IL-1β-induced pentraxin 3 inhibits the proliferation, invasion and cell cycle of trophoblasts in preeclampsia and is suppressed by IL-1β antagonists

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Abstract. Pentraxin 3 (PTX3), a member of the c-reactive protein family, is a long pentraxin protein and a pro-inflammatory marker. However, the role of PTX3 in preeclampsia (PE) remains to be elucidated. Thus, the present study aimed to investigate the biological role and mechanisms underlying PTX3 in PE. In the present study, PTX3 was overexpressed in trophoblasts and the subsequent changes in cell proliferation, cycle distribution and invasion were observed using Cell Counting Kit-8, flow cytometry and Transwell assays, respectively. Moreover, the expression levels of MMP2 and MMP9, proteins associated with the development of PE, were detected using reverse transcription-quantitative PCR and western blot analysis. Following treatment with interleukin (IL)-1β, the expression levels of PTX3 were measured. Furthermore, subsequent changes in cell proliferation, cycle distribution and invasion were investigated following overexpression of PTX3 and treatment with IL-1 receptor antagonist (IL-1Ra). Overexpression of PTX3 inhibited the proliferation, cycle and invasion of HTR-8/SV neo and JEG3 cells. Moreover, treatment with IL-1β increased the expression of PTX3 in HTR-8/SV neo and JEG3 cells, which was suppressed following treatment with the IL-1β antagonist. Following PTX3 overexpression and treatment with IL-1Ra, the inhibitory effects of PTX3 overexpression alone on the invasion of HTR-8/SV neo and JEG3 cells were attenuated. In conclusion, these results indicated that IL-1β could induce PTX3 upregulation, which led to the inhibition of the proliferation, invasion and cell cycle of trophoblasts, thereby promoting the progression of PE.

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Introduction

Preeclampsia (PE) occurs in 4-5% of pregnancies worldwide, accounting for a large number of maternal complications and contributing to a high rate of maternal morbidity (1). PE is characterized by the occurrence of hypertension and proteinuria after 20 weeks of gestation in a patient without a previous history of hypertension (2). In addition, patients with a clinical history of PE are at an increased risk of developing PE in subsequent pregnancies (3). At present, research is focused on further defining and characterizing PE; however, the etiology of PE remains to be elucidated. Moreover, further investigations into predicting PE development are required (2,4).

PE is an inflammatory disease. Previous studies have reported that an altered immune system response and excessive inflammation can contribute to the development of PE (5,6). Inflammatory responses at the utero-placental interface were associated with aberrant extravillous trophoblast invasion during placentation in transgenic preeclamptic rat models and human PE (7,8). Several *in vitro* studies previously suggested that exposure to inflammatory stimuli can trigger the secretion of proinflammatory cytokines from trophoblast cells to mediate PE (9,10). Inflammation occurs in healthy pregnancies in women, yet increased levels of inflammation are often a characteristic of PE (11). Regular nurse appointments are critical for discovering complications in pregnancy (12). Checks during regular nurse appointments include blood pressure measurement, PE diagnosis and surveillance, and determination of optimal timing of delivery (13).

Pentraxin 3 (PTX3), a member of the c-reactive protein family, is a long pentraxin protein and a pro-inflammatory marker (14). Under inflammatory conditions, PTX3 is secreted by vascular endothelial cells and monocytes (15). A number of previous studies have reported elevated expression levels of PTX3 in the serum of pregnant women with PE (16-19). However, the specific role of PTX3 in gestational trophoblast cells is yet to be fully elucidated. It has previously been reported that PTX3 is induced by interleukin (IL)-1β in human endometrial stem cells (20). Mature IL-1β cytokine is a key pro-inflammatory cytokine involved in the induction of a placental inflammatory response (21). Thus, the present study

aimed to explore the effects of PTX3 on the proliferation and invasion of trophoblasts, and the effects of IL-1β on PTX3 expression.

Materials and methods

Cell culture and treatment. HTR-8/SV neo and JEG3 cells were provided by Procell Life Science & Technology Co., Ltd. Cells were grown in DMEM/nutrient mixture F12 (DMEM/F12; HyClone; Cytiva) with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin at 37˚C with 5% CO_2. Untransfected HTR-8/SV neo and JEG3 cells were used as the control group. The vector overexpressing PTX3 (Ov-PTX3) at a concentration of 20 μM was purchased from Wuhan GeneCreate Biological Engineering Co., Ltd. by utilizing pcDNA3.1 vector containing the full-length PTX3 cDNA sequence, regarding the empty vector (Ov-NC) as the corresponding negative control. These plasmids were transfected into HTR-8/SV neo and JEG3 cells ($1x10^6$ cells/well) by using Lipofectamine® 3000 (cat. no. L3000015; Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 48 h. Transfection efficacy was evaluated by performing reverse transcription-quantitative (RT-q)PCR and western blot analysis 48 h post-transfection. When ~80% confluence was reached, cells were cultured in serum-starved DMEM/F12 with or without IL-1β (R&D Systems, Inc.) or 1 μg/ml IL-1 receptor antagonist (IL-1Ra; Swedish Orphan Biovitrum AB) for 24, 48 or 72 h at 37˚C. IL-1Ra was used to pretreat cells at 37˚C for 1 h before transfection.

Cell Counting Kit-8 (CCK-8) assay. For the detection of cell proliferation, cell suspensions were inoculated in a 96-well plate at $5x10^3$ cells/well and incubated at 37˚C with 5% CO_2. CCK-8 reagent (Shanghai Yeasen Biotechnology Co., Ltd.) was subsequently added into each well, and cells were cultured for a further 3 h. The absorbance was read using a microplate reader at 450 nm.

RT-qPCR assay. Total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA by using the iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Inc.) in accordance with the manufacturer's protocol. qPCR was performed using SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.) on a 7900HT Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: Initial denaturation at 95˚C for 2 min; followed by 40 cycles of 95˚C for 20 sec and 72˚C for 30 sec. The sequences of the primers designed were: PTX3 forward, 5'-CATCTCCTTGCGATTCTGTTTTG-3' and reverse, 5'-CCATTCCGAGTGCTCCTGA-3'; MMP2 forward, 5'-TACAGGATCATTGGCTACACACC-3' and reverse, 5'-GGTCACATCGCTCCAGACT-3'; MMP9 forward, 5'-TGTACCGCTATGGTTACACTCG-3' and reverse, 5'-GGCAGGGACAGTTGCTTCT-3'; and GAPDH forward, 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3'. The relative mRNA expression of target genes was calculated using the $2^{-\Delta\Delta Cq}$ method (22) and data were normalized to the GAPDH expression levels detected in the same sample.

Western blot analysis. The treated cells were harvested, lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology) and proteins were quantified using a BCA kit (Thermo Fisher Scientific, Inc.). An equal amount of protein lysates (20 μg) were separated via 10% SDS-PAGE and then transferred to PVDF membranes. The membranes were subsequently blocked with 5% non-fat milk for 1.5 h at room temperature and incubated with primary antibodies overnight at 4˚C at dilutions of 1:1,000, according to the manufacturer's protocol. Primary antibodies for PTX3 (cat. no. ab90806), MMP2 (cat. no. ab92536) and MMP9 (ab76003) were obtained from Abcam. After washing with PBS three times, the membranes were incubated with HRP-conjugated goat anti-rabbit secondary antibody (A0208) at room temperature for 1 h (Beyotime Institute of Biotechnology). Proteins bands were visualized using enhanced chemiluminescence (Thermo Fisher Scientific, Inc.). Band intensity was semi-quantified using ImageJ software (version 1.48v; National Institutes of Health).

Cell cycle detection via flow cytometry. Flow cytometry was conducted to determine the cell distribution in phases. Cells were collected 48 h post-transfection, washed with PBS and fixed in 70% ethanol at -20˚C. Following overnight fixation, cells were washed with PBS and stained with propidium iodide (Beckman Coulter, Inc.) for 30 min at 37˚C. Cell cycle analysis was performed by using an Attune™ NxT Acoustic Focusing Cytometer (Invitrogen; Thermo Fisher Scientific, Inc.).

Transwell invasion assay. Transwell 24-well culture plates with a 8-μm pore insert precoated with Matrigel (BD Biosciences) at 37˚C for 30 min were obtained. A total of $3x10^4$ cells in 100 ml FBS-free DMEM/F12 were placed in the upper chamber. DMEM/F12 containing 20% FBS was added to the lower chamber. After 24 h of incubation at 37˚C, cells were fixed with 4% paraformaldehyde for 20 min at 37˚C and then stained with crystal violet for 30 min at 37˚C. The stained cells were photographed under an inverted light microscope (magnification, x100; Olympus Corporation). ImageJ software (version 1.48v; National Institutes of Health) was used to calculate the numbers of cells invaded in five random images.

Statistical analysis. All experiments were repeated independently three times. Data are indicated as the mean ± standard deviation. The statistical differences among multiple groups were measured by one-way analysis of variance followed by Tukey's post hoc test. Graph Prism 6.0 software (GraphPad Software, Inc.) was used for data analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Overexpression of PTX3 inhibits the proliferation of HTR-8/SV neo and JEG3 cells. It has previously been reported that PTX3 is elevated in the plasma of patients with PE (16-19). Thus, PTX3 was overexpressed in HTR-8/SV neo and JEG3 cells. As presented in Fig. 1A and B, PTX3 expression was significantly upregulated in the Ov-PTX3 group compared with the control or Ov-NC group ($P<0.001$) in HTR-8/SV neo cells. Consistently, Ov-PTX3 transfection

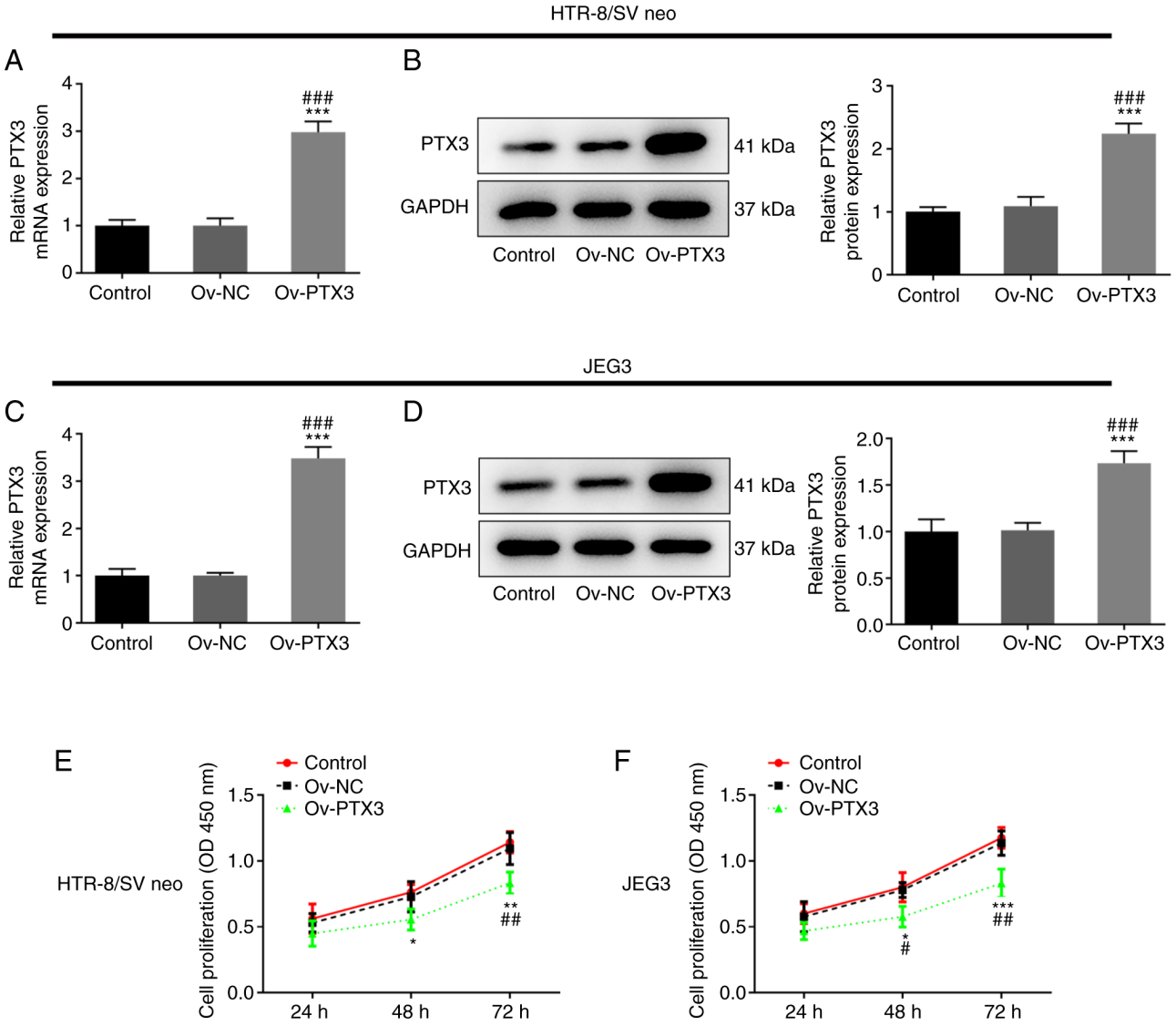

Figure 1. Overexpression of PTX3 inhibits the proliferation of HTR-8/SV neo and JEG3 cells. Expression levels of PTX3 following transfection with PTX3 plasmid in HTR-8/SV neo cells were evaluated by using (A) RT-qPCR and (B) western blot assay. Expression of PTX3 following transfection with PTX3 plasmid in JEG3 cells was determined by using (C) RT-qPCR and (D) western blot assay. Proliferation of (E) HTR-8/SV neo and (F) JEG3 cells following PTX3 overexpression were examined with Cell Counting Kit-8 assay. *P<0.05, **P<0.01, ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. Ov-NC. PTX3, pentraxin 3; RT-qPCR, reverse transcription-quantitative PCR; Ov, overexpression vector; NC, negative control.

also led to upregulated PTX3 expression in JEG3 cells when compared with the control or Ov-NC group (P<0.001; Fig. 1C and D). A key characteristic of PE development is altered trophoblast proliferation (23); thus, the proliferation of HTR-8/SV neo and JEG3 cells was detected following PTX3 overexpression. Notably, the proliferation of HTR-8/SV neo (P<0.05, P<0.01; Fig. 1E) and JEG3 (P<0.05, P<0.01, P<0.001; Fig. 1F) cells was significantly reduced following PTX3 overexpression. These results revealed that overexpression of PTX3 inhibited the proliferation of HTR-8/SV neo and JEG3 cells.

Overexpression of PTX3 inhibits the cell cycle promotion and invasion of HTR-8/SV neo and JEG3 cells. Following PTX3 overexpression, the G_0/G_1 phase of HTR-8/SV neo and JEG3 cells was prolonged, while the S phase was reduced compared with the control or Ov-NC group, suggesting that the cell cycle was arrested (P<0.001; Fig. 2A and B). Furthermore, the invasion of HTR-8/SV neo and JEG3 cells in the Ov-PTX3 group was decreased compared with the control or Ov-NC group (P<0.001; Fig. 3A and B). MMPs, which are zinc-dependent proteases involved in the uterine and vascular tissue remodeling during healthy pregnancy (24,25), are associated with the invasion of trophoblasts (26). Thus, the expression levels of MMP2 and MMP9 were detected following PTX3 overexpression. Notably, expression levels of MMP2 and MMP9 mRNA were reduced following PTX3 overexpression in both HTR-8/SV neo (P<0.001; Fig. 3C) and JEG3 (P<0.001; Fig. 3D) cells compared with the control or Ov-NC group. Meanwhile, significantly upregulated MMP2 and MMP9 protein expression was observed in HTR-8/SV neo (P<0.01, P<0.001; Fig. 3E) and JEG3 (P<0.001; Fig. 3F) cells compared with the control or Ov-NC group. Collectively, these results demonstrated that PTX3 overexpression inhibited the cell cycle promotion and invasion of HTR-8/SV neo and JEG3 cells.

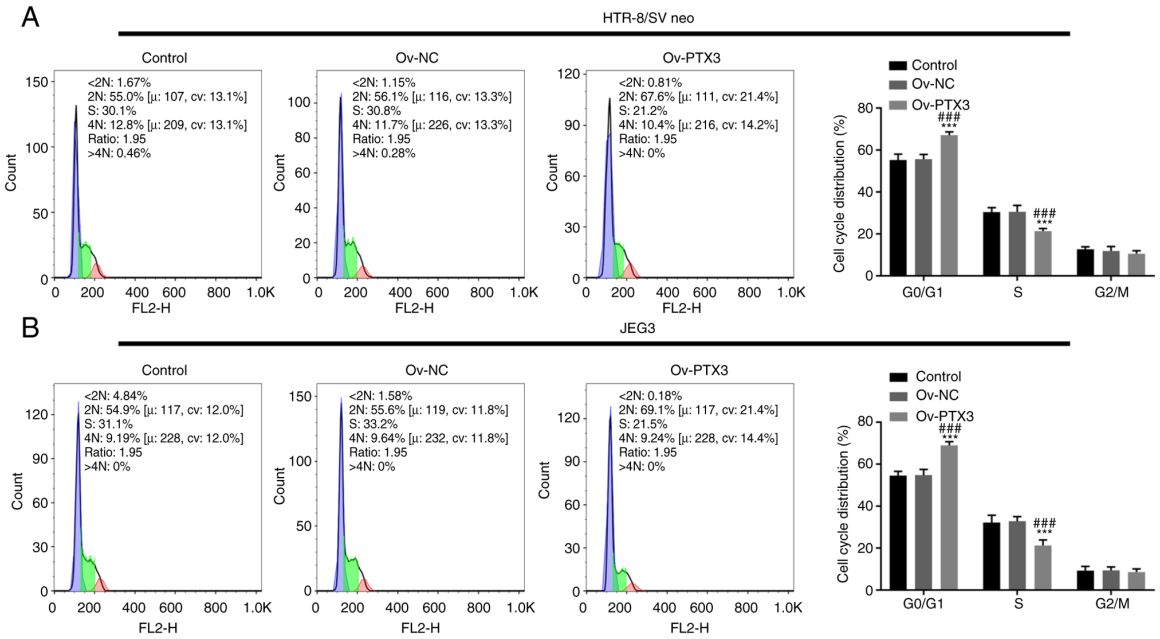

Figure 2. Overexpression of PTX3 inhibits the cell cycle promotion of HTR-8/SV neo and JEG3 cells. Cell cycle distributions in (A) HTR-8/SV neo and (B) JEG3 cells following PTX3 overexpression were tested by flow cytometry. ***P<0.001 vs. Control; $^{###}$P<0.001 vs. Ov-NC. PTX3, pentraxin 3; Ov, overexpression vector; NC, negative control.

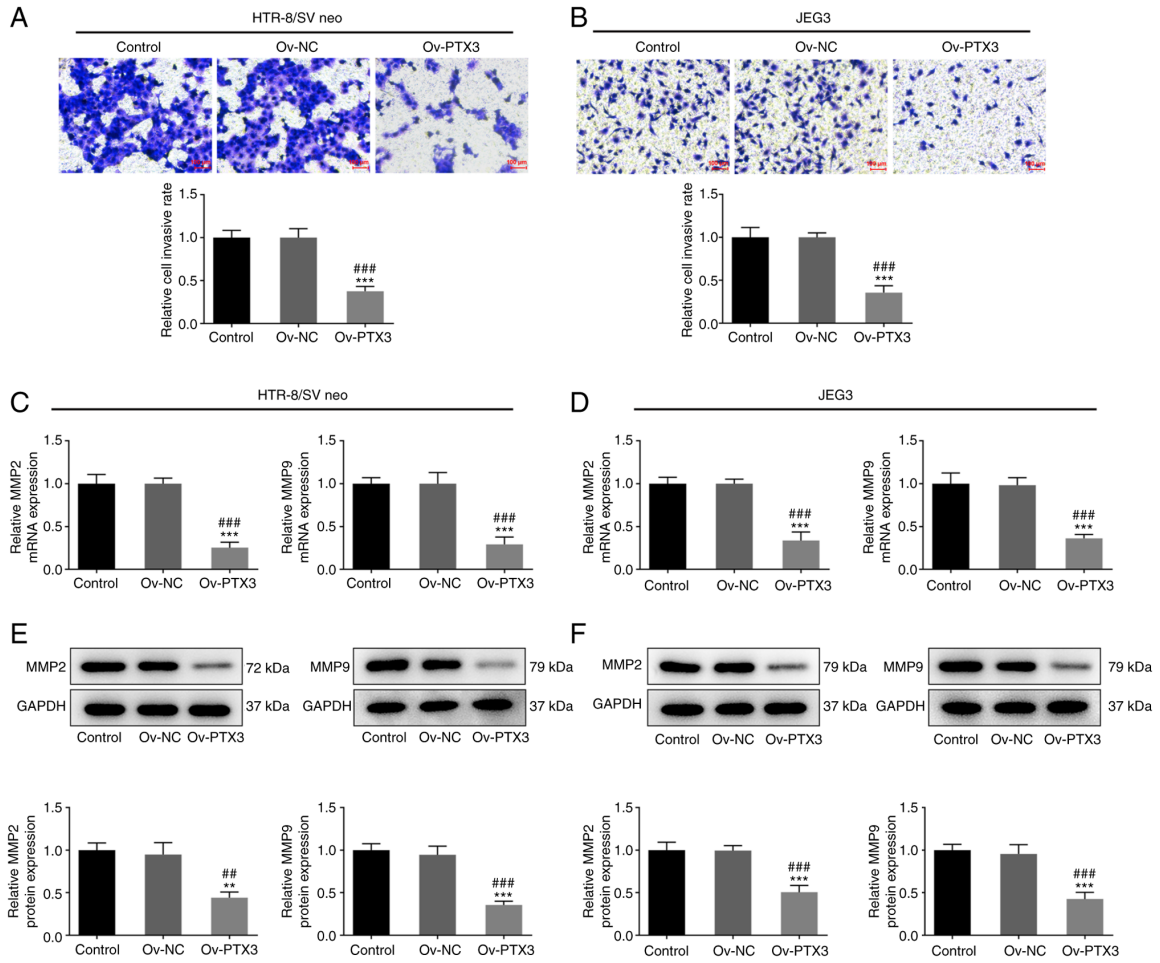

Figure 3. Overexpression of PTX3 inhibits the invasion of HTR-8/SV neo and JEG3 cells. Cell invasion in (A) HTR-8/SV neo and (B) JEG3 cells following PTX3 overexpression were assessed by using a Transwell invasion assay. The mRNA expression of MMP2 and MMP9 in (C) HTR-8/SV neo and (D) JEG3 cells following PTX3 overexpression was detected via reverse transcription-quantitative PCR. The protein expression of MMP2 and MMP9 in (E) HTR-8/SV neo and (F) JEG3 cells following PTX3 overexpression was determined using western blotting. **P<0.01, ***P<0.001 vs. Control; $^{##}$P<0.01, $^{###}$P<0.001 vs. Ov-NC. PTX3, pentraxin 3; Ov, overexpression vector; NC, negative control.

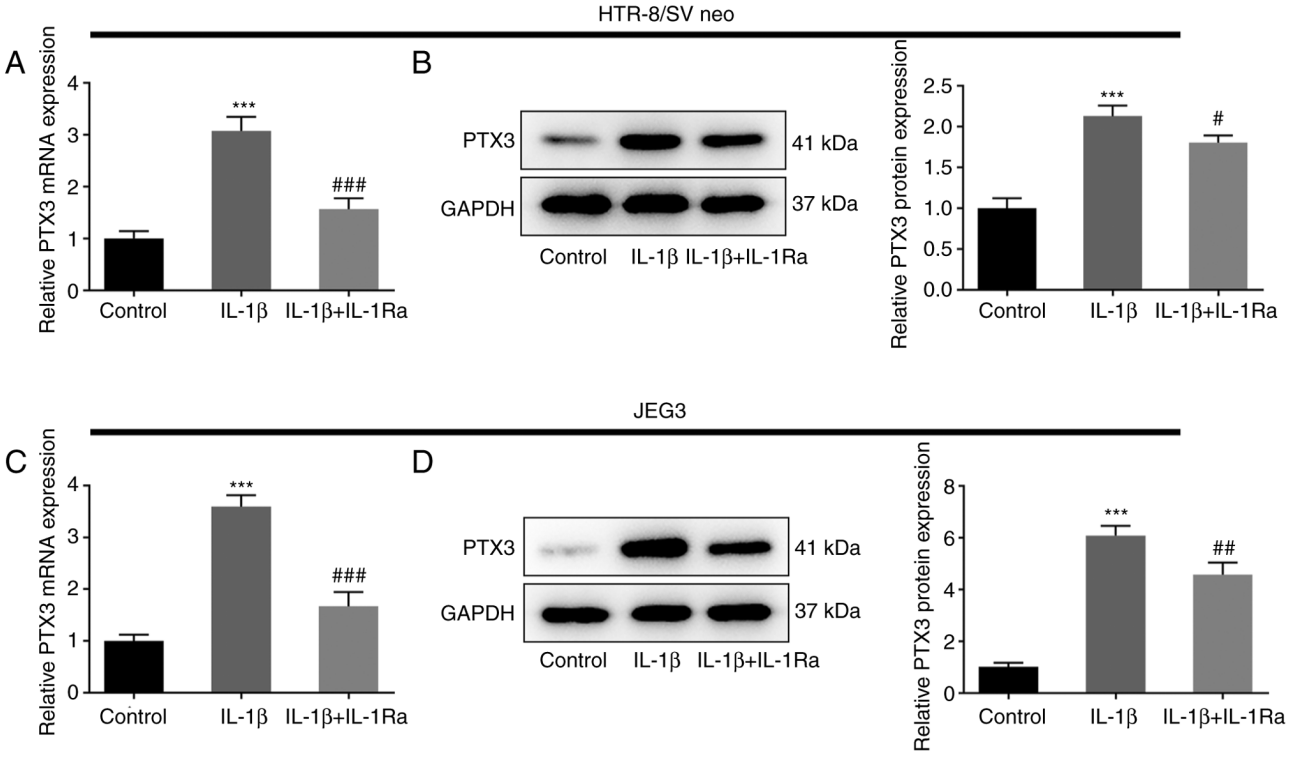

Figure 4. IL-1β induces the expression of PTX3 in HTR-8/SV neo and JEG3 cells, which is suppressed by the IL-1β antagonist. Expression levels of PTX3 in HTR-8/SV neo cells treated with IL-1β and IL-1Ra were evaluated via (A) RT-qPCR and (B) western blot analysis. Expression levels of PTX3 in JEG3 cells treated with IL-1β and IL-1Ra were evaluated via (C) RT-qPCR and (D) western blot analysis. ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. IL-1β. PTX3, pentraxin 3; RT-qPCR, reverse transcription-quantitative PCR; IL-1β, interleukin-1β; IL-1Ra, interleukin-1 receptor antagonist.

IL-1β induces the expression of PTX3 in HTR-8/SV neo and JEG3 cells, which is suppressed by the IL-1β antagonist. IL-1β, an essential inflammatory cytokine involved in the mediation of inflammatory responses, promotes the differentiation and motility of extravillous trophoblasts (27). Thus, the effects of IL-1β on the functions of extravillous trophoblasts were detected following PTX3 overexpression in HTR-8/SV neo and JEG3 cells. Following treatment with IL-1β, the expression of PTX3 was significantly increased in HTR-8/SV neo and JEG3 cells, compared with the control group (P<0.001). However, co-treatment with IL-1β and IL-1Ra attenuated the expression of PTX3, compared with cells treated with IL-1β alone (P<0.05, P<0.01, P<0.001; Fig. 4A-D). Collectively, these results revealed that IL-1β induced the expression of PTX3 in HTR-8/SV neo and JEG3 cells, which was suppressed following treatment with an IL-1β antagonist.

IL-1Ra reverses the inhibitory effects of PTX3 overexpression on proliferation, cell cycle, and invasion of HTR-8/SV neo and JEG3 cells. As the results of the present study demonstrated that IL-1Ra inhibited the promoting effects of PTX3 overexpression in HTR-8/SV neo and JEG3 cells, both cell lines were treated with Ov-PTX3 and IL-1Ra to detect any changes in the activities of cells. Results of the CCK-8 analysis indicated that IL-1Ra treatment increased the levels of proliferation suppressed by Ov-PTX3 (P<0.05; Fig. 5A and B). Moreover, compared with the Ov-PTX3 group, treatment with IL-1Ra in HTR-8/SV neo and JEG3 cells caused a shortened G_0/G_1 phase and prolonged S phase, indicated by the results of flow cytometry (P<0.05, P<0.01, P<0.001; Fig. 5C and D). Furthermore, the levels of invasion in both HTR-8/SV neo (P<0.01; Fig. 6A) and JEG3 (P<0.01; Fig. 6B) cells were increased following treatment with IL-1Ra and Ov-PTX3, compared with the Ov-PTX3 group. Consistently, the mRNA expression of MMP2 and MMP9 in HTR-8/SV neo (P<0.01, P<0.001; Fig. 6C) and JEG3 (P<0.001; Fig. 6D) cells was significantly elevated following treatment with IL-1Ra and Ov-PTX3, compared with the Ov-PTX3 group. The MMP2 and MMP9 protein expression levels exhibited the same trends in HTR-8/SV neo (P<0.01; Fig. 6E) and JEG3 (P<0.001; Fig. 6F) cells following treatment with IL-1Ra and Ov-PTX3, compared with the Ov-PTX3 group. These findings suggested that IL-1Ra reversed the inhibitory effects of PTX3 overexpression on proliferation, cell cycle and invasion of HTR-8/SV neo and JEG3 cells.

Discussion

The results of the present study demonstrated that compared with the control group, the Ov-PTX3 group in both HTR-8/SV neo and JEG3 cells exhibited lower proliferation, invasion and cell cycle promotion of trophoblasts in PE. Results of a previous study demonstrated high levels of PTX3 expression in a number of diseases, including inflammatory diseases. Moreover, high PTX3 plasma levels were observed in mice fed with a high-fat diet, which contributed to the development of obesity (28). High expression levels of PTX3 were also revealed in critically ill patients and those who were infected with sepsis or septic shock, which were associated with high rates of mortality (29,30). Thus, PTX3 has the ability to act as a successful biological marker for

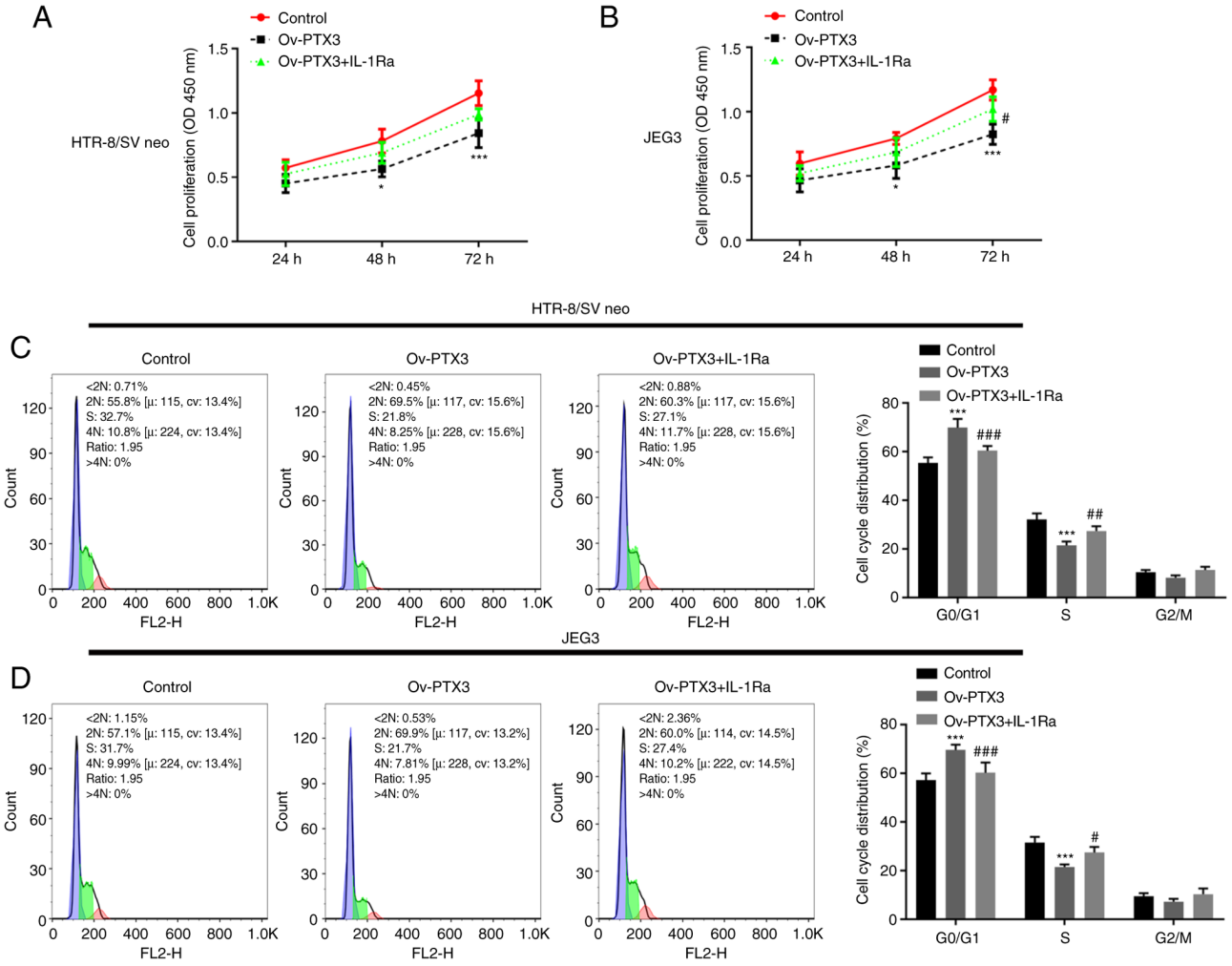

Figure 5. IL-1Ra suppresses the inhibitory effects of PTX3 overexpression on the proliferation and cell cycle of HTR-8/SV neo and JEG3 cells. The proliferation of (A) HTR-8/SV neo and (B) JEG3 cells treated with Ov-PTX3 and IL-1Ra was tested by Cell Counting Kit-8 assay. Cell cycle distributions of (C) HTR-8/SV neo and (D) JEG3 cells treated with Ov-PTX3 and IL-1Ra were assessed by flow cytometry. *P<0.05, ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. Ov-PTX3. IL-1Ra, interleukin-1 receptor antagonist; PTX3, pentraxin 3; Ov, overexpression vector.

the diagnosis of disease mortality and severity, in contrast with other representative markers (29,31).

Although investigations into PE are ongoing, it is classified into two subtypes: Early-onset PE (delivery before 34 weeks of gestation) and late-onset PE (delivery after 34 weeks of gestation) (32). This disease is characterized by inadequate trophoblast invasion in the uterus, poor remodeling of the spiral arteries and redundant trophoblast apoptosis in the placenta (33). A growing body of evidence suggests that MMP2 and MMP9 play a role in endometrial tissue remodeling during the menstrual cycle and pregnancy (34,35). MMP2 and MMP9 are abundantly expressed in invading extravillous trophoblast cells, and the expression of MMP2 and MMP9 is highly related to trophoblast cell invasiveness (36,37). Additionally, it is likely that other MMPs may also be involved in the progression of PE, therefore the expression of additional MMPs will be examined in further studies. Thus, the alteration of MMP2 and MMP9 expression was investigated in the present study; mRNA and protein levels of MMP2 and MMP9 in HTR-8/SV neo and JEG3 cells were suppressed following PTX2 overexpression, highlighting a role of PTX3 in trophoblast migration and invasion in PE development.

PTX3 expression is induced by stimuli, including inflammatory cytokines, Toll-like receptor agonists, distinct microbial moieties and a number of microorganisms (38). Furthermore, the IL-1β pathway plays an essential role in pregnancy (39). The results of a previous study demonstrated that TNF-α and IL-1β are crucial in abnormal extravillous trophoblast invasion in PE (40). It has been reported that plasma level of IL-1β is significantly increased in preeclampsia (41). Luppi and Deloia (42) also demonstrated that the spontaneous intracellular synthesis of IL-1β in monocytes of preeclamptic women was higher than in normal pregnant and non-pregnant women. As PE is an inflammatory disease in which PTX3 is highly expressed, it was hypothesized in the current study that IL-1β played a role in inducing the expression of PTX3 for the promotion of PE development. Thus, IL-1β was used to induce both HTR-8/SV neo and JEG3 cells in the present study. The expression level of PTX3 was significantly increased following IL-1β treatment, which was partially reversed by the further addition of IL-1Ra, suggesting that there may be an incomplete inhibitory effect of IL-1Ra or additional pathways induced by IL-1β may be implicated. This result was consistent with those of a previous study, which highlighted that

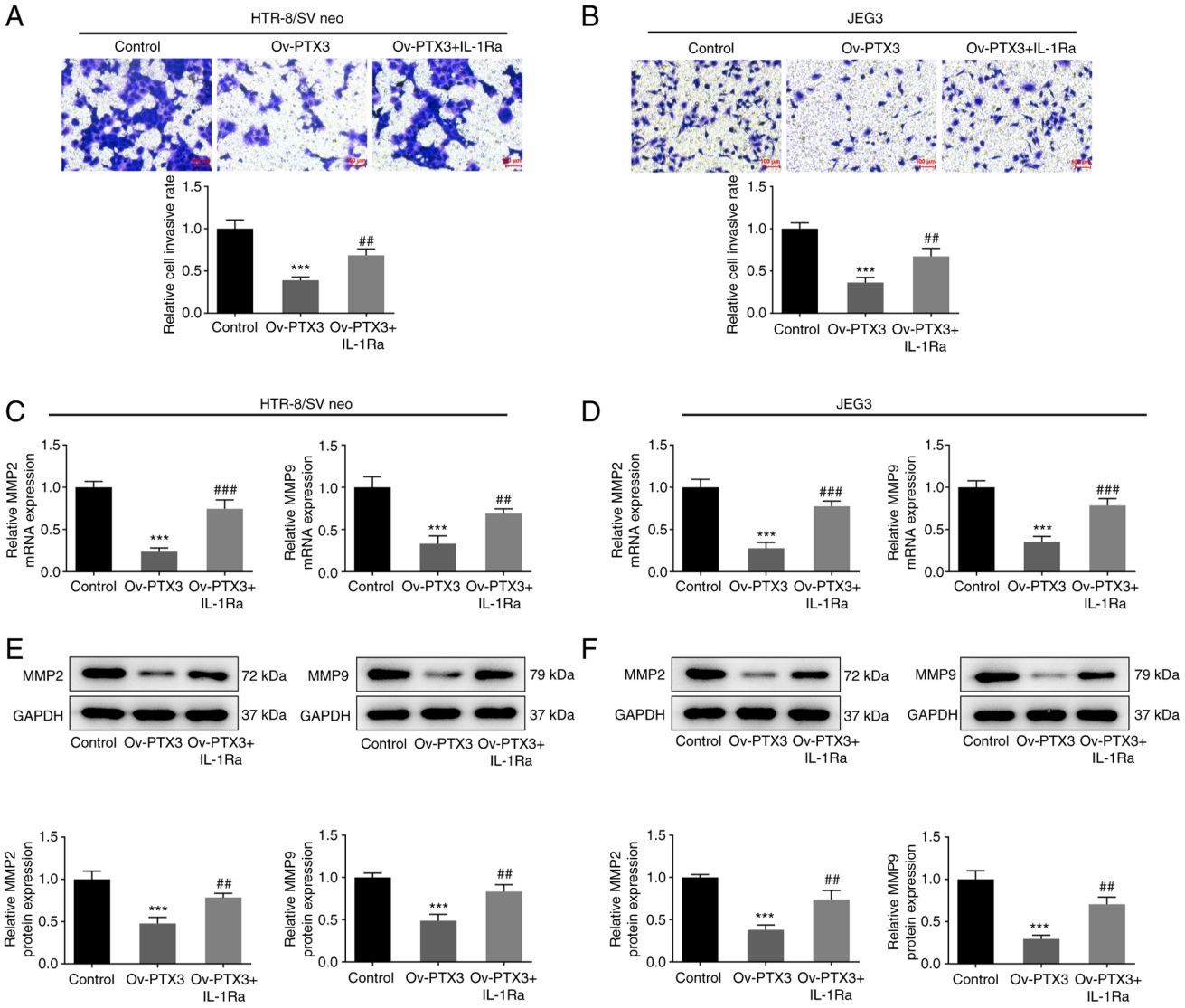

Figure 6. IL-1Ra suppresses the inhibitory effects of PTX3 overexpression on the invasion of HTR-8/SV neo and JEG3 cells. The invasion of (A) HTR-8/SV neo and (B) JEG3 cells treated with Ov-PTX3 and IL-1Ra was assessed by using Transwell invasion assay. The mRNA expression of MMP2 and MMP9 in (C) HTR-8/SV neo and (D) JEG3 cells following treatment with Ov-PTX3 and IL-1Ra was detected via reverse transcription-quantitative PCR. The protein expression of MMP2 and MMP9 in (E) HTR-8/SV neo and (F) JEG3 cells following treatment with Ov-PTX3 and IL-1Ra was determined via western blotting. ***P<0.001 vs. Control; $^{##}$P<0.01, $^{###}$P<0.001 vs. Ov-PTX3. IL-1Ra, interleukin-1 receptor antagonist; PTX3, pentraxin 3; Ov, overexpression vector.

a single cytokine or a single element of the immune system would not account for all normal trophoblastic activities (43). IL-1Ra binds to IL-1R1 with a high affinity and inhibits IL-1α or IL-1β function (44). In the present study, based on the result that IL-1Ra treatment reduced PTX3 level in IL-1β-induced HTR-8/SV neo and JEG3 cells, we speculated that IL-1Ra treatment would also reduce the level of PTX3 in PTX3-overexpressing cells. In the subsequent experiments, both HTR-8/SV neo and JEG3 cells were treated with Ov-PTX3 and IL-1Ra, and cell proliferation was subsequently increased, compared with those treated with Ov-PTX3 alone. Furthermore, Ov-PTX3-induced decreases in invasion and cell cycle promotion were restored following treatment with IL-1Ra. Since IL-1Ra binds to IL-1R1 (common receptor for both IL-1β and IL-1α) (45), it may inhibit the function of IL-1β and IL-1α cytokines. Therefore, in future studies, whether IL-1α also affects PTX3 expression and PE development may require further research.

However, this experiment has some limitations. In this study, the effect of PTX3 overexpression on cell cycle pathways and caspase activity were not explored, which is an aim of our future studies. Besides, lacking the use of additional groups (mutant PTX3, Ov-NC + IL-1Ra or IL-Rα alone treatment groups) and further mechanistic experiments to unravel the pathways mediating the effect of PTX3 on PE development (including proliferation, invasion and cell cycle of trophoblasts) are potential limitations of the present study. Additionally, this study only discussed the effects of PTX3 on PE in HTR-8/SV neo and JEG3 cells by PTX3 overexpression, but had no the data concerning PTX3 silencing or clinical data to show the relationship between PTX3, IL-1β expression and PE development, which is also a potential limitation of this paper.

Collectively, the results of the present study and previous investigations using *in vitro* models revealed that the elevated expression levels of PTX3 induced by IL-1β inhibited the proliferation, invasion and cell cycle of trophoblasts,

thus triggering the progression of PE. Therefore, PTX3 knockout by targeting IL-1β may lead to the prevention of PE development.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XW and JZ designed the study and analyzed the data. JJ performed the experiments. XW and JZ drafted the manuscript and interpreted the data. XW and JJ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

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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