

# Mechanistic study of LncRNA UCA1 promoting growth and cisplatin resistance in lung adenocarcinoma

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## Primary research

**Keywords:** LncRNA, UCA1, cisplatin resistance, tumor development, lung adenocarcinoma

**Posted Date:** March 18th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-317734/v1>

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# Abstract

**Background:** The main objective of the current research was to explore the mechanism of LncRNA UCA1 promoting cisplatin resistance in lung adenocarcinoma (LUAD).

**Method:** The UCA1 expression level of LUAD cell lines was herein detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). We overexpressed UCA1 in A549 cell and downregulated UCA1 in A549/DDP cell by lentivirus-mediated technique. We analyzed their biological differences by cell function experiments, RNA pulldown, protein mass spectrometry (MS), and RNA immunoprecipitation technique (RIP). Tumor formation in nude mice was used to investigate the effect of UCA1 on the proliferation and cisplatin sensitivity of A549/DDP *in vivo*.

**Result:** The results revealed that a higher expression level of UCA1 was expressed in the A549/DDP cell and LUAD tissues than that in A549 cell and adjacent cancer tissues. UCA1 was significantly associated with M stage and clinical stage of LUAD patients from The Cancer Genome Atlas (TCGA) database. Patients with high UCA1 expression had a shorter survival time. After UCA1 overexpressed, the cells capability of proliferation, migration, invasion, clone formation, cisplatin resistance, and the expression level of proteins related to proliferation and drug resistance PCNA, ERCC1 were enhanced, while these trends were mostly reversed in the cells knockdown with UCA1 expression. Tumorigenic assays in nude mice showed that knockdown of UCA1 significantly inhibited tumor growth and reduced cisplatin resistance. It confirmed Enolase 1 (ENO1) was one of RNA binding protein of UCA1.

**Conclusion:** Based on these results, we concluded that UCA1 promoted LUAD progression and cisplatin resistance by binding ENO1 and UCA1 could be a potential diagnostic marker and therapeutic target of LUAD patients.

## Introduction

With an lung cancer was the second most diagnosed cancer and the leading cause of cancer death and there were estimated 2.2 million new cancer cases and 1.8 million deaths in 2020[1]. Due to the absence of validity for diagnosis at the early stage and the lack of effective therapies for advanced lung cancer, the current five-year survival rate of lung cancer patients was lower than 20%[2]. Lung adenocarcinoma (LUAD) was the most common histological type of NSCLC.

Notably, platinum-based chemotherapy was still the first-line treatment of advanced NSCLC, which is an efficient method to improve the survival rate and life quality of patients[3].Inevitably, platinum-based drugs and targeted drugs, which achieved significant effect in the beginning of treatment, had limitations due to the development of resistance with long-term use[4]. The mechanism of resistance to cisplatin was extremely complex, involving multiple genes, and was currently thought to be achieved primarily through multiple mechanisms[5–7]. Despite of many advances in genomic and proteomic studies, the mechanism of cisplatin resistance remained elusive.

Meanwhile, evidences were accumulating to indicate the involvement of Long noncoding RNA (LncRNA) in cancer development, progression, and drug resistance[8–15]. LncRNA urothelial carcinoma-associated 1 (UCA1) was first reported in the research of Wang[16]. These investigators found UCA1 belongs to the human endogenous retrovirus H family, with a full-length of 1439 bp and without protein translation and it was one of the most distinctive genes for bladder cancer[16].

Currently, there were some reports regarding the role of UCA1 in chemoresistance. UCA1/miRNA204-5p/CREB1 axis[17], UCA1/miRNA-143/FOSL2 axis[18], UCA1/miRNA-196a-5p/CREB axis[19] were involved in enhancing the chemoresistance of colorectal, ovarian and bladder cancer. In NSCLC, Li[20] *et al.* discovered that there were complementary binding sites between UCA1 and miRNA-495, and indicated that UCA1 promoted cisplatin resistance of NSCLC by regulating miRNA-495/NRF2 axis by as ceRNA. Liu[21] *et al.* found that after UCA1 knockdown, the expression of EMT-related proteins had changed and LUAD cell lines restored the sensitivity of cisplatin, which meant that UCA1 promoted cisplatin resistance by participating EMT signal pathway. However, the specific regulatory relationship between UCA1 and chemoresistance in NSCLC was still unclear.

In our previous experiments, differentially expressed LncRNAs between LUAD cisplatin sensitive cell line A549 and LUAD cisplatin resistant cell line A549/DDP were screened by LncRNA chip. We found that UCA1 was highly expressed in A549/DDP cell. Hence, we speculated that UCA1 would play a vital role in cisplatin resistance of LUAD. In this study, we furtherly uncovered mechanistic study of LncRNA UCA1 promoting cisplatin resistance in LUAD.

## Materials And Methods

### Human LUAD tissue samples

The clinicopathological features and UCA1 expression in 433 cases of LUAD were downloaded from the Cancer Genome Atlas (TCGA) database. The age of patients ranged from 33 to 88 years (median age 66 years), including 198 males and 235 females. The expression and survival curve of UCA1 in LUAD and normal tissues in TCGA database were obtained through the analysis of UALCAN website (<http://ualcan.path.uab.edu/index.html>) [22].

### Cell culture

A549 and A549/DDP cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and placed in an incubator at 37 °C, with 5% CO<sub>2</sub>. The cell culture medium was changed every 2–3 days. When near confluence, original culture medium was discarded and 2 ml PBS was added to rinse. Cells were digested by pancreatic enzymes, made into single cell suspension, and were sub-cultured at a ratio of 1:3. A549/DDP cells were cultured in the medium containing 1 µg/ml cisplatin to maintain cisplatin resistance capability.

## **Cisplatin sensitivity test**

A single-cell suspension was prepared and adjusted to  $8 \times 10^4$  cells/ml. Cells were seeded into 96-well plates ( $4 \times 10^3$  cells/well) and cultured overnight. The next day, the original liquid in the well was removed. 100  $\mu$ l of complete medium (containing cisplatin) was added to each well. The concentration of cisplatin (Beyotime, Shanghai, China) was 0, 1, 2, 4, 8  $\mu$ g/ml, and only the zeroing hole of the medium and the control hole of the single-cell suspension was set to zero. After 48 h of cultivation, the culture medium was replaced with complete medium containing 10% CCK8. The absorbance value was measured at 450 nm after incubation at 37 °C for 1 hour. A microplate reader detected the absorbance at 450 nm wavelength, cell viability % =  $(A_{\text{plus}} - A_{\text{blank}}) / (A_0_{\text{plus drug}} - A_{\text{blank}}) \times 100\%$ , using the SPSS21.0 software profit regression model to calculate the IC50 of the cell.

## **Cell viability assay**

A single-cell suspension was prepared and adjusted to  $4 \times 10^4$  cells/ml. Cells were seeded into 96-well plates ( $2 \times 10^3$  cells/well) and the cisplatin-containing medium of cisplatin treatment groups was replaced after cells were attached completely. According to the IC50 value of cells, the cisplatin concentration of medium of UCA1 overexpression groups was 2  $\mu$ g/ml, and that of UCA1 knockdown groups was 4  $\mu$ g/ml. The next day, the original liquid in the well was removed. 100  $\mu$ l culture medium containing 10% CCK8 was added into each well. The absorbance value was measured at 450 nm after incubation at 37 °C for 1 hour. This procedure was repeated for five consecutive days.

## **Cell migration and invasion assays**

Migration and invasion assay were performed with 8.0  $\mu$ m pore inserts in a 24-well plate. For migration assay,  $5 \times 10^4$  cells were seeded into the upper compartment of the Transwell inserts. The invasion assay was performed with Matrigel-coated filters. In the cisplatin treatment groups, medium containing 2  $\mu$ g/ml, 4  $\mu$ g/ml cisplatin was added into the upper and lower chambers. Cells could incubate for 24 and 48 h, respectively. Migrated and invaded were fixed by methanol and stained by 0.1% (w/v) crystal violet. Finally, five fields were randomly taken under the microscope to perform cell counting and statistical results. Each experiment was performed three times.

## **Colony formation assay**

300 cells were seeded onto 12-well plates. In the cisplatin treatment groups, medium containing 2  $\mu$ g/ml, 4  $\mu$ g/ml cisplatin was added. After 14 days of incubation, the cells were fixed with 4% paraformaldehyde for 15 mins and stained with 0.1% (w/v) crystal violet for 15 mins. The number of cell colonies (more than 50 cells) was counted and it indicated the ability of cell clone formation. The assay was conducted for three independent times.

## **Constructed lentivirus-mediated overexpression and siRNA vector**

The overexpression vector targeting UCA1 as well as a negative control (NC) transfected A549 cell. A549/DDP cell was transfected siRNA vector targeting UCA1 and negative control shNC (Genechem, Shanghai, China). Transfection was performed with seeding  $2 \times 10^5$  cells into a six-well plate, and after 24 h the medium was aspirated and incubated with transfection complex according to the manufacturer's protocol and MOI value (MOI=10). The A549/DDP and A549 cells were infected by lentivirus for 72 h and treated with 2 ug/ml puromycin, and the overexpression efficiency was detected by RT-qPCR. Detailed target sequences of siRNA and shNC were followed: CTCCTGGAAGCCACAAGATTA and TTCTCCGAACGTGTACGT.

### **Real time RT-PCR**

Total RNA was extracted using TRIzol reagent and reversely-transcribed into cDNA using a PrimeScript RT Reagent Kit (Takara, Dalian, China), in accordance with the manufacturer instructions. The levels of genes were measured Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The detailed primer sequences were shown in Table 1. A 10  $\mu$ l PCR system contained 1  $\mu$ l cDNA template, 0.4  $\mu$ l 10  $\mu$ M preserve primers, 0.4  $\mu$ l 10  $\mu$ M forward primers, 5  $\mu$ l TaKaRa TB Green™ Premix Ex Taq™ II (Takara, Dalian, China), 0.2  $\mu$ l ROX II (Takara, Dalian, China), and 3  $\mu$ l ddH<sub>2</sub>O.

### **Western blot analysis**

For harvesting the protein, cells were washed twice with PBS and lysed with 100  $\mu$ l ice cold 1 $\times$  RIPA lysis buffer (Beyotime, Shanghai, China). after growing to 80% density. For each sample, a total of 20  $\mu$ g protein was conducted electrophoresis in SDS-PAGE gel at 70 V for 40 minutes, 120 V for 60 min and then transferred to PVDF membrane at 300 mA for 60-120 minutes by ice-bath. The membrane was incubated with antibodies ERCC1, PCNA, Survivin, and  $\beta$ -actin (Proteintech, Chicago, USA) overnight at 4 °C. After washing with PBST, the PVDF membrane was incubated with second antibody for 1 h at room temperature. ECL kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to visualize and analyze the expression of indicated proteins. Finally, the protein bands were scanned and analyzed in Alpha View software for gray value. The greyscale of targeted bands was normalized to the greyscale of  $\beta$ -actin, and relative greyscale was analyzed using SPSS software.

### **RNA pulldown**

The primers of sense chain and antisense chain of LncRNA UCA1 were designed for PCR amplification. The PCR products were used as templates for in vitro transcription to obtain pure RNA. The RNA was labeled with biotin. The labeled RNA was mixed with magnetic beads. The cells with 90% growth density were rinsed with pre cooled PBS for three times. The mixture of RNA and magnetic beads was added to the cell lysate and incubated to form RNA-protein complex. The supernatant was purified and analyzed by protein mass spectrometry.

### **RNA immunoprecipitation**

Cells with 90% growth density were added into RIP lysate to prepare cell lysate. The magnetic beads were resuspended with RIP wash buffer and then incubated with 5 µg of corresponding antibody at room temperature for 30 min. The antibody-magnetic bead complex was added to the cell lysate to form RNA-protein-magnetic bead complex. The EP tube contained complex was placed on the magnetic frame and the supernatant was collected, and the RNA was purified and stored at -80 °C for standby.

### ***In vivo* tumorigenic ability**

24 BALB/c nude mice (male, 4 weeks) were purchased from the experimental animal center at Wenzhou Medical University (Wenzhou, China) and the protocols of animal studies were approved by Animal Experimental Ethical Inspection of Laboratory Animal Centre, Wenzhou Medical University (WYDW2020-0380). The mice were maintained and treated under specific pathogen-free conditions and were randomly divided into four groups (A549/DDP-Lenti-shNC, A549/DDP-Lenti-shUCA1, A549/DDP-Lenti-shNC+cislatin and A549/DDP-Lenti-shUCA1+cisplatin).  $1 \times 10^7$  cells were implanted into the right flank of mice via subcutaneous injection. Tumour sizes were calculated using a vernier calliper as follows: tumour volume ( $\text{mm}^3$ ) =  $(L \times W^2)/2$ , where L = long axis and W = short axis. When the tumor size reached 50  $\text{mm}^3$ , cisplatin (0.35 mg/kg) was injected intraperitoneally twice a week for four weeks. The nude mice were sacrificed at the end of experiment, and excised xenograft tumors were weighed.

### **Statistical analysis**

Statistical analyses were performed using SPSS 21.0 software and carried out using the two-sample t-test. The clinical data were analyzed using chi-square test or Fisher's exact test. Values of  $P < 0.05$  were considered statistically significant. NS was not significant; \* was  $P < 0.05$ ; \*\* was  $P < 0.01$ ; \*\*\* was  $P < 0.001$ .

## **Result**

### **UCA1 was highly expressed in LUAD tissues and cells and was related to the prognosis**

In the previous study, we screened the LncRNAs between A549/DDP cell and A549 cell through a high-throughput microarray. We found 984 LncRNAs were up-regulated more than two-fold in the A549/DDP group compared to A549 group, while 559 LncRNAs were down-regulated through the LncRNAs expression profiles and qRT-PCR. Among these, UCA1 was one of the significantly up-regulated genes. To further confirm this result, we detected the expression of UCA1 in A549 and A549/DDP cells, and found that UCA1 was high expressed in A549/DDP cells ( $t=10.35$ ,  $P < 0.001$ , Fig. 1A), which change trend was consistent with that of our previous study. Next, we investigated the UCA1 expression levels in LUAD tissues and normal adjacent tissues through TCGA database, and found that the expression of UCA1 was greatly higher in cancer tissues than in adjacent tissues (<http://ualcan.path.uab.edu/cgi-bin/TCGAExResultNew2.pl?genenam=UCA1&ctype=LUAD>,  $P < 0.001$ , Fig. 1B).

In addition, to investigate the correlation between UCA1 expression and clinical characteristics of LUAD patients, the 433 patients from TCGA database were divided into two groups according to gene expression, and expression levels higher than the median were classified into the high expression group; otherwise, they were classified into the low expression group. The result showed that no correlations were noted between UCA1 expression and age, gender, T stage, N stage, smoking history, and tumor location. While UCA1 was significantly associated with M stage ( $P=0.019$ ) and clinical stage ( $P=0.046$ , Table 2). Kaplan-Meier analysis showed that the overall survival time of patients with high UCA1 expression was significantly shorter than those with low expression level of UCA1 (<http://ualcan.path.uab.edu/cgi-bin/TCGA-survival1.pl?genenam=UCA1&ctype=LUAD>,  $P=0.021$ , Figure 1C).

These results shown the role of UCA1 in LUAD cancer development and cisplatin resistance and the potential as a biomarker to predict poor prognosis and cisplatin resistance in LUAD patients.

### **UCA1 promoted cell proliferation and reduced sensitivity to cisplatin in LUAD cell line**

RT-PCR showed that the RNA expression of UCA1 in overexpression group A549-Lenti-UCA1 was markedly elevated compared with the negative control group A549-Lenti-NC ( $t=54.71$ ,  $P<0.001$ , Fig. 2A). The expression level of UCA1 decreased significantly in A549/DDP-Lenti-shUCA1 group than that in A549/DDP-Lenti-shNC group ( $t=95.10$ ,  $P<0.001$ , Fig. 2B).

It was showed that the absorbance at 450 nm of A549-Lenti-UCA1 group was higher than that of A549-Lenti-NC cells at 24 h ( $t=15.03$ ,  $P=0.0044$ ), 48 h ( $t=7.248$ ,  $P=0.0185$ ), 72 h ( $t=3.390$ ,  $P=0.0275$ ), 96 h ( $t=4.477$ ,  $P=0.0465$ ), 120 h ( $t=12.00$ ,  $P=0.0069$ ) after overexpression of UCA1 (Fig. 2C). Under the effect of 2  $\mu\text{g/ml}$  cisplatin, the absorbance values of A549-Lenti-UCA1 group were still higher than that of A549-Lenti-NC group ( $t=4.578$ ,  $P=0.0102$ ;  $t=10.33$ ,  $P=0.0005$ ;  $t=3.817$ ,  $P=0.0316$ ;  $t=5.391$ ,  $P=0.0057$ ;  $t=4.357$ ,  $P=0.0121$ , Fig. 2C). In contrast, the absorbance of A549/DDP-Lenti-shUCA1 group at 450 nm at 48 h ( $t=5.358$ ,  $P=0.059$ ), 72 h ( $t=12.58$ ,  $P=0.002$ ), 96 h ( $t=10.21$ ,  $P=0.0005$ ) and 120 h ( $t=10.21$ ,  $P=0.0005$ ) were lower than those in A549/DDP-Lenti-shNC group (Fig. 2D). After 4  $\mu\text{g/ml}$  cisplatin treatment, except for 24 h the absorbance, the proliferation levels of A549/DDP-Lenti-shNC cells were still stronger than that of UCA1 knockdown cell lines ( $t=4.165$ ,  $P=0.0141$ ;  $t=8.800$ ,  $P=0.0009$ ;  $t=4.935$ ,  $P=0.0078$ ;  $t=3.756$ ,  $P=0.0198$ ; Fig. 2D). Proliferating cell nuclear antigen (PCNA) was an auxiliary protein involved in DNA replication and has been confirmed to be an indicator to evaluate the proliferation status of tumor cells[23-25]. PCNA protein expression was significantly increased after UCA1 overexpression ( $t=2.990$ ,  $P=0.0403$ , Fig. 2E) and was decreased after UCA1 knockdown ( $t=5.847$ ,  $P=0.0043$ , Fig. 2F). These results demonstrated that UCA1 could promote the proliferation of LUAD cells and reduce sensitivity to cisplatin in LUAD cell lines.

### **UCA1 promoted migration and invasion of LUAD cells**

We found that the number of A549-Lenti-UCA1 migrating cells passing through Transwell chamber was significantly higher than that in control group ( $t=2.596$ ,  $P=0.0318$ , Fig. 3A). After treated with 2  $\mu\text{g/ml}$  cisplatin, this trend was still obvious ( $t=8.352$ ,  $P<0.0001$ , Fig. 3A). On the contrary, A549/DDP-Lenti-shUCA1 group had fewer migration cells than the A549/DDP-Lenti-shNC group ( $t=5.754$ ,  $P=0.0004$ , Fig.

3B). The knockdown cells were treated with 4 µg/ml cisplatin, and the result was the same as without cisplatin treatment group ( $t=3.307$ ,  $P=0.0107$ , Fig. 3B).

The results of the Matrigel invasion assays also showed that UCA1 significantly enhanced the cell invasion capability in A549 cells ( $t=7.537$ ,  $P<0.0001$ ;  $t=3.173$ ,  $P=0.0131$ , Fig. 3C). However, the results were reversed after the knockdown of UCA1 ( $t=5.568$ ,  $P=0.0005$ ;  $t=3.325$ ,  $P=0.0105$ , Fig. 3D). These results suggested that overexpression of UCA1 promoted the migration and invasion of LUAD cells, whether under the influence of cisplatin drug.

### **UCA1 enhanced the clonogenic capability of LUAD cells**

Figure 4A showed that UCA1 overexpression group owed the higher colony forming ability than the negative control group ( $t=8.766$ ,  $P=0.0009$ ;  $t=5.935$ ,  $P=0.0040$ ; Fig. 4A). This result was reversed in UCA1 knockdown group ( $t=6.649$ ,  $P=0.0027$ ;  $t=5.375$ ,  $P=0.0058$ ; Fig. 4B). These results demonstrated that UCA1 markedly enhanced the colony forming ability of LUAD cells.

### **UCA1 enhanced cisplatin resistance of LUAD cells by ERCC1**

Cell IC<sub>50</sub> assay showed that the IC<sub>50</sub> value to cisplatin of A549 cells increased from  $0.865\pm 0.071$  µg/ml to  $1.878\pm 0.037$  µg/ml ( $t=21.92$ ,  $P<0.001$ , Fig. 5A). After UCA1 knockdown, the IC<sub>50</sub> value of A549/DDP cells decreased from  $5.135\pm 0.472$  µg/ml to  $4.021\pm 0.377$  µg/ml ( $t=3.193$ ,  $P=0.0331$ , Fig. 5B).

Excision repair cross complementing gene 1 (ERCC1) protein have been confirmed to be highly correlated with cisplatin resistance in non-small cell lung cancer[26]. We detected the expression of ERCC1 in the cells, and found that ERCC1 increased with the increase of UCA1 expression ( $t=4.010$ ,  $P=0.016$ , Fig. 5C) and decreased with the decrease of UCA1 expression ( $t=6.911$ ,  $P=0.0023$ , Fig. 5D). According to the results, as ERCC1 involved in DNA repair pathway, we inferred that UCA1 might improve cisplatin induced DNA damage by activating the DNA repair pathway to enhance cell cisplatin resistance of LUAD cells.

### **UCA1 promoted proliferation and cisplatin resistance of LUAD cells in vivo**

The tumor formation ability of A549/DDP-Lenti-shNC and A549/DDP-Lenti-shUCA1 cells was checked by nude mice inoculation experiment. As shown in Fig. 6A, after 8 days, the transplanted xenograft tumors were constructed. There was no significant difference in the volume of transplanted tumor except the 10th day. At other time points, such as Day 8 ( $t=2.704$ ,  $P=0.0221$ ), Day 14 ( $t=3.581$ ,  $P=0.0050$ ), Day 16 ( $t=3.566$ ,  $P=0.0051$ ), Day 18 ( $t=3.711$ ,  $P=0.005$ ), Day 22 ( $t=4.110$ ,  $P=0.0021$ ), Day 22 ( $t=4.315$ ,  $P=0.0015$ ), Day 27 ( $t=4.670$ ,  $P<0.001$ ), Day 32 ( $t=6.207$ ,  $P<0.001$ ), Day 35 ( $t=8.725$ ,  $P<0.001$ ) and Day 38 ( $t=6.295$ ,  $P<0.001$ ), the tumor volume of A549/DDP-Lenti-shNC group was significantly higher than that of and A549/DDP-Lenti-shUCA1 cells. The results showed that the weight of nude mice in A549/DDP-Lenti-shNC group ( $20.300\pm 2.326$  g) was slightly lower than that in A549/DDP-Lenti-shUCA1 group ( $21.533\pm 2.482$  g), but the difference was not statistically significant (Fig.6B). While, the weight of transplanted tumor in A549/DDP-Lenti-shUCA1 group ( $0.074\pm 0.042$  g) was significantly lower than that in A549/DDP-Lenti-shNC group ( $0.310\pm 0.066$ g) ( $t=7.354$ ,  $P<0.001$ , Fig. 6CD). The tumor growth was most significantly

inhibited in mice following UCA1 knockdown compared with the NC groups, which indicated that UCA1 played an important role in regulating the growth of LUAD cells *in vivo*.

With cisplatin treatment, the mice body weight of A549/DDP-Lenti-shUCA1+DDP group decreased from  $19.417 \pm 1.137$  g to  $17.067 \pm 0.784$  g and that of A549/DDP-Lenti-shNC+DDP group changed from  $20.383 \pm 0.947$  g to  $18.000 \pm 1.643$  g, but there were no significant difference between the two groups both before and after chemotherapy (Fig 7A). After intraperitoneal injection of cisplatin, the inhibition degree of transplanted tumor in A549/DDP-Lenti-shUCA1+DDP group was significantly higher than that in the control group. On the day 1 of chemotherapy ( $t=2.992$ ,  $P=0.0281$ ), day 3 ( $t=7.976$ ,  $P<0.001$ ), day 6 ( $t=4.886$ ,  $P=0.0038$ ), day 11 ( $t=10.42$ ,  $P<0.001$ ), day 14 ( $t=9.197$ ,  $P<0.001$ ), day 17 ( $t=10.53$ ,  $P<0.001$ ), day 21 ( $t=12.26$ ,  $P<0.001$ ), day 24 ( $t=8.150$ ,  $P<0.001$ ), day 27 ( $t=9.027$ ,  $P<0.001$ ), day 29 ( $t=7.794$ ,  $P<0.001$ ), the tumor volume of A549/DDP-Lenti-shUCA1+DDP group was significantly lower than that of A549/DDP-Lenti-shNC+DDP group (Fig.7B). After chemotherapy, the tumor weight of A549/DDP-Lenti-shUCA1+DDP group was  $0.025 \pm 0.009$  g, which was significantly lower than that of A549/DDP-Lenti-shNC+DDP group ( $0.285 \pm 0.071$ g,  $t=0.885$ ,  $P<0.001$ , FIG. 7CD). These results demonstrated that Knockdownnce with UCA1 restored the sensitivity of A549/DDP cells to cisplatin. Taken together, UCA1 promoted proliferation and cisplatin resistance of LUAD cells *in vivo*.

### The RNA binding protein ENO1 of UCA1 was obtained

Through RNA pulldown and protein mass spectrometry (MS), we analyzed the proteins of RNA pulldown products of the UCA1\_sense and UCA1\_antisense. A total of 441 proteins were identified, among which 219 proteins were identified in both 2 samples, while 75 proteins were identified unique in UCA1\_sense and 147 unique proteins in UCA1\_antisense (Fig. 8A). Detail information of some protein of the MS results were shown in Table 3.

According to the results of protein mass spectrometry, was selected for subsequent experiments. Enolase 1 (ENO1), known as coding enolization enzyme 1, played a key role in in glucose metabolism and tumor development[27]. It was reported that ENO1 was highly expressed in lung cancer tissues and promoted LUAD progression by regulating the glycolytic pathway[28]. We explored whether ENO1 was bound to UCA1 by RIP experiment. The results confirmed that UCA1 was highly expressed in ENO1 immunoprecipitated ( $t=6.859$ ,  $P=0.0024$ , Fig. 8B), indicating that ENO1 possibly was one of the RNA binding proteins of UCA1. It was quite possible that UCA1 might co-engage with ENO1 in regulating cisplatin resistance mechanisms in LUAD.

## Discussion

By LncRNA microarray, we analyzed the differentially expressed genes between cisplatin sensitive cells A549 and cisplatin resistant cells A549/DDP, in which UCA1 expression was up-regulated. Consistent with the results from TCGA database, we discovered that UCA1 in LUAD tissues and A549/DDP cells were highly expressed by RT-QPCR. UCA1 was correlated with metastasis, worse clinical staging and prognosis and were consistent with the previous studies[29–31]. The results suggested that UCA1 was

involved in the development and cisplatin resistance of LUAD and could be used as a biomarker to predict the prognosis and cisplatin resistance.

We found that after UCA1 overexpressed, the capability of cell proliferation, migration and invasion were enhanced. The existing relevant research results showed that UCA1 could participate in cell proliferation[32–34], migration and invasion[35, 36] to regulate tumor progression. Therefore, we detected the IC50 value of LUAD cell lines, and confirmed that IC50 value to cisplatin increased after UCA1 overexpression. This suggested that UCA1 was likely to increasing cisplatin resistance ability of LUAD. Nevertheless, the specific mechanism still needs further research, and remains to be explored.

RNA-binding proteins (RBPs) could bind to their target RNA, form ribonucleoprotein complexes, and regulate gene expression after transcription[37]. And a total of 441 proteins were identified. We selected ENO1 protein to perform RIP experiments. The RIP result showed that expression of UCA1 was increased in the ENO1 immunoprecipitation complex, which indicated that ENO1 was likely to be the RNA binding protein of UCA1. ENO1 has been identified to be involved in the process of drug resistance in many types of tumor cells[38–40]. Therefore, UCA1 may bind with ENO1 and affect the expression of ENO1 and its target genes.

## Conclusion

In summary, UCA1 was involved in the regulation of cell proliferation, migration and invasion, enhanced cell cisplatin resistance in LUAD. High expression of UCA1 predicted a poor prognosis and was associated with tumor distant metastasis and high tumor grade in LUAD patients. ENO1, as one of the RNA binding proteins of UCA1, was regulated by UCA1 to exert its function (Fig. 9). This provided a potential diagnostic marker and therapeutic target for lung cancer and cisplatin resistance of LUAD, which was expected to effectively prolong the survival time and improve the quality of life of LUAD patients.

## Abbreviations

LUAD: lung adenocarcinoma; LncRNA: long noncoding RNA; UCA1: urothelial carcinoma-associated 1; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; RT-qPCR: reverse transcription-quantitative polymerase chain reaction; TCGA: The Cancer Genome Atlas; RPMI-1640: Roswell Park Memorial Institute-1640; FBS: fetal bovine serum; CCK8: cell count kit-8; NC: negative group; Lenti: lentivirus; RIP: RNA immunoprecipitation technique; MS: mass spectrometry; WB: western blotting; ENO1: Enolase 1; ERCC1: Excision repair cross complementing gene 1; PCNA: Proliferating cell nuclear antigen.

## Declarations

## Acknowledgements

Not applicable.

### **Ethics approval and consent to participate**

The protocols of animal studies were approved by Animal Experimental Ethical Inspection of Laboratory Animal Centre, Wenzhou Medical University (WYDW2020-0380).

### **Funding information**

This study was financially supported by the National Natural Science Foundation of China (81672088), Zhejiang Provincial Natural Science Foundation (LY19H200002), the Wenzhou Municipal Science and Technology Bureau of China (Y20190461), Zhejiang Provincial Research Center for Cancer Intelligent Diagnosis and Molecular Technology (JBZX-202003).

### **Authors' contributions**

WMW and KTH designed the study; JLF, JJP, and XY performed the experiments and prepared the figures; YZ, FGS, and JC contributed to drafting the manuscript. All authors read and approved the final manuscript

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors confirm that there are no conflicts of interest.

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41. Legend.

## Tables

Table 1. The detailed primer sequences for real time RT-PCR.

Gene	Primer sequences	
	Forward	Reverse
UCA1	ACGCTAACTGGCACCTTGTT	CTCCGGACTGCTTCAAGTGT
β-actin	CCTGGCACCCAGCACAAT	GCTGATCCACATCTGCTGGAA
ENO1	ACCCAGTGGCTAGAAGTTCAC	CCATGGGCTGTGGGTTCTAA

Table 2. Relationship between expression of UCA1 and clinicopathological characteristics in LUAD patients. N(%)

Group	Total [n=433]	UCA1		P value
		high expression	low expression	
Gender				0.229
Male	198	93(46.97)	105 (53.03)	
Female	235	124 (52.77)	111 (47.23)	
Age (year)				0.598
>65	224	115 (51.34)	109 (48.66)	
≤65	209	102 (48.80)	107 (51.20)	
N stage				0.706
N0	281	142 (50.53)	139 (49.47)	
N1~3	142	69 (48.59)	73 (51.41)	
NX#	10	6 (60.00)	4 (40.00)	
T stage				0.383
T1~2	376	185 (49.20)	191 (50.80)	
T3~4	54	30 (55.56)	24 (44.44)	
TX#	3	2 (66.67)	1 (33.33)	
M stage				0.019*
M0	286	137 (47.90)	149 (52.10)	
M1	20	15 (75.00)	5 (25.00)	
MX#	127	65 (51.18)	62 (48.82)	
Clinical stage				0.046*
I, II	344	164 (47.67)	180 (52.32)	
III, IV	89	53 (59.55)	36 (40.45)	
Smoking				0.434
No	66	36 (54.55)	30 (45.45)	
Yes	277	181 (49.32)	186 (50.68)	
Tumor location				0.217
superior lobe of left lung	108	61 (56.48)	47 (43.52)	
inferior lobe of left lung	65	28 (43.08)	37 (56.92)	
superior lobe of right lung	159	72 (45.28)	87 (54.72)	
middle lobe of right lung	17	10 (58.82)	7 (41.18)	
inferior lobe of right lung	84	46 (54.76)	38 (45.23)	

#: "X" means that the tumor could not be evaluated or measured. The clinicopathological data of these patients was not included in statistical tests.

\*: The difference was statistically significant.

Table 3. Detail information of some proteins of . UCA1\_sense and UCA1\_antisense detected by protein MS after RNA pulldown.

Protein ID	Coverage (%)	Mass (Da)	Unique Peptide	Identified by
sp P11498 PYC_HUMAN	53.9900004863739	129632.6	76	UCA1_antisense
sp Q13085 ACACA_HUMAN	19.2699998617172	265551.7	39	
sp P08238 HS90B_HUMAN	42.960000038147	83263.5	22	UCA1_sense
sp P05783 K1C18_HUMAN	55.5800020694733	48057.4	34	
sp P63261 ACTG_HUMAN	74.9300003051758	41792.5	2	UCA1_sense
sp P11498 PYC_HUMAN	39.5599991083145	129632.6	54	
sp Q13085 ACACA_HUMAN	11.0399998724461	265551.7	25	UCA1_sense
sp P60709 ACTB_HUMAN	49.329999089241	41736.4	2	
sp P08238 HS90B_HUMAN	22.5099995732307	83263.5	11	UCA1_sense
sp P68371 TBB4B_HUMAN	33.0300003290176	49830.7	4	

# Figures

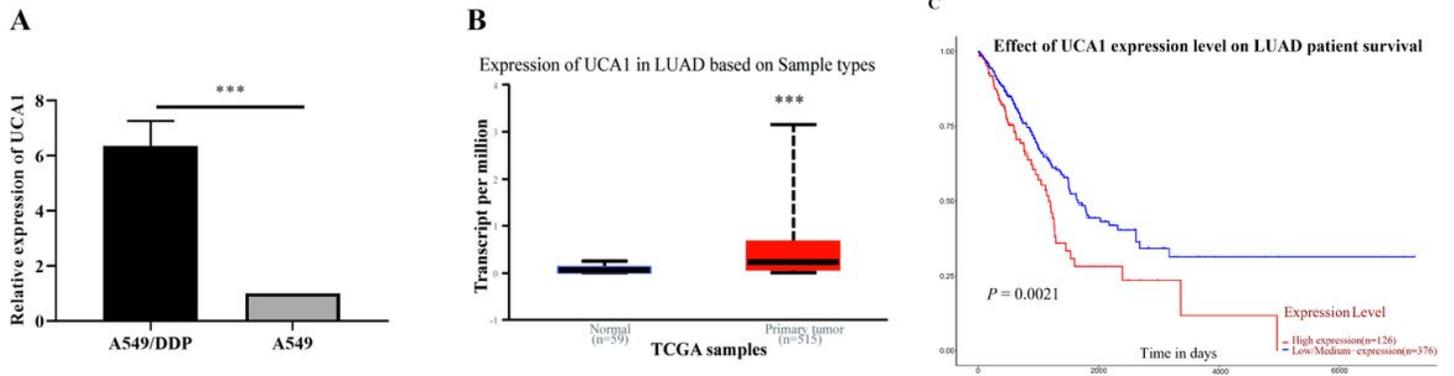
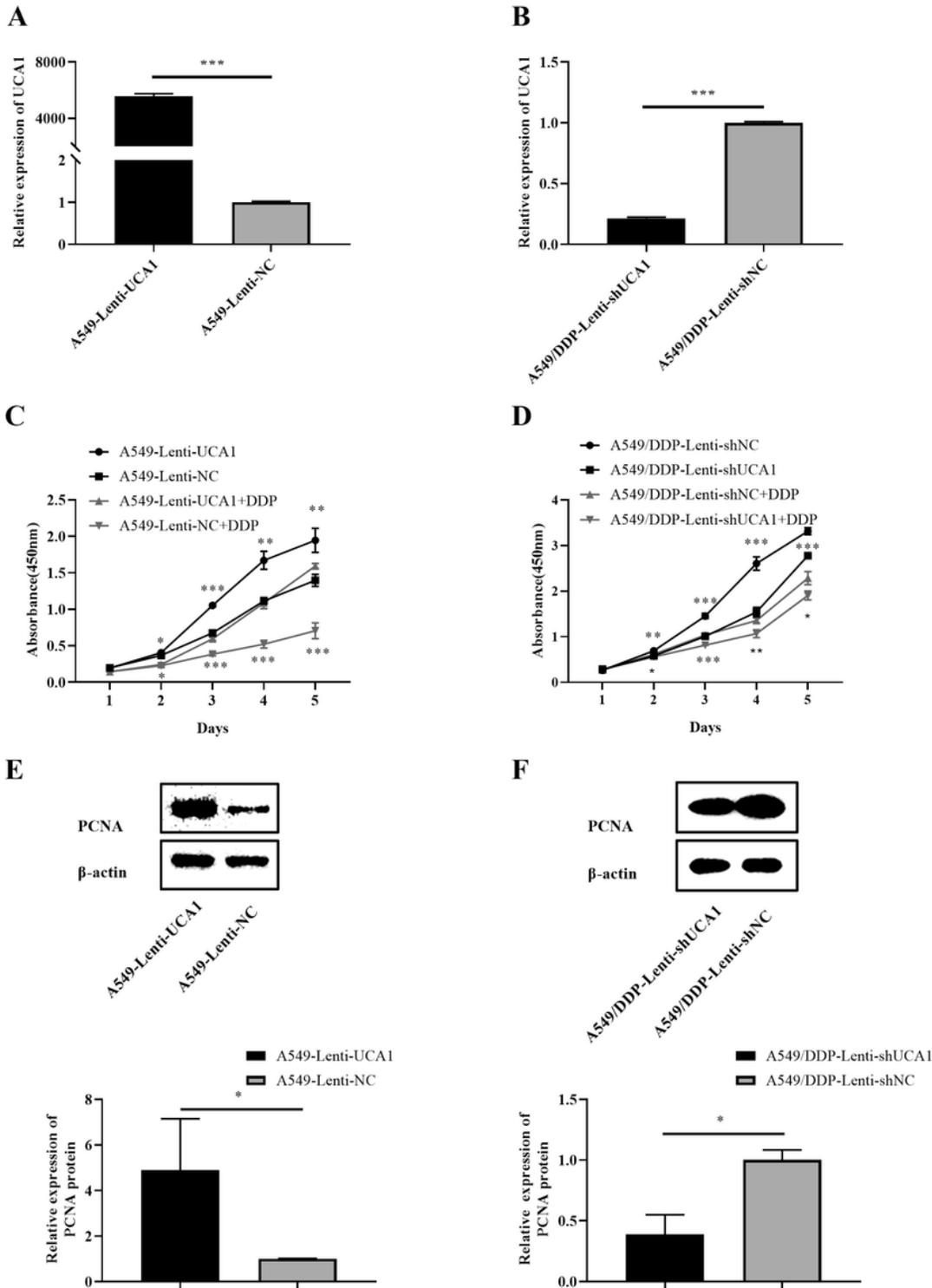


Figure 1

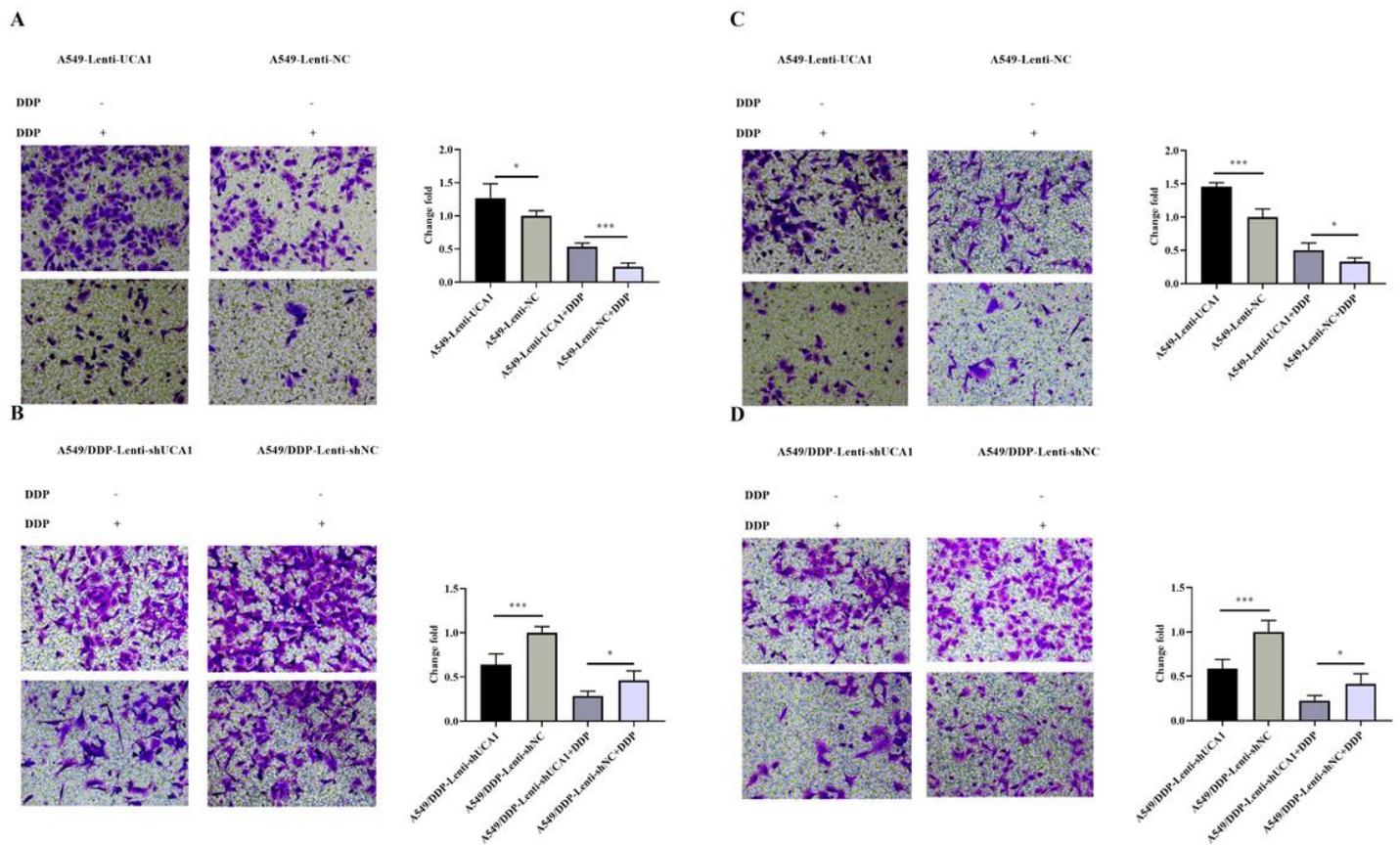
Expression of UCA1 in LUAD and LUAD cell lines and its role in poor prognosis LUAD patients. (A) The expression level of UCA1 in A549/DDP was significantly higher than in A549 cells ( $t=10.35$ ,  $P<0.001$ ). (B) UCA1 was highly expressed in LUAD cancer tissues than corresponding normal tissues (NT) by RT-qPCR ( $t=2.021$ ,  $P=0.0491$ ). (C) The TCGA database indicated that UCA1 expression was higher in tumor tissues than in adjacent normal tissues ( $P<0.001$ ). (D) Kaplan-Meier analysis showed that LUAD patients with higher UCA1 expression had a shorter survival time ( $P=0.021$ ). NT: normal tissue; \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .



**Figure 2**

UCA1 contributed to the proliferative capacity of LUAD cells. (A) The expression of UCA1 in overexpression group A549-Lenti-UCA1 cells was remarkably increased comparing with control group A549-Lenti-NC cells ( $t=54.71$ ,  $P<0.001$ ). (B) The knockdown group A549/DDP-Lenti-shUCA1 cells showed obviously decreased expression of UCA1 than control group A549/DDP-Lenti-shNC ( $t=95.10$ ,  $P<0.001$ ). (C) Under the same conditions, the absorbance values of overexpression group A549-Lenti-UCA1 cell were

higher than that of control group A549-Lenti-NC cells, the difference was statistically significant (Without cisplatin treatment: 24 h:  $t=15.03$ ,  $P=0.0044$ ; 48 h:  $t=7.248$ ,  $P=0.0185$ ; 72 h:  $t=3.390$ ,  $P=0.0275$ ; 96 h:  $t=4.477$ ,  $P=0.0465$ ; 120 h:  $t=12.00$ ,  $P=0.0069$ . With cisplatin treatment: 24 h:  $t=4.578$ ,  $P=0.0102$ ; 48 h:  $t=10.33$ ,  $P=0.0005$ ; 72 h:  $t=3.817$ ,  $P=0.0316$ ; 96 h:  $t=5.391$ ,  $P=0.0057$ ; 120 h:  $t=4.357$ ,  $P=0.012$ ). (D) Without cisplatin treatment, the absorbance values of A549/DDP-Lenti-shUCA1 cells were lower than A549/DDP-Lenti-shNC cells at 450 nm (48 h:  $t=5.358$ ,  $P=0.059$ ; 72 h:  $t=12.58$ ,  $P=0.002$ ; 96 h:  $t=10.21$ ,  $P=0.0005$ ; 120 h:  $t=10.21$ ,  $P=0.0005$ ). Under the action of cisplatin, the trend was still the same (48 h:  $t=4.165$ ,  $P=0.0141$ ; 72 h:  $t=8.800$ ,  $P=0.0009$ ; 96 h:  $t=4.935$ ,  $P=0.0078$ ; 120 h:  $t=3.756$ ,  $P=0.0198$ ). (E) The protein expression and of PCNA was enhanced upon UCA1 overexpression ( $t=2.990$ ,  $P=0.0403$ ). (F) After UCA1 knockdown, the protein expression of PCNA was decreased ( $t=5.847$ ,  $P=0.0043$ ). \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .

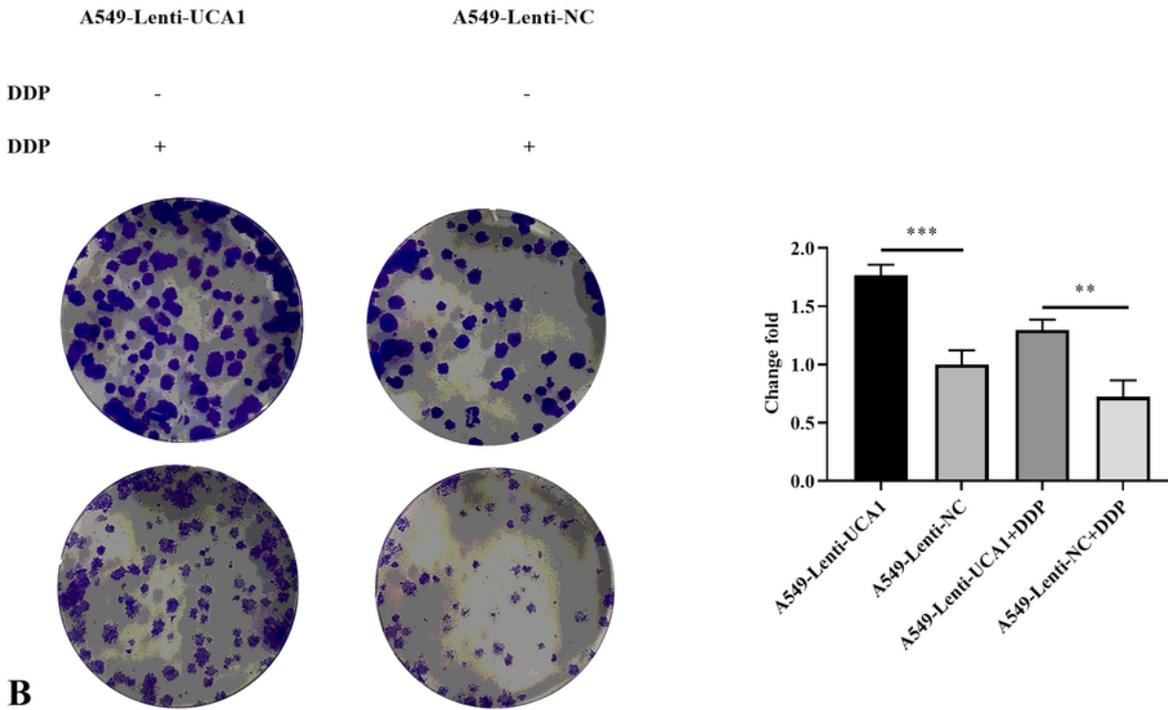


**Figure 3**

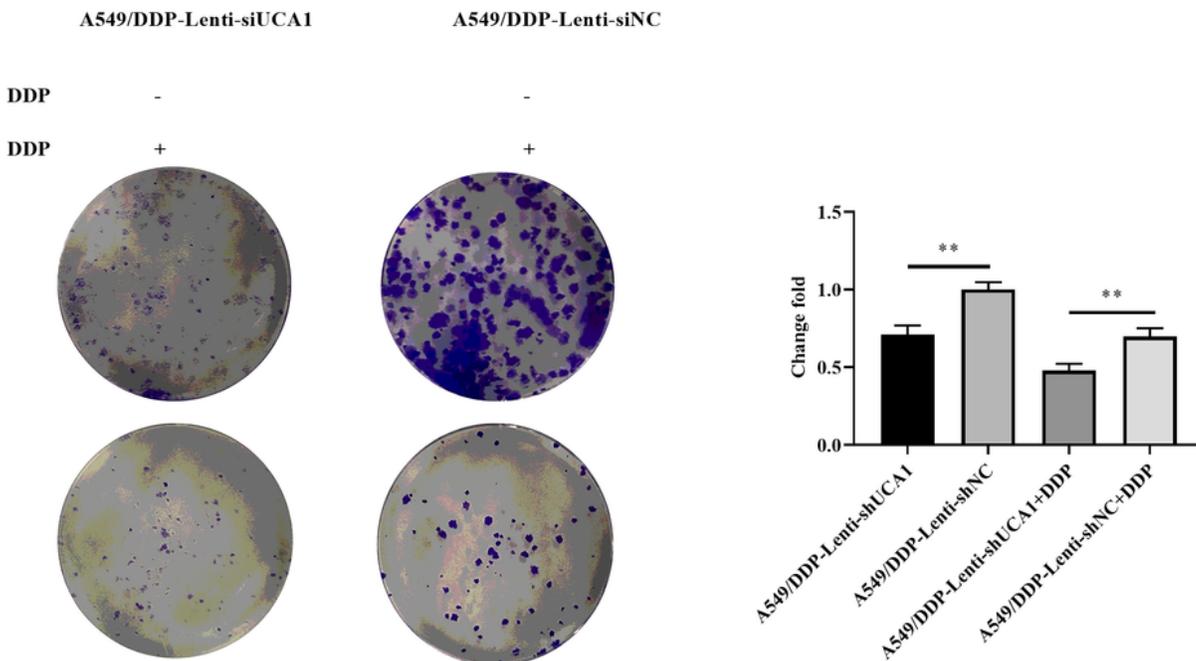
Changes of LUAD cells migration and invasion after UCA1 overexpression and knockdown. (A) Following overexpression of UCA1, the migrating cells passing through Transwell chamber of A549-Lenti-UCA1 group was significantly higher than that in control group ( $t=2.596$ ,  $P=0.0318$ ). After treated with 2  $\mu\text{g/ml}$  cisplatin, this trend was still obvious ( $t=8.352$ ,  $P<0.0001$ ). (B) The migration ability of A549/DDP-Lenti-shUCA1 group was decreased than the A549/DDP-Lenti-shNC group ( $t=5.754$ ,  $P=0.0004$ ). Treated with 4  $\mu\text{g/ml}$  cisplatin, and the result was the same as without cisplatin treatment group ( $t=3.307$ ,  $P=0.0107$ ). (C) An increase of invasion ability was seen after overexpression of UCA1 ( $t=7.537$ ,  $P<0.0001$ ;

t=3.173, P=0.0131). (D) The decrease in cells invasion ability was significant for A549/DDP-Lenti-shUCA1 (t=5.568, P=0.0005; t=3.325, P=0.0105). \*: P<0.05, \*\*: P<0.01, \*\*\*: P<0.001.

**A**



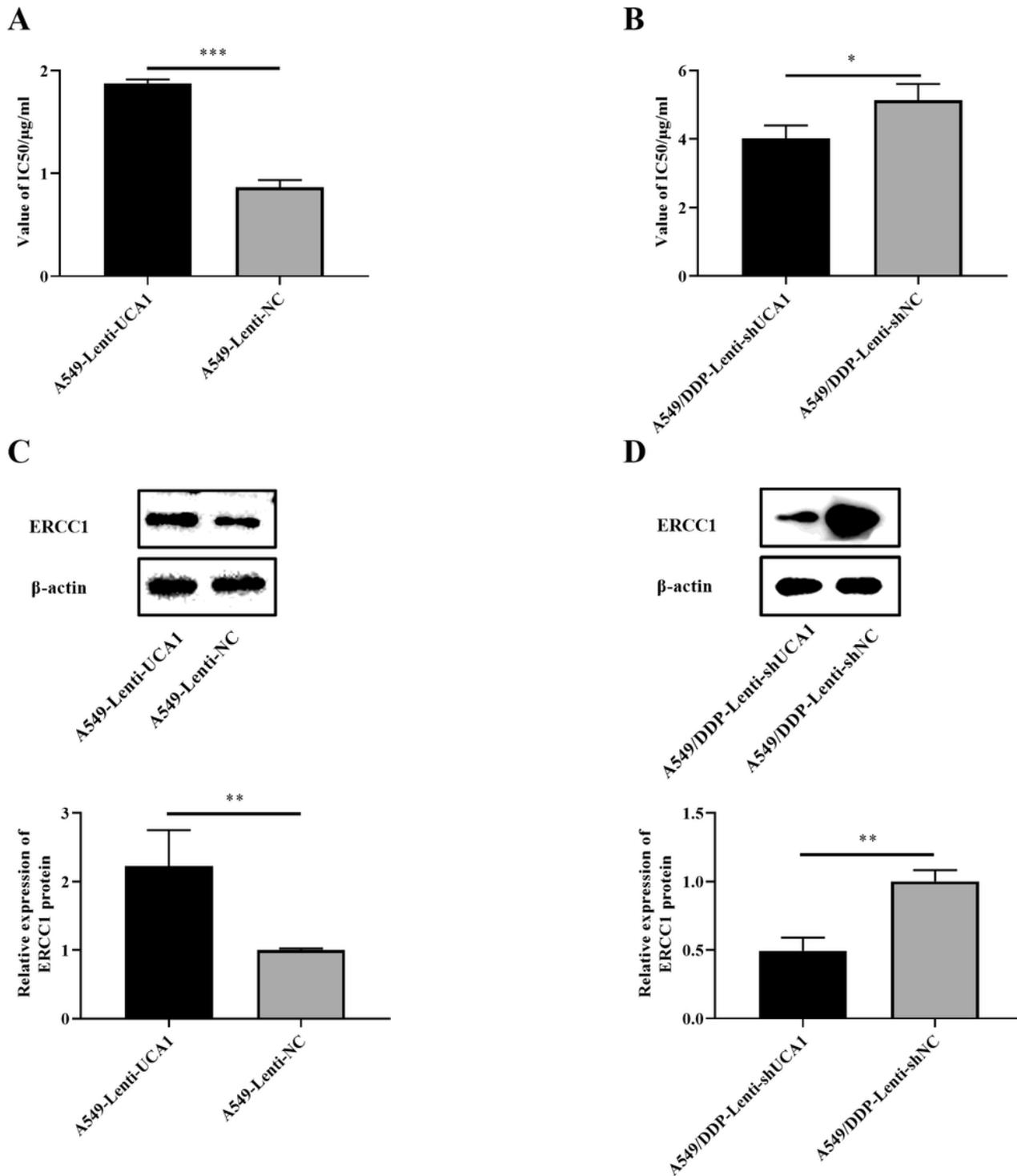
**B**



**Figure 4**

The colony-forming assay showed significantly more colonies after overexpression of UCA1 in A549 cells compared with negative control and UCA1 knockdownnce reversed the increase of cloning ability. (A) The number of clones of A549-Lenti-UCA1 cells was increased than A549-Lenti-NC cells both without cisplatin

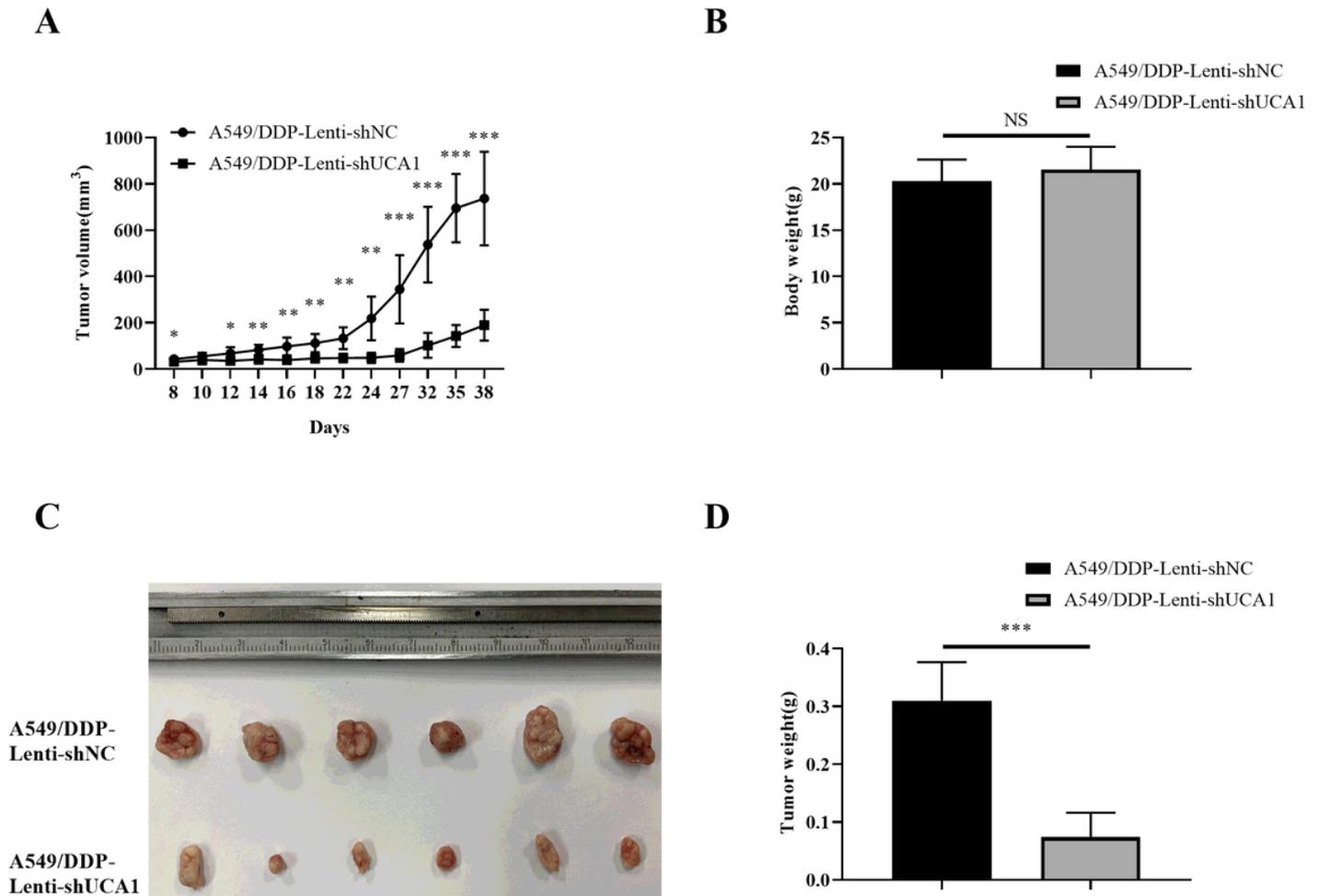
and treated with 2  $\mu\text{g/ml}$  cisplatin ( $t=8.766$ ,  $P=0.0009$ ;  $t=5.935$ ,  $P=0.0040$ ). (B) Less cell colonies in A549/DDP-Lenti-shUCA1 than control group ( $t=6.649$ ,  $P=0.0027$ ;  $t=5.375$ ,  $P=0.0058$ ). \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .



**Figure 5**

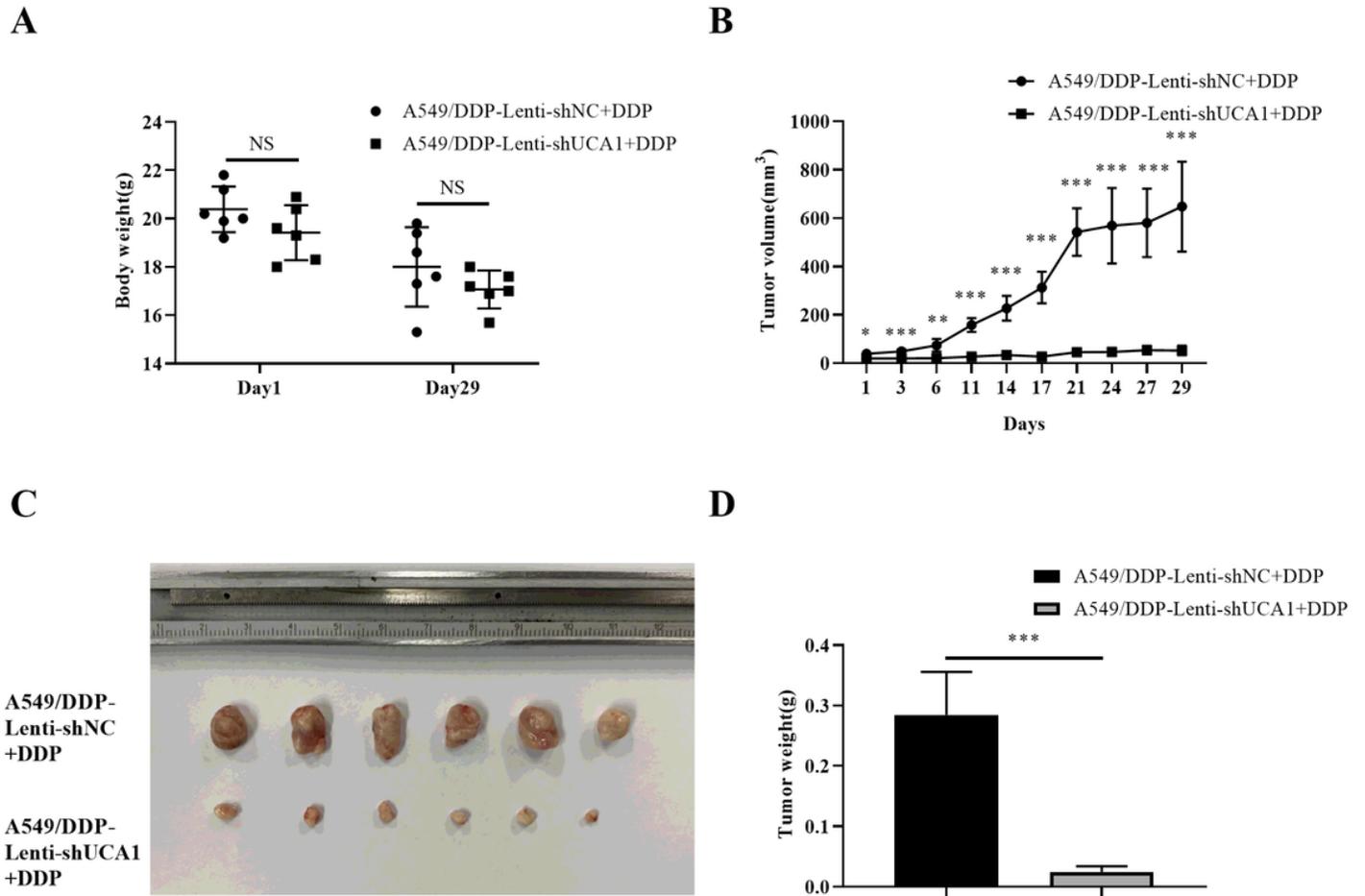
UCA1 enhanced the cisplatin resistance ability of LUAD cell lines. (A) After overexpression of UCA1, the IC50 value of cisplatin was increased. The IC50 value of A549-Lenti-UCA1 group was  $1.878 \pm 0.037$

$\mu\text{g/ml}$ , the control group was  $0.865 \pm 0.071 \mu\text{g/ml}$  ( $t=21.92$ ,  $P<0.001$ ). (B) After overexpression of UCA1, the  $\text{IC}_{50}$  value of cisplatin was decreased. The  $\text{IC}_{50}$  value of A549/DDP-Lenti-shUCA1 group was  $4.021 \pm 0.377 \mu\text{g/ml}$ , A549/DDP-Lenti-shNC group was  $5.135 \pm 0.472 \mu\text{g/ml}$  ( $t=3.193$ ,  $P=0.0331$ ). (C) In UCA1 overexpression group, the protein expression of ERCC1 was obviously increased ( $t=4.010$ ,  $P=0.016$ ). (D) After UCA1 knockdown, the protein expression of ERCC1 was significantly reduced ( $t=6.911$ ,  $P=0.0023$ ). \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .



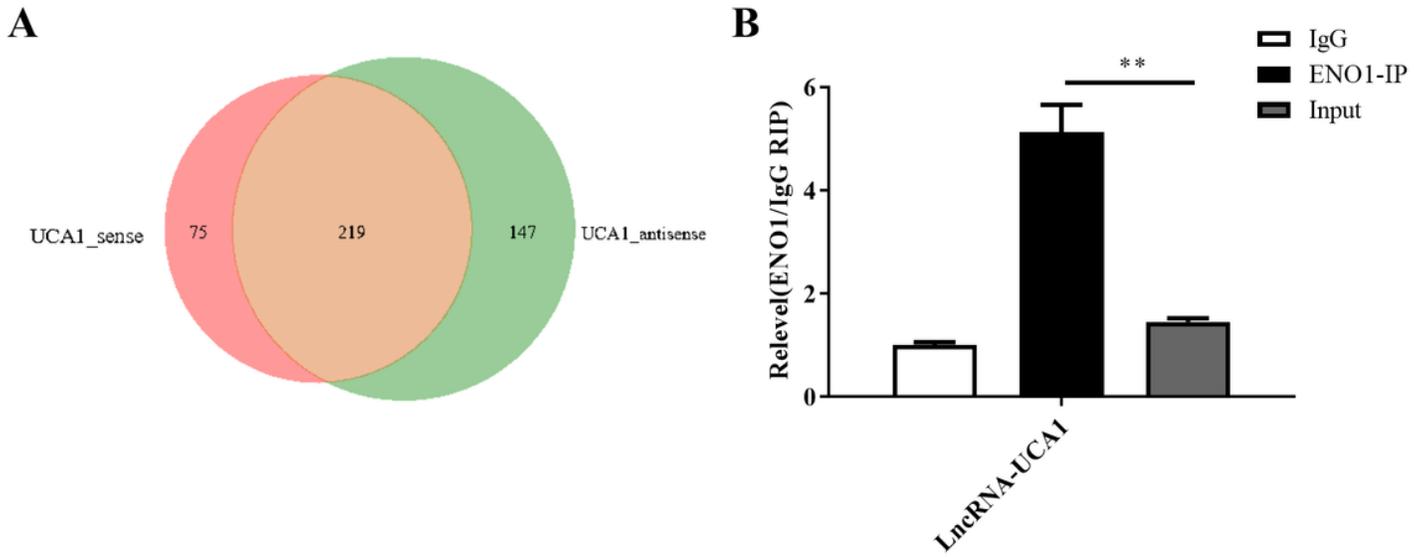
**Figure 6**

UCA1 promoted the growth of cisplatin resistant lung adenocarcinoma cells in vivo. (A) The growth rate of transplanted tumor in UCA1 knockdown group was significantly slower than that in control group; (B) the average weight of nude mice in UCA1 knockdown group and control group had no significant difference between the two groups; (C) the volume of transplanted tumor in A549/DDP-Lenti-shUCA1 group was significantly smaller than that in control group; (D) the weight of transplanted tumor in A549/DDP-Lenti-shNC group was significantly higher than that in UCA1 knockdown group. NS means no statistical difference. NS: not significant, \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .



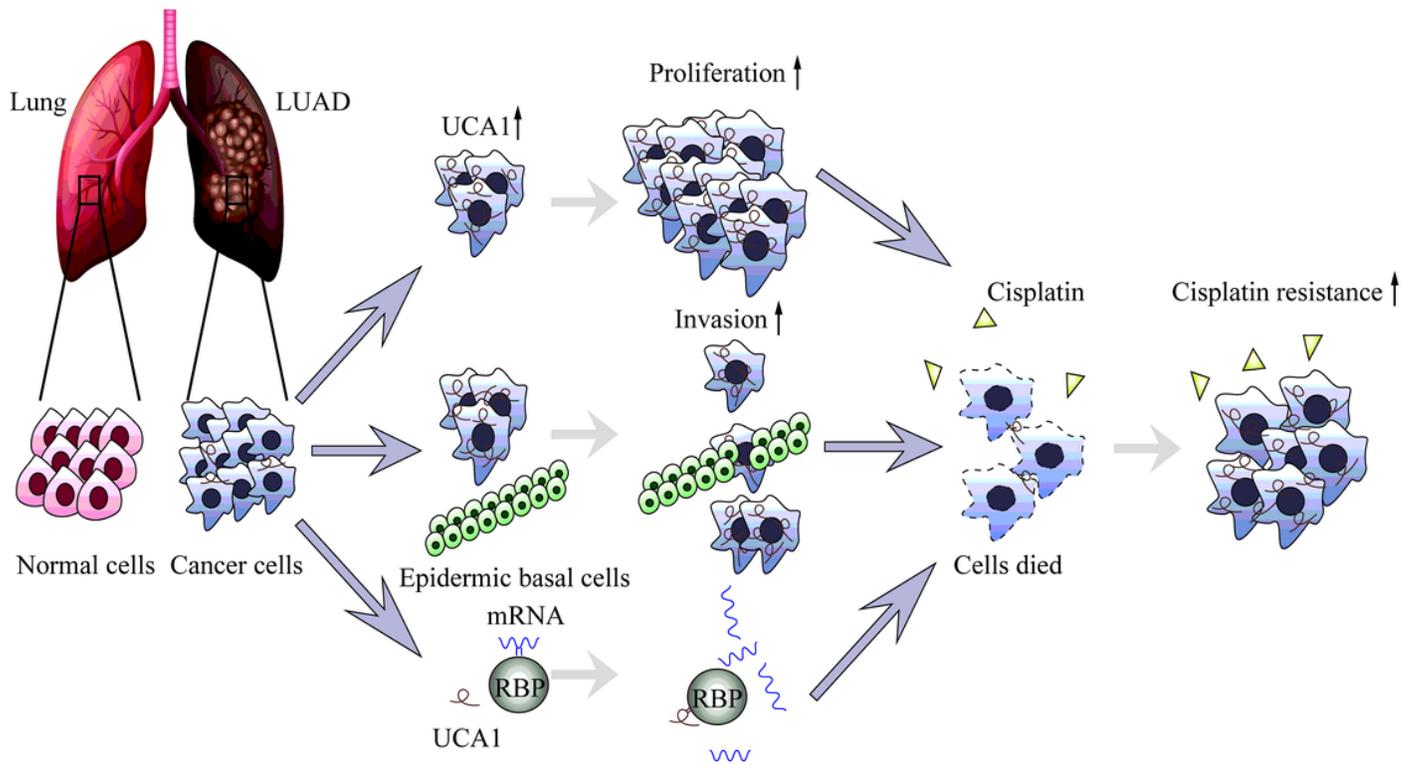
**Figure 7**

Knockdownnce with UCA1 restored cisplatin sensitivity of cisplatin resistant cell lines in vivo. (A) The two groups of nude mice treated with cisplatin showed different degrees of weight loss; (B) The growth of transplanted tumor in UCA1 knockdownnce group was significantly inhibited by cisplatin; (C) The transplanted tumor of A549/DDP-Lenti-shNC+DDP and A549/DDP-Lenti-shUCA1+DDP groups; (D) the weight of transplanted tumor in A549/DDP-Lenti-shUCA1+DDP group was significantly lower than that in A549/DDP-Lenti-shNC+DDP. NS: not significant, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .



**Figure 8**

The RNA binding protein ENO1 of UCA1 was obtained. (A) A Venn diagram showed the proteins of UCA1\_sense and UCA1\_antisense detected by protein MS after RNA pulldown. 219 proteins were identified in both 2 samples, while 75 proteins were identified unique in UCA1\_sense and 147 unique proteins in UCA1\_antisense. (B) The RIP experiment showed that the expression of UCA1 in ENO1 immunoprecipitate was significantly higher than that in control ( $t=6.859$ ,  $P=0.0024$ ). \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .



**Figure 9**

UCA1 participated in LUAD progression and chemoresistance. UCA1 was highly expressed in LUAD tissues in comparison to the adjacent normal lung tissues. After up-regulated UCA1 expression, the proliferation, migration and invasion of LUAD cells were significantly improved. UCA1 bound with RNA binding proteins like ENO1 to regulate the expression of RNP target mRNA. Overall, UCA1 overexpression cells had an enhanced capacity of cisplatin resistance.