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ORIGINAL RESEARCH

RETRACTED ARTICLE: LncRNA LINC01116 Contributes to Cisplatin Resistance in Lung Adenocarcinoma

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Background: Long non-coding RNAs (lncRNAs) have been found to contribute to cisplatin resistance in several cancers; however, the role of lncRNA LINC01116 in cisplatin resistance remains unknown in non-small-cell lung cancer. This study aimed to examine the contribution of LINC01116 to cisplatin resistance in lung adenocarcinoma (LAD).

Materials and Methods: Cisplatin-resistant A549/DDP cells were generated by treatment with cisplatin by dose escalation. LINC01116 expression was compared between A549 and A549/DDP cells, and between cisplatin-resistant and non-resistant LAD specimens. The cell viability, colony formation, proliferation, migration and invasion were measured using MTT and Transwell assays, and cell apoptosis and cell cycle were detected using flow cytometry. The expression of E-cadherin and Vimentin was quantified. LAD xenografts were modeled in nude mice to investigate the role of LINC01116 on the resistance of LAD to cisplatin.

Results: MTT assay measured the IC_{50} values of 13.49 ± 1.62 and 3.52 ± 1.33 μg/mL for A549/DDP and A549 cells, respectively. LINC01116 was overexpressed in cisplatin-resistant LAD specimens and A549/DDP cells ($P < 0.05$). Knockdown of LINC01116 inhibited cell viability, proliferation, migration and invasion, promoted apoptosis and enhanced the sensitivity to cisplatin in A549/DDP cells, while LINC01116 overexpression promoted cell viability, proliferation, migration and invasion, inhibited apoptosis and reduced the sensitivity to cisplatin in A549 cells. LINC01116 knockdown resulted in a 2.1-fold increase in E-cadherin expression and a 56% reduction in Vimentin expression in A549/DDP cells, and LINC01116 overexpression resulted in a 45% reduction in E-cadherin expression and a 1.82-fold increase in Vimentin expression in A549 cells.

Conclusion: Dysregulation of lncRNA LINC01116 expression results in resistance of LAD to cisplatin via the EMT process. Our findings support the oncogenic role of LINC01116 to promote the development of cisplatin resistance in LAD, and LINC01116 may be a novel predictor of poor response to cisplatin.

Keywords: lung adenocarcinoma, cisplatin, chemotherapy resistance, LINC01116, epithelial–mesenchymal transition

Introduction

Lung cancer is the leading cause of cancer morbidity and mortality globally.[1] It was estimated that 2.09 million new cases were diagnosed with lung cancer and 1.76 million people died from lung cancer in the world in 2018.[2] Non-small-cell lung cancer (NSCLC) accounts 80% to 85% of all lung cancers, and lung adenocarcinoma (LAD), the predominantly histological subtype of NSCLC, accounts for approximately 40% of all lung cancers.[3]

Although great progresses have been achieved in the diagnosis and therapy of NSCLC,[4] the prognosis of this malignancy remains unsatisfactory due to high

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recurrence and metastasis, and the five-year survival rate is below 15%, since most cases are diagnosed at an advanced or metastasized stage.[5] Currently, platinum-based combination chemotherapy like cisplatin and carboplatin, is the standard treatment for NSCLC, which has been proved to effectively prolong the overall survival (OS).[6] However, the chemotherapy efficacy is greatly limited by the drug resistance, and a majority of the patients may experience disease progression, resulting in chemotherapy failure.[7] Despite multiple attempts to illustrate the molecular mechanisms underlying cisplatin resistance, the exact mechanisms of cisplatin resistance have not been fully demonstrated in NSCLC until now.[8–10] A better understanding of the molecular mechanisms involved in the development of resistance to cisplatin is of great significance to overcome drug resistance and improve the clinical outcomes in patients with LAD.

Long non-coding RNA (lncRNA), a type of transcripts with greater than 200 nucleotides in length, has shown an important role in carcinogenesis and has been linked with cancer progression.[11–13] In addition, lncRNAs are found to be involved in chemotherapy resistance in multiple human cancers, including LAD.[14,15] LncRNA LINC01116, a novel lncRNA located on chromosome 2q31.1 with 838 nt in length, is reported to contribute to cancer progression. siRNA-induced LINC01116 knockdown was found to decrease prostate cancer PC-3 cell proliferation, and disruption of the LINC01116 gene using a CRISPR/CAS9 system resulted in a four-fold decrease in the ability of PC-3 cells, indicating the oncogenic role of LINC01116 in PC-3 cells.[16] In addition, LINC01116 silencing was found to suppress the development of oral squamous cell carcinoma and inhibit the cell migration and invasion in head and neck squamous cell carcinoma.[17,18] Previous studies have also demonstrated that LINC01116 promotes the progression of nasopharyngeal carcinoma, gastric cancer, osteosarcoma, glioma and epithelial ovarian cancer.[19–23] Furthermore, a recent study showed LINC01116 contributed to gefitinib resistance in NSCLC through affecting *IFI44* expression.[24] However, the involvement of LINC01116 in chemoresistance of LAD remains unknown until now.

In this study, we generated a cisplatin-resistant A549/DDP cell line, and detected LINC01116 overexpression in cisplatin-resistant LAD specimens and A549/DDP cells, and siRNA-induced LINC01116 knockdown was found to inhibit LAD cell viability, proliferation, migration and invasion, promote apoptosis and enhanc the sensitivity to cisplatin in A549/DDP cells, while LINC01116 overexpression promoted cell viability, proliferation, migration and invasion, inhibited apoptosis and reduced the sensitivity to cisplatin in A549 cells. We found LINC01116 knockdown resulted in elevated E-cadherin expression and reduced Vimentin expression in A549/DDP cells, and LINC01116 overexpression resulted in reduced E-cadherin expression and elevated Vimentin expression in A549 cells. Our data support the oncogenic role of LINC01116 to promote the development of cisplatin resistance in LAD, and suggest that LINC01116 may be a novel marker of poor response to cisplatin.

Materials and Methods

Cell Lines and Culture

The parental human lung adenocarcinoma epithelial A549 cell line was purchased from the cancer institute, Chinese Academy of Sciences. The cisplatin-resistant A549/DDP cells were generated by treatment with cisplatin by dose escalation from 0 to 1.0 μg/mL. Both types of cell lines were cultured in RPMI 1640 medium (GIBCO-BRL; Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin under an air atmosphere containing 5% CO_2 at 37°C. Exponential-phase cells were harvested and used for the subsequent experiments.

Tissue Samples

We obtained 42 paired LAD tissues and cisplatin-resistant tissues from patients undergoing surgery and aspiration biopsy at the First and Second Affiliated Hospital of Nanjing Medical University (Nanjing, China) during the period between 2013 and 2016. In this study, patients with complete or partial response following treatment with platinum-based chemotherapy were defined cisplatin sensitive, while those with stable disease or disease progression following platinum-based chemotherapy were considered cisplatin resistant. The patients were diagnosed with LAD (stages I, II, and III) based on the histopathological evaluation. All collected tissue samples were immediately snap-frozen in liquid nitrogen and stored at −80° C until RNA extraction.

Cell Transfection

A549/DDP cells were seeded onto six-well plates for 24 h, transfected with siRNAs (si-NC, si-LINC01116 1# and 2#) using Lipofectamine 2000 (Invitrogen; Carlsbad, CA, USA) and then incubated for 48 h. The LINC01116 sequence was synthesized and subcloned into the pcDNA3.1 vector (Invitrogen; Shanghai, China) to generate the pcDNA-LINC01116 vector for overexpression in cells. Plasmid vectors

(pcDNA3.1-LINC01116 and empty vector) were transfected into A549 cells by Lipofectamine 2000 according to the manufacturer's instructions.

MTT Assay

The half-maximal inhibitory concentration (IC_{50}) was measured using an MTT assay. Briefly, the transfected cells were seeded onto 96-well plates at a density of 3.0 × 10^3 cells/well and harvested in standard medium overnight. Cells were treated with a graded series of cisplatin (0, 0.5, 1, 5, 10, 15, 20, 25, 30 and 35 μg/mL) of. Following incubation for 48 h, MTT solutions (0.5 mg/mL; Sigma-Aldrich; St. Louis, MO, USA) were transferred and incubated for further 4 h. The medium was then substituted with 150 μL dimethyl sulfoxide (Sigma-Aldrich; St. Louis, MO, USA) and vortexed for 10 min. The absorbance of each well was measured at 490 nm. In addition, the cell viability was evaluated at 0, 24, 48, 72 and 96 h using 0.5 mg/mL MTT solution without cisplatin treatment. Each assay was repeated at least in triplicate.

Colony Formation Assay and Cell Migration and Invasion Assays

For colony formation assay, transfected cells were placed in each well of 6-well plates at a density of 0.5 [illegible] cells/well and maintained in RIPA1640 medium containing 10% FBS for approximately two weeks, replacing the medium once every four days. After two weeks, cells were fixed with methanol and stained with 0.1% crystal violet (Sigma-Aldrich; St. Louis, MO, USA) and then visible colonies were counted.

To assess cell migration and invasion, Transwell cell migration and invasion assays were performed, and migration chambers with 8.0 μm pore size (Millipore; Bedford, MA, USA) were placed into a 24-well plate were used in assays. For the migration assay, 4×10^4 transfected cells were transferred to 300 μL of RIPA 1640 medium supplemented with 1% FBS in the upper chamber. For the invasion assay, 1×10^5 transfected cells in 300 μL of RIPA 1640 medium supplemented with 1% FBS were transferred into the upper chamber, with an insert covered with Matrigel (Corning; Corning, OH, USA). RIPA 1640 medium supplemented with 10% FBS was added to the lower chamber. After incubation for 24 h, cells migrated or invaded through the membrane were stained with methanol and 0.1% crystal violet, imaged, and counted by an IX71 inverted microscope (Olympus; Tokyo, Japan). All assays were independently repeated in triplicate.

Flow Cytometry

A549/DDP and A549 cells transfected with siRNA-LINC01116 and pcDNA-LINC01116 were harvested for 48 h. For apoptosis assays, transfected cells were double-stained with Annexin V-FITC and propidium iodide (PI) and analyzed on a FACScan flow cytometer (BD Biosciences; San Diego, CA, USA) equipped with the CellQuest software (BD Biosciences; Franklin Lakes, NJ, USA) following the manufacturer's protocol. Cells were classified into viable, dead, early apoptotic and apoptotic, and then the relative percentages of early apoptotic cells were counted and compared with that of the control transfectant for each assay or compared between cells receiving different treatment. For the cell cycle analysis, cells were single-stained with PI with the BD Cycletest plus DNA Reagent Kit (BD Biosciences; Franklin Lakes, NJ, USA) and analyzed on a FACScan flow cytometer. Cells at the G0/G1, S and G2/M phase were counted and compared with controls. Each experiment was repeated in triplicate.

qPCR Assay

Total RNA was extracted from tissues or transfected cells with the TRIZOL reagent (Invitrogen; Carlsbad, CA, USA) following the manufacturer's instructions, and reversely transcribed into cDNA in a final volume of 20 μL using random primers under standard conditions with the PrimeScript RT Reagent Kit (TaKaRa; Dalian, China). To analyze LINC01116 expression, qPCR assay was performed on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems; Foster City, CA, USA) with the SYBR Premix Ex Taq (TaKaRa) following the manufacturer's instructions. The LINC01116 expression was quantified using qPCR assay with the following specific primers: forward, 5′-GCTTTGCTGAAGACGAGCAG-3′; and reverse, 5′-GGGTGATGGCAGAGTGAGAC-3′. All qPCR assays were performed in triplicate, and the expression level was analyzed using the $2^{-\Delta\Delta CT}$ method. High LINC01116 expression was defined as threefold or higher changes between cisplatin non-resistant and resistant cancer specimens, while low expression was defined as less than threefold changes between cisplatin non-resistant and resistant cancer specimens.

Western Blotting Assay

Transfected cells were lysed with RIPA extraction reagent (Solarbio; Beijing, China) supplemented with a protease inhibitor cocktail (Solarbio; Beijing, China) and phenylmethylsulfonyl fluoride (Solarbio, Beijing, China). Total protein was

separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the immunoblots were transferred to the PVDF membranes with 0.22 μm in pore size (Millipore; Billerica, MA, USA). Then, the immunoblots were incubated with specific antibodies against E-cadherin (GeneTex; Irvine, CA, USA) and Vimentin (GeneTex; Irvine, CA, USA), while GAPDH (CMCTAG, Inc.; Shanghai, China) served as a loading control. ECL chemiluminescence substrate (Millipore; Billerica, MA, USA) was used to quantify the protein expression by densitometry with the Quantity One software (Bio-Rad Laboratories, Inc.; Hercules, CA, USA).

In vivo Chemosensitivity Assay

Four-week-old male athymic BALB/c nude mice that were purchased from Model Animal Research Center of Nanjing University (Nanjing, China) and maintained under specific pathogen-free conditions were used for the in vivo chemosensitivity assay. A549/DDP cells were transiently transfected with si-LINC01116 1# or si-NC, incubated in 6-well culture plates for 48 h, washed with PBS, and resuspended at a concentration of 2.0×10^7 cells/mL. Resuspended cells (0.1 mL) were subcutaneously injected into a single side of the groin region of each mouse. Tumor growth was examined once every three days, and tumor volume was calculated using the following formula: $V = 0.5 \times D \times d^2$, while V indicates tumor volume, D indicates the longitudinal diameter of the tumor and d indicates the transverse diameter. If the mean tumor volume reached approximately 50 mm^3, cisplatin was administered by intraperitoneal injection at a dose of 3 mg/kg, once every three days, for a total of three doses. Mice were sacrificed 4 weeks post-injection, and the primary tumors were excised, paraffin-embedded, formalin fixed, and subjected to H&E staining and immunostaining analysis for Ki-67, E-cadherin and Vimentin protein expression. Hereby, the E-cadherin and Vimentin expression was qualitatively assessed using immunostaining analysis. Positive E-cadherin expression was defined as presence of E-cadherin staining and positive Vimentin expression was defined as presence of Vimentin staining.

Ethics Statement

This study was approved by the Ethics Review Committee of the Second Affiliated Hospital of Nanjing Medical University prior to the commencement of the study (approval no.: [2014]KY-011). All experiments were performed in accordance with the Declaration of Helsinki and National Regulations for the Management of Laboratory Animals released by the central government of China on October 31, 2017. Written informed consent was obtained from all participants involved in the study, following a detailed description of the purpose of the study.

Statistical Analysis

All measurement data are expressed as mean ± standard error (SE) and processed using the software GraphPad Prism version 5.0. Data were tested for statistical significance with Student's *t*-test, one-way analysis of variance (ANOVA) and Mann–Whitney *U*-test. All statistical analyses were performed using the statistical software SPSS version 20.0 (SPSS, Inc.; Chicago, IL, USA), and a P value of < 0.05 were considered statistically significant.

Results

LINC01116 Expression is Upregulated in Cisplatin-Resistant Human LAD Specimens and A549/DDP Cells

The A549/DDP cells appeared large swelling or spindle-shaped cells (Figure 1A). To verify the contribution of LINC01116 to the acquired cisplatin resistance, the relative LINC01116 expression was quantified in LAD specimens and A549 cells. qPCR assay showed significantly greater LINC01116 expression in cisplatin-resistant LAD specimens than in non-resistant specimens (Figure 1B), and higher LINC01116 expression was detected in A549/DDP cells than in A549 cells (Figure 1C). MTT assay measured 13.49 ± 1.62 and 3.52 ± 1.33 μg/mL IC_{50} values of cisplatin against A549/DDP and A549 cells ($P = 0.0089$) (Figure 1D), indicating that A549/DDP cells were 3.84 times more resistant to cisplatin than the parental A549 cells. These data suggest that high LINC01116 expression may correlate with cisplatin resistance in LAD.

Knockdown of LINC01116 Suppresses Proliferation and Inhibits Migration and Invasion of A549/DDP Cells

qPCR assay showed lower LINC01116 expression in A549/DDP cells transfected with si-LINC01116 1# or 2# than in those transfected with si-NC 48 h post-transfection (Figure 2A), and MTT assay revealed an approximately 43.3% reduction in the IC_{50} value of cisplatin against si-LINC01116 1# or 2# transfected

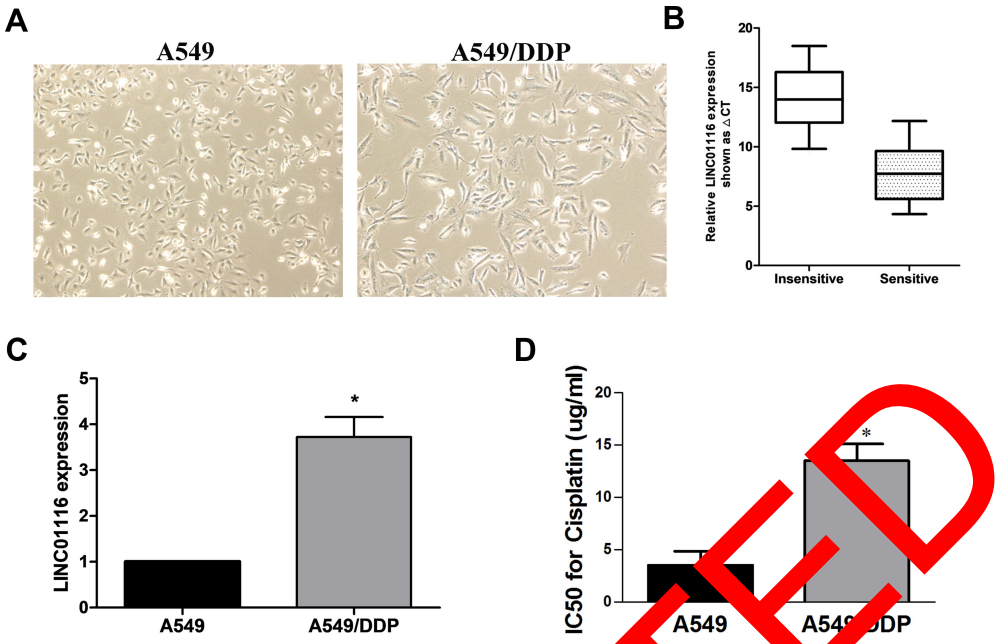

Figure 1 LINC01116 expression is significantly up-regulated in cisplatin-resistant A549/DDP cells as compared that in parental A549 cells. (**A**) Morphologies of A549 and A549/DDP cells (× 20). (**B**) qPCR assay detected higher LINC01116 expression in cisplatin-resistant LAD specimens than in non-resistant specimens. (**C**) qPCR assay detected higher LINC01116 expression in A549/DDP cells than in A549 cells. (**D**) The IC_{50} of cisplatin against A549/DDP cells is significantly greater than that against A549 cells. All experiments are repeated in triplicated, and data are shown as mean ± S. *$P < 0.05$, **$P < 0.01$.

A549/DDP cells relative to si-NC transfected cells (Figure 2B). In addition, MTT and colony formation assays showed that siRNA-induced knockdown of LINC01116 expression inhibited A549/DDP cell proliferation (Figure 2C and D), and Transwell migration and invasion assays showed knockdown of LINC01116 expression resulted in a reduction in A549/DDP cell migration and invasion (Figure 2E). Taken these findings together, LINC01116 down-regulation is found to suppress the proliferation and inhibit the migration and invasion of A549/DDP cells.

Knockdown of LINC01116 Promotes Apoptosis and Induces G1 Phase Arrest of A549/DDP Cells

Flow cytometry detected a significant rise in the apoptotic rate of A549/DDP cells transfected with si-LINC01116 1# or 2# relative to those transfected with si-NC (Figure 3A) and a more percentage of si-LINC01116 1# or 2# transfected cells at the G0/G1 phase and a lower proportion of si-LINC01116 1# or 2# transfected cells at the S phase as compared with si-NC transfected cells (Figure 3B). These data demonstrate that knockdown of LINC01116 promote the apoptosis and induces G1 cell cycle arrest in A549/DDP cells.

Knockdown of LINC01116 Increases the Sensitivity of A549/DDP Cells to Cisplatin

To examine the effect of LINC01116 knockdown on the sensitivity of A549/DDP cells to cisplatin, A549/DDP cells were transfected with si-NC or si-LINC01116 1# and exposed to cisplatin at concentrations of 0.0, 1.0 and 2.0 μg/mL, respectively. Flow cytometry revealed higher apoptotic rates of LINC01116 1# transfected A549/DDP cells than those of si-NC transfected cells regardless of cisplatin treatment (Figure 4A), and the percentage of si-LINC01116 1# transfected A549/DDP cells gradually increased at G0/G1 phase and gradually decreased at S phase with the rise in the cisplatin concentration (Figure 4B). These data demonstrate that silencing LINC01116 increase the sensitivity to cisplatin in A549/DDP cells through inducing apoptosis and promoting cell cycle arrest at G0/G1 phase.

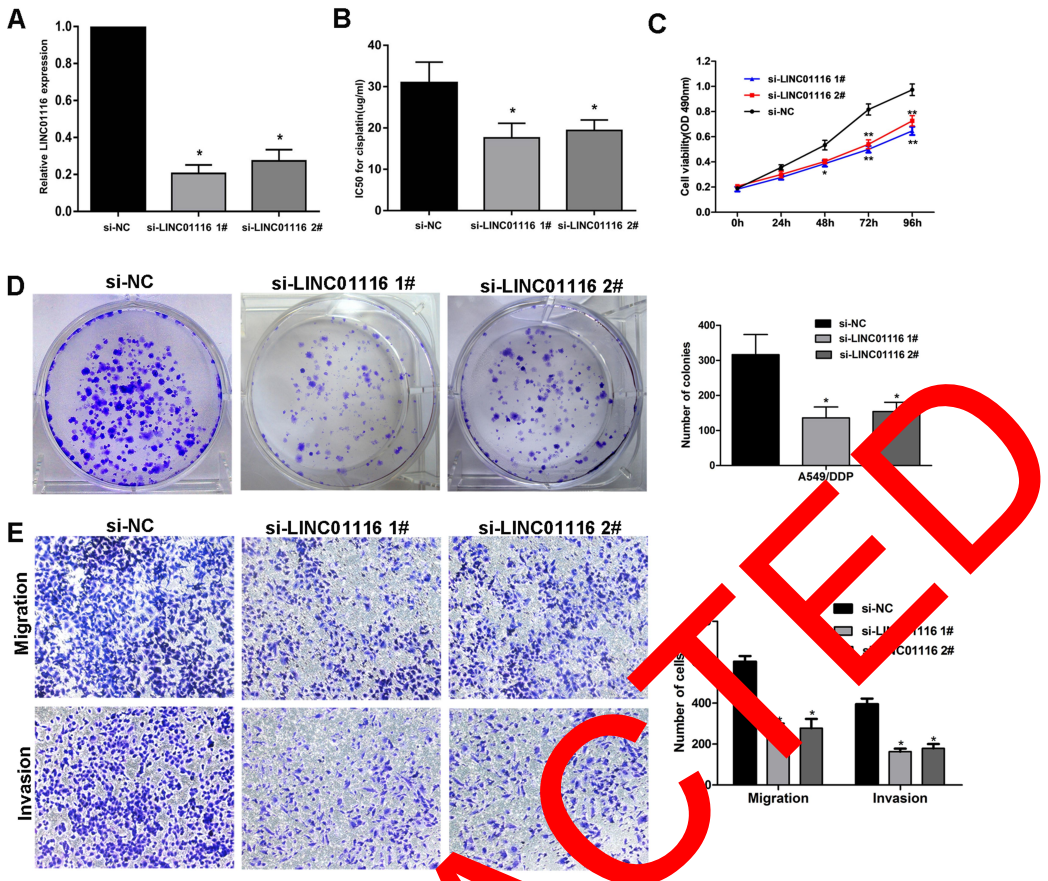

Figure 2 siRNA-induced knockdown of LINC01116 suppresses A549/DDP cell proliferation and invasion. A549/DDP cells were transfected with si-NC, si-LINC01116 1# or 2#. (**A**) qPCR assay detects lower LINC01116 expression in A549/DDP cells transfected with si-LINC01116 1# or 2# than in those transfected with si-NC 48 h post-transfection. (**B**) MTT assay measures the IC_{50} values of cisplatin against si-NC, si-LINC01116 1# and 2# transfected A549/DDP cells. (**C**) MTT assay measures the viability of A549/DDP cells transfected with si-NC, si-LINC01116 1# and 2#. (**D**) Colony formation assays were conducted to determine the cell proliferation ability for transfected LAD cells. (**E**) Knockdown of LINC01116 suppresses A549/DDP cell migration and invasion. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$.

Overexpression of LINC01116 Promotes A549 Cell Proliferation, Migration and Invasion

To further explore the role of LINC01116 in LAD, we constructed an empty vector and a pcDNA-LINC01116 plasmid, and conducted a series of functional assays. qPCR assay detected higher LINC01116 expression in pcDNA-LINC01116-transfected A549 cells than in empty vector-transfected cells 48 h post-transfection (Figure 5A). Then, overexpression with the pcDNA-LINC01116 plasmid was found to promote the proliferation (Figure 5B), and MTT assay measured a higher IC_{50} value of cisplatin against pcDNA-LINC01116-transfected A549 cells than against empty vector-transfected cells (Figure 5C), while colony-forming assay showed a higher colony formation ability of A549 cells transfected with the pcDNA-LINC01116 plasmid than those transfected with the empty vector (Figure 5D). In addition, Transwell migration and invasion assays revealed more pcDNA-LINC01116-transfected A549 cells that had migrated or invaded through the membrane than empty vector-transfected cells (Figure 5E). Our findings demonstrate that LINC01116 overexpression promotes the proliferation, migration and invasion of A549 cells.

Overexpression of LINC01116 Inhibits Apoptosis and Induces S Phase Arrest of A549 Cells

Flow cytometry showed a lower apoptotic rate of pcDNA-LINC01116-transfected A549 cells than that of empty vector-transfected cells (Figure 6A), and the proportion of pcDNA-LINC01116-transfected A549 cells was significantly lower at the G0/G1 phase and greater at the S phase relative of empty vector-transfected cells (Figure 6B). Our

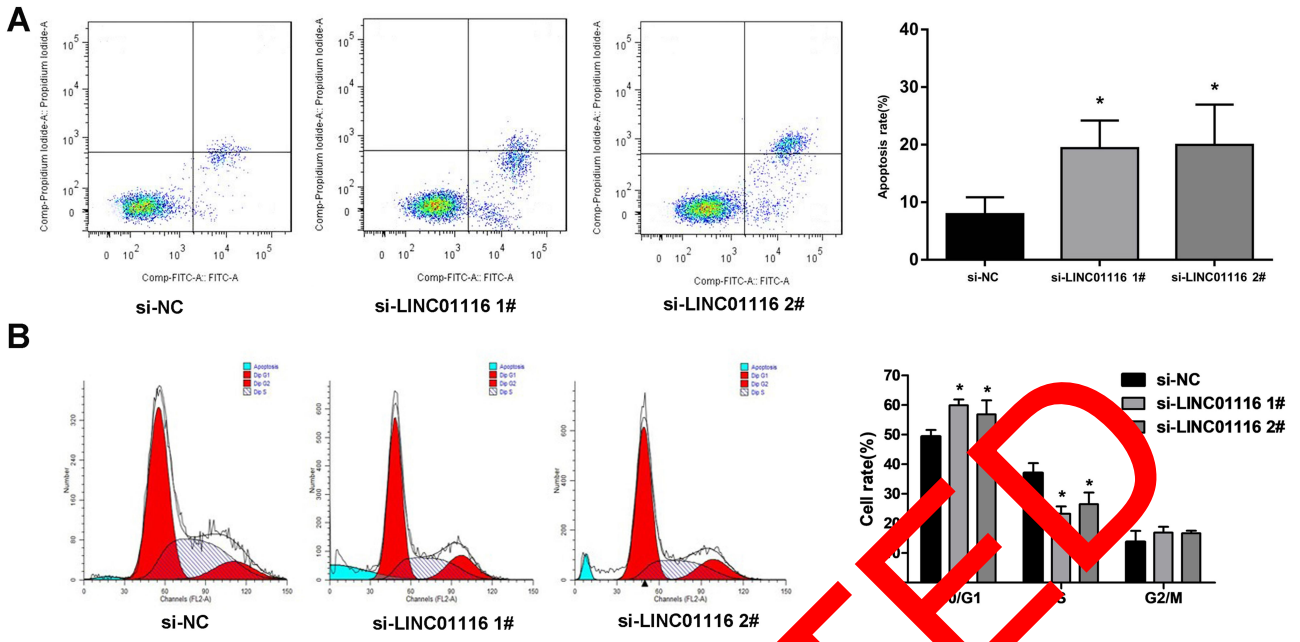

Figure 3 LINC01116 knockdown promotes apoptosis and induces G1 cell cycle arrest in A549/DDP cells. A549/DDP cells are transfected with si-NC, si-Linc01116 1# or 2#. (**A**) Flow cytometry detects a significant rise in the apoptotic rate of A549/DDP cells transfected with si-LINC01116 1# or 2# relative to those transfected with si-NC. (**B**) Flow cytometry detects a more percentage of si-LINC01116 1# or 2# transfected cells in the G0/G1 phase and a lower proportion of si-LINC01116 1# or 2# transfected cells at the S phase as compared with si-NC transfected cells. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$.

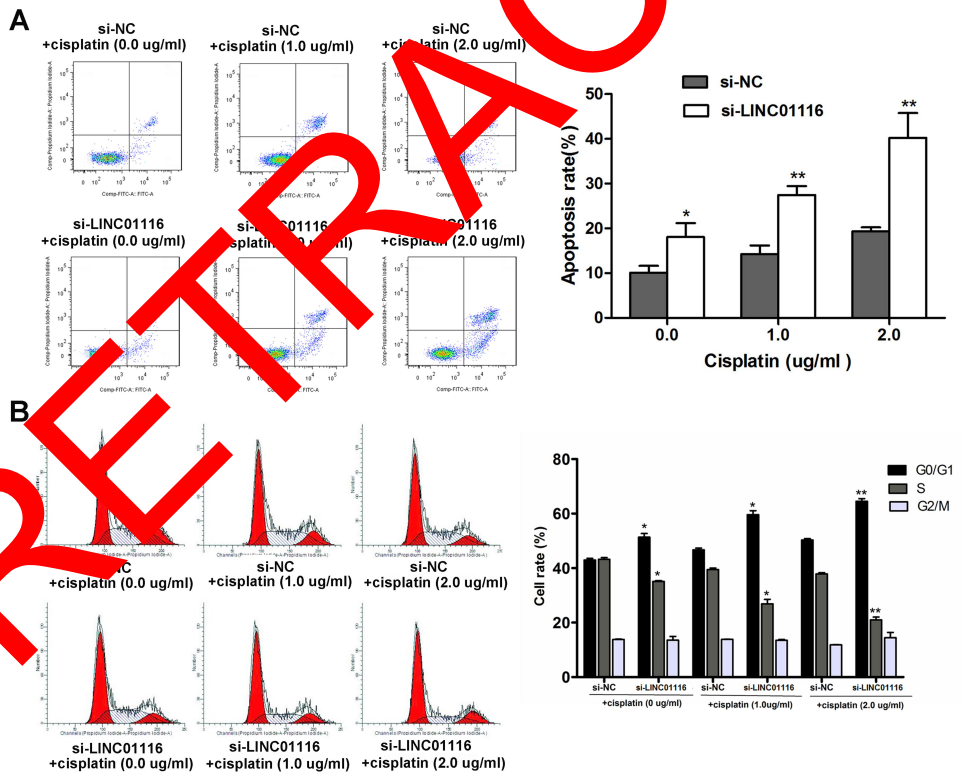

Figure 4 Knockdown of LINC01116 increases the sensitivity of A549/DDP cells to cisplatin. (**A**) Flow cytometry detects higher apoptotic rates of LINC01116 1# transfected A549/DDP cells than those of si-NC transfected cells regardless of cisplatin treatment. (**B**) The percentage of si-LINC01116 1# transfected A549/DDP cells gradually increases at G0/G1 phase and gradually decreased at S phase with the rise in the cisplatin concentration, as revealed by flow cytometry. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$.

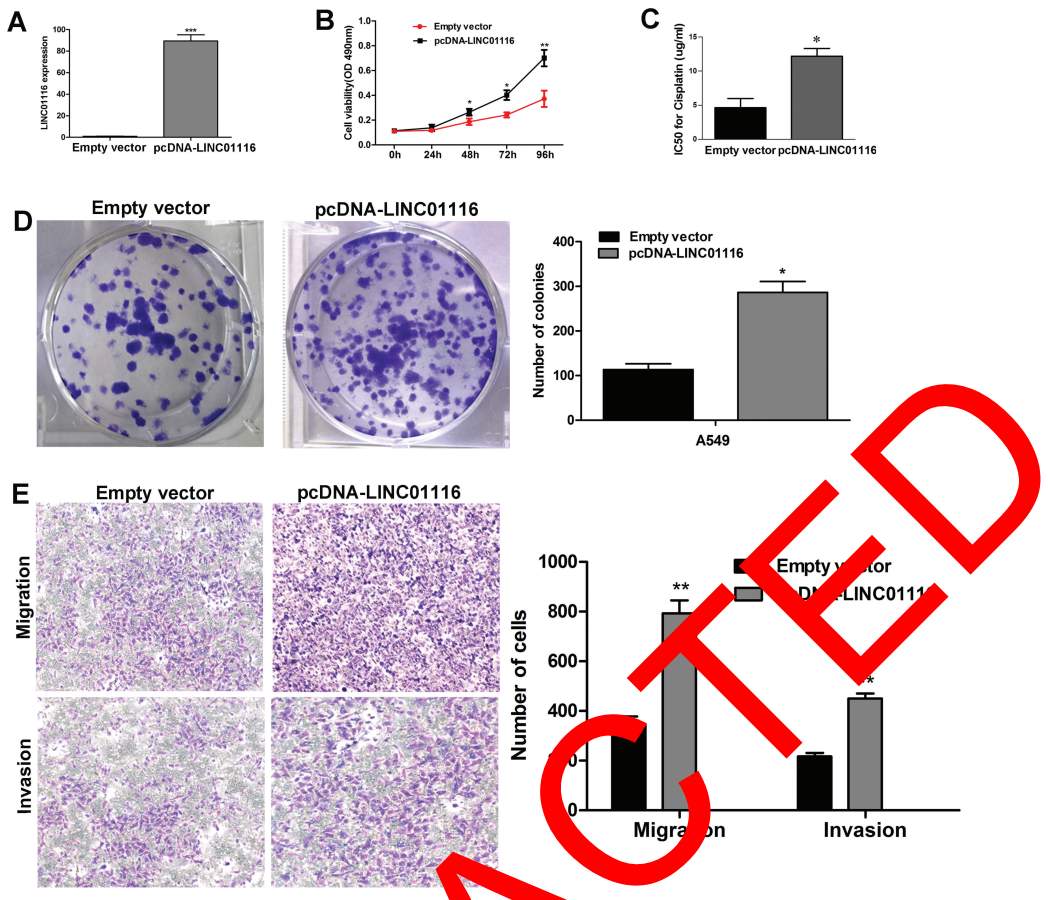

Figure 5 Overexpression of LINC01116 promotes A549 cell proliferation, migration and invasion. A549 cells are transfected with the empty vector or pcDNA-Linc01116 plasmid. (**A**) qPCR assay quantifies higher LINC01116 expression in pcDNA-LINC01116-transfected A549 cells than in empty vector-transfected cells 48 h post-transfection. (**B**) MTT assay measures the viability of A549 cells transfected with the pcDNA-LINC01116 plasmid and empty vector. (**C**) MTT assay measures a higher IC_{50} value of cisplatin against pcDNA-LINC01116-transfected A549 cells than against empty vector-transfected cells. (**D**) Colony-forming assay detects the proliferation of A549 cells transfected with the pcDNA-LINC01116 plasmid and empty vector. (**E**) Transwell migration and invasion assays reveal more pcDNA-LINC01116-transfected A549 cells that had migrated or invaded through the membrane than empty vector-transfected cells. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

findings indicate that LINC01116 overexpression suppresses A549 cell apoptosis and induces cell cycle arrest at S phase.

Overexpression of LINC01116 Reduces the Sensitivity of A549 Cells to Cisplatin

To assess the effect of LINC01116 overexpression on the resistance of A549 cells to cisplatin, the parental A549 cells were transfected with the pcDNA-LINC01116 plasmid and exposed to cisplatin at concentrations of 0.0, 1.0 and 1.5 μg/mL, respectively. Flow cytometry revealed lower apoptotic rates of pcDNA-LINC01116-transfected A549 cells than those of empty vector-transfected cells regardless of cisplatin treatment (Figure 7A), and the percentage of pcDNA-LINC01116-transfected A549 cells gradually increased at G0/G1 phase and gradually decreased at S phase with the increase in the cisplatin concentration (Figure 7B). These data demonstrate that overexpression of LINC01116 reduces the sensitivity to cisplatin in A549 cells through decreasing apoptosis and the percentage of A549 cells at G0/G1 phase.

Knockdown of LINC01116 Improves in vivo Sensitivity to Cisplatin

To further investigate the effect of LINC01116 knockdown on tumor growth, nude mice were inoculated subcutaneously with si-LINC01116 #1 or si-NC transfected A549/DDP cells, followed by cisplatin treatment, and the volume and weight of lung tumor xenografts were recorded 28 d post-inoculation (Figure 8A). The weight of the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells was significantly lower than those derived from si-NC transfected A549/DDP cells (Figure 8B). qPCR assay detected down-regulation of LINC01116 expression in the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP

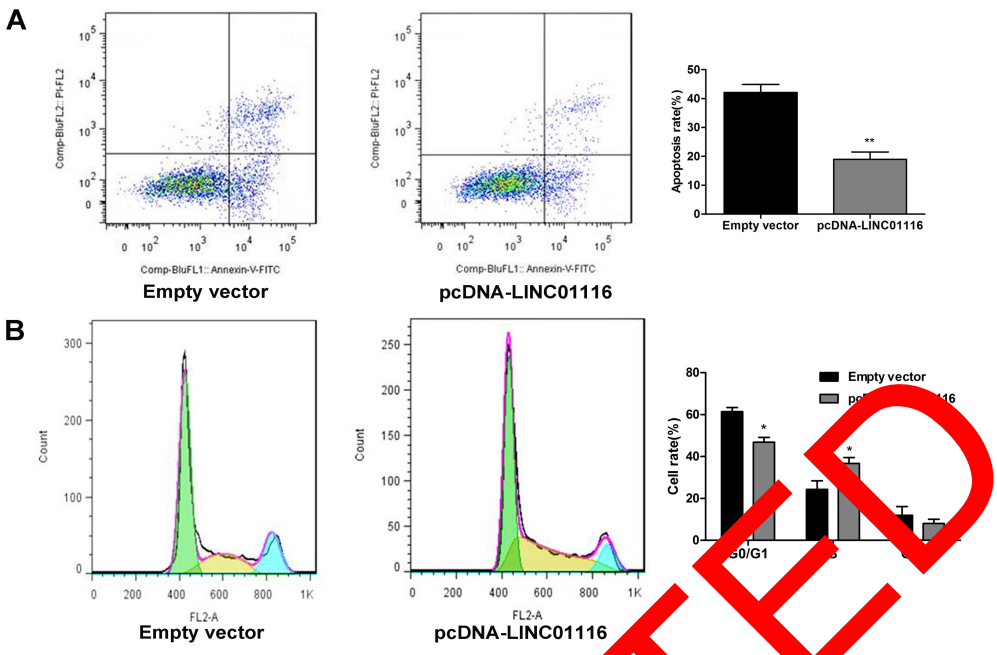

Figure 6 Overexpression of LINC01116 inhibits apoptosis and induces S phase arrest of A549 cells. (**A**) Flow cytometry detects a lower apoptotic rate of pcDNA-LINC01116-transfected A549 cells than that of empty vector-transfected cells. (**B**) Flow cytometry detects a lower proportion of pcDNA-LINC01116-transfected A549 cells at the G0/G1 phase and a greater proportion at the S phase relative of empty vector-transfected cells. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$.

cells than in that from si-NC transfected A549/DDP cells (Figure 8C). Following isolation of lung tumor xenografts from nude mice 28 days post-inoculation, the volume of the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells was significantly lower than those derived from si-NC transfected A549/DDP cells (Figure 8D and E). Immunohistochemical analysis detected lower Ki-67 expression in the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells than in that from si-NC transfected A549/DDP cells (Figure 8F). In addition, higher E-cadherin expression and lower Vimentin expression were found in the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells than in that from si-NC transfected A549/DDP cells (Figure 8F). These data demonstrate that knockdown of LINC01116 expression inhibits LAD progression by promoting the in vivo sensitivity of A549/DDP cells to cisplatin.

Effect of LINC01116 Expression on Epithelial–Mesenchymal Transition (EMT) Process

To investigate the effect of LINC01116 expression on the EMT process, the expression of two EMT markers, E-cadherin and Vimentin, was quantified using qPCR and Western blotting assays. qPCR assay revealed that knockdown of LINC01116 expression resulted in elevated *E-cadherin* expression and reduced *Vimentin* expression in A549/DDP cells (Figure 9A), overexpression of LINC01116 expression resulted in reduced *E-cadherin* expression and elevated *Vimentin* expression in A549 cells (Figure 9B). Western blotting showed that LINC01116 knockdown resulted in elevated E-cadherin and reduced Vimentin expression in A549/DDP cells (Figure 9C), and LINC01116 overexpression resulted in reduced E-cadherin expression and elevated Vimentin expression in A549 cells (Figure 9D). These data demonstrate that LINC01116 knockdown or overexpression affects LAD cell proliferation and migration through regulating the EMT process.

LINC01116 Expression Correlates with Chemotherapy Sensitivity in LAD Patients

Kaplan–Meier survival analysis revealed a longer OS in LAD patients with low LINC01116 expression than those with high LINC01116 expression (Figure 10A, Supplementary Table 1). Then, immunohistochemical analysis showed lower E-cadherin expression and higher Vimentin expression in cisplatin-resistant LAD specimens than in non-resistant specimens (Figure 10B). These data indicate that LINC01116

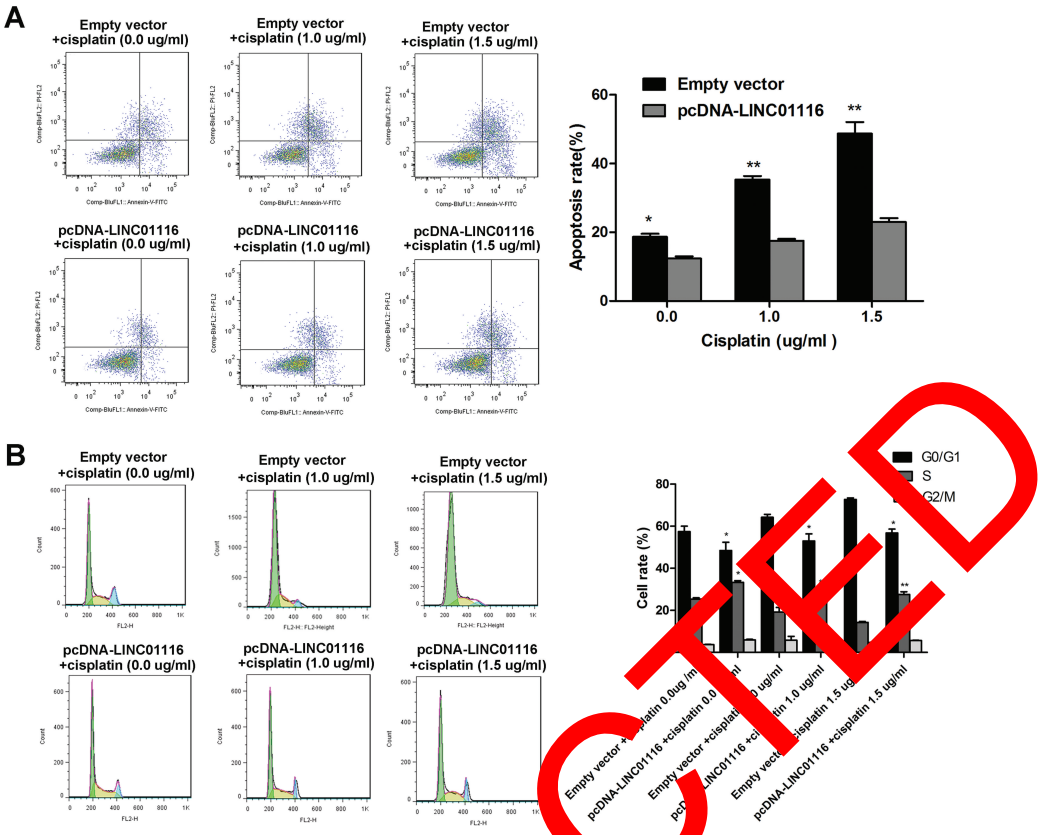

Figure 7 Overexpression of LINC01116 reduces the sensitivity of A549 cells to cisplatin. (**A**) Flow cytometry reveals lower apoptotic rates of pcDNA-LINC01116-transfected A549 cells than those of empty vector-transfected cells regardless of cisplatin treatment. (**B**) The percentage of pcDNA-LINC01116-transfected A549 cells gradually increases at G0/G1 phase and gradually decreases at S phase with the increase in the cisplatin concentration, as revealed by the flow cytometry. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$.

expression correlates with the response to cisplatin-based chemotherapy in LAD patients.

Discussion

As the most common histological subtype of NSCLC, LAD is characterized by aggressive invasion, high metastatic potential and poor prognosis.[25] Although great advances have been achieved for the management of NSCLC,[26–28] chemotherapy resistance remains a major obstacle to improve the prognosis in LAD patients.[29–31] Cisplatin, which inhibits DNA replication and damages cell membrane structures, is currently the most common chemotherapeutic agent used for the treatment of advanced NSCLC.[32–34] However, the sensitivity of NSCLC to cisplatin decreases with the disease progression, and the emergence of cisplatin resistance will eventually result in chemotherapy failure.[35] Understanding of cisplatin resistance is a prerequisite for the management of chemotherapy resistance in NSCLC.

Previous studies have extensively investigated the mechanisms responsible for resistance to cisplatin in NSCLC. Alpha-1 antitrypsin (A1AT), a member of the serpin (serine protease inhibitor) family, was recently reported to induce cisplatin resistance in NSCLC,[36] and large tumour suppressor (LATS) kinases were found to contribute to cisplatin resistance in advanced NSCLC.[37] FAM83D, an oncoprotein in multiple human cancer, was identified to promote cisplatin resistance in NSCLC via the AKT/mTOR pathway,[38] and CLEC4M, a Ca^{2+}-dependent C-type lectin, was reported to promote cisplatin resistance in NSCLC.[39] Recently, glucose-6-phosphate dehydrogenase (G6PD), a critical enzyme of the pentose phosphate pathway, was found to contribute to cisplatin resistance in NSCLC.[40] In addition, the role of microRNAs in cisplatin resistance has also been examined in NSCLC. For example, miR-103a-3p was revealed to potentiate chemoresistance to cisplatin in NSCLC by targeting neurofibromatosis 1,[41] and miR-324-5p contributed to cisplatin resistance in NSCLC by targeting FBXO11 signalling.[42] Previous studies also identified the involvement of miR-608 and miR-328 in the development of cisplatin resistance in NSCLC.[43,44] Furthermore, some circular RNAs, such as hsa_circ_0085131, circ_0076305 and

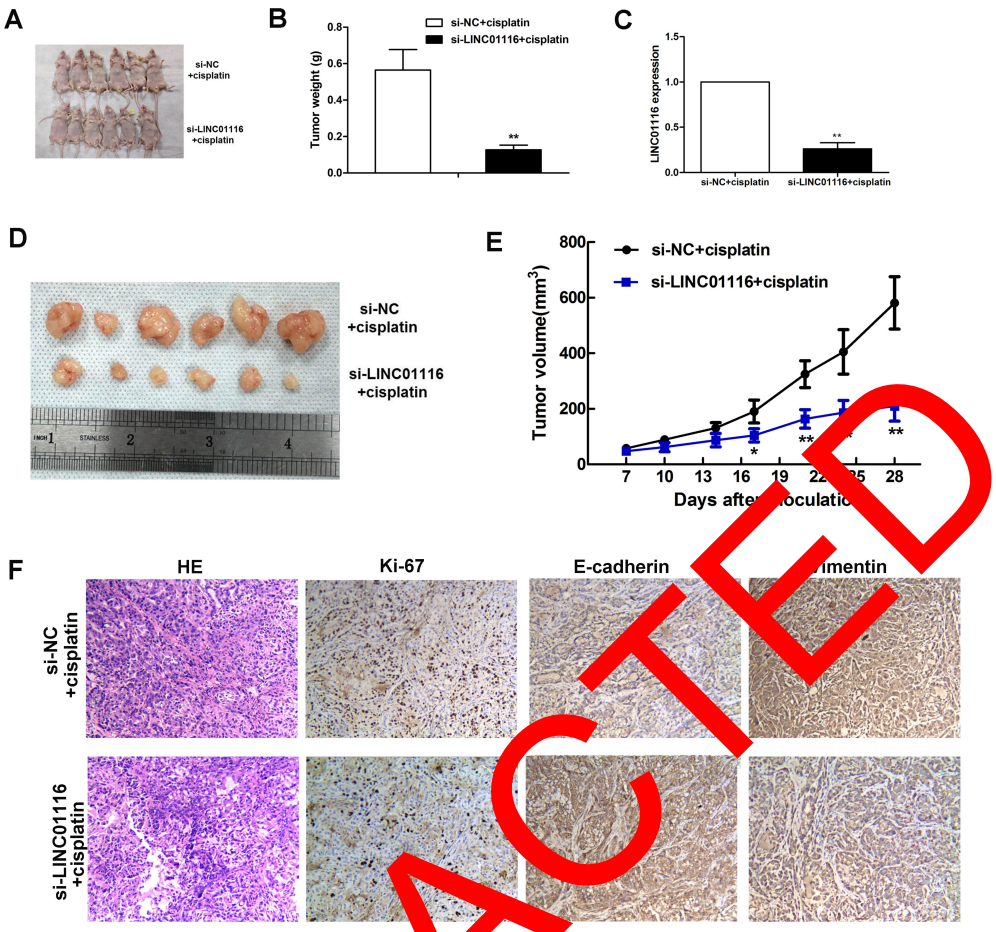

Figure 8 LINC01116 knockdown improves the in vivo sensitivity of A549/DDP cells to cisplatin. (**A**) Nude mice that are inoculated subcutaneously with si-LINC01116 #1 or si-NC transfected A549/DDP cells are sacrificed 28 days post-inoculation. (**B**) The tumor weight of the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells is significantly lower than those derived from si-NC transfected A549/DDP cells. (**C**) qPCR assay detects down-regulation of LINC01116 expression in the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells as compared to that from si-NC transfected A549/DDP cells. (**D**) Lung tumor xenografts are isolated from nude mice inoculated subcutaneously with si-LINC01116 #1 or si-NC transfected A549/DDP cells 28 days post-inoculation. (**E**) The volume of the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells is significantly lower than those derived from si-NC transfected A549/DDP cells. (**F**) Immunohistochemical analysis detects lower Ki-67 expression, higher E-cadherin expression and lower Vimentin expression in the lung tumor xenograft derived from si-LINC01116 #1 transfected A549/DDP cells than that from si-NC transfected A549/DDP cells (HE staining, × 100). * All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.0$ **$P < 0.01$.

hsa_circ_0001946, have been linked with the resistance to cisplatin in NSCLC.[8,] These findings urge the attempt to investigate the role of lncRNAs in cisplatin resistance in NSCLC.

LncRNAs are non-coding protein transcripts that have been proved to play a key regulatory role in tumorigenesis, metastasis, and chemotherapy resistance.[11–14] Increasing evidence has demonstrated the involvement of LncRNA in cisplatin resistance in multiple human cancers.[47–51] In NSCLC, LncRNA SPRY4-IT1 was reported to reverse cisplatin resistance partially through downregulating MPZL-1 via EMT,[52] and LncRNA NORAD was found to increase cisplatin resistance via the miR-129-1-3p/SOX4 axis.[53] A recent study concluded that LncRNA-XIST contributes to cisplatin resistance through down-regulating miRNA-144-3p.[54] Knockdown of LncRNA HOXA-AS3 was reported to enhance the sensitivity of NSCLC to cisplatin in vitro and in vivo through mediating homeobox A3 (HOXA3) expression,[55] and LncRNA FOXD2-AS1 was found to confer cisplatin resistance in NSCLC via the miR185-5p-SIX1 axis.[56] In addition, there are some other LncRNAs that have been implicated in cisplatin resistance in NSCLC;[57–62] however, the role of LncRNA LINC01116 in cisplatin resistance remains unknown in NSCLC.

LncRNA LINC01116 has been shown to play an oncogenic role in multiple human cancers.[17–21] qPCR assay detected up-regulation of LINC01116 expression in prostate cancer cells as compared to that in normal human prostate epithelial cells, which encouraged the subsequent

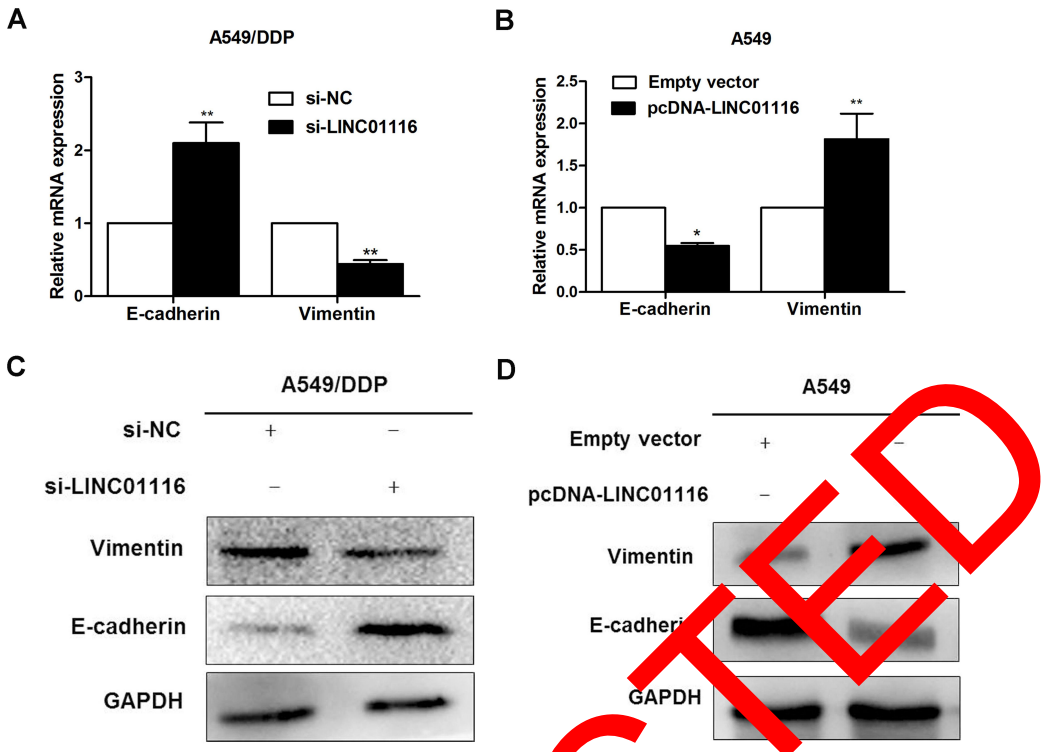

Figure 9 Effect of LINC01116 expression on EMT. (**A**) qPCR assay detects elevated *E-cadherin* expression and reduced *Vimentin* expression in A549/DDP cells transfected with si-LINC01116 #1 relative to A549/DDP cells transfected with si-NC. (**B**) qPCR assay detects lower *E-cadherin* expression and higher *Vimentin* expression in A549/DDP cells transfected with the pcDNA-Linc01116 plasmid than in those transfected with the empty vector. (**C**) Western blotting determined higher E-cadherin expression and lower Vimentin expression in A549/DDP cells transfected with si-LINC01116 #1 than in those transfected with si-NC, while GAPDH serves a loading control. (**D**) Western blotting determined lower E-cadherin expression and high Vimentin expression in A549/DDP cells transfected with pcDNA-Linc01116 plasmid than in those transfected with the empty vector, while GAPDH serves a loading control. All experiments are repeated in triplicated, and data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$.

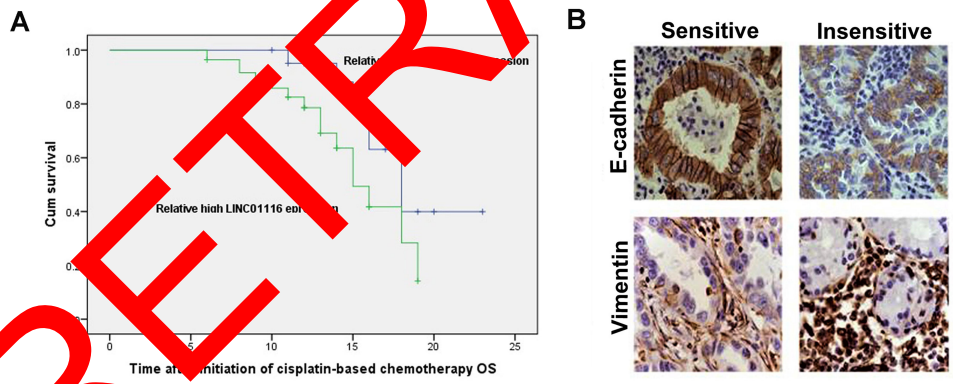

Figure 10 LINC01116 expression correlates with chemotherapy sensitivity in LAD patients. (**A**) Kaplan–Meier survival analysis reveals a longer OS in LAD patients with low LINC01116 expression than those with high LINC01116 expression. (**B**) Immunohistochemical analysis shows lower E-cadherin expression and higher Vimentin expression in cisplatin-resistant LAD specimens than in non-resistant specimens (HE staining, × 100).

functional assays.[16] siRNA-induced knockdown of *LINC01116* decreased prostate cancer cell proliferation, and the disruption of the *LINC01116* gene with a CRISPR/CAS9 system resulted in a four-fold reduction in the number of prostate cancer cell colony formation, which supporting the oncogenic role of LINC01116 in prostate cancer.[16] Based on the breast cancer data set GSE54002 captured from the public database Gene Expression Omnibus (GEO), LncRNA LINC01116 expression was found to up-regulate in breast cancer tissues as compared to in paracancerous tissues, and a further qPCR assay detected higher LINC01116 expression in 64 clinical breast cancer specimens than in 30 normal breast specimens; in addition, LINC01116 expression was

reported to correlate with OS, tumor size, TNM stage in patients with breast cancer, suggesting the prognostic value of LINC01116 in breast cancer.[63] Zhang et al[64] detected up-regulation of LINC0116 in osteosarcoma, and found that LINC0116 promoted osteosarcoma cell viability and migration, and LINC0116 knockdown suppressed tumor growth in nude mice. In addition, LINC01116 was reported to overexpressed and contributed to the progression of nasopharyngeal carcinoma, gastric cancer, osteosarcoma, glioma, epithelial ovarian cancer and head and neck squamous cell carcinoma.[17–23] However, there is little knowledge on the role of LINC01116 in NSCLC.

In this study, we detected that LncRNA LINC01116 was overexpressed in cisplatin-resistant LAD specimens than in non-resistant specimens, and significantly up-regulated in cisplatin-resistant A549/DDP cells relative to in parental A549 cells, which was in agreement with previous findings in other cancers.[63,64] The findings from the qPCR assay encouraged our subsequent functional assays. Knockdown of LINC01116 inhibited the viability, proliferation, migration, and invasion of A549/DDP cells, promoted apoptosis and enhanced the sensitivity to cisplatin in A549/DDP cells, while LINC01116 overexpression promoted the viability, proliferation, migration, and invasion of A549 cells, inhibited apoptosis and reduced the sensitivity to cisplatin in A549 cells, which was consistent with previous reports.[24,64] These data further support the oncogenic role of LINC01116 in LAD, and demonstrate that LINC01116 may increase the sensitivity of LAD cells to cisplatin through promoting cell apoptosis.

The mechanisms of action for LINC011116 have been reported in different cancer settings. LINC01116 was found to promote the proliferation and migration of osteosarcoma cells through targeting miR-520a-3p and upregulating IL6R via the Jak-stat signaling pathway,[64] and was reported to promote epithelial ovarian cancer progression through regulating cell apoptosis.[23] In addition, LINC01116 was identified to regulates angiogenesis of glioma by targeting vascular endothelial growth factor A (VEGFA),[22] and LINC01116 was shown to promote gastric cancer cell invasion and migration by positively interacting with lncRNA CASC11.[20] A recent study showed that LINC01116 contributed to gefitinib resistance in NSCLC cells by regulating IFI44 expression.[24] However, the mechanism underlying the contribution of LINC01116 to cisplatin resistance remains unknown in NSCLC until now.

EMT, which is characterized by loss of epithelial markers like E-cadherin, and acquisition of mesenchymal markers including N-cadherin and Vimentin, is a critical biological process involved in tumor progression and metastasis,[65–67] and contributes to chemotherapy resistance in NSCLC.[68–70] LncRNAs have been found to correlate with tumor metastasis via EMT.[71] Knockdown of LncRNA BANCR was reported to promote NSCLC cell metastasis by regulating EMT,[72] and up-regulation of LncRNA MALAT1 was reported to promote lung cancer brain metastasis by inducing EMT.[73] In the present study, LINC01116 knockdown resulted in an increase in E-cadherin expression and a reduction in Vimentin in A549/DDP cells at both translational and transcriptional levels, and LINC01116 overexpression resulted in reduced E-cadherin expression and elevated Vimentin expression in A549 cells. It is therefore hypothesized that LINC01116 may contribute to cisplatin resistance in LAD cells partly via the EMT process. However, the exact molecular mechanisms of LINC01116 for targeting EMT to mediate the resistance to cisplatin require further investigations.

The present study has some limitations. First, this study was performed in a single cell line, which may limit the strength of our work. Second, most of our study focused on the role of LINC01116 in the biological behaviors of NSCLC, and the mechanism explaining the contribution of LINC01116 to cisplatin resistance in NSCLC was little studied. Third, we performed a xenograft tumor growth assay to examine the effect of LINC01116 knockdown on the in vivo sensitivity to cisplatin; however, the PDX animal model may be a better assay to assess the effect of silencing LINC01116 on the in vivo sensitivity to cisplatin. Further mechanistic studies in multiple cell lines with a PDX animal assay to validate the findings from the present study and explore the mechanisms underlying the role of LINC01116 in cisplatin resistance in NSCLC seem justified.

Conclusions

In summary, the results of the present study demonstrate that dysregulation of lncRNA LINC01116 expression results in resistance of LAD to cisplatin via the EMT process, suggesting that LINC01116 knockdown may target EMT to enhance cisplatin chemosensitivity by regulating apoptosis and cell cycle distribution, and LINC01116 overexpression inhibits the sensitivity to cisplatin in LAD cells. Our findings support the oncogenic role of LINC01116 to promote the development of cisplatin resistance in LAD, and LINC01116 may be a novel marker of poor response to cisplatin. It is considered that LINC01116 may be a potential therapeutic target for LAD chemotherapy.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Neal RD, Sun F, Emery JD, Callister ME. Lung cancer. *BMJ*. 2019;365:l1725.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424.
3. Lambe G, Durand M, Buckley A, Nicholson S, McDermott R. Adenocarcinoma of the lung: from BAC to the future. *Insights Imaging*. 2020;11(1):69.
4. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature*. 2018;553(7689):446–454.
5. Asselain B, Barrière JR, Clarot C, et al. Metastatic NSCLC: clini molecular, and therapeutic factors associated with long-term survi *Respir Med Res*. 2019;76:38–44.
6. Chen R, Manochakian R, James L, et al. Emerging therapeutic agents for advanced non-small cell lung cancer. *J Hematol Oncol*. 2 ;13 :58.
7. Giglio A, Nuvola G, Baldini C. Clinical consider on for c osing combination therapies in advanced non-small-c lung ca Eastern Cooperative Organization performa sta 2, roids and antibiotics. *Future Oncol*. 2020.
8. Kong R. Circular RNA hsa_cir 35131 is in lved in cisplatin-resistance of non-small ce ung c er cells by re ulating autophagy. *Cell Biol Int*. 2020.
9. Wang L, Ma L, Xu F, et al ole of long non-co g RNA in drug resistance in non-small c lung cancer. *Thorac Cancer*. 2018;9 (7):761–768.
10. Fan CC, Tsai ST, Lin CY, l HD2 con butes to non-small cell lung cancer cisp resista by the tivation of NOX4-ROS-ABCC1 axis. *edox B* . 2020; 101 1.
11. Papoutsog P, Mous kas A. Lo non-coding RNAs and TGF-β signaling ancer 2020.
12. Chatterjee M, upta S. Emerging roles of long non-coding RNAs in cancer. *J Biosci*. 2019;44(1):22.
13. Chen M, Fan M, Ya l, Lang J. Identification of potential oncogenic long non-coding RNA set as a biomarker associated with colon cancer prognosis. *J Environ Pathol Toxicol Oncol*. 2020;39(1):39–49.
14. Chen QN, Wei CC, Wang ZX, Sun M. Long non-coding RNAs in anti-cancer drug resistance. *Oncotarget*. 2017;8(1):1925–1936.
15. Ayers D, Vandesompele J. Influence of microRNAs and long non-coding RNAs in cancer chemoresistance. *Genes*. 2017;8(3):E95.
16. Beaver LM, Kuintzle R, Buchanan A, et al. Long noncoding RNAs and sulforaphane: a target for chemoprevention and suppression of prostate cancer. *J Nutr Biochem*. 2017;42:72–83.
17. Wu J, Chen Z, Zhang L, et al. Knockdown of LINC01116 inhibits cell migration and invasion in head and neck squamous cell carcinoma through epithelial-mesenchymal transition pathway. *J Cell Biochem*. 2020;121(1):867–875.
18. Chen Z, Tao Q, Qiao B, Zhang L. Silencing of LINC01116 suppresses the development of oral squamous cell carcinoma by up-regulating microRNA-136 to inhibit FN1. *Cancer Manag Res*. 2019;11:6043–6059.
19. Xing H, Sun H, Du W. LINC01116 accelerates nasopharyngeal carcinoma progression based on its enhancement on MYC transcription activity. *Cancer Med*. 2020;9(1):269–277.
20. Su X, Zhang J, Luo X, et al. LncRNA LINC01116 promotes cancer cell proliferation, migration and invasion in gastric cancer by positively interacting with lncRNA CASC11. *Onco Targets Ther*. 2019;12:8117–8123.
21. Zhang ZF, Xu HH, Hu WH, Hu TY, Wang XB. LINC01116 promotes proliferation, invasion and migration of oste cells by silencing p53 and EZH2. *Eur Rev Med Pharmacol* . 2019;23(6813–6823.
22. Ye J, Zhu J, Chen H, et al. A nov ncRNA-LINC 16 regulates tumorigenesis of glioma by targeting V FA. *Int J Can* . 2020;146 (1):248–261.
23. Fang YN, Huang ZL, Li H t al. LINC0111 ompr es the progression of epithelial ovari cancer v regulating ell apoptosis. *Eur Rev Med Pharmacol Sci*. 2 18;2 16):5127–5133.
24. Wang H, Lu B, n S, et Long non ding RNA LINC01116 contributes to tinib resista in on-small cell lung cancer through re ating 44. *Mol Ther cleic Acids*. 2019;19:218–227.
25. Balzer BWR, Loo Lewis CR, Trahair TN, Anazodo AC. Aden oma of th lung in childhood and adolescence: a stematic review. *J Thorac Oncol*. 2018;13(12):1832–1841.
26. ck M, Rabe K Precision diagnosis and treatment for advanced n -small-cell lu cancer. *N Engl J Med*. 2017;377(9):849–861.
27. T hi S, Kasso E, Rassy EE, et al. Management of stage III non- ll cell g cancer. *Semin Oncol*. 2017;44(3):163–177.
28. Gridelli C, Rossi A, Carbone DP, et al. Non-small-cell lung cancer. *Nat Rev Dis Primers*. 2015;1:15009.
29. Jancalkova D, Pesek M, Benesova L, Topolcan O, Jr HL, Minarik M. Acquired resistance of pulmonary adenocarcinoma to initially successful targeted therapy due to EGFR mutation T790M. *Anticancer Res*. 2007;27(4A):1879–1882.
30. Wu SG, Liu YN, Tsai MF, et al. The mechanism of acquired resistance to irreversible EGFR tyrosine kinase inhibitor-afatinib in lung adenocarcinoma patients. *Oncotarget*. 2016;7(11):12404–12413.
31. Chang CC, Hsieh TL, Tiong TY, et al. Regulation of metastatic ability and drug resistance in pulmonary adenocarcinoma by matrix rigidity via activating c-Met and EGFR. *Biomaterials*. 2015;60:141–150.
32. Nguyen CTT, Petrelli F, Scuri S, Nguyen BT, Grappasonni I. A systematic review of pharmacoeconomic evaluations of erlotinib in the first-line treatment of advanced non-small cell lung cancer. *Eur J Health Econ*. 2019;20(5):763–777.
33. Fennell DA, Summers Y, Cadranel J, et al. Cisplatin in the modern era: the backbone of first-line chemotherapy for non-small cell lung cancer. *Cancer Treat Rev*. 2016;44:42–50.
34. Wang G, Reed E, Li QQ. Molecular basis of cellular response to cisplatin chemotherapy in non-small cell lung cancer (review). *Oncol Rep*. 2004;12(5):955–965.
35. Amable L. Cisplatin resistance and opportunities for precision medicine. *Pharmacol Res*. 2016;106:27–36.
36. Wu DM, Liu T, Deng SH, et al. Alpha-1 antitrypsin induces epithelial-to-mesenchymal transition, endothelial-to-mesenchymal transition, and drug resistance in lung cancer cells. *Onco Targets Ther*. 2020;13:3751–3763.
37. Luo SY, Kwok HH, Yang PC, Ip MS, Minna JD, Lam DC. Expression of large tumour suppressor (LATS) kinases modulates chemotherapy response in advanced non-small cell lung cancer. *Transl Lung Cancer Res*. 2020;9(2):294–305.

38. Yin C, Lin X, Wang Y, et al. FAM83D promotes epithelial-mesenchymal transition, invasion and cisplatin resistance through regulating the AKT/mTOR pathway in non-small-cell lung cancer. *Cell Oncol*. 2020;43(3):395–407.
39. Tan LM, Li X, Qiu CF, et al. CLEC4M is associated with poor prognosis and promotes cisplatin resistance in NSCLC patients. *J Cancer*. 2019;10(25):6374–6383.
40. Zhang R, Tao F, Ruan S, et al. The TGFβ1-FOXM1-HMGA1-TGFβ1 positive feedback loop increases the cisplatin resistance of non-small cell lung cancer by inducing G6PD expression. *Am J Transl Res*. 2019;11(11):6860–6876.
41. Zhu H, Yang J, Yang S. MicroRNA-103a-3p potentiates chemoresistance to cisplatin in non-small cell lung carcinoma by targeting neurofibromatosis 1. *Exp Ther Med*. 2020;19(3):1797–1805.
42. Ba Z, Zhou Y, Yang Z, Xu J, Zhang X. miR-324-5p upregulation potentiates resistance to cisplatin by targeting FBXO11 signalling in non-small cell lung cancer cells. *J Biochem*. 2019;166(6):517–527.
43. Wang Y, Li F, Ma D, Gao Y, Li R, Gao Y. MicroRNA-608 sensitizes non-small cell lung cancer cells to cisplatin by targeting TEAD2. *Mol Med Rep*. 2019;20(4):3519–3526.
44. Wang C, Wang S, Ma F, Zhang W. miRNA-328 overexpression confers cisplatin resistance in non-small cell lung cancer via targeting of PTEN. *Mol Med Rep*. 2018;18(5):4563–4570.
45. Dong Y, Xu T, Zhong S, et al. Circ_0076305 regulates cisplatin resistance of non-small cell lung cancer via positively modulating STAT3 by sponging miR-296-5p. *Life Sci*. 2019;239:116984.
46. Huang MS, Liu JY, Xia XB, et al. Hsa_circ_0001946 inhibits lung cancer progression and mediates cisplatin sensitivity in non-small cell lung cancer via the nucleotide excision repair signaling pathway. *Front Oncol*. 2019;9:508.
47. Guo J, Pan H. Long noncoding RNA LINC01125 enhances cisplatin sensitivity of ovarian cancer via miR-1972. *Med Sci Monit*. 2019;25:9844–9854.
48. Zhang S, Ma H, Zhang D, et al. LncRNA KCNQ1OT1 regulates proliferation and cisplatin resistance in tongue cancer via miR-211-5p mediated Ezrin/Fak/Src signaling. *Cell Death Dis*. 2017;8(7):742.
49. Liu P, Li X, Cui Y, et al. LncRNA-MALAT1 mediates cisplatin resistance via miR-101-3p/VEGF-C pathway in bladder cancer. *Acta Biochim Biophys Sin (Shanghai)*. 2019;51(11):1148–1157.
50. Ye Y, Yang S, Han Y, et al. HOXD-AS1 confers cisplatin resistance in gastric cancer through epigenetic silencing PLD4 via recruiting EZH2. *Open Biol*. 2019;9(5):190021.
51. Cheng FH, Zhao ZS, Liu WD. Long non-coding RNA ROR regulated ABCB1 to induce cisplatin resistance in osteosarcoma by sponging miR-153-3p. *Eur Rev Med Pharmacol Sci*. 2019;23(17):7256–7265.
52. Ye Y, Gu J, Liu P, et al. Long non-coding RNA SPRY4-IT1 reverses cisplatin resistance by down-regulating PTPRZL-1 via suppressing EMT in NSCLC. *Onco Targets Ther*. 2020;13:2783–2793.
53. Huang X, Xin S, Pei Y, Yu Z. NORAD accelerates chemoresistance of non-small-cell lung cancer via targeting at miR-[illegible]-3p. *Biosci Rep*. 2020;40(1):BSR20193489.
54. Tian LJ, Li P, Wang D, et al. Upregulation of long noncoding RNA (lncRNA) X-Inactive Specific Transcript (XIST) is associated with cisplatin resistance in Non-Small Cell Lung Cancer (NSCLC) by down-regulating microRNA-144-3p. *Med Sci Monit*. 2019;25:8095–8104.
55. Lin S, Zhang R, An X, et al. LncRNA HOXA-AS3 confers cisplatin resistance by interacting with HOXA3 in non-small-cell lung carcinoma cells. *Oncogenesis*. 2019;8(11):60.
56. Ge P, Cao L, Yao YJ, Jing RJ, Wang W, Li HJ. lncRNA FOXD2-AS1 confers cisplatin resistance of non-small-cell lung cancer via regulation of miR185-5p-SIX1 axis. *Onco Targets Ther*. 2019;12:6105–6117.
57. Tong L, Wu W. Effects of long non-coding RNA (lncRNA) cancer susceptibility candidate 2c (CASC2c) on proliferation, metastasis and drug resistance of non-small cell lung cancer (NSCLC) cells through ERK1/2 and β-catenin signaling pathways. *Pathol Res Pract*. 2019;215(9):152522.
58. Wang W, Dong ML, Zhang W, Liu T. Long noncoding LUCAT1 promotes cisplatin resistance of non-small cell lung cancer by promoting IGF-2. *Eur Rev Med Pharmacol Sci*. 2019;23(12):5229–5234.
59. Shi SL, Zhang ZH. Long non-coding RNA SNHG1 contributes to cisplatin resistance in non-small cell lung cancer by regulating miR-140-5p/Wnt/β-catenin pathway. *Neoplasma*. 2019;66(5):756–765.
60. Shang J, Xu YD, Zhang YY, Li M. Long noncoding RNA OR3A4 promotes cisplatin resistance of non-small cell lung cancer by upregulating CDK1. *Eur Rev Med Pharmacol Sci*. 2019;23(10):4220–4225.
61. Tang H, Han X, Li M, Li T, Han Y. Linc00221 modulates cisplatin resistance in non-small-cell lung cancer via sponging miR-519a. *Biochimie*. 2019;162:132–143.
62. Gao BB, Wang SX. lncRNA PVT1 200 regulates the cell proliferation and cisplatin resistance in non-small cell lung cancer via PI3K/AKT pathway. *Eur Rev Med Pharmacol Sci*. 2019;23(3):1093–1101.
63. Hu HB, Chen Q, Ding [illegible]. LncRNA LINC01116 competes with miR-145-5p for the regulation of ESR1 expression in breast cancer. *Eur Rev Med Pharmacol Sci*. 2018;22(7):1987–1993.
64. Zhang B, Yu L, Fu N, et al. LINC01116 targets miR-520a-3p and affects IL6R to promote the proliferation and migration of osteosarcoma cells through the Jak-stat signaling pathway. *Biomed Pharmacother*. 2018;107:270–282.
65. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420–1428.
66. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest*. 2009;119(6):1429–1437.
67. Thiery JP, Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. *J Clin Invest*. 2009;119(6):1438–1449.
68. Jiang GB, Fang HY, Tao DY, Chen XP, Cao FL. COX-2 potentiates cisplatin resistance of non-small cell lung cancer cells by promoting EMT in an AKT signaling pathway-dependent manner. *Eur Rev Med Pharmacol Sci*. 2019;23(9):3838–3846.
69. He Y, Xie H, Yu P, Jiang S, Wei L. FOXC2 promotes epithelial-mesenchymal transition and cisplatin resistance of non-small cell lung cancer cells. *Cancer Chemother Pharmacol*. 2018;82(6):1049–1059.
70. Weng CH, Chen LY, Lin YC, et al. Epithelial-mesenchymal transition (EMT) beyond EGFR mutations per se is a common mechanism for acquired resistance to EGFR TKI. *Oncogene*. 2019;38(4):455–468.
71. Gugnoni M, Ciarrocchi A. Long noncoding RNA and epithelial mesenchymal transition in cancer. *Int J Mol Sci*. 2019;20(8):E1924.
72. Sun M, Liu XH, Wang KM, et al. Downregulation of BRAF activated non-coding RNA is associated with poor prognosis for non-small cell lung cancer and promotes metastasis by affecting epithelial-mesenchymal transition. *Mol Cancer*. 2014;13:68.
73. Shen L, Chen L, Wang Y, Jiang X, Xia H, Zhuang Z. Long noncoding RNA MALAT1 promotes brain metastasis by inducing epithelial-mesenchymal transition in lung cancer. *J Neurooncol*. 2015;121(1):101–108.

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is