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Expression of PIK3IP1 in the murine uterus during early pregnancy

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Abstract

The ovarian steroid hormones, estrogen (E2) and progesterone (P4), are essential regulators of uterine functions necessary for development, embryo implantation, and normal pregnancy. ARID1A plays an important role in steroid hormone signaling in endometrial function and pregnancy. In previous studies, using high density DNA microarray analysis, we identified phosphatidylinositol-3-kinase interacting protein 1 (*Pik3ip1*) as one of the genes up-regulated by ARID1A. In the present study, we performed real-time RT-PCR and immunohistochemistry analysis to investigate the regulation of PIK3IP1 by ARID1A and determine expression patterns of PIK3IP1 in the uterus during early pregnancy. The expression of PIK3IP1 was strong at the uterine epithelial and stromal cells of the control mice. However, expression of PIK3IP1 was remarkably reduced in the $Pgr^{cre/+}Arid1a^{f/f}$ mice and progesterone receptor knock-out (PRKO) mice. During early pregnancy, PIK3IP1 expression was strong at day 2.5 of gestation (GD 2.5) and then slightly decreased at GD 3.5 at the epithelium and stroma. After implantation, PIK3IP1 expression was detected at the secondary decidualization zone. To determine the ovarian steroid hormone regulation of PIK3IP1, we examined the expression of PIK3IP1 in ovariectomized control, $Pgr^{cre/+}Arid1a^{f/f}$, and PRKO mice treated with P4 or E2. P4 treatment increased the PIK3IP1 expression at the luminal and glandular epithelium of control mice. However, the PIK3IP1 induction was decreased in both the $Pgr^{cre/+}Arid1a^{f/f}$ and PRKO mice, compared to controls. Our results identified PIK3IP1 as a novel target of ARID1A and PGR in the murine uterus.

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Keywords

PIK3IP1; ARID1A; Uterus; Progesterone receptor

1. Introduction

The uterine epithelial and stromal compartments undergo dynamic molecular and morphological changes to prepare for development and pregnancy. Estrogen (E2) and progesterone (P4) meditate these changes by activating transcription of target genes through binding their cognate receptors. E2 stimulates proliferation of uterine epithelial cells, and P4 inhibits E2-mediated proliferation of uterine epithelial cells. The progesterone receptor (PGR) and estrogen receptors (ESRs) are the steroid hormone receptors that bind P4 and E2 respectively, and are critical in the regulation of the female reproductive system [1]. Both receptors are expressed within the uterus, and allow actions of P4 and E2 to commence [2,3].

Signaling of P4 through PGR is crucial for successful pregnancy [4]. PGR is encoded in humans by one gene, and is found in two isoforms, PR-A and PR-B [5,6]. The PR-A isoform is associated with ovarian and uterine function, whereas the PR-B isoform is necessary for proper mammary gland development [7,8]. The pivotal role of PGR within reproductive tissues causes it to be an area of focus within reproductive diseases. Specifically, reduced expression of PGR has been associated with endometriosis, a prevalent disease in women's health [9]. Overall, aberrations of steroid hormone signaling can be found in many uterine disorders including infertility [10], endometriosis [11], endometrial cancer [12], and uterine leiomyoma [13].

AT-rich interaction domain 1a (ARID1A) is one subunit within the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex [14]. It is located in chromosomal region 1p36, which is a region that is often deleted within many different forms of cancer [14,15]. A high frequency of mutation of ARID1A in cancers, specifically endometrioid cancer of the uterus and ovarian clear cell and endometrioid cancer, has revealed the potential of ARID1A to be defined as a tumor suppressor [16,17]. ARID1A is able to inhibit tumor growth and cellular proliferation [18]. Several studies have linked SWI/SNF and AR ID1A to transcriptional regulation, particularly nuclear hormone-induced transcription and expression of cell cycle regulators [19, 21]. Previously, we have shown that ARID1A protein levels are lower during both the proliferative and secretory phases, in epithelial and stromal cells of women with endometriosis compared to those without [22]. Ablation of *Arid1a* in the murine uterus results in the inability to inhibit E2-induced epithelial cell proliferation and E2-responsive target gene expression [22]. However, the molecular mechanism of ARID1A action in steroid hormone regulation and pregnancy is not well studied.

Phosphatidylinositol-3-kinase interacting protein 1 (PIK3IP1) is a transmembrane protein that is known to negatively regulate the PI3K pathway [23]. This down-regulation occurs due to PIK3IP1's shared homology to p85, the regulatory subunit of the PI3K pathway [25]. p85 binds to the p110 catalytic subunit of PI3K, and PI3KIP1's shared domain allows it to bind here as well, ultimately attenuating the pathway [25]. The PI3K pathway is involved

with many cellular processes, including angiogenesis, cell proliferation, motility, differentiation, and survival [4]. All of these processes are imperative for tumorigenesis, thus connecting the PI3K pathway to possible cancer development [4]. In this regard, PIK3IP1 can be seen as a tumor suppressor in that is down-regulates the PI3K pathway. Mutation of ARID1A results in a lower expression of PIK3IP1 [15]. PIK3IP1 has also been studied within the context of uterine and ovarian function, however results are limited. One study has revealed PIK3IP1 is able to induce apoptosis within some ovarian cancer subtypes [15]. Due to PIK3IP1's direct effect on the PI3K pathway, further study in relation to uterine and ovarian function is important for the pathogenesis of possible corresponding diseases.

PI3K is a well-known regulator of cell division, motility, and survival in most cell types. PIK3IP1 binds to the p110 catalytic subunit of PI3K and reduces its activity in vitro. However, the function and regulation of PIK3IP1 in the uterus have not been reported to date, and the biologic processes in which it is involved are unclear. Within the present study, we examined the spatiotemporal expression during early pregnancy and regulation of PIK3IP1 in the response to P4 and E2 in the uterus.

2. Materials and Methods

2.1 Animals and tissue collection

Mice were cared for and used in the designated animal care facility according to Michigan State University's institutional guidelines. All animal procedures were approved by the Institutional Animal Care and Use Committee of Michigan State University. For the early pregnancy study, C57BL/6 female mice at 8 weeks of age were mated with C57BL/6 male mice and uterine samples from pregnant mice were obtained at different days of pregnancy. The morning of vaginal plug observation was designated as day 0.5 of gestation (GD 0.5).

For the study of steroid hormone regulation, C57BL/6 female mice at 8 weeks of age were first ovariectomized at day 1. After this, there was a 2-week period of rest, enabling the mice to heal. The ovariectomized mice were utilized for injection, in which mice received vehicle (sesame oil; Sigma–Aldrich, St. Louis, MO), estradiol (E2, 1μg/mL; Sigma–Aldrich, St. Louis, MO), P4 (10mg/mL; Sigma–Aldrich, St. Louis, MO), or both E2 and P4 (1 μg/mL E2, 10 mg/mL P4). Following the time line (either 6 hour treatment, or 3 day treatment), mice were euthanized, and uterine tissues were immediately frozen at the time of dissection for real time qPCR or fixed with 4% (v/v) paraformaldehyde for immunohistochemistry.

2.2 Quantitative real-time PCR

The RNeasy total RNA isolation kit (Qiagen, Valencia, CA, USA) was utilized in order to extract RNA from uterine tissues. The mRNA levels of *Pik3ip1* were measured through real-time PCR TaqMan analysis, utilizing the Applied Biosystems StepOnePlus system (Applied Biosystems, Foster City, CA, USA). Pre-validated proves, primers, 18S RNA, and Universal Master mix reagent were purchased from Applied Biosystems (Applied Biosystems, Carlsbad, CA). The template cDNA was made with MMLV Reverse Transcriptase (Invitrogen Corp., Carlsbad, CA) and 1 μg of total RNA with use of random hexamers. The real-time PCR was all done with three independent RNA sets, and mRNA quantities were

normalized against the 18S RNA with use of the ABI rRNA control reagents. Statistical analyses were performed using Student's t-tests using the Instat package from GraphPad (San Diego, CA). $p<0.05$ was considered statistically significant.

2.3 Immunohistochemistry

Uterine tissue samples that were paraffin-embedded were sectioned at 6 μm and were mounted on glass slides. Slides sat overnight to dry. Slides were placed on a slide dryer for 15 minutes, then deparaffinized through exposure to a graded alcohol series [Xylene 3x5min (Fisher, Pittsburgh, PA), 100% ethanol 3 × 3min (Fisher, Pittsburgh, PA), 95% ethanol 2 × 2min, 70% ethanol 2 × 2min] and exposed to an antigen activator (1:100 antigen activator to distilled water; Vector Laboratories, Burlingame, CA). Slides were soaked in water, then incubated with a solution of 3% hydrogen peroxide (Sigma, St. Louis, MO) in methanol (Fisher, Pittsburgh, PA). After being rinsed, tissue samples were circled with a mini pap pen (Invitrogen, Carlsbad, CA) and exposed to a 10% normal goat serum (Vector Laboratories, Burlingame, CA) in PBS solution. The primary antibody (Santa Cruz, Dallas, Texas) was applied after removal of the NGS/PBS serum, and left to sit overnight at 4 degrees C, at the following solutions: 1:200 PIK3IP1, 1:1000 PGR. The following day, slides were rinsed, then exposed to a secondary antibody solution (Vector Laboratories, Burlingame, CA). Following this exposure, slides were again rinsed, and then exposed to a 1:1000 solution of dilute Streptavidin HRP (Vector Laboratories, Burlingame, CA) with PBS. Slides were rinsed, and a DAB solution (Vector Laboratories, Burlingame, CA) was applied. Slides were rinsed, then dipped with a following rinse, in the following reagents: hematoxylin (Biocare Medical, Pacheco, CA), hydrogen chloride (Sigma–Aldrich, St. Louis, MO), and lithium carbonate (Poly scientific, Bay Shore, NY). The steps of dehydration [2 × 2min 100% ethanol, 5min 100% ethanol, 2 × 2min Xylene, 5min Xylene] were followed, and cover slips were glued to the slides with permount (Fisher Scientific).

3. Results

3.1 PIK3IP1 as a target of ARID1A and PGR in the murine uterus

To determine transcriptional regulation of *Pik3ip1* by ARID1A, real-time qPCR was performed in the uteri of control (*Arid1a* $^{f/f}$) and *Pgr*$^{cre/+}$*Arid1a* $^{f/f}$ (*Arid1a*$^{d/d}$) mice [22] at GD 3.5. The results revealed that there was a significant decrease in the mRNA expression in the *Arid1a*$^{d/d}$ mice uterus compared to the control mice (Fig. 1A). This result was extended through immunohistochemistry to examine spatial expression of PIK3IP1 protein in the *Arid1a*$^{d/d}$ mice and progesterone receptor knock-out (PRKO) [26] (Fig. 1B). Control samples at GD 3.5 showed strong PIK3IP1 expression within the uterine epithelial, glandular, and stromal cells. However, *Arid1a*$^{d/d}$ mice showed a remarkable reduction in PIK3IP1 expression throughout when compared to control mice. PRKO mice showed a down-regulation of PIK3IP1 only in the stromal cells, but exhibited a comparable expression within the uterine epithelium and glands to control mice. These data suggest that the expression of PIK3IP1 is regulated by ARID1A and PGR.

3.2 PIK3IP1 expression in the uterus during early pregnancy

To investigate the expression profiles of PIK3IP1 in mouse uterus during early pregnancy, immunohistochemistry was performed in the uterus from GD 0.5 to GD 7.5 (Fig. 2). The initiation of pregnancy was marked by the presence of the postcoital vaginal plug (GD 0.5). At GD 0.5, PIK3IP1 expression was present within uterine epithelial and gland cells, however expression was not seen within stromal cells. At GD 2.5, expression increased, and was also present within stromal cells. At GD 3.5, PIK3IP1 levels showed a slight decrease, however remained strong in all three areas. At GD 4.5, embryos are attached to uterine epithelial cells at implantation sites. PIK3IP1 expression was seen within the inner cytoplasmic regions of the embryos throughout, however there was not any nuclear staining within the embryo itself. Weak expression was seen within the primary decidual zone, though expression was strong within the secondary decidual zone. At GD 5.5, the area surrounding the embryo showed an increase in PIK3IP1 expression. The primary decidual zone showed no PIK3IP1 expression, while the secondary decidual zone continued to show strong expression. This pattern was similarly found in GD 7.5 mice. These data indicate that PIK3IP1 is tightly regulated in the uterus during early pregnancy.

3.3 The regulation of PIK3IP1 by P4 in the uterus

In order to evaluate PIK3IP1 expression in response to P4 treatment, immunohistochemistry was performed on ovariectomized control (wild type and *Arid1a*$^{f/f}$), *Arid1a*$^{d/d}$, and PRKO mice treated with either vehicle or P4 for 6 hours (Fig. 3). Within the control mice, PGR expression was present in only the epithelial cells, showing no expression in stroma. After P4 treatment in control mice, epithelial expression of PGR decreased. Interestingly, PGR expression was completely gone within the uterus of *Arid1a*$^{d/d}$ mice. PRKO mice were used as a negative control and did not show PGR expression in the uterus. These results suggest that PGR expression is also regulated by ARID1A.

PIK3IP1 expression was relatively low within the ovariectomized control mice treated with vehicle, showing weak gland and epithelial staining. After P4 treatment, a strong expression was induced within the epithelial and gland cells of control mice. Both the *Arid1a*$^{d/d}$ and the PRKO mouse models showed no expression of PIK3IP1 after vehicle treatment. PIK3IP1 expression also did not increase within these models after P4 treatment. This data further reveals that PIK3IP1 is regulated by PGR and ARID1A.

3.4 The regulation of PIK3IP1 by E2 in the uterus

To determine whether PIK3IP1 expression is regulated by E2, immunohistochemistry was performed in the uteri from ovariectomized female mice with E2 treatment (Fig. 4). Vehicle treated samples showed relatively weak expression patterns, with expression presence in the luminal and glandular epithelium only, as we observed in Fig. 3. After 6 hours of E2 treatment, expression within the luminal and glandular epithelium increased, and there was a slight increase in stromal expression (Fig. 4B). This increase in expression was even more drastic after 3 day treatment, with very strong expression in both the luminal and glandular epithelium, however expression within the stroma was not stronger after 3 day treatment (Fig. 4E).

To determine whether P4 antagonizes the effect of E2 in PIK3IP1 expression, immunohistochemistry was performed in the uteri from ovariectomized female mice with E2 plus P4. E2+P4 treatment showed a similar trend: 6-hour treatment resulted in increased expression in the epithelial and glandular cells, and 3 day treatment resulted in an increase in epithelial, stromal, and glandular expression. There was no observed difference between PIK3IP1 expression patterns after E2 treatment compared to expression patterns after both E2 and P4 treatment. The results suggest that PIK3IP1 expression is induced by E2.

4. Discussion

For the first time, this study looks at the regulation of *Pik3ip1* by ARID1A and PGR, and determined the expression patterns of PIK3IP1 in the uterus during early pregnancy and in response to ovarian steroid hormone treatment. In the present study, we report that PIK3IP1 expression levels are significantly lower in mice with conditional ablation of *Arid1a* (*Arid1a*$^{d/d}$) (Fig 1). In response to a lack of *Arid1a*, mice exhibited a lessened expression of PIK3IP1, further confirming PIK3IP1 is a target of *Arid1a*.

Throughout implantation and early pregnancy, fluctuations in steroid hormone expression are exhibited. GD 0.5 and 1.5 in murine pregnancy are the days in which pre-ovulatory ovarian E2 is secreted, and proliferation occurs. From GD 2.5 on, proliferation shifts from the luminal and glandular epithelial cells to the stromal cells. Overall, we observed fluctuation of PIK3IP1 expression (Fig. 2). At GD 2.5 expression levels were the highest overall. E2 stimulated proliferation occurs during the first two days of pregnancy in the mouse uterus [28]. Our result then allows us to conclude PIK3IP1 is regulated by E2 during early pregnancy. On GD 3.5, the uterus shifts from a pre-receptive state to a P4 induced receptive state, allowing for implantation [28]. At GD 4.5, implantation occurs, as stromal cells transition into decidual cells in response to a blastocyst [27]. In the present study, results showed low levels of PIK3IP1 expression in the primary decidual zone (PDZ), whereas its expression was high in the secondary decidual zone (SDZ). PDZ will eventually degenerate by day 8, whereas SDZ, stemming from differentiated PDZ cells into polyploidy decidual cells, will be slowly replaced by placental and embryonic growth after GD 7.5 [28,29]. This allows for a potential connection between placental and embryonic growth development and PIK3IP1 expression.

PRKO is useful in assessing P4's role in gene and protein expression. Past research has revealed that the ablation of PGR results in abnormalities in the reproductive biology of the murine mouse model, which includes an increase in the response to both P4 and E2, and also a defect in the implantation process [30]. Within this study, we utilized the PRKO mouse model in order to analyze the expression of PIK3IP1 in response to the ablation of both PGR isoforms (Fig. 1 and Fig. 3). Results showed a difference in only stromal expression of PIK3IP1 in the PRKO mouse model in comparison to controls. Stromal cells are associated with the production of paracrine factors, mediated through the PGR receptors, which are critical in enhancing epithelial differentiation and growth within the uterus [31]. These results suggest that stromal PIK3IP1 expression is regulated by PGR.

P4 is a steroid hormone that is critical in reproductive processes, and has been utilized for therapeutic treatment in peri-menopausal women who experience aberrant bleeding or menstrual problems [32,33]. In order to analyze P4's effect on PIK3IP1 expression, we examined PIK3IP1 expression within control, *Arid1a*$^{d/d}$, and PRKO mice, with both vehicle and 6-hour P4 treatment (Fig. 3). In this analysis we found that P4 treatment increased expression in the control mice. This result allows us to hypothesize P4's role in PIK3IP1 expression, and also allows for a connection of P4 treatment in the two different mouse models. Within the *Arid1a*$^{d/d}$ mouse model we see no PIK3IP1 expression in the vehicle treatment, however we are able to see a slight increase in expression after P4 treatment for 6 hours. Seeing as how PIK3IP1 is able to inhibit PI3K stimulated cell growth, proliferation, and survival, this increased expression after ovarian hormone treatment allows for a possible connection to therapeutic treatments necessary for inhibition of this very process.

Within the context of our previous results, we are already able to see that ovarian steroid hormones E2 and P4 have an illustrated effect on PIK3IP1 expression. However, to further confirm this effect, we observed treatment of ovariectomized mice with E2, P4, and a combination of both E2 and P4 (Fig. 4). Our results showed very low expression of PIK3IP1 within the vehicle treated ovariectomized mice, revealing that ovarian hormones do not singularly control PIK3IP1. However, after P4, E2, and E2 + P4 treatments PIK3IP1 was significantly expressed, revealing a direct effect of all hormone treatments on PIK3IP1. This increase in expression was seen after 6-hour treatment, although after 3-day treatment there was a greater increase in expression. Through this revelation, we are able to make a possible connection from P4, E2, or E2 + P4 treatment within these cells to potential therapeutic treatment.

Our results demonstrate that PIK3IP1 is a novel target of ARID1A and PGR in the murine uterus. Additional research analysis within this context would provide for greater insight into the role of PIK3IP1 within the context of reproductive biology, and provide for a new possible therapeutic target within uterine disorder.

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References

1. Large MJ, DeMayo FJ. The regulation of embryo implantation and endometrial decidualization by progesterone receptor signaling. Mol Cell Endocrinol. 2012; 358:155–165. [PubMed: 21821095]
2. Acconcia F, Kumar R. Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Lett. 2006; 238:1–14. [PubMed: 16084012]
3. Li X, O'Malley BW. Unfolding the action of progesterone receptors. J Biol Chem. 2003; 278:39261–39264. [PubMed: 12893816]
4. Wetendorf M, DeMayo FJ. Progesterone receptor signaling in the initiation of pregnancy and preservation of a healthy uterus. Int J Dev Biol. 2014; 58:95–106. [PubMed: 25023675]
5. Grandi G, Toss A, Cortesi L, Botticelli L, Volpe A, Cagnacci A. The Association between Endometriomas and Ovarian Cancer: Preventive Effect of Inhibiting Ovulation and Menstruation during Reproductive Life. Biomed Res Int. 2015; 2015:751571. [PubMed: 26413541]

6. Broi MGD, Rocha CVJ, Meola J, Martins WP, Carvalho FM, Ferriani RA, Navarro PA. Expression of PGR, HBEGF, ITGAV, ITGB3 and SPP1 genes in eutopic endometrium of infertile women with endometriosis during the implantation window: a pilot study. JBRA Assist Reprod. 2017; 21:196–202. [PubMed: 28837027]
7. Conneely OM, Mulac-Jericevic B, DeMayo F, Lydon JP, O'Malley BW. Reproductive functions of progesterone receptors. Recent Prog Horm Res. 2002; 57:339–355. [PubMed: 12017551]
8. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci U S A. 2003; 100:9744–9749. [PubMed: 12897242]
9. Grandi G, Mueller MD, Bersinger NA, Facchinetti F, McKinnon BD. The association between progestins, nuclear receptors expression and inflammation in endometrial stromal cells from women with endometriosis. Gynecol Endocrinol. 2017; 33:712–715. [PubMed: 28412861]
10. Han SJ, O'Malley BW. The dynamics of nuclear receptors and nuclear receptor coregulators in the pathogenesis of endometriosis. Hum Reprod Update. 2014; 20:467–484. [PubMed: 24634322]
11. Patel BG, Rudnicki M, Yu J, Shu Y, Taylor RN. Progesterone resistance in endometriosis: origins, consequences and interventions. Acta Obstet Gynecol Scand. 2017; 96:623–632. [PubMed: 28423456]
12. Yoo JY, Yang WS, Lee JH, Kim BG, Broaddus RR, Lim JM, Kim TH, Jeong JW. MIG-6 negatively regulates STAT3 phosphorylation in uterine epithelial cells. Oncogene. 2017
13. Islam MS, Ciavattini A, Petraglia F, Castellucci M, Ciarmela P. Extracellular matrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics. Hum Reprod Update. 2017:1–27. [PubMed: 29190351]
14. Chunder N, Mandal S, Basu D, Roy A, Roychoudhury S, Panda CK. Deletion mapping of chromosome 1 in early onset and late onset breast tumors--a comparative study in eastern India. Pathol Res Pract. 2003; 199:313–321. [PubMed: 12908521]
15. Takeda T, Banno K, Okawa R, Yanokura M, Iijima M, Irie-Kunitomi H, Nakamura K, Iida M, Adachi M, Umene K, Nogami Y, Masuda K, Kobayashi Y, Tominaga E, Aoki D. ARID1A gene mutation in ovarian and endometrial cancers (Review). Oncol Rep. 2016; 35:607–613. [PubMed: 26572704]
16. Guan B, Mao TL, Panuganti PK, Kuhn E, Kurman RJ, Maeda D, Chen E, Jeng YM, Wang TL, Shih Ie M. Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. Am J Surg Pathol. 2011; 35:625–632. [PubMed: 21412130]
17. Jones S, Wang TL, Shih Ie M, Mao TL, Nakayama K, Roden R, Glas R, Slamon D, Diaz LA Jr, Vogelstein B, Kinzler KW, Velculescu VE, Papadopoulos N. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010; 330:228–231. [PubMed: 20826764]
18. Guan B, Wang TL, Shih Ie M. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. Cancer Res. 2011; 71:6718–6727. [PubMed: 21900401]
19. Mao TL, Shih Ie M. The roles of ARID1A in gynecologic cancer. J Gynecol Oncol. 2013; 24:376–381. [PubMed: 24167674]
20. Samartzis EP, Noske A, Dedes KJ, Fink D, Imesch P. ARID1A mutations and PI3K/AKT pathway alterations in endometriosis and endometriosis-associated ovarian carcinomas. Int J Mol Sci. 2013; 14:18824–18849. [PubMed: 24036443]
21. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? Cancer Discov. 2013; 3:35–43. [PubMed: 23208470]
22. Kim TH, Yoo JY, Wang Z, Lydon JP, Khatri S, Hawkins SM, Leach RE, Fazleabas AT, Young SL, Lessey BA, Ku BJ, Jeong JW. ARID1A Is Essential for Endometrial Function during Early Pregnancy. PLoS Genet. 2015; 11:e1005537. [PubMed: 26378916]
23. DeFrances MC, Debelius DR, Cheng J, Kane LP. Inhibition of T-cell activation by PIK3IP1. Eur J Immunol. 2012; 42:2754–2759. [PubMed: 22706993]
24. He X, Zhu Z, Johnson C, Stoops J, Eaker AE, Bowen W, DeFrances MC. PIK3IP1, a negative regulator of PI3K, suppresses the development of hepatocellular carcinoma. Cancer Res. 2008; 68:5591–5598. [PubMed: 18632611]

25. Song HK, Kim J, Lee JS, Nho KJ, Jeong HC, Kim J, Ahn Y, Park WJ, Kim DH. Pik3ip1 modulates cardiac hypertrophy by inhibiting PI3K pathway. PLoS One. 2015; 10:e0122251. [PubMed: 25826393]

26. Fernandez-Valdivia R, Jeong J, Mukherjee A, Soyal SM, Li J, Ying Y, Demayo FJ, Lydon JP. A mouse model to dissect progesterone signaling in the female reproductive tract and mammary gland. Genesis. 2010; 48:106–113. [PubMed: 20029965]

27. Huet-Hudson YM, Andrews GK, Dey SK. Cell type-specific localization of c-myc protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. Endocrinology. 1989; 125:1683–1690. [PubMed: 2667965]

28. Das SK. Regional development of uterine decidualization: molecular signaling by Hoxa-10. Mol Reprod Dev. 2010; 77:387–396. [PubMed: 19921737]

29. Paria BC, Zhao X, Das SK, Dey SK, Yoshinaga K. Zonula occludens-1 and E-cadherin are coordinately expressed in the mouse uterus with the initiation of implantation and decidualization. Dev Biol. 1999; 208:488–501. [PubMed: 10191061]

30. Vasquez YM, DeMayo FJ. Role of nuclear receptors in blastocyst implantation. Semin Cell Dev Biol. 2013; 24:724–735. [PubMed: 23994285]

31. Pabona JM, Zeng Z, Simmen FA, Simmen RC. Functional differentiation of uterine stromal cells involves cross-regulation between bone morphogenetic protein 2 and Kruppel-like factor (KLF) family members KLF9 and KLF13. Endocrinology. 2010; 151:3396–3406. [PubMed: 20410205]

32. Czyzyk A, Podfigurna A, Genazzani AR, Meczekalski B. The role of progesterone therapy in early pregnancy: from physiological role to therapeutic utility. Gynecol Endocrinol. 2017; 33:421–424. [PubMed: 28277122]

33. Prior JC. Progesterone for Symptomatic Perimenopause Treatment - Progesterone politics, physiology and potential for perimenopause. Facts Views Vis Obgyn. 2011; 3:109–120. [PubMed: 24753856]

34. Wei JJ, William J, Bulun S. Endometriosis and ovarian cancer: a review of clinical, pathologic, and molecular aspects. Int J Gynecol Pathol. 2011; 30:553–568. [PubMed: 21979592]

35. Dowlut-McElroy T, Strickland JL. Endometriosis in adolescents. Curr Opin Obstet Gynecol. 2017

- PIK3IP1 is tightly regulated in the murine uterus during early pregnancy.
- PIK3IP1 is a target of ARID1A and PGR in the murine uterus.
- PGR expression is regulated by ARID1A.
- PIK3IP1 expression is induced by progesterone and estrogen.

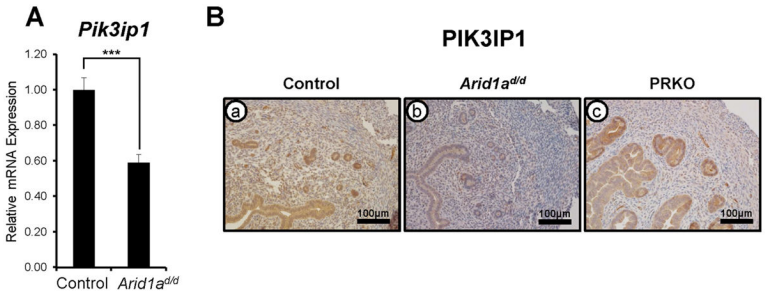

Figure 1. The expression of PIK3IP1 in the uteri of control, *Arid1a*$^{d/d}$, and PRKO mice

(A) The expression pattern of *Pik3ip1* from control and *Arid1a*$^{d/d}$ mice uteri by real-time qPCR. Total RNA used for the real-time qPCR assay was prepared from control and *Arid1a*$^{d/d}$ mice uteri at GD 3.5. The results represent the mean ± SEM of three independent RNA sets. $p < 0.05$. (B) The localization pattern of PIK3IP1 proteins by immunohistochemistry in the uteri of control (a), *Arid1a*$^{d/d}$ (b), and PRKO (c) mice. Uterine sections were collected from control, *Arid1a*$^{d/d}$, and PRKO mice at GD 3.5.

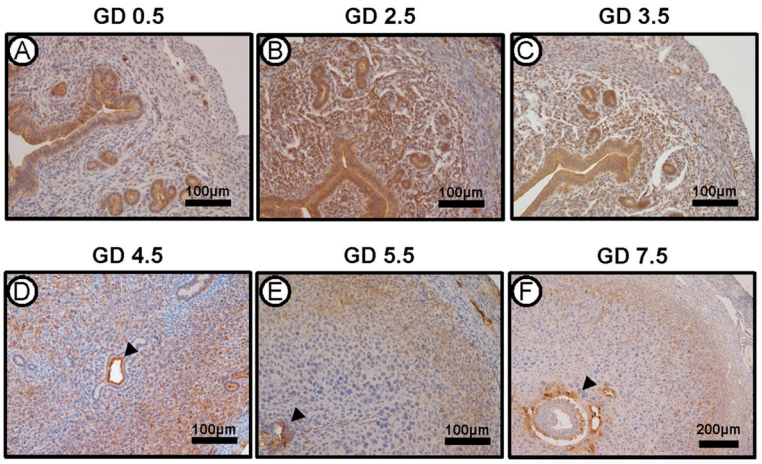

Figure 2. The localization pattern of PIK3IP1 during early pregnancy

Representative photomicrographs show immunohistochemical staining of PIK3IP1 proteins in the uterus from GD 0.5 to GD 7.5. The arrow indicates embryo.

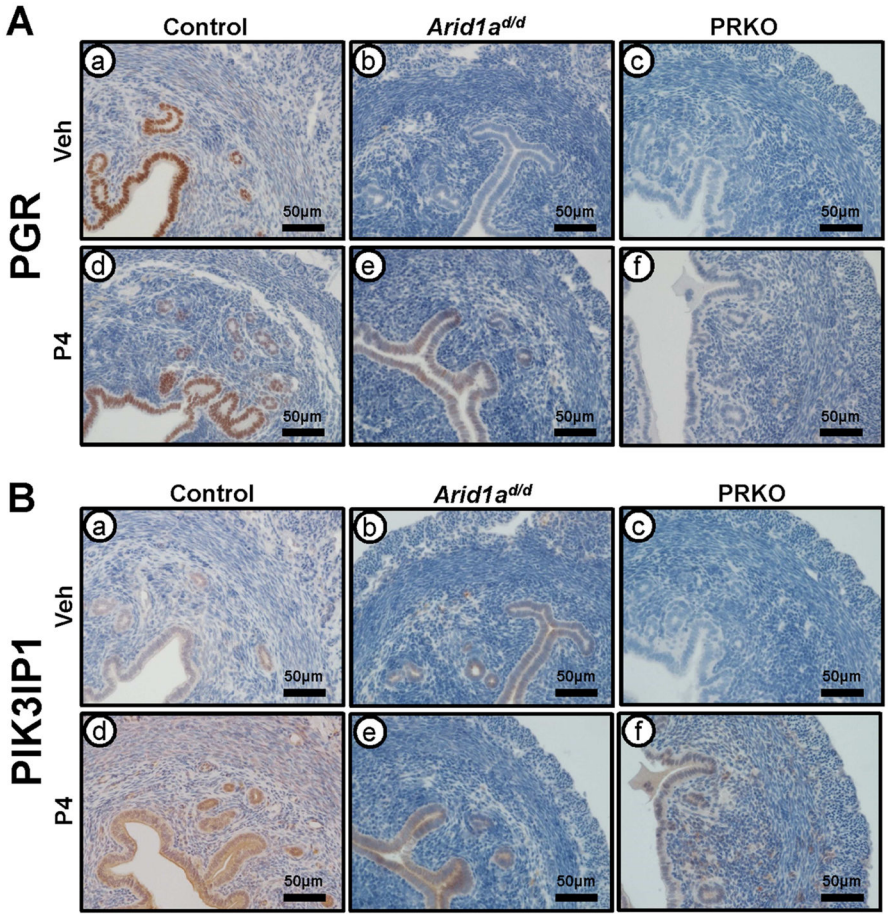

Figure 3. The regulation of PGR and PIK3IP1 expression by P4 treatment in the uterus

Representative photomicrographs show the localization pattern of PGR (A) and PIK3IP1 (B) by immunohistochemistry in the vehicle or P4-treated uteri from control (a and d), *Arid1a*$^{d/d}$ (b and e), and PRKO (c and f). Uterine sections were collected from ovariectomized control, *Arid1a*$^{d/d}$, and PRKO that were treated with P4 or vehicle (sesame oil) for 6 hours.

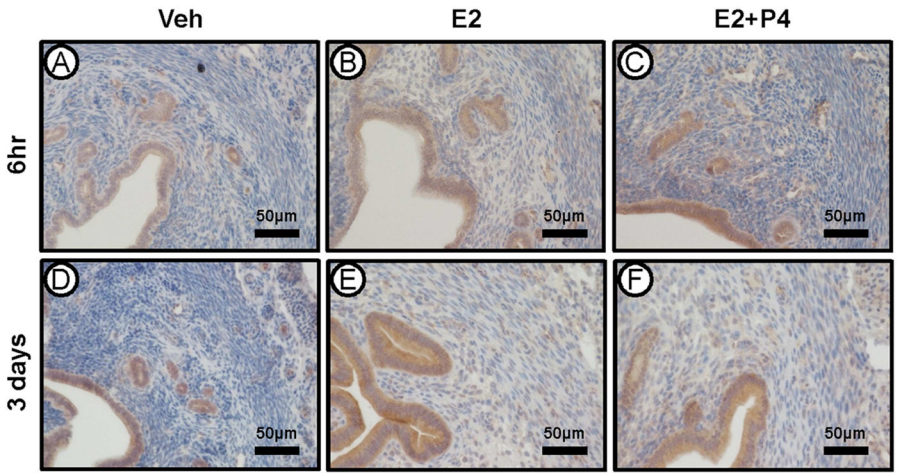

Figure 4. The expression of PIK3IP1 by E2

Representative photomicrographs show immunohistochemical staining of PIK3IP1 proteins in the uterus from mice treated with vehicle or E2. Uterine sections were collected from ovariectomized wild-type mice treated with vehicle (A and D), E2 (B and E), and E2 + P4 (C and F) for 6 hours (A–C) and 3 days (D–F).