Platycodin D inhibits the proliferation, invasion and migration of endometrial cancer cells by blocking the PI3K/Akt signaling pathway via ADRA2A upregulation

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Abstract. Endometrial cancer (EC) is a complex disease that affects the reproductive health of females worldwide. Platycodin D (PD) is known to exert numerous anticancer effects, markedly inhibiting cell proliferation, inducing apoptosis and causing cell cycle arrest in several types of cancer. The present study aimed to explore the mechanisms underlying the effects of PD in EC cells. The viability and proliferation of human endometrial stromal cells (ESCs) and RL95-2 EC cells following treatment with PD were evaluated using Cell Counting Kit-8, MTT and colony formation assays. Wound healing and Transwell assays were also performed to assess the migration and invasion of EC cells following treatment with PD. The expression levels of α2A-adrenergic receptor (ADRA2A) were measured using reverse transcription-quantitative PCR and western blotting assays with and without PD treatment and following transfection with short hairpin (sh) RNAs targeting ADRA2A2. Moreover, western blot analysis was performed to measure the expression levels of Ki67, PCNA, MMP2 and MMP9 and the phosphorylation of proteins of the PI3K/Akt signaling pathway. The results demonstrated that treatment with PD markedly decreased the proliferation, invasion and migration of EC cells, and reduced activation of the PI3K/Akt signaling pathway in EC cells. Moreover, transfection with sh-ADRA2A attenuated the effects of PD. ADRA2A expression was downregulated in EC cells compared with ESCs, and ADRA2A expression was elevated in EC cells following treatment with PD. In conclusion, the present study indicates that PD blocked the PI3K/Akt signaling pathway via the upregulation of ADRA2A expression, thereby inhibiting the proliferation, invasion and migration of EC cells.

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Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy worldwide, with an incidence estimated at 5.9% and continuing to rise (1,2). EC ranks as the fourth most frequently occurring cancer among females and exerts a high psychological and physical burden on patients (3). The standard treatment for metastatic endometrial carcinoma includes chemotherapy based on platinum drugs and hormone therapy (4). Although EC is usually diagnosed at an early stage and the prognosis is generally good, some patients experience metastatic or recurrent EC, and their 5-year survival rate is only 10-20% (5). Therefore, the discovery of novel drugs for the treatment of EC is required.

Platycodin D (PD), a triterpenoid saponin extracted from the roots of *Platycodin grandiflorum*, has been reported to possess anticancer effects in several cancer cell lines, including lung, gastric and prostate cancer cells (6). PD has been shown to suppress the tumor growth of breast cancer cells *in vitro* and *in vivo* via the inhibition of mouse double minute 2 homolog and mutated p53 in MDA-MB-231 cells (7). In addition, PD has been reported to inhibit the proliferation, migration and invasion of MDA-MB-231 human breast cancer cells via the suppression of EGF-induced activation of the EGFR, MAPK and PI3K/Akt signaling pathways (8). Furthermore, PD markedly impacts the proliferation of prostate cancer cells via the induction of apoptosis and cell cycle arrest (9). However, the mechanisms underlying the effects of PD on EC cells are yet to be fully elucidated.

A preliminary analysis performed for the present study using the ENCORI database (10), revealed that the α2A-adrenergic receptor (ADRA2A) is downregulated in EC tissues, and the low expression of ADRA2A is associated with

poor outcomes in patients with EC. In addition, a previous study showed that ADRA2A expression is markedly downregulated in cervical cancer tissue and cell lines, while its overexpression suppresses the proliferation, migration and invasion of cervical cancer cells. Moreover, the study also demonstrated that ADRA2A overexpression promotes cell aging and apoptosis, and decreases the phosphorylation levels of PI3K, Akt and mTOR in cervical cancer cells (11). Therefore, it is hypothesized that ADRA2A may block PI3K/Akt signaling pathways in other diseases.

Notably, the SwissTargetPrediction webtool (12) predicts that PD targets ADRA2A, and therefore may regulate its expression. However, whether ADRA2A inhibits the proliferation, migration and invasion of EC cells remains to be fully elucidated. Therefore, the present study aimed to investigate the effects and underlying mechanisms of PD and the roles of ADRA2A in the proliferation, migration and invasion of EC cells, in particular, whether they involve the PI3K/Akt signaling pathway.

Materials and methods

Bioinformatics tools. The ENCORI database (https://starbase.sysu.edu.cn/index.php) was used to detect ADRA2A expression in endometrial cancer tissues. The SwissTargetPrediction webtool (http://www.swisstargetprediction.ch/) predicted the interaction between PD and ADRA2A.

Cell culture. The human endometrial stromal cell (HESCs; cat. no. CRL-4003) line and RL95-2 human EC cell line were obtained from the American Type Culture Collection. The ESCs were cultured in a 1:1 mixture of DMEM/F12 (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Beyotime Institute of Biotechnology) in a T25 culture flask with 5% CO_2 and 95% air at 37˚C. The RL95-2 cells were grown in DMEM/F12 containing 10% FBS, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2. PD was purchased from Shanghai Yuanye Bio-Technology Co., Ltd.

Cell Counting Kit-8 (CCK-8) assay. ESCs and RL95-2 cells were seeded into 96-well plates at a density of $4x10^4$ cells/well and incubated for 24 h at 37˚C. Following cell attachment, 5, 10, 20 and 40 μM PD solution was added into each well. Untreated cells served as a control. Subsequently, the plate was incubated for 24, 48 and 72 h at 37˚C, and 10 μl CCK-8 reagent (Beyotime Institute of Biotechnology) was added to each well. A microplate reader was used to measure the optical density at 450 nm.

Cell transfection. Short hairpin (sh)RNA vectors with a plasmid backbone of pRNA-U6.1 targeting ADRA2A (sh-ADRA2A-1, 5'-GGATCAAGACATAAGTAAA-3' and sh-ADRA2A-2, 5'-GCTCAAGATTCAAGATACA-3') and a negative control (sh-NC, 5'-CCGGCAACAAGATGAAGAGCACCAACTC-3') were synthesized by ChemicalBook. RL95-2 cells were transfected with 100 nM sh-ADRA2A or sh-NC at 37˚C for 48 h. All transfection experiments were carried out using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Cells were collected for subsequent experiments at 48 h post-transfection.

Colony formation assay. RL95-2 cells in the logarithmic growth phase were cultured in culture dishes (diameter, 60 mm) at a concentration of $1x10^3$ cells/100 μl at 37˚C for 10 days with or without 5, 10 or 20 μM PD. Subsequently, the medium was removed and the cells were rinsed in PBS. The cells were then fixed with methanol for 15 min at room temperature and stained with Giemsa for 10 min at room temperature. Clusters containing >50 cells were identified as a colony. The colonies were counted manually and images captured under a microscope (Olympus Corporation). All experiments were performed in triplicate.

Western blotting. Total proteins were obtained from RL95-2 cells treated with or without 5, 10 or 20 μM PD for 24 h, or from ESCs by lysis using cold RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentration was determined using a BCA Protein Assay kit (Beijing Solarbio Science & Technology Co., Ltd.) and protein samples (20 μg per lane) were separated on 10% gels by SDS-PAGE (Thermo Fisher Scientific, Inc.), transferred onto PVDF membranes (MilliporeSigma) and incubated for 1 h at room temperature with 5% skimmed milk. The membranes were then incubated at 4˚C overnight with the following primary antibodies: Ki67 (1:1,000; ab92742), proliferating cell nuclear antigen (PCNA; 1:1,000; ab92552), MMP2 (1:1,000; ab92536), MMP9 (1:1,000; ab76003), ADRA2A (1:1,000; ab85570), phospho (p)-PI3K (1:1,000; ab182651), p-Akt (1:1,000; ab192623), PI3K (1:1,000; ab191606), Akt (1:1,000; ab179463) and GAPDH (1:2,500; ab9485), all from Abcam. After washing with TBST (0.1% Tween), the membranes were incubated with Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (1:2,000; ab6721; Abcam) at 37˚C for 1 h. The immunoreactive protein bands were visualized using an enhanced chemiluminescence detection system (Amersham; Cytiva) according to the manufacturer's instructions. Subsequently, protein brands were observed using ImageJ (v1.8.0; National Institutes of Health) and quantified by densitometry (QuantityOne 4.5.0 software; Bio-Rad Laboratories, Inc.). GAPDH served as an internal reference.

Wound healing assay. RL95-2 cells treated with 5, 10 or 20 μM concentrations of PD were seeded into 6-well plates (500 cells/well) and cultured until 90% confluence was reached. Five scratches were made in each well using a 200-μl pipette tip. The wells were washed with PBS three times to remove the detached cells from the wound. Subsequently, following the application of serum-free medium, the cells were incubated at 37˚C in 5% CO_2 for 24 h. Cell migration was evaluated using a light microscope (Olympus Corporation). The relative migration rate (%)=(wound width at 0 h-wound width at 48 h)/wound width at 0 h x100.

Transwell assay. RL95-2 cells were suspended at a final concentration of $2x10^5$ cells/ml. DMEM/F12 (200 μl) was placed into the upper compartment of a Transwell chamber, precoated with Matrigel (Sigma-Aldrich; Merck KGaA) at 37˚C for 30 min, following treatment with 5, 10 or 20 μM

concentrations of PD. Subsequently, medium supplemented with 10% FBS was added to the lower chamber. Following 24 h of incubation at 37˚C, a cotton swab was used to remove the non-invasive cells. The remaining invaded cells were fixed with 4% paraformaldehyde for 5 min at room temperature and stained using 0.1% crystal violet for 20 min at room temperature. A light microscope (Olympus Corporation) was used to observe and count the stained cells.

Reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted from RL95-2 cells treated with or without 5, 10 or 20 μM PD for 24 h, and from ESCs using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Subsequently, cDNA was obtained by reverse transcription of the extracted RNA using a PrimeScript™ RT reagent kit (cat. no. RR037Q; Takara Bio, Inc.). The temperature protocol was 15 min at 37˚C, 5 sec at 85˚C and 30 min at 4˚C. qPCR was conducted using a SYBR green qPCR kit (Takara Bio, Inc.) in a Roche LightCycler®96 system (Roche Diagnostics GmbH) following the manufacturer's protocol. The primer sequences for PCR were as follows: ADRA2A: 5'-ATCCTGGCCTTGGGA GAGAT-3' (forward) and 5'-TCTCAAAGCAGGTCCGTG TC-3' (reverse); GAPDH: 5'-GGGAAACTGTGGCGTGAT-3' (forward) and 5'-GAGTGGGTGTCGCTGTTGA-3' (reverse). The PCR thermocycling program was 95˚C for 3 min, followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 1 min. A final extension step at 72˚C for 7 min was performed in each qPCR assay. The $2^{-\Delta\Delta Cq}$ method was used for quantification (13).

MTT assay. An MTT assay was carried out to assess the proliferation of EC cells. Briefly, RL95-2 cells were seeded into 96-well plates ($2x10^3$/well) for 24, 48 and 72 h incubation with or without 20 μM PD. Following incubation, 10 μl MTT (5 mg/ml) was added to each well and incubation was continued for a further 4 h. Subsequently, the culture medium was discarded, 150 μl DMSO (Thermo Fisher Scientific, Inc.) was added and the plates were shaken for 10 min for full dissolution. The absorbance was read at 490 nm using a Microplate Autoreader (Omega Bio-Tek, Inc.).

Statistical analysis. All experiments were repeated three times, with three replicates each time. Statistical analysis was carried out using SPSS 19.0 statistical software (IBM Corp). Data are presented as the mean ± standard deviation. One-way ANOVA followed by Bonferroni's post hoc test was used to assess differences among multiple groups, and an unpaired Student's t-test was used to analyze differences between two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PD inhibits the viability of EC cells. The effects of PD on the viability of ESCs and RL95-2 cells treated with different concentrations of PD for 24 h were investigated. The chemical structure of PD is shown in Fig. 1A. No change was observed in the viability of ESCs treated with 5, 10 and 20 μM PD, while the viability of ESCs treated with 40 μM PD was slightly decreased compared with that of the untreated control, as displayed in Fig. 1B. The results indicate that 40 μM PD exerted an inhibitory effect on normal endometrial cell viability. However, the viability of RL95-2 cells treated with higher concentrations of PD (10-40 μM) was significantly reduced compared with that of the untreated control group. These results indicate that various concentrations of PD inhibited the viability of EC cells. On the basis of these results, 5, 10 and 20 μM concentrations of PD were selected for use in subsequent experiments.

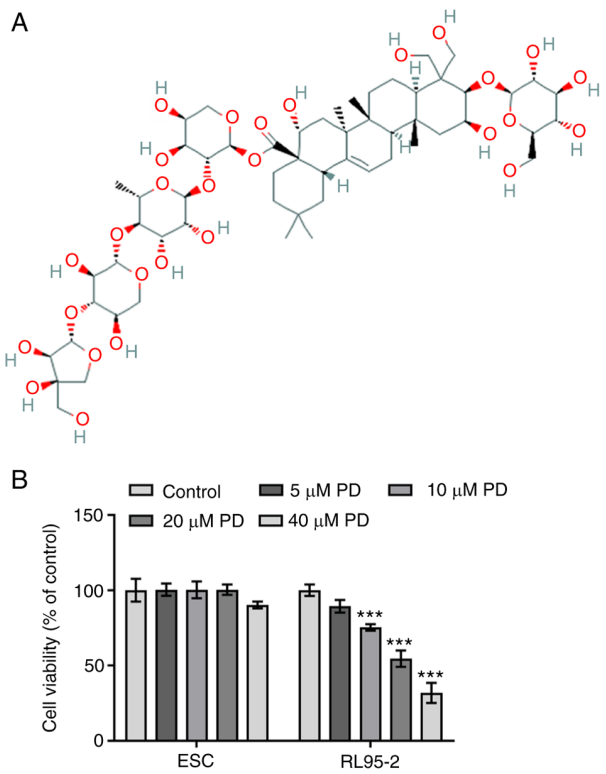

Figure 1. PD inhibits the viability of endometrial cancer cells. (A) The chemical structure of PD. (B) ESCs and RL95-2 cells were seeded into 96-well plates and incubated with PD for 24 h. Cell viability was detected using a Cell Counting Kit-8 assay. Data are expressed as the mean ± SD. ***P<0.001 vs. control. PD, platycodin D; ESC, endometrial stromal cell.

PD suppresses the proliferation of EC cells. To verify the inhibitory effect of PD on the proliferation of EC cells, CCK-8 and colony formation assays were performed and the expression levels of Ki67 and PCNA were examined in PD-treated EC cells. The results demonstrate that in comparison with the control group, cell proliferation decreased as the PD concentration increased, and the reduction in proliferation was maintained over a prolonged time period (Fig. 2A). Moreover, the results of the colony formation assay reveal that the number of colonies gradually decreased as the PD concentration increased (Fig. 2B). In addition, the western blotting results shown in Fig. 2C demonstrate that the expression levels of Ki67 and PCNA also decreased following treatment with PD. Collectively, the aforementioned results indicate that PD exerted a significant inhibitory effect on EC proliferation.

PD inhibits the migration and invasion of EC cells. The effect of PD on EC cell migration and invasion was evaluated

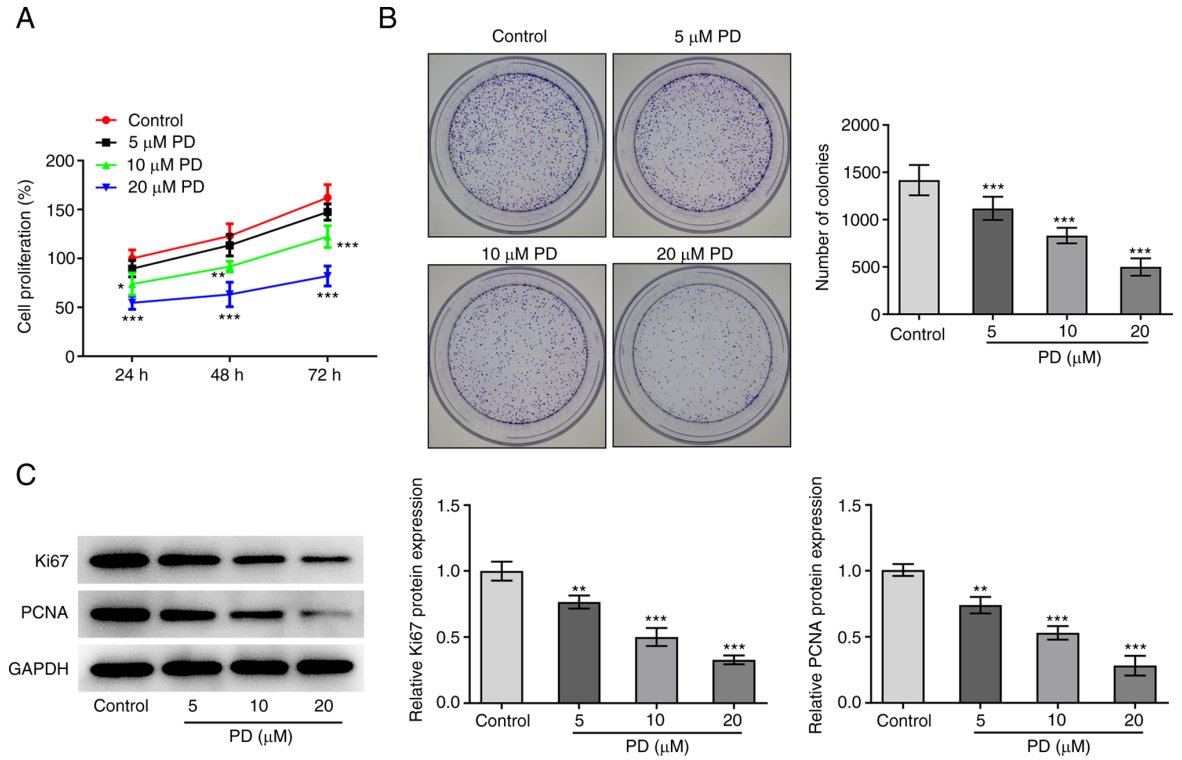

Figure 2. PD suppresses the proliferation of RL95-2 cells. (A) Cell proliferation of RL95-2 cells was evaluated by Cell Counting Kit-8 assay. (B) Colony formation by RL95-2 cells. (C) The protein levels of Ki67 and PCNA in RL95-2 cells treated with PD for 24 h were evaluated by western blot assay. Data are expressed as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 vs. control. PD, platycodin D; PCNA, proliferating cell nuclear antigen.

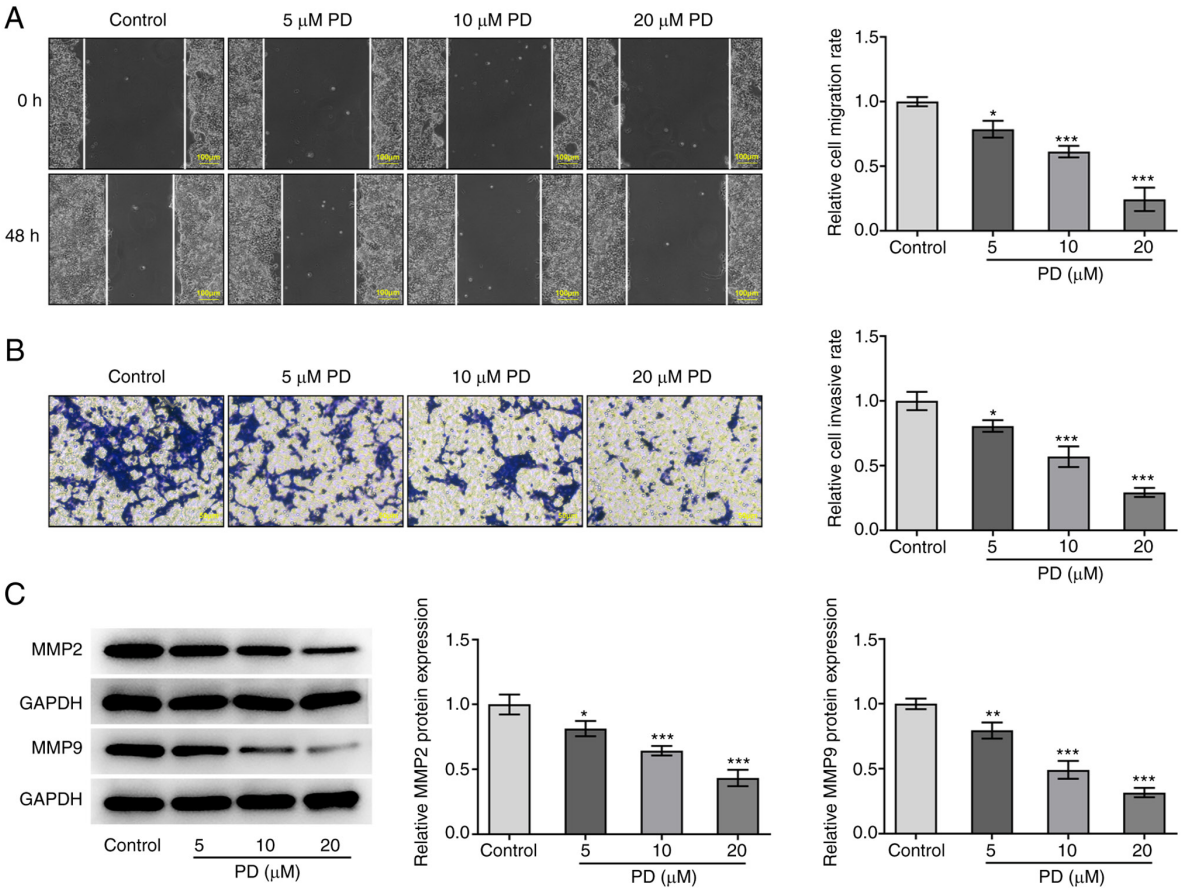

Figure 3. PD inhibits the migration and invasion of RL95-2 cells. (A) Cell migration and (B) invasion were evaluated using wound healing and Transwell assays, respectively. Wound image magnification x100; Transwell image magnification, x200. (C) Expression of MMP2 and MMP9 in RL95-2 cells treated with PD for 24 h was detected by western blot assay. Data are expressed as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 vs. control. PD, platycodin D.

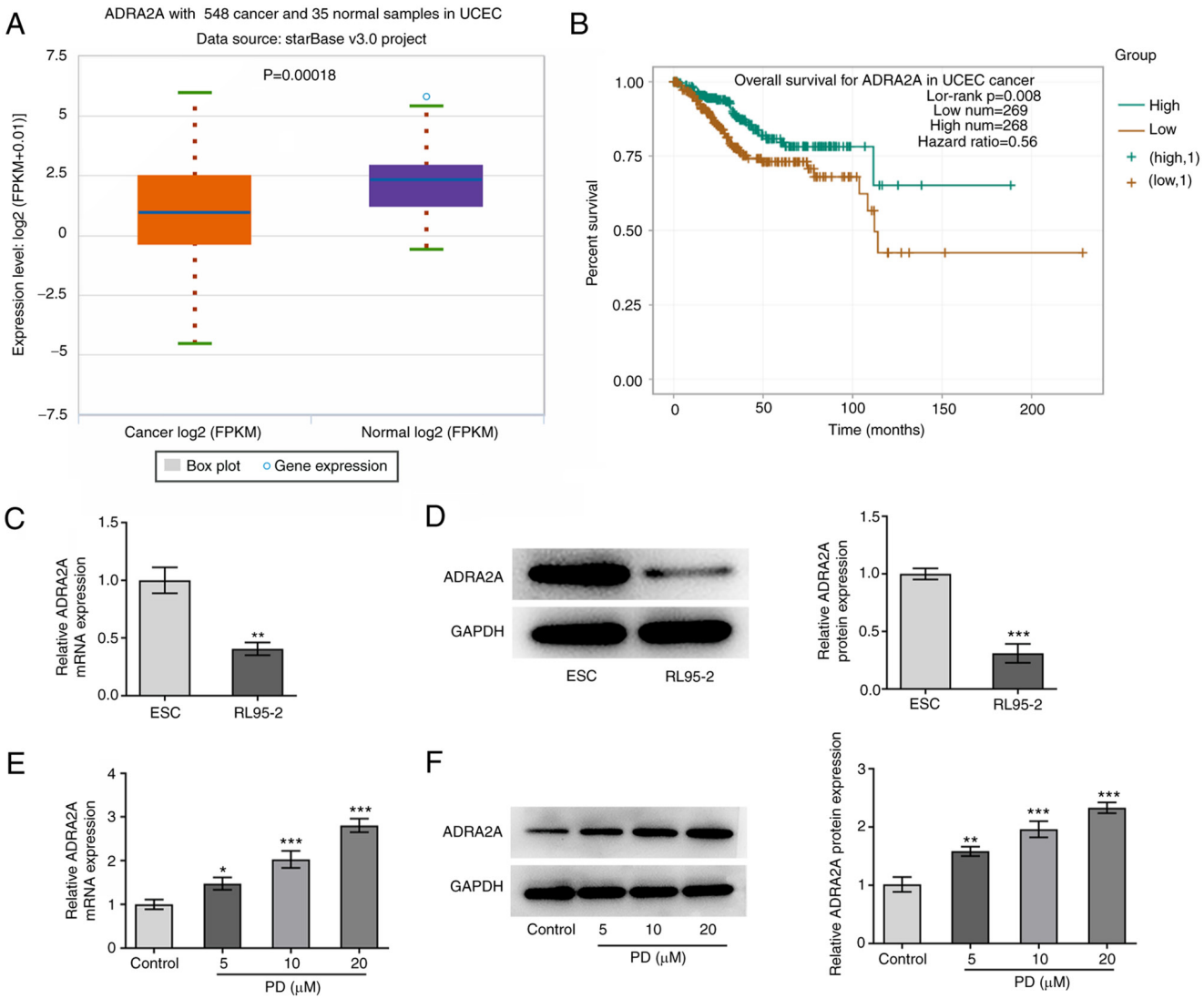

Figure 4. PD upregulates ADRA2A expression in RL95-2 cells. ENCORI database results showing (A) the expression of ADRA2A and (B) the association between ADRA2A expression and the overall survival level of patients with endometrial cancer. (C) RT-qPCR and (D) western blot assays were performed to determine the expression of ADRA2A in RL95-2 cells and ESCs. The expression of ADRA2A in RL95-2 cells treated with PD for 24 h was tested using (E) RT-qPCR and (F) western blot assays. Data are expressed as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 vs. control. PD, platycodin D; ADRA2A, α2A-adrenergic receptor; UCEC, uterine corpus endometrial carcinoma; RT-qPCR, reverse transcription-quantitative PCR; ESC, endometrial stromal cell.

using wound healing and Transwell assays, respectively, and the protein expression levels of MMP2 and MMP9 in EC cells were detected following PD treatment. As displayed in Fig. 3A and B, treatment with PD significantly reduced the migration and invasion of the EC cells. Moreover, the expression levels of MMP2 and MMP9 were reduced by PD compared with those in the control group, as shown in Fig. 3C. These results demonstrate that PD inhibited the migration and invasion of EC cells.

PD increases the expression of ADRA2A in EC cells. Gene expression data obtained from the ENCORI database demonstrate that ADRA2A is expressed at a lower level in EC patient samples compared with normal samples, and low expression levels of ADRA2A are associated with poorer overall survival in patients with EC (Fig. 4A and B). The effect of PD treatment on the expression of ADRA2A in EC cells was then investigated. The RT-qPCR and western blotting results in Fig. 4C and D demonstrate that the expression of ADRA2A in RL-95 cells was markedly reduced compared with that in healthy ESCs. As displayed in Fig. 4E and F, the treatment of RL-95 cells with PD significantly increased the expression levels of ADRA2A compared with those in the untreated control group. These findings indicate that PD increases ADRA2A expression in EC cells.

PD inhibits the proliferation, invasion and migration of EC cells via the upregulation of ADRA2A expression. The ADRA2A, Ki67, PNCA, MMP2 and MMP9 expression levels and cell proliferation, colony formation, invasion and migration abilities of EC cells were determined following PD treatment, to assess whether PD exerts its effects on EC cells via the upregulation of ADRA2A expression. As displayed in Fig. 5A and B, ADRA2A expression in EC cells was significantly decreased following transfection with shADRA2A-1 and -2. Moreover, the expression levels of ADRA2A were decreased to a higher

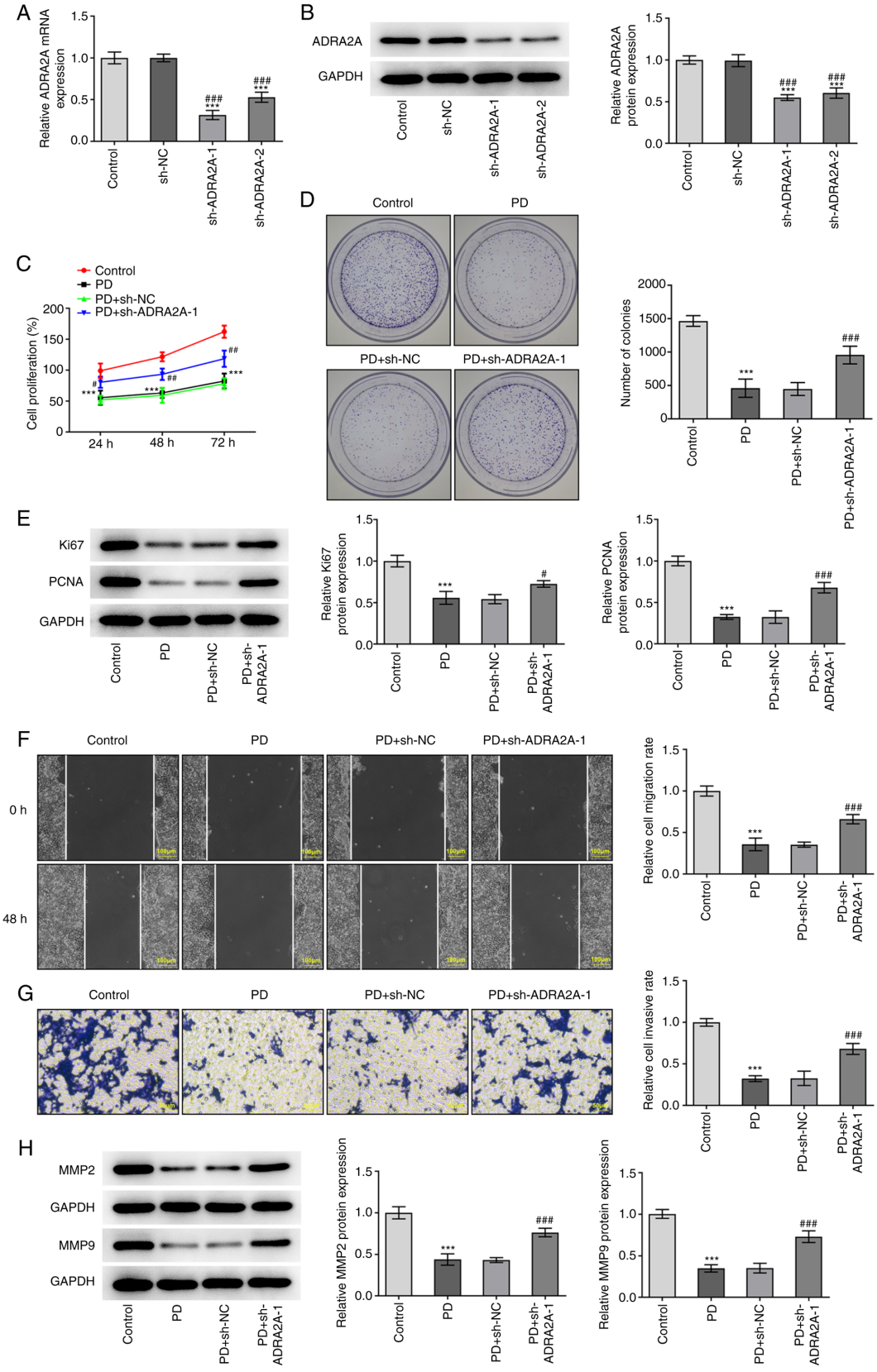

Figure 5. PD inhibits the proliferation, invasion and migration of RL95-2 cells via the upregulation of ADRA2A expression. (A) RT-qPCR and (B) western blot assays were carried out to detect the expression of ADRA2A in RL95-2 cells after transfection with sh-ADRA2A-1 and sh-ADRA2A-2. (C) Cell proliferation was evaluated by MTT assay after transfection with sh-ADRA2A-1. (D) The colony formation of RL95-2 cells following transfection with sh-ADRA2A-1 was investigated. (E) The protein levels of Ki67 and PCNA were examined by western blot assay after treatment with PD and/or transfection with sh-ADRA2A-1. (F) Cell migration and (G) invasion were measured using wound healing and Transwell assays, respectively, after treatment with PD and/or transfection with sh-ADRA2A-1. Wound healing assay magnification, x100; Transwell assay magnification, x200. (H) Western blotting was carried out to assess the expression of MMP2 and MMP9 in RL95-2 cells treated with PD for 24 h after transfection with sh-ADRA2A-1. Data are expressed as the mean ± SD. ***P<0.001 vs. control. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. PD + sh-NC. PD, platycodin D; ADRA2A, α2A-adrenergic receptor; RT-qPCR, reverse transcription-quantitative PCR; sh, short hairpin; NC, negative control; PCNA, proliferating cell nuclear antigen.

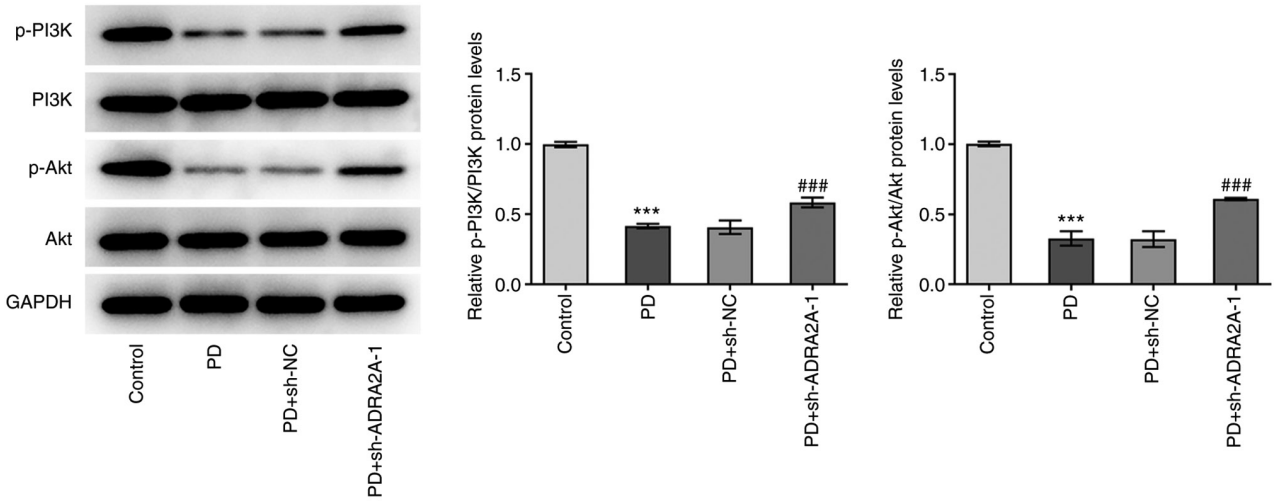

Figure 6. PD blocks the PI3K/Akt signaling pathway via the upregulation of ADRA2A expression. The protein levels of p-PI3K and p-Akt in RL95-2 cells treated with PD for 24 h were detected by western blot assay after transfection with sh-ADRA2A-1. Data are expressed as the mean ± SD. ***P<0.001 vs. control. ###P<0.001 vs. PD + sh-NC. PD, platycodin D; ADRA2A, α2A-adrenergic receptor; p-, phospho-; sh, short hairpin; NC, negative control.

extent following transfection with sh-ADRA2A-1 compared with sh-ADRA2A-2. Consequently, sh-ADRA2A-1 was selected for use in subsequent experiments, along with 20 μM PD. The results of MTT assays demonstrate that cell proliferation following treatment with PD was markedly reduced compared with that of the untreated control (Fig. 5C), and the PD-induced reduction in cell proliferation was significantly attenuated by sh-ADRA2A-1 in the PD + sh-ADRA2A-1 group. The results of the colony formation assay were consistent with this, and demonstrate that while treatment with PD reduced the number of colonies, the PD-induced reduction in colony formation was attenuated in the PD + sh-ADRA2A-1 group (Fig. 5D). In addition, the protein expression levels of Ki67 and PCNA were significantly decreased in the PD group compared with the control group, and these reductions were attenuated in the PD + sh-ADRA2A-1 group, as displayed in Fig. 5E. The migration and invasion of EC cells treated with PD were also significantly reduced compared with those of the untreated control, and significantly increased in the PD + sh-ADRA2A-1 transfection group compared with the PD group (Fig. 5F and G). Moreover, the knockdown of ADRA2A attenuated the reduction in MMP2 and MMP9 expression in EC cells that was induced by PD treatment (Fig. 5H). Collectively, the aforementioned results indicate that PD inhibited the proliferation, invasion and migration of EC cells via the upregulation of ADRA2A expression.

PD blocks the PI3K/Akt signaling pathway via the upregulation of ADRA2A expression. To clarify the effects of PD on the PI3K/Akt signaling pathway, the phosphorylation levels of PI3K and Akt were investigated by western blotting. As shown in Fig. 6, the p-PI3K/PI3K and p-Akt/Akt ratios in EC cells treated with PD were significantly decreased compared with those in the control cells, while transfection with sh-ADRA2A-1 significantly attenuated the PD-induced changes in the p-PI3K and p-Akt levels. These findings demonstrate that PD blocked activation of the PI3K/Akt signaling pathway via the upregulation of ADRA2A expression.

Discussion

EC is regarded as one of the most serious diseases in females, with rising incidence and mortality rates (14), and a poor prognosis with regard to recurrence or metastasis (15). Despite advances in the biological understanding of EC, numerous aspects of the current treatment options remain controversial, including the surgical assessment of the lymph nodal status in EC, which may determine the specific therapeutic method, and the selection of patients for adjuvant radiation or chemotherapy, which may impact the therapeutic effect and prognosis of the patients (16). A previous study demonstrated that PD may serve as an effective antitumor drug (17). However, the antitumor effect of PDs in EC has not been fully elucidated. In the present study, the inhibitory effects of PD on the viability, proliferation, invasion and migration of EC cells were explored. In addition, the present study demonstrated that ADRA2A expression levels were lower in EC cells than in normal ESCs. The results of various assays in the present study indicated that PD suppressed the proliferation, invasion and migration of EC cells via the upregulation of ADRA2A expression. Moreover, the results confirmed that ADRA2A blocks the PI3K/Akt signaling pathway and reduces its activation. These findings indicate that PD and the expression levels of ADRA2A have an association with EC.

The results of previous studies have shown that PD has antitumor effects in several types of cancer, including lung (18), gastric (19) and bladder cancer (17). PD has also been reported to target cancer cells by inducing apoptosis, arresting the cell cycle and inhibiting angiogenesis, invasion and metastasis via multiple signaling pathways (20). For example, the results of a previous study indicated that PD exerts antitumor effects by inducing apoptosis, suppressing invasion and migration, and arresting the cell cycle of gallbladder cancer cells (21). Moreover, the results of another study demonstrated that PD inhibited cell proliferation and induced apoptosis in BEL-7402 human hepatocellular carcinoma cells (22). A study by Wu *et al* (23) demonstrated that PD inhibited the

proliferation and migration of various multiple myeloma cell lines by activating the NF-κB and Janus kinase 2/STAT3 pathways. In another study, Zhang *et al* (24) demonstrated that PD exerted inhibitory effects on the growth and invasion of human oral squamous cell carcinoma via inactivation of the NF-κB pathway. The results of the present study revealed that the viability of EC cells was decreased following treatment with PD. In addition, high expression levels of the proliferation marker Ki67 are associated with reduced EC-specific survival (25), and the present study detected reduced protein expression levels of Ki67 and PCNA in EC cells following treatment with PD, which indicated that PD has the ability to inhibit cell proliferation in EC.

In a previous study, PD was shown to markedly downregulate the expression levels of MMP2 and MMP9 in rats with cardiac hypertrophy (26). The expression levels of MMP2 and MMP9 in EC cells were measured in the present study, and the results demonstrated that they were decreased following treatment with PD, as were the invasion and migration of the cells. The aforementioned results indicate the ability of PD to inhibit the invasion and migration of EC cells.

Analyses conducted using the ENCORI database revealed that the expression of ADRA2A is downregulated in the tumor tissues of patients with EC, and low ADRA2A expression is associated with a poor prognosis. In the present study, the expression of ADRA2A was observed to be downregulated in EC cells compared with ESCs. However, the expression levels of ADRA2A in EC cells were elevated following PD treatment, which suggests that ADRA2A is a target gene that is regulated by PD. This finding is in accordance the predicted binding between PD and ADRA2A obtained using the SwissTargetPrediction webtool. In addition, a previous study reported that the downregulation of ADRA2A gene expression in prostate cancer was associated with aggregation, proliferation and migration (27). Moreover, another study identified that high ADRA2A expression is associated with the inhibition of tumor cell proliferation (28). Consistent with these previous findings, the present study demonstrated that the proliferation, invasion and migration of EC cells were reduced following PD treatment, and these effects were attenuated following ADRA2A knockdown, which suggests that PD inhibits the proliferation, invasion and migration of EC cells via the upregulation of ADRA2A expression.

As reported, PD and ADRA2A are pivotal regulators of the PI3K/AKT pathway in tumors (8,10,29). PI3K/AKT signaling is a growth-regulating cellular signaling pathway, the activation of which is increased in numerous human cancers (30). It may also participate in the apoptosis and migration of tumor cells (31). Notably, the PI3K/Akt signaling pathway is a key mechanism by which the growth, migration, proliferation and metabolism of mammalian cells are controlled (32). For example, microRNA-936 has been shown to promote the proliferation and invasion of gastric cancer cells by downregulating fibroblast growth factor 2 expression and activating the PI3K/Akt signaling pathway (33), while TRIM29 has been reported to promote the progression of thyroid carcinoma via activation of the PI3K/Akt signaling pathway (34). The western blotting results in the present study demonstrated that the protein levels of p-PI3K and p-Akt were significantly reduced following PD treatment; however, these PD-induced reductions in phosphorylation levels were increased following ADRA2A knockdown, which suggests that ADRA2A regulates the PI3K/Akt signaling pathway. Collectively, these findings suggest that PD inhibits the PI3K/Akt signaling pathway via the upregulation of ADRA2A expression. Notably, the results of the present study show that ADRA2A knockdown only partially reversed the antitumor effects of PD, suggesting that other PD-mediated antitumor pathways may exist in EC. Thus, other potential PD-mediated mechanisms will be investigated in the future. For example, a previous study demonstrated that NLRC5 promotes cell migration and invasion in EC via activation of the PI3K/Akt signaling pathway (35). Therefore, this is a specific mechanism that requires further investigation with regard to the effects of PD treatment on EC cells.

In conclusion, the results of the present study suggest that PD inhibits the proliferation, migration and invasion of EC cells via the upregulation of ADRA2A and inhibition of the PI3K/Akt signaling pathway.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ZN and ZDa designed the study, drafted and revised the manuscript. ZN, ZDa, DS, PQ and ZL performed the experiments. QP and ZDe analyzed the data and searched the literature. All authors read and approved the final manuscript. ZN and ZDe confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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