressiveness, and chemotherapy resistance, suggest the gain function of mutant *P53* in gastric cancer (12, 13). Post-translational modifications regulate the activity of *P53* mainly including ubiquitination, phosphorylation, and acetylation (14). The *P53* protein levels were regulated by the ubiquitin-proteasome pathway (15).

Lung cancer causes cancer deaths of the highest degree, with more than 1.5 million each year estimated to die from lung cancer. Approximately 85% of patients have histological subtypes referred to as non-small cell lung cancer (NSCLC). Lung cancer-associated transcription 1(*LUCAT1*), a nickname for tobacco cancer-associated lncRNA (SCAL1), was firstly reported to be associated with lung cancer related to smoking (16). *LUCAT1* is known to be linked with cisplatin resistance in NSCLC colorectal cancer, and ovarian cancer (17-19). Since smoking is the etiology of non-small cell lung cancer, *LUCAT1* may also be involved in the development of non-small cell lung cancer.

Therefore, we investigated the role of *LUCAT1* in NSCLC cancer progression. Our findings identified a prominent role for *LUCAT1* in regulating *P53* ubiquitination and as a predictor of survival in NSCLC.

Materials and Methods

Ethics statement

All experiments were carried out in accordance with the standards approved by the Ethics Committee of Zhongkegengjiu Hospital of Anhui and each patient signed informed consent before surgery.

Patient samples

Primary normal and NSCLC tissues of 30 surgical patients were selected at Zhongkegengjiu Hospital in Anhui province from May 2022 to March 2023. Tissue samples were preserved at -80 °C.

Cell culture and transfection

Human SPC-A1 and A549 cell lines were cultured in Roswell Park Memorial Institute (RPMI) medium containing 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2 which was acquired from ATCC (Manassas, VA, USA). The siRNA sequences for *LUCAT1* and negative control (NC) were synthesized by Genepharma (Shanghai, China). Cells were transfected with siRNA using Lipofectamine 3000 transfection reagent (Invitrogen). The efficiency of the knockdown was determined by RT-PCR assay. *Si-LUCAT1* sense, CAGAAGAUUCAGAAGAUAAGGAUI and anti-sense, AUCCUUAUCUUCUGACAUCUUCUG.

Quantitative RT-PCR assay

GoScript Reverse Transcription (RT) System (Promega) kit and GoTaq qPCR master mix were used for reverse transcription. The primers were shown in Table 1, and the reaction was put in effect with the instructions with Step One Plus Fluorescence Quantitative PCR (ABI) instrument. Gene expression levels were determined by the $2^{-\Delta\Delta Ct}$ quantitative analysis method.

Cell viability test

Cell viability was measured according to the instructions with the CCK-8 kit. Simply, 10 μl CCK-8 reagent was added into the cell culture medium and incubated for 2-3 h. Subsequently, the absorbance of CCK-8 was measured at 450 nm OD using an Epoch digitizer (BioTek).

Table 1: The primer sequences

Gene		*Sequence (5' to 3')*
Si-LUCAT1	Sense	CCCAUCAGAAGAUGUCAGAAGAUAA
	Antisense	UUAUCUUCUGACAUCUUCUGAUGGG
LUCAT1	Forward	ACCAGCTGTCCCTCAGTGTTCT
	Reverse	AGGCCTTTATCCTCGGGTTGCCT
si-*p53*		GAATGAGGCCTTAGAGTTA
p53	Forward	GAACCGCCGACCTATCCTTA
	Reverse	GGCAGGCACAAACACGAAC
GAPDH	Forward	CCGGGAAACTGTGGCGTGATGG
	Reverse	AGGTGGAGGAGTGGGTGTCGCTGTT

EdU assay

Click-iT® Plus EdU Imaging Kits according to the instructions EdU assay was performed. Simply, in the culture medium, 10 μM EdU for 2 h was added. Hoechst® 33342 were used to stain nuclei at room temperature for 30 min. Finally, results were determined microscopically and quantified by counting 10 random fields.

Transwell assay

A549 and SPC-A1 cells were inoculated in a stromal gel-coated upper cavity with pores (50 LL stromal gel, BD Bioscience, USA). The medium with 10% FBS was added to the upper and lower compartments. The migratory and invasive cells were incubated for 24h on the surface of the inferior membrane, then fixed and stained with 20% Giemsa solution. Five random fields in each chamber were calculated using an inverted microscope (Olympus, Japan).

Cell cycle analysis

Transfected or non-transfected NSCLC cells were gathered and fixed in 70% ethanol at 4 °C overnight. Cells were then treated with FxCycle™ PI/RNase staining solution (Thermo Scientific) at 37°C in the dark for 30 min. The cell cycle was analyzed using an AccuriC6 cytometer (BD Biosciences).

Cell apoptosis analysis

Apoptosis analysis was measured by Annexin V/propidium iodide (PI) apoptosis detection kit (eBioscience, San Diego, CA) following the instructions. Simply, human A549 and SPC-A1 cells (40,000) were suspended into the binding buffer. Then, cells were stained with APC-conjugated Annexin V for 5 min followed treated with PI for 15 min. Flow cytometry analysis assessed cell apoptosis rate.

Western blotting

The total protein of the tissue or cells was extracted using the BCA method to determine the protein con-centration, and the protein was denatured to prepare a sample. SDS-PAGE was performed, and the membrane was transferred, blocked with 5% milk on a shaker for 1 h, and incubated with the following primary anti-bodies: anti-CDK4 (ab108357, 1:1000), anti-Cyclin D1 (ab134175, 1:1000), anti-cleaved caspase 3 (ab32042, 1:500), anti-cleaved caspase 9 (ab2324, 1:500) and GAPDH (ab8245, 1:1000). The membrane was shaken at 4'C overnight, washed, incubated with the secondary antibody for 1 h at room temperature, washed, and exposed (20).

RNA pull down assay

Briefly, *LUCAT1* and its antisense RNA were biotinylated by using MEGAscript™ T7/SP6 Transcription Kit (Life Technologies, USA) according to the manufacturer's instructions. The biotinylated RNAs were then incubated with cell lysate at 4 °C for two hours. Proteins that interact with *LUCAT1* were precipitated by Dynabeads™ M-280 Streptavidin beads (Life Technologies, USA) by incubating at 4 °C for one hour.

RIP assay

Following the protocols with the Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, USA), first, A549 cells were lysed. Then, the cell extraction was incubated with RNAs magnetic beads conjugated with a human anti-Ago2 antibody or with the ordinary rabbit IgG (Abcam, Cambridge, UK) as the NC. Finally, the RNA purified with Proteinase K was assayed by RT-PCR assay.

Ubiquitination assay

Cell lysate supernatants were incubated with *p53* antibodies and protein A-Sepharose beads (Santa Cruz Biotechnology, USA) overnight at 4 °C. Precipitated proteins were washed boiled, and separated by SDS-PAGE. The ubiquitination levels of *p53* were determined with an anti-ubiquitin antibody (Proteintech, USA).

Statistical analysis

All data were presented with mean ± standard deviation (SD). Student *t*-test was used to determine the significant differences between different groups. Differences were considered significant at $P < 0.05$.

Results

Highly expressed LUCAT1 is positively correlated with poor prognosis

To investigate whether *LUCAT1* is concerned with the etiopathogenesis of NSCLC, we checked out the expression of *LUCAT1* carefully and methodically in NSCLC using the gene Expression profile Interaction analysis (GEPIA) tool based on TCGA. *LUCAT1* expression was higher in NSCLC tissues (Fig. 1A-B). To verify the results, the *LUCAT1* expression levels in clinical specimens and NSCLC cell lines were spotted by the qRT-PCR assay. As shown in Fig. 1C, *LUCAT1* was meaningfully upregulated in lung tumors. In addition, *LUCAT1* expression in NSCLC lines was increased compared with normal bronchial epithelial cells, especially in A549 and SPC-A1 (Fig. 1D). To make clear and comprehensive the relationship between expression of *LUCAT1* and overall survival time, a shorter overall survival time was found in patients with higher *LUCAT1* levels (Fig. 1E).

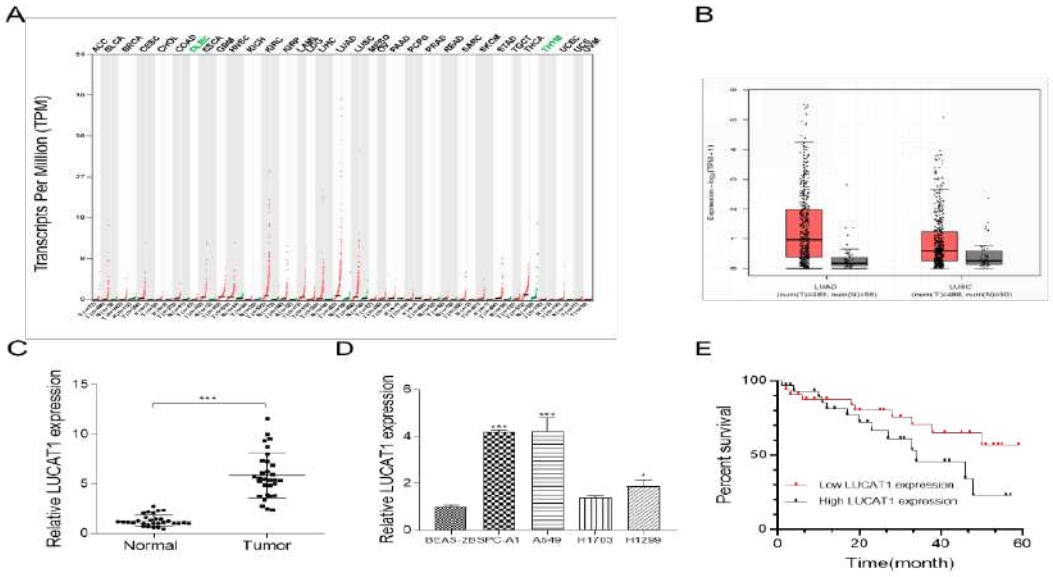

Fig. 1: Highly expressed *LUCAT1* is positively correlated with poor prognosis. (A&B) Gene Expression Profiling Interactive Analysis (GEPIA) indicated the expression of *LUCAT1* in various tumors. RT-PCR analysis showed the expression of *LUCAT1* in NSCLC tissues (C) and cells (D). (E) Kaplan-Meier analysis for OS of NSCLC patients with low and high *LUCAT1* expression. Data represent the mean ± SD. $^{*}P<0.05$, $^{***}P<0.001$. n=3

Knockdown of *LUCAT1* inhibits cell growth of NSCLC *in vitro*

To investigate whether dysregulation of *LUCAT1* regulated the proliferation of NSCLC cells, siRNA targeting endogenous *LUCAT1* was transfected into SPC-A1 cells and A549 cells, respectively. the qRT-PCR assay detected transfection efficiency and results verified beyond doubt that knockdown of *LUCAT1* remarkedly restrained the *LUCAT1* expression (Fig. 2A). CCK-8 and EdU assay showed that inhibition of endogenous *LUCAT1* significantly restrained proliferation of NSCLC cells in vitro (Fig. 2B&2C). Furthermore, transwell experiments showed that down-regulation of *LUCAT1* greatly reduced the invasive and migration abilities of SPC-A1 and A549 cells (Fig. 2D).

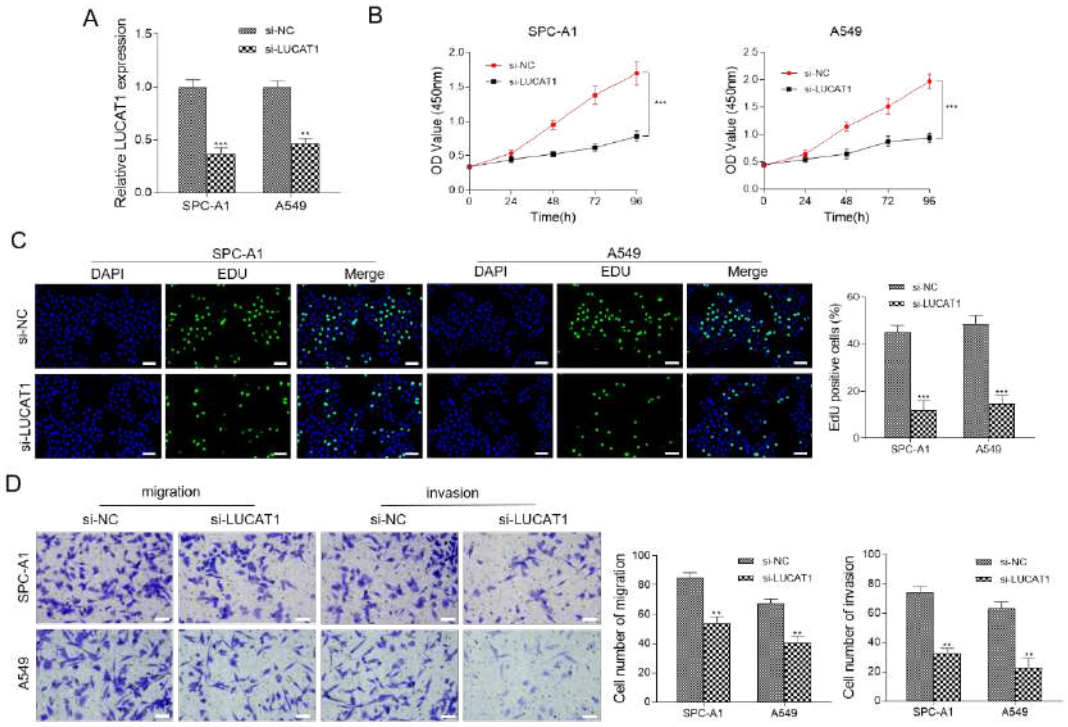

Fig. 2: Knockdown of *LUCAT1* inhibits cell growth of NSCLC *in vitro*. (A) The transfection efficiency of *LUCAT1* knockdown was verified by RT-PCR analysis. (B) CCK-8 assay showed cell proliferation. (C) Cell proliferation was detected by EdU assay. (D) Transwell analysis assessed cell migration and invasion. Data represent the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$. n=5

Knockdown of LUCAT1 regulates cell-cycle progression and apoptosis in NSCLC cells

To further strengthen the theatrical role of *LUCAT1* in cell cycle mastery progression, and apoptosis in NSCLC, we knocked out *LUCAT1* expression and analyzed its function by flow cytometry. Knockdown of *LUCAT1* promoted G0/G1 phase cells, while decreased S and G2/M phase cells (Fig. 3A). In addition, silencing of *LUCAT1* dramatically enhanced the apoptosis rate of SPC-A1 and A549 cells (Fig. 3B). Meanwhile, cleaved caspase 3 and cleaved caspase 9 proteins which are known as apoptosis-related proteins, their levels were elevated in NSCLC cells transfected with the si-*LUCAT1* group, while CDK4 and cyclin D1 proliferation-related proteins were decreased in NSCLC cells transfected with the *si-LUCAT1* group (Fig. 3C). These results suggest that *LUCAT1* regulates cell cycle progression in NSCLC.

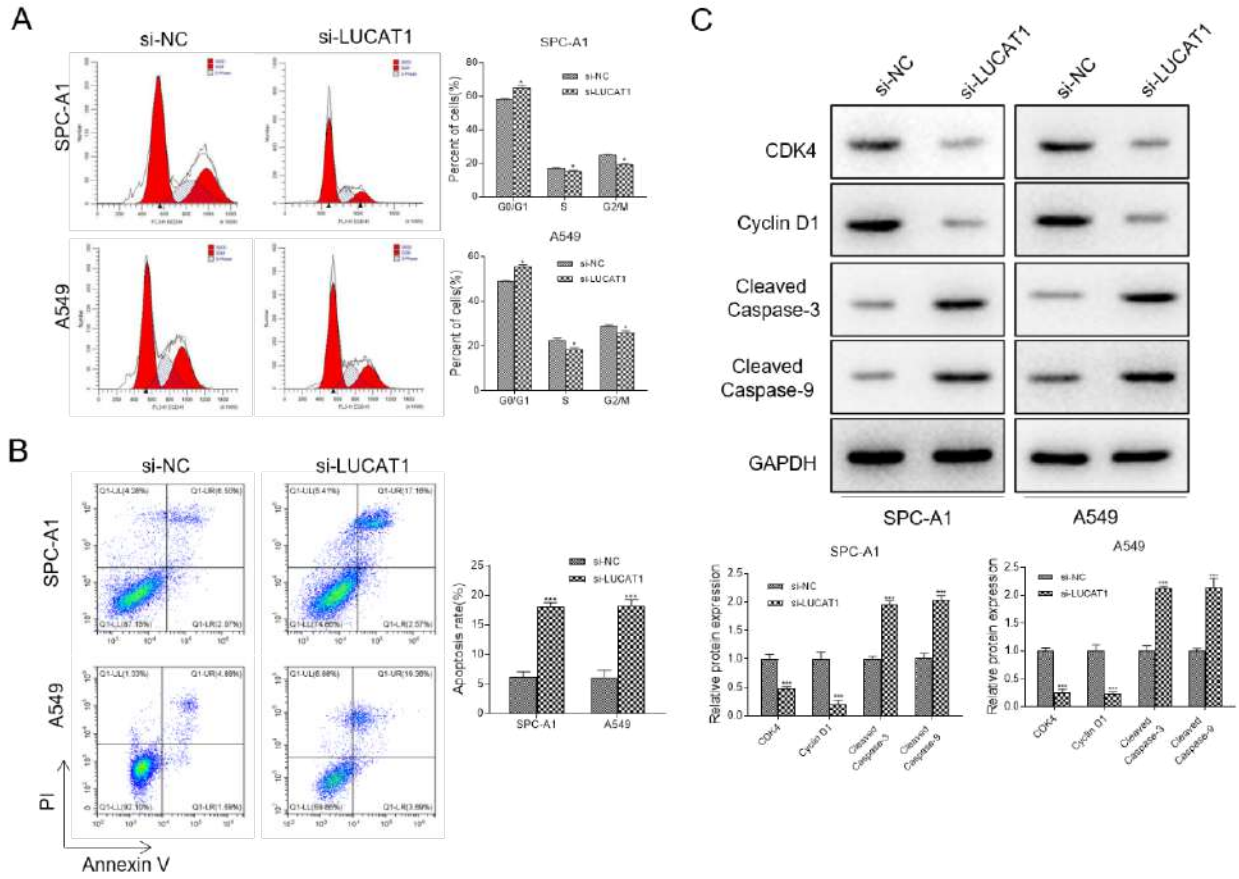

Fig. 3: Knockdown of *LUCAT1* regulates cell-cycle progression and apoptosis in NSCLC cells. (A-B) Flow cytometry analysis indicated cell-cycle progression and apoptosis of NSCLC cells. (C) Western blot analyses showed the expression of cell-cycle and apoptosis-related protein. Data represent the mean ± SD. $^{*}P<0.05$, $^{***}P<0.001$. n=3

LUCAT1 interacts with p53

To understand the molecular mechanism of *LUCAT1* in NSCLC progress, we predicted the targets of *LUCAT1* through the tool (http://pridb.gdcb.iastate.edu/RPISeq/). The anticipation score of the RF algorithm was 0.8, and that of the SMV algorithm was 0.96, suggesting that *p53* may be the protein interacting with *LUCAT1*. The results showed that *LUCAT1* was principally placed in the nucleus of A549 and SPC-A1 cell lines (Fig. 4A). *LUCAT1* pulled down *p53* successfully, while the antisense sequence of it failed (Fig. 4B). In addition, RIP experiments were performed to detect whether endogenous *LUCAT1* could bind *P53* protein in A549 cells, results indicated that *LUCAT1* was in a statistically significant way concentrated in *p53* antibody captured settlings compared with IgG control, which confirmed the interaction between *LUCAT1* and *p53* (Fig. 4C). The negative correlation between *LUCAT1* transcription and *p53* protein in NSCLC tumors was analyzed with Person's coefficient (Fig. 4D). Therefore, we speculated that *LUCAT1* was involved in the ubiquitination mediated degradation of *p53*.

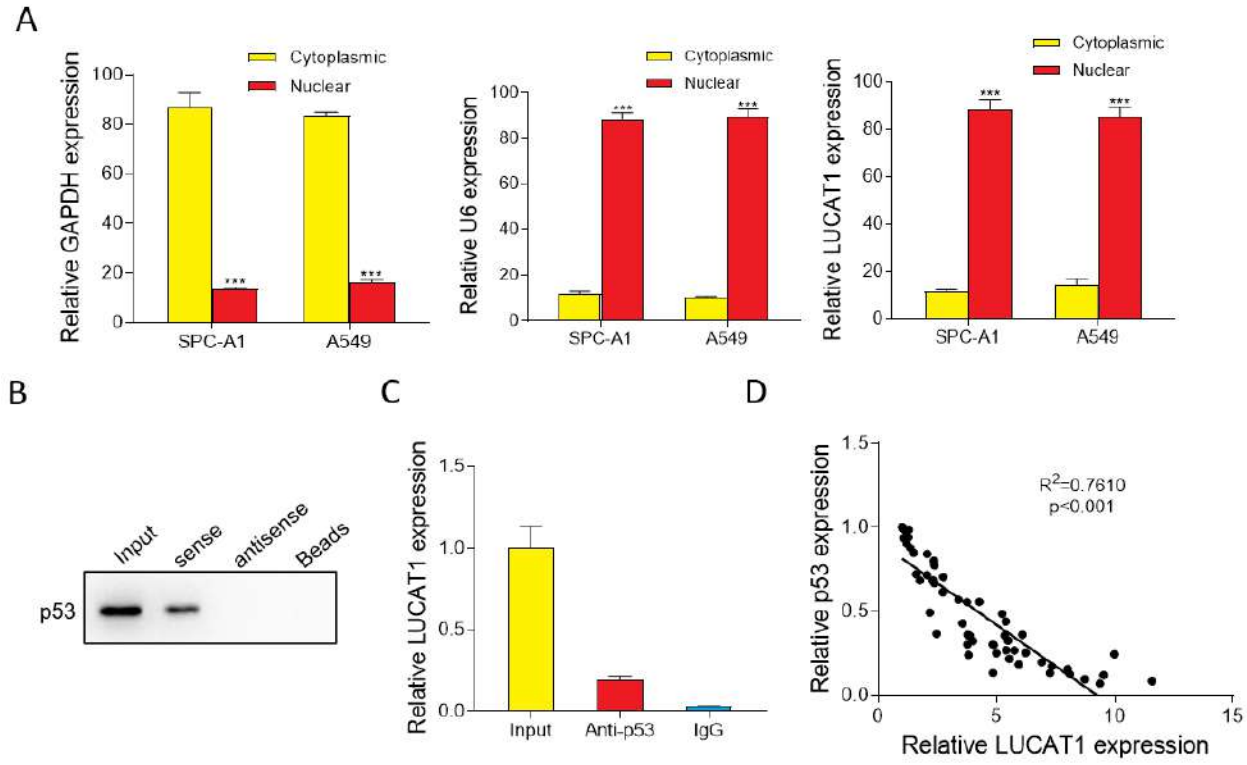

Fig. 4: *LUCAT1* interacts with *p53*. (A) The distribution of *LUCAT1* was detected by nuclear-cytoplasmic separation. (B-C) The interaction between *LUCAT1* and *p53* was detected by RNA pull-down and RIP analysis. (D) Correlation analysis of *LUCAT1* and *P53* expression in clinical samples. Data represent the mean ± SD. ***P<0.001. n=3

LUCAT1 promotes the degradation and ubiquitination of p53

Interestingly, the *p53* protein level was significantly increased while the *p53* mRNA level remained constant when *LUCAT1* was silenced (Fig. 5A), resulting in this result that may be attributed to proteasomal degradation. A549 cells transfected with *si-LUCAT1* or si-NC were incubated with the protein synthesis inhibitor CHX, and proteasome inhibitor MG132, respectively. The difference in *p53* protein expression results showed that *LUCAT1* knockdown enhanced the firmness of *p53* protein in the presence of protein synthesis inhibitor CHX (Fig. 5B), and the accumulation level of *p53* in *LUCAT1* knockdown cells after MG132 treatment was comparable to that of control cells (Fig. 5C). The reduction of *p53* abundance by *LUCAT1* was not owing to transcriptional effects. To further prove the effectiveness of *LUCAT1* on the ubiquitination of *p53*, a ubiquitination assay was performed in A549 cells. The knockdown of *LUCAT1* led to the decrease of *p53* ubiquitination (Fig. 5D).

Knockdown of *LUCAT1* inhibits malignant phenotypes of NSCLC by regulating *p53* expression

Thinking about the above effects of *LUCAT1* on *p53* protein degradation and ubiquitination, we next evaluated whether *LUCAT1* regulates the NSCLC process by acting on *p53*. A549 and SPC-A1 cells were treated with si-*p53* and its control. Knockdown of *p53* remarkedly inhibited the expression of *p53* protein (Fig. 6A), indicating successful transfection. Functionally, *p53* silencing partly conversed the effects of *LUCAT1* knockdown on cell proliferation, invasion, and apoptosis (Fig. 6B-D).

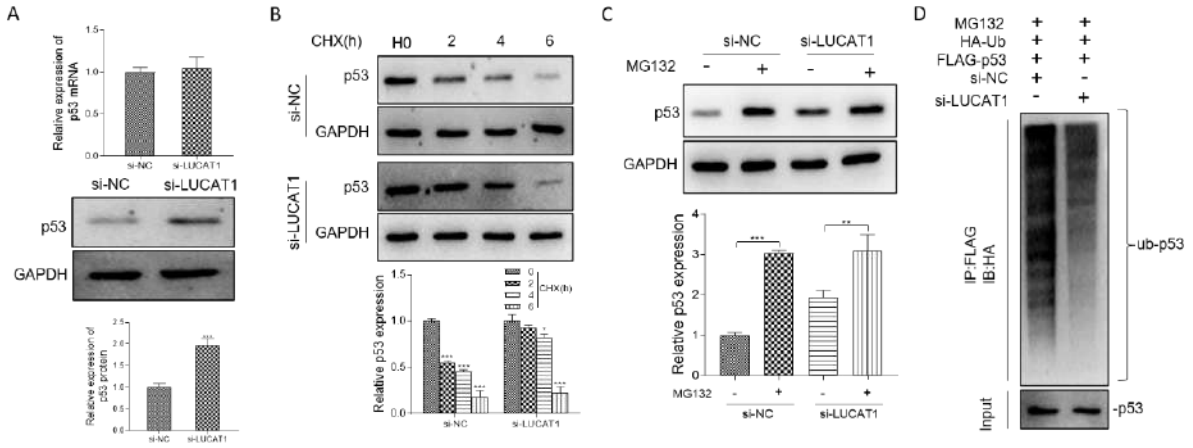

Fig. 5: *LUCAT1* promotes the degradation and ubiquitination of *p53*. (A&B) The expression of *p53* mRNA and protein levels in *si-LUCAT1*-transfected cells. A549 cells were transfected with *LUCAT1* siRNA or its negative control (NC), and then treated with 50 μg/ml cycloheximide (CHX) or 20 μM MG132 for 24 h. (C) A549 cells were co-transfected with HA-Ub, Flag-*p53*, *LUCAT1* siRNA, or negative control, and then treated with 20 μM MG132 for 24 h. (D) Immunoprecipitation was performed using anti-Flag antibody. IgG served as a negative control. Ubiquitinated *p53* protein used anti-HA antibody. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. n=3

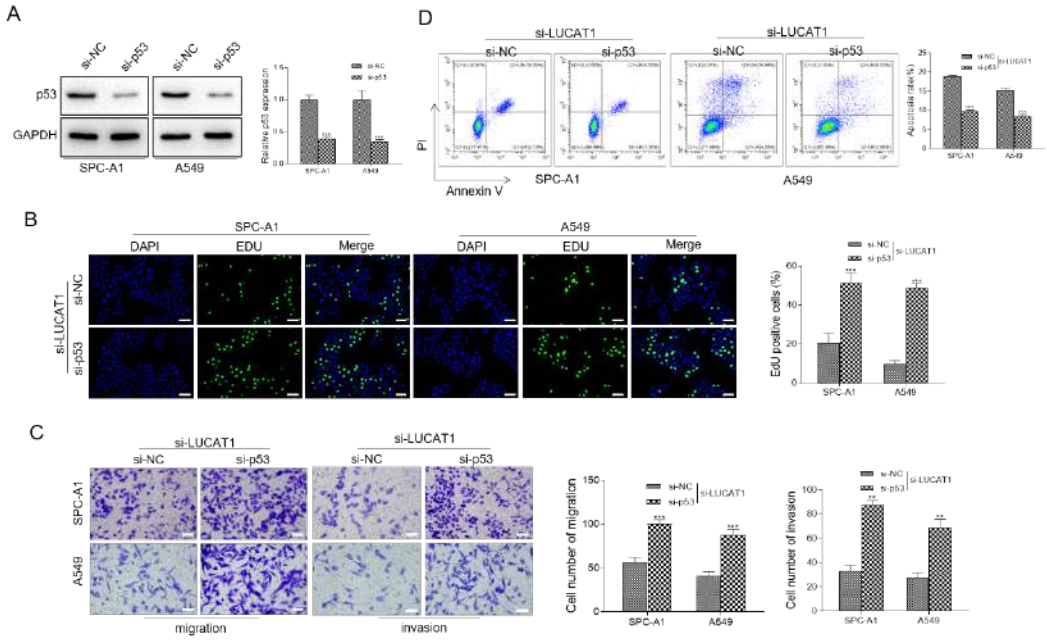

Fig. 6: Knockdown of *LUCAT1* inhibits malignant phenotypes of NSCLC by regulating *p53* expression. (A) The efficiency of *p53* knockdown was verified by RT-PCR analyses. (B) EdU assay showed cell proliferation. (C) Transwell analysis detected cell migration and invasion. (D) Flow cytometry analysis assessed apoptosis of cancer cells. Data represent the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$. n=3

Discussion

Adding studies intimate that unnatural expression of lncRNAs is nearly connected with the circumstance of assorted human diseases, peculiarly tumorigenesis and cancer progression (21-23). Despite the numerous studies on lncRNAs in cancer, only a few reports have judged the particular mechanisms of RNAs in regulating cancer. We found that *LUCAT1* was accompanying the de-

velopment and progression of NSCLC in this study. The survival rate of NSCLC patients was associated with the expression of *LUCAT1*. Functionally, knockdown of *LUCAT1* meaningfully inhibited the viability, proliferation, invasion, and migration of NSCLC cells, while promoting apoptosis.

Mechanistically, the sustained activated cell cycle signaling is a most noticeable biological feature for malignant tumor progression, and anti-tumor drugs ground on objecting cell-cycle regulators, which have been shown to be efficacious (24, 25). By contrast, acquired or constitutional resistance to CDK inhibitors of cancer cells has become a major obstruction to their clinical practical application (26, 27). Therefore, an alternative location strategy in cancer cells to inflect cell cycle regulators is expected hopefully.

Here, we verified beyond doubt that *LUCAT1* contributes to the progress or growth of cell cycle progression of NSCLC cells by affecting *p53*, CDK4, and Cyclin D1. As newly emerging ideas have disclosed extra activities of *P53* in the cytoplasm, where it triggers off apoptosis and inhibits autophagy. As a significant anti-oncogene, its deletion oftentimes issues tumorigenesis (28). The nuclear versus cytoplasmic outcomes of *P53* is dictated by multiple posttranslational modifications which affected interactions with other proteins, shuttlecock between the cytoplasm and the nucleus. More precisely, *LUCAT1* upgrades the unstableness of *p53* protein in NSCLC cells.

Although up to the present time we have yet to elucidate other cell cycle regulators in the pathways involved in *LUCAT1*-mediated, our findings bring out a refreshing mechanism of lncRNA-mediated post-translational modification in NSCLC cells.

Conclusion

LUCAT1 promotes *LUCAT1*-mediated ubiquitination and degradation of *p53*, and the combination of the two further accelerates this process. Our findings revealed a driving role of *LUCAT1* in NSCLC tumorigenesis. *LUCAT1* interacted with *p53* protein and facilitated its instability. The *p53*/*LUCAT1* axis promoted NSCLC growth, providing cogent evidence of concept for a novel therapeutic susceptibility of NSCLC.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

No funding was received in this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Iyer MK, Niknafs YS, Malik R, et al (2015). The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet,* 47(3): 199-208.
2. Schmitt AM, Chang HY (2016). Long Noncoding RNAs in Cancer Pathways. *Cancer Cell,* 29(4): 452-463.
3. Arun G, Diermeier SD, Spector DL (2018). Therapeutic Targeting of Long Non-Coding RNAs in Cancer. *Trends Mol Med,* 24(3): 257-277.
4. Niknafs YS, Han S, Ma T, Speers C, et al (2016). The lncRNA landscape of breast cancer reveals a role for DSCAM-AS1 in breast cancer progression. *Nat Commun,* 7: 12791.
5. Malik R, Patel L, Prensner JR, et al (2014). The lncRNA PCAT29 inhibits oncogenic phenotypes in prostate cancer. *Mol Cancer Res,* 12(8): 1081-7.
6. Statello L, Guo CJ, Chen LL, Huarte M (2021). Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol,* 22(2): 96-118.
7. Feretzaki M, Pospisilova M, Valador Fernandes R,

Lunardi T, Krejci L, Lingner J (2020). RAD51-dependent recruitment of TERRA lncRNA to telomeres through R-loops. *Nature,* 587(7833): 303-308.

8. Slack FJ, Chinnaiyan AM (2019). The Role of Non-coding RNAs in Oncology. *Cell,* 179(5): 1033-1055.
9. Li G, Kryczek I, Nam J, et al (2021). LIMIT is an immunogenic lncRNA in cancer immunity and immunotherapy. *Nat Cell Biol,* 23(5): 526-537.
10. Kandoth C, McLellan MD, Vandin F, et al (2013). Mutational landscape and significance across 12 major cancer types. *Nature,* 502(7471): 333-339.
11. Chen J (2016). The Cell-Cycle Arrest and Apoptotic Functions of *p53* in Tumor Initiation and Progression. *Cold Spring Harb Perspect Med,* 6(3): a026104.
12. Mantovani F, Collavin L, Del Sal G (2019). Mutant *p53* as a guardian of the cancer cell. *Cell Death Differ,* 26(2): 199-212.
13. Shin YJ, Kim Y, Wen X, Cho NY, Lee S, Kim WH, Kang GH (2019). Prognostic implications and interaction of L1 methylation and *p53* expression statuses in advanced gastric cancer. *Clin Epigenetics,* 11(1): 77.
14. Dai C, Gu W (2010). *p53* post-translational modification: deregulated in tumorigenesis. *Trends Mol Med,* 16(11): 528-36.
15. Pant V, Lozano G (2014). Limiting the power of *p53* through the ubiquitin proteasome pathway. *Genes Dev,* 28(16): 1739-51.
16. Thai P, Statt S, Chen CH, Liang E, Campbell C, Wu R (2013). Characterization of a novel long noncoding RNA, SCAL1, induced by cigarette smoke and elevated in lung cancer cell lines. *Am J Respir Cell Mol Biol,* 49(2): 204-11.
17. Sun Y, Jin SD, Zhu Q, et al (2017). Long non-coding RNA *LUCAT1* is associated with poor prognosis in human non-small lung cancer and regulates cell proliferation via epigenetically repressing p21 and p57 expression. *Oncotarget,* 8(17): 28297-28311.
18. Chen Y, Yu X, Xu Y, Shen H (2017). Identification of dysregulated lncRNAs profiling and metastasis-associated lncRNAs in colorectal cancer by genome-wide analysis. *Cancer Med,* 6(10): 2321-2330.
19. Zheng ZG, Xu H, Suo SS, et al (2016). The Essential Role of H19 Contributing to Cisplatin Resistance by Regulating Glutathione Metabolism in High-Grade Serous Ovarian Cancer. *Sci Rep,* 6: 26093.
20. Wen Y, Wu J, Pu Q, et al (2023). ABT-263 exerts a protective effect on upper urinary tract damage by alleviating neurogenic bladder fibrosis. *Ren Fail*, 45(1): 2194440.
21. Dey BK, Mueller AC, Dutta A (2014). Long non-coding RNAs as emerging regulators of differentiation, development, and disease. *Transcription,* 5(4): e944014.
22. Zhang K, Shi H, Xi H, et al (2017). Genome-Wide lncRNA Microarray Profiling Identifies Novel Circulating lncRNAs for Detection of Gastric Cancer. *Theranostics,* 7(1): 213-227.
23. Fattahi S, Kosari-Monfared M, et al (2020). LncRNAs as potential diagnostic and prognostic biomarkers in gastric cancer: A novel approach to personalized medicine. *J Cell Physiol,* 235(4): 3189-3206.
24. Wang L, Shao X, Zhong T, et al (2021). Discovery of a first-in-class CDK2 selective degrader for AML differentiation therapy. *Nat Chem Biol,* 17(5): 567-575.
25. Patnaik A, Rosen LS, Tolaney SM, et al (2016). Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non-Small Cell Lung Cancer, and Other Solid Tumors. *Cancer Discov,* 6(7): 740-53.
26. Fassl A, Brain C, Abu-Remaileh M, et al (2020). Increased lysosomal biomass is responsible for the resistance of triple-negative breast cancers to CDK4/6 inhibition. *Sci Adv,* 6(25): eabb2210.
27. Kumarasamy V, Vail P, Nambiar R, Witkiewicz AK, Knudsen ES (2021). Functional Determinants of Cell Cycle Plasticity and Sensitivity to CDK4/6 Inhibition. *Cancer Res,* 81(5): 1347-1360.
28. Green DR, Kroemer G (2009). Cytoplasmic functions of the tumour suppressor *p53*. *Nature,* 458(7242): 1127-30.