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Long non-coding RNA UCA1 promotes the progression of paclitaxel resistance in ovarian cancer by regulating the miR-654-5p/SIK2 axis

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Abstract. – OBJECTIVE: Ovarian cancer (OC) is a common tumor in women, and the development of chemoresistance is the major obstacle to its treatment. Long non-coding RNAs (LncRNAs) have been linked to chemoresistance in many cancers. However, the function of lncRNA urothelial carcinoma associated1 (UCA1) in paclitaxel (PTX) resistance of OC is not well elucidated.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of UCA1, microRNA-654-5p (miR-654-5p) and salt inducible kinase 2 (SIK2). Cell PTX resistance and proliferation were evaluated by 3-(4, 5-dimethyl-2 thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay. The abilities of apoptosis, migration and invasion were measured by Flow cytometry and Transwell assays, respectively. Dual-luciferase reporter assay was used to verify the interaction among UCA1, miR-654-5p and SIK2. Besides, Western blot analysis was performed to assess the protein level of SIK2.

RESULTS: UCA1 was markedly upregulated in OC tissues and PTX-resistant OC cells. Silencing of UCA1 restrained the PTX resistance, reduced the proliferation, migration, invasion and enhanced the apoptosis of PTX-resistant OC cells. MiR-654-5p could be sponged by UCA1, and the inhibitory effect of its overexpression on the progression of PTX-resistant OC cells could be reversed by overexpressed-UCA1. Moreover, SIK2 was a target of miR-654-5p. Silencing of SIK2 could hinder the PTX resistance and suppress the progression of PTX-resistant OC cells, while miR-654-5p inhibitor could invert this inhibitory effect. Also, the expression of SIK2 was regulated by miR-654-5p and UCA1 expression.

CONCLUSIONS: LncRNA UCA1 plays an active role in PTX resistance of OC and is crucial to maintain the development of PTX resistance in OC, which provides a new therapeutic target for the study of OC chemoresistance.

Key Words:
Ovarian cancer, UCA1, MiR-654-5p, SIK2, PTX resistance.

Introduction

Ovarian cancer (OC) is one of the most common malignant tumors of the female reproductive system, among which epithelial OC (EOC) has the highest mortality rate among all kinds of female tumors, seriously threatening the life safety of the women[1,2]. According to statistics, the United States had 22,240 new cases of OC patients in 2018, and more than 14,000 women died of OC[3]. At present, surgery combined with chemotherapy is the main treatment for OC, among which paclitaxel (PTX) combined with platinum is the first-line chemotherapy for OC[4,5]. Despite many efforts, the occurrence of chemoresistance has seriously hindered the process of OC treatment. Therefore, the search for biomarkers affecting OC resistance is expected to provide a theoretical basis for solving the problem of OC resistance.

Long non-coding RNAs (lncRNAs) are a kind of non-coding protein RNAs with a length of 200 nucleotides, which have been proved to be used as biomarkers to participate in the development of many diseases, including OC[6,7]. Moreover, abnormally expressed lncRNAs are implicated with the development of chemoresistance in a variety of tumors[8,9]. In fact, lncRNA CCTT1 could en-

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hance the drug resistance of esophageal cancer and act as a biomarker[10]. Also, lncRNA ROR could induce the cisplatin resistance of osteosarcoma[11]. Urothelial carcinoma associated1 (UCA1) is a lncRNA found in bladder carcinoma[12]. As research has progressed, it has been found that UCA1 is abnormally expressed in many cancers and is involved in the regulation of cancer chemoresistance, including colorectal cancer, bladder cancer and gastric cancer[13-15]. However, its role in the chemoresistance of OC is still worth further exploration.

Salmena et al[16] have shown that lncRNAs could function as competitive endogenous RNAs (ceRNAs) to sponge microRNAs (miRNAs) to regulate downstream gene expression. MiR-654-5p is a miRNA located at chromosome 14q and has been verified to be involved in the progression of breast cancer[17]. Also, Majem et al[18] reported that miR-654-5p could inhibit the development of OC. Nevertheless, little research has been done on the role of miR-654-5p in chemoresistance of OC.

Salt inducible kinase 2 (SIK2) is a member of the adenosine 5'-monophosphate-activated protein kinase (AMPK) sub-family, which is implicated in metabolic regulation and cancer progression[19,20]. In OC, SIK2 promoted the metabolism and metastasis of OC[21,22]. Also, SIK2 could be targeted by miR-874-3p and miR-874-5p to participate in the development of EOC[23]. Hence, SIK2 was expected to be a good target for OC treatment.

The purpose of this study was to investigate the role of UCA1 in OC resistance and clarify its mechanism through bioinformatics prediction and experimental verification. The discovery of the UCA1/miR-654-5p/SIK2 axis provided a promising new perspective on the function of UCA1 promoting chemoresistance in OC.

Patients and methods

Tissue Samples Collection and Cell Culture

The present study was approved by the Ethics Committee of Gansu Provincial Maternity and Child-Care Hospital. OC tissues and adjacent normal tissues were collected from 31 cases of OC patients in the Gansu Provincial Maternity and Child-care Hospital. All patients had not received any other treatment and signed informed consents.

OC cells (A2780, OAW42, OVCAR4, SKOV3 and HeyA8) and human ovarian epithelial cells (IOSE-386) were purchased from the National for Typical Culture Collection (NTCC, Beijing, China). SKOV3 and HeyA8 cells were treated with increasing concentrations of PTX to establish PTX-resistant OC cells (SKOV3/PTX and HeyA8/PTX). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; HyClone, South-Logan, UT, USA) containing 10% fetal bovine serum (FBS; HyClone), Penicillin-Streptomycin solution (10 U/mL-10 μg/mL; at 37°C with 5% CO_2.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNAs were extracted using TRIzol reagent (TaKaRa, Dalian, China) and reverse transcribed complementary DNA (cDNA) using the cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). SYBR Green real-time PCR (TaKaRa) was used to perform a qRT-PCR assay to measure the relative expression of UCA1, miR-654-5p and SIK2 with $2^{-\Delta\Delta Ct}$ methods. Also, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were considered as internal controls, respectively. The primer sequences were as follows: UCA1: F, 5'-CTCTCCATTGGGTTCACCATTC-3', R, 5'-GCGGCAGGTCTTAAGAGATGAG-3'; miR-654-5p: F, 5'-TGGTGGGCCGCAGAACATGTGC-3', R, 5'-ACATGTTCTGCGGCCCACGAAT-3'; SIK2: F, 5'-TGAGCAGGTTCTTCGACTGAT-3', R, 5'-AGATCGCATCAGTCTCACGTT-3'; GAPDH:F, 5'-TGTTCGTCATGGGTGTGAAC-3', R, 5'-ATGGCATGGACTGTGGTCAT-3'; U6: F, 5'-CTCGCTTCGGCAGCACA-3', R, 5'-AACGCTTCACGAATTTGCGT-3'.

Cell Transfection

UCA1 small interfering RNA (siRNA) and overexpression plasmid (si-UCA1 and UCA1) or their negative controls (si-NC and vector), miR-654-5p mimic and inhibitor (miR-654-5p and anti-miR-654-5p) or their negative controls (miR-NC and anti-miR-NC), siRNA against SIK2 (si-SIK2) and its negative control (si-NC) were bought from Geneseed (Guangzhou, China). The above plasmid vectors were transfected into SKOV3/PTX and HeyA8/PTX cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

Cell PTX Resistance and Proliferation Assays

3-(4,5-dimethyl-2thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) Assay Kit (Beyotime, Shanghai, China) was used to assess the PTX resistance and proliferation of cells.

Briefly, SKOV3/PTX and HeyA8/PTX cells were seeded into 96-well plates. On the one hand, cells were treated with PTX at different concentrations (0 nM, 0.5 nM, 1.0 nM, 1.5 nM, 2.0 nM and 2.5 nM) for 48 h, and then MTT solution was added and incubated for 4 h. Next, dimethyl sulfoxide (DMSO) was added into cells and incubated for 15 min. Determination of absorbance at 560 nm and the calculation of half-maximal inhibitory concentration (IC50) were used to evaluate the PTX resistance of cells. On the other hand, SKOV3/PTX and HeyA8/PTX cells were treated with MTT and DMSO at the specified time point after transfection, which was the same as the above incubation time. Then, the absorbance was detected at 490 nm to evaluate the proliferation of cells.

Flow Cytometry

SKOV3/PTX and HeyA8/PTX cells were harvested after transfection for 48 h and stained using the Annexin V-fluorescein isothiocyanate (FITC)/Propidium iodide (PI) Apoptosis Detection Kit (Yeasen, Shanghai, China). Flow cytometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to collect fluorescence signals and determine the apoptosis rate of SKOV3/PTX and HeyA8/PTX cells.

Transwell Assay

The migration and invasion assays were conducted using Transwell chambers with an 8-μm pore size (Millipore, Billerica, MA, USA). The difference was that the invasion assay was pre-coated Matrigel (Millipore) in the upper chambers, while the migration assay was not coated. SKOV3/PTX and HeyA8/PTX cells were re-suspended with serum-free medium and seeded into the upper chambers, while the lower chambers were added DMEM containing 10% FBS. After incubation for 24 h, SKOV3/PTX and HeyA8/PTX cells were fixed with paraformaldehyde and stained with crystal violet. Cell images were collected at a magnification of 200 × and the number of migrated and invaded cells was counted in five randomly fields.

Dual-Luciferase Reporter Assay

The sequences of UCA1 or SIK2 3'UTR containing the miR-654-5p target binding sites and mutant binding sites were inserted into pGL3 promoter vector (Promega, Madison, WI, USA) to construct WT/MUT-UCA1 or WT/MUT-SIK2 reporter vectors. SKOV3/PTX and HeyA8/PTX cells were co-transfected with miR-654-5p mimic or miR-NC and the above reporter vectors using Lipofectamine 3000 (Invitrogen). After transfection for 48 h, SKOV3/PTX and HeyA8/PTX cells were collected and the luciferase activities were measured using Dual-Luciferase Reporter Assay Kit (Transgen, Beijing, China).

Western Blot (WB) Analysis

Tissues and cells were lysed with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore), and blocked with 5% non-milk for 1 h. Then, the membranes were incubated with primary antibody against SIK2 (1:2,000, Invitrogen) or GAPDH (1:1,000, Invitrogen) at 4°C overnight. After the membranes were washed with Tris-Buffered Saline Tween-20 (TBST), they were incubated with secondary antibody (1:2,000, Invitrogen) for 1 h. Protein signals were measured by enhanced chemiluminescence (ECL) solution (Beyotime) using iBright 1500 System.

Statistical Analysis

All statistical analysis was carried out using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). Student's *t*-test was used to compare the statistical differences between the two groups. One-way analysis of variance was used to compare the differences among multiple groups, followed by Tukey's test. Differences were defined as statistically significant for $p < 0.05$.

Results

UCA1 Expression was Increased in OC Tissues and Cells

Firstly, we tested the expression of UCA1 in OC. As shown in Figure 1A, UCA1 expression was higher in OC tissues than that in adjacent normal tissues. Besides, we also found that the expression of UCA1 was upregulated in all five OC cells, especially in SKOV3 and HeyA8 cells, compared with IOSE-386 cells (Figure 1B). Furthermore, UCA1 expression in SKOV3/PTX and HeyA8/PTX cells was more than that in SKOV3 and HeyA8 cells (Figure 1C), suggesting that UCA1 might be related to PTX resistance of OC.

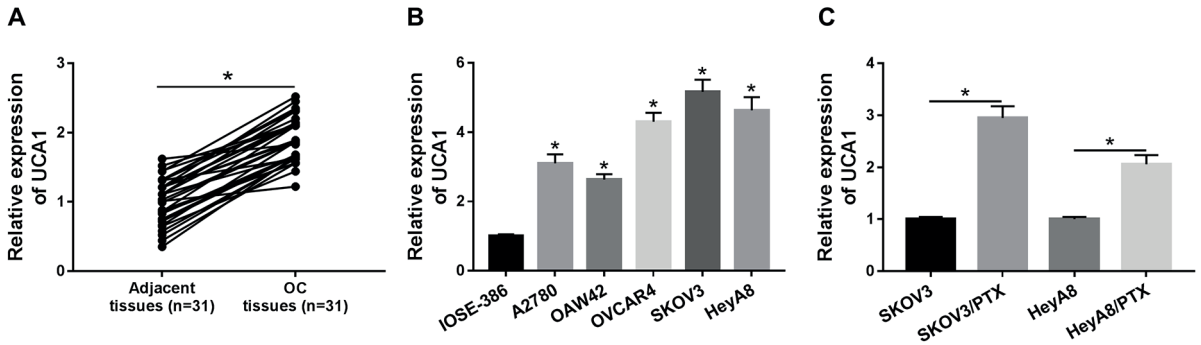

Figure 1. UCA1 expression was upregulated in OC tissues and cells. **(A)** QRT-PCR was used to measure the expression of UCA1 in OC tissues (n= 31) and adjacent normal tissues (n= 31). **(B)** UCA1 expression was detected by qRT-PCR in human ovarian epithelial cells (IOSE-386) and OC cells (A2780, OAW42, OVCAR4, SKOV3 and HeyA8). **(C)** The expression of UCA1 in OC cells (SKOV3 and HeyA8) and PTX-resistant OC cells (SKOV3/PTX and HeyA8/PTX) was assessed by qRT-PCR. *$p< 0.05$.

Knockdown of UCA1 Inhibited the Progression of PTX-Resistant OC Cells

To further confirm our results, we transfected si-UCA1 and si-NC into SKOV3/PTX and HeyA8/PTX cells. The decrease of UCA1 expression in SKOV3/PTX and HeyA8/PTX cells indicated that the transfection of si-UCA1 was successful (Figure 2A). Through detecting cell viability, we found that the IC50 value of cells was significantly decreased after UCA1 silencing, indicating that PTX resistance of SKOV3/PTX and HeyA8/PTX cells was suppressed (Figure 2B). Also, the proliferation of SKOV3/PTX and HeyA8/PTX cells was hindered by UCA1 knockdown (Figure 2C). At the same time, we also examined the apoptotic capacity (Figure 2D) and the number of apoptotic cells was increased significantly after silenced-UCA1 in SKOV3/PTX and HeyA8/PTX cells. Furthermore, Transwell assay indicated that reduced-UCA1 restrained the migration and invasion of SKOV3/PTX and HeyA8/PTX cells (Figure 2E-F). Hence, all data verified that UCA1 played an active role in PTX resistance of OC cells.

UCA1 Directly Targeted miR-654-5p

To clarify that the function of UCA1 acted as a ceRNA, we used LncBase v.2 tool to predict the miRNA that could interact with UCA1. As shown in Figure 3A, miR-654-5p has complementary sites that bind to UCA1. In order to confirm this interaction, WT-UCA1 and MUT-UCA1 reporter vectors were constructed to perform the Dual-luciferase reporter assay. The results showed that miR-654-5p mimic markedly decreased the luciferase activity of WT-UCA1, while had no effect on MUT-UCA1 in SKOV3/PTX and HeyA8/PTX cells (Figure 3B). Also, we detected miR-654-5p expression in OC tissues and cells and found that it was lower expressed in OC tissues and PTX-resistant OC cells (Figure 3C-D). In addition, we detected the effect of UCA1 expression on miR-654-5p expression to further confirmation. The increased expression of UCA1 indicated that the transfection efficiency of UCA1 overexpression plasmid was excellent in SKOV3/PTX and HeyA8/PTX cells (Figure 3E). Through qRT-PCR, we discovered that miR-654-5p expression was improved by UCA1 silencing, while suppressed by UCA1 overexpression in SKOV3/PTX and HeyA8/PTX cells (Figure 3F). Therefore, we hypothesized that UCA1 could sponge miR-654-5p in OC cells.

Overexpressed-UCA1 Could Partially Reverse the Inhibition Effects of miR-654-5p Overexpression on the Progression of PTX-Resistant OC Cells

To explore the function of miR-654-5p in OC and evaluate whether UCA1 regulated PTX-resistant OC cell progression through miR-654-5p, we co-transfected miR-654-5p and UCA1 overexpression plasmid into SKOV3/PTX and HeyA8/PTX cells. MiR-654-5p was increased by miR-654-5p mimic and inhibited by UCA1 overexpression, suggesting that the transfection efficiency of miR-654-5p and UCA1 overexpression was excellent in SKOV3/PTX and HeyA8/PTX cells (Figure 4A). The statistical results of IC50 values showed that overexpression of miR-654-

5p remarkably reduced the resistance of SKOV3/PTX and HeyA8/PTX cells to PTX, and this effect could be recovered by overexpressed-UCA1 (Figure 4B). Besides, MTT and Flow cytometry assays showed that the inhibition of proliferation and the promotion of apoptosis by miR-654-5p mimic could be partially reversed by elevated expression of UCA1 in SKOV3/PTX and HeyA8/PTX cells (Figure 4C-D). In addition, the migration and invasion of SKOV3/PTX and HeyA8/PTX cells were hindered by miR-654-5p overexpression, while overexpression of UCA1 could

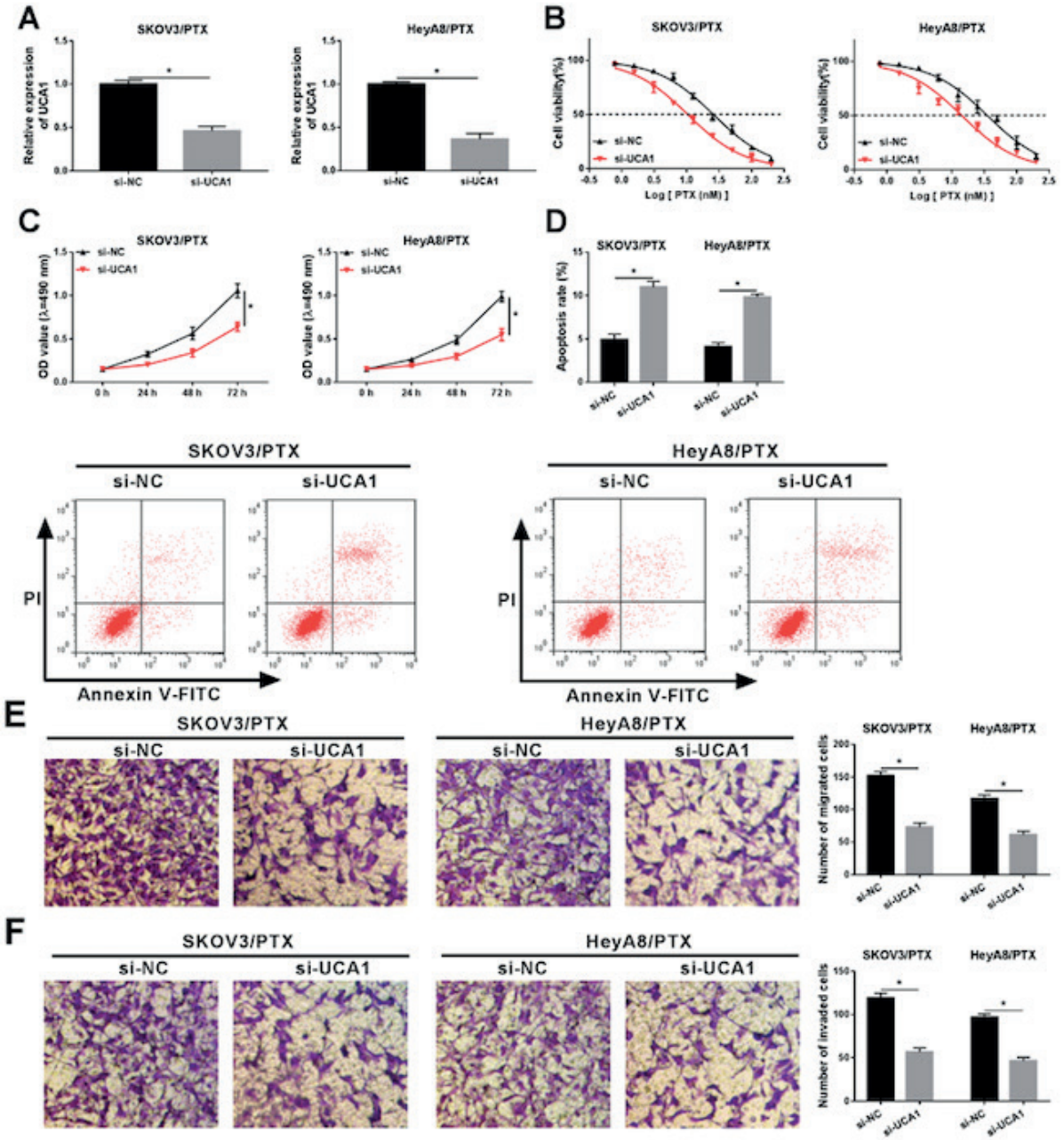

Figure 2. Effects of UCA1 expression on the progression of PTX-resistant OC cells. SKOV3/PTX and HeyA8/PTX cells were transfected with si-UCA1 and si-NC. **(A)** The expression of UCA1 was detected by qRT-PCR to evaluate the transfection efficiency of si-UCA1 inSKOV3/PTX and HeyA8/PTX cells. **(B-C)** MTT assay was performed to measure the PTX resistance and proliferation of SKOV3/PTX and HeyA8/PTX cells. **(D-F)** Flow cytometry **(D)** and Transwell assay **(E-F)** were used to evaluate the abilities of apoptosis, migration and invasion in SKOV3/PTX and HeyA8/PTX cells, respectively. All images were collected at a magnification of 200 ×. * $p< 0.05$.

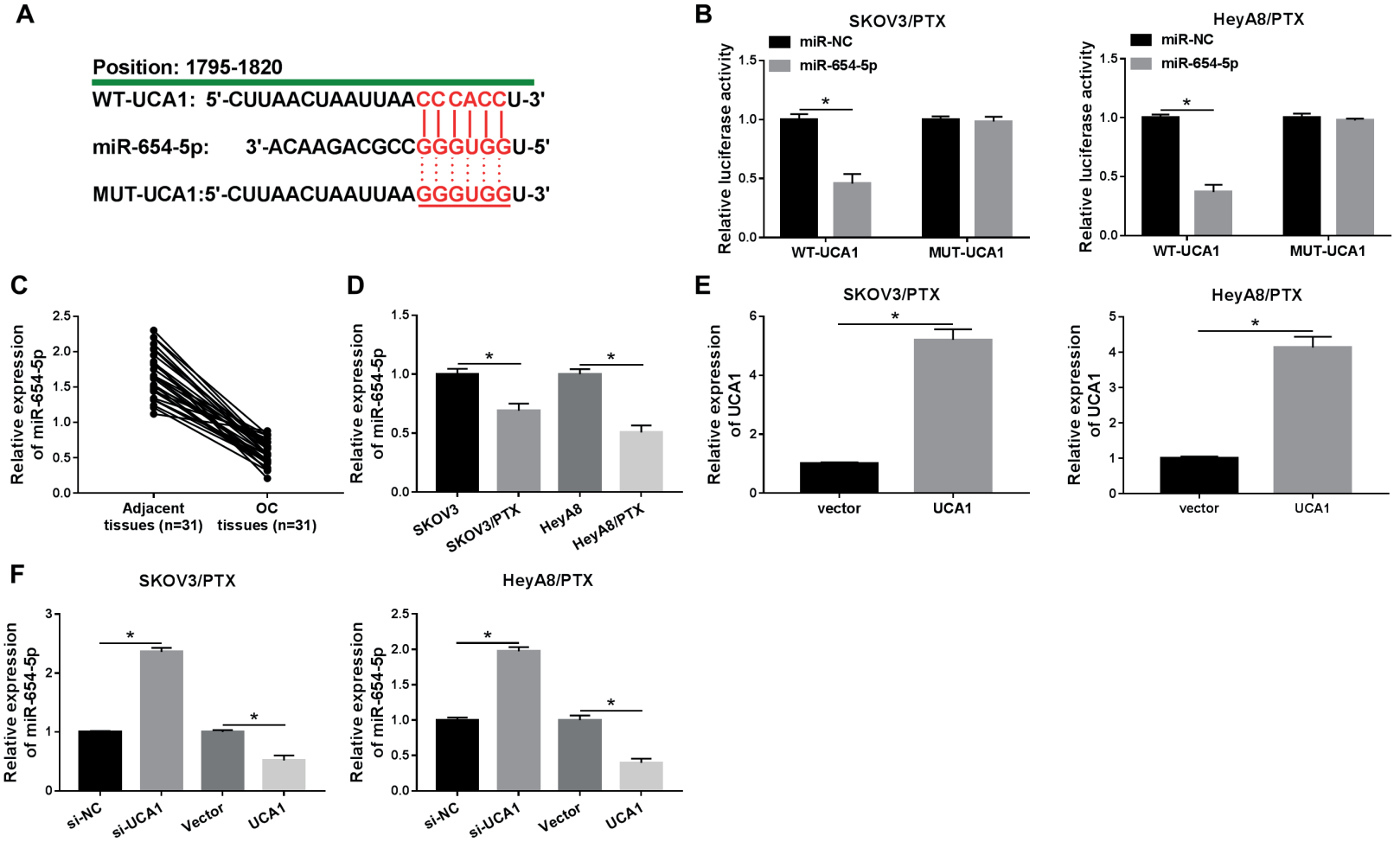

Figure 3. UCA1 was a sponge of miR-654-5p. **(A)** The predicted target regions of UCA1 with the miR-654-5p binding sites and mutant binding sites were shown. **(B)** Dual-luciferase reporter assay was used to verify the targeted binding relationship between miR-654-5p and WT-UCA1 or MUT-UCA1 in SKOV3/PTX and HeyA8/PTX cells. **(C-D)** The expression of miR-654-5p was increased in OC tissues and PTX-resistant OC cells (SKOV3/PTX and HeyA8/PTX) tested by qRT-PCR. **(E)** UCA1 expression in SKOV3/PTX and HeyA8/PTX cells was detected by qRT-PCR to evaluate the transfection efficiency of UCA1 overexpression plasmid. **(F)** QRT-PCR was performed to detect the effect of UCA1 expression on miR-654-5pexpression. *$p< 0.05$.

invert this effect (Figure 4E-F). These results suggested that miR-654-5p played a vital role in the regulation of UCA1 on the PTX resistance of OC.

SIK2 was a Target of miR-654-5p

To investigate the molecular mechanism of UCA1 in OC, we used TargetScan tool to predict the target gene of miR-654-5p and found a putative miR-654-5p binding site located in the 3'UTR of SIK2 (Figure 5A). Dual-luciferase reporter assay results revealed that miR-654-5p overexpression markedly inhibited the luciferase activity of WT-SIK2 in SKOV3/PTX and HeyA8/PTX cells, while did not affect MUT-SIK2 (Figure 5B). Moreover, the mRNA and protein levels of SIK2 were highly expressed in OC tissues and PTX-resistant OC cells (Figure 5C-F). Also, we investigated the effect of miR-654-5p expression on SIK2 expression in SKOV3/PTX and HeyA8/PTX cells. The decreased expression of miR-654-5p in SKOV3/PTX and HeyA8/PTX cells revealed that the transfection efficiency of miR-654-5p inhibitor was good (Figure 5G). Through the detection of the mRNA and protein expression, we discovered that overexpression of miR-654-5p hindered SIK2 expression, while its knockdown promoted the expression of SIK2 in SKOV3/PTX and HeyA8/PTX cells (Figure 5H-I). These results indicated that miR-654-5p targeted SIK2 in OC.

MiR-654-5p Inhibitor Partially Restored the Suppression Effects of Silenced-SIK2 on the Progression of PTX-Resistant OC Cells

To investigate the function of SIK2, we transfected si-SIK2 and anti-miR-654-5p into SKOV3/PTX and HeyA8/PTX cells. By detecting the mRNA and protein levels of SIK2, we determined that the inhibitory effect of si-SIK2 on SIK2 could be reversed by anti-miR-654-5p, suggesting that si-SIK2 and anti-miR-654-5p had good transfection efficiency and could be used for follow-up tests (Figure 6A-B). The detection of IC50 values in SKOV3/PTX and HeyA8/PTX cells revealed that knockdown of SIK2 markedly inhibited the PTX resistance of cells, while miR-654-5p inhibitor could partially invert these effects (Figure 6C). Moreover, miR-654-5p inhibitor also reversed the suppression effects of SIK2 knockdown on the proliferation, migration and invasion, as well as the acceleration effect of it on the apoptosis in SKOV3/PTX and HeyA8/PTX cells (Figure 6D-G). All data determined that SIK2 expression was crucial to the occurrence of PTX resistance of OC cells.

UCA1 and miR-654-5p Regulated SIK2 Expression

To determine the regulatory relationship between SIK2 and UCA1 or miR-654-5p, we measured the mRNA and protein expression of SIK2 in SKOV3/PTX and HeyA8/PTX cells transfected miR-654-5p mimic and UCA1 overexpression plasmid. The results indicated that miR-654-5p overexpression inhibited the expression of SIK2 in SKOV3/PTX and HeyA8/PTX cells, while the overexpressed-UCA1 could improve its expression (Figure 7A-B). These results revealed that SIK2 expression was regulated by miR-654-5p and UCA1 expression.

Discussion

At present, there are many studies on lncRNA regulating OC chemoresistance. Li et al[24] suggested that the dysregulation of lncRNA expression was related to cisplatin resistance of OC and could be used as a potential target for the detection of OC resistance. Besides, Yu et al[25] indicated that the downregulation of HOTAIR expression could increase the sensitivity of OC to cisplatin. Therefore, elucidating the mechanisms affecting OC resistance could help develop new clinical treatment strategies for OC. In previous studies, UCA1 was thought to be related to the development of cisplatin resistance of OC[26]. Herein, we discovered that UCA1 expression was improved in OC tissues and PTX-resistant OC cells, which was consistent with the conclusion of Wang et al[27]. The knockdown of UCA1 suppressed the resistance of OC cells to PTX, inhibited the proliferation, migration, invasion, and promoted the apoptosis of PTX-resistant OC cells. This confirmed the positive effect of UCA1 on OC resistance and provided a theoretical basis for UCA1 to become a biomarker for clinical OC resistance diagnosis and treatment.

Numerous studies have shown that miRNA regulatory networks play an essential role in the progression and chemoresistance of cancer[28,29]. Xu et al[30] showed that miR-375-3p inhibited tumorigenesis and chemoresistance in colorectal cancer. Also, Chai et al[31] revealed that miR-101 reduced chemoresistance of liver cancer to cisplatin. Although miR-654-5p has been shown to play an important role in a variety of cancers, this is the first study

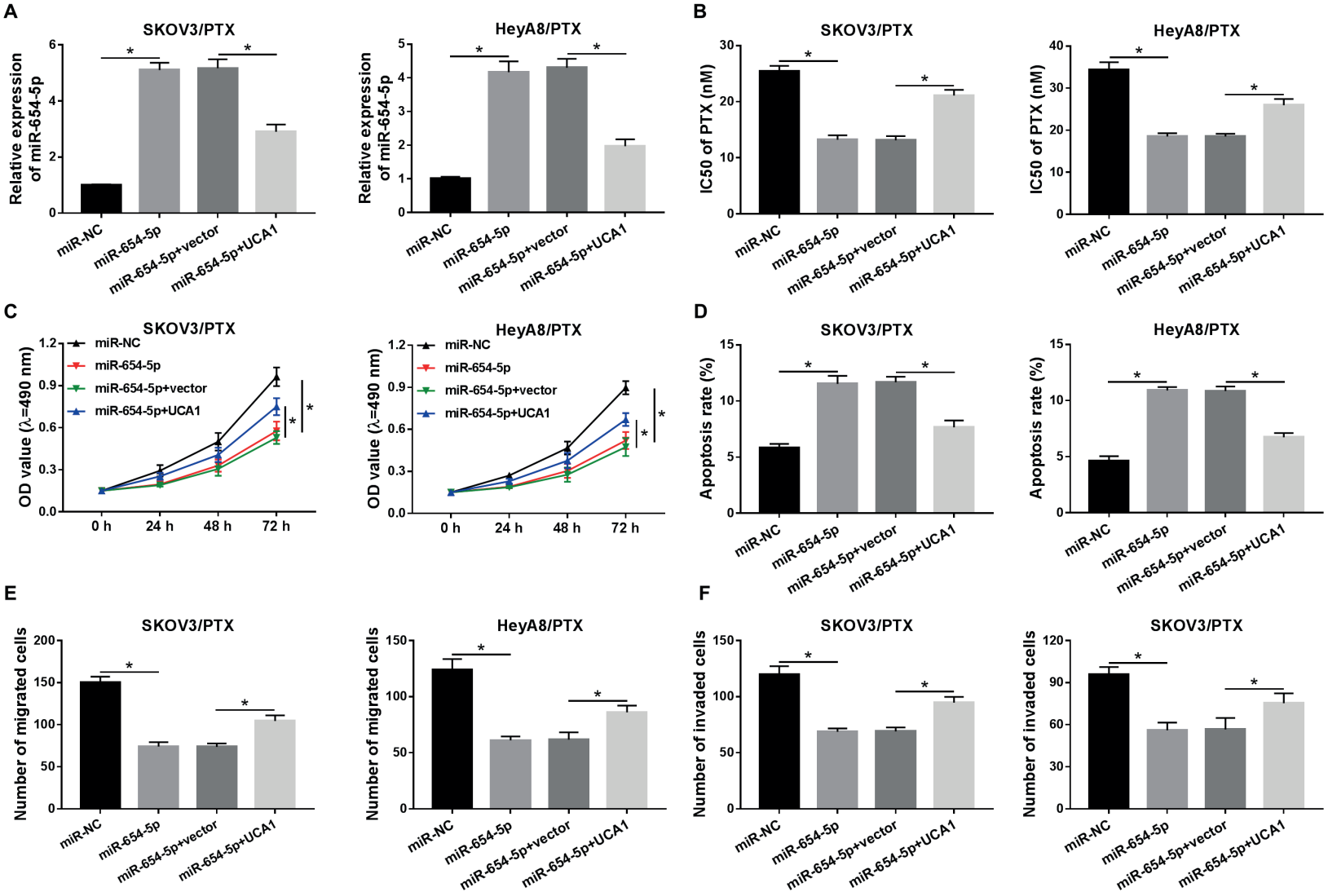

Figure 4. Effects of miR-654-5p overexpression on the progression of PTX-resistant OC cells. SKOV3/PTX and HeyA8/PTX cells were co-transfected with miR-654-5p mimic (miR-654-5p) and UCA1 overexpression plasmid (UCA1) or their negative controls (miR-NC and vector). **(A)** The expression of miR-654-5p in SKOV3/PTX and HeyA8/PTX cells was evaluated by qRT-PCR to assess the transfection efficiency of miR-654-5p mimic and UCA1 overexpression plasmid. **(B-C)** The PTX resistance and proliferation of SKOV3/PTX and HeyA8/PTX cells were measured by MTT assay. (D-F) Flow cytometry **(D)** and transwell assay **(E-F)** were performed to assess the abilities of apoptosis, migration and invasion in SKOV3/PTX and HeyA8/PTX cells, respectively. *$p< 0.05$.

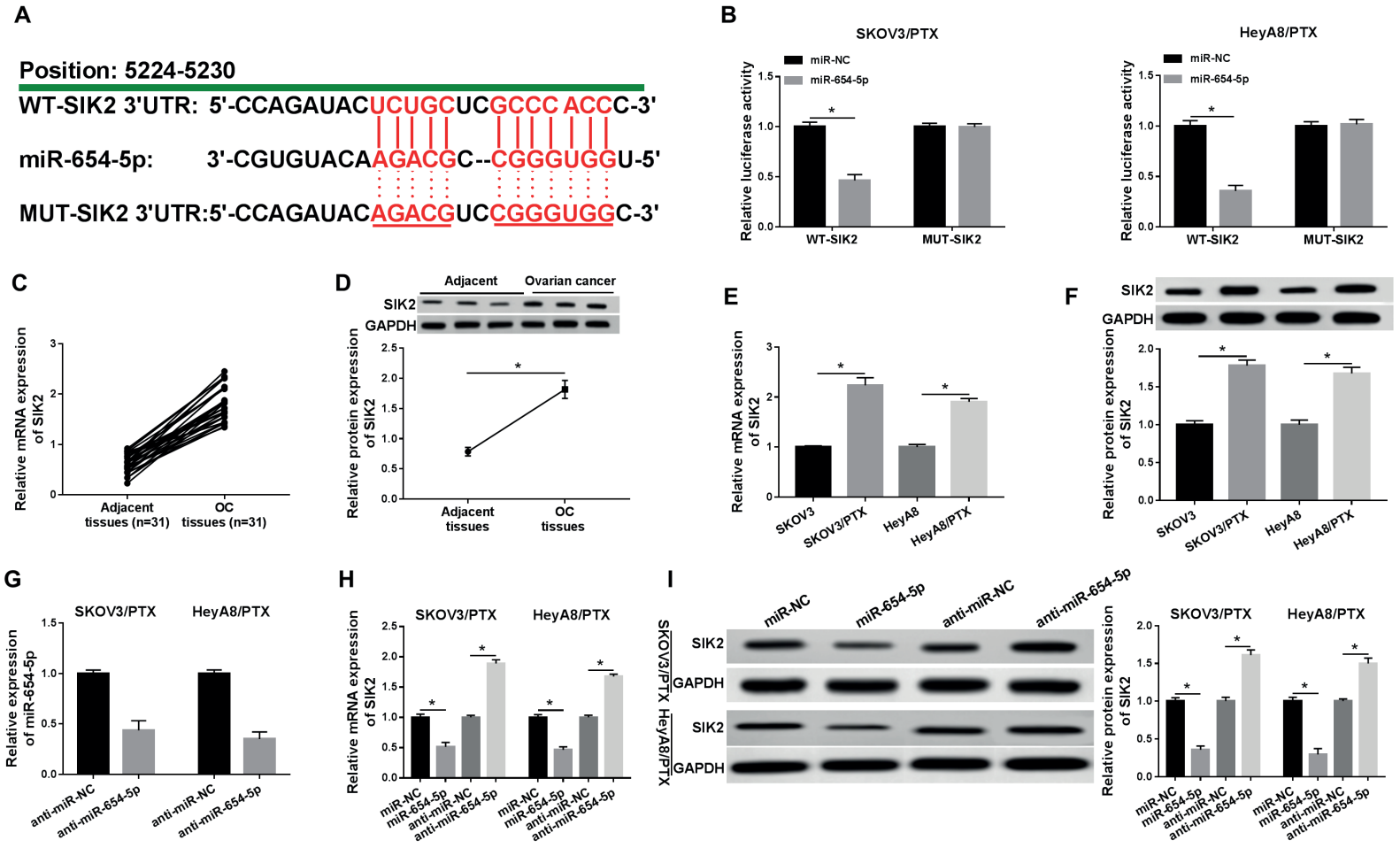

Figure 5. SIK2 was a target of miR-654-5p. **(A)** The predicted target regions of SIK2 3'UTR with the miR-654-5p binding sites and mutant binding sites were shown. **(B)** Dual-luciferase reporter assay was used to detect the interaction between miR-654-5p and SIK2 in SKOV3/PTX and HeyA8/PTX cells. **(C-D)** The mRNA and protein expression of SIK2 in OC tissues (n = 31) and adjacent normal tissues (n = 31) were examined by qRT-PCR and WB analysis. **(E-F)** QRT-PCR and WB analysis were performed to assess the mRNA and protein expression of SIK2 in OC cells (SKOV3 and HeyA8) and PTX-resistant OC cells (SKOV3/PTX and HeyA8/PTX). **(G)** The expression of miR-654-5p was detected by qRT-PCR to evaluate the transfection efficiency of anti-miR-654-5p. (H-I) QRT-PCR and WB analysis were used to measure the effect of miR-654-5p expression on the mRNA and protein expression of SIK2. *$p< 0.05$.

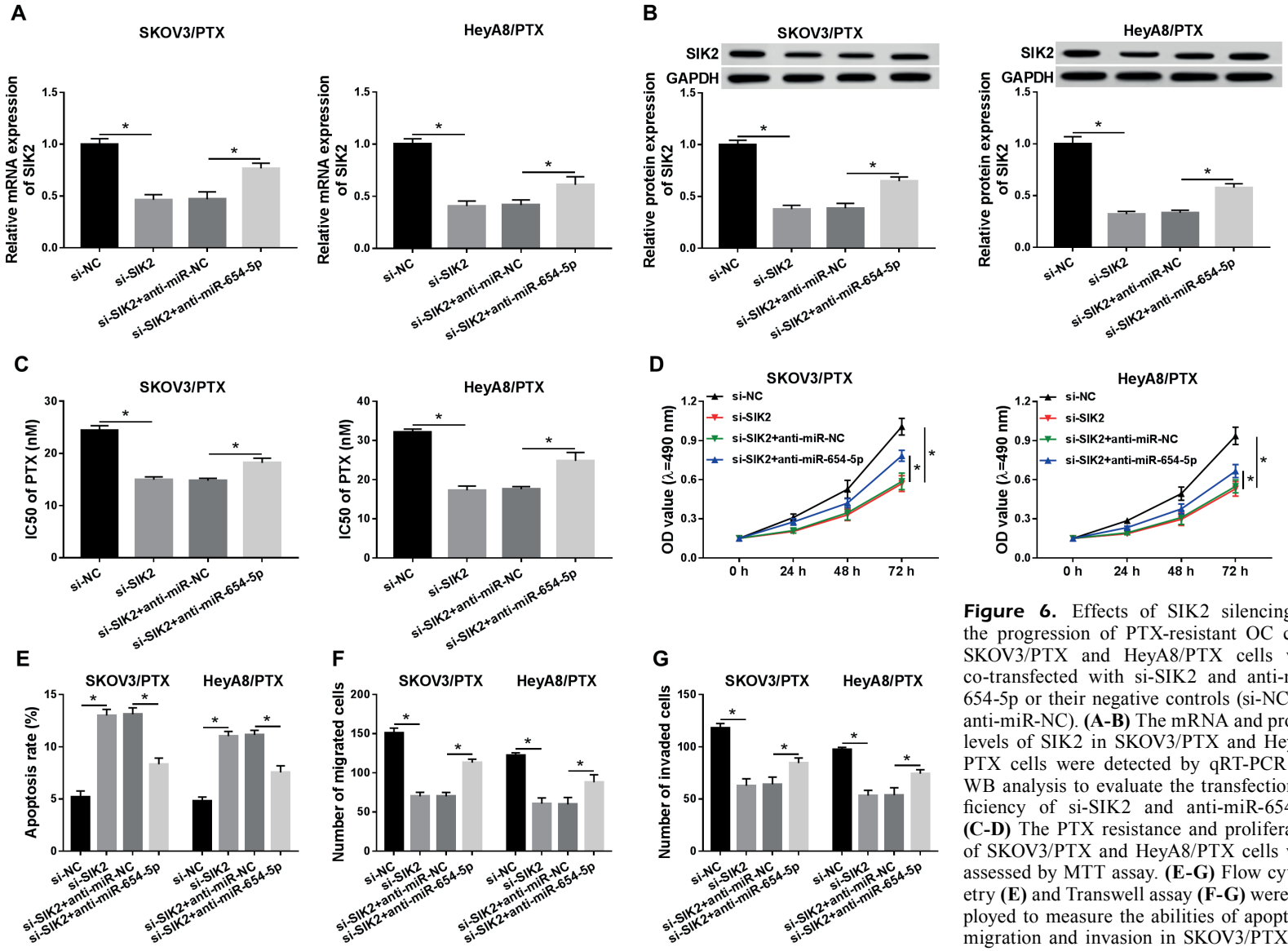

Figure 6. Effects of SIK2 silencing on the progression of PTX-resistant OC cells. SKOV3/PTX and HeyA8/PTX cells were co-transfected with si-SIK2 and anti-miR-654-5p or their negative controls (si-NC and anti-miR-NC). **(A-B)** The mRNA and protein levels of SIK2 in SKOV3/PTX and HeyA8/PTX cells were detected by qRT-PCR and WB analysis to evaluate the transfection efficiency of si-SIK2 and anti-miR-654-5p. **(C-D)** The PTX resistance and proliferation of SKOV3/PTX and HeyA8/PTX cells were assessed by MTT assay. **(E-G)** Flow cytometry **(E)** and Transwell assay **(F-G)** were employed to measure the abilities of apoptosis, migration and invasion in SKOV3/PTX and HeyA8/PTX cells, respectively. * $p < 0.05$.

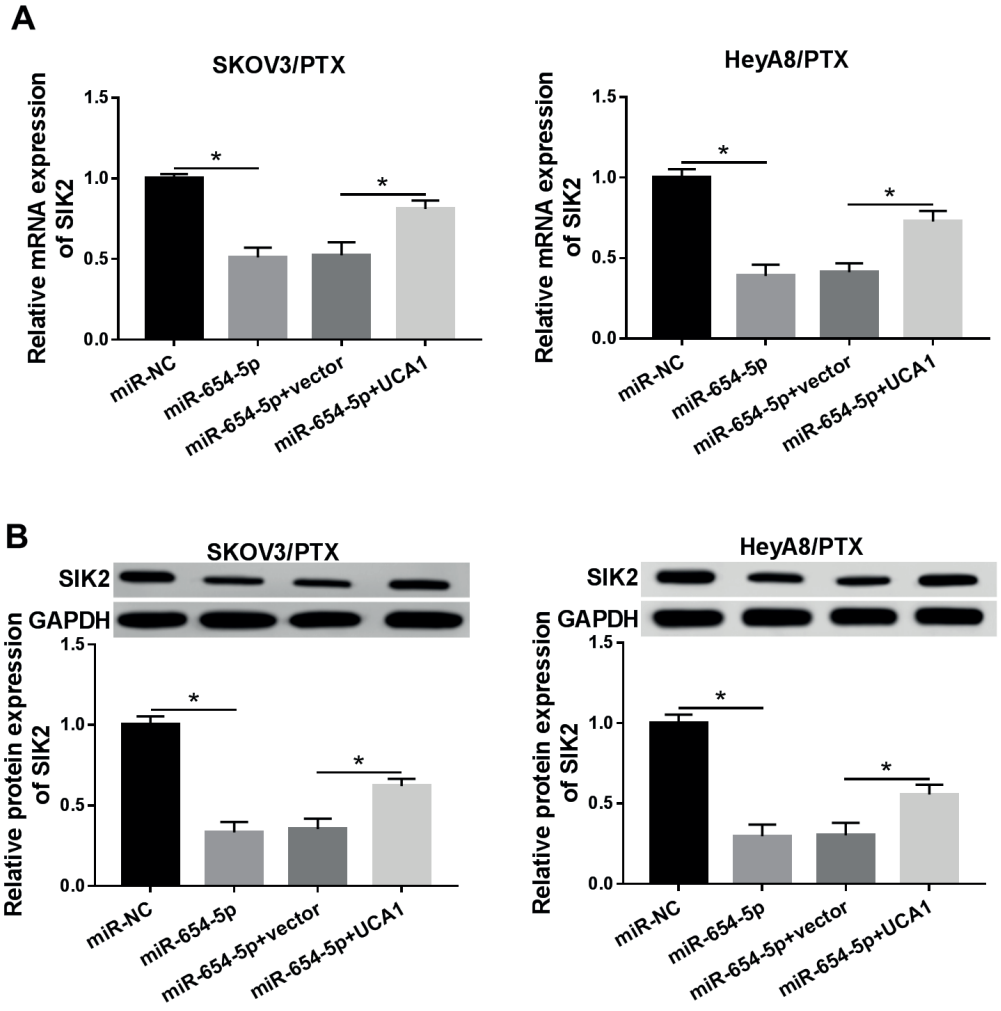

Figure 7. Effects of UCA1 and miR-654-5p expression on SIK2 expression in PTX-resistant OC cells. SKOV3/PTX and HeyA8/PTX cells were co-transfected with miR-654-5p mimic (miR-654-5p) and UCA1 overexpression plasmid (UCA1) or their negative controls (miR-NC and vector). **(A)** The expression of SIK2 was examined by qRT-PCR. **(B)** WB analysis was performed to determine the protein level of SIK2. * $p< 0.05$.

to identify its association with OC resistance. In the present study, we discovered that miR-654-5p could be sponged by UCA1. Consistent with previous studies[18], we also found that miR-654-5p expression was significantly decreased in OC tissues and PTX-resistance OC cells. Through functional verification, we concluded that miR-654-5p overexpression could hinder the resistance of OC cells to PTX and suppress the progression of PTX-resistant OC cells. This inhibition could be alleviated by overexpression of UCA1. Therefore, these results uncovered that miR-654-5p played a negative role in UCA1-regulated OC resistance. At the same time, we predicted that SIK2 was a target of miR-654-5p.

Investigations on the involvement of SIK2 in OC chemoresistance have been reported. Ahmed et al[32] found that the absence of SIK2 prevented the separation of centrosomes during mitosis, thereby making cancer cells sensitive to PTX. Besides, Zhou et al[33] suggested that SIK2 promoted OC metastasis and resistance to PTX, which we also confirmed. Here, we verified that SIK2 was upregulated in OC and its silencing inhibited resistance to PTX and hindered the progression of PTX-resistant OC cells. These results indicate the need for SIK2 expression to maintain PTX resistance in OC cells. Furthermore, the effect of SIK2 silencing on the progression of PTX-resistant OC cells could be reversed by miR-654-5p inhibitor, and its ex-

pression was regulated by miR-654-5p and UCA1, which confirmed the existence of SIK2 function as a target gene of UCA1/miR-654-5p. Our findings highlighted the biological role of UCA1 in OC resistance, and the correlation between UCA1 and PTX resistance also suggested potential ways to improve PTX sensitivity in OC patients. Further research is needed to overcome challenges such as specificity and uptake rates before it can be applied to clinical treatment.

Although there are many reports on lncRNA, more potential functions of lncRNA in cancer still need to be improved. Our study first proposed that UCA1 affected PTX resistance of OC by regulating the miR-654-5p/SIK2 axis, and this new molecular mechanism enriched the role of UCA1 in OC. In addition, the function of miR-645-5p in OC resistance was also first discovered, providing a new reference for studying the function of miR-645-5p in other cancers.

Conclusions

Our research revealed that lncRNA UCA1 promoted PTX resistance of OC through regulating the miR-654-5p/SIK2 axis. This study showed that we had taken a significant step forward in the study of OC chemoresistance and the identification of its biomarkers.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Romero I, Bast RC Jr. Minireview: human ovarian cancer: biology, current management, and paths to personalizing therapy. Endocrinology 2012; 153: 1593-1602.
2) Vogell A, Evans ML. Cancer screening in women. Obstet Gynecol Clin North Am 2019; 46: 485-499.
3) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.
4) Pashaei-Asl F, Pashaei-Asl R, Khodadadi K, Akbarzadeh A, Ebrahimie E, Pashaiasl M. Enhancement of anticancer activity by silibinin and paclitaxel combination on the ovarian cancer. Artif Cells Nanomed Biotechnol 2018; 46: 1483-1487.
5) Xiao J, Xu M, Hou T, Huang Y, Yang C, Li J. Dasatinib enhances antitumor activity of paclitaxel in ovarian cancer through Src signaling. Mol Med Rep 2015; 12: 3249-3256.
6) Chandra Gupta S, Nandan Tripathi Y. Potential of long non-coding RNAs in cancer patients: from biomarkers to therapeutic targets. Int J Cancer 2017; 140: 1955-1967.
7) Zhan L, Li J, Wei B. Long non-coding RNAs in ovarian cancer. J Exp Clin Cancer Res 2018; 37: 120.
8) Xia H, Hui KM. Mechanism of cancer drug resistance and the involvement of noncoding RNAs. Curr Med Chem 2014; 21: 3029-3041.
9) Dai E, Yang F, Wang J, Zhou X, Song Q, An W, Wang L, Jiang W. ncDR: a comprehensive resource of non-coding RNAs involved in drug resistance. Bioinformatics 2017; 33: 4010-4011.
10) Hu M, Zhang Q, Tian XH, Wang JL, Niu YX, Li G. lncRNA CCAT1 is a biomarker for the proliferation and drug resistance of esophageal cancer via the miR-143/PLK1/BUBR1 axis. Mol Carcinog 2019 Sep 22. doi: 10.1002/mc.23109. [Epub ahead of print]
11) 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: 7256-7265.
12) Wang XS, Zhang Z, Wang HC, Cai JL, Xu QW, Li MQ, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ, Chen WF. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clin Cancer Res 2006; 12: 4851-4858.
13) Sun F, Liang W, Qian J. The identification of CRNDE, H19, UCA1 and HOTAIR as the key lncRNAs involved in oxaliplatin or irinotecan resistance in the chemotherapy of colorectal cancer based on integrative bioinformatics analysis. Mol Med Rep 2019; 20: 3583-3596.
14) Wu J, Li W, Ning J, Yu W, Rao T, Cheng F. Long noncoding RNA UCA1 targets miR-582-5p and contributes to the progression and drug resistance of bladder cancer cells through ATG7-mediated autophagy inhibition. Onco Targets Ther 2019; 12: 495-508.
15) Shang C, Guo Y, Zhang J, Huang B. Silence of long noncoding RNA UCA1 inhibits malignant proliferation and chemotherapy resistance to adriamycin in gastric cancer. Cancer Chemother Pharmacol 2016; 77: 1061-1067.
16) Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146: 353-358.
17) Tan YY, Xu XY, Wang JF, Zhang CW, Zhang SC. MiR-654-5p attenuates breast cancer progression by targeting EPSTI1. Am J Cancer Res 2016; 6: 522-532.
18) Majem B, Parrilla A, Jimenez C, Suarez-Cabrera L, Barber M, Marin A, Castellvi J, Tamayo G, Moreno-Bueno G, Ponce J, Matias-Guiu X, Alameda F, Romero I, Sanchez JL, Perez-Benavente A, Moran S, Esteller M, Reventos J, Rigau M, Gil-Moreno A, Segura MF, Santamaria A. MicroRNA-654-5p suppresses ovarian

cancer development impacting on MYC, WNT and AKT pathways. Oncogene 2019; 38: 6035-6050.

19) Sakamoto K, Bultot L, Goransson O. The salt-inducible kinases: emerging metabolic regulators. Trends Endocrinol Metab 2018; 29: 827-840.

20) Du WQ, Zheng JN, Pei DS. The diverse oncogenic and tumor suppressor roles of salt-inducible kinase (SIK) in cancer. Expert Opin Ther Targets 2016; 20: 477-485.

21) Miranda F, Mannion D, Liu S, Zheng Y, Mangala LS, Redondo C, Herrero-Gonzalez S, Xu R, Taylor C, Chedom DF, Karaminejadranjbar M, Albukhari A, Jiang D, Pradeep S, Rodriguez-Aguayo C, Lopez-Berestein G, Salah E, Abdul Azeez KR, Elkins JM, Campo L, Myers KA, Klotz D, Bivona S, Dhar S, Bast RC, Jr., Saya H, Choi HG, Gray NS, Fischer R, Kessler BM, Yau C, Sood AK, Motohara T, Knapp S, Ahmed AA. Salt-inducible kinase 2 couples ovarian cancer cell metabolism with survival at the adipocyte-rich metastatic niche. Cancer Cell 2016; 30: 273-289.

22) [No authors listed]. SIK2 promotes ovarian cancer spread. Cancer Discov 2016; 6: OF1.

23) Xia B, Lin M, Dong W, Chen H, Li B, Zhang X, Hou Y, Lou G. Upregulation of miR-874-3p and miR-874-5p inhibits epithelial ovarian cancer malignancy via SIK2. J Biochem Mol Toxicol 2018; 32: e22168.

24) Li Q, Zhang J, Zhou J, Yang B, Liu P, Cao L, Jing L, Liu H. lncRNAs are novel biomarkers for differentiating between cisplatin-resistant and cisplatin-sensitive ovarian cancer. Oncol Lett 2018; 15: 8363-8370.

25) Yu Y, Zhang X, Tian H, Zhang Z, Tian Y. Knockdown of long non-coding RNA HOTAIR increases cisplatin sensitivity in ovarian cancer by inhibiting cisplatin-induced autophagy. J BUON 2018; 23: 1396-1401.

26) Li Z, Niu H, Qin Q, Yang S, Wang Q, Yu C, Wei Z, Jin Z, Wang X, Yang A, Chen X. lncRNA UCA1 mediates resistance to cisplatin by regulating the miR-143/FOSL2-signaling pathway in ovarian cancer. Mol Ther Nucleic Acids 2019; 17: 92-101.

27) Wang J, Ye C, Liu J, Hu Y. UCA1 confers paclitaxel resistance to ovarian cancer through miR-129/ABCB1 axis. Biochem Biophys Res Commun 2018; 501: 1034-1040.

28) Wei X, Shen X, Ren Y, Hu W. The roles of microRNAs in regulating chemotherapy resistance of non-small cell lung cancer. Curr Pharm Des 2018; 23: 5983-5988.

29) Abba ML, Patil N, Leupold JH, Moniuszko M, Utikal J, Niklinski J, Allgayer H. MicroRNAs as novel targets and tools in cancer therapy. Cancer Lett 2017; 387: 84-94.

30) Xu X, Chen X, Xu M, Liu X, Pan B, Qin J, Xu T, Zeng K, Pan Y, He B, Sun H, Sun L, Wang S. miR-375-3p suppresses tumorigenesis and partially reverses chemoresistance by targeting YAP1 and SP1 in colorectal cancer cells. Aging (Albany NY) 2019; 11: 7357-7385.

31) Chai Z, Yin X, Chen J, Shi J, Sun J, Liu C, Liu F, Cheng S. MicroRNA-101 modulates cisplatin chemoresistance in liver cancer cells via the DNA-PKcs signaling pathway. Oncol Lett 2019; 18: 3655-3663.

32) Ahmed AA, Lu Z, Jennings NB, Etemadmoghadam D, Capalbo L, Jacamo RO, Barbosa-Morais N, Le XF; Australian Ovarian Cancer Study Group, Vivas-Mejia P, Lopez-Berestein G, Grandjean G, Bartholomeusz G, Liao W, Andreeff M, Bowtell D, Glover DM, Sood AK, Bast RC Jr. SIK2 is a centrosome kinase required for bipolar mitotic spindle formation that provides a potential target for therapy in ovarian cancer. Cancer Cell 2010; 18: 109-121.

33) Zhou J, Alfraidi A, Zhang S, Santiago-O'Farrill JM, Yerramreddy Reddy VK, Alsaadi A, Ahmed AA, Yang H, Liu J, Mao W, Wang Y, Takemori H, Vankayalapati H, Lu Z, Bast RC Jr. A novel compound ARN-3236 inhibits salt-inducible kinase 2 and sensitizes ovarian cancer cell lines and xenografts to paclitaxel. Clin Cancer Res 2017; 23: 1945-1954.