Cyclophilin A plays an important role in embryo implantation through activating Stat3

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Abstract

Embryo implantation is a crucial step for the successful establishment of mammalian pregnancy. Cyclophilin A (CYPA) is a ubiquitously expressed intracellular protein and is secreted in response to inflammatory stimuli to regulate diverse cellular functions. However, there are currently no reports about the role of CYPA in embryo implantation. Here, we examine the expression pattern of CYPA during mouse early pregnancy and explore the potential role of CYPA during implantation. CYPA is expressed in the subluminal stroma surrounding the implanting blastocyst on day 5 of pregnancy, but not at inter-implantation sites. In ovariectomized mice, estrogen and progesterone significantly stimulate CYPA expression. When pregnant mice are injected intraperitoneally with CYPA inhibitor, the numbers of implantation sites are significantly reduced. Using an in vitro stromal cell culture system, Ppia siRNA knockdown of CYPA and CYPA-specific inhibitor treatment partially inhibits levels of CD147, MMP3 and MMP9. Decreased CYPA expression also significantly inhibits Stat3 activity and expands estrogen responsiveness. Taken together, CYPA may play an important role during mouse embryo implantation.

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Introduction

Successful implantation is a critical step for good pregnant outcome, and it requires synchronization between an implantation-competent blastocyst and a receptive uterine endometrium. In humans, about 75% of failed pregnancies are considered to be due to implantation failure (Norwitz et al. 2001), and two-thirds of implantation failure is attributed to the defective uterine receptivity (Achache & Revel 2006). In human-assisted reproduction, the in vitro fertilization (IVF) success rate is strongly dependent on uterine endometrium status. During the implantation period, the uterus undergoes morphological and dynamic molecular changes to allow for uterine components and embryo implantation. These changes are tightly regulated by ovarian hormones together with locally produced signaling molecules. Using transgenic mouse models and in vitro stromal cell lines, numerous signaling molecules and pathways as key regulators in embryo implantation have been identified (Zhang et al. 2013). Activation of transcription factor STAT3 in response to cytokines and growth factors is critical for uterine receptivity, whose conditional knockout in mouse uterus results in implantation failure (Sun et al. 2013). Matrix metalloproteinases (MMPs), a family of zinc-dependent proteases, whose activity in the mouse uterus is critical for degrading extracellular matrix (ECM) and promoting trophoblast invasion (Alexander et al. 1996). Still, the potential gene regulatory network during the embryo implantation remains largely unexplored.

Cyclophilin A (CYPA), the Ppia gene product, is a ubiquitously expressed protein in eukaryotic cells. CYPA was first identified as a major intracellular receptor of the immunosuppressive drug cyclosporin A (CsA) (Handschumacher et al. 1984). It has peptidyl prolyl isomerase activity which catalyzes cis-trans isomerization of peptidyl–prolyl bonds (Fischer et al. 1989) and regulates many biological functions, including protein folding and trafficking and T-cell activation (Nigro et al. 2013). Besides its intracellular role, CYPA can be secreted from cells in response to inflammatory stimuli such as hypoxia, infection, and oxidative stress. Secreted CYPA (eCYPA) can mediate intercellular communication to act as an autocrine/paracrine factor (Sherry et al. 1992, Jin et al. 2000). Embryo implantation
can be considered an inflammatory process due to the numerous leukocytes that infiltrate the uterus (Dekel et al. 2014). During the peri-implantation period, mouse endometrial cells widely proliferate, and leads to insufficient oxygen content in the uterus (Daikoku et al. 2003). These studies suggest that the uterus is exposed to a microenvironment of hypoxia and inflammation during implantation. Therefore, we speculate CYPA is in a secreted form in mice uterus during implantation. The autocrine/paracrine signal is transduced by a cell surface receptor-CD147. CYPA/CD147 signal can regulate cell proliferation, apoptosis, migration and chemotaxis in many cell types (Zhao et al. 2008). Previous studies showed that embryos lacking CD147 gene died around the time of implantation (Igakura et al. 1998), and WT blastocysts transferred to pseudopregnant CD147-null females failure to implant (Kuno et al. 1998). However, the expression, regulation, and function of CYPA in mouse uterus during embryo implantation are still unknown. The purpose of the current study was to detect the expression pattern of CYPA in the mouse uterus during early pregnancy and to investigate the potential roles of CYPA during implantation.

Materials and methods

Animal treatments

Adult CD-1 mice were caged in a controlled environment (12 h of light and 12 h of dark cycle) with free access to regular food and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of Anhui Medical University. Adult female mice were mated with fertile male mice to induce pregnancy (day 1 is the day of vaginal plug). Pregnancy was confirmed by recovering embryos from the oviduct or uterus on days 1–4. The implantation sites on day 5 were visualized after i.v. injection of 0.1 mL of 1% Chicago blue dye (Sigma Aldrich) in saline.

To examine the effects of steroid hormones on Ppia expression, ovariecotomized mice were injected intraperitoneally with estriol-17β (100 ng/mouse) or progesterone (1 mg/mouse). The control mice were injected subcutaneously with sesame oil (100 µL/mouse). Mice were killed and the uteri were collected after the hormone injection for different time points. Each group had at least three mice.

TMN 355 (40 µg/0.1 mL/mouse; R&D Systems) was dissolved in DMSO and injected intraperitoneally with saline at day 1 of pregnancy and killed on day 5 (9:00 h) to check the implantation status. Embryos at the blastocyst stage were recovered by flushing uterus on day 4 (22:00 h). At least three mice were used for each group.

In situ hybridization

Total RNAs from mouse uteri on day 8 of pregnancy were reverse transcribed and amplified with the specific primers for mouse Ppia. The PCR fragment of Ppia was cloned into pGEM-T plasmid and verified by sequencing. Digoxigenin-labeled sense or antisense cRNA probes were transcribed in vitro using a digoxigenin RNA labeling kit (Roche Applied Science). As described previously (Lin et al. 2018), frozen uterine sections (10 µm) were mounted onto 3-aminopropyltriethoxysilane (Sigma Aldrich)-coated slides and fixed in 4% parafomaldehyde solution in PBS. Hybridization was performed at 55°C for 16 h. Digoxigenin-labeled Ppia sense probe was used as a negative control. Then, the sections were incubated with anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche Applied Science). All of the sections were counterstained with methyl green (1%).

Isolation and culture of uterine stromal cells

Mouse uterine stromal cells were obtained as described previously (Lin et al. 2018). Briefly, pregnancy on days 4 uteri were split longitudinally and digested with 1% (w/v) trypsin (Amresco) and 6 mg/mL dispase (Sigma Aldrich) in HBSS for 1 h at 4°C followed by 1 h at room temperature and 10 min at 37°C to remove luminal epithelial cells. The remaining tissues were incubated in 5 mL of HBSS containing 0.15 mg/mL collagenase I (Invitrogen) at 37°C after rinsing three times with HBSS. To collect the stromal cells, the supernatants were shaken and filtered through a 70 µm gauze filter and centrifuged. The obtained cells were grown in DMEM/F-12 medium containing 10% heat-inactivated fetal bovine serum (FBS, Biological Industries). The medium was changed to remove unattached epithelial cells after 1 h.

To perform TMN355 treatment, the stromal cells were treated with 5 µM TMN355 in DMEM/F12 containing 2% cFBS for different time points.

siRNA transfection

The siRNA for mouse Ppia and control siRNA were designed and synthesized by General Biosystems (Heifei, China). According to the manufacturer’s protocol, stromal cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen). Briefly, for cells cultured in a well of a 12-well culture plate, 50 pmol siRNA and 2 µL Lipofectamine 2000/well were diluted in 250 µL OPTI-MEM (Invitrogen), respectively. After a 5 min of incubation, the diluted siRNA was mixed with diluted Lipofectamine 2000 and incubated for 20 min at room temperature. The siRNA-Lipofectamine 2000 mixture was added onto cultured stromal cells and replace with fresh medium after 6 h. Transfected cells were harvested for real-time RT-PCR or Western blot 48 h after transfection.

Real-time PCR

Total RNAs from mouse uteri or cultured cells were extracted using RIZOL (TaKaRa) and synthesized into cDNA following the cDNA reverse transcriptase reagent kit (TaKaRa) according to the manufacturer's protocol. Then real-time PCR was performed with TB Green Premix Ex Taq II kit (TaKaRa) on the Stepone plus instrument (Applied biosystems). The 2ΔΔCt threshold cycle method was employed to analyze relative changes of gene expression compared with reference gene
Rpl7. All primers used for real-time PCR in this study were listed in Table 1.

Western blot analysis

As described previously (Lin et al. 2018), Proteins were extracted from cultured stromal cells with lysis buffer. The BCA reagent kit (Applygen, Beijing, China) was used to measure the concentration of proteins. The protein samples were separated by 12% SDS-PAGE gel and transferred onto PVDF membranes (Millipore). PVDF membranes were blocked with 5% non-flat milk (Sangon, Shanghai, China) and followed by probing with the corresponding antibodies for CYPA (final concentration: 1 μg/mL; Abcam), p-Stat3 (final concentration: 12 ng/mL; Cell signaling), T-Stat3 (final concentration: 24 ng/mL; Cell signaling), Vimentin (final concentration: 0.27 μg/mL; Prositech), Cytokeratin 18 (CK18, final concentration: 0.3 μg/mL; Proteintech), β-Tubulin (final concentration: 0.1 μg/mL; Proteintech) and Gapdh (final concentration: 0.2 μg/mL; Santa Cruz) overnight at 4°C. After washing, the matched secondary antibodies conjugated with HRP (final concentration: 0.1 μg/mL; Elabscience) were incubated PVDF membranes. Signals were developed with the ECL kit (Sangon, Shanghai, China).

Immunofluorescence

Paraffin-embedded uteri sections (5 μm thick) were deparaffinized and dehydrated. Sections were blocked in 10% horse serum for 1 h at 37°C and then incubated with anti-CyPA antibody (final concentration: 10 μg/mL; Abcam) overnight at 4°C. After washing, the sections were incubated with anti-goat Alexa Fluor 488 (final concentration: 10 μg/mL, Invitrogen) as the second antibody for 1 h and counterstained with DAPI for nuclei. Finally, the fluorescence signals were observed under fluorescence microscopy (Leica).

Statistical analysis

All of the experiments were independently repeated at least three times. Each experiment included at least three independent samples. Comparison between two groups was made by Student’s t-test. The multiple comparisons were performed with one-way ANOVA. Results are presented as the mean ± s.e. In all cases, P < 0.05 was considered significantly different.

Results

Localization of CYPA in mouse uterus during early pregnancy

In situ hybridization was performed to examine the spatial and temporal distribution of Ppia mRNA in mouse uteri during early pregnancy (Fig. 1). On day 1 of pregnancy, the uteri presented basal levels of Ppia mRNA signal. On day 2 of pregnancy, Ppia mRNA signals were strongly detected in the glandular and luminal epithelium. On days 3 and 4 of pregnancy, Ppia mRNA signals were still strong in the glandular and luminal epithelium, moreover, Ppia mRNA was strongly detected in the subluminal stroma. On day 5 of pregnancy, strong Ppia mRNA signals were detected in the luminal epithelium and subluminal stroma surrounding the implanting blastocyst. However, there was a much weaker signal at inter-implantation sites compared to implanting sites. From day 6 to 8 of pregnancy, Ppia mRNA was strongly expressed in the embryos and secondary decidual zone. There was no detectable signal when the Ppia antisense probe was replaced with the sense probe.

Immunofluorescence was performed to detect CYPA protein localization. From day 1 to day 4 of pregnancy, the expression pattern of CYPA protein was similar to that of Ppia mRNA (Fig. 2). However, on the day of 5 pregnancy, CYPA protein was strongly detected in these stromal cells surrounding decidual layer. On day 6 to 8 of pregnancy, CYPA was weakly detected in the decidualized cells and mainly found in undifferentiated stromal cells adjacent to the myometrium and decreased as the decidua were developing (Fig. 2). CYPA could also be detected in embryos on days 7 and 8 of pregnancy.

Table 1 Primer used in this study.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Accession number</th>
<th>Primer sequences</th>
<th>Application</th>
</tr>
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<tr>
<td>Ppia</td>
<td>NM_008907</td>
<td>AGGATTTGGGTATAAGGGTT</td>
<td>In situ hybridization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAAATGGTTGATGGATTAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCAAGCATGTCGCTTTGGG</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>CD147</td>
<td>NM_001077184</td>
<td>TTGATGGTTAATGCCCACCC</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>MMP3</td>
<td>NM_010809</td>
<td>TGCCGGTCTTCACATTGATCT</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>MMP9</td>
<td>NM_013599</td>
<td>ACCACAGCGCAACTGACCA</td>
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</tr>
<tr>
<td>Ltf</td>
<td>NM_008522</td>
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<tr>
<td>Muc1</td>
<td>NM_013605</td>
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<tr>
<td>Rpl7</td>
<td>NM_29016</td>
<td>CAGATGGACACCGACAGGATTC</td>
<td>Real-time PCR</td>
</tr>
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</table>

There was no observed immunostaining in day 1 uterus when normal rabbit IgG was used to replace the primary antibody of CYPA as a negative control (Fig. 2).

Regulation of ovarian steroid hormones on CYPA expression

Both estrogen and progesterone are crucial for mouse embryo implantation. Therefore, ovariectomized mice were used to examine whether Ppia expression was regulated by ovarian steroid hormones. In situ hybridization results showed that there was the only basal level of Ppia mRNA in the luminal and glandular epithelium in the ovariectomized mice. Estrogen stimulated a marked Ppia mRNA expression in the luminal and glandular epithelium (Fig. 3A). The elevated Ppia mRNA expression peaked at 12 h after estrogen treatment and then declined (Fig. 3A). Data from Real-time PCR also demonstrated a similar Ppia mRNA expression pattern with in situ hybridization (Fig. 3B). After ovariectomized mice were treated with progesterone, a low level of Ppia mRNA was seen in the luminal, glandular epithelium and stromal cells near the lumen at 6 h and 12 h (Fig. 3A). However, Ppia mRNA signals were mainly localized in the luminal, glandular epithelium and stromal cells near the lumen at 24 h (Fig. 3A). Real-time PCR showed that the level of Ppia mRNA was significantly increased at 24 h after progesterone treatment (Fig. 3C).

Effects of CYP A on implantation

Based on the localization and expression of CYPA during mouse early pregnancy, we assumed that CYPA should be important for mouse implantation. To test the influence of CYPA on implantation and pregnancy, a potent inhibitor for CYPA-TMN355 was injected intraperitoneally on days 3 and 4 of pregnancy twice a day at 9:00 and 21:00 h. After pregnant mice were treated with TMN355, the implantation sites were still observed on day 5 of pregnancy; however, the number of implantation sites was significantly reduced in treated mice compared with control mice (Fig. 4A and B). This indicates CYPA is indeed important for implantation. Meanwhile, morphologically normal blastocysts can be recovered by flushing uteri of TMN355 treatment (Supplementary Fig. 2, see section on supplementary materials given at the end of this article). These results indicated that uterine CYPA may be indispensable for embryo implantation.

To reveal the molecular basis of the effect of CYPA on implantation, the protein of implantation sites on day 5 is extracted from control and TMN355 treated mice. Western blot results showed an active form of STAT3 (Phospho-STAT3, p-STAT3), p-STAT3 expression was remarkably reduced following TMN355 treatment compared with control (Fig. 4C and E). The regulation of CYPA on p-STAT3 was further confirmed within an in vitro stromal cell culture system. The purity of the stromal cells was verified by the expression of Vimentin (stromal cell marker) and Cytokeratin 18 (epithelial cell marker) by Western blot. In the stromal cells, Vimentin was strongly detected, but Cytokeratin 18 was not detected (Supplementary Fig. S1), showing the stromal cells were not contaminated in the luminal epithelial sheets. When we down-regulated CYPA expression by Ppia siRNA or inhibited its activity by TMN355 in the cultured cells, the expression of p-STAT3 was significantly downregulated (Fig. 4F, G, H, I, J and K). In Stat3-inactivated mouse uteri, estrogen-responsive gene, Lactoferrin (Ltf), and Mucin 1 (Muc1) protein (also known to be markers of uterine receptive) were up-regulated (Sun et al. 2013). We then analyzed the expression of Ltf and Muc1 in uteri of TMN355 treatment by qRT-PCR, the expression of Ltf and Muc1 mRNA were also significantly upregulated compared with control (Fig. 4L and M). These results suggested that decrease of CYPA expression in the uterus.
may exaggerate estrogen responsiveness by reducing Stat3 activity, and eventually affected the embryo implantation.

**Effects of CyPA on CD147, MMP-3 and MMP-9 expression**

CD147, a receptor for CyPA, is crucial for signal transduction by CyPA. According to previous studies, we noticed that the protein expression pattern of CyPA and CD147, MMP-3 and MMP-9 were similar during early pregnancy (Das *et al.* 1997, Xiao *et al.* 2002, Chen *et al.* 2009). To further investigate the molecular mechanisms of CyPA in regulating CD147 and MMP gene expression in stromal cells, the expression of *Ppia* mRNA was silenced by the specific siRNA in cultured stromal cells (Fig. 5A). Upon *Ppia* knockdown, the expression levels of CD147, MMP-3 and MMP-9 mRNA remarkably reduced (Fig. 5B). When stromal cells were treated with TMN355, the level of MMP-3 and MMP-9 mRNA had an obvious decrease compared with control (Fig. 5C).

**Discussion**

In this study, we firstly show that the localization of *Ppia* mRNA and CyPA protein in mouse uterus during early pregnancy. The results are shown in Figs 1 and 2 demonstrate that CyPA on days 1 to 2 of pregnancy predominantly localizes in the uterine epithelium cells and is firstly detected in the subluminal stromal cells on day 3 of pregnancy. Mouse uterine cells undergo profound proliferation and differentiation during the peri-implantation period (Li *et al.* 2011). On day 1 to 2 of pregnancy, estrogen (E2) from ovarian mainly stimulates the proliferation of uterine epithelium cells. On day 3 of pregnancy, progesterone (P4) derived from the formation of corpus luteum induces uterine stromal cell proliferation. By using the ovariectomized mice model, our results show that E2 significantly stimulates the expression of *Ppia* mRNA in uterine epithelium cells, while P4 stimulates its expression in the uterine stromal cell. Some studies show that CyPA promotes the proliferation and migration of VSMC as well as the activation of MMPs in VSMC (Satoh *et al.* 2008). Moreover, several reports have shown that CyPA overexpression promotes cancer cell proliferation in many types of cancers (Feng *et al.* 2015, Zhu *et al.* 2015). These data suggest CyPA may be involved in the E2 and P4-mediated proliferation of uterine epithelium and stromal cells to prepare the uteri to enter into the receptive status conducive to blastocyst implantation.

This dynamic uterine expression pattern of CyPA motivated us to study its potential roles in the peri-
implantation events. In our study, when pregnant mice are treated with CYP A inhibitor, TMN355, the numbers of implantation sites are significantly reduced compared with controls. Decreased CYP A activity in mice uterus and isolated uterine stromal cells by TMN355 treatment significantly inhibit STAT3 activity. STAT3 is well known as a marker of uterine receptivity. Uterine deletion of STAT3 leads to increased estrogenic responses, impeding uterine differentiation to the receptive state and ultimately causes embryo implantation failing (Sun et al. 2013). To search for the underlying molecular mechanisms, we further demonstrate that the decline of CYP A activity in the mice uterus could markedly enhance the expression of epithelial estrogen-regulated genes including Ltf and Muc1 which serve as the cell-surface barrier to hinder embryo implantation (Aplin et al. 2001), and there are some similar features of implantation failure between CYP A inhibition in mice and STAT3 conditional knockout mice. Based on the above results, we speculate that decreased CYP A expression in the uterus may expand estrogen responsiveness by reducing STAT3 activity and impair normal uterine receptivity, resulting in embryo implantation failure. Collectively, our findings concerning CYP A add a potentially new regulatory pathway contributing to the complexity of implantation.

With the onset of implantation on day 5, the uterine stromal cells surrounding the invading embryo began to undergo decidualization, strong Ppia mRNA signals are detected in these stromal cells, but strong CYP A protein signals are detected in the stromal cells surrounding decidual layer. On days 6–8 of pregnancy, when these decidual stromal cells have transformed into mature decidual cells and form the secondary decidual zone (SDZ). The expression of Ppia mRNA is strongly detected in SDZ. But CYP A protein expression disappears in the SDZ, and more intense signals of CyPA protein are detected in undifferentiated stromal cells close to the myometrium. The data from CYP A immunostaining are not reconciled to that from in situ hybridization. The different localization of RNA and protein expression may be due to the microenvironment of hypoxia (Daikoku et al. 2003) and inflammation (Dekel et al. 2014) in the uterus during implantation, leading to the secretion of CYP A (eCyPA) from stromal cells, and then spread to the undifferentiated stromal cells adjacent to the myometrium. Finally, the expression of CYP A in the decidual cells was downregulated. Indeed, CD147 (also known as Basigin or EMMPRIN), the receptor of eCyPA, is also an inducer of MMP-3 and MMP-9 expression in mice uterine stromal fibroblasts (Chen et al. 2009a). Implantation in the mouse is a highly invasive process that requires the activity of MMPs expressed by stromal fibroblasts. But high levels of MMPs expression can lead to the embryo uncontrolled invasion into the uterus. Therefore, the localized control of MMP expression within the uterus is critical for successful implantation. Our results showed in Fig. 2 demonstrate that the expression pattern for CYP A within the pregnant mouse uterus correlates closely with those previously reported for CD147, MMP-3 and MMP-9 (Alexander et al. 1996, Bany et al. 2000, Chen et al. 2009a). This led us to hypothesize that CYP A may regulate CD147, MMPs expression in mouse uterine fibroblasts. We use a uterine stromal cell culture system in which CYP A is markedly reduced by Ppia siRNA and CYP A-specific inhibitor confirmed the regulation of CYP A on CD147, MMP3 and MMP9. Together with our results, these data suggest that the downregulation of CYP A in the decidual cells in vivo provided a possible mechanism...
Figure 4 Regulation and function of CYPA during embryo implantation. (A) The morphology of uteri on day 5 after the mice were treated with TMN355 on day 3 and day 4 of pregnancy. (B) The numbers of implantation sites were used to show the impact of TMN355 on implantation. (C) Western blot analysis of CYPA, p-Stat3 and T-Stat3 protein level in mouse uterus after the mice were treated with TMN355 on days 3 and 4 of pregnancy. (D) The quantitative analysis of CYPA protein in Fig. 4C. (E) The quantitative analysis of p-Stat3 protein in Fig. 4C. Western blot analysis of CYPA, p-Stat3 and T-Stat3 protein level after stromal cells were treated with TMN355 for 6h. (G) The quantitative analysis of CYPA protein in Fig. 4F. (H) The quantitative analysis of p-Stat3 protein in Fig. 4F. (I) Western blot analysis of CYPA, p-Stat3 and T-Stat3 protein level after stromal cells were transfected with mouse Ppia siRNA (Si-Ppia) for 48 h. (J) The quantitative analysis of CYPA protein in Fig. 4H. (K) The quantitative analysis of p-Stat3 protein in Fig. 4H. (L) Real-time PCR analysis of Ltf mRNA expression in mouse uterus after the mice were treated with TMN355 on days 3 and 4 of pregnancy. N, the number of mice. All experiments were repeated at least three times. Error bars represent standard errors. *P<0.05.

Figure 5 Regulation of CYPA in mouse endometrium stromal cells. (A) Real-time PCR analysis of Ppia mRNA expression after stromal cells were transfected with mouse Si-Ppia for 48 h. (B) Real-time PCR analysis of CD147, MMP3 and MMP9 mRNA expression after stromal cells were transfected with mouse Si-Ppia for 48 h. (C) The level of CD147, MMP3 and MMP9 mRNA were detected by real-time PCR after stromal cells were treated with TMN355 for 6 h. All experiments were repeated at least three times. Error bars represent standard errors. *P<0.05.
to limit MMP expression by regulating CD147 during implantation and prevent the uncontrolled invasion of embryonic cytotrophoblast cells into the uterus.

In conclusion, our data demonstrate that Pdia mRNA and CYP A protein are strongly expressed in mouse uterus during early pregnancy and regulated by estrogen or progesterone. The inhibition of CYP A reduces the rate of implantation and affects some implantation-related factors. To further clarify the mechanism of CYP A action during implantation in vivo, future studies will require the use of CYP A-knockout mouse model.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/REP-20-0187.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
T Y and S L designed and performed major experiments, analyzed the data, and wrote the manuscript. R X performed cell culture and treatments. T X D performed mouse model experiments. Y L and H G performed western blot. H L D and X H Z initiated, organized, and designed the study, analyzed the data, and wrote the manuscript. All authors commented on the manuscript.

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