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Silencing LINC01116 suppresses the development of lung adenocarcinoma via the AKT signaling pathway

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Abstract

Background: A growing body of evidence has proven that long noncoding ribonucleic acids (lncRNAs) are important epigenetic regulators that play crucial parts in the pathogenesis of human cancers. Previous studies have shown that long intergenic nonprotein coding RNA 01116 (LINC01116) is a carcinogen in several carcinomas; however, its function in lung adenocarcinoma (LUAD) has not been clarified. Here, we aimed to investigate the role of LINC01116 in LUAD.

Methods: The relative expression levels of LINC01116 in LUAD cell lines and tissues were detected by quantitative reverse transcription polymerase chain reaction. A Kaplan–Meier survival analysis was performed using patient information from the Gene Expression Profiling Interactive Analysis (GEPIA) database. LUAD proliferation, invasion, migration, and apoptosis were measured by performing cell counting kit-8, colony formation, transwell, wound healing, and flow cytometric assays. A xenograft animal experiment was performed to investigate the effect of LINC01116 in vivo. Protein kinase B (AKT) signaling pathway-related protein expressions were tested by Western blot assay.

Results: LINC01116 expression was upregulated in LUAD cells and tissues. The loss-of-function experiments on LUAD cells revealed that silencing LINC01116 expression could decrease cell viability both in vitro and in vivo. Furthermore, silencing LINC01116 inhibited LUAD cell invasion and migration and induced cell apoptosis. Mechanically, silencing LINC01116 significantly decreased p-AKT protein levels, and an AKT pathway stimulator could rescue the suppressive effects of small interfering LINC011116-specific RNAs on LUAD development.

Conclusions: Our study demonstrated that silencing LINC01116 suppresses the development of LUAD via the AKT signaling pathway.

KEYWORDS
AKT pathway, cell proliferation, LINC01116, LUAD, migration

BACKGROUND

Lung cancer is one of the most common aggressive malignancies with high mortality and morbidity worldwide.[1] Non-small cell lung cancer (NSCLC) is the most common type of lung cancer among affected patients, and lung adenocarcinoma (LUAD) is the predominant pathological type of NSCLC.[2] Despite advances in comprehensive therapy, patients who are diagnosed with LUAD still have a poor prognosis due to the aggressiveness of the tumor, resistance to treatments, and recurrence over time.[3] Therefore, there is an urgent need and high demand for the identification of key molecules involved in LUAD to develop early diagnostic strategies and to improve the clinical outcome.

Long noncoding ribonucleic acids (lncRNAs) are functional noncoding RNA molecules that are more than 200 nt

in length and do not possess the protein-coding capacity.[4] LncRNAs play an important role in gene regulation and, thus, have the potential to affect many aspects of cellular homeostasis, including proliferation, migration, and genomic stability.[5] Growing evidence has suggested that the dysregulation of lncRNA is related to the occurrence and progression of numerous cancers. For example, the lncRNA, HOX antisense intergenic RNA (HOTAIR), influences cell viability, invasion, migration, and apoptosis in breast carcinoma.[6] LncRNA regenerating family member 1 gamma (REG1CP) promotes cancer cell differentiation and tumorigenesis, and its upregulation is associated with poor prognosis in patients with colorectal cancer.[7] LncRNA-D16366 has been proven to be downregulated in tissues and serum and might be a potential tumor marker for the diagnosis and prognosis of patients with hepatocellular carcinoma.[8] Although the presence of a great number of human lncRNAs has been reported so far, the physiological function of most lncRNAs is poorly understood.

Recently, it has been reported that long intergenic nonprotein coding RNA 01116 (LINC01116) promotes nasopharyngeal carcinoma development[9] and accelerates tumor proliferation in glioma.[10] Silencing LINC01116 may inhibit the progression of oral squamous cell carcinoma via microRNA (miR)-136-mediated fibronectin 1 (FN1) inhibition.[11] Additionally, LINC01116 has been found to exacerbate lung squamous cell carcinoma progression via targeting the miR-744-5p/ sodium voltage-gated channel beta subunit 1 (SCN1B) axis.[12] However, the biological function of LINC01116 and its underlying mechanism in LUAD are still unknown.

Therefore, in this study, we aimed to investigate the role of LINC01116 in LUAD.

We observed that LINC01116 was highly expressed in LUAD cells and tissues. LUAD patients with lower LINC01116 expression levels exhibited better survival outcomes than those with higher LINC01116 expression levels. Silencing LINC01116 suppressed cell proliferation, invasion, and migration, while promoting the apoptosis of LUAD cells. In exploring the underlying mechanisms, we identified that LINC01116 promoted LUAD progression through the regulation of the protein kinase B (AKT) signaling pathway. Based on these observations, we concluded that LINC01116 plays key roles in the progression of LUAD. LINC01116 might serve as a new diagnostic tumor marker as well as a potential therapeutic target for LUAD treatment.

METHODS

Cell lines

Normal human bronchial epithelial cells (HBE) and four LUAD cell lines, SPC-A-1, A549, H1975, and H1299, were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). All cell lines were raised in Dulbecco's modified Eagle medium (DMEM; Gibco; Thermo Fisher Scientific Inc.) containing 100 U/ml of penicillin, 100 μg/ml of streptomycin (Gibco; Thermo Fisher Scientific Inc.), and 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific Inc.). An incubator (Thermo Fisher Scientific Inc.) was maintained in a damp environment supplied with 5% of CO_2 at 37°C.

Tissue samples

In the study, GEPIA was preliminary used to explore the different expression of LINC01116 in LUAD comparing to normal lung tissues. A total of 10 pairs of LUAD tissue and tumor adjacent lung tissue samples (> 2.0 cm from the edge of the tumor tissue) were collected from patients between September 2019 and November 2019 at Shandong Provincial Hospital Affiliated to Shandong First Medical University. Among them, two were women and eight were men, with a median age of 56 years (range: 36–71 years). None of the patients underwent radiotherapy or chemotherapy before surgery. The tissue specimens obtained were quickly frozen and stored in liquid nitrogen until analysis to avoid RNA degradation. This study was approved by the Institutional Review Board (IRB) of Shandong Provincial Hospital Affiliated to Shandong First Medical University and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the participating patients before the study commenced.

Cell transfection

Small interfering RNAs (siRNAs) specific to LINC01116 (si-LINC01116) and scrambled oligonucleotides (si-NC) were obtained from a commercial supplier (Olinger Biotechnology Co. Ltd.). The following four target sequences were selected for the inhibition of LINC01116: (1) 5′-GC AGTGTATTAGAAGACAA-3′, (2) 5′-GACCGAGTCTC AACTATAT-3′, (3) 5′-GGTAACATCAGAATGGCAA-3′, and (4) 5′-CCGCATAGTGTAACTTTAA-3′. H1299 and A549 cells were seeded in six-well plates at a density of 1×10^5/ml. Cells were transfected when the cell confluence reached 60%–80% according to the manufacturer's instructions of Lipofectamine 2000 (Invitrogen).

Real-time reverse-transcription polymerase chain reaction (RT-qPCR)

TRIzol reagent (Invitrogen) was used to extract the total RNA, and a reverse transcription kit (Takara) was used to reverse-transcribe the total RNA into complementary deoxyribose nucleic acid (cDNA). The relative expression levels of the genes were quantified via the $2 - \Delta\Delta Ct$ method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference. The sense and antisense primer sequences encoding LINC01116 and GAPDH

messenger (m)RNA were as follows: LINC01116 sense primer, 5′-CTCACTCTGCCATCACCCAG-3′; LINC01116 antisense primer, 5′-CACACCTGCAGTTCCAGCTA-3′; GAPDH sense primer, 5′-GATTTGGTCGTATTGGGCGC-3′; and GAPDH antisense primer, 5′-AGTGATGGC ATGGACTGT GG-3′.

Cell counting kit-8 (CCK-8) assay

A CCK-8 assay kit was used to evaluate the cellular proliferative ability according to the manufacturer's instructions. The transfected cells were seeded into a 96-well plate with 1.5×10^3 cells per well. In total, a 100 μl cell suspension was incubated into each well. To identify the rate of cellular proliferation, the cells in each well were incubated with 10 μl of CCK-8 reagent (Dojindo Laboratory), following which the plates were incubated in 5% CO_2 at 37°C for another 1.5 h. A microplate reader (Bio-Rad Laboratories) was used to record the absorbance in each well at 450 nm. Cell viability curves were plotted based on the absorbance at 24, 48, 72, and 96 h after incubation.

Colony formation assay

A total of 500 cells were seeded in a 60 mm culture dish and grown until visible colonies were formed (approximately two weeks). The cell colonies were fixed with 4% paraformaldehyde for 30 min and then stained with crystal violet for 30 min. Subsequently, the colonies were washed, air-dried, photographed, and counted. Each colony that contained more than 50 cells was counted as a clone.

Flow cytometry for measuring cell apoptosis

FITC Annexin V Apoptosis Detection Kit I (BD Biosciences) was used to perform the apoptosis analysis. The transfected cells were resuspended in 1× binding buffer at a concentration of 2×10^6 cells/ml. A total volume of 100 μl of the solution was transferred to a 5 ml culture tube. Subsequently, 5 μl of propidium iodide (PI) and 5 μl of FITC Annexin V were added. After incubation in darkness at room temperature for 5 min, 400 μl of 1 × binding buffer was added to each tube. The cells were divided into viable cells, dead cells, early apoptotic cells, and apoptotic cells. The FACS Calibur Flow Cytometer (BD Biosciences) was used to analyze the stained cells.

Migration and invasion assay

The cells that were suspended in serum-free medium were placed into the upper transwell chamber (8 μm membrane pore size; BD Biosciences) of a 24-well plate at a density of 5×10^4 cells in each well. For the invasion assays, 1×10^4 cells in the serum-free medium were seeded into the upper chamber of an insert coated with Matrigel (Sigma-Aldrich). The basolateral chamber was imbued with 600 μl of culture media containing 10% FBS as a chemoattractant. After incubation for 24 h, we removed any cells remaining on the upper membrane with cotton wool. Cells that had invaded or migrated through the membrane were fixed with 4% paraformaldehyde for 15 min and then stained with 0.1% crystal violet for 5 min. The CKX53 inverted microscope (Olympus) was used to photograph and count the cells.

Wound healing assay

For the wound healing assay, the cells were incubated until the fusion rate reached 100% in the six-well plate. The cells were scratched with a sterilized 200 μl pipette tip in the wells. The cells were cultured with serum-free DMEM medium after they were washed three times with phosphate buffered saline (PBS). Wound closure was observed and photographed at 0 and 24 h after incubation. Image J software was used to measure the wound healing area.

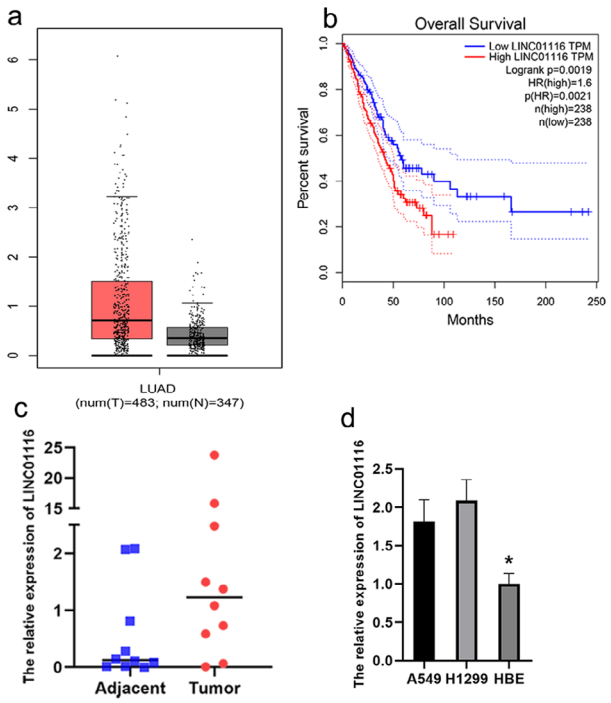

FIGURE 1 LINC01116 is highly expressed in LUAD and associated with a poor prognosis. (a) Data from the GEPIA database showed that LINC01116 expression was significantly increased in 483 LUAD tissues (red box) compared with 347 normal adjacent tissues (black box). (b) Data from the GEPIA database showed that the mean OS time of LUAD patients with a high expression level of LINC01116 was significantly lower compared with patients with a low expression level. (c, d) LINC01116 was highly expressed in LUAD tissue samples or cells compared with matched normal tissues or HBE cell line. Data are presented as the mean ± SD of three independent experiments. *$p < 0.05$. LUAD, lung adenocarcinoma; RT-qPCR, real-time reverse-transcription polymerase chain reaction; T, LUAD tumor tissue; N, adjacent normal lung tissue; OS, overall survival

RNA fluorescence in situ hybridization (RNA-FISH)

An RNA-FISH analysis was used to detect the subcellular localization of LINC01116 using a fluorescein (FAM)-labeled RNA probe (Service Biotechnology Co., Ltd.). RNA-FISH was carried out using the Ribo Fluorescent In Situ Hybridization Kit (Ribo) according to the manufacturer's instructions. A confocal fluorescence microscope (NIKON ECLIPSE TI-SR, Japan) was used to detect the hybridization signal.

Western blotting

The mammalian protein extraction reagent, RIPA (Beyotime), supplemented with a protease inhibitor cocktail (Roche) was used to lyse the cells. A protein assay kit (Bio-Rad) was used to measure the protein concentrations. A total of 20 μg of the protein extractions was separated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to 0.22 mm nitrocellulose membranes, and cultured with specific antibodies. Afterwards, primary and horseradish peroxidase (HRP)-labeled secondary antibodies (Affinity Biosciences) were used to incubate the membranes overnight at 4°C after washing with tris-buffered saline and tween 20 (TBST). The aforementioned antibodies were purchased from Affinity Biosciences. Signals were developed using an enhanced chemiluminescence (ECL) detection reagent (Thermo Fisher). The gray values of the protein bands were computed using the ImageJ software with normalization to GAPDH.

Tumor xenografts in nude mice

A549-shLINC01116 and A549-shNC (Oligobio) were used for the in vivo tumorigenicity experiment. A total of 20 specific pathogen-free (SPF) BALB/c female nude mice (4–6 weeks old, 18 to 22 g) were randomly assigned to the sh-LINC01116 or shNC group. A total of 1×10^7 A549 cells were subcutaneously injected into the right flank of the BALB/c nude mice. Subsequently, tumor length and width were measured every seven days. The nude mice were euthanized 30 days after injection. The tumor xenografts were resected and weighed. Tumor size was calculated using the formula (length × width × width)/2 and recorded in mm^3. The experiment was approved by the ethics committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University.

Statistical analysis

The measurement data from the three independent experiments were expressed as mean ± standard deviation (SD). Student's *t*-test or one-way analysis of variance (ANOVA) was used to compare the differences between the groups. The different expression levels of LINC01116 and their association with the overall survival (OS) of LUAD

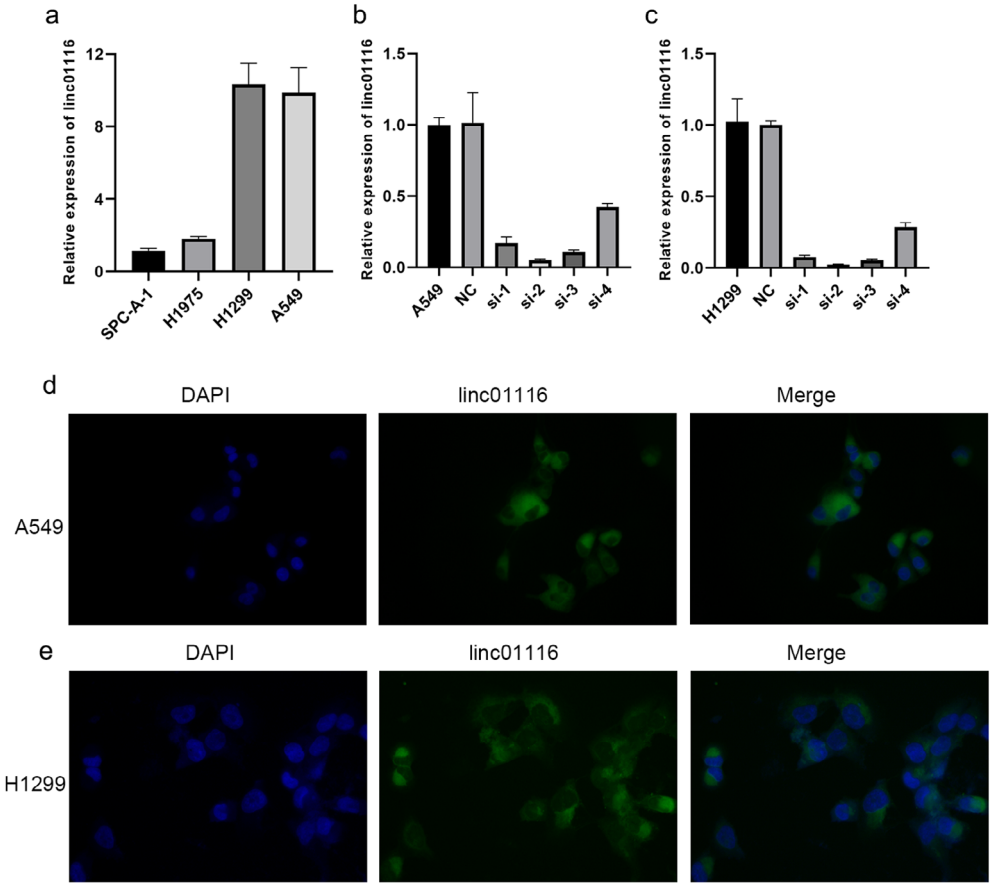

FIGURE 2 The expressions of LINC01116 in LUAD cell lines. (a) The expressions of LINC01116 in LUAD cell lines SPC-A-1, H1975, H1299, and A549 were detected by RT-qPCR. (b, c) The knockdown efficacy of si-LINC01116 was examined by RT-qPCR in LUAD cells H1299 and A549 undergoing treatment with si-LINC01116 or si-NC. (d, e) LINC01116 expression is located in cytoplasm of LUAD cells, as revealed by FISH assay. Data are presented as the mean ± SD of three independent experiments. NC, normal control; LUAD, lung adenocarcinoma; RT-qPCR, real-time reverse-transcription polymerase chain reaction

patients were presented as Kaplan–Meier plots. A log-rank test was performed to identify the differences among the OS curves. The statistical analyses were performed using SPSS (version 22.0, SPSS Inc.) and GraphPad Prism 8 statistical software. Differences were considered statistically significant when the p-value was <0.05.

RESULTS

LINC01116 is highly expressed in LUAD and high LINC01116 expression levels indicates an unfavorable prognosis

First, based on data from the Gene Expression Profiling Interactive Analysis (GEPIA) database, we uncovered that LINC01116 is upregulated in LUAD, indicating that LINC01116 plays an important role in LUAD development (Figure 1(a)). Therefore, we analyzed the prognostic value of LINC01116 in LUAD using patient data from the GEPIA database and found that high LINC01116 expression levels indicate an unfavorable prognosis in patients with LUAD ($p = 0.0019$) (Figure 1(b)). To explore the expression patterns of LINC01116 in LUAD, we analyzed LINC01116 expression in LUAD tissues and cell lines (HBE, SPC-A-1, H1975, H1299, and A549) by performing RT-qPCR. Consistent with the aforementioned findings, the RT-qPCR results revealed that LINC01116 was highly expressed in the ten LUAD tissue samples from our department and in the LUAD cell lines compared with in-matched normal tissue samples or in the HBE cell line (Figure 1(c), (d); $p < 0.05$).

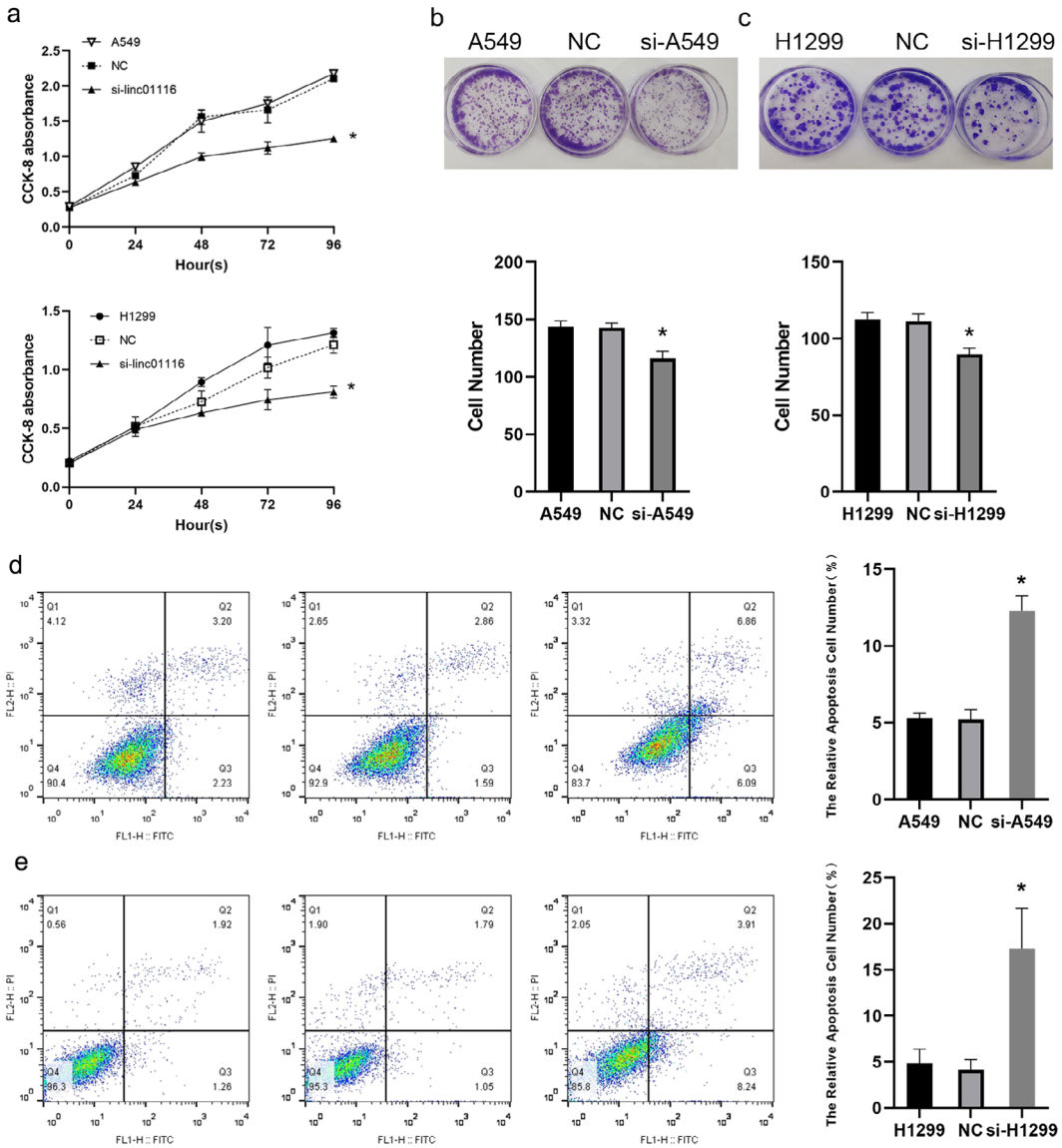

FIGURE 3 Silencing LINC01116 inhibits LUAD cell proliferation and induces cell apoptosis in vitro. (a–c) The proliferation of LUAD cells treated with si-LINC01116 was assessed by CCK-8 and colony formation assays. (d, e) The apoptosis of LINC01116-deficient H1299 and A549 cells was determined by flow cytometric assay. Data are presented as the mean ± SD of three independent experiments. *$p < 0.05$. NC, normal control; LUAD, lung adenocarcinoma

Silencing LINC01116 inhibits proliferation and induces apoptosis of LUAD cells in vitro

In vitro functional analyses were performed to examine whether the elevated expression of LINC01116 is related to the onset and occurrence of LUAD. We selected four LUAD cell lines and found that the expression levels of LINC01116 were higher in the H1299 and A549 cell lines (Figure 2(a)). Therefore, the H1299 and A549 cell lines were selected for further study. The FISH assay was used to detect the location of LINC01116 in LUAD cells, and we found that LINC01116 was mainly expressed in the cytoplasm (Figure 2(d), (e)). Subsequently, H1299 and A549 cells were treated with si-LINC01116 or siNC, and an RT-qPCR was performed to detect LINC01116 expression. As shown in Figure 2(b), (c), the expression of LINC01116 was significantly downregulated in the si-LINC01116-2 group compared with in the other groups. Therefore, si-LINC01116-2 was selected as the LINC01116 inhibitor for further study.

The results of the CCK-8 assay revealed that silencing LINC01116 reduced the proliferation of H1299 and A549 cells (Figure 3(a); $p < 0.01$); these results were further tested by performing the colony formation assay (Figure 3(b), (c); $p < 0.05$). To examine whether the effect of LINC01116 on the proliferation of LUAD cells is mediated via alterations in cell apoptosis, we conducted a flow cytometric assay to determine the level of cell apoptosis in LUAD cells. The results revealed that the knockdown of LINC01116 induced cell apoptosis (Figure 3(d), (e); $p < 0.01$). Therefore, these data indicate that LINC01116 promotes LUAD cell proliferation by facilitating DNA synthesis.

Silencing LINC01116 inhibits LUAD cell migration and invasion in vitro

Metastasis is a characteristic of malignant tumors and the leading cause of death among cancer patients.[13] Tumor metastasis begins with the invasion of tumor cells into the extracellular matrix and their migration toward the blood stream.[14] To determine the effect of LINC01116 on the ability of LUAD cells to migrate and invade, we performed wound healing assays and transwell assays. Compared with the control cells, the migration rate of the H1299 and A549 cells was significantly restrained in LINC01116 knockdown cells 24 h after they were scratched (Figure 4(a), (b); $p < 0.01$). The transwell assays revealed that the migratory ability of LINC01116-transduced cells was significantly lower compared with that of the control cells (Figure 5(a), (b); $p < 0.01$). Furthermore, the invasion potential of the invading cells was also reduced after the LINC01116 knockdown (Figure 5(c), (d); $p < 0.05$). These results indicate that silencing LINC01116 could significantly decrease the LUAD cell migration and invasion potential compared with that of the control cells.

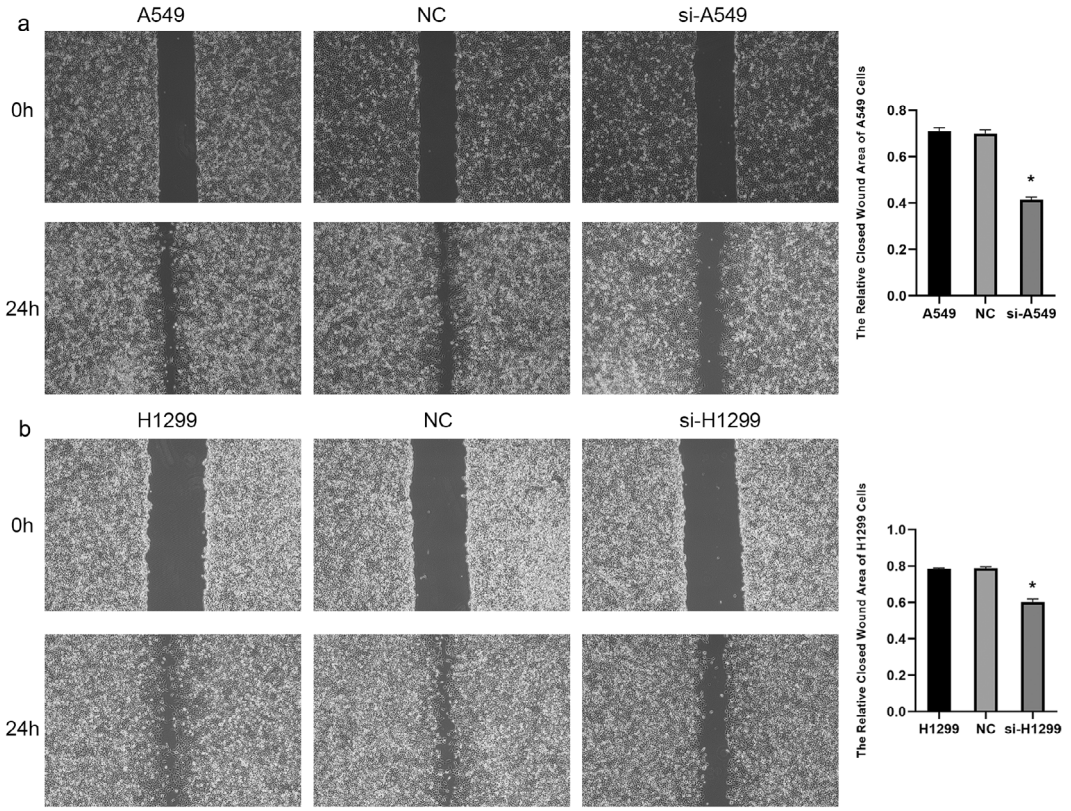

FIGURE 4 Influence of the LINC01116 knockdown on the migratory abilities of A549 (a) and H1299 (b) cells was assessed by wound healing assays. Data are presented as the mean ± SD of three independent experiments. *$p < 0.05$. NC, normal control

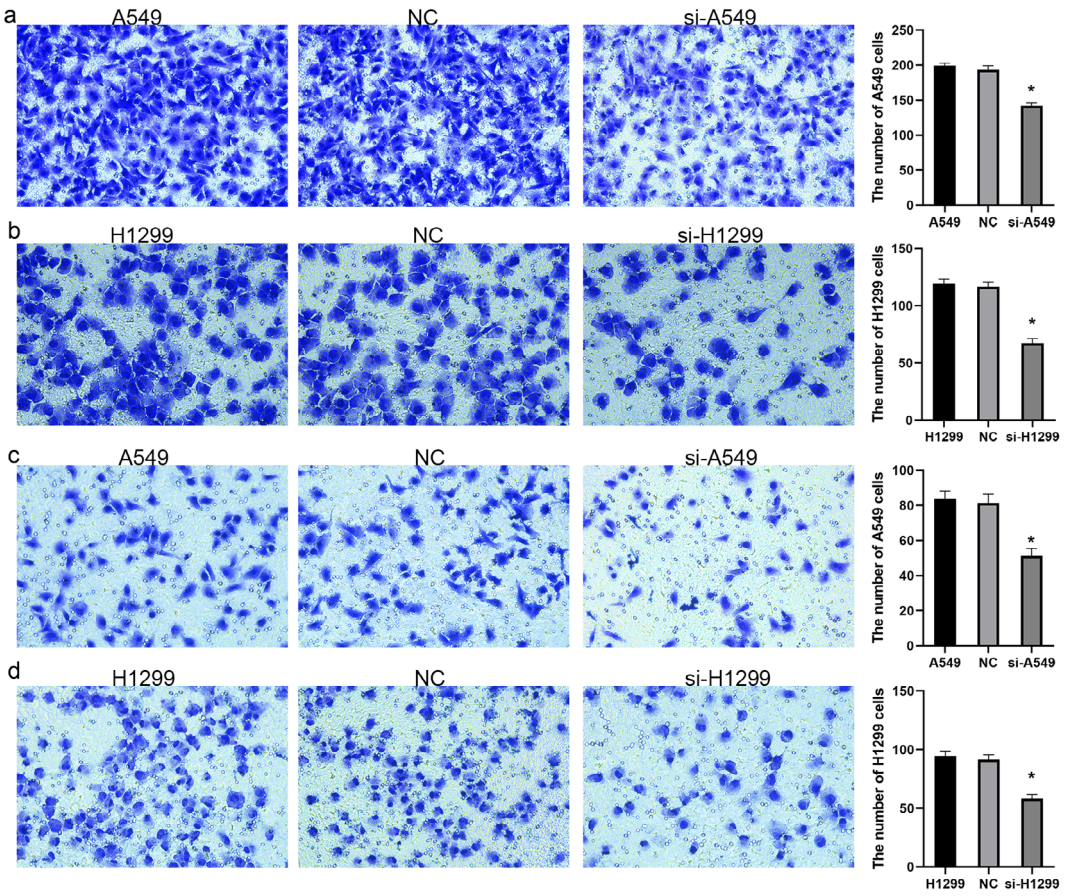

FIGURE 5 Influence of the LINC01116 knockdown on the migratory and invasive abilities of A549 (a, c) and H1299 (b, d) cells was assessed by transwell migration and invasion assays. *$p < 0.05$. NC, normal control

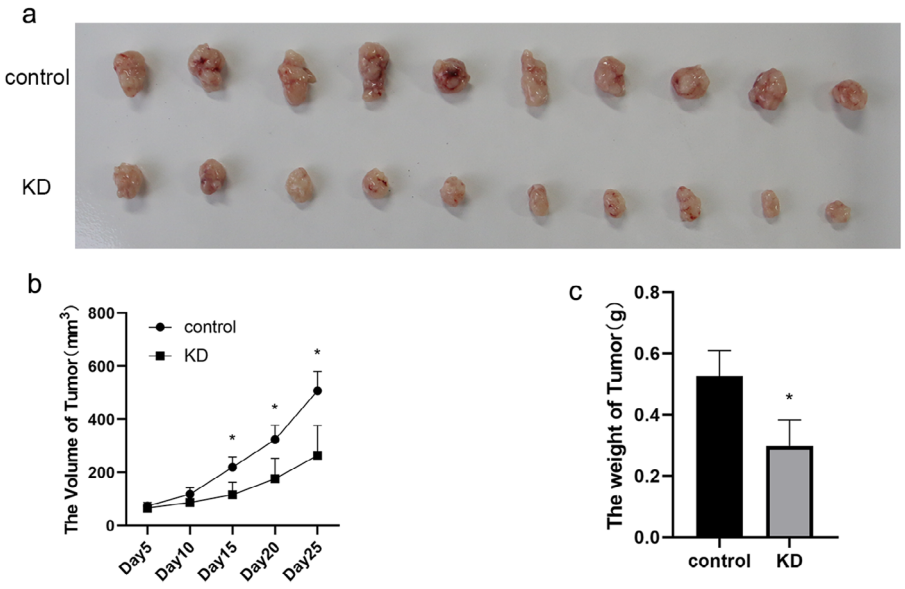

FIGURE 6 Silencing LINC01116 inhibits LUAD growth in vivo. (a) Heterotopic xenograft images. (b, c) Tumor volume and weight in the xenograft mice from the LINC01116 knockdown group and the control group. Data are presented as the mean ± SD of three independent experiments. *$p < 0.05$. NC, normal control; KD, knockdown, LUAD, lung adenocarcinoma

Silencing LINC01116 inhibits LUAD growth in vivo

In addition to the aforementioned procedures, we developed a xenograft nude mouse model to determine the tumorigenicity of LINC01116 in vivo. The resected tumors from nude mice were analyzed to compare the differences in tumor size and weight (Figure 6(a)). We observed that the sizes of the subcutaneous xenografts formed in nude mice by the A549 cells transfected with sh-LINC01116 and the

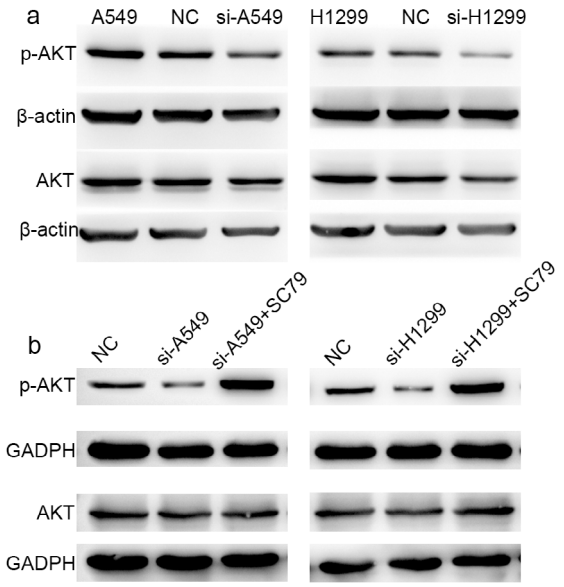

FIGURE 7 Western blot analyses of the protein level. (a) Western blot analyses of the protein level of AKT, pAKT and GAPDH in LUAD cells. (b) SC79 could activate the AKT signaling pathway. NC, normal control

control cells (sh-NC group) were 264.36 ± 112.52 mm^3 and 507.61 ± 73.7 mm^3, respectively (Figure 6(b)). The tumor size was significantly smaller in the sh-LINC01116 group than in the control group ($p < 0.01$). The weights of the xenografts of the subcutaneous tumors in the sh-LINC01116 and control groups were 0.298 ± 0.085 g and 0.526 ± 0.084 g, respectively (Figure 6(c)). The xenograft weight was significantly lower in the sh-LINC01116 group than in the control group ($p < 0.001$). These results suggest that the knockdown of LINC01116 suppresses LUAD tumor growth in a xenograft mouse model.

LINC01116-induced LUAD cell proliferation and invasion via the AKT signaling pathway

The phosphoinositide-3-kinase (PI3K)/AKT signaling pathway is at the core of cell signaling. Activated AKT regulates the function of numerous substrates that are involved in the modulation of cell survival, cell cycle progression, and cellular growth.[15] We speculated that LINC01116 regulates LUAD progression via the AKT signaling pathway. To test our hypothesis, we determined the protein expression levels of the key molecules in the AKT signaling pathway. As shown in Figure 7(a), the knockdown of LINC01116 significantly decreased the p-AKT protein level, while the change in the AKT protein level was not obvious.

We used a specific stimulator, SC79 (Abcam), which activates the AKT signaling pathway (Figure 7(b)), to examine the LINC01116-induced modulation of AKT activity in LUAD cell lines. Rescue assays were conducted to determine whether LINC01116 modulates the development of LUAD via the AKT signaling pathway. As shown in Figure 8(a)–(c), the viability of LINC01116-depleted H1299 and A549 cells was significantly enhanced ($p < 0.01$), and the apoptosis was inhibited ($p < 0.05$) after treatment with SC79. Furthermore, SC79 treatment enhanced the migration and invasion ability in both the LINC01116-transduced cell lines (Figure 8(d), (e); $p < 0.05$). Above all, these data suggest that silencing LINC01116 inhibits the development of LUAD via the AKT signaling pathway.

DISCUSSION

Despite advances in surgical techniques[16] and comprehensive therapy,[17,18] the survival rates of patients with LUAD are still low. The vast majority of these patients are initially asymptomatic, and the disease is typically discovered at advanced stages.[19] An estimated 733 000 new lung cancer cases and 610 000 lung cancer deaths occurred in China in 2015.[20] Therefore, there is an urgent need to identify a novel diagnostic tumor marker as well as a potential therapeutic target in LUAD. Increasing evidence has proved that lncRNAs play an important role in the carcinogenesis of malignant tumors, as they regulate the genes involved in cell proliferation, apoptosis, and metastasis.[21,22] Although several lncRNAs have been identified in LUAD,[23–25] the underlying tumorigenicity of LUAD remains unknown.

LINC01116, also known as tumor-associated long noncoding RNA expressed on chromosome 2 (TALNEC2), is a newly recognized lncRNA that is located in the 2q31.1 region. Previous studies have revealed that LINC01116 is a causative gene in several cancers.[9,12,26–28] However, its role in LUAD is still unknown. Consistent with the results of former studies, in this study, we proved that LINC01116 expression significantly increased in LUAD tissues and cell lines. Silencing LINC01116 suppresses LUAD cell proliferation, migration, and invasion and induces cell apoptosis. Moreover, our in vivo assay demonstrated that silencing LINC01116 surely inhibits the carcinogenesis of LUAD cells in a nude mouse xenograft model. These data indicate that LINC01116 might serve as a novel diagnostic tumor marker as well as a potential therapeutic target for LUAD treatment.

The PI3K/AKT pathway is one of the most important intracellular signaling pathways, which modulates cell proliferation, differentiation, cellular metabolism, and survival.[29] AKT is one of the major proteins involved in this signaling pathway. The phosphorylation of PI3K and AKT is essential for pathway activation. Once phosphorylated, PI3K activates and phosphorylates downstream AKT in order to induce a cascade reaction.[30] Previous studies have shown that AKT signaling pathways are dysregulated in LUAD. For instance, neuregulin 1 (NRG1) might be an inhibitor in the progression of LUAD, and its function is related to the AKT and extracellular signal-regulated protein kinase (ERK)1/2 pathways.[31] Flotillin-1 (FLOT1) facilitates LUAD cell proliferation, invasion, and migration and inhibits cell apoptosis. In addition, FLOT1 induces epithelial–mesenchymal transition (EMT) and regulates the LUAD cell cycle via activating

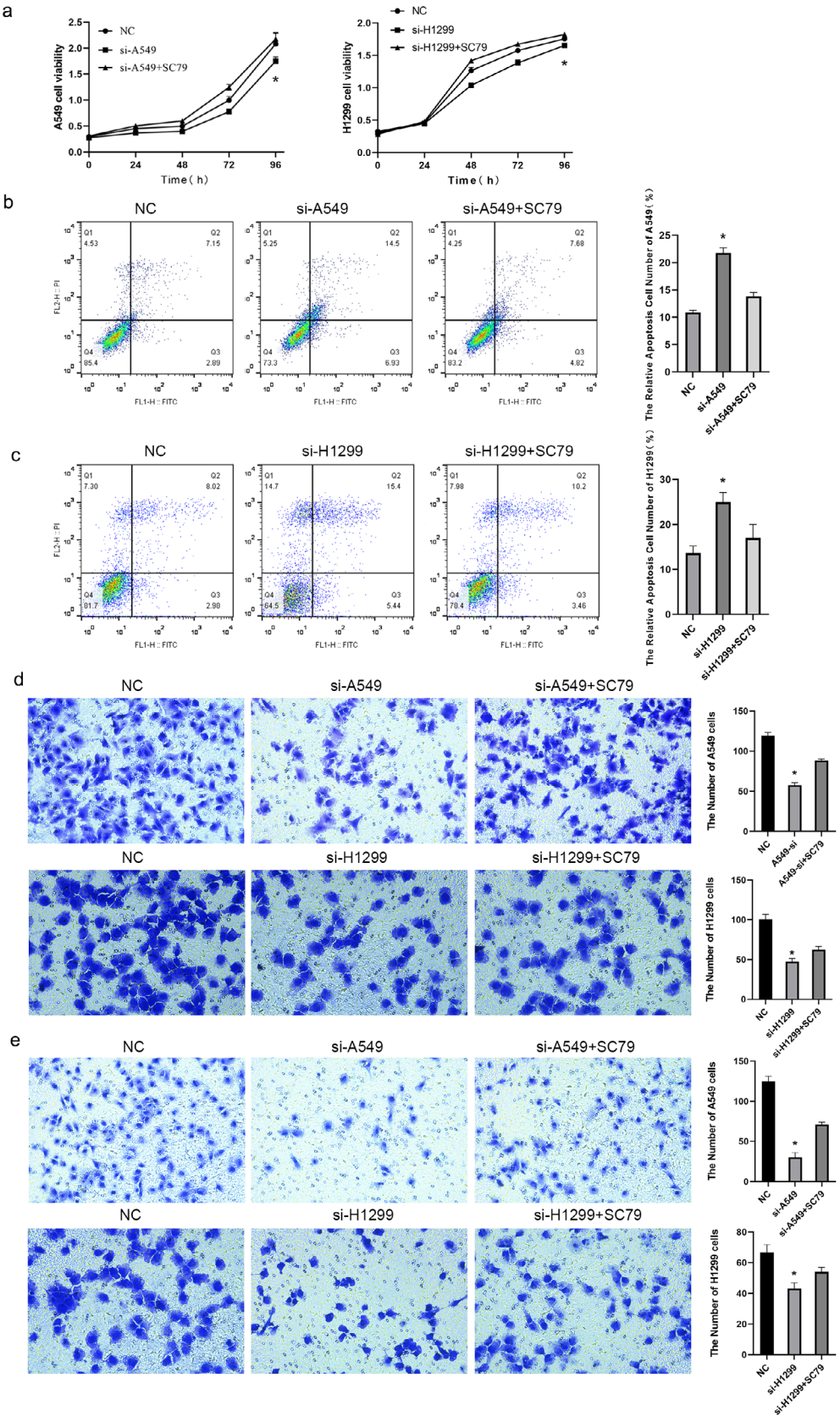

FIGURE 8 LINC01116 induced LUAD cell proliferation and invasion by regulating the AKT signaling pathway. (a) Cell proliferation of si-NC, si-LINC01116 and si-LINC01116 + SC79 in H1299 and A549 cells was evaluated through CCK-8 assays. (b, c) Apoptosis in each group was determined by flow cytometric assays. (d, e) The migratory and invasive abilities in each group were assessed by transwell assays. SC79, a specific AKT signaling pathway stimulator. Data are presented as the mean ± SD of three independent experiments. *$p < 0.05$. NC, normal control

ERK/AKT cascades.[32] Agmatinase promotes LUAD tumorigenesis by activating the mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling pathways.[33] In the current study, we found that the protein level of p-AKT was reduced after LINC01116 silencing, while the total protein level remained unchanged, indicating that LINC01116

positively regulates the AKT signaling pathway. Furthermore, we performed rescue assays using a highly specific AKT pathway activator, SC79, to determine whether LINC01116 regulates LUAD development via the AKT signaling pathway. Sc79 treatment significantly enhanced the proliferation and inhibited the apoptosis of LINC01116-depleted H1299 and A549 cells. Furthermore, the migration and invasion abilities were enhanced in both the LINC01116-depleted cell lines. These findings indicate that silencing LINC01116 suppresses the development of LUAD via the AKT signaling pathway.

In conclusion, our study revealed that LINC01116 is upregulated in LUAD tissues and cell lines. To our knowledge, this is the first report to indicate that silencing LINC01116 suppresses LUAD cell proliferation, migration, and invasion and induces cell apoptosis. Our findings also suggest that LINC01116 contributes to the malignant behavior of LUAD cells at least partly through activating the AKT signaling pathway; this is a meaningful insight that could aid in exploring LINC01116 as a novel diagnostic tumor marker as well as a potential therapeutic target for LUAD treatment.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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