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ORIGINAL RESEARCH

Downregulation of circNRIP1 Suppresses the Paclitaxel Resistance of Ovarian Cancer via Regulating the miR-211-5p/HOXC8 Axis

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Background: Circular RNA (circRNA) has an essential regulatory role in the chemotherapy resistance of cancers. Nevertheless, the role of circRNA nuclear receptor-interacting protein 1 (circNRIP1) in the paclitaxel (PTX) resistance of ovarian cancer (OC) remains unclear.

Material and Methods: The circNRIP1, miR-211-5p and homeobox C8 (HOXC8) expression levels were assessed using qRT-PCR. The PTX resistance of cells was measured by 3-(4, 5-dimethylthiazolyl-2-yl)-2-5 diphenyl tetrazolium bromide (MTT) assay. Furthermore, cell proliferation, apoptosis, migration and invasion were detected by colony formation assay, flow cytometry and transwell assay, respectively. Moreover, the protein levels of proliferation, apoptosis, metastasis-related markers and HOXC8 were determined by Western blot (WB) analysis. Tumor xenograft models were constructed to explore the influence of circNRIP1 on OC tumor growth. The interaction between miR-211-5p and circNRIP1 or HOXC8 was confirmed by dual-luciferase reporter assay and RNA immunoprecipitation (RIP) assay.

Results: CircNRIP1 was highly expressed in PTX-resistant OC tissues and cells. Silencing of circNRIP1 repressed the PTX resistance of OC cells in vitro and OC tumor in vivo. Furthermore, circNRIP1 sponged miR-211-5p, and miR-211-5p inhibitor could reverse the inhibitory effect of circNRIP1 knockdown on the PTX resistance of OC cells. In addition, miR-211-5p targeted HOXC8, and HOXC8 overexpression could reverse the suppression effect of miR-211-5p on the PTX resistance of OC cells. Additionally, the expression of HOXC8 was regulated by circNRIP1 and miR-211-5p.

Conclusion: CircNRIP1 silencing could inhibit the PTX resistance of OC via regulating the miR-211-5p/HOXC8 axis, showing that circNRIP1 might be a potential target for OC resistance treatment.

Keywords: ovarian cancer, PTX resistance, circNRIP1, miR-211-5p, HOXC8

Introduction

Ovarian cancer (OC) is a malignant tumor produced by the abnormal growth of cells in or near the ovary.[1] Early lesions are often not easy to detect, so most of the confirmed diagnosis of OC patients have reached the advanced stage.[2,3] Although the treatment of OC continues to improve, the 5-year survival rate of advanced OC patients is only 20–40%.[4] Surgical treatment combined with chemoradiotherapy is an important way to treat OC.[5,6] However, the occurrence of chemotherapy resistance has raised new problems for the clinical treatment of OC.[7] Therefore, elucidating the mechanisms affecting chemotherapy resistance is of great significance for improving the cure rate of OC patients.

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Circular RNA (circRNA) is a kind of non-coding RNA (ncRNA) with a closed ring structure.[8,9] In recent years, the role of circRNA in a variety of diseases has been well documented.[10,11] Some studies have indicated that circRNA can function as an important biomarker for cancer treatment, including OC.[12] Paclitaxel (PTX) is one of the most common chemotherapy drugs used for OC treatment.[13] A recent study indicated that circCELSR1 could promote the PTX resistance of OC in mediating the miR-1252/FOXR2 axis.[14] Therefore, circRNA is also expected to be a potential target for overcoming cancer resistance. Researches have shown that circRNA nuclear receptor-interacting protein 1 (circNRIP1, also known as circ_0002711) may act as an oncogene to promote the progression of renal carcinoma and gastric cancer.[15,16] Wang et al used bioinformatics to analyze the differentially expressed circRNAs in OC patients and normal people and found that circNRIP1 was significantly overexpressed in OC patients.[17] However, the role of circNRIP1 in OC progression and whether it participates in the regulation of OC resistance has not been reported so far.

The molecular mechanism of circRNA can act as a competitive endogenous RNA (ceRNA) of microRNA (miRNA) has also been widely recognized.[18] MiR-211-5p is a miRNA with significantly low expression in many cancer, which has been reported to be involved in the regulation of cancer progression as a tumor suppressor.[19,20] Zhang et al showed that miR-211-5p inhibitor could restrain the cisplatin resistance of tongue cancer.[21] In OC, the results of Wang et al indicated that miR-211 was lowly expressed in OC, and its upregulation accelerated the chemotherapy sensitivity of OC.[22] Homeobox C8 (HOXC8) is a gene that is significantly highly expressed in cancer and is closely related to cancer progression, including OC.[23,24] Xu et al reported that knockdown of HOXC8 repressed the resistance of hepatocellular carcinoma to oxaliplatin.[25]

The purpose of our study was to investigate the role of circNRIP1 in the PTX resistance of OC. In addition, through bioinformatics analysis and experimental verification, we confirmed that circNRIP1 could sponge miR-211-5p to regulate HOXC8 expression. Our study proposed a new regulatory axis for the regulation of OC resistance, providing a new biomarker for the improvement of OC resistance.

Materials and Methods

Tissues Collection

Our study was approved by the Ethics Committee of Xingtai People's Hospital, and 28 PTX-sensitive OC patients and 28 PTX-resistant OC patients were recruited from Xingtai People's Hospital. All OC tissues (28 PTX-sensitive OC tissues (S) and 28 PTX-resistant OC tissues (R)) and 56 adjacent normal tissues (N) were collected and stored at −80°C. All patients signed written informed consent.

Cell Culture

Human ovarian epithelial cell line (HOEC), OC cell lines (A2780 and SKOV3) and PTX-resistant OC cell lines (A2780/PTX and SKOV3/PTX) were obtained from China Center for Type Culture Collection (CCTCC, Wuhan, China). HOEC cells were grown in DMEM (Hyclone, Logan, UT, USA) and all OC cell lines were cultured in RPMI-1640 medium (Hyclone). The medium was added with 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (Hyclone), and all cells were cultured in an atmosphere with 5% CO_2 at 37°C. The medium used for PTX-resistant OC cell lines was additionally added with 5 nM PTX (Meilun Biotech, Dalian, China) for maintaining the PTX-resistant phenotype.

RNA Extraction and qRT-PCR

RNAiso Plus reagent (Takara, Dalian, China) was used for total RNA extraction, and PrimeScript RT Reagent Kit (Takara) was used to reverse-transcribe RNA into cDNA. QRT-PCR could be performed using the SYBR Green (Takara). GAPDH and U6 were used as the normalized controls. Primers were presented as below: circNRIP1, F 5′-CAGATTCAGAGCAGTCCCACA-3′, R 5′-TCTGGCTGTGTTTCTCCCAA-3′; NRIP1, F 5′-GAGCACTCCACCTTTACTTACAT-3′, R 5′-CAATCATACCTATCGGTTTATCTG-3′; miR-211-5p, F 5′-CTCGAGTAACCGTATTGTTCGCGTCATGCCAGCA-3′, R 5′-GCGGCCGCCAGACCATGTGTCCCATTTG-3′; HOXC8, F 5′-CACGTCCAAGACTTCTTCCACCACGGC-3′, R 5′-CACTTCATCCTTCGATTCTGGAACC-3′; GAPDH, F 5′-TCAAGAAGGTGGTGAAGCAG-3′, R 5′-GAGGGGAGATTCAGTGTGGT-3′; U6, F 5′-CTCGCTTCGGCAGCACATATACT-3′, R 5′-ACGCTTCACGAATTTGCGTGTC-3′.

Identification of the Circular Characteristic and Stability of circRNA

Extracted RNA was treated with Ribonuclease R (Rnase R; Epicentre, Madison, WI, USA). Then, the expression levels of circNRIP1 and NRIP1 mRNA were detected using qRT-PCR to assess the circular characteristic of circNRIP1. For

evaluating the stability of circNRIP1, the cells were incubated with Actinomycin D (ActD; Thermo Fisher Scientific, Rockford, IL, USA) for 0, 2, 4, 8 and 12 h, respectively. After that, the expression levels of circNRIP1 and NRIP1 mRNA were measured by qRT-PCR.

3-(4, 5-Dimethylthiazolyl-2-Yl)-2-5 Diphenyl Tetrazolium Bromide (MTT) Assay

The PTX resistance of cells was measured using MTT Kit (Beyotime, Shanghai, China). The cells were treated with different concentrations of PTX (0.125, 0.5, 2, 8, 32, and 128 μM) for 48 h, followed by incubation with MTT solution for 4 h. After treated with DMSO for 10 min, the absorbance was detected at 560 nm. Cell viability was determined and half-maximal inhibitory concentration (IC50) was calculated to evaluate the PTX resistance of cells.

Cell Transfection

All plasmids and oligonucleotides were obtained from RiboBio (Guangzhou, China), including small interference RNA targeting circNRIP1 (si-circNRIP1#1/#2) or HOXC8 (si-HOXC8), lentiviral short hairpin RNA against circNRIP1 (sh-circNRIP1), miR-211-5p mimic (miR-211-5p), miR-211-5p inhibitor (anti-miR-211-5p), circNRIP1 and HOXC8 overexpression plasmids (circNRIP1 and HOXC8), and corresponding negative controls (si-NC, sh-NC, miR-NC, anti-miR-NC, pCD-ciR and pcDNA). Transfection was performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA).

Colony Formation Assay

After transfection, A2780/PTX and SKOV3/PTX cells were plated into 6-well plates and cultured for 2 weeks. After fixed with paraformaldehyde and stained with crystal violet, the colony numbers of A2780/PTX and SKOV3/PTX cells were counted using a microscope.

Flow Cytometry

The apoptosis of A2780/PTX and SKOV3/PTX cells was detected using Annexin V- FITC/PI Apoptosis Detection Kit (Qcbio Science & Technologies, Shanghai, China). After transfection, the cells were collected and re-suspended with 1 × binding buffer. Then, the cells were stained with Annexin V-FITC and PI. The apoptotic cells were determined by a flow cytometer.

Transwell Assay

24-well plates of transwell chambers were obtained from Corning Inc., (Corning, NY, USA). Matrigel-coated membrane (Corning) was used for invasion assay. A2780/PTX and SKOV3/PTX cells were seeded in the upper chambers, which were filled with serum-free medium. The lower chambers were added with complete medium. After 24 h, the lower chamber cells were fixed and stained. The migrated and invaded cells were observed and counted by a microscope (100 ×).

Western Blot (WB) Analysis

Protein was extracted by RIPA buffer (Beyotime), and separated using 10% SDS-PAGE gel. After electrophoretically transferred onto PVDF membranes (Bio-Rad, Hercules, CA, USA), the membranes were incubated with the primary antibodies against proliferating cell nuclear antigen (PCNA; 1:3,000, Genetex, SA, Texas, USA), CyclinD1 (1:2,000, Genetex), B-cell lymphoma-2 (Bcl-2; 1:3,000, Genetex), cleaved-caspase 3 (c-caspase 3, 1:1,000, Genetex), metal matrix proteinase 2 (MMP2; 1:3,000, Genetex), MMP9 (1:2,000, Genetex), HOXC8 (1:3,000, Genetex) or GAPDH (1:5,000, Genetex), followed by incubation with secondary antibody (1:2,000, Genetex). Finally, protein signals were detected using the Supersignal West Femto Kit (Thermo Fisher Scientific).

Tumor Xenograft Models

SKOV3/PTX cells transfected sh-circNRIP1 or sh-NC were injected subcutaneously into the flank of BALB/c nude mice (Southern Medical University, Guangzhou, China). After injection for 7 days, the length and width of subcutaneous tumors in mice were measured to calculate tumor volume, and then PBS or PTX was injected intraperitoneally into mice at 3 mg/kg. Thereafter, injections and measurements were taken every 4 days up to 27 days. After the mice were euthanized, the tumors were taken out and weighed, and circNRIP1 expression was detected. This study was approved by the Animal Committee of Xingtai People's Hospital and performed according to the Guide for the Care and Use of Laboratory Animals.

Localization of circRNA

Cytoplasmic & Nuclear RNA Purification Kit (Norgen Biotek Corp, Ontario, Canada) was employed to isolate and extract the cytoplasm and nucleus RNA of cells. Then, the circNRIP1, U6 and GAPDH expression levels in the cytoplasm and

nucleus of A2780/PTX and SKOV3/PTX cells were detected using qRT-PCR. U6 and GAPDH were used as the nucleus control and cytoplasm control, respectively.

Dual-Luciferase Reporter Assay

Wild-type and mutant-type circNRIP1 and HOXC8 (circNRIP1-WT/MUT and HOXC8 3′UTR-WT/MUT) reporter vectors were constructed by Fenghui (Hunan, China), and then they were co-transfected with miR-211-5p mimic or miR-NC into A2780/PTX and SKOV3/PTX cells. After 48 h, the luciferase activity was determined using Luc-Pair™ Duo-Luciferase HS Assay Kit (GeneCopoeia, Rockville, MD, USA).

RNA Immunoprecipitation (RIP) Assay

A2780/PTX and SKOV3/PTX cells were lysed by RIP buffer (Yingbiotech, Shanghai, China). The lysate was incubated with magnetic beads (Yingbiotech) coupled with the antibodies against Ago2 or IgG at 4°C overnight. Then, the circNRIP1, miR-211-5p and HOXC8 expression levels were examined by qRT-PCR.

Statistical Analysis

GraphPad Prism 7.0 software (GraphPad, La Jolla, CA, USA) was the main tool for statistical analysis. All data were expressed as mean ± SD. Student's *t*-test or one-way ANOVA was responsible for P values. $P < 0.05$ was considered to be statistically significant.

Results

CircNRIP1 Was Upregulated in OC

Compared to adjacent normal tissues, we uncovered that circNRIP1 was markedly increased in OC tissues, and its expression in PTX-resistant OC tissues was significantly higher than that in PTX-sensitive OC tissues (Figure 1A). Similarly, circNRIP1 also was upregulated in OC cells compared with that in HOEC cells, and its expression in PTX-resistant OC cells (A2780/PTX and SKOV3/PTX) was remarkably higher than that in normal OC cells (A2780 and SKOV3) (Figure 1B). Also, circNRIP1 showed strong resistance to Rnase R in A2780/PTX and SKOV3/PTX cells, confirming that circNRIP1 was a circular transcript (Figure 1C-D). Meanwhile, ActD assay results indicated that circNRIP1 had better resistance to ActD compared with linear NRIP1 mRNA, revealing that circNRIP1 was more stable than NRIP1 mRNA (Figure 1E-F). Furthermore, MTT assay was used to confirm the resistance of A2780/PTX and SKOV3/PTX cells to PTX, and the results revealed that with the increase of PTX concentration, the viabilities of A2780/PTX and SKOV3/PTX cells were remarkably higher than that of A2780 and SKOV3 cells, and their IC50 values were also markedly enhanced compared with A2780 and SKOV3 cells, suggesting that PTX-resistant OC cells had good resistance to PTX (Figure 1G-I).

Knockdown of circNRIP1 Reduced the Resistance of A2780/PTX and SKOV3/PTX Cells to PTX in vitro

To explore the role of circNRIP1 on the PTX resistance of OC cells, we transfected si-circNRIP1#1/#2 into A2780/PTX and SKOV3 cells. The decreasing expression of circNRIP1 indicated that the transfection efficiencies of si-circNRIP1#1 and si-circNRIP1#2 were excellent (Figure 2A). Through measuring the resistance of A2780/PTX and SKOV3/PTX cells to PTX, we discovered that silenced circNRIP1 could suppress the viability of A2780/PTX and SKOV3/PTX cells, and the IC50 values were significantly repressed (Figure 2B-D). Moreover, circNRIP1 knockdown inhibited the proliferation and promoted the apoptosis of A2780/PTX and SKOV3/PTX cells (Figure 2E-G). Additionally, the number of migrated and invaded A2780/PTX and SKOV3/PTX cells also was suppressed by circNRIP1 silencing (Figure 2H-I). Furthermore, WB analysis showed that the protein levels of proliferation markers (PCNA and CyclinD1), anti-apoptosis marker (Bcl-2), and metastasis markers (MMP2 and MMP9) were repressed by circNRIP1 silencing, and apoptosis marker (c-caspase 3) was promoted by circNRIP1 knockdown (Figure 2J-L). Our data suggested that circNRIP1 knockdown could enhance the sensitivity of OC cells to PTX.

CircNRIP1 Silencing Promoted the Sensitivity of OC Tumor to PTX in vivo

For further confirming the function of circNRIP1 in OC, OC tumor xenograft model for SKOV3/PTX cells stably transfected with sh-circNRIP1 or sh-NC was constructed. Through measuring the tumor volume and weight, we found that the tumor volume and weight of the sh-circNRIP1 group were significantly decreased compared to the sh-NC group, and the tumor volume and weight of sh-circNRIP1 + PTX group were lower than the sh-NC + PTX group, suggesting that circNRIP1 knockdown markedly enhanced the sensitivity of OC tumor to PTX (Figure 3A-B). In addition, by detecting circNRIP1 expression in OC tumor tissues, we confirmed that the transfection of sh-circNRIP1 was successful, Besides,

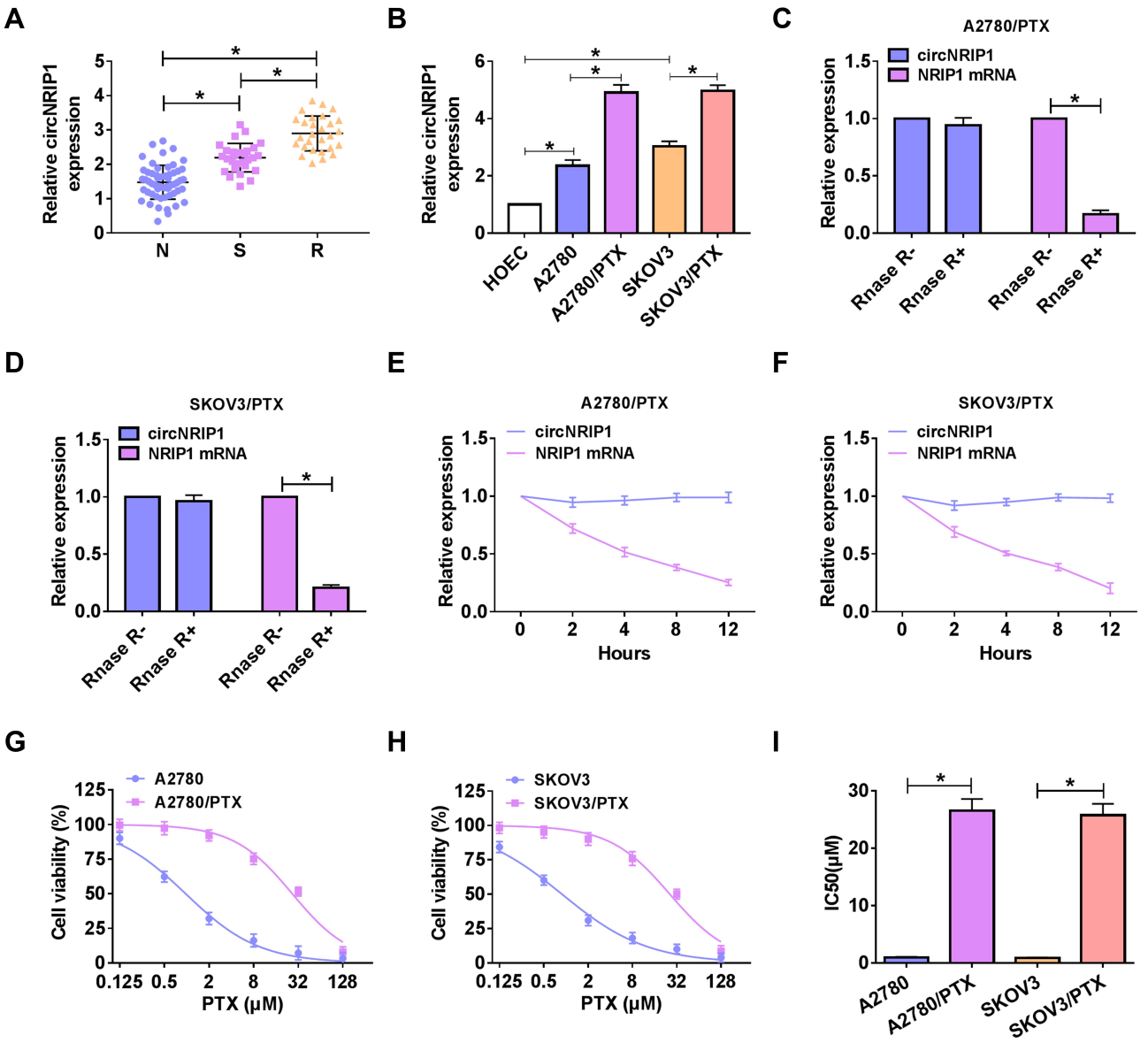

Figure 1 The expression and circular characteristics identification of circNRIP1 in OC. (**A**) The expression of circNRIP1 was detected by qRT-PCR in adjacent normal tissues (N), PTX-sensitive OC tissues (S) and PTX-resistant OC tissues (R). (**B**) QRT-PCR was used to measure the circNRIP1 expression in HOEC cells, normal OC cells and PTX-resistant OC cells. (**C–D**) Rnase R assay was used to evaluate the resistance of circNRIP1 and NRIP1 mRNA to the digestion of RNase R in A2780/PTX and SKOV3/PTX cells. (**E–F**) ActD assay was performed to assess the stable of circNRIP1 and NRIP1 mRNA in A2780/PTX and SKOV3/PTX cells. (**G–I**) The viabilities and IC50 values of A2780, A2780/PTX, SKOV3 and SKOV3/PTX cells were detected by MTT assay to reflect the PTX resistance of cells. *$P < 0.05$.

circNRIP1 expression in the sh-NC + PTX group was increased compared with the sh-NC group, indicating that PTX could promote the expression of circNRIP1 (Figure 3C). Therefore, we confirmed that the interference of circNRIP1 could restrain the resistance of OC tumor to PTX.

CircNRIP1 Could Sponge miR-211-5p in OC

For investigating the potential molecular mechanism of circNRIP1 in the PTX resistance of OC, we used the LncBase Predicted tool to predict the miRNA targets for circNRIP1. As presented in Figure 4A, miR-211-5p was found to be complementary to circNRIP1. Moreover, we discovered that circNRIP1 was mainly accumulated in the cytoplasm of A2780/PTX and SKOV3/PTX cells, which confirmed that circNRIP1 might serve as a ceRNA for miR-211-5p (Figure 4B-C). The results of dual-luciferase reporter assay presented that miR-211-5p suppressed the luciferase activity of circNRIP1-WT rather than circNRIP1-MUT (Figure 4D-E), and RIP assay suggested that the expression of circNRIP1 and miR-211-5p was markedly

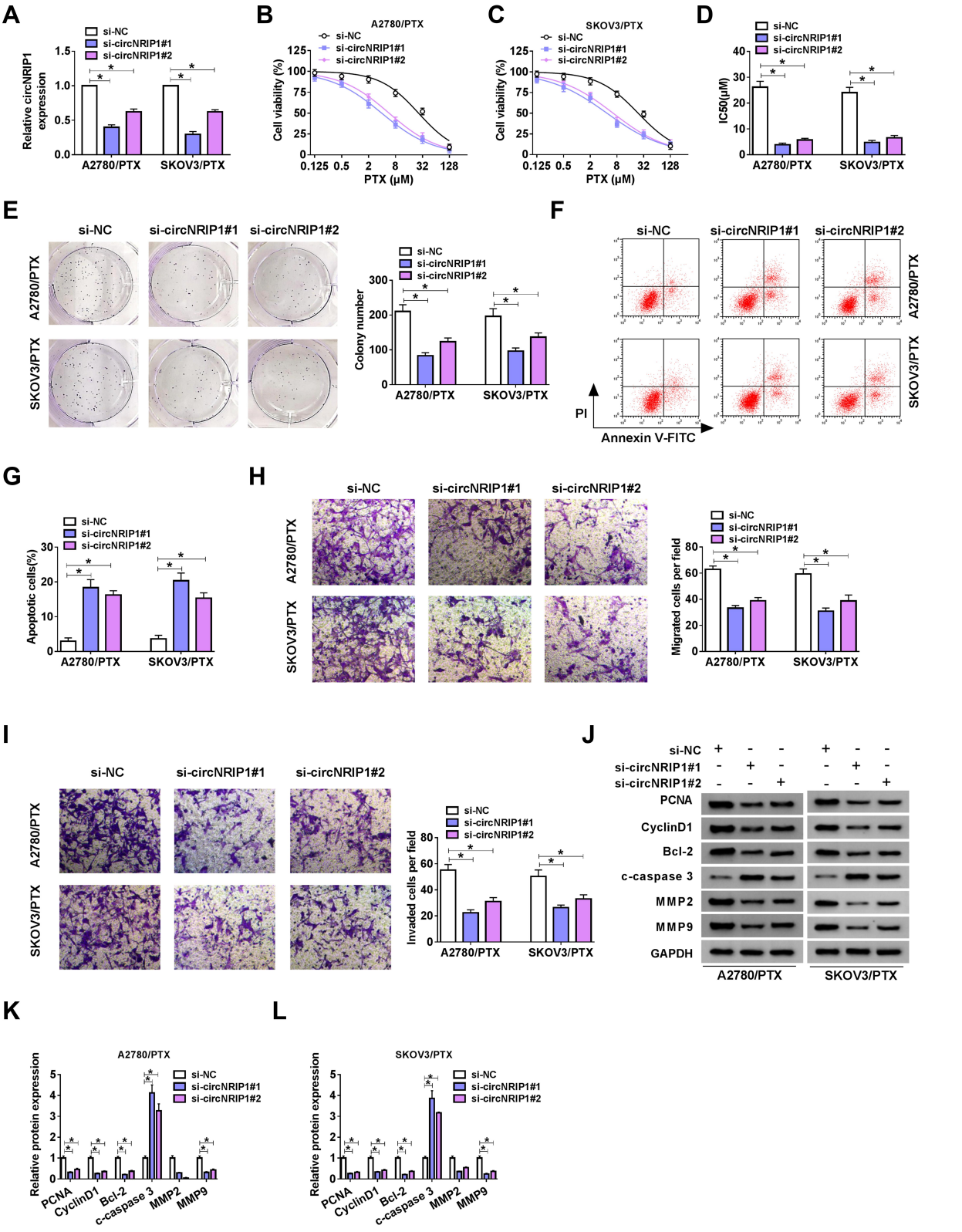

Figure 2 Knockdown of circNRIP1 reduced the PTX resistance of OC cells. A2780/PTX and SKOV3/PTX cells were transfected with si-NC, si-circNRIP1#1 or si-circNRIP1#2. (**A**) The circNRIP1 expression was measured by qRT-PCR. (**B–D**) The viabilities and IC50 values of cells were determined using MTT assay. Colony formation assay (**E**), flow cytometry (**F–G**) and transwell assay (**H–I**) were used to detect the proliferation, apoptosis, migration and invasion, respectively. (**J–L**) WB analysis was employed to measure the protein levels of PCNA, CyclinD1, Bcl-2, c-caspase 3, MMP2 and MMP9. * $P < 0.05$.

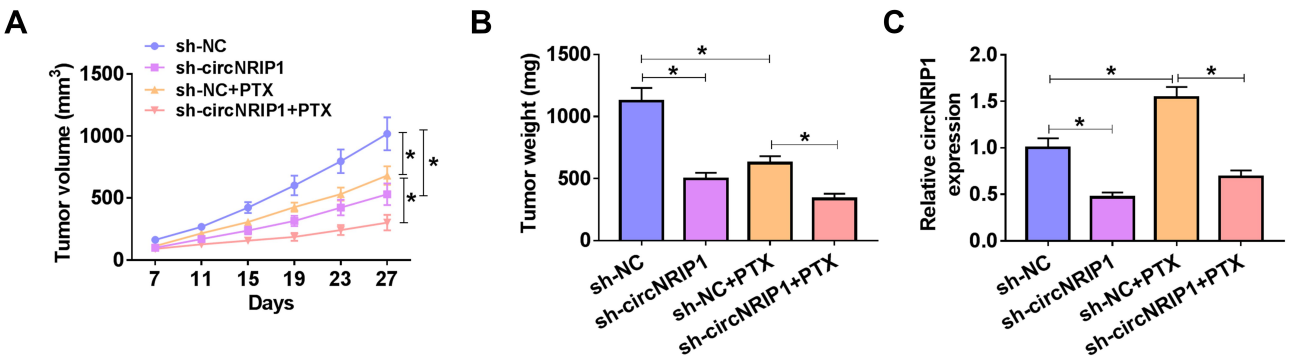

Figure 3 CircNRIP1 silencing promoted the sensitivity of OC tumor to PTX in vivo. SKOV3/PTX cells transfected with sh-NC or sh-circNRIP1 were injected into nude mice, and then treated with PTX. Tumor volume (**A**) and tumor weight (**B**) were detected in OC tumor xenograft model. (**C**) The circNRIP1 expression was measured by qRT-PCR. * $P < 0.05$.

enriched in Ago2 compared to IgG (Figure 4F-G). Additionally, the expression of miR-211-5p could be promoted by circNRIP1 knockdown, while inhibited by circNRIP1 overexpression (Figure 4H-I). These results revealed that circNRIP1 could directly target miR-211-5p in OC cells.

CircNRIP1 Regulated the PTX Resistance of OC Cells by Targeting miR-211-5p

Through detecting miR-211-5p expression, we discovered that miR-211-5p expression in OC tissues was obviously decreased compared to that in adjacent normal tissues, and was lower in PTX-resistant OC tissues than in PTX-sensitive OC tissues (Figure 5A). Correlation analysis revealed that there was a negative correlation between miR-211-5p and circNRIP1 expression in OC tissues (Figure 5B). Similarly, miR-211-5p was downregulated in OC cells, and was markedly lowly expressed in PTX-resistant OC cells compared with that in normal OC cells (Figure 5C). To explore whether circNRIP1 regulated the PTX resistance of OC cells via sponging miR-211-5p, we co-transfected si-circNRIP1#1 and anti-miR-211-5p into A2780/PTX and SKOV3/PTX cells to perform the rescue experiments. QRT-PCR results suggested that anti-miR-211-5p could reverse the promoting effect of circNRIP1 silencing on miR-211-5p expression, revealing that the transfection of si-circNRIP1#1 and anti-miR-211-5p was successful (Figure 5D). The results of MTT assay indicated that miR-211-5p inhibitor partially reversed the reduction effect of circNRIP1 knockdown on the PTX resistance of A2780/PTX and SKOV3/PTX cells (Figure 5E-G). Meanwhile, the suppressive effect of circNRIP1 silencing on the proliferation, migration and invasion, and the promotion effect on the apoptosis of A2780/PTX and SKOV3/PTX cells also could be partially reversed by miR-211-5p knockdown (Figure 5H-K). Furthermore, miR-211-5p inhibitor also inverted the decreasing effect of circNRIP1 knockdown on the protein levels of PCNA, CyclinD1, Bcl-2, MMP2 and MMP9, and the increasing effect on the protein level of c-caspase 3 (Figure 5L-O).

MiR-211-5p Directly Targeted HOXC8 in OC

The StarBase software predicted that HOXC8 had binding sites with miR-211-5p (Figure 6A). Besides, dual-luciferase reporter assay results revealed that miR-211-5p overexpression could repress the luciferase activity of HOXC8 3′UTR-WT, while had no effect on the luciferase activity of HOXC8 3′UTR-MUT (Figure 6B and C). Also, RIP assay results indicated that the enrichment of miR-211-5p and HOXC8 in Ago2 was remarkably higher than that in IgG (Figure 6D and E). Meanwhile, qRT-PCR results suggested that the HOXC8 mRNA and protein levels in A2780/PTX and SKOV3/PTX cells could be inhibited by miR-211-5p overexpression and promoted by miR-211-5p inhibitor (Figure 6F-I). All data showed that HOXC8 could be targeted by miR-211-5p in OC cells.

HOXC8 Participated in miR-211-5p Regulated the PTX Resistance of OC Cells

By measuring the mRNA and protein levels of HOXC8 in OC tissues, we uncovered that HOXC8 expression was elevated in OC tissues compared to adjacent normal tissues, and was higher in PTX-resistant OC tissues (Figure 7A and B). Moreover, the results of correlation analysis suggested that HOXC8 expression was negatively correlated with miR-211-5p expression in OC tissues (Figure 7C). In OC cells, we

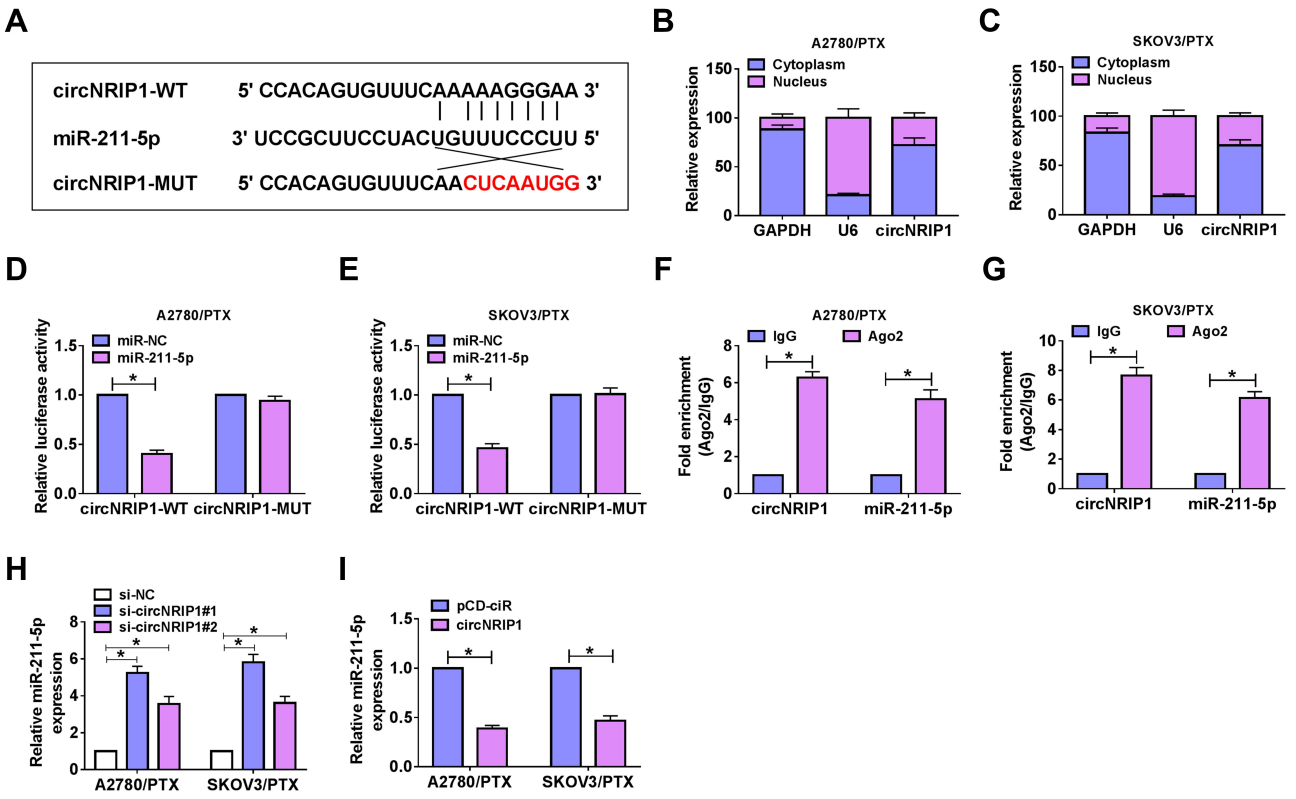

Figure 4 CircNRIP1 could sponge miR-211-5p in OC. (**A**) The sequences of circNRIP1-WT and circNRIP1-MUT were shown. (**B–C**) QRT-PCR was used to measure the expression of circNRIP1, GAPDH and U6 in the nucleus and cytoplasm of A2780/PTX and SKOV3/PTX cells. Dual-luciferase reporter assay (**D–E**) and RIP assay (**F–G**) were performed to verify the interaction relationship between miR-211-5p and circNRIP1 in A2780/PTX and SKOV3/PTX cells. (**H–I**) The expression of miR-211-5p was measured by qRT-PCR to evaluate the effect of circNRIP1 expression on miR-211-5p expression. * $P < 0.05$.

found that HOXC8 expression was remarkably promoted compared to HOEC cells, and also were upregulated in PTX-resistant OC cells compared to normal OC cells (Figure 7D and E). To further confirm that miR-211-5p regulated the PTX resistance of OC cells by HOXC8, we performed the rescue experiments. By detecting the IC50 values at different concentrations of miR-211-5p, we determined that the inhibition effect of miR-211-5p on the PTX resistance of cells was strongest when the concentration of miR-211-5p mimic was 50 nM, so we selected 50 nM miR-211-5p for subsequent tests (Supplementary Fig 1). Subsequently, we co-transfected miR-211-5p mimic and HOXC8 overexpression plasmid into A2780/PTX and SKOV3/PTX cells. The increasing mRNA and protein levels of HOXC8 in the miR-211-5p + HOXC8 group compared to the miR-211-5p group revealed that the transfection efficiencies of miR-211-5p mimic and HOXC8 overexpression plasmid were good (Figure 7F and G). Through evaluating the viabilities and IC50 values of A2780/PTX and SKOV3/PTX cells, we found that miR-211-5p overexpression decreased the resistance of A2780/PTX and SKOV3/PTX cells to PTX, while HOXC8 could partially reverse this effect (Figure 7H-J). Besides, the inhibitory effect of miR-211-5p on the proliferation, migration, invasion and the promotion effect on the apoptosis of A2780/PTX and SKOV3/PTX cells also could be partially reversed by HOXC8 overexpression (Figure 7K-N). Moreover, upregulation of HOXC8 also inverted the suppressive effect of miR-211-5p on the protein levels of PCNA, CyclinD1, Bcl-2, MMP2 and MMP9, and the accelerating effect on the protein level of c-caspase 3 in A2780/PTX and SKOV3/PTX cells (Figure 7O-R). In addition, we examined the effect of the knockdown of HOXC8 on the PTX resistance of OC cells. The results showed that when the expression of HOXC8 was successfully decreased (Supplementary Fig 2A-B), the viabilities and IC50 values of A2780/PTX and SKOV3/PTX cells were significantly inhibited (Supplementary Fig 2C-E). Furthermore, silenced HOXC8 also repressed the proliferation, migration, invasion, and promoted apoptosis in A2780/PTX and SKOV3/PTX cells (Supplementary Fig 2F-I). These results suggested that the knockdown of HOXC8 could inhibit the PTX resistance of OC cells, which was similar to the role of miR-211-5p. Our results illuminated that miR-211-5p could target HOXC8 to inhibit the PTX resistance of OC cells.

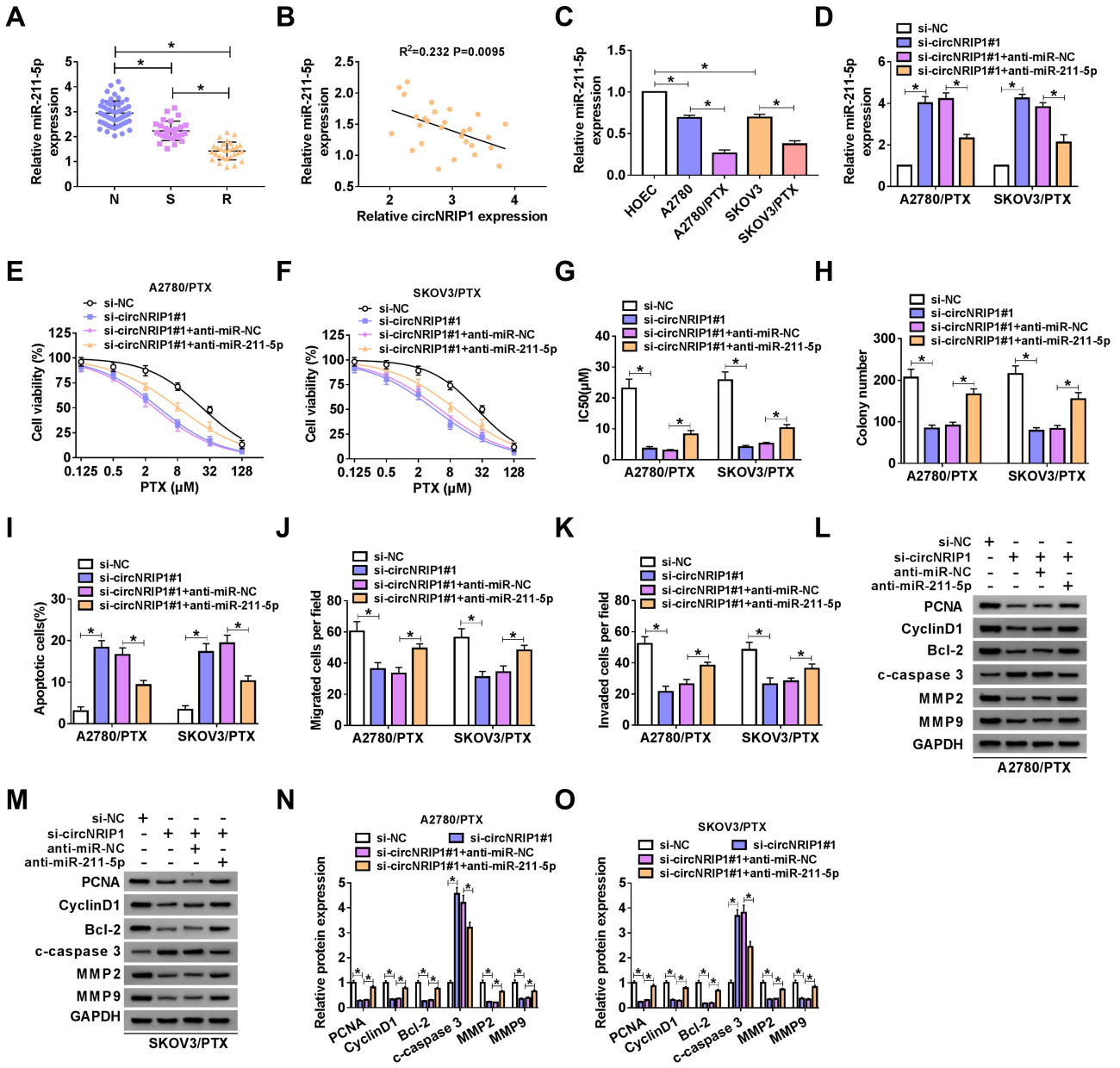

Figure 5 CircNRIP1 regulated the PTX resistance of OC cells by targeting miR-211-5p. (**A**) The expression of miR-211-5p was determined by qRT-PCR in adjacent normal tissues (N), PTX-sensitive OC tissues (S) and PTX-resistant OC tissues (R). (**B**) Pearson correlation analysis was used to evaluate the correlation between circNRIP1 and miR-211-5p in OC tissues. (**C**) QRT-PCR was employed to detect the miR-211-5p expression in HOEC cells, normal OC cells and PTX-resistant OC cells. (**D–O**) A2780/PTX and SKOV3/PTX cells were transfected with si-NC, si-circNRIP1#1, si-circNRIP1#1 + anti-miR-NC, or si-circNRIP1#1 + anti-miR-211-5p, respectively. (**D**) MiR-211-5p expression was detected by qRT-PCR. (**E–G**) MTT assay was performed to measure cell viability and IC50 values. Colony formation assay (**H**), flow cytometry (**I**) and transwell assay (**J–K**) were employed to determine the proliferation, apoptosis, migration and invasion, respectively. (**L–O**) The protein levels of PCNA, CyclinD1, Bcl-2, c-caspase 3, MMP2 and MMP9 were measured by WB analysis. * $P < 0.05$.

CircNRIP1 Positively Regulated HOXC8 Expression by Sponging miR-211-5p

For further confirming the circNRIP1/miR-211-5p/HOXC8 axis existed in OC, we explored the effect of circNRIP1 expression on HOXC8 expression. The results of correlation analysis indicated that HOXC8 expression was positively correlated with circNRIP1 in OC (Figure 8A). Furthermore, qRT-PCR and WB analysis results revealed that circNRIP1 silencing could inhibit the mRNA and protein levels of HOXC8 in A2780/PTX and SKOV3/PTX cells, while this effect was reversed by miR-211-5p inhibitor (Figure 8B-C). These data suggested that circNRIP1 could sponge miR-211-5p to regulate HOXC8 expression in OC.

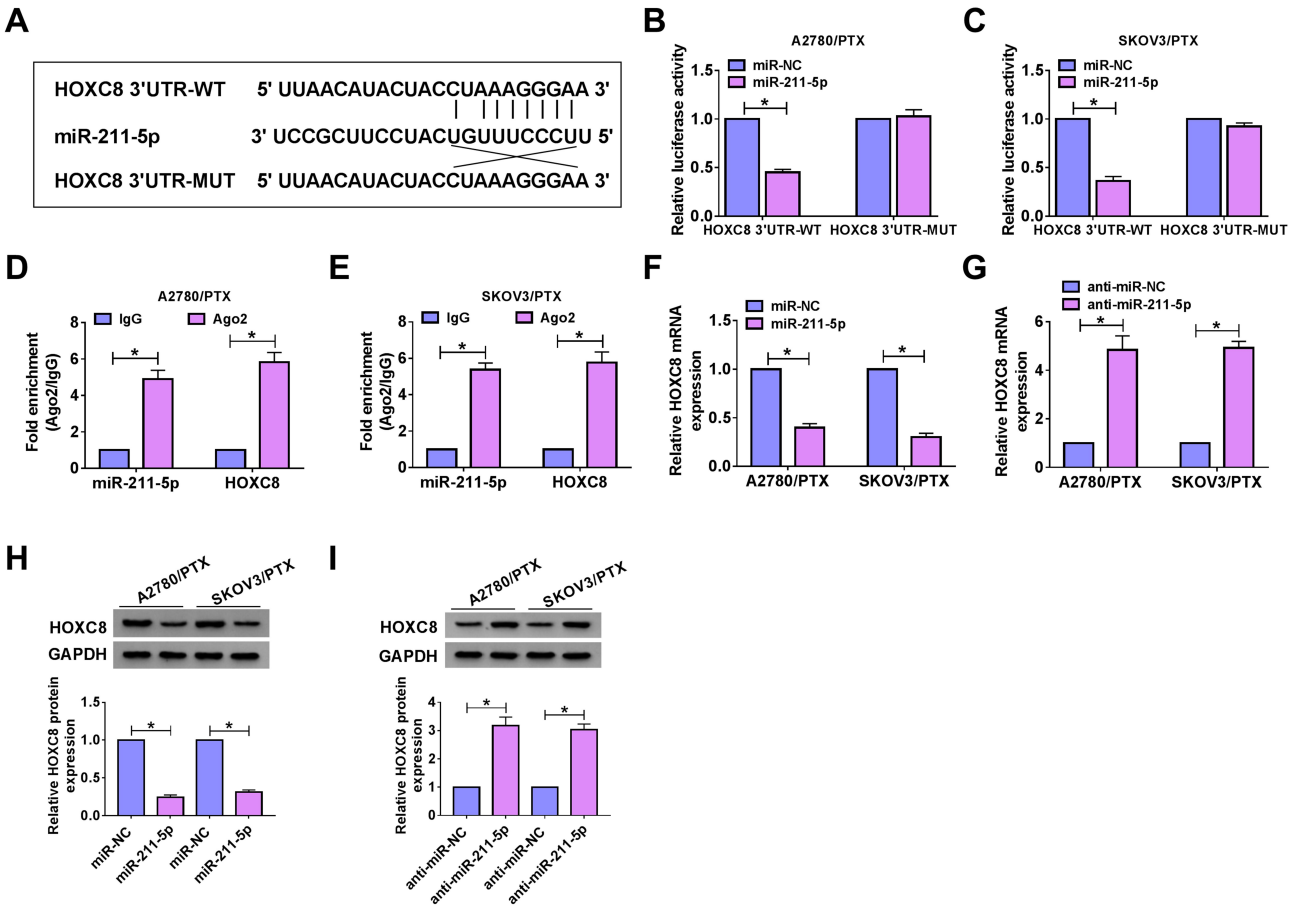

Figure 6 MiR-211-5p directly targeted HOXC8 in OC. (**A**) The fragments of HOXC8 3′UTR-WT and HOXC8 3′UTR-MUT were presented. Dual-luciferase reporter assay (**B–C**) and RIP assay (**D–E**) were used to confirm the interaction relationship between miR-211-5p and HOXC8 in A2780/PTX and SKOV3/PTX cells. QRT-PCR (**F–G**) and WB analysis (**H–I**) were employed to detect the mRNA and protein levels of HOXC8 in A2780/PTX and SKOV3/PTX cells transfected with anti-miR-NC or anti-miR-211-5p. * $P < 0.05$.

Discussion

The mechanism of chemotherapy resistance may be manifold and extremely complex, including the interactions of many molecules.[26,27] The great potential of circRNA in regulating the chemotherapy resistance of cancer has been demonstrated. For example, the study of Liu et al suggested that circ-PVT1 enhanced the PTX resistance of gastric cancer by regulating the miR-124-3p/ZEB1 axis,[28] and Ma et al showed that circAMOTL1 might have a positive effect on the PTX resistance of breast cancer via targeting AKT.[29] Here, we focused on exploring the circNRIP1 role in the PTX resistance of OC. Our results revealed that circNRIP1 was upregulated in OC tissues and cells, especially in those with PTX resistance, which was similar with the previous study.[12] Besides, we confirmed that circNRIP1 was a circular, stable circRNA and located in the cytoplasm of cells.

Currently, a large number of studies on circRNA in the chemotherapy resistance of cancer suggest that different circRNAs may also have different roles in cancer resistance.[30,31] In view of the high expression of circNRIP1 in OC, we investigated the influence of circNRIP1 downregulation on the chemotherapy resistance of OC. The results revealed that circNRIP1 silencing suppressed the PTX resistance of OC cells to restrain the proliferation, metastasis, and accelerate the apoptosis of OC cells in vitro. In vivo experiments suggested that interference of circNRIP1 also repressed the resistance of OC tumor to PTX. These results revealed that circNRIP1 might have an active role in the PTX resistance of OC, and circNRIP1 knockdown might be an important method to overcome chemotherapy resistance in OC patients.

Further experiments revealed that circNRIP1 could sponge miR-211-5p to regulate HOXC8 expression. Consistent with the previous findings,[22] our results indicated that miR-211-5p expression was decreased in PTX-resistant OC tissues and cells, and the reversal effect of miR-211-5p inhibitor on the function of circNRIP1

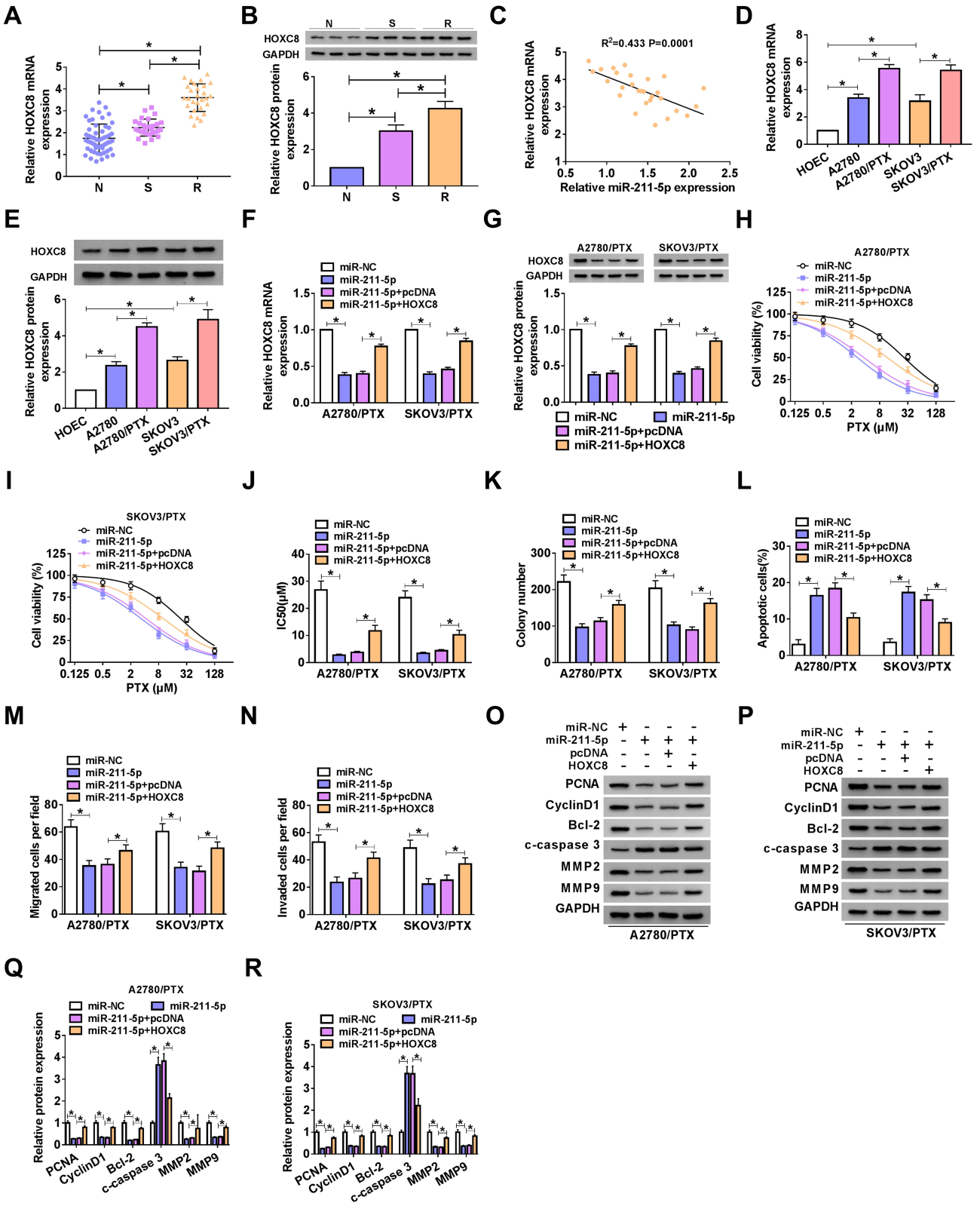

Figure 7 MiR-211-5p regulated the PTX resistance of OC cells via targeting HOXC8. (**A–B**) The mRNA and protein levels of HOXC8 in adjacent normal tissues (**N**), PTX-sensitive OC tissues (**S**) and PTX-resistant OC tissues (**R**) were measured by qRT-PCR and WB analysis. (**C**) Pearson correlation analysis was performed to assess the correlation between HOXC8 and miR-211-5p in OC tissues. (**D–E**) QRT-PCR and WB analysis were used to determine the mRNA and protein levels of HOXC8 in HOEC cells, normal OC cells and PTX-resistant OC cells. (**F–R**) A2780/PTX and SKOV3/PTX cells were transfected with miR-NC, miR-211-5p, miR-211-5p + pcDNA, or miR-211-5p + HOXC8, respectively. (**F–G**) The mRNA and protein levels of HOXC8 in A2780/PTX and SKOV3/PTX cells were detected by qRT-PCR and WB analysis. (**H–J**) The viabilities and IC50 values were determined by MTT assay. Colony formation assay (**K**), flow cytometry (**L**) and transwell assay (**M–N**) were used to evaluate the proliferation, apoptosis, migration and invasion, respectively. (**O–R**) WB analysis was employed to assess the protein levels of PCNA, CyclinD1, Bcl-2, c-caspase 3, MMP2 and MMP9. * $P < 0.05$.

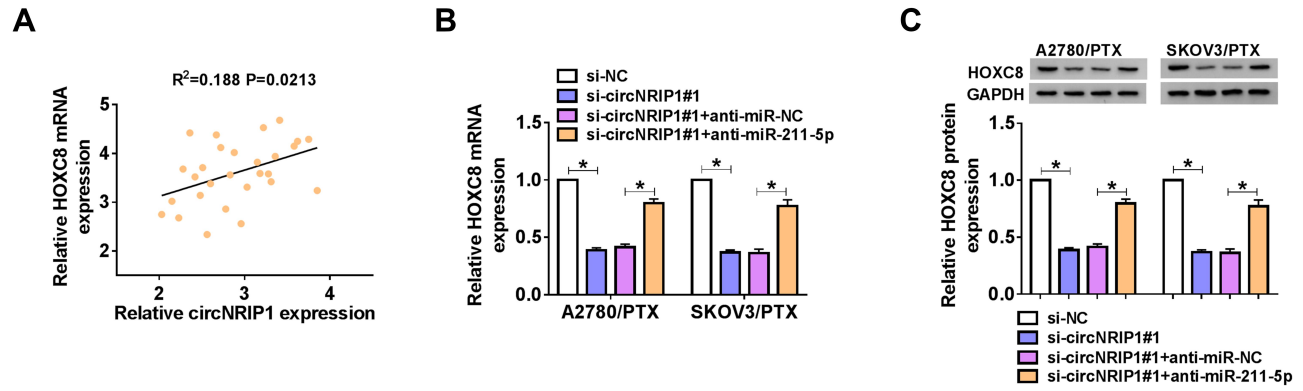

Figure 8 CircNRIP1 positively regulated HOXC8 by sponging miR-211-5p. (**A**) The correlation between HOXC8 and circNRIP1 in OC tissues was analyzed by Pearson correlation analysis. QRT-PCR (**B**) and WB analysis (**C**) were performed to measure the mRNA and protein levels of HOXC8 in A2780/PTX and SKOV3/PTX cells transfected with si-NC, si-circNRIP1#1, si-circNRIP1#1 + anti-miR-NC, or si-circNRIP1#1 + anti-miR-211-5p, respectively. * $P < 0.05$.

knockdown confirmed that circNRIP1 could sponge miR-211-5p to promote the PTX resistance of OC. Our study found that HOXC8 had elevated expression in OC tissues and cells with PTX-resistant, which was similar to the previous study.[25] Function experiments revealed that miR-211-5p hindered the resistance of OC cells to PTX by targeting HOXC8. Furthermore, HOXC8 expression was positively regulated by circNRIP1 and negatively regulated by miR-211-5p. Our study provided a new mechanism for circNRIP1 to regulate cancer resistance.

Conclusions

In summary, our results revealed that silencing of circNRIP1 inhibited the PTX resistance of OC by regulating the miR-211-5p/HOXC8 axis. Our findings provide new targets for overcoming chemotherapy resistance in OC. In addition, the introduction of the circNRIP1/miR-211-5p/HOXC8 regulatory axis provides a new reference for exploring the role of circNRIP1 in other cancers.

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Disclosure of Interest

The authors declare that they have no conflicts of interest for this work.

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