European Review for Medical and Pharmacological Sciences 2018; 22: 8333-8342

LINC00324 exerts tumor-promoting functions in lung adenocarcinoma via targeting miR-615-5p/AKT1 axis

Z.-H. PAN[1], X.-Q. GUO[1], J. SHAN[1], S.-X. LUO[2]

[1]Department of Radiation Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China
[2]Department of Medical Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China

Abstract. – OBJECTIVE: The underlying mechanism of long non-coding RNA (lncRNA) in lung adenocarcinoma (LAC) has not been fully understood yet. Hence, this study aimed to determine the biological function of LINC00324 in LAC and to provide a novel diagnostic and therapeutic target for it.

PATIENTS AND METHODS: The expression level of LINC00324 in 87 paired LAC tumor tissues and matched para-tumor tissues was detected using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Cell counting kit-8 (CCK-8) assay was employed to analyze the cell proliferative ability, whereas flow cytometry was performed to detect cell apoptotic rate. Cell metastasis change was measured using wound-healing assay and transwell assay. Luciferase reporter gene assay and Western blotting analysis were utilized to investigate the underlying mechanism of LINC00324 in LAC.

RESULTS: LINC00324 was highly expressed in LAC tissues compared with the para-tumor samples. Identically, the expression level of LINC00324 was significantly higher in LAC cell lines. The overexpression of LINC00324 promoted cell proliferation and inhibited cell apoptosis of LAC cells, while knockdown of LINC00324 presented the opposite effect. Up-regulation of LINC00324 accelerated cell migration and invasion, but down-regulation of LINC0324 decreased cell metastasis of LAC cells. Furthermore, miR-615-5p was found to be regulated by LINC00324 and inhibited AKT1 expression, indicating that LINC00324 promoted cell progression *via* affecting the miR-615-5p/AKT1 pathway.

CONCLUSIONS: LINC00324 was significantly over-expressed in LAC tissues and cells. It promoted proliferation and metastasis but inhibited cell apoptosis of LAC cells via sponging miR-615-5p to promote AKT1 expression. Our results demonstrated LINC00324 as a novel diagnostic and therapeutic target for LAC.

Key Words
LINC00324, Proliferation, Metastasis, LAC, MiR-615-5p, AKT1.

Introductions

Lung cancer is a common tumor in humans; its incidence ranks first among various malignant tumors. Lung cancer is the number one killer in cancer-related deaths, seriously threatening human life[1]. Lung adenocarcinoma (LAC) has become the most common type of lung cancer, accounting for about 40% of all types of lung cancer cases[2]. The pathogenesis of LAC is extremely complicated involving multiple factors. Current researches have confirmed that genetic level changes are one of the important factors in the development of LAC[3]. The activation of oncogenes or mutations and deletions of tumor-suppressor genes may be involved in the process of tumor cell invasion and metastasis[4].

Long non-coding RNAs are a type of non-coding RNAs with longer than 200 nt in length. Many studies have confirmed the biological function of lncRNA in tumor development and progression[5]. For example, lncRNA TP73-AS1 functioned as oncogenic lncRNA and predicted poor prognosis in osteosarcoma[6]. LncRNA NEAT1 interacted with DDX5 and promoted colorectal cancer progression *via* activating the Wnt/β-catenin pathway[7]. LINC00963 was up-regulated in melanoma and facilitated disease progression *via* miR-608/NACC1 axis[8]. LncRNA FAL1 increased BMI1 expression, accelerated cell proliferation and metastasis *via* repressing PTEN in NSCLC[9].

LINC00324, located in 17p13.1, was confirmed as an oncogene in gastric cancer. It promoted cell proliferation by binding with HuR and stabilizing FAM83B expression[10]. However, LINC00324 functions in LAC have not been mentioned before.

Here in our study, we first detected LINC00324 expression in LAC tumor tissues and adjacent

Corresponding Author: Suxia Luo, MD; e-mail: luosxrm@163.com

para-tumor tissues, as well as in LAC cell lines. By the overexpression or knockdown of LINC00324 in A549 or H1299 cells, biological performances of LAC cells were investigated. Furthermore, miR-615-5p/AKT1 was verified as a direct target for LINC00324. Our work demonstrated the LINC00324 expression and function in LAC for the first time, providing a novel target for diagnosis and therapy for LAC.

Patients and Methods

Clinical Tissues

Eighty-seven pairs of surgically resected LAC and matched adjacent para-tumor tissue samples were obtained from The Affiliated Cancer Hospital of Zhengzhou University. None of the patients in this study were treated with preoperative chemotherapy or radiotherapy. This experiment got the approval of the Ethics Committee of The Affiliated Cancer Hospital of Zhengzhou University. The informed consents were signed by all patients before surgery. Tissue samples were stored in liquid nitrogen until use.

Cells

LAC-derived cell lines (A549, PC-9, H1650, SPCA1, H1299) and human normal lung bronchial epithelial cell line 16HBE were purchased from the Academy of Sciences of China (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) was used to maintain the cells. Cells were cultured in the atmosphere of 5% humidified environment of CO_2 at 37°C.

Cell Transfection

Plasmids containing LINC00324 full length (pcDNA3.1-LINC00324) and controls (pcDNA3.1-NC) were synthesized and cloned into lentiviral vectors (LV-LINC00324 and LV-NC). MiR-615-p mimics and controls (miR-NC) were obtained from Genechem (Shanghai, China). Three siRNAs targeting LINC00324 were got from GenePharma Biotechnology Co. Ltd. (Shanghai, China). Lentiviral vectors were transfected using polybrene (GenePharma Biotechnology Co. Ltd., Shanghai, China) according to the manufacturer's instructions. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was applied for transfection of siRNAs and miR-615-5p mimics. The transfection efficiency was detected using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR).

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

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was employed to isolate the total RNA. The concentration and quality of isolated RNA were detected with using a Nanodrop Spectrophotometer (ND-3000, Thermo-Fisher Scientific, Waltham, MA, USA). MiRNA cDNA synthesis kit (Ribobio, Guangzhou, China) and PrimeScriptTM RT reagent Kit (TaKaRa, Dalian, China) were used to reverse transcription of cDNA. QRT-PCR was realized using SYBR Green (TaKaRa, Dalian, China) and performed in ABI-7500 (ABI, NY, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for mRNA levels of LINC00324 or AKT1 and U6 was served as an endogenous control for miRNA-615-5p. The relative expressions of lncRNA and miRNA were calculated using the $2^{-\Delta\Delta Ct}$ method. All the Primers were synthesized by GeneWiz Co. (Suzhou, China). Primer sequences used in this study were as follows: LINC00324, F: 5'-CCCCCAGGAACTCCTTACTC-3', R: 5'-TGTGTCCTAGGGACGAAGGA-3'; AKT1, F: 5'-GTAAACATCGGGGTCTCGACTG-3', R: 5'-AGTGTCGTGTTGCGAGTCG-3'; miRNA-615-5p, F: 5'-GCATTTAGCAGCGAGACAA-3', R: 5'-AGCGACACGTGCGAATGTTCT-3'. U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Cell Counting Kit-8 (CCK-8) Assay

Cells transfected with lentivirus or siRNA were prepared for suspension and plated in 96-well plates. At different time points (0 h, 24 h, 48 h, 72 h), 10 μL of CCK-8 reagent (Dojindo, Kumamoto, Japan) was added into the 96-well plate, followed by incubation in the cell culture incubator for 2 h. The absorbance of each well of the 96-well plate was measured at the wavelength of 470 nm using a spectrophotometer. Each experiment was repeated at least three times.

Cell Apoptosis Detection

Cell apoptosis was tested using an Annexin V-FITC/PI Kit (Vazyme, Nanjing, China) according to the manufacturer's instructions. Cells transfected with lentivirus or siRNAs were cultured in a 6-well plate (1×10^5 cells/well) and cultured for 48 h. Cells

were harvested into the flow tubes. A total of 500 μL binding buffer containing 50 μL Annexin V-FITC and 50 μL Propidium Iodide (PI) were added into the tube. Finally, a FACScan flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was used to detect the apoptotic rate.

Wound-Healing Assay

Cells were plated in 6-well plates and maintained in the culture medium containing 10% FBS until cells were fully confluent. After washing with phosphate-buffered saline (PBS), three scratches were vertically drawn at the bottom of the 6-well plate using a 200 μL tip. After PBS washing, the cells were cultured for 48 h in serum-free medium. Healing of the cell scratches was observed under a microscope and photographed.

Transwell Assay

For the invasion assay, 8-μm chambers (Millipore, Billerica, MA, USA) were purchased and pre-coated with Matrigel (BD, Franklin Lakes, NJ, USA). A549 or H1299 cells after transfection were suspended in serum-free medium and seeded in the top chamber with 3×10^4 cells per well. A total of 500 μL medium containing 10% FBS was added in the bottom chamber. After incubation for 36 h, un-penetrating cells on the top chamber were wiped off using cotton swabs. Cells that penetrated to the bottom chamber were fixed with methanol and stained with crystal violet. Five randomly selected fields in each sample were recorded under a microscope (ZEISS, Oberkochen, Germany) at 400 × magnification.

Luciferase Reporter Gene Assay

PGL3-LINC00324-Wild-Type (or Mutant type) or AKT1 3'-untranslated region (3'-UTR)-Wild-Type (or Mutant type) reporter plasmids were constructed by Genepharma Biotechnology Co., Ltd. (Shanghai, China). Dual-Luciferase Reporter Assay kit (Promega, Madison, WI, USA) was obtained to measure the change of Luciferase activity. Cells co-transfected with constructed reporter plasmids and miR-615-5p mimics or miR-control were cultured and lysed according to the manufacturers' instructions. Luciferase activity was finally measured.

Western Blotting

Total proteins from the cells and tissues were isolated using ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) containing protease inhibitors (Beyotime, Shanghai, China). Proteins were added onto 10% SDS-PAGE for electrophoresis within 2 h. Protein samples were electrophoretically transferred to a PVDF (polyvinylidene difluoride) membrane (Millipore, Billerica, MA, USA). Subsequently, membranes were immersed in 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS) buffer at room temperature for 2 h, followed by incubation with the appropriate primary antibodies for AKT1 or GAPDH (CST, Danvers, MA, USA) overnight at 4°C. The horseradish peroxidase-labeled secondary antibody was then used to bind the primary antibody. Protein quantification was done using an ECL kit (Millipore, Billerica, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) were used for statistical analysis. Data were expressed as mean ± SD (standard deviation). The Student's *t*-test was utilized to assess the difference between the two groups and p-value <0.05 was considered statistically significant.

Results

LINC00324 Was Overexpressed in LAC Tissues and Cell Lines

To verify the expression of LINC00324 in LAC, we detected LINC00324 expression in 87 paired of LAC tissues and matched para-tumor tissues using qRT-PCR. Figure 1A showed higher expression of LINC00324 in LAC samples than the para-tumor group. Identically, expressions of LINC00324 in LAC cell lines (A549, PC-9, H1650, SPCA1, H1299) were higher than that of the normal human lung bronchial epithelial cells 16HBE (Figure 1B). These results indicated the potential role of LINC00324 in LAC progression and may act as a tumor-promoting gene in LAC.

To further study the function of LINC00324 in LAC, we over-expressed LINC00324 in A549 cells by LV-LINC00324 transfection. LINC00324 expression markedly increased after transfection of LV-LINC00324 in A549 cells (Figure 1C). Three siRNAs for LINC00324 were synthesized and their transfection efficacies were verified in H1299 cells. Among them, transfection efficacy of siRNA-LINC00324-3 was the most pronounced and was chosen for following experiments (Figure 1D).

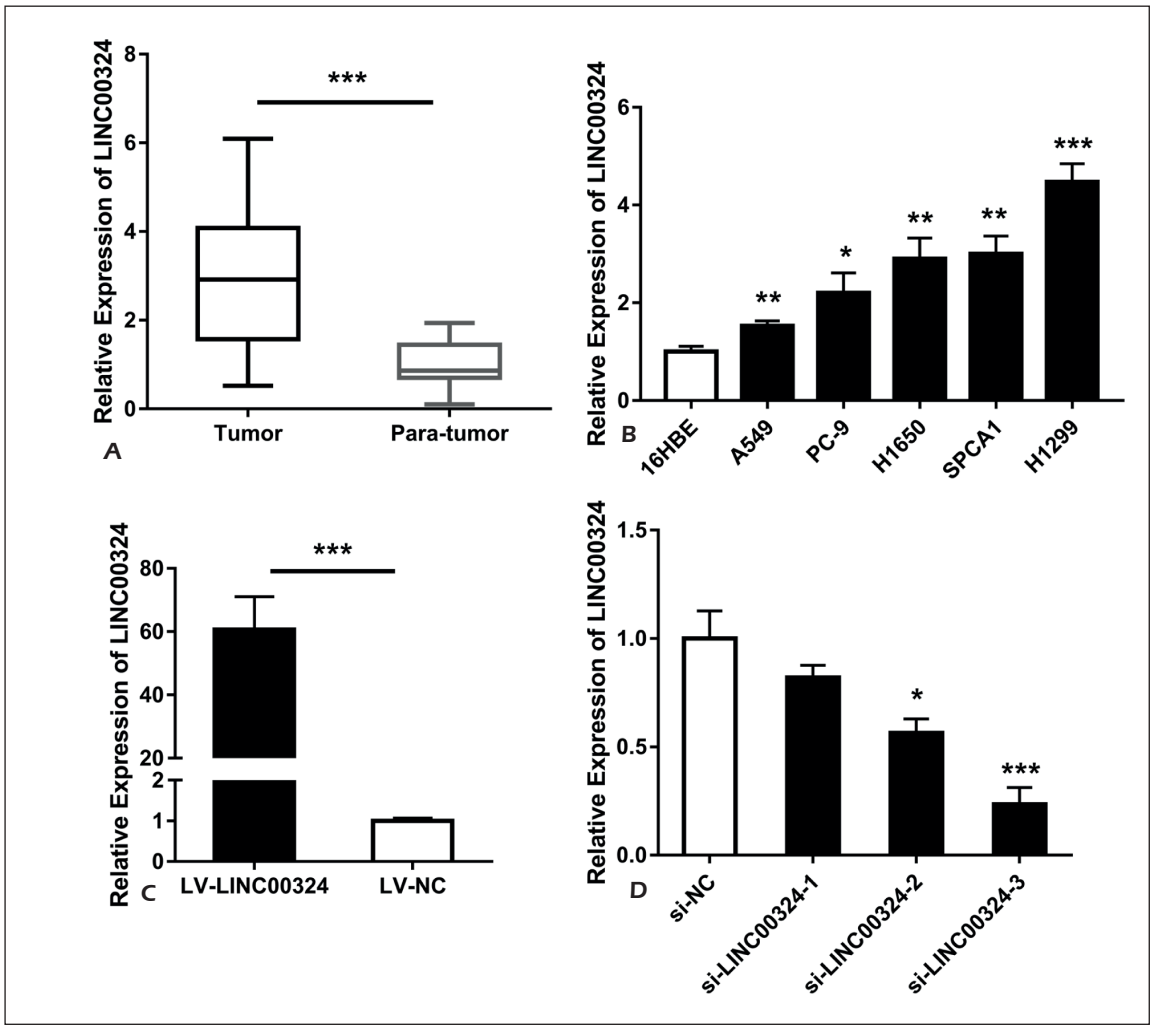

Figure 1. LINC00324 was overexpressed in LAC tissues and cells. **A**, QRT-PCR showed the lncRNA LINC00324 expression in a total of 87 paired LAC tissues and adjacent para-tumor tissues. **B**, LINC00324 expression in LAC cell lines (A549, PC-9, H1650, SPCA1, SPCA1) and human normal bronchial epithelial cell line (16HBE). **C**, LV-LINC00324 or LV-NC were transfected into A549 cells. **D,** Three siRNAs targeting LINC00324 (siRNA-LINC00324-1, siRNA-LINC00324-2, siRNA-LINC00324-3) or negative controls (siRNA-NC) were transfected into SPCA1 cells. ***p<0.001, **p<0.01 *p<0.05 compared to the control group.

LINC00324 Promoted Cell Proliferation and Inhibited Cell Apoptosis in LAC

Next, the CCK-8 assay was conducted to detect the influence of LINC00324 on cell growth. LINC00324 overexpression markedly promoted the proliferation of A549 cells, while LINC00324 knockdown decreased the proliferation of H1299 cells (Figure 2A, 2B). Furthermore, the apoptosis of established cells was measured using flow cytometry. The over-expression of LINC00324 in A549 cells reduced apoptotic rate significantly. However, the knockdown of LINC00324 accelerated apoptosis in H1299 cells (Figure 2C, 2D). These data indicated that LINC00324 could promote proliferation but inhibit apoptosis of LAC cells.

Ectopic Expression of LINC00324 Influenced Cell Migration and Invasion of LAC

Furthermore, we detected the change of cell metastasis ability *via* wound-healing assay and transwell assay. As shown in Figure 3A, the extent of wound healing of A549 cells transfected with LV-LINC00324 was much greater than that of the control group. However, the knockdown of LINC00324 slowed down the speed of wound healing in H1299 cells. Transwell assay also validated that LV-LINC00324 transfection enhanced the invasive ability of A549 cells, while LINC00324 knockdown reduced the invasive ability of H1299 cells (Figure 3B). These results

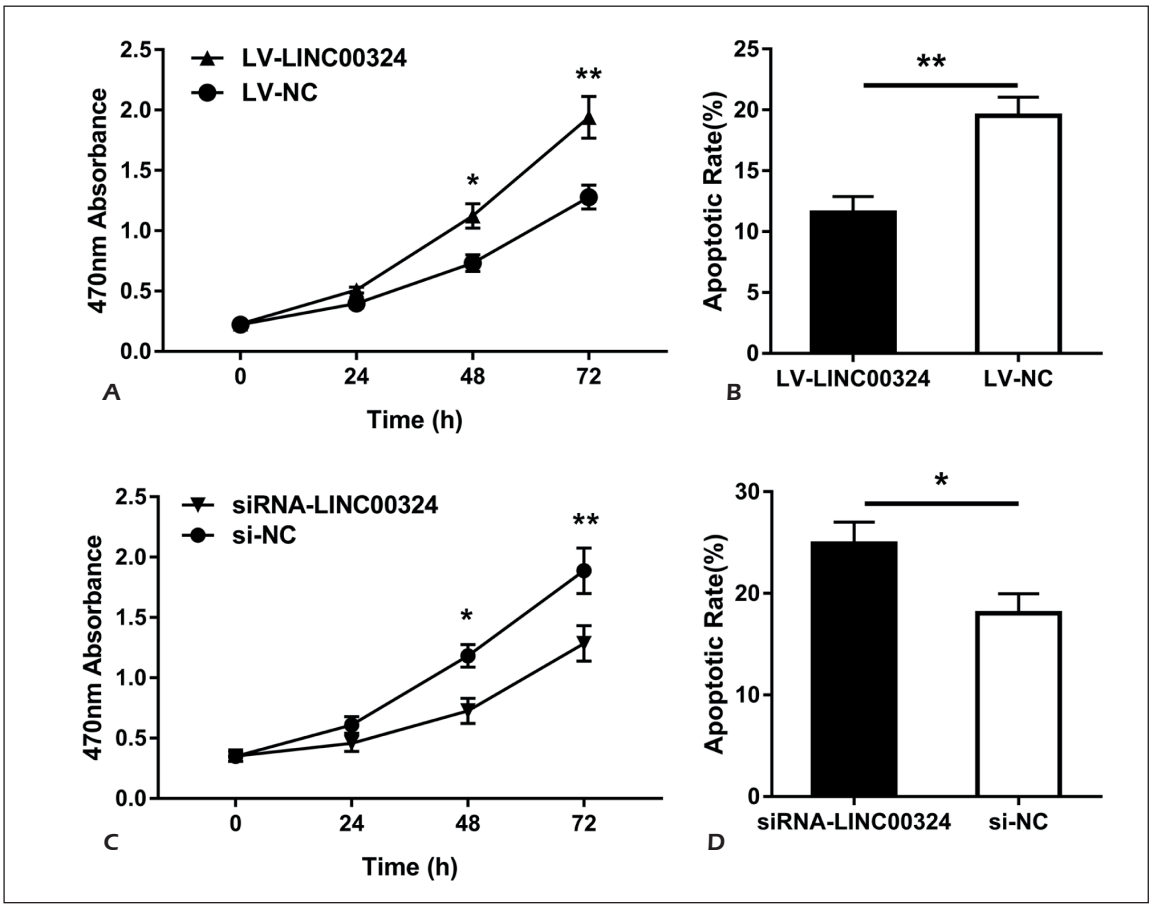

Figure 2. LINC00324 affected the proliferation of LAC cells *in vitro*. **A-B**, CCK-8 assays showed the proliferation ability of A549 cells transfected with LV-LINC00324 and LV-NC or SPCA1 cells transfected with siRNA-LINC00324 and siRNA-NC. **C-D,** Cell apoptosis detection showed the apoptotic rate of A549 cells transfected with LV-LINC00324 and LV-NC or SPCA1 cells transfected with siRNA-LINC00324 and siRNA-NC. $^{**}p<0.01$, $^{*}p<0.05$ compared to the control group.

suggested LINC00324 promoted cell migration and invasion of LAC cells.

LINC00324 Sponged MiR-615-5p in LAC

We have identified LINC00324 function as an oncogene in LAC. We further studied the underlying mechanism of LINC00324 in LAC. Considering the ceRNA role of lncRNAs, miR-615-5p was found to be a target for LINC00324 through database searching (Figure 4A). We constructed Dual-Luciferase reporter assay to verify our speculation. Cells co-transfected with PGL3-LINC00324-Wild-Type and miR-615-5p mimics showed a significant decrease in Luciferase activity, but the PGL3-LINC00324-Mutant showed no difference (Figure 4B). The expression level of miR-615-5p was detected using qRT-PCR. MiR-615-5p expression was markedly reduced in A549 cells overexpressing LINC00324, but increased in H1299 cells with LINC00324 knockdown (Figure 4C, 4D). These data demonstrated LINC00324 could act as a sponge for miR-615-5p in LAC.

LINC00324 Promoted LAC Progression Via MiR-615-5p/AKT1 Axis

Furthermore, we found that AKT1 can function as a target gene for LINC00324/miR-615-5p through bioinformatics prediction. MiR-615-5p could bind to the 3'-UTR of AKT1 (Figure 5A). To identify the binding effect, we next measured the Luciferase activity in PGL3-AKT1 3'-UTR wild-type group and PGL3- AKT1 3'-UTR mutant group. The wild-type group showed a remarkable decrease in Luciferase activity, whereas the mutant group had no significant change compared to the control group (Figure 5B). It is suggested AKT1 may act as a direct target for miR-615-5p in LAC cells. Next, the protein of AKT1 in LV-LINC00324 transfected A549 cells and siRNA-

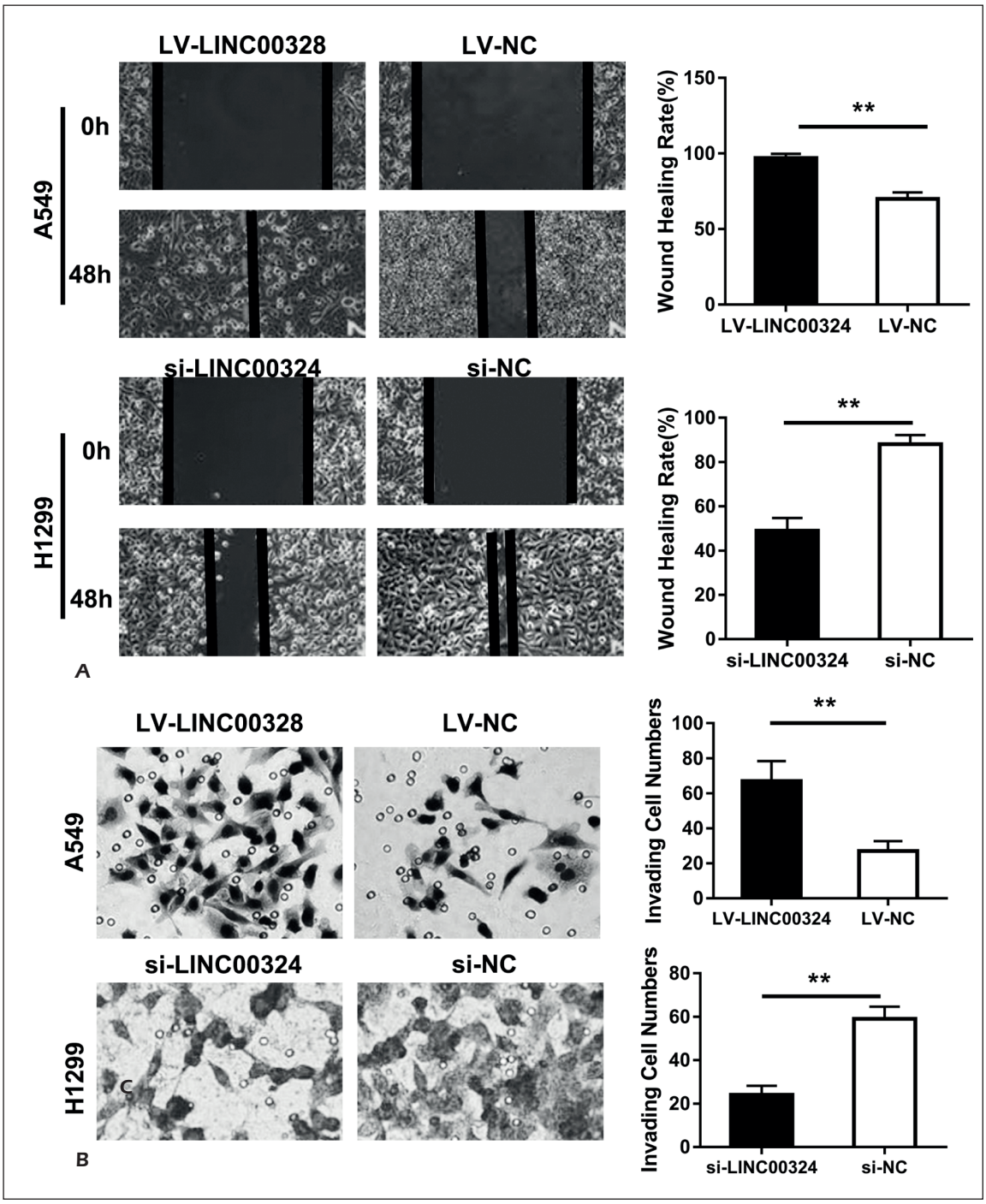

Figure 3. LINC00324 effected the migration and invasion of LAC cells. **A**, Wound-healing assay indicated the migration ability change in established A549 cells and SPCA1 cells. **B**, Transwell assay showed the invaded cell number in established A549 cells and SPCA1 cells. $^{**}p<0.01$, $^{*}p<0.05$ compared to the control group.

LINC00324 transfected H1299 cells was detected using Western blotting. AKT1 expression was higher in LV-LINC00324 transfected A549 cells but lower in siRNA-LINC00324 transfected H1299 cells compared to the negative control group, relatively (Figure 5C, 5D). All these elucidated that LINC00324 promoted LAC progression *via* miR-615-5p/AKT1 axis.

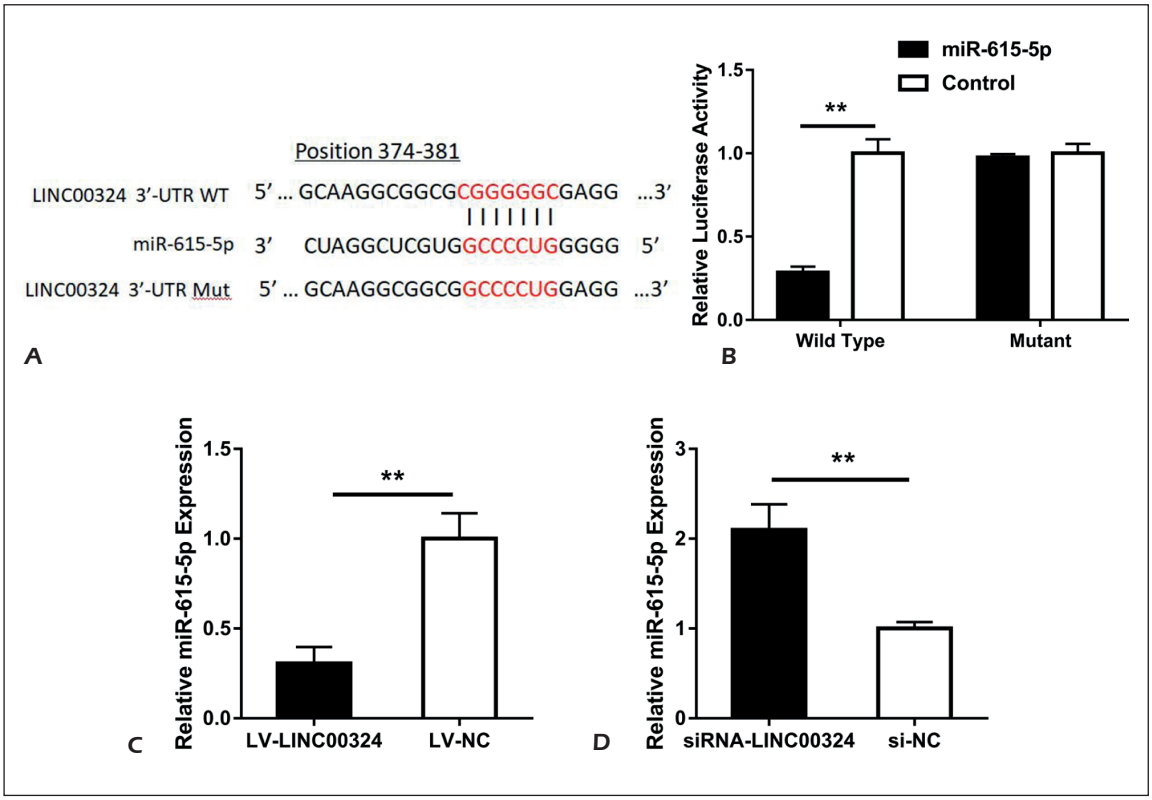

Figure 4. LINC00324 functioned as a sponge of miR-615-5p. **A**, The sequences of LINC00324 with miR-615-5p, including wild-type and mutant binding sites. **B**, Luciferase reporter gene assay verified the relationship within LINC00324 and miR-615-5p. **C-D,** QRT-PCR showed the miR-615-5p expression level in A549 or SPCA1 cells transfected with LV-LINC00324 or siRNA-LINC00324. ***p<0.001, **p<0.01 compared to the control group.

Discussion

Lung cancer is still the malignancy with the highest mortality rate worldwide. Lung cancer has a high degree of malignancy without significant early symptoms, leading to the low 5-year survival rate. Non-small cell lung cancer mainly includes squamous cell carcinoma (LSCC), adenocarcinoma (LAC) and large cell carcinoma. The incidence of LAC has gradually occupied the highest proportion of lung cancer in recent years[1,2,11]. Therefore, the diagnosis and treatment of early LAC are particularly important, urgently requiring sensitive and specific lung cancer molecular markers. In recent years, molecular targeted therapy for cancer has been widely recognized. Among them, diagnosis and treatment of tumor-based gene levels have become a new hot spot[3,5,12].

With the development of genomics, many non-coding genes that do not have gene coding functions have been found to be involved in the regulation of many biological processes. Many non-coding RNAs (ncRNAs), including miRNAs and lncRNAs, regulate the occurrence and progression of tumors at pre-transcriptional and post-transcriptional levels[3,4,12]. In this work, we first examined the expression level of LINC00324 in LAC. Although that the promotive role of LINC00324 in the proliferation and invasion of gastric cancer cells has been reported, its role in lung cancer has not been elucidated yet. The expression level of LINC00324 was significantly elevated in LAC tissues and cells, suggesting the tumor-promoting effect of LINC00324 on LAC.

To further reveal the role of LINC00324 in LAC, we constructed LINC00324 overexpressing A549 cell line and LINC00324 deficiency H1299 cell line for a series of functional tests. CCK-8 assay confirmed that over-expression of LINC00324 significantly promoted the proliferation of LAC cells, while knockdown showed an opposite effect. Further, we found that LINC00324 inhibited cell apoptosis, indicating that LINC00324 has a role in promoting growth in LAC cells. Similarly, we found that the over-

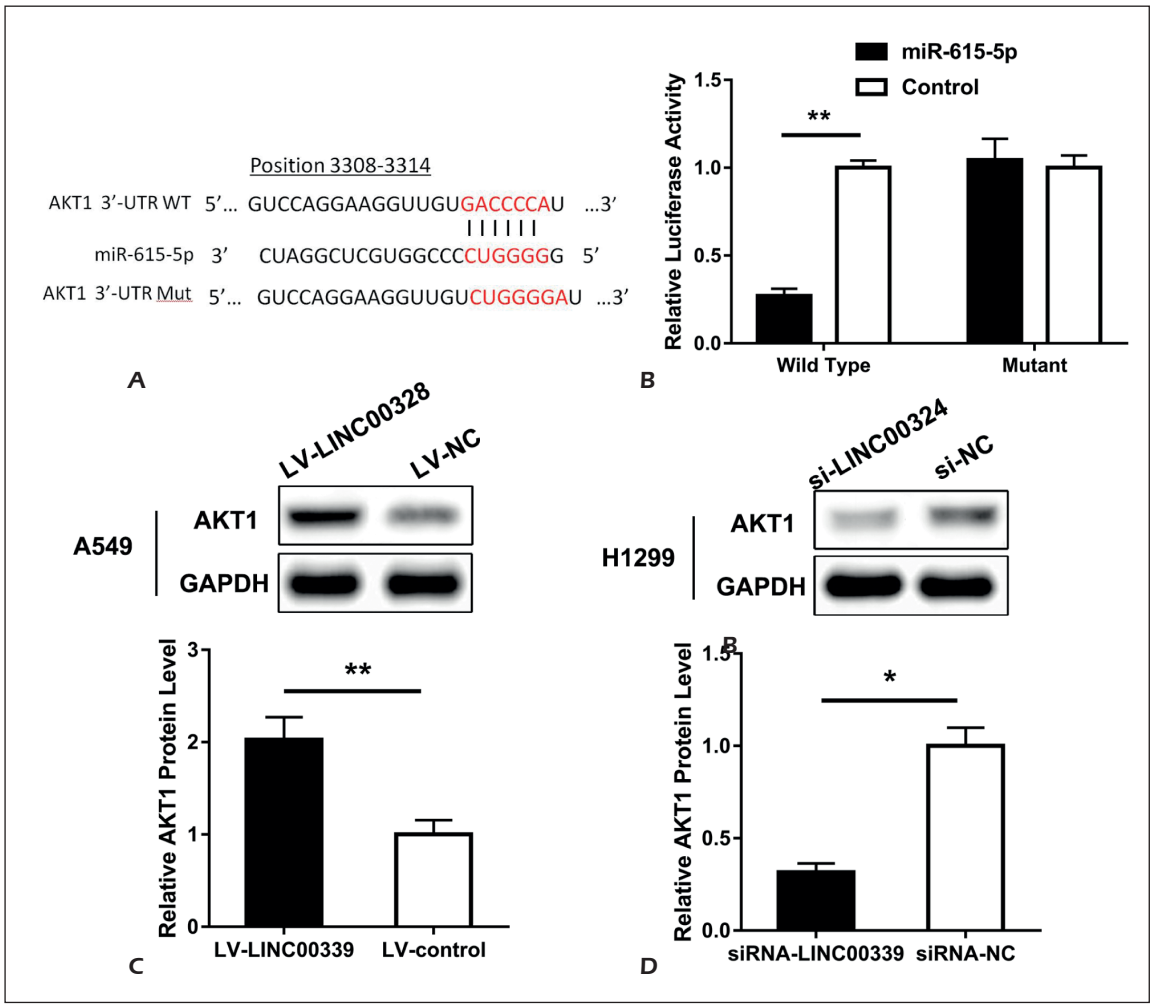

Figure 5. AKT1 was identified as the target gene of miR-615-5p. **A**, The sequences of AKT1 mRNA 3'-UTR and miR-615-5p, including wild-type and mutant type. **B**, Luciferase reporter gene assay indicated the relationship between AKT1 and miR-615-5p. **C-D,** Western blotting indicated the AKT1 protein expression in established A549 cells and SPCA1 cells. $^{**}p<0.01$ $^{*}p<0.05$ compared to the control group.

expression of LINC00324 significantly promoted migration and invasion of A549 cells by the wound-healing assay and transwell assay. On the contrary, LINC00324 knockdown significantly inhibited migration and invasion of H1299 cells. It is indicated that LINC00324 also has a promotive effect on the metastasis of LAC cells.

Previous studies have shown that lncRNA exerts its role through sponging specific miRNAs[13]. Hence, we searched several databases and found that miR-615-5p was a target gene for LINC00324. Through the Luciferase reporter gene assay, we confirmed that LINC00324 could directly bind to miR-615-5p. QRT-PCR further confirmed that miR-615-5p expression was negatively regulated by LINC00324 in LAC cells. AKT1 was found to be a target gene of miR-615-5p through biological analysis, which was able to promote the development of LAC. MiR-615-5p has been reported to participate in the progression of many tumor diseases as an anti-cancer miRNA. It targeted IGF2 in human esophageal squamous cell carcinoma and inhibited tumor-suppressing function[14]. MiR-615-5p mediated natural killer cells cytotoxicity *via* down-regulating IGF-1R and slowed down cell growth and migration *via* repressing SHMT2 in hepatocellular carcinoma[15,16]. Furthermore, in pancreatic adenocarcinoma, miR-615-5p was regulated by CDX2 and inhibited cell proliferation by suppressing AKT2 expression[17-19]. MiR-615-5p acted as a promoter in carcinogenesis of LAC[20]. AKT1 was an oncogene in several

cancer diseases including osteosarcoma, pancreatic ductal adenocarcinoma, colorectal cancer, and breast cancer. It could be regulated by miR-520d-3p, miR-637, miR-422a, miR-409-3p, thereafter promoting cell proliferation and metastasis[21-24].

Luciferase reporter gene assay confirmed that miR-615-5p could directly bind to the 3'-UTR of AKT1 and inhibited AKT1 expression. Western blotting analysis showed that the over-expression of LINC00324 upregulated AKT1 expression in A549 cells, while the expression of the AKT1 protein in H1299 cells was significantly reduced with LINC00324 knockdown. We demonstrated that LINC00324 regulated the protein expression of the AKT1 by sponging miR-615-5p, eventually promoting the proliferation and development of LAC cells.

Conclusions

We explored the biological function of LINC00324 in promoting LAC cell proliferation, migration and invasion. Moreover, LINC00324 could sponge and inhibit miR-615-5p, and then up-regulate AKT1 to exert its tumor-promoting effect. We revealed the role of LINC00324 in the development of LAC, providing a feasible target for LAC diagnosis and its targeted therapy.

Conflict of Interests:

The authors declare that they have no conflict of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
2) Torre LA, Siegel RL, Jemal A. Lung cancer statistics. Adv Exp Med Biol 2016; 893: 1-19.
3) Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-463.
4) Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev 2016; 96: 1297-1325.
5) Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.
6) Li X, Wang X, Mao L, Zhao S, Wei H. LncRNA TP73AS1 predicts poor prognosis and promotes cell proliferation in ovarian cancer via cell cycle and apoptosis regulation. Mol Med Rep 2018; 18: 516-522.
7) Zhang M, Weng W, Zhang Q, Wu Y, Ni S, Tan C, Xu M, Sun H, Liu C, Wei P, Du X. The lncRNA NEAT1 activates Wnt/beta-catenin signaling and promotes colorectal cancer progression via interacting with DDX5. J Hematol Oncol 2018; 11: 113.
8) Jiao H, Jiang S, Wang H, Li Y, Zhang W. Upregulation of LINC00963 facilitates melanoma progression through miR-608/NACC1 pathway and predicts poor prognosis. Biochem Biophys Res Commun 2018; 504: 34-39.
9) Pan C, Yao G, Liu B, Ma T, Xia Y, Wei K, Wang J, Xu J, Chen L, Chen Y. Long noncoding RNA FAL1 promotes cell proliferation, invasion and epithelial-mesenchymal transition through the PTEN/AKT signaling axis in non-small cell lung cancer. Cell Physiol Biochem 2017; 43: 339-352.
10) Zou Z, Ma T, He X, Zhou J, Ma H, Xie M, Liu Y, Lu D, Di S, Zhang Z. Long intergenic non-coding RNA 00324 promotes gastric cancer cell proliferation via binding with HuR and stabilizing FAM83B expression. Cell Death Dis 2018; 9: 717.
11) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
12) Kopp F, Mendell JT. Functional classification and experimental dissection of long noncoding RNAs. Cell 2018; 172: 393-407.
13) Militello G, Weirick T, John D, Doring C, Dimmeler S, Uchida S. Screening and validation of lncRNAs and circRNAs as miRNA sponges. Brief Bioinform 2017; 18: 780-788.
14) Yang B, Xie R, Wu SN, Gao CC, Yang XZ, Zhou JF. MicroRNA-615-5p targets insulin-like growth factor 2 and exerts tumor-suppressing functions in human esophageal squamous cell carcinoma. Oncol Rep 2018; 39: 255-263.
15) Rahmoon MA, Youness RA, Gomaa AI, Hamza MT, Waked I, El Tayebi HM, Abdelaziz AI. MiR-615-5p depresses natural killer cells cytotoxicity through repressing IGF-1R in hepatocellular carcinoma patients. Growth Factors 2017; 35: 76-87.
16) Wu X, Deng L, Tang D, Ying G, Yao X, Liu F, Liang G. miR-615-5p prevents proliferation and migration through negatively regulating serine hydromethyltransferase 2 (SHMT2) in hepatocellular carcinoma. Tumour Biol 2016; 37: 6813-6821.
17) Gao W, Gu Y, Li Z, Cai H, Peng Q, Tu M, Kondo Y, Shinjo K, Zhu Y, Zhang J, Sekido Y, Han B, Qian Z, Miao Y. miR-615-5p is epigenetically inactivated and functions as a tumor suppressor in pancreatic ductal adenocarcinoma. Oncogene 2015; 34: 1629-1640.
18) Jiang Y, Zhang Y, Li F, Du X, Zhang J. CDX2 inhibits pancreatic adenocarcinoma cell proliferation via promoting tumor suppressor miR-615-5p. Tumour Biol 2016; 37: 1041-1049.
19) Sun Y, Zhang T, Wang C, Jin X, Jia C, Yu S, Chen J. MiRNA-615-5p functions as a tumor suppressor in pancreatic ductal adenocarcinoma by targeting AKT2. PLoS One 2015; 10: e0128257.

20) Zhao ZG, Jin JY, Zhang AM, Zhang LP, Wang XX, Sun JG, Chen ZT. MicroRNA profile of tumorigenic cells during carcinogenesis of lung adenocarcinoma. J Cell Biochem 2015; 116: 458-466.
21) He TD, Zhu JK, Wei ZX, Wang YM. MicroRNA-520d-3p inhibits osteosarcoma progression by degradation of Akt1. Eur Rev Med Pharmacol Sci 2018; 22: 2315-2320.
22) Wei WT, Nian XX, Wang SY, Jiao HL, Wang YX, Xiao ZY, Yang RW, Ding YQ, Ye YP, Liao WT. miR-422a inhibits cell proliferation in colorectal cancer by targeting AKT1 and MAPK1. Cancer Cell Int 2017; 17: 91.
23) Xu RL, He W, Tang J, Guo W, Zhuang P, Wang CQ, Fu WM, Zhang JF. Primate-specific miRNA-637 inhibited tumorigenesis in human pancreatic ductal adenocarcinoma cells by suppressing Akt1 expression. Exp Cell Res 2018; 363: 310-314.
24) Zhang G, Liu Z, Xu H, Yang Q. miR-409-3p suppresses breast cancer cell growth and invasion by targeting Akt1. Biochem Biophys Res Commun 2016; 469: 189-195.