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Rev Invest Clin. 2024;76(2):103-15 ORIGINAL ARTICLE

PITPNA-AS1 Inhibits Cell Proliferation and Migration in Ovarian Cancer by Regulating the MIR-223-3p/RHOB Axis

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ABSTRACT

Background: Ovarian cancer is a fatal gynecologic malignancy. Long non-coding RNA (lncRNA) has been verified to serve as key regulator in ovarian cancer tumorigenesis. **Objective:** The aim of the study was to study the functions and mechanism of lncRNA PITPNA-AS1 in ovarian cancer cellular process. **Methods:** Clinical ovarian cancer samples were collected and stored at an academic medical center. Cellular fractionation assays and fluorescence *in situ* hybridization were conducted to locate PITPNA-AS1 in OC cells. TUNEL staining, colony-forming assays, and Transwell assays were performed for evaluating cell apoptosis as well as proliferative and migratory abilities. Western blot was conducted for quantifying protein levels of epithelial-mesenchymal transition markers. The binding relation between genes was verified by RNA pulldown, RNA immunoprecipitation, and luciferase reporter assays. Gene expression levels in ovarian cancer tissues and cells were subjected to RT-qPCR. **Results:** PITPNA-AS1 level was downregulated in ovarian cancer samples and cells. PITPNA-AS1 overexpression contributed to the accelerated ovarian cancer cell apoptosis and inhibited cell migration, proliferation, and epithelial-mesenchymal transition process. In addition, PITPNA-AS1 interacted with miR-223-3p to regulate RHOB. RHOB knockdown partially counteracted the repressive impact of PITPNA-AS1 on ovarian cancer cell activities. **Conclusion:** PITPNA-AS1 inhibited ovarian cancer cellular behaviors by targeting miR-223-3p and regulating RHOB. (REV INVEST CLIN. 2024;76(2):103-15)

Keywords: PITPNA-AS1. miR-223-3p. RHOB. Ovarian cancer.

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INTRODUCTION

Ovarian cancer (OC) is reported to be the third commonest gynecological malignant tumor in the world[1]. The approaches currently adopted for OC treatment include surgery, cytotoxic chemotherapy, and radiotherapy[2]. In the last decades, standard treatments for OC have contributed to the improvement in the outcome for OC patients. Nonetheless, the occurrence of acquired drug resistance and metastasis usually results in adverse prognosis in patients at advanced stage[3]. Thus, exploration of novel OC-related biomarkers is important for OC diagnosis and target therapy.

Long non-coding RNA (lncRNA) refers to a poorly conserved endogenous RNA with over 200 nucleotides[4]. Previous research has verified that lncRNAs are key players in modulating malignant behaviors of cancer cells, such as cell growth, apoptosis, migration, and invasiveness[5,6]. Emerging evidence has demonstrated the significance of lncRNAs in regulating OC tumorigenesis[7,8]. LncRNA PITPNA-AS1 is a relatively novel lncRNA and is aberrantly expressed in diverse human cancers[9,10]. Silenced PITPNA-AS1 restrains the epithelial-mesenchymal transition (EMT) process, metastasis, and proliferation of lung cancer cell lines through binding with miR-32-5p[11]. PITPNA-AS1 aggravates the malignant processes in hepatocellular carcinoma by abrogating the repressive impact of miR-223-3p on WNT5A[12]. PITPNA-AS1 facilitates the tumorigenicity of cervical cancer via modulating cell cycle and apoptosis[13]. Nevertheless, research on PITPNA-AS1 is still quite limited and the significance of PITPNA-AS1 in OC biological process remains unclear. Different from the tumor-promoting role of PITPNA-AS1 in other types of cancer, PITPNA-AS1 is predicted to be downregulated in ovarian serous cystadenocarcinoma, the most common histological subtype of OC, based on bioinformatics analysis. According to RNA-seq of tissue-specific genes in normal tissues collected from human individuals (source: NCBI database-gene), PITPNA-AS1 shows a high expression level in normal ovarian samples, while its expression in other normal human organs such as lung, stomach, and liver is relatively low. That may explain why previous studies reported that PITPNA-AS1 acts as a tumor promoter in lung cancer, gastric cancer, and hepatocellular carcinoma but PITPNA-AS1 is predicted to have anti-tumor potential in OC according to bioinformatics analysis. The present work was designed to reveal the role of PITPNA-AS1 in regulating OC cell activities.

LncRNA can participate the regulation of cancer occurrence acting as a competing endogenous RNA (ceRNA) to competitively bind to microRNA and elevate messenger RNA (mRNA) level[14]. Mounting studies showed that lncRNAs modulate OC tumorigenesis through the ceRNA network. For example, lncRNA KTN1-AS1 promotes OC cell growth and metastasis by upregulating the oncogenic gene ZNF326 via interaction with miR-505-3p[15]. LncRNA HCG18 binds with miR-29a/b to elevate TRAF4/TRAF5 level, thereby contributing to the migratory ability and EMT process of epithelial OC[16]. LncRNA GAS5 inhibits the invasive capacity of OC cells by regulating miR-96-5p and PTEN as a ceRNA[17]. The ceRNA networks mediated by PITPNA-AS1 in other types of cancer were confirmed; however, whether PITPNA-AS1 can regulate OC cell activities through ceRNA mechanism remains unclarified.

In this study, functional experiments were applied to determine the biological significance of PITPNA-AS1 in OC. In addition, ceRNA pattern mediated by PITPNA-AS1 in OC was investigated. The findings might provide a new clue in improving the therapeutic methods for OC.

METHODS

Tissue sampling

OC tissue samples (n = 24) and healthy samples (n = 24) were obtained from patients with OC at the hospital. The ethics committee of the hospital approved the protocol, and informed consents were obtained from all participants. No participants had received cancer therapy before admission. After surgery, the tissue samples were kept at -80°C in a freezer.

In situ hybridization

The collected tissues were embedded in paraffin and then dewaxed, rehydrated, and digested with protease K. Next, the samples were rinsed with phosphate-buffered saline (PBS). The *in situ* hybridization kit (Boster, Wuhan, China) was utilized to evaluate

PITPNA-AS1 expression in clinical tissues. In brief, the buffer containing the probe of PITPNA-AS1 (Bersin-Bio, Guangzhou, China) was added to the samples for 12 h of incubation at 65°C. The coverslip was then washed with PBS and fixed with 4% paraformaldehyde.

Cell lines and cell culture

IOSE80, a normal ovarian epithelial cell line, and three human OC cell lines (OVCAR-3, SK-OV-3, and CaoV-3) were obtained from ATCC. All cells were incubated in DMEM (Invitrogen, USA) containing 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) in moist atmosphere at 37°C with 5% CO_2.

Transfection

The pcDNA vectors containing full sequence of PITPNA-AS1 were used for amplifying PITPNA-AS1 level in OC cells, with the empty pcDNA vector as the control. Ras homolog family member B (RHOB) expression was silenced by short hairpin RNA targeting RHOB (sh-RHOB#1/2) and the negative control (NC) plasmid was named sh-NC. The plasmids of miR-223-3p mimics were used for miR-223-3p overexpression, with the control plasmid named NC mimics. All plasmids were obtained from GenePharma (Shanghai, China). For cell transfection, OVCAR-3 and SK-OV-3 cells were transfected with these plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) for 48 h as instructed.

RT-qPCR

TRIzol reagent (Takara, Japan) was employed for extraction of the total RNA from OC samples and cells. RNA (5 μg) was used to reverse transcribe cDNAs utilizing a cDNA Synthesis Kit (Sangon, Biotech). The qPCR was conducted with the FastStart Universal SYBR Green Master Kit (4913850001; Roche, Switzerland) on the ABI RT-PCR System (ABI, Foster City, USA). RNA level was calculated with $2^{-\Delta\Delta Ct}$ method. PITPNA-AS1 and mRNA expression were normalized to GAPDH level, while miRNA expression was normalized to U6.

Western blotting

Proteins in OC cells were obtained using RIPA buffer (P0013B, Beyotime, China). The BCA method was used for the determination of protein concentration. Protein contents were fractionated by 10% SDS-PAGE and then were loaded onto PVDF membranes. Afterward, these membranes were blocked using 5% fat-free milk for 1 hr and then incubated with anti-E-cadherin (#ab40772, 1:1000; Abcam, USA), anti-N-cadherin (#ab245117; 1:1000), anti-Vimentin (#ab8978, 1:1000), anti-RHOB (#ab155149; 1:500) and GAPDH (#ab9485; 1:2500) overnight at 4°C. Then the secondary antibody was incubated with the membranes at 37°C for 2 h without light exposure. ECL Kit (#P0018AS, Beyotime) was employed to visualize the immunoblots, and ImageJ software was utilized for quantification.

Colony forming assay for cell proliferation determination

For the colony forming assay, indicated OC cells (3×10^3) were plated on 6-well plates. The colony containing over 50 cells was considered as a significant one. Cells were cultured for 2 weeks and then fixed with 100% methanol for 30 min. Next, cells were dyed with 0.1% crystal violet solution (1 mL). During cell culture, fresh medium was provided every 3 days. Images of colonies were obtained using an Epson scanner GT-X970 (Epson, Japan).

TUNEL staining for cell apoptosis detection

OC cells were fixed by paraformaldehyde (4%) after PBS washing. After that, 1% Triton X-100 was utilized for cell permeabilization on ice for 2 min. Next, TUNEL test solution (50 μL) was used to treat the cells for 1 h. Cell nuclei were dyed with DAPI solution. Finally, a microscope was used to capture the images of the representative sections.

Transwell assay for cell migration evaluation

Transwell inserts (8 μm pore size; Corning, USA) were utilized for cell migration determination. The upper chamber was added with OC cells cultured in FBS-free RPMI. The medium with 10% FBS was placed to the lower chamber. Cells were cultured for 24 h followed by PBS washing and were fixed with 4% paraformaldehyde for 10 min. Finally, crystal violet (0.05%) was utilized to stain the migrated cells

(passed through the membranes of the upper chamber) for 15 min. Images were taken with a light microscope (Olympus).

Luciferase reporter assay

The wild-type or mutated sequences of PITPNA-AS1 or RHOB 3' UTR were subcloned to pmirGLO reporters (Promega, USA) for the establishment of pmirGLO-PITPNA-AS1-Wt/Mut or pmirGLO-PITPNA-AS1-Wt/Mut. The established vectors were co-transfected with miR-223-3p mimics or NC mimics into OC cells. Lipofectamine 2000 was applied to perform the transfection. At 48 h post-transfection, a luciferase reporter assay system was used to examine the luciferase activities.

RNA pulldown assay

Biotinylated PITPNA-AS1-Wt/-Mut and the biotinylated control (Bio-NC) were obtained from Sangon (Shanghai, China) followed by cell transfection for 24 h. Next, cell lysate was prepared and incubated with the Dynabeads (Thermo Fisher Scientific, USA) for 15 min. RNA on the beads was quantified by RT-qPCR.

RNA immunoprecipitation assay

Magna RNA Immunoprecipitation Kit (Millipore) was applied to the assay. In brief, cell lysates were incubated with anti-Ago2 antibody (1:30, #ab186733, Abcam) precoated on the magnetic beads, with anti-IgG (1:16, #ab182931) antibody as the control group. RNA in the precipitates was analyzed by qPCR.

Subcellular fraction assay

The nuclear and cytoplasmic parts were separated using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) as instructed. Then, PITPNA-AS1, GAPDH (cytoplasmic control), and U6 (nuclear control) were extracted from the nuclear and cytoplasmic parts using TRIzol (Invitrogen) and subjected to qPCR.

Fluorescence *in situ* hybridization (FISH)

Subcellular distribution of PITPNA-AS1 was also detected using fluorescence *in situ* hybridization (FISH) according to previously introduced methods[18]. In brief, after 4% paraformaldehyde treatment and PBS washing, OC cell permeabilization on ice was performed using PBS with 0.5% Triton X-100 for 5 min. Then, OC cells were incubated with PITPNA-AS1 probes (RiboBio, Guangzhou, China) in hybridization buffer at 37°C overnight. After DAPI staining, Olympus IX83 (Olympus, Japan) was used to capture the immunofluorescent images.

Statistical analysis

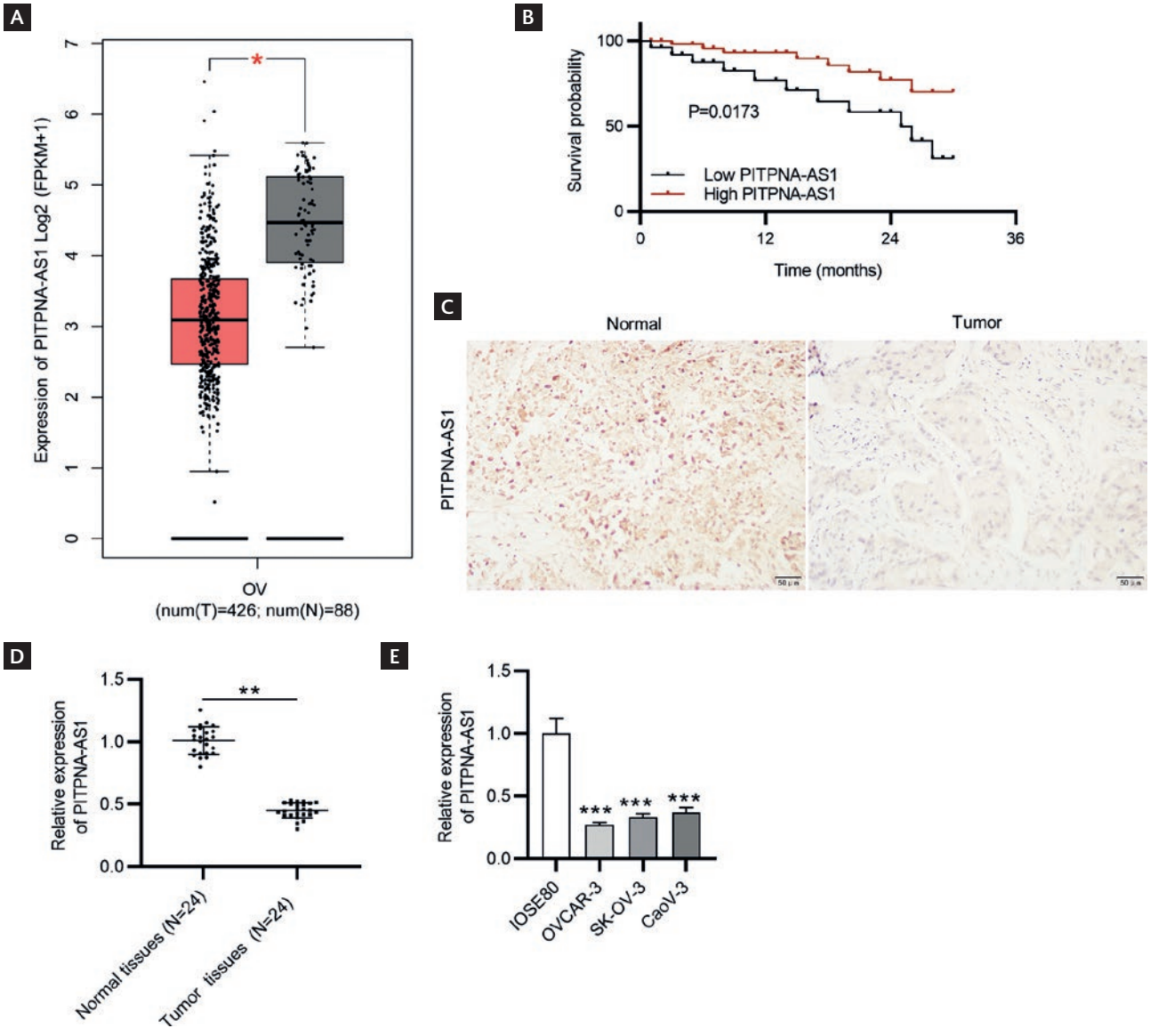
All experiments were conducted 3 times. Data analysis was performed using SPSS 13.0 and graphs were constructed by applying GraphPad Prism 5 (La Jolla, CA, USA). The collected data are shown as the mean ± SD. The significance of differences among groups was evaluated using Student's t-test and ANOVA followed by Tukey's *post hoc* test. Correlations between genes were identified by Spearman's correlation analysis. $p < 0.05$ was deemed as the threshold for statistically significance.

RESULTS

PITPNA-AS1 showed low expression in ovarian cancer and correlated with poor outcome in patients

According to the GEPIA website (data source: TCGA)[19], PITPNA-AS1 shows a low level in OC samples (Fig. 1A). To determine PITPNA-AS1 expression in OC, clinical samples were collected for analysis. The correlation between PITPNA-AS1 expression and the survival outcome of patients enrolled in the study was analyzed and shown in Fig. 1B. As can be seen from the plot, a low PITPNA-AS1 level was associated with poor survival probability while high PITPNA-AS1 expression predicted better survival outcome (Fig. 1B). *In situ* hybridization was performed to detect PITPNA-AS1 expression in tumor and normal samples collected from patients. Consistent with the results of bioinformatics analysis, our results showed that PITPNA-AS1 was downregulated in cancerous samples (Fig. 1C). According to results of RT-qPCR, a low PITPNA-AS1 level was detected in OC samples compared to that in healthy samples (Fig. 1D). In addition, a decreased PITPNA-AS1 level was shown in OC cells (OVCAR-3, CaoV-3, and SK-OV-3) compared to that in IOSE80 cells (Fig. 1E).

Figure 1. PITPNA-AS1 suppressed malignant cellular behaviors in OC. **A:** the bioinformatics tool, GEPIA, was used to analyze PITPNA-AS1 expression in ovarian serous cystadenocarcinoma (OV), the most common histological subtype of OC. **B:** the correlation between PITPNA-AS1 expression and the survival outcome of patients enrolled in the study was illustrated using a plot. **C:** *in situ* hybridization was performed to detect PITPNA-AS1 expression in tumor and normal samples collected from patients. **D:** RT-qPCR for detecting PITPNA-AS1 level in OC samples and healthy tissues. **E:** RT-qPCR for detection of PITPNA-AS1 level in LSCC cell lines and IOSE80 cells. OC: ovarian cancer.

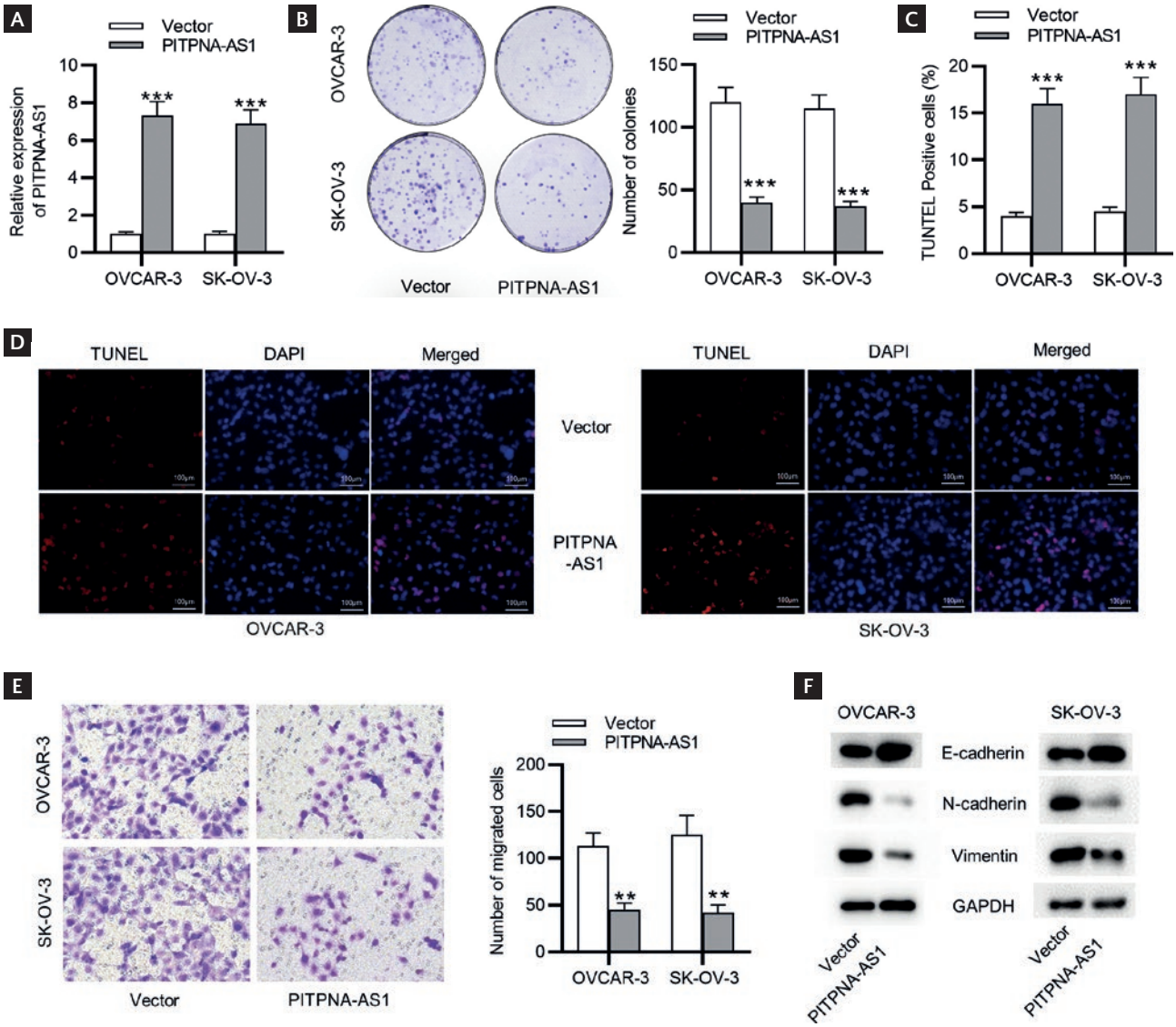


Overexpression of PITPNA-AS1 inhibited malignant behaviors of ovarian cancer cells

Then, pcDNA vectors containing PITPNA-AS1 sequence were transfected into OC cell line SK-OV-3 and OVCAR-3 to explore the role of PITPNA-AS1 in the regulation of OC cell process. Data indicated that PITPNA-AS1 expression was successfully increased after pcDNA-PITPNA-AS1 transfection (Fig. 2A). Colony forming assays delineated that PITPNA-AS1 impaired the proliferative ability of OC cells (Fig. 2B). TUNEL staining was applied to evaluate apoptosis, and the results manifested that the rate of apoptotic OC cells was elevated after overexpressing PITPNA-AS1 (Fig. 2C and D). According to Transwell assays, PITPNA-AS1 overexpression greatly weakened OC cell migration (Fig. 2E). In addition, PITPNA-AS1

Figure 2. Overexpression of PITPNA-AS1 inhibited malignant behaviors of OC cells. **A:** PITPNA-AS1 level in OC cells after pcDNA-PITPNA-AS1 transfection was subjected to PCR analysis. **B:** colony forming assays for measuring the influence of PITPNA-AS1 on the proliferation of two OC cell lines. **C and D:** TUNEL staining of the apoptotic cells after transfection of pcDNA vectors. **E:** Transwell assays for evaluation of OC cell migration in two indicated groups. **F:** Western blotting for quantification of protein expression of EMT markers in OC cells. OC: ovarian cancer.

**p < 0.01.
***p < 0.001.



hampered the EMT process in OC cells by declining Vimentin and N-cadherin protein levels while upregulating E-cadherin protein level (Fig. 2F).

PITPNA-AS1 interacted with miR-223-3p in ovarian cancer cells

Afterward, the regulatory mechanisms mediated by PITPNA-AS1 in OC were explored. First, a cellular fractionation assay was required for determining the location of PITPNA-AS1 in OC cells, and the results revealed that PITPNA-AS1 was predominantly distributed in cytoplasm (Fig. 3A). In addition, FISH also verified that PITPNA-AS1 mostly existed in the cytoplasmic part of OC cells (Fig. 3B). These findings suggest that PITPNA-AS1 may regulate genes post-transcriptionally in OC. Subsequently, based on StarBase prediction[20], three potential miRNAs having binding

sites with PITPNA-AS1 were subjected to RNA pull-down assays. It was denoted that miR-223-3p gained the most obvious enrichment in Bio-PITPNA-AS1-Wt among all candidates (Fig. 3C). Furthermore, RT-qPCR illustrated that miR-223-3p was upregulated in OC cells and samples (Fig. 3D and E). A potential binding site of miR-223-3p to PITPNA-AS1 was displayed in Fig. 3F. MiR-223-3p level was obviously increased in OC cells after transfection with miR-223-3p mimics (Fig. 3G). It was discovered that miR-223-3p enhancement remarkably weakened the luciferase activity of wild-type PITPNA-AS1 vector, but the luciferase activity of mutant-type PITPNA-AS1 vector exhibited no obvious changes (Fig. 3H), validating the binding relationship of miR-223-3p and PITPNA-AS1. Moreover, data from Spearman's correlation analysis manifested that PITPNA-AS1 was inversely associated with miR-223-3p in 24 OC samples (Fig. 3I).

MiR-223-3p targeted RHOB in ovarian cancer cells

Next, we resorted to Targetscan[21] and identified the top 5 targets of miR-223-3p. It was discovered that elevated miR-223-3p level resulted in the most distinct reduction of RHOB expression in OC cells (Fig. 4A). In addition, the binding area between miR-223-3p and RHOB is conserved among many species (Fig. 4B), while the binding area between miR-223-3p and TBC1D17 (or FBXW7) is not conserved in some species, such as mouse, rat, rabbit, and cow (Supplementary Figure), a reason why it may be impossible to verify of the regulatory axis using animal experiments in the future. Therefore, the target RHOB was finally identified for the present study. The binding area, predicted with Targetscan, between miR-223-3p and RHOB is shown in Fig. 4C. The luciferase activity of RHOB-Wt plasmids was notably abated by overexpressed miR-223-3p, while that of RHOB-Mut was not efficiently altered, which demonstrated that miR-223-3p bound to RHOB (Fig. 4D). Moreover, overexpressing miR-223-3p declined RHOB mRNA expression and protein level in OC cells, as reflected in Fig. 4E and F. However, RHOB levels were surged in response to PITPNA-AS1 overexpression (Fig. 4G). This finding substantiated that RHOB was inversely regulated by miR-223-3p while being positively regulated by PITPNA-AS1 in OC cells. Furthermore, results from RNA immunoprecipitation assays validated that miR-223-3p, PITPNA-AS1, and RHOB were all enriched in the Ago2 pellet compared with those in the IgG control pellet (Fig. 4H), which revealed that miR-223-3p, PITPNA-AS1, and RHOB jointly formed the RNA-induced silencing complex and further confirmed the regulatory relation of the three players.

RHOB expression was decreased in ovarian cancer

RHOB expression was decreased in both OC tissues and cells (Fig. 5A and B) according to qPCR analyses, and the findings were in line with the conclusion generated from bioinformatics analysis (Fig. 5C). Moreover, Spearman's correlation analysis showed the positive relationship between PITPNA-AS1 expression and RHOB level (Fig. 5D) as well as the inverse relation between miR-223-3p level and RHOB association (Fig. 5E) in 24 OC samples.

RHOB knockdown antagonized the inhibitory influence of PITPNA-AS1 interference on ovarian cancer cell activities

OC cell line OVCAR-3 was transfected with sh-RHOB to silence RHOB levels (Fig. 6A). In addition, the increase in RHOB protein level mediated by PITPNA-AS1 overexpression was countervailed by sh-RHOB (Fig. 6B). Then, data of colony forming assays revealed that RHOB deficiency abolished the prohibitory impact of PITPNA-AS1 overexpression on OVCAR-3 cell proliferation (Fig. 6C). TUNEL staining revealed that the enhancement of cell apoptotic ratio induced by PITPNA-AS1 was partly reversed by inhibiting RHOB level (Fig. 6D). Moreover, the inhibition of RHOB level countervailed the suppressive impact of PITPNA-AS1 interference on OVCAR-3 cell migration (Fig. 6E). Meanwhile, we observed that RHOB deficiency partially offset the suppression on EMT process induced by PITPNA-AS1, as evidenced by the alterations of EMT markers (Fig. 6F).

DISCUSSION

OC is a lethal gynecologic malignancy worldwide, seriously threatening the health of women[22]. Multiple investigations have verified the critical roles of lncRNAs in modulating OC. The biological significance of PITPNA-AS1 in OC has not been reported yet.

Figure 3. PITPNA-AS1 interacted with miR-223-3p in OC cells. **A and B:** cellular fractionation assays and FISH for determination of the distribution of PITPNA-AS1 in OC cells. **C:** the potential miRNAs binding to PITPNA-AS1 were obtained from starBase with the screening condition of CLIP Data: medium stringency (≥ 2), and RNA pulldown assays were utilized to select the most suitable one by determining the binding ability. **D and E:** RT-qPCR for detecting miR-223-3p expression in OC cells and samples. **F:** a binding site between PITPNA-AS1 and miR-223-3p (from starBase). **G:** miR-223-3p expression in OC cells with transfection of miRNA mimics was subjected to PCR analysis. **H:** luciferase reporter assays for evaluation of the binding possibility of PITPNA-AS1 and miR-223-3p. **I:** expression correlation of PITPNA-AS1 and miR-223-3p using Spearman's correlation analysis. OC: ovarian cancer.

**p < 0.01.
***p < 0.001.

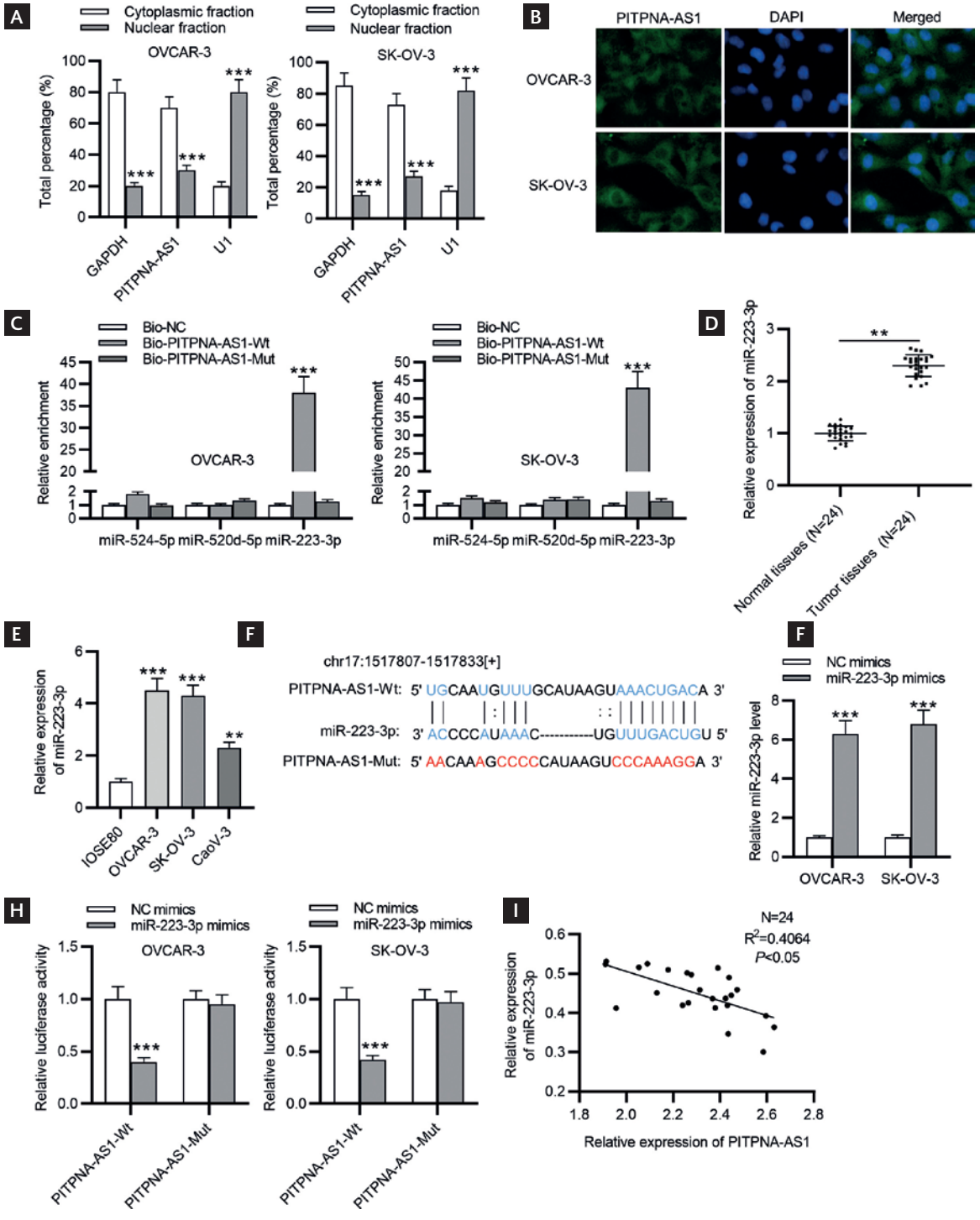


Figure 4. MiR-223-3p targeted RHOB in OC cells. **A:** target scan was adopted for finding possible targets of miR-223-3p. RT-qPCR was performed to select the best target by measuring gene expression in the context of miR-876-5p overexpression. **B:** the binding area of miR-223-3p and RHOB is conserved among many species. **C:** a binding area of RHOB and miR-223-3p (from Targetscan). **D:** luciferase reporter assays for evaluation of the binding possibility of RHOB and miR-223-3p. **E-G:** Western blot and qPCR for evaluation of RHOB protein and mRNA levels in OC cells overexpressing miR-223-3p or ITPNA-AS1. **H:** the relationship of PITPNA-AS1, miR-223-3p and RHOB was validated using RNA immunoprecipitation assays. OC: ovarian cancer; RHOB: Ras homolog family member B.

*p < 0.05.
**p < 0.01.
***p < 0.001.

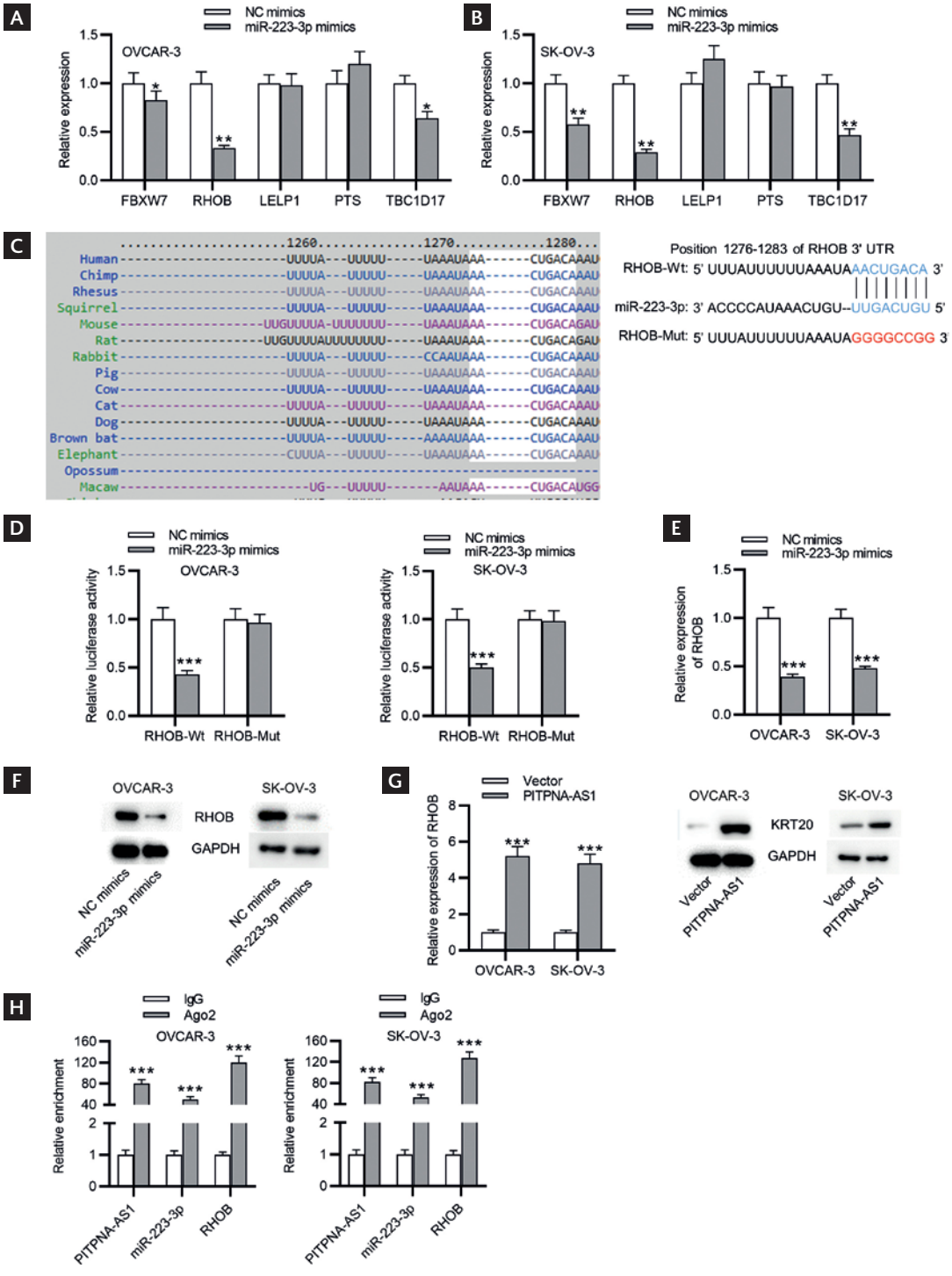
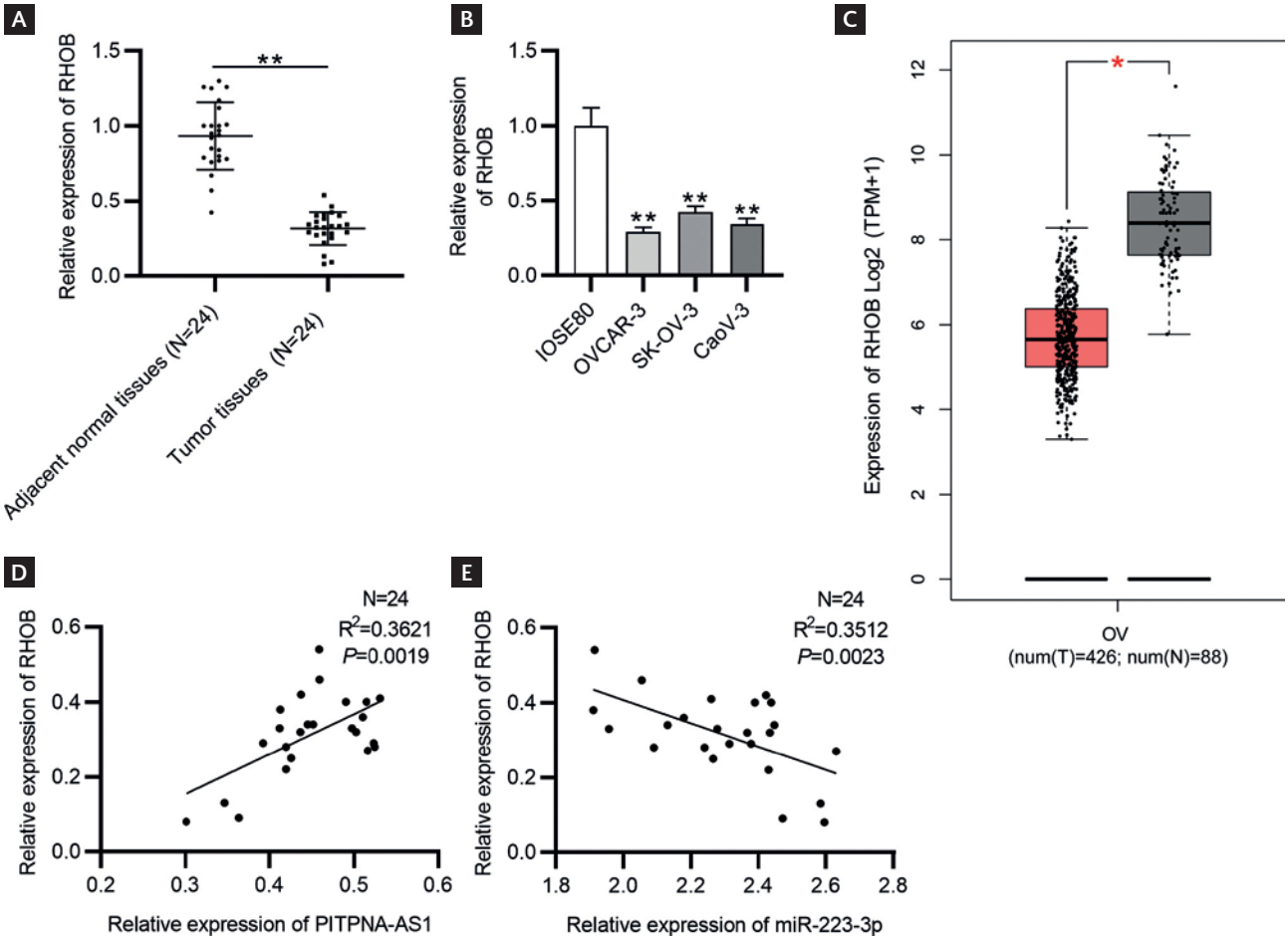


Figure 5: RHOB expression was reduced in OC. **A and B:** RHOB expression in OC cells and samples were measured using qPCR. **C:** bioinformatics analysis of RHOB expression in OV tissues using the tool GEPIA. **D and E:** expression association between RHOB and PITPNA-AS1 (or miR-223-3p) was identified with Spearman coefficient. OC: ovarian cancer; RHOB: Ras homolog family member B.

**p < 0.01.

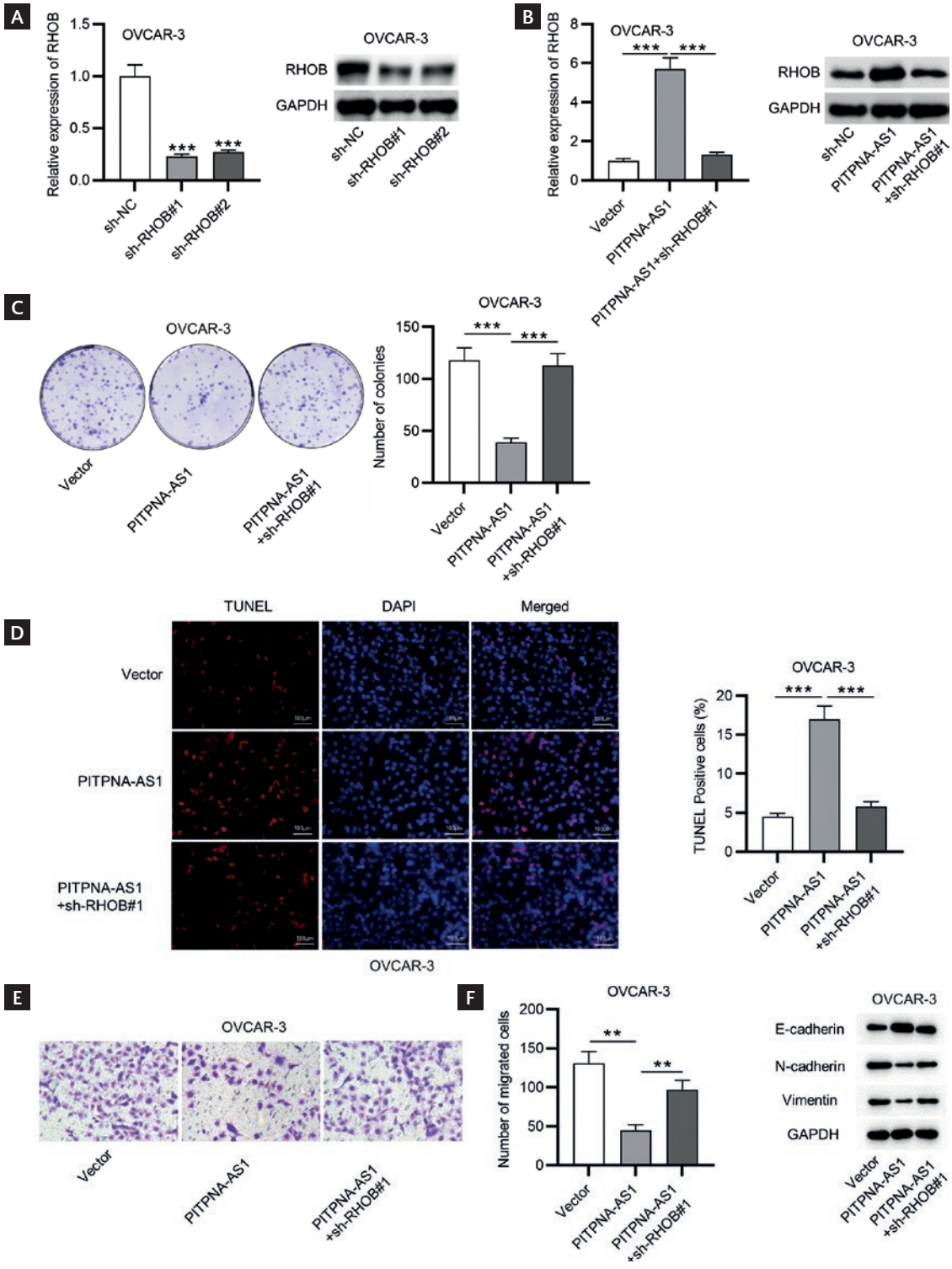


Interestingly, it is discovered that PITPNA-AS1 serves as an oncogene in other cancer types. In this report, our results demonstrated that PITPNA-AS1 was downregulated in OC cells and samples. Moreover, PITPNA-AS1 mitigated OC cell malignancies by hindering cell proliferation, migration, EMT, and boosting cell apoptosis, indicating the anti-oncogenic function of PITPNA-AS1 in OC. Previous reports showed that lncRNA exerts different regulatory roles in accordance with their subcellular distribution[23]. In this study, it was found that PITPNA-AS1 is mainly distributed in the cytoplasmic part in OC cells.

MicroRNAs are also members of non-coding RNA family, and they have and estimated 22 nucleotides in length[24,25]. LncRNAs in the cytoplasm can regulate the stability and translation of downstream mRNAs by interacting with miRNAs according to ceRNA hypothesis[26]. In this work, miR-223-3p was validated to be targeted by PITPNA-AS1 in OC cell lines. The tumor-promoting impact of miR-223-3p in OC has been confirmed. MiR-223-3p was shown to target LARP4 and circBNC2 interacted with miR-223-3p to alter LARP4 expression in epithelial OC cells[23]. miR-223-3p is also implicated in the ceRNA network of circBNC2/miR-223-3p/FBXW7[27]. Overexpressing miR-223-3p facilitates OC cell growth and migration by inversely regulating SOX11[28]. MiR-223-3p, in the present study, was also highly expressed in OC tissues and cells.

Figure 6. PITPNA-AS1 contributed to OC cell malignancies through the regulation of RHOB. **A:** RT-qPCR was applied to measure RHOB knockdown efficiency. **B:** RHOB expression in OC cells with empty pcDNA vector, pcDNA-PITPNA-AS1, and PITPNA-AS1+sh-RHOB#1 was subjected to qPCR analysis. **C:** cancer cell proliferation was measured using colony forming assays. **D:** apoptosis was detected by TUNEL staining, and **E:** migration was measured using Transwell assays. **F:** Western blotting for assessment of protein levels of EMT markers in the three groups. RHOB: Ras homolog family member B.

**p < 0.01.
***p < 0.001.



RHOB, also called ARH6, MST081, or RHOH6, can hamper cell survival and metastasis, and its level is commonly weakened in malignant progression[29]. The involvement of RHOB in the tumorigenesis of different cancers has been previously identified. For example, miR-223 facilitates gastric cancer progression by targeting RHOB[26]. The roles of miR-223 and RHOB in tumorigenesis are in line with our findings. In our paper, RHOB was observed to be a target of miR-223-3p (previously named miR-223) in OC cells. Furthermore, RHOB showed low expression in OC cells and samples and was positively regulated by PITPNA-AS1. Moreover, RHOB knockdown abolished the suppressive impact on OC cell process exerted by PITPNA-AS1. Furthermore, it was reported that RHOB inversely regulates the PI3K/Akt signaling and suppresses gemcitabine resistance in pancreatic cancer[30]. The signaling pathway mediated by the PITPNA-AS1/miR-223-3p/RHOB axis was not explored in this work but could be a direction for future investigations.

In conclusion, this study suggested that PITPNA-AS1 functioned as an anti-oncogene in OC cells. PITPNA-AS1 hampers cell proliferation, migration, and the EMT process, while promoting cell apoptotic rate by regulating the miR-223-3p/RHOB axis. This study first revealed the function of PITPNA-AS1 in OC and may expand the understanding of PITPNA-AS1 in tumorigenesis. PITPNA-AS1 might be a promising marker for targeted therapy and a potential predicator of treatment response in the future but there is a long way to go before its clinical application.

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The ethics committee of the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology approved the protocol.

SUPPLEMENTARY DATA

Supplementary data are available at DOI: 10.24875/RIC.23000235. These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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