

Research Article

Long non-coding RNA PVT1-mediated miR-543/SERPINI1 axis plays a key role in the regulatory mechanism of ovarian cancer

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Purpose: To investigate the regulation mechanism of long non-coding RNA (lncRNA) plasmodioma variant translocation 1 (PVT1) in ovarian cancer (OC).

Methods: The levels of PVT1, microRNA (miR)-543, serpin peptidase inhibitor-clade I (neuroserpin)-member 1 (SERPINI1) in OC tissues and OVCAR-3, A2780, TOV-112D of OC cell lines were detected by quantitative real-time PCR (qRT-PCR) and Western Blot (WB). Cell proliferation, migration, invasion, apoptosis and the regulatory relationship between genes and target genes were analyzed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Transwell, flow cytometry and dual luciferase reporter (DLR). The OC patients were followed up for 5 years to analyze the relationship between PVT1 and 5-year overall survival (OS).

Results: In contrast with miR-543, PVT1 and SERPINI1 were highly expressed in OC tissues and cell lines, and high levels of PVT1 were significantly associated with lower 5-year OS of patients. Down-regulating PVT1 not only inhibited the malignant proliferation, migration and invasion of OC cells, but promoted cell apoptosis. PVT1 regulated miR-543 in a targeted manner, and its overexpression could attenuate the anticancer effect of miR-543 on OC cells. In addition, miR-543 also directly targeted SERPINI1, and miR-543 knockdown weakened the inhibitory effect of down-regulated SERPINI1 on OC progression. Furthermore, we found that PVT1 acted as a competitive endogenous RNA to sponge miR-543, thereby regulating the expression of SERPINI1.

Conclusion: PVT1 can mediate the molecular mechanism of OC by miR-543/SERPINI1 axis regulatory network, which is a new therapeutic direction for OC.

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Introduction

Ovarian cancer (OC) is the eighth leading cause of cancer-related deaths worldwide, and its risk increases with the number of ovulation cycles in women [1,2]. According to epidemiological statistics of OC, in 2018, this disease plagued approximately 22240 new cases and accounted for 14070 deaths in the U.S.A., and the 5-year relative survival rate of advanced OC was only 29% [3,4]. Currently, the mainstream treatment approaches for OC are constantly optimized, which however, will inevitably trigger recurrence and chemotherapy resistance that cannot be ignored, resulting in unsatisfactory improvement of OC patients' condition [5,6]. Therefore, in-depth understanding of the molecular mechanism of OC and further elucidating its pathological process is essential for the treatment and prevention of this disease.

As molecular regulators that have attracted increasing attention in recent years, long non-coding RNAs (lncRNAs) can control basic biological processes and tumor progression, and research on them is conducive to better understanding the gene regulatory network of cancer [7,8]. The lncRNA family not only plays a role in the development of gynecological tumors such as breast cancer, cervical cancer and endometrial cancer, but participates in the pathological mechanism of OC [9–12]. For example, CACS15 can promote the malignant development of OC by recruiting EZH2 to suppress the expression of APC, and DANCR can mediate tumor growth and angiogenesis of OC by targeting regulation of microRNA (miR)-145 [13,14]. Plasmocytoma variant translocation 1 (PVT1), which is the focus of this study, is often used as a carcinogen to induce the aggravation of the disease in cancer, and can regulate the tumor microenvironment through the negative regulation of miRNA. Currently, it has been found to be tumorigenic in breast cancer, OC and other gynecological cancers [15]. Through Starbase, we predicted the potential target sites between PVT1 and miR-543, of which the latter serves as a tumor inhibitor in cervical cancer while playing a malignant role in promoting tumor growth and metastasis in prostate cancer [16,17]. In addition, miR-543 shows a low level in OC tissues and cells, which can play an anticancer role in OC by targeting TWIST1, and can also be regulated by placental growth factors and silenced to promote MMP7-mediated tumor invasion in OC [18,19]. Besides, we found via TargetScan that miR-543 had potential binding sites with serpin peptidase inhibitor-clade I (neuroserpin)-member 1 (SERPINI1). As a double-sided secreted protein, SERPINI1 has been found to be abnormally highly expressed in hepatocellular carcinoma and colorectal cancer, while abnormally down-regulated in gastric cancer, which may be carcinogenic or anticancer in different cancers [20,21]. In addition, SERPINI1 also mediates the mechanism of brain metastasis, in which pathological changes promote the survival of tumor cells and vascular combination, and is also closely associated with the brain recurrence of primary tumors, suggesting that SERPINI1 may be a threat to human health in most cases [22].

We hypothesized that the PVT1/miR-543/SERPINI1 axis regulatory network played a key part in the pathological development of OC, and hereby we are here to explore it.

Materials and methods

Tissue sample collection

Forty-two patients with OC admitted to China-Japan Union Hospital of Jilin University from January 2013 to January 2015 were selected, with an average age of (49.66 ± 5.41) years. After obtaining the consent of the patients, cancer tissues and adjacent tissues were collected as the research samples during tumor resection, and then stored in liquid nitrogen tanks for future use. Inclusion criteria: patients diagnosed with OC [23] by pathological diagnosis, who received treatment for the first time, without any medications such as hormones within the past 6 months, and with normal cognition and unobstructed communication. Exclusion criteria: patients with other ovarian diseases, those with other malignant tumors, severe complicated diseases of heart, lung, kidney and other organs or severe infectious diseases, or those who refused to provide laboratory specimens. The present study was carried out under the guidance of the Helsinki Declaration of the World Medical Association and the approval of the Ethics Committee of China-Japan Union Hospital of Jilin University. All the enrolled patients were informed and who agreed to participate in the present study had given written informed consent.

Follow-up

The patients were followed up once every 3 months by telephone, visit, medical records or other means. The overall survival (OS) started from the day of diagnosis and ended with the death of the patient or the last follow-up over the 5-year follow-up period.

Cell culture and transfection

Human OC cell lines OVCAR-3, A2780, TOV-112D and normal ovarian epithelial cell line HOSEpiC (Zishi Biotechnology Co., Ltd., Shanghai, China, os101565, os100739, zs101819, As100539) were purchased and placed in DMEM (Xinfan Biological Technology Co., Ltd., Shanghai, China, 1273) containing 10% PBS and 100 U/ml penicillin plus 100 µg/ml streptomycin (Lianshuo Biotechnology Co., Ltd., Shanghai, China, AMEKO) at 37°C and 5% CO₂.

By constructing the recombinant plasmids pEGFP-PVT1/SERPINI1 and pSilencer-PVT1/SERPINI1, the expression levels were up-regulated and down-regulated, respectively. PVT1 low expression plasmids pSilencer-PVT1 (si-PVT1), PVT1 high expression plasmids pEGFP-PVT1 (PVT1), empty vector plasmids (vector), SERPINI1 low expression plasmids pSilencer-SERPINI1 (si-SERPINI1), SERPINI1 high expression plasmids pEGFP-SERPINI1 (sh-SERPINI1), negative control (NC) of RNA (si-NC), miR-543 mimics (miR-543), miR-543 inhibition sequence (anti-miR-543), miR-NC and anti-miR NC (anti-miR-NC) were respectively transfected with the cells with the aid of

Lipofectamine™ 2000 kit (Biomag Biotechnology Co., Ltd., Wuxi, China, 11668019) as described in the instructions provided with the kit.

Quantitative real-time PCR

First, the total RNA was isolated from tissues and cells with TRIzol reagent (EK-Bioscience Co., Ltd., Shanghai, China, 5007050). Then 5 µg of the total RNA were processed for reverse transcription of cDNA as instructed by the kit instructions (Qiming Biotechnology Co., Ltd., Shanghai, China, OX02700). After transcription, 1 µl of synthetic cDNA was amplified. GAPDH was used as the internal reference for PVT1 and SERPINI1, while U6 for miR-543, and the data were analyzed by $2^{-\Delta\Delta C_t}$.

Western Blot detection

The total protein was extracted from OC tissues and cultured OC cells by RIPA lysis, and the protein concentration was determined by BCA kit (Rongbai Biotechnology Co., Ltd., Shanghai, China, LCB004). After adjusting the concentration to 4 µg /µL, the protein was separated by 12% SDS-PAGE electrophoresis and transferred to a membrane for sealing in 5% blocking solution. The dilution ratio of SERPINI1, β-Actin and other primary antibodies was 1:1000. The antibodies were obtained from Shanghai Xiyuan Biotechnology Co., Ltd., among which all the primary antibodies were detected with rabbit polyclonal antibodies. Each membrane was rinsed three times with PBS (Zhenyu Biotechnology Co., Ltd., Shanghai, China, 130-070-525) for 15 min each, and then incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibody. After blotting the excess liquid on the membrane, it was developed by ECL, and the gray value was analyzed.

MTT assay for cell viability

After 24 h of transfection, the cells were harvested and adjusted to 4×10^3 cells per well. Then the cells were inoculated on 96-well plates and incubated at 37°C for 0, 24, 48 and 72 h, at which time point, 20 µl 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) (Zhenyu Biotechnology Co., Ltd., Shanghai, China, CT0025) was added for another 4-h culture at 37°C. After that, dimethyl sulfoxide was added at 200 µl/well, and the optical density (OD) value of each group was measured by Nabi spectrophotometer (Xiyuan Biotechnology Co., Ltd., Shanghai, XY-381-07341) at the wavelength of 450 nm.

Transwell for cell migration and invasion

Cells were collected 24 h after transfection, adjusted to 3×10^4 cells/well, and inoculated on 24-well plates. Then trypsin digestion was performed before the cells were transferred to the upper chamber, where 200 µl RPMI1640 (Shuangru Biotechnology Co., Ltd., Shanghai, China, LS115-001) was added, while the lower chamber was added with 600 ml RPMI1640 (containing 10% FBS). After 48 h of culture at 37°C, the upper chamber was swabbed and washed three times with PBS, fixed with paraformaldehyde for 10 min, followed by three times of rinsing with double steam water. After being dried, the cells were stained with 0.5% Crystal Violet, and the number of cell migration was observed and recorded under a microscope. For the determination of cell invasion, the bottom membrane was diluted with 50 mg/l Matrigel (1:8; Hengfei Biotechnology Co., Ltd., Shanghai, China, M8370) on the surface of the upper chamber, and the rest of the steps were the same as above.

Flow cytometry for apoptosis

After digestion with 0.25% trypsin, the transfected cells were washed twice with PBS, and mixed with 100 µl binding buffer to prepare a 1×10^6 cells/ml suspension. AnnexinV-FITC and propidium iodide (PI) were then added in turn with 10 µl each, incubated at room temperature in darkness for 5 min, and tested by NovoCyte flow cytometer (FC) (Ranger Apparatus Co., Ltd., Shanghai, China). The experiment was repeated three times to average the value.

Dual luciferase reporter assay

The cDNA fragments of PVT1 wild-type (PVT1-Wt) and PVT1 mutant (PVT1-Mut) fragments, SERPINI1 wild-type (SERPINI1-Wt) and corresponding mutant (SERPINI1-Mut) fragments were subcloned into the luciferase gene downstream of the luciferase reporter vector, respectively. MiR-543 mimics were co-transfected with PVT1-Wt or PVT1-Mut (SERPINI1-Wt or SERPINI1-Mut). Forty-eight hours later, firefly and renin luciferase activities in cell lysates were continuously determined by the dual luciferase reporter (DLR) kit (Huzhen Biotechnology Co., Ltd., Shanghai, China, 11402HZ60).

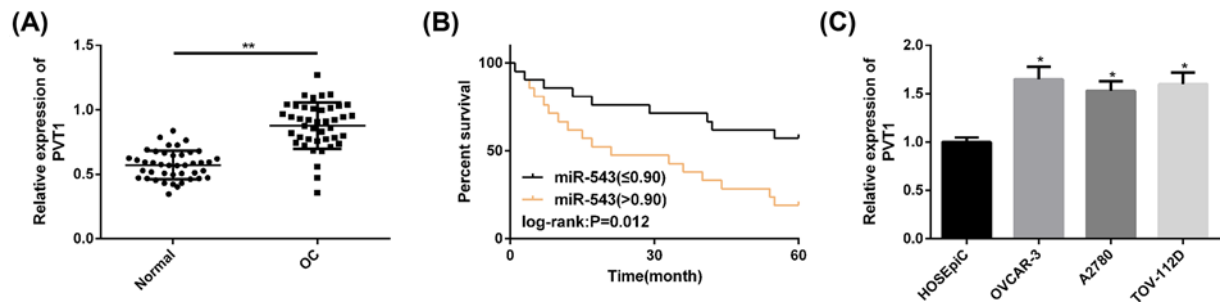


Figure 1. PVT1 expression in OC samples and cell lines

(A) The PVT1 expression in cancer tissues of OC patients was markedly higher than that in adjacent tissues. (B) High expression of PVT1 was greatly associated with poor prognosis of OC. (C) In OC cell lines, PVT1 was more noticeably up-regulated in OVCAR-3 and TOV-112D. Compared with HOSEpic or between the two groups, $*P < 0.05$, $**P < 0.01$.

RNA immunoprecipitation

RNA immunoprecipitation (RIP) was utilized to verify the interaction between PVT1 and miR-543, and the experiment was conducted strictly following the instructions of the EZMagna RIP kit (Goy Biotechnology Co., Ltd., Shanghai, China, GOY-E5944). After lysis, the cells were incubated with protein A magnetic beads and conjugated with antibodies at 4°C. Six hours later, the beads were washed with buffer and then incubated with 0.1% SDS/0.5 mg/ml proteinase K at 55°C for 30 min to discard the protein. Finally, quantitative real-time PCR (qRT-PCR) analysis was performed on the immunoprecipitated RNA to verify the presence of PVT1 and miR-543 using specific primers.

RNA pull-down experiment

The template PVT1 DNA was prepared by PCR, and the product was ligated to pGEM-T Easy Vector and transformed into competent cells. The plasmid prepared by 5 µg was purified with QIAquick PCR purification kit (Huishen Biotechnology Co., Ltd., Shanghai, China, 28104). The transcription reaction of PVT1 was labeled with biotin-UTP (Yage Biotechnology Co., Ltd., Shanghai, China, YS-10258R) *in vitro*, and OC cells were transfected with biotinylated miR-543-Wt, miR-543-Mut and negative control Bio-NC, respectively. Forty-eight hours later, the cell lysate were incubated with M-280 *Streptomyces* magnetic beads according to the production instructions. The washed beads were then placed in an RNase-free solution, followed by a 10-min incubation with equal volume of biotinylated miR-543 in binding and washing buffer on the rotator at room temperature, and then the beads with fixed miR-543 fragments were cultivated with 10 mM EDTA pH 8.2 and 95% formamide at 65°C for 5 min. TRIzol was used to purify the bound RNA and qRT-PCR was utilized to detect the PVT1 level in the bead-bound RNA complex.

Statistical methods

In the present study, GraphPad 6 was used for data analysis and picture drawing. Intergroup comparisons were performed by the independent sample *t* test, while multigroup comparisons were conducted by one-way ANOVA, and post-hoc pairwise comparisons were done by LSD *t* test. Multi-time profiles were analyzed by repeated measures analysis of variance, and the post-hoc test was conducted by Bonferroni. Pearson test was utilized for correlation analysis, Kaplan–Meier method was applied to draw the survival curve, and Log-rank test was used to evaluate the difference in survival time between groups. A statistically significant difference was assumed at $P < 0.05$.

Results

Up-regulated PVT1 in OC samples and cell lines

PVT1 was dramatically up-regulated in OC patients' cancer tissue samples. The follow-up was successfully completed, and the 5-year OS was 38.10% (16/42). We used the median (0.90) as the cut-off point of high and low expression, and found that high PVT1 expression was significantly correlated with the lower 5-year OS in OC patients. In addition, PVT1 up-regulation was also observed in OC cell lines, and the up-regulation was more pronounced in OVCAR-3 and TOV-112D cell lines, so the two were selected for further analysis. All the above results were statistically significant ($P < 0.05$) (Figure 1).

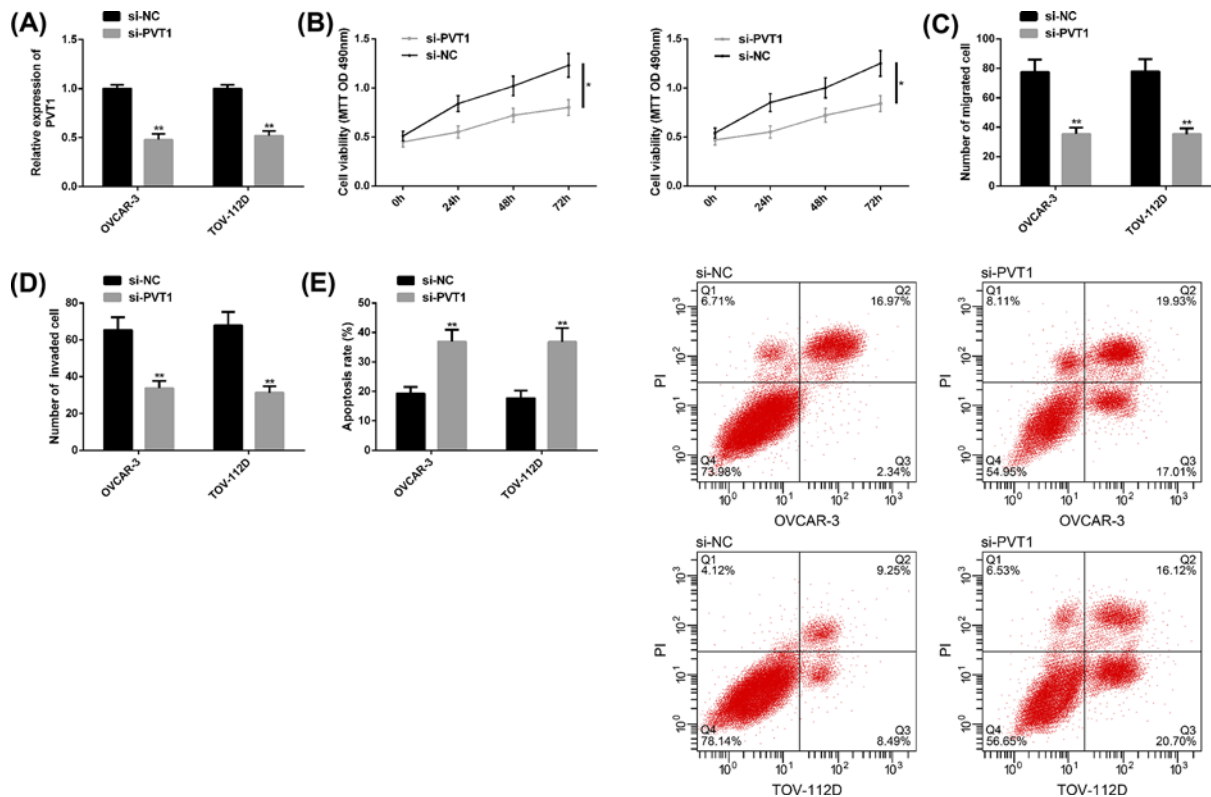


Figure 2. Effects of knocking down PVT1 on OC cell behaviors

(A) After knocking down PVT1, the expression of PVT1 in OC cells was markedly down-regulated. (B–D) After knocking down PVT1, the proliferation, migration, and invasion of OC cells were obviously inhibited. (E) The promoted apoptosis of OC cells after PVT1 knockdown, as well as the FC diagram. Compared with si-NC or between the two groups, * $P < 0.05$, ** $P < 0.01$. Abbreviation: si, short interfering.

Knocking down PVT1 was not conducive to OC cell proliferation, migration and invasion, but induced apoptosis

After knocking down PVT1 and transfecting it into OC cells, we found that PVT1 was evidently down-regulated, cell proliferation, migration and invasion behaviors were remarkably suppressed, and apoptosis was noticeably induced, with statistically significant differences ($P < 0.05$) (Figure 2).

Targeting relationship between PVT1 and miR-543

We found through Star Base (<http://starbase.sysu.edu.cn/index.php>) that PVT1 and miR-543 had potential target sites. After up-regulation of miR-543, the PVT1-Wt luciferase activity was noticeably decreased ($P < 0.05$), while the PVT1-Mut luciferase activity changed little ($P > 0.05$). In the RIP experiment, PVT1 and miR-543 levels precipitated by Argonaute2 (Ago2) antibodies were evidently elevated than those of IgG, and in RNA pull-down experiments, PVT1 was only pulled down by biotin-labeled miR-543-WT. Subsequently, clinical findings revealed a significantly lower level of miR-543 in OC patients' cancer tissues, and a marked negative correlation between PVT1 and miR-543 ($r = -0.638$, $P < 0.001$). In OC cell lines, we noticed that miR-543 was also notably reduced, especially in OVCAR-3 and TOV-112D, so further analysis was performed on these two cell lines. Besides, PVT1 was identified to be enhanced and miR-543 was blocked in OC cells transfected with PVT1, while miR-543 was enhanced after transfection of si-PVT1. All the above results were statistically significant ($P < 0.05$) (Figure 3).

PVT1 reversed the anticancer effect of miR-543 on OC cells

MiR-543 expression was enhanced by transfection of miR-543 mimics in OC cells, while its level was attenuated by transfection of PVT1 on this basis. In addition, it was found that the proliferation, migration and invasion of OC cells

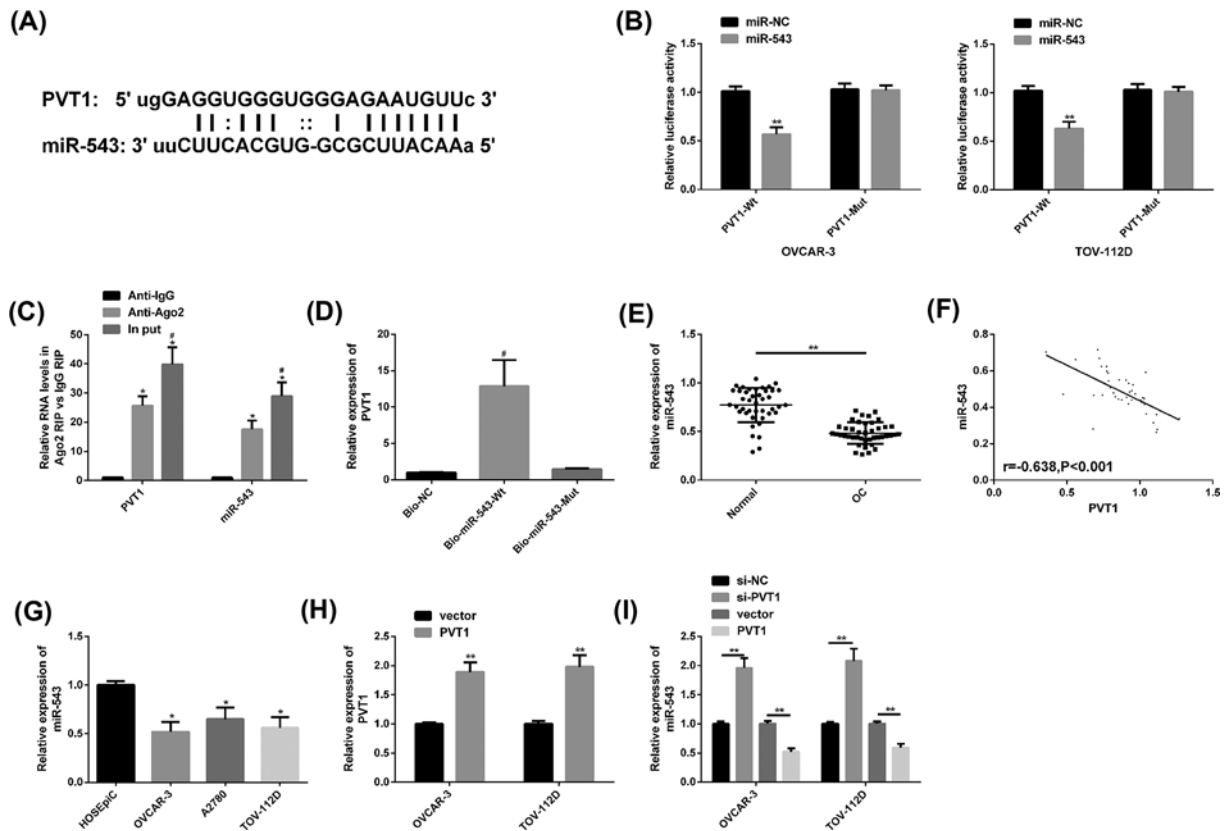


Figure 3. Targeting relationship between PVT1 and miR-543

(A) Potential targeting sites of PVT1 and miR-543. (B) Relative luciferase activity-DLR assay. (C) RIP experiment. (D) RNA pull-down experiment. (E) The miR-543 expression in OC cancer tissues was remarkably lower than that in adjacent tissues. (F) PVT1 was notably negatively correlated with miR-543 ($r = -0.638, P < 0.001$). (G) The miR-543 level in OC cell lines OVCAR-3 and TOV-112D was markedly lower. (H) OC cells transfected with PVT1 had higher PVT1 level than those transfected with the vector. (I) OC cells had a higher miR-543 level after transfection with si-PVT1, while those transfected with PVT1 had a lower miR-543 level. Compared with miR-NC/Anti-IgG/HOSEpic/vector or between the two groups, $*P < 0.05$, $**P < 0.01$; compared with Anti-Ago2/Bio-NC, $\#P < 0.05$. Abbreviation: Anti, antisense; IgG, Immunoglobulin G; Mut, mutant; si, short interfering; Wt, wild-type;.

were inhibited and apoptosis was induced after miR-543 mimics transfection, but after further transfection of PVT1, the effect of miR-543 on OC cells was reduced. All the above results were statistically significant ($P < 0.05$) (Figure 4).

Targeted regulation of SERPINI1 by miR-543

Through TargetScan (http://www.targetscan.org/vert_72/), it was found that there were potential binding sites between miR-543 and SERPINI1, and the up-regulation of miR-543 only significantly reduced the SERPINI1-Wt luciferase activity. The expression and protein levels of SERPINI1 in OC cancer tissues were dramatically elevated than those in adjacent tissues, and were markedly negatively correlated with miR-543 ($r = -0.707, P < 0.001$). SERPINI1 expression and protein levels were also obviously increased in OC cells, and they were the most notably in OVCAR-3 and TOV-112D, which were hereby used for further analysis. After anti-miR-543 transfection, miR-543 was inhibited, but the expression and protein levels of SERPINI1 were increased, while the opposite result was obtained by transfecting miR-543 mimics. All the above results were statistically significant ($P < 0.05$) (Figure 5).

Down-regulation of miR-543 attenuated si-SERPINI1's inhibitory effect on OC progression

Si-SERPINI1 mediated the reduction in SERPINI1 messenger RNA (mRNA) and protein levels, inhibited the proliferation, migration and invasion of OC cells, and promoted apoptosis. When miR-543 was further down-regulated, the above results were evidently reversed. All the above results were statistically significant ($P < 0.05$) (Figure 6).

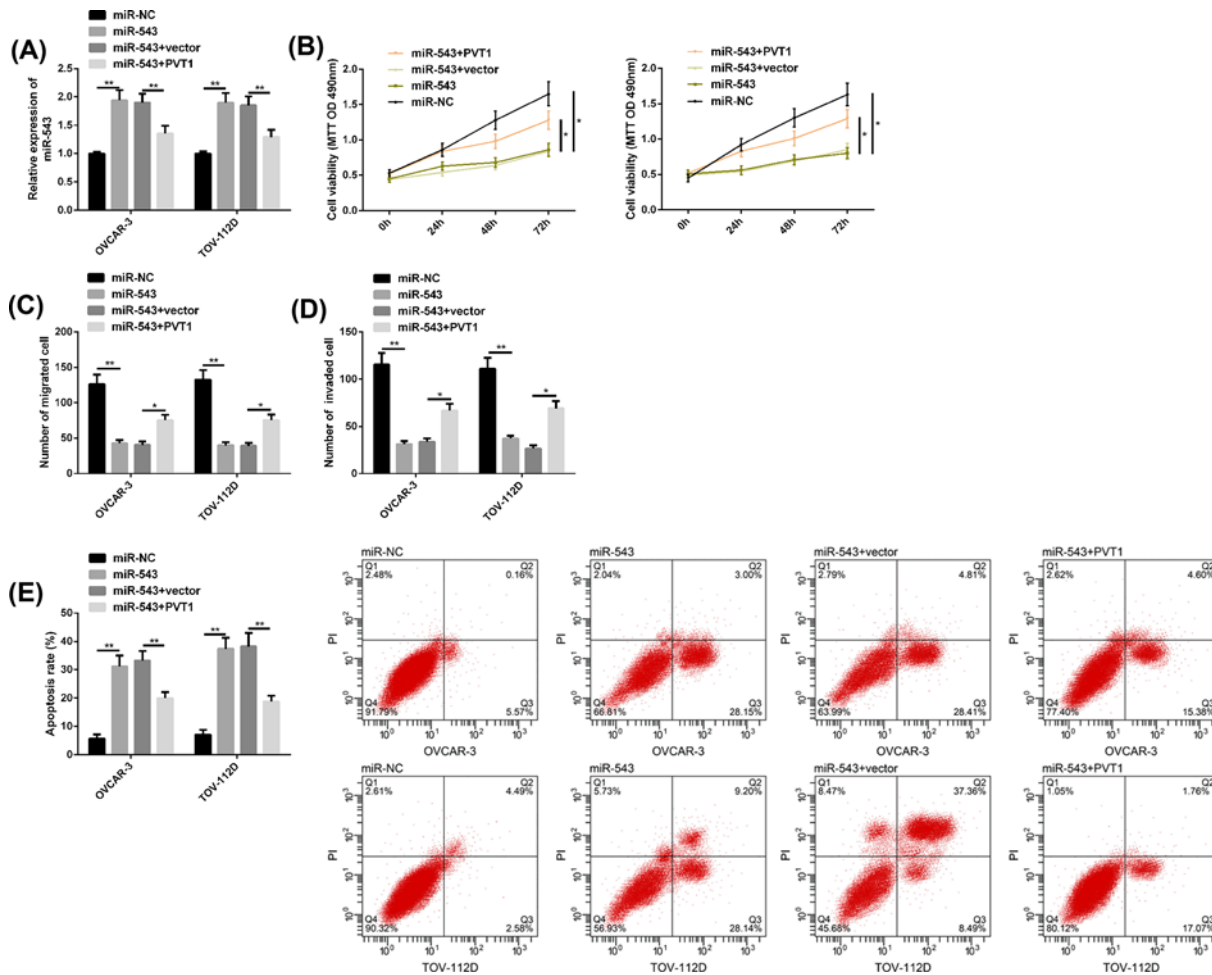


Figure 4. PVT1 reversed the anticancer effect of miR-543 on OC cells

(A) MiR-543 expression after transfection of miR-543 mimics and PVT1. (B–D) Proliferation, migration and invasion of OC cells after transfection of miR-543 mimics and PVT1. (E) Apoptosis of OC cells after transfection of miR-543 mimics, PVT1 and FC diagram. Compared between the two groups, * $P < 0.05$ and ** $P < 0.01$.

SERPINI1 expression regulated by PVT1 and miR-543

The mRNA and protein levels of SERPINI1 were significantly inhibited after transfection of miR-543 mimics, while those were markedly increased after further transfection of PVT1, both with statistically significant differences ($P < 0.05$) (Figure 7).

Discussion

OC is a fatal gynecological tumor, and patients with metastatic OC often present high mortality and short survival [24]. lncRNAs show abnormal dysregulation in the body and are crucial in regulating the occurrence and development of tumors [25]. In this study, we focused on the role of PVT1 in OC and tapped its underlying regulatory mechanisms.

Located on human chromosome 8q24.21, PVT1 is carcinogenic in various diseases such as liver cancer and colorectal cancer [26]. In studies such as Gou et al. [27], PVT1 regulated the progression of liver cancer by targeting miR-214. And as reported by Wang et al. [28], PVT1 could participate in the metastatic mechanism of colorectal cancer through sponging miR-145. In the present study, we first evaluated the expression of PVT1 in OC tissues and cells. It was found that PVT1 showed high levels in both OC tissues and cell lines, suggesting that it may be the key in the disease progression of OC. The prognostic results demonstrated that high PVT1 level was remarkably associated with lower 5-year OS of patients, indicating that high PVT1 level may predict poor prognosis of patients. While cell analysis of PVT1 exhibited that PVT1 down-regulation inhibited OC cells' proliferation, migration and invasion, and induced apoptosis, which suggested that the down-regulating PVT1 is helpful to the improvement of OC.

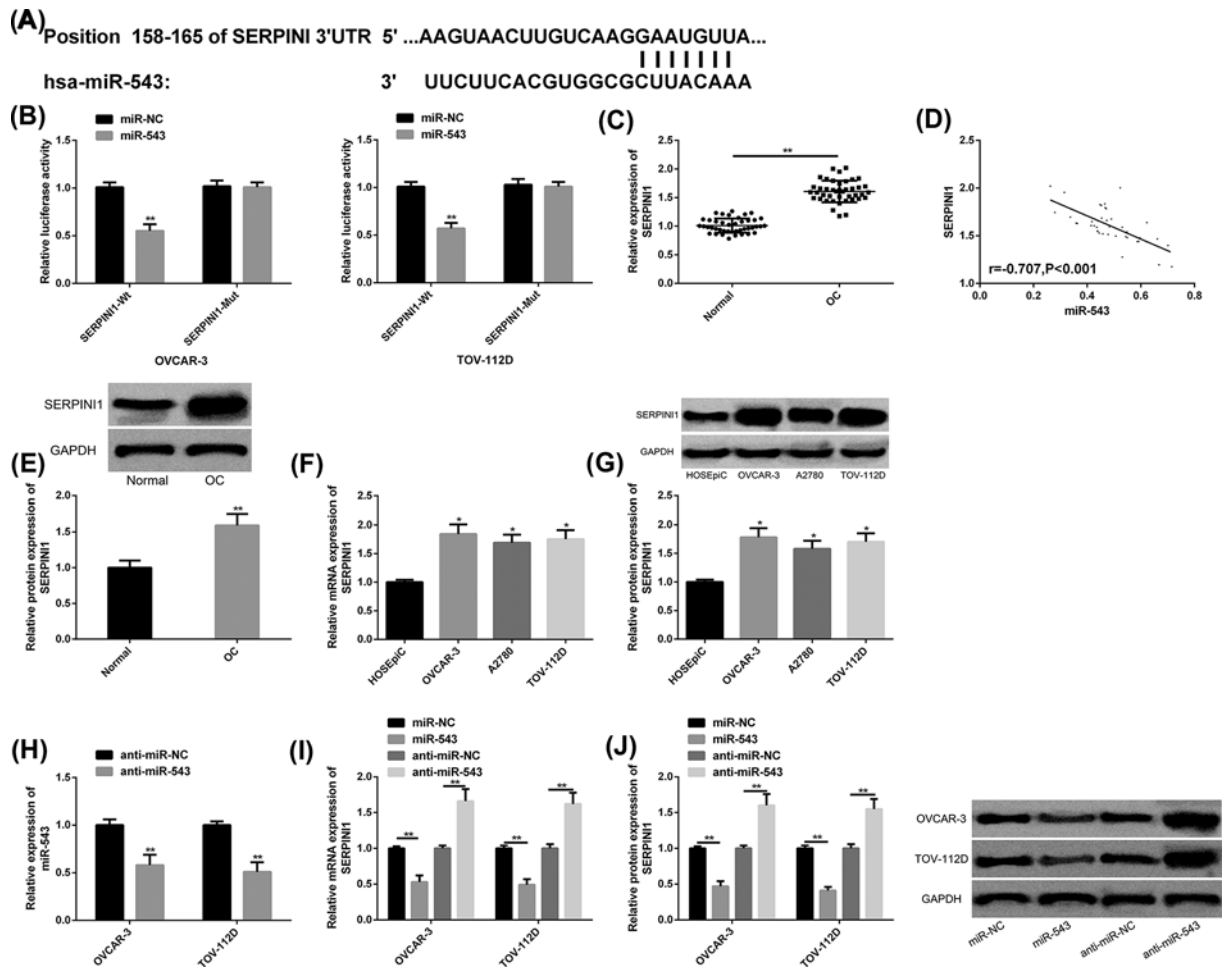


Figure 5. Targeted regulation of SERPIN1 by miR-543

(A) MiR-543 had binding sites with SERPIN1. (B) Relative luciferase activity-DLR assay. (C) SERPIN1 was up-regulated in OC cancer tissues. (D) MiR-543 was negatively correlated with SERPIN1 ($r = -0.707$, $P < 0.001$). (E) Higher protein level of SERPIN1 in OC cancer tissues, as well as protein maps. (F,G) SERPIN1 mRNA levels, protein levels and protein maps in OC cell lines. (H) MiR-543 expression was reduced after transfection with anti-miR-543. (I,J) SERPIN1 mRNA levels, protein levels, and protein maps after transfection of miR-543 mimics and anti-miR-543. Compared with miR-NC/Normal/HOSEpiC/anti-miR-NC or between the two groups, $*P < 0.05$, $**P < 0.01$. Abbreviations: anti, antisense; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mRNA, messenger RNA.

LncRNA can act as a molecular sponge of miRNA to negatively regulate its expression and cause miRNA targets to be repressed, thus regulating biological functions [29]. Therefore, we explored whether PVT1 functioned as a molecular sponge for miRNA. DLR assay, RIP and RNA pull-down experiments all confirmed the target-controlled relationship between PVT1 and miR-543. Additionally, we detected the high miR-543 expression in OC tissues and cells, and the marked negative correlation between PVT1 and miR-54 through correlation analysis. Further investigation into the role of miR-543 in OC cell behaviors revealed that overexpressed miR-543 greatly prevented the proliferation, migration and invasion of OC cells, and enhanced the apoptosis ability, indicating that miR-543 had anti-tumor activity in OC. Furthermore, up-regulating PVT1 was found to be able to eliminate the positive effect of miR-543 on OC cells, suggesting that the anti-tumor activity of miR-543 might be affected by PVT1.

We further explored the ways in which miR-543 played a part in OC progression. The potential binding sites between SERPIN1 and miR-543 were identified by TargetScan, and later their binding relationship was confirmed by DLR. SERPIN1 belongs to the family of serine protease inhibitors and has been reported to present differential expression in the probe group of epithelial OC cell lines [30,31]. Our research found that inhibiting SERPIN1 was not conducive to the proliferation, migration or invasion of OC cells, but was beneficial to apoptosis. However, when

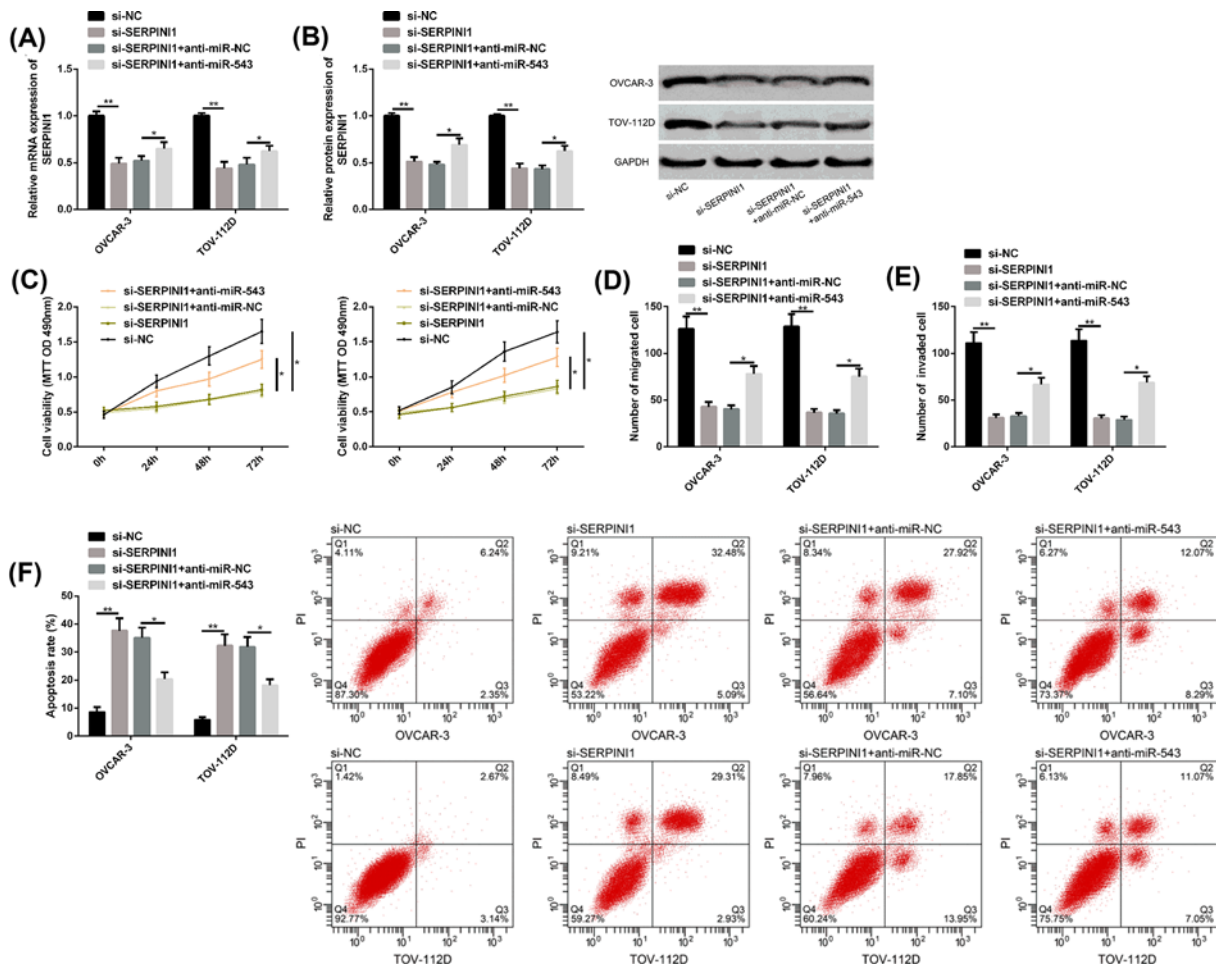


Figure 6. Down-regulation of miR-543 attenuated si-SERPINI1's inhibitory effect on OC progression (A,B) SERPINI1 mRNA level, protein level and protein map of OC cells after transfection of si-SERPINI1 and anti-miR-543. (C–E) Proliferation, migration, and invasion of OC cells after transfection with si-SERPINI1 and anti-miR-543. (F) Apoptosis of OC cells after transfection with si-SERPINI1, anti-miR-543, as well as FC diagram. Compared between the two groups, * $P < 0.05$ and ** $P < 0.01$. Abbreviations: anti, antisense; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; si, short interfering.

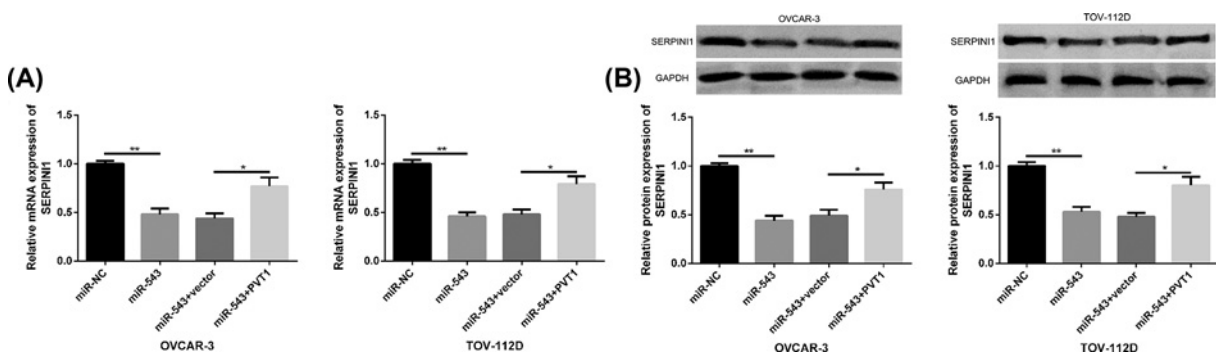


Figure 7. SERPINI1 expression regulated by PVT1 and miR-543 (A) SERPINI1 mRNA level in OC cells transfected with miR-543 mimics and PVT1. (B) SERPINI1 protein level in OC cells transfected with miR-543 and PVT1, as well as protein maps. Compared between the two groups, * $P < 0.05$ and ** $P < 0.01$. Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

we further down-regulated miR-543, the above results were obviously reversed, suggesting that although SERPINI1 exerted a regulatory effect on OC cell behaviors, this effect was also affected by miR-543. Finally, we found that SERPINI1 was positively regulated by PVT1 and negatively regulated by miR-543.

Although the present study confirmed that PVT1, as an endogenous RNA, positively regulates SERPINI1 through sponging miR-543, there is still some room for improvement. First of all, whether PVT1 is an independent prognostic factor of OC, and whether PVT1 has the potential to predict the prognosis of OC remain to be explored. Secondly, the influence of PVT1 on OC chemical resistance still need to be discussed to clarify its potential regulatory mechanism on OC chemical sensitivity. Furthermore, the potential value of PVT1 in predicting the recurrence of OC can be supplemented in the future studies. Last but not the least, we can supplement the correlation diagram of star database for evidence.

Conclusion

To sum up, we propose for the first time the molecular mechanism by which PVT1-mediated miR-543/SERPINI1 axis regulates the progression of OC, which may be beneficial to the treatment of OC patients.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

Chong Qu and Chunmei Dai contributed to the conception or design of the work. Yahua Guo contributed to the acquisition, analysis, or interpretation of data for the work. Rui Qin and Junbao Liu drafted the manuscript, and critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Abbreviations

APC, adenomatous polyposis coli; DANCR, differentiation antagonizing non-protein coding RNA; DLR, dual luciferase reporter; EZH2, enhancer of zeste homolog 2; GADPH, glyceraldehyde-3-phosphate dehydro-genase; lncRNA, long non-coding RNA; LSD, line segment detector; miR, microRNA; MMP7, matrix metalloproteinase-7; mRNA, messenger RNA; NC, negative control; OC, ovarian cancer; OS, overall survival; PI, propidium iodide; PVT1, plasmocytoma variant translocation 1; qRT-PCR, quantitative real-time PCR; RIP, RNA immunoprecipitation; SERPINI1, serpin peptidase inhibitor-clade I (neuroserpin)-member 1.

References

- Webb, P.M. and Jordan, S.J. (2017) Epidemiology of epithelial ovarian cancer. *Best Pract. Res. Clin. Obstet. Gynaecol.* **41**, 3–14, <https://doi.org/10.1016/j.bpobgyn.2016.08.006>
- Trabert, B., Tworoger, S.S., O'Brien, K.M. et al. (2020) The risk of ovarian cancer increases with an increase in the lifetime number of ovulatory cycles: an analysis from the Ovarian Cancer Cohort Consortium (OC3). *Cancer Res.* **80**, 1210–1218, <https://doi.org/10.1158/0008-5472.CAN-19-2850>
- Torre, L.A., Trabert, B., DeSantis, C.E. et al. (2018) Ovarian cancer statistics, 2018. *CA Cancer J. Clin.* **68**, 284–296, <https://doi.org/10.3322/caac.21456>
- Reid, B.M., Permut, J.B. and Sellers, T.A. (2017) Epidemiology of ovarian cancer: a review. *Cancer Biol. Med.* **14**, 9–32
- Matulonis, U.A., Sood, A.K., Fallowfield, L., Howitt, B.E., Sehouli, J. and Karlan, B.Y. (2016) Ovarian cancer. *Nat. Rev. Dis. Primers* **2**, 16061, <https://doi.org/10.1038/nrdp.2016.61>
- Moore, K., Colombo, N., Scambia, G. et al. (2018) Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* **379**, 2495–2505, <https://doi.org/10.1056/NEJMoa1810858>
- Ong, M.S., Cai, W., Yuan, Y. et al. (2017) 'Lnc'-ing Wnt in female reproductive cancers: therapeutic potential of long non-coding RNAs in Wnt signalling. *Br. J. Pharmacol.* **174**, 4684–4700, <https://doi.org/10.1111/bph.13958>
- Zhan, L., Li, J. and Wei, B. (2018) Long non-coding RNAs in ovarian cancer. *J. Exp. Clin. Cancer Res.* **37**, 120, <https://doi.org/10.1186/s13046-018-0793-4>
- Li, Y.L., Wang, X.M., Qiao, G.D. et al. (2020) Up-regulated lnc-lung cancer associated transcript 1 enhances cell migration and invasion in breast cancer progression. *Biochem. Biophys. Res. Commun.* **521**, 271–278, <https://doi.org/10.1016/j.bbrc.2019.08.040>
- Song, W., Wang, J., Liu, H. et al. (2019) Effects of lncRNA lnc-LIF-AS on cell proliferation, migration and invasion in a human cervical cancer cell line. *Cytokine* **120**, 165–175, <https://doi.org/10.1016/j.cyto.2019.05.004>
- Li, S., Shan, Y., Li, X. et al. (2019) lncRNA SNHG5 modulates endometrial cancer progression via the miR-25-3p/BTG2 axis. *J. Oncol.* **2019**, 7024675, <https://doi.org/10.1155/2019/7024675>

- 12 Chai, Y., Liu, J., Zhang, Z. and Liu, L. (2016) HuR-regulated lncRNA NEAT1 stability in tumorigenesis and progression of ovarian cancer. *Cancer Med.* **5**, 1588–1598, <https://doi.org/10.1002/cam4.710>
- 13 Liu, Y., Sun, J., Yu, J. et al. (2019) lncRNA CACS15 accelerates the malignant progression of ovarian cancer through stimulating EZH2-induced inhibition of APC. *Am. J. Transl. Res.* **11**, 6561–6568
- 14 Lin, X., Yang, F., Qi, X. et al. (2019) lncRNA DANCER promotes tumor growth and angiogenesis in ovarian cancer through direct targeting of miR-145. *Mol. Carcinog.* **58**, 2286–2296, <https://doi.org/10.1002/mc.23117>
- 15 Wang, W., Zhou, R., Wu, Y. et al. (2019) PVT1 promotes cancer progression via microRNAs. *Front. Oncol.* **9**, 609, <https://doi.org/10.3389/fonc.2019.00609>
- 16 Liu, X., Gan, L. and Zhang, J. (2019) miR-543 inhibits cervical cancer growth and metastasis by targeting TRPM7. *Chem. Biol. Interact.* **302**, 83–92, <https://doi.org/10.1016/j.cbi.2019.01.036>
- 17 Du, Y., Liu, X.H., Zhu, H.C., Wang, L., Ning, J.Z. and Xiao, C.C. (2017) MiR-543 promotes proliferation and epithelial-mesenchymal transition in prostate cancer via targeting RKIP. *Cell. Physiol. Biochem.* **41**, 1135–1146, <https://doi.org/10.1159/000464120>
- 18 Yu, Q., Zhang, Z., He, B., Wang, H., Shi, P. and Li, Y. (2020) MiR-543 functions as tumor suppressor in ovarian cancer by targeting TWIST1. *J. Biol. Regul. Homeost. Agents* **34**, 101–110
- 19 Song, N., Liu, H., Ma, X. and Zhang, S. (2015) Placental growth factor promotes metastases of ovarian cancer through MiR-543-regulated MMP7. *Cell. Physiol. Biochem.* **37**, 1104–1112, <https://doi.org/10.1159/000430235>
- 20 Yamanaka, S., Olaru, A.V., An, F. et al. (2012) MicroRNA-21 inhibits Serpin1, a gene with novel tumour suppressive effects in gastric cancer. *Dig. Liver Dis.* **44**, 589–596, <https://doi.org/10.1016/j.dld.2012.02.016>
- 21 Matsuda, Y., Miura, K., Yamane, J. et al. (2016) SERPIN1 regulates epithelial-mesenchymal transition in an orthotopic implantation model of colorectal cancer. *Cancer Sci.* **107**, 619–628, <https://doi.org/10.1111/cas.12909>
- 22 Valiente, M., Obenaus, A.C., Jin, X. et al. (2014) Serpins promote cancer cell survival and vascular co-option in brain metastasis. *Cell* **156**, 1002–1016, <https://doi.org/10.1016/j.cell.2014.01.040>
- 23 Doubeni, C.A., Doubeni, A.R. and Myers, A.E. (2016) Diagnosis and management of ovarian cancer. *Am. Fam. Physician* **93**, 937–944
- 24 Liu, S.P., Yang, J.X., Cao, D.Y. and Shen, K. (2013) Identification of differentially expressed long non-coding RNAs in human ovarian cancer cells with different metastatic potentials. *Cancer Biol. Med.* **10**, 138–141
- 25 Yang, G., Lu, X. and Yuan, L. (2014) lncRNA: a link between RNA and cancer. *Biochim. Biophys. Acta* **1839**, 1097–1109, <https://doi.org/10.1016/j.bbagr.2014.08.012>
- 26 Yang, Y.R., Zang, S.Z., Zhong, C.L., Li, Y.X., Zhao, S.S. and Feng, X.J. (2014) Increased expression of the lncRNA PVT1 promotes tumorigenesis in non-small cell lung cancer. *Int. J. Clin. Exp. Pathol.* **7**, 6929–6935
- 27 Gou, X., Zhao, X. and Wang, Z. (2017) Long noncoding RNA PVT1 promotes hepatocellular carcinoma progression through regulating miR-214. *Cancer Biomark* **20**, 511–519, <https://doi.org/10.3233/CBM-170331>
- 28 Wang, Z., Su, M., Xiang, B., Zhao, K. and Qin, B. (2019) Circular RNA PVT1 promotes metastasis via miR-145 sponging in CRC. *Biochem. Biophys. Res. Commun.* **512**, 716–722, <https://doi.org/10.1016/j.bbrc.2019.03.121>
- 29 Zhou, M., Wang, X., Shi, H. et al. (2016) Characterization of long non-coding RNA-associated ceRNA network to reveal potential prognostic lncRNA biomarkers in human ovarian cancer. *Oncotarget* **7**, 12598–12611, <https://doi.org/10.18632/oncotarget.7181>
- 30 Chen, P.Y., Chang, W.S., Chou, R.H. et al. (2007) Two non-homologous brain diseases-related genes, SERPIN1 and PDCD10, are tightly linked by an asymmetric bidirectional promoter in an evolutionarily conserved manner. *BMC Mol. Biol.* **8**, 2, <https://doi.org/10.1186/1471-2199-8-2>
- 31 Cody, N.A., Zietarska, M., Filali-Mouhim, A., Provencher, D.M., Mes-Masson, A.M. and Tonin, P.N. (2008) Influence of monolayer, spheroid, and tumor growth conditions on chromosome 3 gene expression in tumorigenic epithelial ovarian cancer cell lines. *BMC Med. Genomics* **1**, 34, <https://doi.org/10.1186/1755-8794-1-34>