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Original Article

Pregnancy-associated plasma protein A up-regulated by progesterone promotes adhesion and proliferation of trophoblastic cells

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Abstract: Embryo implantation and development is a complex biological process for the establishment of the successful pregnancy. Progesterone is a critical factor in the regulation of embryo adhesion to uterine endometrium and proliferation. Although it has been reported that pregnancy-associated plasma protein A (PAPPA) is increased in pregnant women, the relationship between progesterone and PAPPA, and the effects of PAPPA on embryo adhesion and proliferation are still not clear. The present results showed that the serum level of progesterone and PAPPA was closely correlated by ELISA assay ($p < 0.01$). PAPPA was detected in the villi of early embryo by RT-PCR, Western blot, immunohistochemistry and immunofluorescent staining. Moreover, PAPPA was significantly up-regulated by progesterone in trophoblastic (JAR) cells by Real-time PCR and ELISA assay ($p < 0.01$); while the expression was decreased by the progesterone receptor inhibitor RU486. The down-regulation of PAPPA by siRNA transfection or up-regulation of PAPPA by progesterone treatment significantly decreased or increased the adhesion rate of trophoblastic cells to human uterine epithelial cell lines (RL95-2 and HEC-1A), respectively ($p < 0.01$), as well as the proliferation of trophoblastic cells. In conclusion, PAPPA is up-regulated by progesterone, which promotes the adhesion and proliferation potential of trophoblastic cells.

Keywords: PAPPA, progesterone, adhesion, proliferation, embryo

Introduction

Embryo implantation and development is a complex biological process. The zygote develops into morula, then the early blastocyst, which transfers from the oviduct to the uterine cavity. In the uterine cavity, embryo implantation typically includes blastocyst apposition, adhesion to the uterine epithelium and penetration into the stroma of the endometrium [1]. The molecular recognition and interaction between the embryonic cells and the uterine epithelial cells play vital roles during embryo implantation. Trophoblasts play crucial roles in embryo implantation [2]. As the outermost layer of blastocyst, trophoblasts proliferate, develop and evolve to the different grades of villus. The attachment and proliferation of the embryo is the symbol of maturated embryo and pivotal to establish and maintain the successful pregnancy in mammals.

During embryo implantation and development, embryo has not only the morphological alteration, but also expresses and secretes a variety of molecules which are involved in the process of attachment and proliferation of embryonic cells, such as hormones, cytokines and growth factors and its receptors, etc [3, 4]. Among these factors, progesterone secreted mainly by ovary and placenta, is a necessary one in the stimulation of embryo implantation and development. During implantation window period, progesterone can maintain the receptivity of endometrium and allows the matured blastocysts be adhered on to it [5-7]. After embryo implantation, the endometrium is changed into decidua [8]. In addition, progesterone promotes

embryo development by increasing cell growth. P. Bowman et al. reported that progesterone elevated the cleavage of blastocysts in mice [9, 10]. Besides, increases of progesterone advanced embryonic cell proliferation in sheep [11]. During the preceding of pregnancy, progesterone level in serum has been gradually increasing [12]. Progesterone is required for successful embryo implantation [13]. In the first-trimester of pregnancy, the low serum level of progesterone in the pregnant woman may lead to the risk of abortion. Therefore, it is necessary to supply with the progesterone until the serum level of progesterone reaches to the normal level. Progesterone regulates the expression of many target molecules in embryo implantation and development [14, 15]. However, the relationship between progesterone and pregnancy-associated plasma protein A (PAPPA) has not been examined.

PAPPA was first identified in the serum from pregnant women in 1974, and mainly secreted by placenta [16]. Now it has been found that PAPPA is expressed in many other tissues [17-19]. PAPPA could be detected in serum after the fifth week of pregnancy. It is significantly increased during the first trimester of pregnancy, and continuously elevates until the end of pregnancy. It showed that low maternal serum level of PAPPA was associated with fetal Down's syndrome (trisomy 21 and trisomy 18) in the first trimester of pregnancy [20]. In early pregnancy, it has been reported that higher serum levels of PAPPA predicted a better fetal growth [21, 22]. In contrast, lower serum level of PAPPA indicates a high risk of delivery of a small-for-gestational age (SGA) infant [23, 24].

The mechanism related to progesterone regulation and the functions of PAPPA in embryo implantation and development is not clear. Here, we identified that the PAPPA level was closely correlated with the progesterone level in serum. PAPPA was highly expressed in the villi and trophoblastic cells. PAPPA was up-regulated by progesterone, which promoted the embryo adhesion *in vitro* implantation model and proliferation of trophoblastic cells.

Materials and methods

Cell culture

The human trophoblastic (JAR) cell line was obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's modified Eagle's medium/Ham's nutrient mixture F12 medium (DMEM/F12; Hyclone, USA) supplemented with 10% fetal bovine serum (PAA, Austria) and 100 U/ml penicillin and 50 μg/μl streptomycin at 37°C under 5% CO_2 in cell incubator.

The human uterine epithelial cell lines, RL95-2 cells were grown in DMEM/F12 supplemented with 10% FBS, 5 μg/ml insulin, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C under 5% CO_2 in cell incubator; HEC-1A cells were grown in McCoy's 5A (Hyclone, USA) supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C under 5% CO_2 in cell incubator. The growth medium was renewed every 2-3 days.

Serum and tissue samples

The protocols for human study were in accordance with the Institutional Review Board of Dalian Medical University. Samples were obtained from Yingkou Central Hospital and the Secondary Affiliated Hospital of Dalian Medical University from 2012 to 2013. Women serum samples used in this study were obtained from the women at the ages of 25 to 35. The non-pregnant control group (n=28) was excluded from other gynecological abnormalities. The pregnant women were confirmed by ultrasound detection at 10 to 12 gestational weeks. The serum samples collected from normal pregnant group (n=31) and threatened abortion group (n=29) were used to analyze progesterone and PAPPA level. These paraffin-embedded human villi and decidua tissue blocks were used for PAPPA and keratin 7 (KRT-7) immunohistochemical staining. The fresh human villi and decidua tissues were collected from the non-drug abortion women.

ELISA assay

Commercial enzyme-linked immunosorbent assay (ELISA) kits (Westang Biotech Company, China) were used to detect the serological values of progesterone and PAPPA according to manufacturer's instructions. The absorbance was measured at 450 nm using an ELISA microplate reader (Thermo Fisher Scientific, USA). Three samples were tested in each group for each time.

Transient transfection

Cells were seeded onto six-well plates. When cells reached 70% confluence, PAPPA siRNA

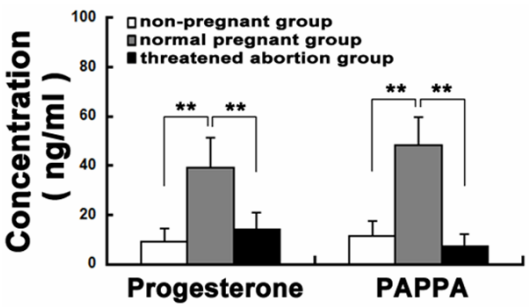

Figure 1. Serum level of PAPPA positively correlates with progesterone. ELISA was used to analyze the level of progesterone and PAPPA. Non-pregnant: serum from control women; normal pregnant: serum from normal pregnant women; threatened abortion: serum from women with threatened abortion. **, $p < 0.01$.

was transiently transfected into the cells with Lipofectamine™ reagent (Life Technologies, USA), following the manufacturer's instructions. The transfection was terminated after 5 h. RNA and protein was harvested after 48 h or 72 h for Real-time PCR or ELISA detection. Each experiment was repeated three times. PAPPA siRNA sequences were as follows: PAPPA siRNA-1 (5'-GUG CCC UGA AUC ACA ACU ATT-3', and 5'-UAG UUG UGA UUC AGG GCA CTT-3'); PAPPA siRNA-2 (5'-GAG GCC UUC AAG CAA UAC ATT-3', and 5'-UGU AUU GCU UGA AGG CCU CTT-3'); PAPPA siRNA-3 (5'-GCG ACG ACA UGA AUA AGA UTT-3', and 5'-AUC UUA UUC AUG UCG UCG CTT-3'). The scrambled control sequences were 5'-UUC UUC GAA CGU GCU ACG UTT-3', and 5'-ACG UGA CAC GUU CGG AGA ATT-3'.

RT-PCR and Real-time PCR

Villi and decidua tissues were treated with RNAiso Plus reagent (TaKara, Japan) for RNA extraction. Takara RNA PCR Kit (TaKara, Japan) was used for obtaining the cDNA. The primers of PAPPA were 5'-ATA TCT CAC GTG ACC GAG GA-3' (forward), and 5'-AGC TGA TGG TGC TGG AAG TC-3' (reverse). The product length is 702 bp. The primers of GAPDH were 5'-TCC TGT TCG ACA GTC AGC CGC AT-3' (forward), and 5'-TGC AAA TGA GCC CCA GCC TTC TCC A-3' (reverse). The product length is 402 bp. PCR reactions were performed as follows: pre-denaturation at 95°C for 5 min, 30 cycles of 95°C for 45 s, 57°C for 45 s, 72°C for 1 min and a final extension for 10 min at 72°C in 25 μl reaction mixture. Agarose gel electrophoresis was used to check the PCR product.

Trophoblastic cells were cultured in six-well plates and treated with progesterone (10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L), RU486 (10^{-5} mol/L), combination RU486 with progesterone (10^{-5} mol/L), or PAPPA siRNAs, respectively, for 48 h. After treatment or transient transfection, RNA extraction was performed as mentioned before, and PrimeScript™ RT reagent Kit with gDNA Eraser kit (Takara, Japan) was used for synthesizing cDNA. SYBR® Premix Ex Taq™ (Takara, Japan) was used for quantitative Real-time PCR. Primers for Real-time PCR were as follows: PAPPA: 5'-CCA ACG CTT GAC ACA AAG TCC-3' (forward), and 5'-CCT TGA AAT ATC AAA CAA GCA CTC C-3' (reverse). GAPDH: 5'-GCA CCG TCA AGG CTG AGA AC-3' (forward), and 5'-TGG TGA AGA CGC CAG TGG A-3' (reverse). The reactions were performed with Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies, USA).

Western blot

Villi and decidua tissues were washed in phosphate-buffered saline (PBS) for three times before incubation with lysis buffer (PBS with 1% NP-40 and 1 mmol/L PMSF) at 4°C for 2 h. The tissue lysates were clarified by centrifugation at 8000 ×g for 10 min, and boiled with 5× loading buffer (250 mmol/L Tris-HCl pH 6.8, 10% SDS, 50% glycerol, 5% β-mercaptoethanol) for 10 min. Total protein was quantified with the Coomassie Protein Assay Reagent (Bio-Rad, USA) using bovine serum albumin (BSA) as a standard. Total proteins (30 μg) were separated by 12% SDS-PAGE. After electrophoresis, the proteins were transferred to a nitrocellulose membrane and blocked with 5% fat-free milk powder diluted in Tris-buffered saline with 0.05% Tween 20 (TTBS) for 2 h, and incubated with rabbit anti-human PAPPA antibody (1:200, Abcam, USA) in TTBS overnight at 4°C. After washing three times the next day, membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (1:1000, Santa Cruz. USA) for 45 min at room temperature, and immunoreactive proteins were visualized with enhanced chemiluminescence (ECL) detection system (Amersham Biosciences, Piscataway, NJ).

Immunohistochemical and immunofluorescent staining

Tissue slides were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 15 min. Slides were blocked with 10% normal goat serum for 10 min, and incubated with anti-

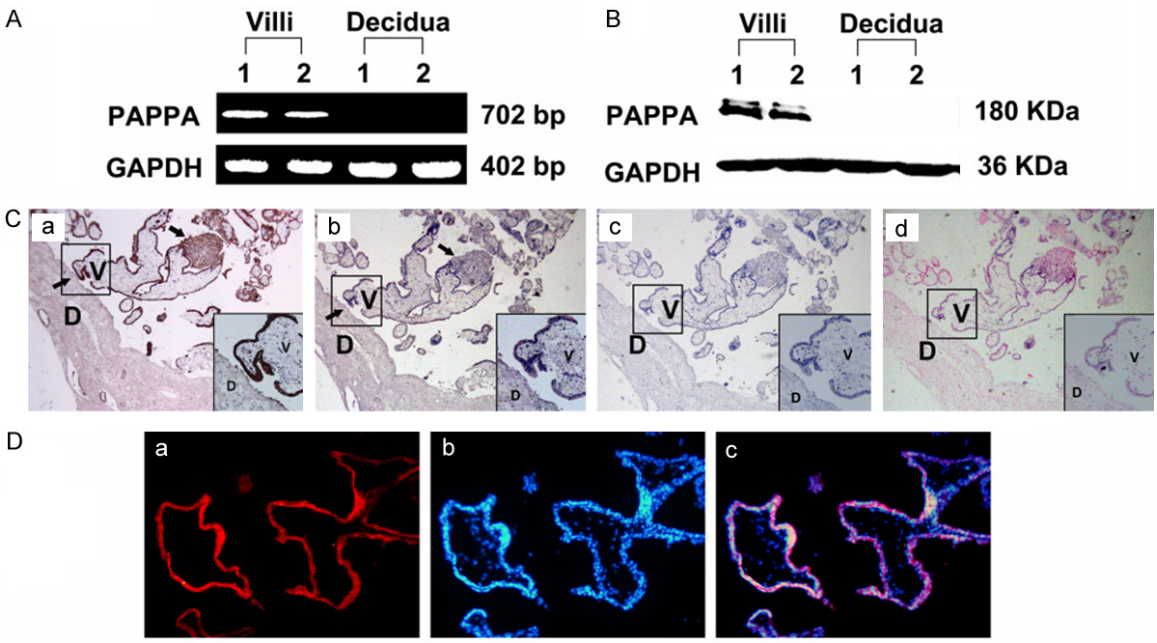

Figure 2. Detection of PAPPA expression in human villi and decidua tissues. A. RT-PCR was used to analyze PAPPA expression identified on 1% agarose gel in human villi (Lane 1, 2) and decidua tissue (Lane 3, 4). GAPDH was an internal control. B. PAPPA expression in human villi (Lane 1, 2) and decidua (Lane 3, 4) tissue was detected by Western blot. C. Immunohistochemistry was performed for KRT-7 and PAPPA expression in human villi and decidua tissue (40×). a. KRT-7. b. PAPPA. c. Negative control. d. HE staining. D: decidua tissues; V: villi tissues. D. PAPPA expression in villi tissue was detected by immunofluorescence (100×). a. PAPPA (red). b. DAPI (blue). c. Merged.

human PAPPA antibody (1:50, Santa Cruz. USA) and anti-human keratin 7 antibody (1:50, Protein Tech, USA) at 4°C for 12 h. The slides were incubated with biotinylated secondary antibody at 37°C for 15 min, and reacted with a streptavidin-peroxidase conjugate at 37°C for 10 min, then used 3, 3'-diaminobenzidine as a chromogen substrate. Meyer's hematoxylin was used as a counterstained dye. A negative control was obtained by replacing the primary antibody with PBS. Images were captured with the Olympus fluorescence microscope (Olympus, Japan).

Frozen sections were fixed with 4% paraformaldehyde for 48 h at room temperature, and then treated with 20% sucrose for 48 h. Tissues were embedded with OCT and sliced to 18 μm at -20°C. After blocking with goat serum for 2 h at room temperature, the primary anti-human PAPPA antibody (1:50) was incubated at 4°C for 12 h. The next day, after 1 h incubation with phycoerythrin (PE)-conjugated anti-goat IgG (1:200, Santa Cruz. USA) at 37°C, slides was washed 3 times for 5 min with PBS, and then treated with DAPI (Santa Cruz. USA) for 5 min at 37°C. PAPPA immunofluorescent staining was photographed with the inverted microscope (Olympus, Japan).

Cell adhesion assay

RL95-2 or HEC-1A cells were grown on 96-well plates to form a confluent monolayer. Trophoblastic cells of differently treated were stained with CellTracker™ Green CMFDA (Life Technologies, USA) 1 h before adhesion assay. The cells were gently seeded onto RL95-2 or HEC-1A cell monolayers in trophoblastic culture medium. After 1 h, unbound trophoblastic cells were removed by washing with PBS. The attached cells were detected by multimode plate reader (PerkinElmer, USA) and photographed on a fluorescent phase microscope (Olympus, Japan) .

Cell proliferation assay

3×10^3 cells/well were cultured in 96-well plates. After cultured for 1, 2, 3, 4 and 5 days, the supernatant solution was removed and each well was washed with PBS, then added 100 μl DMEM/F12 medium without FBS and 10 μl of Cell Counting Kit-8 (CCK-8, Beyotime Institute of Biotechnology, China) solution. After incubat-

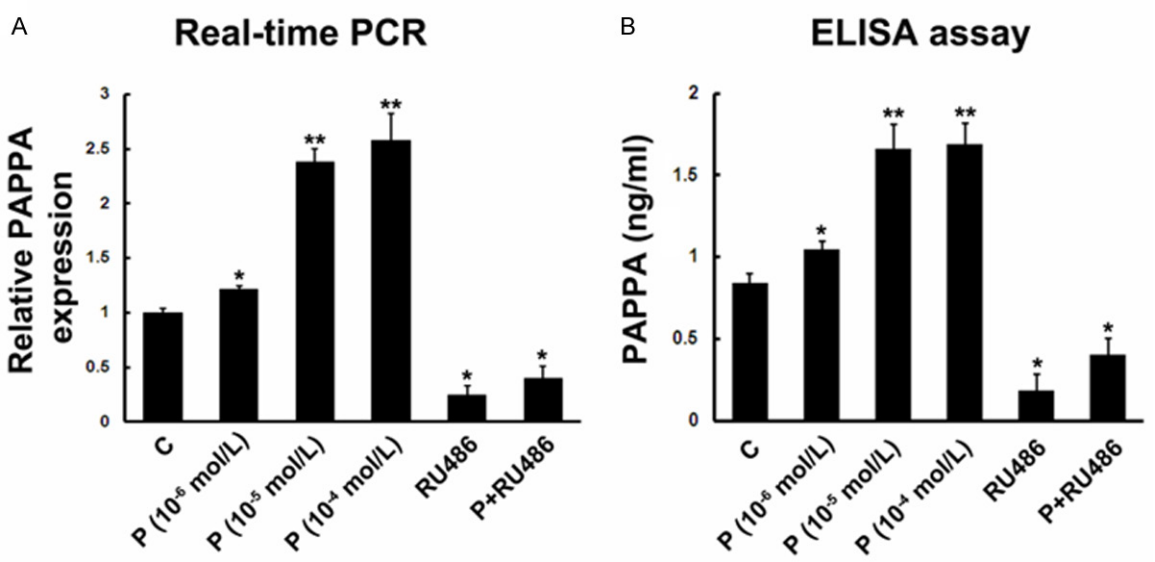

Figure 3. Progesterone stimulates PAPPA expression in trophoblastic cells. Cells were treated with progesterone at the concentration of 10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L, RU486 (10^{-5} mol/L), and combined progesterone (10^{-5} mol/L) with RU486 (10^{-5} mol/L) for 48 h. The mRNA and protein was prepared from the cells after treatment. A. PAPPA gene expression was analyzed by Real-time PCR. B. PAPPA expression in protein level was detected by ELISA. *, $p < 0.05$. **, $p < 0.01$.

ed for 1 h in the incubator, the absorbance was measured at 450 nm using an ELISA microplate reader. Three samples were tested in each group for each incubation time.

Cell cultured as mentioned above, and each well was washed with PBS. Then, 0.25% Trypsin was added, and collected the cells after 3 min. Cell counting was performed after trypan blue staining.

Statistical analysis

All experiments were performed at least 3 times, and statistical analyses were carried out using the SPSS statistical software 17.0. One-way analysis of variance (ANOVA) was used to compare multiple groups. $p < 0.05$ were considered statistically significant.

Results

Serum progesterone and PAPPA level correlated in pregnant women

The relationship between progesterone and PAPPA was analyzed by ELISA in serum samples from non-pregnant control, normal pregnant group and the threatened abortion group, respectively. The level of progesterone and PAPPA in each group was shown (**Figure 1**). We found that the progesterone level was significantly elevated in normal pregnant group compared with the non-pregnant control ($p < 0.01$); while progesterone level in the threatened abortion group significantly decreased compared with the normal pregnant group ($p < 0.01$). Meanwhile, the detection of PAPPA level in serum showed the similar changes as that of the progesterone. The results indicated that PAPPA was closely related with that of progesterone in serum.

Expression of PAPPA in villi and decidua tissues in pregnant women

The expression of PAPPA was detected in villi and decidua tissues by RT-PCR and Western blot. We found that the PAPPA gene and protein expression was high in villi tissue while not detectable in decidua tissue (**Figure 2A** and **2B**). Paraffin sections containing both villi and decidua tissues were used to analyze the expression of PAPPA by immunohistochemical staining. KRT-7 was a molecule marker for villi tissue. We found that PAPPA was observed in villi tissue, but not in decidua tissue (**Figure 2C**). Immunofluorescence was performed to further determine PAPPA expression in villi tis-

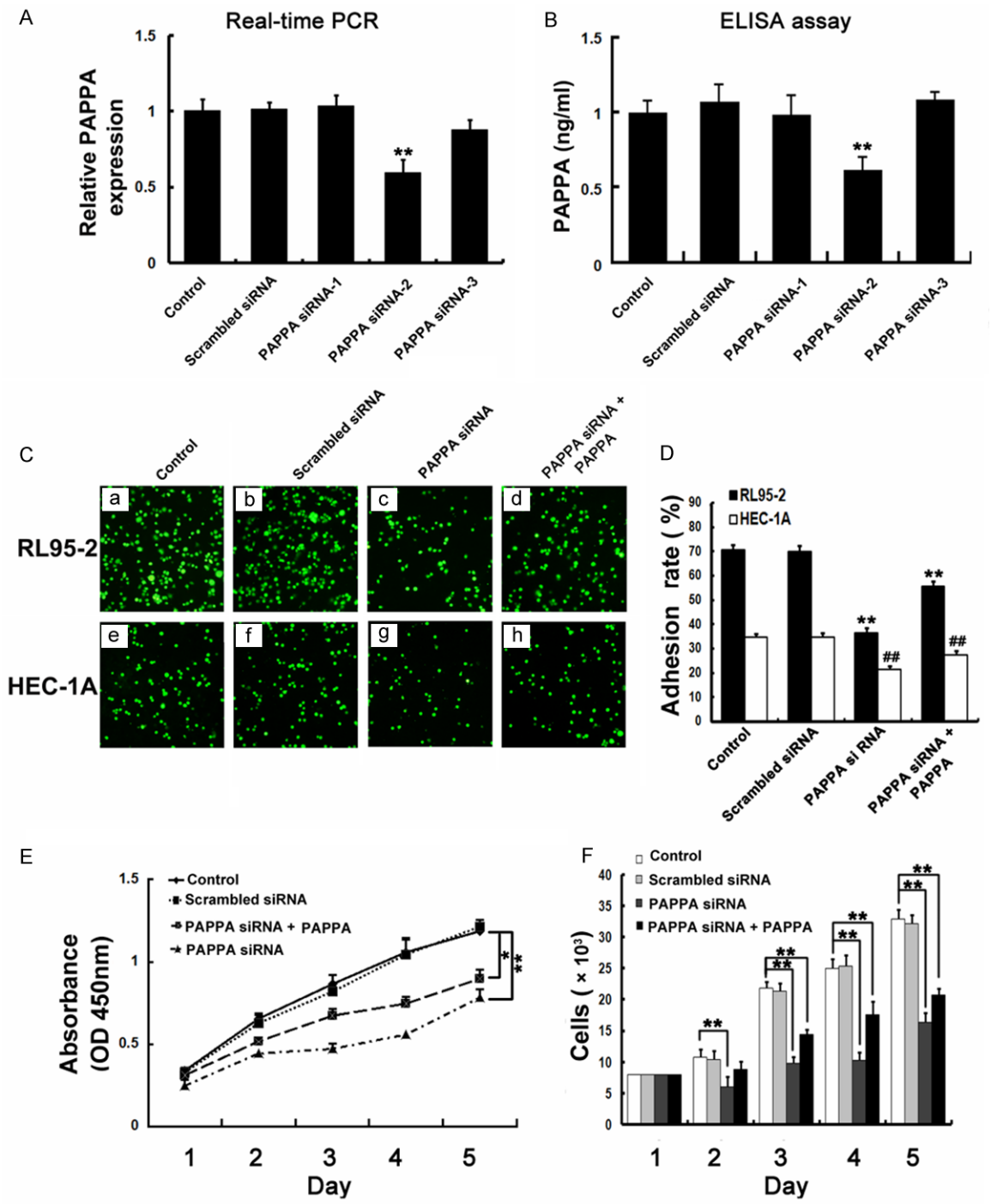

Figure 4. Down-regulated PAPPA expression decreases adhesion of trophoblastic cells to uterine epithelial RL95-2 and HEC-1A cell monolayer and cell proliferation. Trophoblastic cells were treated with PAPPA siRNA-1, siRNA-2, siRNA-3, scrambled siRNA for 48 h. (A) PAPPA mRNA level was measured by Real-time PCR. (B) ELISA assay of PAPPA protein level. (C) Adhesion assay of trophoblastic cells. Cells were stained with CellTracker™ Green CMFDA 1 h before adhesion assay and photoed with fluorescence microscope. Untreated trophoblastic cells on RL95-2 (a) and HEC-1A (e) cell monolayer. Trophoblastic cells treated with scrambled siRNA on RL95-2 (b) and HEC-1A (f) cell monolayers. Trophoblastic cells treated with PAPPA siRNA-2 on RL95-2 (c) and HEC-1A (g) cell monolayer. Trophoblastic cells treated with PAPPA siRNA-2 and PAPPA (100 ng/ml) on RL95-2 (d) and HEC-1A (h) cell monolayers. (D) Adhesion rate was measured using multimode plate reader. (E) CCK-8 assay. The absorbance was measured at 450 nm. (F) Cell counting assay. *, $p < 0.05$; **, $p < 0.01$; for RL95-2. #, $p < 0.05$; ##, $p < 0.01$; for HEC-1A.

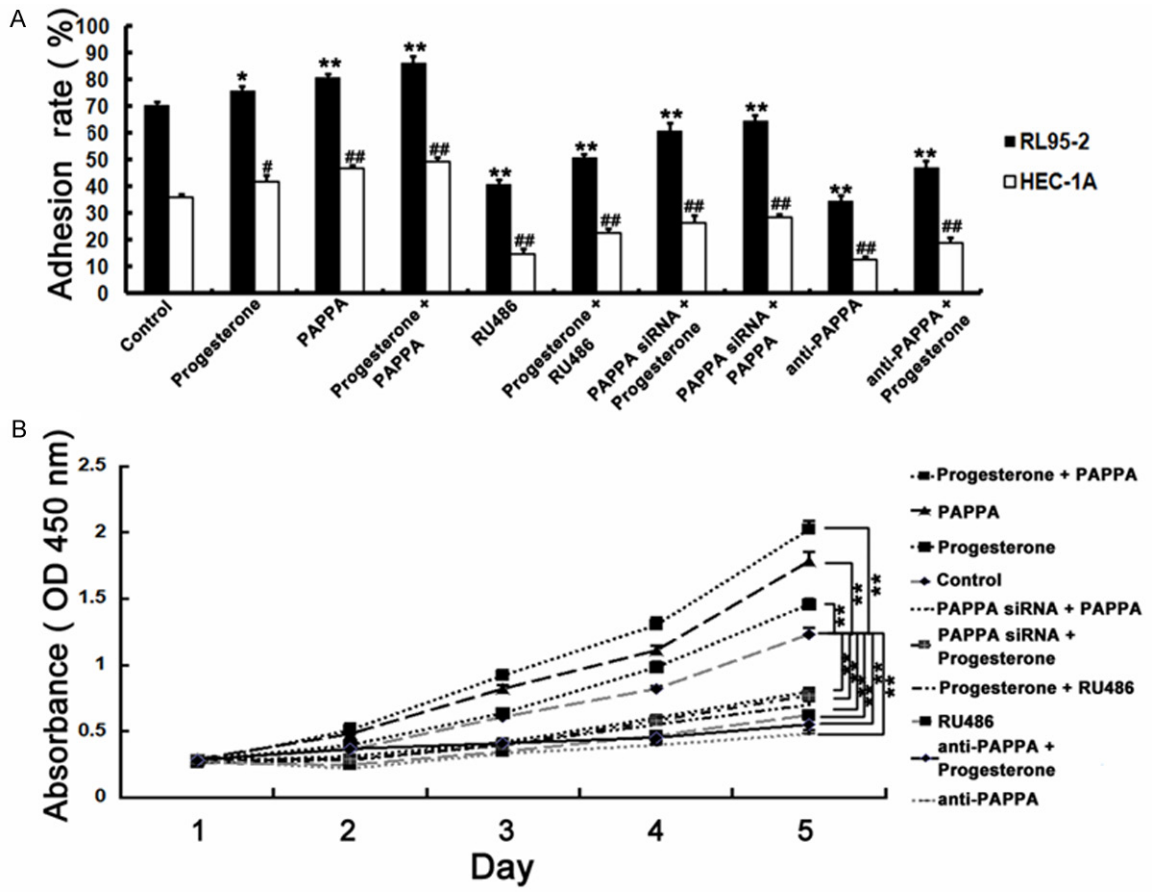

Figure 5. Progesterone promotes trophoblastic cell adhesion and proliferation via up-regulating PAPPA expression. Trophoblastic cells were treated with progesterone (10^{-5} mol/L), PAPPA (100 ng/ml), combined progesterone with PAPPA, RU486 (10^{-5} mol/L), combined PAPPA siRNA with progesterone, combined PAPPA siRNA with PAPPA, anti-PAPPA antibody (2 μg/ml), and combined anti-PAPPA antibody with progesterone for 48 h. A. Trophoblastic cells adhesion rate assay was detected after treatment by multimode plate reader. B. Trophoblastic cell proliferation was assessed with CCK-8 assay after treatment mentioned above. The absorbance was measured at 450 nm. *, $p < 0.05$; **, $p < 0.01$; for RL95-2. #, $p < 0.05$; ##, $p < 0.01$; for HEC-1A.

sue. The nucleus was shown after DAPI staining (blue). The results showed that PAPPA protein labeled with PE (red) was only expressed in villi tissue as compared to decidua tissue (**Figure 2D**).

Progesterone up-regulated the expression of PAPPA in trophoblastic cells

The regulatory effect of progesterone and RU486 on PAPPA was analyzed in trophoblastic cells. As shown in **Figure 3**, the results showed that the gene and protein level of PAPPA was elevated compared to untreated cells by Real-time PCR (**Figure 3A**) and ELISA (**Figure 3B**) after progesterone treatment in different concentrations. The highest expression of PAPPA was present in 10^{-4} mol/L of progesterone ($p < 0.01$). In contrast, lower PAPPA expression was found in RU486 treated cells; while it was recovered by the addition of progesterone. The results showed that progesterone increased PAPPA expression.

Down-regulated PAPPA decreased the adhesion and proliferation of trophoblastic cells

PAPPA siRNAs were used to down-regulate the gene expression of PAPPA. It was showed that PAPPA siRNA-2, but not siRNA-1 and siRNA-3, had the significant inhibitory effect on PAPPA expression, as compared to negative control and scrambled siRNA in gene and protein level (**Figure 4A** and **4B**) ($p < 0.01$).

In vitro implantation model constituting of trophoblastic cells and uterine epithelial RL95-2 cells and HEC-1A cells was used to analyze the role of PAPPA in cell adhesion. The trophoblastic cells separately treated with the scrambled siRNA, PAPPA siRNA-2, or combined PAPPA siRNA-2 and PAPPA. The adhered trophoblastic cells were observed after fluorescent staining and the adhesion rate was analyzed (**Figure 4C** and **4D**). It was showed that PAPPA siRNA markedly decreased the adhesion rate of trophoblastic cells to RL95-2 cells (36.13% ± 2.11%, $p < 0.01$) and HEC-1A cells (21.37% ± 1.23%, $p < 0.01$), compared to the control and scrambled siRNA treatment separately (**Figure 4C** and **4D**). However, when cells were treated with PAPPA , the rate of adhesion was recovered ($p < 0.01$).

CCK-8 and cell counting assay were performed to test the effect of PAPPA on cell proliferation. As shown in **Figure 4E** and **4F**, down-regulated PAPPA expression by PAPPA siRNA-2 inhibited trophoblastic cells growth compared to the control and scrambled siRNA; while cell proliferation increased after PAPPA treatment ($p < 0.05$). The results of cell counting were similar with that of CCK-8 assay ($p < 0.05$).

Progesterone promoted trophoblastic cell adhesion and proliferation via up-regulated PAPPA expression

As shown in **Figure 5A**, compared to the control group, progesterone increased the adhesion rate of trophoblastic cells to HEC-1A and RL95-2 cells, while RU486, the inhibitor of progesterone receptor, decreased the adhesion rate of trophoblastic cells ($p < 0.01$). In the cells treated with combined progesterone and RU486, the adhesion rate was recovered. After PAPPA treatment, the adhesion rate was significantly increased; while the rate was decreased after down-regulated PAPPA (**Figure 4C** and **4D**). After combined PAPPA siRNA with PAPPA or progesterone treatment, the adhesion rate was recovered. When trophoblastic cells was pre-incubated with anti-human PAPPA antibody, the adhesion rate was significantly decreased compared to the control group; while progesterone revived the rate ($p < 0.01$). The highest adhesion rate appeared when combined PAPPA with progesterone. The results showed that PAPPA or progesterone promoted trophoblastic cell adhesion while down-regulated PAPPA expression or inhibited effect of progesterone suppressed adhesion. The trend of cell proliferation was similar with that of cell adhesion ($p < 0.05$) (**Figure 5B**).

Discussion

The specific stages of embryo implantation and development process are under a precise control. The expression and secretion of many molecules play important roles in maintaining the embryo's functional state. In this study, we found that PAPPA level is correlated with progesterone level in serum. It was separately reported that progesterone and PAPPA level increased in pregnancy, indicating that there might be the association with them. Al-Sebai MA et al. reported that low serum level of progesterone resulted in early pregnancy failure, and progesterone is considered as a biomarker of threatened abortion in clinical diagnosis [25, 26]. Meanwhile, low PAPPA level in serum linked to preeclampsia, mall for gestational age at delivery and threatened abortion [27-29]. Here, we detected the PAPPA and progesterone level in non-pregnant control, pregnant and threatened abortion group by ELISA assay. We found that the serum levels of PAPPA and progesterone were both high in pregnant group; while those in non-pregnant control and threatened abortion group were low (**Figure 1**), which provided the direct evidence of relationship between PAPPA and progesterone. Therefore, PAPPA, together with progesterone, can be seen as a novel marker of threatened abortion, as well as an evaluation item of normal developed and matured embryo.

Progesterone regulates many down-stream gene expressions through the receptor mediated way, such as Bmp2, Wnts and ILs, et al, which are involved in embryo implantation and development [30-32]. In addition, PAPPA could be regulated by many factors. For instance, PAPPA expression was increased by TNF-α, TGF-β and IL-1β stimulation in human coronary artery smooth muscle or osteoblastic cells [33 34]. We found PAPPA level was correlated with progesterone level in serum in **Figure 1**. However, it is unknown whether PAPPA can be regulated by progesterone directly. Trophoblastic cells were used to detect the regulatory effect of progesterone on PAPPA. After progesterone treatment, the PAPPA expression was increased in both gene and protein level of trophoblastic cells; while the PAPPA expression decreased after RU486 treatment, the inhibitor of progesterone receptor. However, progester-

one recovered the PAPPA expression by RU486 inhibition (**Figure 3**). The data indicated that PAPPA was up-regulated by progesterone.

PAPPA was secreted by placenta [35]. In **Figure 2**, it showed that PAPPA was expressed only in villi tissue that could develop into placenta in late pregnancy. PAPPA could be seen as an autocrine factor in embryo implantation and development. Although it was reported that the PAPPA level was increased in the pregnancy and abnormal level of PAPPA had relationship with abortion or other pathological processes. However, the direct effect of PAPPA on trophoblastic cells has not been reported. It has been showed that PAPPA promoted the growth of lung cancer and ovarian cancer *in vivo* [36, 37]. In addition, Sahraravand et al reported that PAPPA expression was closely related to the size of the placenta during early pregnancy [35]. As shown in **Figures 4** and **5**, down-regulation of PAPPA by siRNA or anti-PAPPA antibody treatment inhibited cell growth; while supplement with PAPPA enhanced proliferation. PAPPA expression was decreased by RU486, thus RU486 inhibited the cell proliferation. Adhesion associated factors can mediate cell recognition and adhesion in maternal-fetal interface, which are the molecular basis in successful embryo implantation. Embryo expresses and secretes multiple factors to promote its attachment to endometrium. For instance, IL-1α and IL-1β secreted by embryo during implantation process, promoted embryo adhesion [38]. The adhesive effect of PAPPA has not been reported. In **Figures 4** and **5**, decreases of PAPPA reduced the trophoblastic cell adhesion; while addition of PAPPA raised the adhesion of trophoblastic cells. Similar with cell proliferation, RU486 played inhibitory effect on cell adhesion. Moreover, progesterone resisted the effect of depressed PAPPA on decreasing trophoblastic cell adhesion. We found that PAPPA facilitated embryo adhesion and proliferation for the first time.

It is meaningful to find the specific markers to evaluate the development and maturation of embryo, exceptionally in serum assay. This study revealed that PAPPA worked as a regulator in embryo implantation and development. Besides, PAPPA up-regulated by progesterone promoted trophoblastic cell adhesion and proliferation. It provides experimental evidence that PAPPA can be considered as a new diagnostic marker to evaluate the quality of embryo.

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Disclosure of conflict of interest

None.

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