Decidual expression and regulation of fatty acid desaturase 3 during mouse decidualization

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Abstract

Decidualization is required for the successful establishment of pregnancy in rodents and primates. Fatty acid desaturase 3 (Fads3) belongs to the fatty acid desaturase family, which is a crucial enzyme for highly unsaturated fatty acid biosynthesis. However, the expression, regulation and function of Fads3 during early pregnancy in mice are still unknown. In this study, we examined Fads3 expression, regulation and function during mouse decidualization. The expression of Fads3 is detected in the subluminal stromal cells at implantation site on day 5 of pregnancy, but not at inter-implantation site and in day 5 pseudopregnant uteri. Compared to delayed implantation, Fads3 is strongly expressed after delayed implantation is activated by estrogen treatment. From days 6 to 8, Fads3 mRNA signals are significantly detected in the decidua. In ovariectomized mice, estrogen significantly stimulates Fads3 expression. However, estrogen has no effect on Fads3 expression in ovariectomized ER α -deficient mice, suggesting that estrogen regulation on Fads3 expression is ER α dependent. When ovariectomized mice were treated with progesterone, Fads3 expression is significantly increased by progesterone. Progesterone stimulation on Fads3 expression is also detected in cultured stromal cells, which is abrogated by RU486 treatment. These data indicate that progesterone upregulation on Fads3 expression is progesterone receptor-dependent. Fads3 knockdown by siRNA reduces *in vitro* decidualization of mouse stromal cells. Taken together, Fads3 may play an important role during mouse decidualization.

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Introduction

Embryo implantation and decidualization are essential for the establishment of pregnancy in rodents and primates. After embryo implantation occurs, the stromal cells surrounding the implanting blastocyst begin to proliferate and differentiate into large, round decidual cells. This process is known as decidualization. Impaired decidualization can lead to adverse outcomes such as implantation failure and miscarriage (Carson *et al.* 2000). In mice, decidualization is stimulated by the blastocyst and can also be induced by scratching the endometrium or intraluminal injection of oil (Gellersen & Brosens 2014). The establishment of pregnancy is orchestrated by ovarian estrogen and progesterone, which are crucial for implantation and decidualization in mammals (Dey *et al.* 2004). The physiological effects of estrogen and progesterone are mediated by binding to their cognate receptors, estrogen receptors (ER) and progesterone receptor (PR). ER α -knockout mice show ovarian dysfunction and estrogen insensitivity of the uterus (Schomberg *et al.* 1999). PR-knockout mice are infertile due to the defects in embryo implantation and decidualization (Mulac-Jericevic *et al.* 2000). However,

the molecular mechanism during decidualization is still unclear.

Highly unsaturated fatty acids (HUFA), especially arachidonic acid (20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), are metabolically required for many developmental processes. The dysfunction in the metabolism of HUFAs will result in cardiovascular disease, cancers and diabetes (Lee *et al.* 2016). HUFA, a long-chain subgroup of poly unsaturated fatty acids, can be biosynthesized from linoleic acid (LA, 18:2n-6) and α -linolenic acid (ALA, 18:3n-3) (Zhang *et al.* 2017).

Fatty acid desaturases are essential enzymes that mediate the introduction of double bonds at specific positions in a long-chain fatty acid (Nakamura & Nara 2004). Fatty acid desaturase 1 and 2 (FADS1 and FADS2) catalyze the biosynthesis of HUFA. FADS1 introduces a double bond at the $\Delta 5$ position of 20-carbon fatty acids (Kothapalli *et al.* 2016). FADS2 catalyzes the introduction of a double bond at the $\Delta 4$, $\Delta 6$ or $\Delta 8$ position, which is a rate-limiting step for the biosynthesis of eicosapentaenoic acid (DHA) (Park *et al.* 2016). Arachidonic acid is the precursor for the biosynthesis of prostaglandins, prostacyclins and leukotrienes (Lee *et al.*

2016). Arachidonic acid can be synthesized from LA utilizing three enzymatic steps. Two of these steps are catalyzed by FADS1 and FADS2 (Hester *et al.* 2014). FADS3 is the third member of the FADS gene cluster and evolutionarily results from a gene duplication event with *Fads1* and *Fads2* (Zhang *et al.* 2017). However, FADS3 function remains elusive. Based on the 62 and 70% sequence homology with *Fads1* and *Fads2*, *Fads3* may be a new fatty acid desaturase (Blanchard *et al.* 2011). Recent data indicate that *Fads3* plays a role during early development as an enhancer of HUFA biosynthesis and/or regulation (Zhang *et al.* 2017).

Based on our SAGE analysis, *Fads3* expression is significantly higher at the implantation site than inter-implantation in mouse uterus (Ma *et al.* 2006), suggesting a putative involvement of *Fads3* in early pregnancy. However, the expression, regulation and function of *Fads3* in mouse uterus during early pregnancy are still unknown. In this study, we showed that *Fads3* is strongly expressed in mouse decidua and upregulated by progesterone.

Materials and methods

Animal treatments

Mature CD-1 mice were caged in a temperature- and light-controlled environment (12 h of light and 12 h of darkness) with free access to regular food and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of South China Agricultural University.

Adult female mice were mated with fertile or vasectomized males to induce pregnancy or pseudopregnancy (day 1 is the day of vaginal plug). On days 1–4, pregnancy was confirmed by flushing embryos from oviducts or uterus. On day 5, the implantation sites were visualized through intravenous injection of 0.1 mL of 1% Chicago blue dye (Sigma Aldrich) in saline.

Delayed implantation was induced by ovariectomizing pregnant mice at 08:30–09:00 on day 4 of pregnancy. Progesterone was subcutaneously injected (1 mg/mouse, Sigma Aldrich) from days 5 to 7. Estradiol-17 (25 ng/mouse, Sigma Aldrich) was given to progesterone-primed delayed mice to activate blastocyst implantation on day 7 of pregnancy. On day 8 of pregnancy, the mice were killed to collect uteri after estrogen treatment. The activation of implantation sites were identified through intravenous injection of 0.1 mL of 1% Chicago blue dye. Delayed implantation was confirmed by flushing blastocysts from one horn of the uterus on day 8 of pregnancy and uteri were collected for further analysis.

To induce the artificial decidualization, pseudopregnancy mice were infused 10 μ L of sesame oil (S3547; Sigma Aldrich) into one uterine horn on day 4, whereas another uninjected horn served as a control. The uteri were collected on day 8 of pseudopregnancy.

To determine the effects of steroid hormones on *Fads3* expression, the ovariectomized mice rested for 2 weeks to eliminate circulating ovarian steroids. Mice were injected subcutaneously with estradiol-17 β (100 ng/mouse) or

progesterone (1 mg/mouse). The control mice were injected subcutaneously with sesame oil (100 μ L/mouse). Mice were killed and the uteri were collected after the hormone injections for 24 h.

To estimate whether *Fads3* expression was ER α dependent, WT or ER α knockout (ER α KO) mice were ovariectomized and rested for 2 weeks and then injected subcutaneously with estradiol-17 β (100 ng/mouse). The control mice were treated with sesame oil as vehicle. The uteri were collected after the estradiol-17 injections for 24 h.

In situ hybridization

Total RNAs from mouse uteri on day 5 of pregnancy were reverse transcribed and amplified with the specific primers for mouse *Fads3*. The amplified fragment of *Fads3* was cloned into pGEM-T plasmid and verified by sequencing. The primers of T7 and SP6 were used to amplify *Fads3* fragment from the pGEM-*Fads3* plasmids. Digoxigenin-labeled antisense or sense cRNA probes were transcribed *in vitro* using a digoxigenin RNA labeling kit (Roche Applied Science).

As previously described (Ding *et al.* 2018), frozen sections (10 μ m) were mounted on 3-aminopropyltriethoxysilane (A3648; Sigma Aldrich)-treated slides and fixed in 4% paraformaldehyde solution in PBS. Hybridization was performed at 55°C for 16 h. Digoxigenin-labeled *Fads3* sense probe was used as negative control. Following hybridization and post-hybridization washes, sections were incubated with sheep anti-digoxigenin antibody conjugated to alkaline phosphatase (1:5000; Roche Applied Science). Endogenous alkaline phosphatase activity was inhibited with 2 mM levamisole. All the sections were counterstained with 1% methyl green. The positive signal was visualized as a dark brown color.

Isolation and culture of uterine stromal cells

Mouse uterine stromal cells were isolated as described previously (Liang *et al.* 2014). Briefly, uterine horns from day 4 pregnant mice were split longitudinally, washed with Hanks' balanced salt solution (HBSS; Sigma) and digested with 1% (w/v) trypsin (Amresco, Cleveland, USA) and 6 mg/mL dispase (Roche Applied Science) in HBSS for 1 h at 4°C followed by 1 h at room temperature and 10 min at 37°C. After rinsing three times with HBSS, the remaining tissues were incubated in 6 mL of HBSS containing 0.15 mg/mL collagenase I (Invitrogen; 17100-017) at 37°C for 30 min. The digested tissues were shaken and filtered through a 70 μ m wire gauze filter and centrifuged to collect the stromal cells. The isolated cells were grown in DMEM/F-12 medium containing 2% heat-inactivated fetal bovine serum (FBS, Biological Industries, Cromwell, Israel). After an initial culture for 1 h, the medium was changed to remove unattached epithelial cells. The stromal cells were cultured in DMEM/F-12 (D2906; Sigma Aldrich) containing 10% (v/v) charcoal-stripped FBS (cFBS, Biological Industries). To induce *in vitro* decidualization, cells were treated with 10 nM estrogen and 1 μ M progesterone in DMEM/F12 containing 2% cFBS for different time points.

Stromal cells were also treated with 1 μ M of progesterone for 24 h. Cells were pretreated with 1 μ M of RU486 (Cayman) or JNK kinase inhibitor (Cayman) 1 h and then treated with progesterone.

siRNA transfection

The siRNA for mouse *Fads3* and nonspecific siRNA were designed and synthesized by Ribobio Co., Ltd. (Guangzhou, China). The stromal cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After cells were transfected for 6 h, the stromal cells were induced for *in vitro* decidualization for 48 h.

Real-time PCR

Total RNAs were extracted from the whole uteri or cultured cells by using TRIZOL (TaKaRa) and reverse transcribed into cDNA by following the PrimeScript reverse transcriptase reagent kit (TaKaRa). Then real-time PCR was performed with SYBR Premix Ex Taq kit (RR820A TaKaRa) on the CFX96 TOUCHTM (Bio-Rad). The specific primer sequences of each gene used for real-time were provided in Table 1. The $2^{-\Delta\Delta Ct}$ method was used to analyze the data from real-time PCR.

Western blot analysis

Western blot was performed as described previously (Liang et al. 2014). Briefly, proteins for Western blot were extracted from cultured stromal cells by homogenization lysis buffer including 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100 and 0.25% sodium deoxycholate. The concentration of proteins was measured by BCA kit (Applygen, Beijing, China). The samples (10 μ g per lane) were separated by 10% SDS-PAGE gel and transferred onto PVDF membranes (Millipore) followed by blocking with 5% non-fat milk (Sangon, Shanghai, China). PVDF membranes were probed with the corresponding antibodies for FADS3 (ABclonal,

Wuhan, China) and β -Actin (Cell Signaling) overnight at 4°C. Membranes were then incubated with the matched secondary antibodies conjugated with HRP (1:5000). Signals were detected with the ECL kit (Pierce Biotechnology).

Statistical analysis

All of the experiments were independently repeated at least three times. The difference between two groups was compared by Student's *t* test. The multiple comparisons were performed with one-way ANOVA followed by Student's *t*-test. Data are presented as the mean $\pm$ standard error. In all cases, $P < 0.05$ was considered significantly different.

Results

Fads1, Fads2 and Fads3 mRNA expression during early pregnancy

In situ hybridization was used to examine the spatial distribution of *Fads1*, *Fads2* and *Fads3* mRNA in mouse uteri during early pregnancy. There were no visible *Fads1* and *Fads2* mRNA signals in the uteri from days 1 to 4 of pregnancy. On day 5 of pregnancy, *Fads1* and *Fads2* mRNA signals were weakly detected in the subluminal stromal cells surrounding the implanting blastocyst at implantation sites. On days 6 and 8, *Fads1* and *Fads2* mRNA signals were faintly detected in the decidua (Fig. 1).

From days 1 to 4 of pregnancy, there was no visible *Fads3* mRNA signal in the uteri. On day 5 of pregnancy, *Fads3* mRNA was detected specifically in the subluminal stromal cells surrounding the implanting blastocyst at implantation sites (Fig. 1). On days 6 and 8, *Fads3* mRNA signals were highly detected in the decidua (Fig. 1).

Because the mRNA signals for *Fads1* and *Fads2* were weakly detected in mouse uteri from days 1 to 8 of pregnancy, no further analysis was performed on *Fads1* and *Fads2*.

Table 1 Primers used in this study.

Gene	ID	Primer sequences	Size (bp)	Application
<i>Fads1</i>	BC063053	CTCTGGTTGGACGCTTAC TGGAGAGTCAGATAGCCC	364	<i>In situ</i> hybridization
<i>Fads2</i>	BC057189	CCACCTGTTCTTCTTCTC TGCAGGCTCTTTATGTCC	324	<i>In situ</i> hybridization
<i>Fads3</i>	BC071266	ACAAGTGGCTGGTCATCG CCAACAACCTCCATAGCCAG	339	<i>In situ</i> hybridization
<i>Fads3</i>	NM-021890.3	CAGATACCTGCCCTACAACC CCACAGCAAGTCCGTC	132	Real-time PCR
<i>Rpl7</i>	NM29016	GCAGATGTACCGCACTGAGATTC ACCTTTGGGCTTACTCCATTGATA	129	Real-time PCR
<i>Dtprp</i>	NM-010088	AGCCAGAAATCACTGCCACT TGATCCATGCACCCATAAAA	119	Real-time PCR
<i>Pr13c1</i>	NM_001163218	GGTTTGGCACATCTTGGTGTT CACAGAACAAGGCCAAAGGC	162	Real-time PCR
<i>Bmp8a</i>	NM_001256019.1	CGCCTCTATGTGGAACCG CTGGAGCGTGGTGCTTGTC	82	Real-time PCR
<i>Abp1</i>	NM_001161621	GGTGAGCGTGTTCCTAT GGAAAGCATCCAGGAAAGT	178	Real-time PCR

Figure 1 *In situ* hybridization of *Fads1*, *Fads2* and *Fads3* mRNA in mouse uteri during early pregnancy, showing the localization of *Fads1*, *Fads2* and *Fads3* mRNA in mouse uteri on days 1 (D1), 4 (D4), 5 (D5), 6 (D6) and 8 (D8). On each day of pregnancy, 3–5 mice were used. At least three implantation sites per mouse were used for *in situ* hybridization and a representative section was shown. Asterisk (*), embryo. Bar = 300 μ m.

***Fads3* mRNA expression during pseudopregnancy, delayed and activated implantation**

Because *Fads3* was strongly detected at implantation sites, we also checked *Fads3* expression at inter-implantation sites. However, there was no detectable signal at inter-implantation sites (Fig. 2A). Data from RT-PCR also showed that *Fads3* level at implantation site was significantly higher than that at inter-implantation sites on day 5 of pregnancy (Fig. 2B).

To further address whether the expression of *Fads3* is dependent on the embryo, we examined the expression of *Fads3* during pseudopregnancy. There was no detectable *Fads3* signal in mouse uteri on day 5 of pseudopregnancy (Fig. 2A).

Delayed implantation model was applied to assess whether *Fads3* expression is regulated by active blastocyst. When embryo implantation was delayed by ovariectomy, there was no detectable *Fads3* mRNA signal in the uteri. After delayed implantation was

terminated by estrogen, *Fads3* mRNA was detected in the subluminal stromal surrounding the blastocyst of implantation sites (Fig. 2A).

***Fads3* mRNA expression during artificial decidualization**

Because *Fads3* expression was strongly detected in the decidua on day 8 of pregnancy, RT-PCR was used to verify *Fads3* expression. Compared to inter-implantation site, *Fads3* expression level was significantly higher at implantation sites on day 8 of pregnancy (Fig. 3A). Then, artificial decidualization model was used to determine whether *Fads3* is induced by oil injection. There was no detectable *Fads3* mRNA signal in the control uterine horn, while a high level of *Fads3* mRNA expression was detected in the deciduoma of oil-injected uterine horn (Fig. 3B). Real-time PCR results also showed that *Fads3* mRNA signals were strongly upregulated in deciduoma compared with control (Fig. 3C).

Figure 2 Expression of *Fads3* mRNA during pseudopregnancy and under delayed implantation. (A) *In situ* hybridization of *Fads3* expression at inter-implantation site on day 5 of pregnancy (D5 NI), on day 5 of pseudopregnancy and under delayed implantation and activated uteri. D5 NI, inter-implantation site on day 5; PD5, day 5 of pseudopregnancy; Asterisk (*), embryo. Bar = 300 μm. Three mice were used in each group. At least 3–5 sections from each mouse were used for *in situ* hybridization. A representative section was shown. (B) Real-time PCR analysis of *Fads3* expression between inter-implantation and implantation on day 5. D5 IS, implantation site on day 5. Uteri from three mice were used for real-time PCR. Three independent repeats were performed.

Steroid hormonal regulation on *Fads3*

Because estrogen and progesterone are crucial for mouse decidualization (Dey *et al.* 2004), ovariectomized mice were used to determine whether *Fads3* expression was regulated by estrogen and progesterone. After ovariectomized mice were treated with estrogen, *Fads3* expression was weakly detected in the luminal epithelium and stromal cells compared with control (Fig. 4A). Real-time PCR analysis showed that *Fads3* mRNA level was remarkably enhanced compared with control uteri. However, estrogen had no stimulating effect on *Fads3* expression in ovariectomized ERα-knockout mice (Fig. 4B), suggesting that estrogen regulation of *Fads3* expression is ERα-dependent.

When ovariectomized mice were treated with progesterone, *Fads3* mRNA signals were weakly seen in uterine luminal epithelium and stromal cells (Fig. 4A). Data from real-time PCR indicated that *Fads3* expression was significantly increased compared with

Figure 3 Expression of *Fads3* mRNA during artificial decidualization. (A) Real-time PCR analysis of *Fads3* expression between inter-implantation and implantation on day 8. D8-NI, inter-implantation site on day 8; D8-IS, implantation site on day 8. (B) *In situ* hybridization of *Fads3* expression under artificial decidualization. Bar = 300 μm. (C) Real-time PCR analysis of *Fads3* expression under artificial decidualization. Uteri from three mice were used for real-time PCR. Three independent repeats were performed. At least 3–5 sections from each mouse were used for *in situ* hybridization. A representative section was shown.

control (Fig. 4C). Furthermore, *Fads3* expression was also obviously increased when cultured stromal cells were treated with progesterone, which was abrogated by RU486 treatment (Fig. 4D), suggesting that progesterone regulation of *Fads3* expression is progesterone receptor dependent. In the cultured stromal cells, the expression of *Fads3* was inhibited by JNK kinase inhibitor, and progesterone induction on *Fads3* expression was slightly inhibited by the inhibitor for JNK kinase (Fig. 4E).

Fads3 mRNA expression during *in vitro* decidualization

Because *Fads3* is strongly expressed in the decidual cells, *in vitro* decidualization model was used to examine whether *Fads3* is involved in mouse decidualization. After stromal cells were induced for *in vitro* decidualization, there was a significant induction of *Dtprp*, *Prl3c1* and *Bmp8a* (Fig. 5), reliable markers for mouse *in vitro* decidualization (Kimura *et al.* 2001, Bany & Cross 2006, Kelleher *et al.* 2017, Li *et al.* 2017). Meanwhile, *Fads3* expression was also significantly induced under *in vitro* decidualization for 12, 24, 48 and 72 h, respectively (Fig. 5B).

To investigate the function of *Fads3* during decidualization, the expression of *Fads3* was silenced by the specific siRNA. After *Fads3* expression was knocked down by *Fads3* siRNA (Fig. 5C and D), there was a remarkable reduction for the expression levels of *Dtprp*,

Figure 4 Steroid hormones regulation on *Fads3* mRNA expression. (A) *In situ* hybridization of *Fads3* expression after ovariectomized mice were treated with sesame oil, estrogen and progesterone, respectively. (B) Real-time PCR of *Fads3* mRNA expression after WT or ER KO ovariectomized mice were injected with estrogen. (C) Real-time PCR of *Fads3* mRNA expression after WT ovariectomized mice were injected with progesterone. (D) Real-time PCR of *Fads3* mRNA expression after stromal cells were treated with progesterone or progesterone plus RU486. Con, control; E, estrogen; P, progesterone; group P+RU486. Co-treatment with progesterone plus RU486. (E) Effects of JNK inhibitor on progesterone-induced *Fads3* expression. Stromal cells were pretreated with JNK inhibitor for 1 h and then treated with progesterone. Three mice were used in each group. At least 3–5 sections from each mouse were used for *in situ* hybridization. A representative section was shown. All experiments were repeated at least three times.

Prl3c1 and *Bmp8a* (Fig. 5E, F and G). Meanwhile, based on *in situ* hybridization, *Fads3* shares a co-localization with *Abp1*, which is essential to mouse embryo implantation and decidualization (Liang *et al.* 2010). In this study, *Abp1* expression was remarkably reduced following *Fads3* siRNA treatment (Fig. 5H).

Of the prostaglandins synthesized from arachidonic acid, PGE₂ has been proved to be essential to decidualization (Kennedy *et al.* 2007). When stromal cells were treated with PGE₂ under *in vitro* decidualization, PGE₂ had no obvious effects on *Fads3* expression (Fig. 6A). Although PGE₂ had no stimulating effects on both *Dtprp* and *Prl3c1* levels, PGE₂ was able to rescue the inhibitory effect of *Fads3* siRNA on both *Dtprp* and *Prl3c1* levels (Fig. 6B and C).

Discussion

In this study, we showed that *Fads3* is strongly expressed in the decidual cells at implantation sites. Furthermore, *Fads3* expression is not detected at inter-implantation sites, in day 5 pseudopregnant uterus and under delayed uterus, suggesting that *Fads3* expression is embryo dependent during peri-implantation period.

Under *in vitro* decidualization, knockdown of *Fads3* expression by siRNA leads to a significantly reduction of *Dtprp*, *Bmp8a* and *Prl3c1*, which are reliable markers for mouse *in vitro* decidualization (Kimura *et al.* 2001, Bany & Cross 2006, Kelleher *et al.* 2017, Li *et al.* 2017). These data suggest that *Fads3* may play a role during mouse decidualization. Based on the high homology with FADS1 and FADS2, FADS3 should be important for arachidonic acid (Pedrono *et al.* 2010). Our previous study showed that arachidonic acid can activate the cytosolic phospholipase A₂a (cPLA₂a)/cyclooxygenase-2 (COX2) pathway, which is essential during embryo implantation and decidualization in mouse uterus (Zhao *et al.* 2012). cPLA₂a catalyzes arachidonic acid release from membrane phospholipids (Murakami *et al.* 2000). cPLA₂a is crucial for successful pregnancy because of cPLA₂a KO mice show small litters, abnormal uterine spacing of embryos and often exhibit pregnancy failures (Bonventre *et al.* 1997, Song *et al.* 2002). Conditional deletion of mouse uterine Trp53 (p53(d/d)), leads to premature uterine senescence and preterm birth, and also causes an increase in arachidonic and docosahexaenoic acid (Lanekoff *et al.* 2016). These data suggest that a balance in arachidonic acid should be essential for decidual development. It is

Figure 5 Expression and function of *Fads3* under *in vitro* decidualization. (A) Real-time PCR of *Dtprp* mRNA expression under *in vitro* decidualization from 12 h to 72 h. (B) Real-time PCR of *Fads3* mRNA expression during *in vitro* decidualization. *Fads3* expression was obviously upregulated compared to the control from 12 h to 72 h. (C) *Fads3* mRNA expression after stromal cells were transfected with *Fads3* siRNA and the nonspecific siRNA (NC) under *in vitro* decidualization for 48 h. (D) Western blot analysis showed that FADS3 protein level was significantly reduced by *Fads3* siRNA. (E) Effects of *Fads3* siRNA on *Dtprp* expression. (F) Effects of *Fads3* siRNA on *Prl3c1* expression. (G) Effects of *Fads3* siRNA on *Bmp8a* expression. (H) Effects of *Fads3* siRNA on *Abp1* expression. Con, control; group E+P, Co-treatment with estrogen plus progesterone. Stromal cells from 3 to 6 mice were isolated, pooled and used for cell culture and treatments in triplicates. All experiments were repeated at least three times.

possible that *Fads3* is involved in mouse decidualization through arachidonic acid production.

Furthermore, arachidonic acid can be converted to prostaglandin H₂ (PGH₂) by COX2, a crucial rate-limiting enzyme. COX2-null mice resulted infertility with multiple defects in ovulation, embryo implantation

and decidualization in female reproduction. These defects can be restored by exogenous prostaglandin administration in both of cPLA2a- and COX2-null mice (Lim *et al.* 1997, Song *et al.* 2002). Indomethacin, an inhibitor of COX2, can suppress the generation of PGE2 and result in embryo implantation failure (Hamilton & Kennedy 1994). PGE2 is essential to decidualization (Kennedy *et al.* 2007). In this study, PGE2 is able to rescue the inhibitory effect of *Fads3* siRNA on both *Dtprp* and *Prl3c1* levels although PGE2 has no stimulating effect on *Fads3* expression, suggesting that *Fads3* may be involved in PGE2 synthesis. Additionally, our previous study reported that *Abp1* mRNA is highly expressed in the decidual cells in mouse uterus and plays a role during mouse decidualization (Liang *et al.* 2010). Based on data from *in situ* hybridization, *Fads3* is co-localized with *Abp1* in the primary decidual zone in mouse uterus, suggesting that *Fads3* may have a connection with *Abp1* during mouse decidualization. In this study, knockdown of *Fads3* expression by siRNA leads to a significant reduction on *Abp1* expression during *in vitro* decidualization, suggesting that *Fads3* may regulate mouse decidualization through *Abp1*.

In our study, the levels of *Dtprp*, *Prl3c1* and *Bmp8a* are partially inhibited by *Fads3* siRNA. The failure of complete inhibition of decidualization may be caused by the partial inhibition of *Fads3* siRNA on *Fads3* expression and the possible involvement of both *Fads1* and *Fads2*. Both *Fads1* and *Fads2* are also weakly expressed in the primary decidual zone on day 5 of pregnancy. It is known that FADS1 and FADS2 participate in the biosynthesis of arachidonic acid from LA (Hester *et al.* 2014). However, the molecular mechanism of *Fads3* action needs to be further explored.

Estrogen is crucial for blastocyst implantation in rodents (Zhang *et al.* 2013). Conditional knockout ERα in mouse uteri results in implantation failure (Mulac-Jericevic *et al.* 2000). Our study showed that *Fads3* expression level was significantly higher after ovariectomized mice were treated with estrogen. However, estrogen has no effects on *Fads3* expression in ERα-deficient ovariectomized mice, suggesting that estrogen regulates *Fads3* expression via ERα in mouse uteri.

In this study, progesterone stimulated *Fads3* expression in ovariectomized mouse uterus and cultured uterine stromal cells, which was abrogated by progesterone receptor antagonist RU486. These results indicate that progesterone stimulates *Fads3* expression through PR. Recent studies showed that *Fads3* is a target gene for MYCN, NF-κB or p63 transcription factors, which could be involved in cell proliferation, differentiation and apoptosis (Yan *et al.* 2007, Gu *et al.* 2008). Decidualization is characterized by stromal cells proliferation, differentiation and apoptosis. Furthermore, within the mouse mammary gland, NF-κB is upregulated by progesterone. NF-κB is also a regulator of COX2

Figure 6 Effects of PGE2 on *Fads3* action during *in vitro* decidualization. (A) PGE2 has no effects on *Fads3* expression. (B) PGE2 has no stimulating effects on *Dtprp*, but can rescue the inhibitory effect of *Fads3* siRNA on *Dtprp* level. (C) PGE2 has no stimulating effects on *Dtprp*, but can rescue the inhibitory effect of *Fads3* siRNA on *Prl3c1*. Stromal cells from 3 to 6 mice were isolated, pooled and used for cell culture and treatments in triplicates. All experiments were repeated at least three times.

expression in many cell types (Schmedtje *et al.* 1997). It is possible that progesterone may regulate *Fads3* through NF- κ B. We also showed that JNK-1 may be involved in progesterone regulation on *Fads3* because progesterone induction of *Fads3* is decreased by JNK-1 inhibitor.

In conclusion, *Fads3* is strongly expressed in the decidual cells and regulated by estrogen or progesterone. Knockdown of *Fads3* can reduce decidualization.

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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