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Research Article

Interleukin-13 receptor subunit alpha-2 is a target of progesterone receptor and steroid receptor coactivator-1 in the mouse uterus†

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Abstract

The endometrium, composed of epithelial and stromal cell compartments, is tightly regulated by the ovarian steroid hormones estrogen (E2) and progesterone (P4) during early pregnancy. Through the progesterone receptor (PGR), steroid receptor coactivators, and other transcriptional coregulators, progesterone inhibits E2-induced cell proliferation and induces the differentiation of stromal cells in a process called decidualization to promote endometrial receptivity. Although interleukin-13 receptor subunit alpha-2 (*Il13ra2*) is expressed in the human and mouse endometrium, its potential role in the steroid hormone regulation of the endometrium has not been thoroughly examined. In this study, we employed PGR knockout mice and steroid receptor coactivator-1 knockout mice (SRC-1$^{-/-}$) to profile the expression of *Il13ra2* in the murine endometrium and determine the role of these transcriptional regulators in the hormone-responsiveness of *Il13ra2* expression. Furthermore, we utilized a well-established decidualization-inducing steroidogenic cocktail and a siRNA-based knockdown of *IL13RA2* to determine the importance of *IL13RA2* in the decidualization of primary human endometrial stromal cells. Our findings demonstrate that *Il13ra2* is expressed in the subepithelial stroma of the murine endometrium in response to ovarian steroid hormones and during early pregnancy in a PGR- and SRC-1-dependent manner. Furthermore, we show that knockdown of *IL13RA2* before in vitro decidualization of primary human endometrial stromal cells partially compromises the full decidualization response.

We conclude that *Il13ra2* is a downstream target of progesterone through PGR and SRC-1 and plays a role in mediating the stromal action of ovarian steroid hormones.

Summary Sentence

Expression of *Il13ra2* is induced by progesterone in the murine uterine stroma through the progesterone receptor and steroid receptor coactivator-1, and it is involved in human stromal cell decidualization.

Key words: endometrium, progesterone receptor, steroid hormones, *Il13ra2*.

Introduction

The uterus is a complex organ, which is made up of unique, heterogeneous cell types with specialized roles that work together to establish and sustain pregnancy. The myometrium, the outer layer composed primarily of smooth muscle, gives the uterus structure and contracts during parturition [1], while the endometrium comprises the inner lining that is the primary player in uterine receptivity to embryo implantation [2]. Within the endometrium, epithelial cells and surrounding stromal cells must coordinate seamlessly to provide a window of receptivity where the epithelium ceases proliferation to allow blastocyst invasion, at which time stromal cells transition into decidual cells to support and nourish the embryo development [3–5].

Each step of this complex process is temporally and spatially governed by the ovarian steroid hormones estrogen (E2) and progesterone (P4) acting through their cognate receptors (estrogen receptor and progesterone receptor [PGR]) and downstream signaling pathways that induce target gene transcription [6, 7]. Dysregulation of the tightly regulated balance of E2 and P4 commonly results in uterine disorders such as infertility, endometriosis, and endometrial cancer [8–10]. E2 dominates the proliferative phase of the menstrual cycle to allow thickening of the endometrium until P4 causes the transition into the secretory phase, when proliferation ceases, decidualization occurs, and the window of receptivity opens [10]. Decidualization, a key piece of this process, involves the differentiation of endometrial stromal cells to an epithelioid, secretory phenotype, and these supportive cells surround an implanting embryo to facilitate normal pregnancy establishment [7].

P4 is often labeled the "hormone of pregnancy" in part because of how integral expression of its target genes is to successful implantation [6]. Like other steroid hormones, P4's genomic effects are primarily enacted through binding its cognate nuclear receptor, PGR, which induces transcription of its target genes [3, 11, 12]. Analysis of PGR knockout (PRKO) mice revealed the critical nature of PGR in the female reproductive tract because PRKO females had many reproductive abnormalities, including anovulation and a defect of decidualization [12].

When P4 binds PGR, the complex does not carry out its functions independently but instead recruits coregulators that are necessary for proper modification of chromatin and assembling of transcriptional machinery [13]. One family of such coregulators intimately connected with P4 signaling is the steroid receptor coactivator (SRC) family, consisting of SRC-1, SRC-2, and SRC-3 [7, 14]. In the endometrium, SRC-3 dysregulation is primarily associated with increased cancer risk [15, 16], but SRC-1 and SRC-2 are expressed in both the endometrial epithelium and stroma and are critical mediators of P4 action in the decidualization response [7, 14, 17]. SRC-1 loss alone does not render mice infertile [18], but knocking out SRC-2 in the uterus causes fertility defects that are exacerbated by the additional loss of SRC-1, firmly establishing the need for these two coregulators in proper P4 regulation of the endometrium [14, 19].

One role of P4 signaling in the uterus is to downregulate inflammation, which involves discouraging high levels of proinflammatory cytokines [8, 20]. Interleukin-13 (IL-13) is a cytokine linked primarily to immune dysregulation in various autoimmune and allergic diseases [21], but it is also expressed in the uterus throughout the menstrual cycle [22] and responds to ovarian steroid hormones in endometrial stromal and epithelial cells [23]. IL-13 is generally considered anti-inflammatory in that it inhibits the production of proinflammatory cytokines such as IL-1β, IL-6, IL-8, tumor necrosis factor-alpha, granulocyte colony-stimulating factor, and interferon-alpha [24, 25]. In the endometrium, IL-13 is found in all cellular compartments, but its expression is strongest in the luminal epithelium [22]. IL-13 appears to act primarily through binding a heterodimer composed of IL-13 receptor subunit alpha-1 (IL13RA1) and IL-4 receptor alpha (IL4R), but it also recognizes IL-13 receptor subunit alpha-2 (IL13RA2) [26–28]. IL13RA2 was first thought to only act as a decoy receptor to compete for and sequester IL-13; however, more recent findings indicate an active signaling role for IL13RA2 [27, 28] in extracellular signal-regulated kinase (ERK)/activator protein 1 (AP-1)-mediated proliferation [29] and transforming growth factor-beta-mediated fibrosis [30].

The binding of IL-13 by IL13RA2 amplifies the invasion and metastasis of pancreatic [31] and ovarian cancers [29] and has been suggested as a biomarker for ovarian cancer [32]. Increased matrix metalloproteinase expression in response to IL13RA2 binding activates the AP-1 transcription factor pathway and ERKs, which, in turn, encourage proliferative action in cancer cells [29]. Research on the role of IL13RA2 in the endometrium is incomplete. Though IL-13 expression in the endometrium appears to influence inflammatory conditions [23] and is upregulated in women with recurrent spontaneous abortion [22], its biological significance is unclear, and any importance of IL13RA2 in this role of IL-13 is unknown. However, *IL13RA2* was identified by microarray analysis both in human endometrial stromal cells (hESCs), where it was found downregulated in response to P4 treatment [33], and in mice, where it was found downregulated at the implantation site (IS) in one study [34] and upregulated in response to P4 in another study [35]. Furthermore, one study found that *Il13ra2* expression is induced by steroid hormones in the mouse uterus dependent on the presence of SRC-1 [36]. Together, these data indicate a potential role for *IL13RA2* in steroid hormone regulation of the endometrium. Within the present study, we examined the spatiotemporal expression of *Il13ra2* in the mouse uterus during early pregnancy and the regulation of *Il13ra2* in response to P4 and E2 in the uterus. Furthermore, we characterized PGR- and SRC-1-dependent regulation of *Il13ra2* expression in the

mouse uterus and investigated the importance of *IL13RA2* in the in vitro decidualization of primary hESCs.

Materials and methods

Animals and tissue collection

Mice were maintained in the designated animal care facility at Michigan State University according to the institutional guidelines for the care and use of laboratory animals. For hormone response tests, mice at 6 weeks of age were ovariectomized. Two weeks later, ovariectomized wild type, PRKO [12], and SRC-1$^{-/-}$ (SRC-1 knockout) [18] mice were injected with one of the following: vehicle (sesame oil), P4 (1 mg/mouse), E2 (0.1 µg/mouse), or a combination of P4 and E2 at the same doses as administered individually. Mouse uteri were collected at 4, 16, or 40 h of treatment ($n = 3$/group). The injections were repeated every 12 h for the 16 and 40 h samples. Uterine samples from specific stages of pregnancy were obtained by the mating of wild type mice, and the morning of a vaginal plug was designated as gestation day (GD) 0.5. Uterine samples for in situ hybridization at GD 0.5, GD 2.5, and GD 4.5 were taken from pseudopregnant mice after mating with vasectomized males. Samples at GD 5.5 and GD 7.5 were taken from IS regions of normal pregnant uteri. Samples for quantitative real-time polymerase chain reaction (RT-qPCR) were taken from pregnant mice, with GD 5.5 and GD 7.5 samples separated into IS and interimplantation site (I-IS) regions. Uterine tissues were flash frozen at the time of dissection and stored at −80 °C for later RNA extraction or fixed with 10% (*v*/*v*) formalin for in situ hybridization.

Quantitative real-time PCR

RNA was extracted from the uterine tissues or cultured cells using the RNeasy total RNA isolation kit (Qiagen, Valencia, CA). Expression levels of mouse *Il13ra2* mRNA and human stromal cell *IL13RA2* mRNA were measured by RT-qPCR TaqMan analysis using the ABI Prism 7700 Sequence Detector System according to the manufacturer's instructions (PE Applied Biosystems, Foster City, CA). Prevalidated probes and primers were purchased from Applied Biosystems for *Il13ra2* (00515166), *IL13RA2* (00152924), *RPL7* (02596927), *Gapdh* (99999915), and 18S RNA (99999901). cDNA was produced from 1 µg of total RNA using random hexamers and MMLV Reverse Transcriptase (Invitrogen, Carlsbad, CA). RT-qPCR was performed using RT-qPCR Universal Master Mix reagent (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. All RT-qPCR was done by using three independent RNA sets. Target gene mRNA quantities were normalized against 18S (mouse), *Gapdh* (mouse) or *RPL7* (human).

In situ hybridization

The protocol for in situ hybridization was essentially as described previously [37, 38]. Briefly, uterine tissues were fixed at room temperature in 10% (*v*/*v*) formalin then dehydrated using a graded ethanol series before embedding in paraffin. Tissue sections were mounted onto poly-L-lysine-coated slides (VWR Scientific Products, West Chester, PA) before use in in situ hybridization. Generation of riboprobes was accomplished by in vitro transcription of amplified DNA products containing the T7 polymerase promoter sequence flanking the *Il13ra2* nucleotide primer sequence using [^{35}S] UTP (Promega, Madison, WI). Slides were incubated in a buffer containing Proteinase K, Tris, and EDTA, acetylated with acetic anhydride, dehydrated, and exposed to denatured antisense or sense probes in hybridization buffer. Hybridization was performed overnight before exposure to RNase A and washing in a mixture of formamide, saline sodium citrate, and 2-mercaptoethanol, followed by dehydration in a graded series of ethanol in ammonium acetate. The slides were then exposed to Biomax MR film (Kodak, Rochester, NY) overnight before being dipped in autoradiography emulsion (Amersham Biosciences, Piscataway, NJ) and being stored for several days in a refrigerated light proof box. At the completion of the developing process, slides were counterstained with hematoxylin.

Primary hESC cultures and in vitro decidualization

Human stromal cell isolation, culture, siRNA knockdown, and in vitro decidualization were performed based on previously described protocols [39, 40]. Human primary endometrial stromal cells (hESCs) were obtained from Michigan State University's Center for Women's Health Research Female Reproductive Tract Biorepository. Samples were obtained with written informed consent from premenopausal women undergoing hysterectomy for benign indications in the absence of all hormonal therapies for at least 90 days before surgery. Isolation of hESCs from pure endometrial tissue by collagenase digestion and filtration was performed as previously described [41]. Isolated cells were cultured and grown to confluency in phenol red–free RPMI-1640 medium (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS; Gibco), 0.1 mM sodium pyruvate (Gibco), and 1% penicillin streptomycin (P/S; Gibco). For induction of in vitro decidualization, cells were transferred to OPTI-MEM medium (Gibco) with 2% FBS depleted of steroids by pretreatment with dextran-coated charcoal (CS-FBS; Gibco), 10 nM estradiol (E2, Sigma-Aldrich, St. Louis, MO), 1 µM medroxyprogesterone acetate (Sigma-Aldrich), 50 µM cAMP (Sigma-Aldrich), and 1% P/S. Growth media were replaced every 2 days for the course of the 6-day treatment. Transfection with *IL13RA2* siRNA (L-004598-00-0020, Dharmacon, Lafayette, CO) to achieve mRNA knockdown and with nontargeting scrambled siRNA (NC1486135, Dharmacon) as a control was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) based on the manufacturer's instructions before in vitro decidualization. Briefly, siRNAs were diluted in Opti-MEM to 50 nM then mixed with diluted transfection agent. After complex formation, complexes were diluted in Opti-MEM with 2% CS-FBS to make transfection media and added to confluent hESCs. Cells were incubated with transfection media for 6 h before replacing with growth media and incubating for 2 days before in vitro decidualization induction.

Statistical analysis

Statistical analyses were performed using Student's *t*-test for data with only two groups. For tests involving multiple comparisons, we performed one-way ANOVA analysis followed by Tukey's *post hoc* test using the Instat package from GraphPad (GraphPad Software, Inc., San Diego, CA). A value of $P < 0.05$ was considered statistically significant.

Results

Il13ra2 is a target of SRC-1 and PGR in the mouse uterus

To examine whether *Il13ra2* expression is induced by P4, we first analyzed the levels of *Il13ra2* mRNA in ovariectomized wild type mouse uteri 4 h after vehicle or P4 treatment using RT-qPCR. *Il13ra2* expression was significantly upregulated in the P4-treated

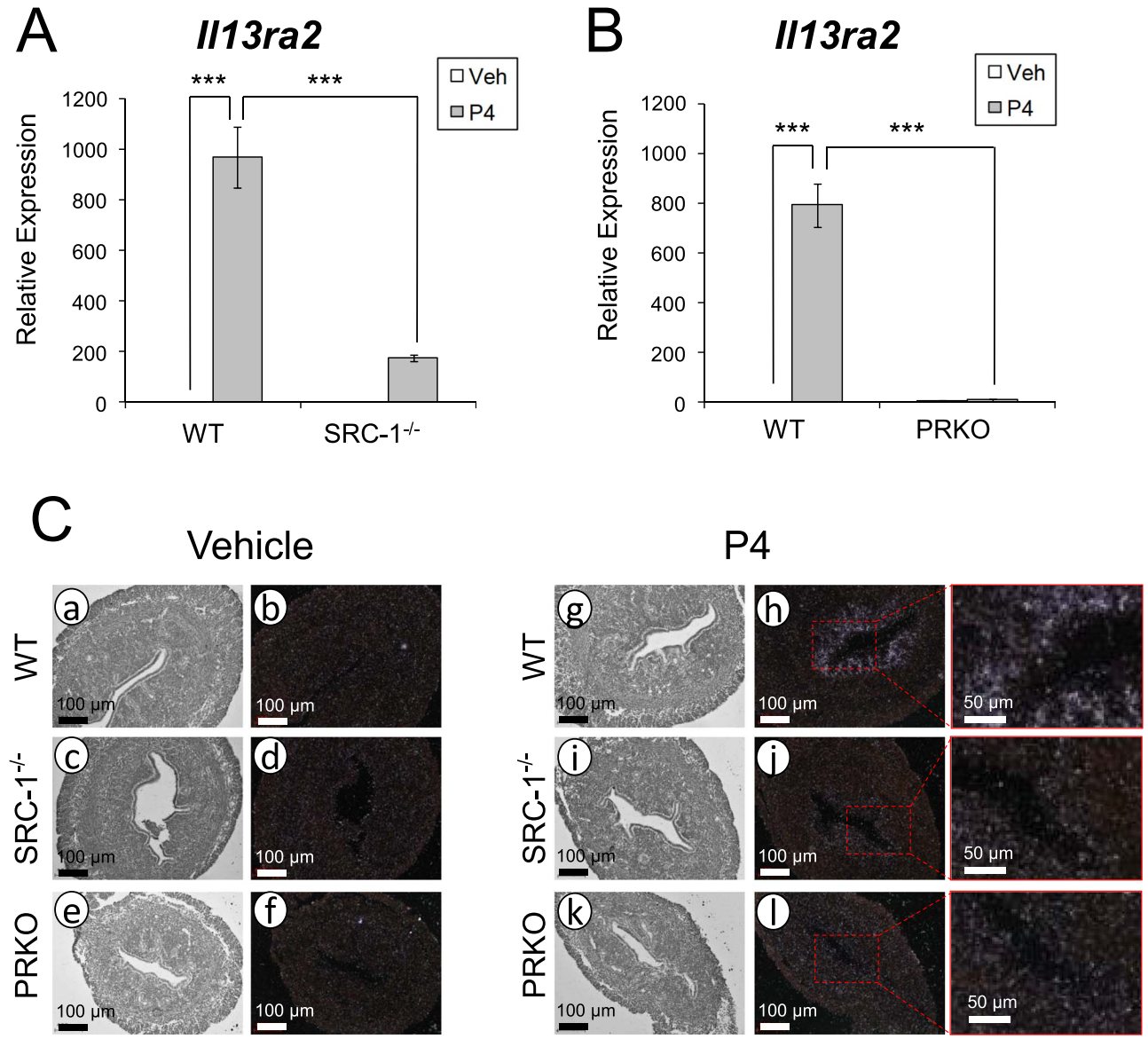

Figure 1. Uterine *Il13ra2* expression in response to P4 treatment. (A and B) Relative *Il13ra2* mRNA expression 4 h after administration of vehicle or P4 as measured by RT-qPCR in whole uterine tissue preparations from ovariectomized wild type (WT), SRC-1$^{-/-}$, and PRKO mice. Graphs represent the mean ± SEM ($n = 3$; ***$P < 0.001$). (C) Bright-field (a, c, e, g, i, k) and dark-field (b, d, f, h, j, l) photomicrographs of WT, SRC-1$^{-/-}$, and PRKO mouse uterine sections after in situ hybridization probing for *Il13ra2* mRNA 4 h after administration of vehicle (a–f) or P4 (g–l; $n = 3$).

group compared to the vehicle-treated group (Figure 1A and B). To test whether this induction is dependent on SRC-1 and PGR, we utilized ovariectomized SRC-1$^{-/-}$ [18] and PRKO [12] mice and again treated with P4 or vehicle. We observed a significant decrease in *Il13ra2* mRNA expression in both SRC-1$^{-/-}$ and PRKO mice compared to wild type mice treated with P4 (Figure 1A and B).

To gain an understanding of the spatial localization of *Il13ra2* mRNA, we performed in situ hybridization on uterine cross-sections from wild type, SRC-1$^{-/-}$, and PRKO mice treated with either vehicle or P4. We observed signal only in the wild type P4-treated group, as expected, but the expression was notably limited to the subepithelial stromal cells (Figure 1C). As shown by these findings, *Il13ra2* expression is induced in a localized region of the murine uterus in response to P4 and mediated by SRC-1 and PGR.

Il13ra2 expression is induced by E2 and P4 in a synergistic and time-dependent manner

In order to observe the broader effect of steroid hormone signaling on *Il13ra2* expression, we treated ovariectomized wild type mice with vehicle, E2, P4, or E2 + P4 and assessed the relative expression of *Il13ra2* in the murine uterus at 16 and 40 h of treatment. Using RT-qPCR, we found that the combination treatment of E2 + P4 synergistically upregulated *Il13ra2* expression significantly higher than any other treatment at both 16 h and 40 h, with the 40-h treatment group's mRNA expression level being the highest (Figure 2A).

Corroborating these results, in situ hybridization performed on uterine tissue sections from the 40-h treatment group revealed slightly increased *Il13ra2* mRNA expression in the P4 only treated group and intensely increased expression in the E2 + P4-treated group (Figure 2B). Expression was again localized to the

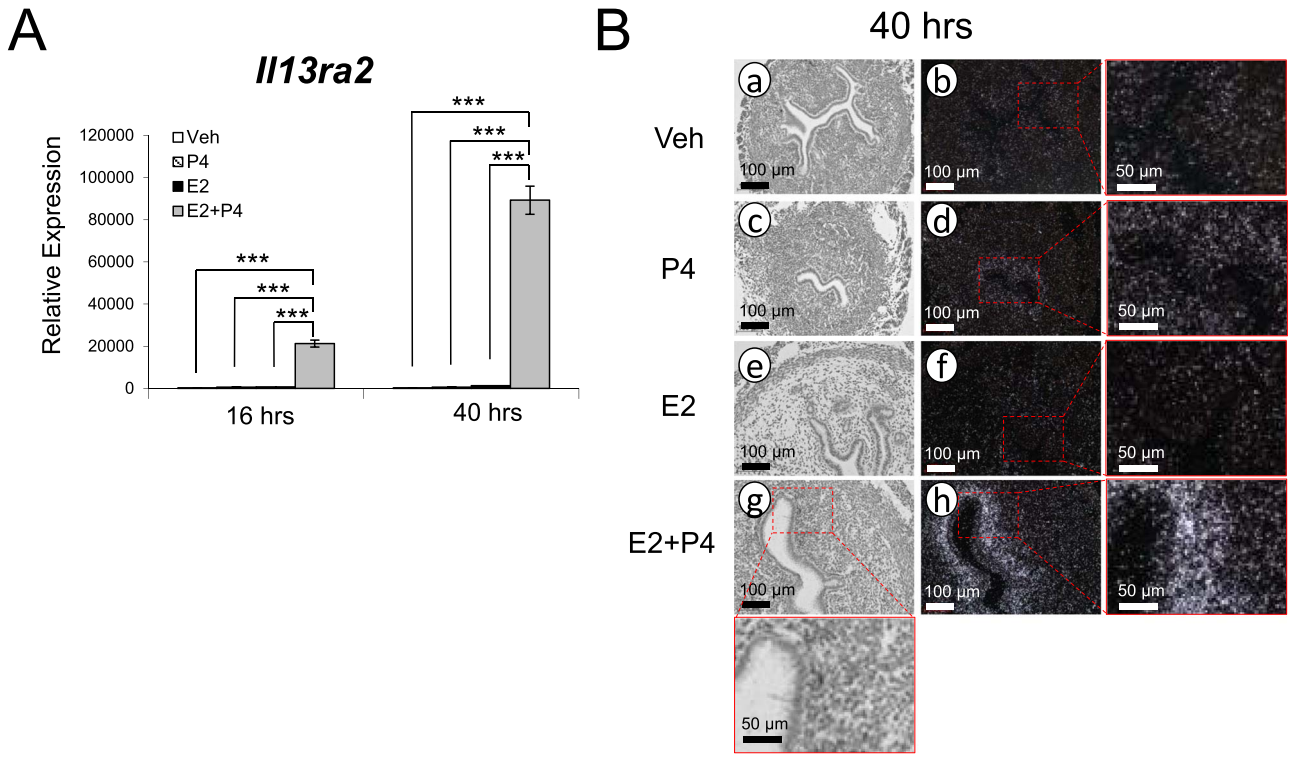

Figure 2. Time course of uterine *Il13ra2* expression in response to ovarian steroid hormones. (A) Relative *Il13ra2* mRNA expression after administration of vehicle, P4, E2, or combined E2 + P4 as measured by RT-qPCR after 16 h and 40 h in whole uterine tissue preparations from ovariectomized WT mice. Graphs represent the mean ± SEM ($n = 3$; $^{***}P < 0.001$). (B) Bright-field (a, c, e, g) and dark-field (b, d, f, h) photomicrographs of WT mouse uterine sections after in situ hybridization probing for *Il13ra2* mRNA after administration of vehicle, P4, E2, or combined E2 + P4 at 40 h after treatment ($n = 3$).

subepithelial stromal cells of the uterus. Overall, these results indicate a time-dependent induction of *Il13ra2* by cooperative action of E2 and P4.

Il13ra2 expression in the uterus during early pregnancy

Steroid hormone signaling is tightly regulated during early pregnancy and critical for pregnancy success, so the expression pattern of *Il13ra2* during this time is of great interest. To characterize its expression over the course of early pregnancy, we performed in situ hybridization and RT-qPCR on mouse uterine tissue collected during early pregnancy or pseudopregnancy from GD 0.5 to GD 7.5. With in situ hybridization, we observed notable signal at GD 4.5 but at no other stage (Figure 3A). With RT-qPCR, we detected significant increases in *Il13ra2* expression at GD 4.5 and at GD 5.5 I-IS regions (Figure 3B). This finding indicates *Il13ra2* is most highly expressed in the uterus at the early implantation stage of early pregnancy and away from the IS postimplantation [4].

IL13RA2 in in vitro decidualization of hESCs

Given the hormonal control and temporal and spatial expression pattern of *Il13ra2* in mice, we asked whether it may be functionally involved in the decidualization process of hESCs. To determine the basal expression level of in *IL13RA2* in hESCs, we utilized the IGROV-1 human ovarian adenocarcinoma cell line [42, 43] as a positive control because ovarian cancer cells are known to consistently express *IL13RA2* [32, 44]. Using RT-qPCR, we found that our cultured hESCs at 38% the level of IGROV-1 cells (Figure 4A). To determine if *IL13RA2* is involved in the differentiation of hESCs to a decidual character, we utilized a common protocol for in vitro decidualization induction using a combination of E2, medroxyprogesterone acetate (a P4 analog), and cAMP, together comprising EPC media [39, 41]. Confirming the successful induction of in vitro decidualization, decidualization marker genes *PRL* and *IGFBP1* were significantly upregulated by day 6 of EPC treatment compared to untreated controls according to RT-qPCR analysis (Figure 4B). *IL13RA2* was also significantly upregulated on day 6 of in vitro decidualization (Figure 4B).

To determine if *IL13RA2* is functionally important for in vitro decidualization of hESCs, we transfected the cells with *IL13RA2* siRNA or with nontargeting control siRNA prior to inducing decidualization. Efficient knockdown of *IL13RA2* mRNA levels was confirmed with RT-qPCR before inducing decidualization (−2.60-fold; $P < 0.001$; Figure 5A). Knockdown of *IL13RA2* prior to EPC treatment significantly diminished the induction of *PRL* and *IGFBP1* on day 6 of in vitro decidualization, indicating a functional role for *IL13RA2* in enabling a full decidualization response in hESCs (Figure 5B).

Discussion

A thorough knowledge of the complex mechanisms of E2 and P4 signaling interplay in the uterus is critical to understanding both healthy uterine function and the pathophysiology of uterine dysfunction [2, 6–9]. P4 action through PGR, in particular, has been repeatedly shown to induce many factors necessary for maintenance of uterine function such as *Ihh* [45, 46], *Hand2* [47], *Areg* [48], *Gata2* [49, 50], and *Sox17* [49, 51]. While much progress has been

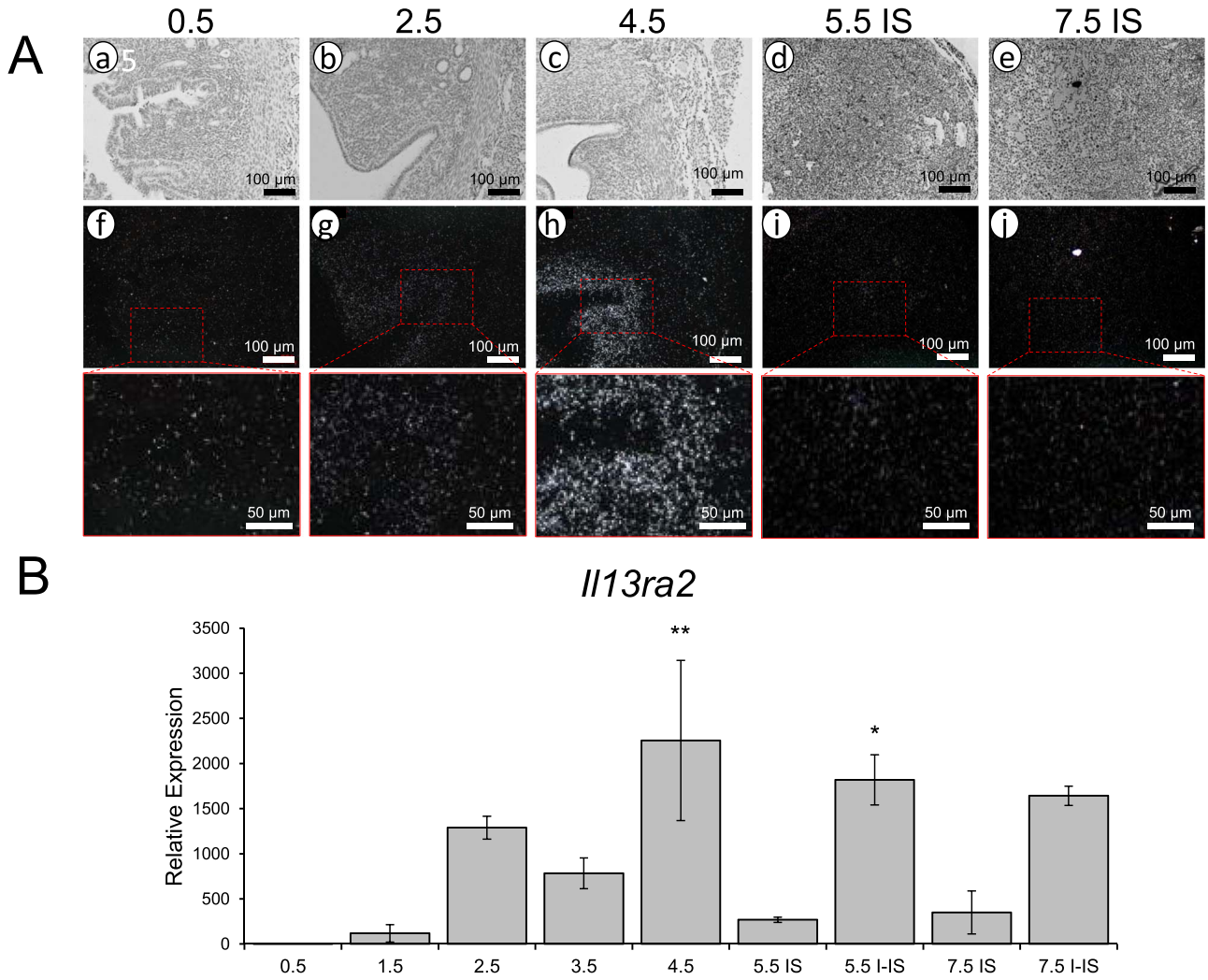

Figure 3. *Il13ra2* expression during early pregnancy. (A) Bright-field (a–e) and dark-field (f–j) photomicrographs of WT mouse uterine sections from GD 0.5 to GD 7.5 showing *Il13ra2* mRNA expression by in situ hybridization ($n = 3$). (B) *Il13ra2* mRNA expression by RT-qPCR in mouse uterine samples from GD 0.5 to GD 7.5, with GD 0.5 and GD 7.5 samples separated into implantation site (IS) and interimplantation site (I-IS) regions. Graphs represent the mean ± SEM ($n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, compared to GD 0.5).

made in recent years unraveling the complex networks regulated by P4 and PGR in the uterus, many players downstream of PGR still need to be explored, particularly because of the complexity of P4 signaling regulation in epithelial–stromal cross-talk [3]. In this study, we have identified *Il13ra2* as a uterine stromal target of PGR and SRC-1.

Previous reports of *Il13ra2* expression in the endometrium have been contradictory and limited in detail. The first noted mention of *Il13ra2* in the uterus by Reese *et al*. was in a large microarray experiment designed to find markers of uterine receptivity in mice, where *Il13ra2* expression was found downregulated at newly forming ISs compared to I-IS regions [34]. Our contrasting finding that *Il13ra2* expression increases in the subepithelial stroma at peri-implantation might be explained by the fact that the previous experiment included all layers of the uterus as well as the blastocyst in the IS analysis. Indeed, uterine epithelial-specific ablation of PGR does not significantly alter *Il13ra2* expression after P4 treatment, highlighting the importance of uterine compartment-specific analysis [52]. Two past studies identified *Il13ra2* expression changes in uterine cells after P4 treatment via microarray analysis [33, 35]. In cultured hESCs, P4 treatment decreased *IL13RA2* expression, although this microarray result was not validated [33]. However, in mouse uteri, P4 treatment induced *Il13ra2* in a PGR-dependent manner [35, 52]. Our data corroborate the previous mouse findings by showing that *Il13ra2* is upregulated in the subepithelial stroma after P4 treatment.

It is important to note the role of immune cells in the endometrial stroma. Specifically, there have been reports of important functions for uterine macrophages [53] and dendritic cells [54] during early pregnancy in mice. While the expression and function of *Il13ra2* in immune cells has not been well characterized, its expression has been reported in the immune cell-rich spleen [55] and specifically in macrophages [30]. Thus, it is possible that *Il13ra2* may be expressed and function in the immune cells of the endometrial stromal bed during early pregnancy.

Our results demonstrate that uterine SRC-1 is necessary for full induction of *Il13ra2* expression in response to P4 treatment, which is

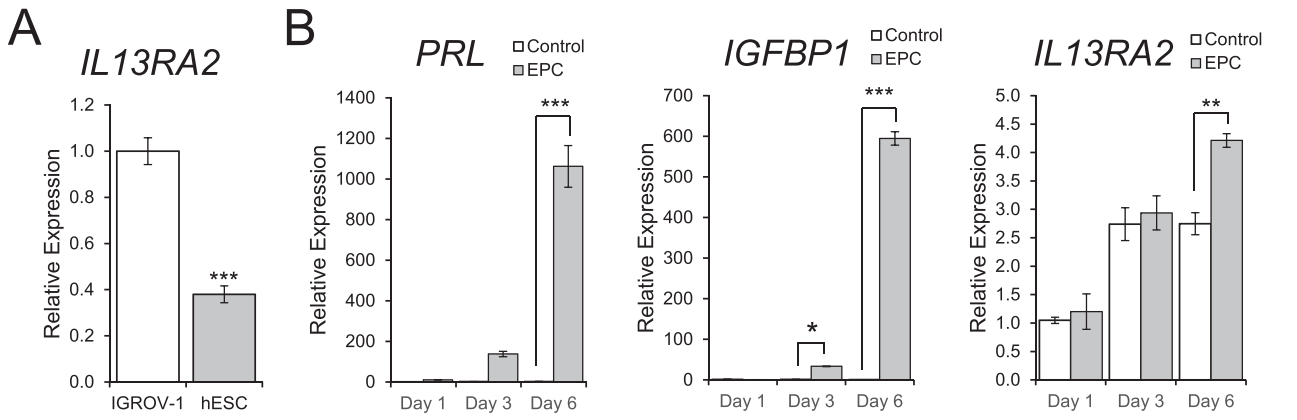

Figure 4. Expression of *IL13RA2* during in vitro decidualization of primary human endometrial stromal cells. (A) RT-qPCR showing the basal expression of *IL13RA2* mRNA in primary human endometrial stromal cells (hESC) relative to the positive control IGROV-1 Human Ovarian Cancer Cell Line. The graph represents the mean ± SEM ($n = 3$; *** $P < 0.001$). (B) RT-qPCR showing the mRNA expression levels of *PRL*, *IGFBP1*, and *IL13RA2* in primary human endometrial stromal cells on days 1, 3, and 6 of treatment with control or in vitro decidualization induction (EPC) media. Graphs represent the mean ± SEM ($n = 3$; * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$).

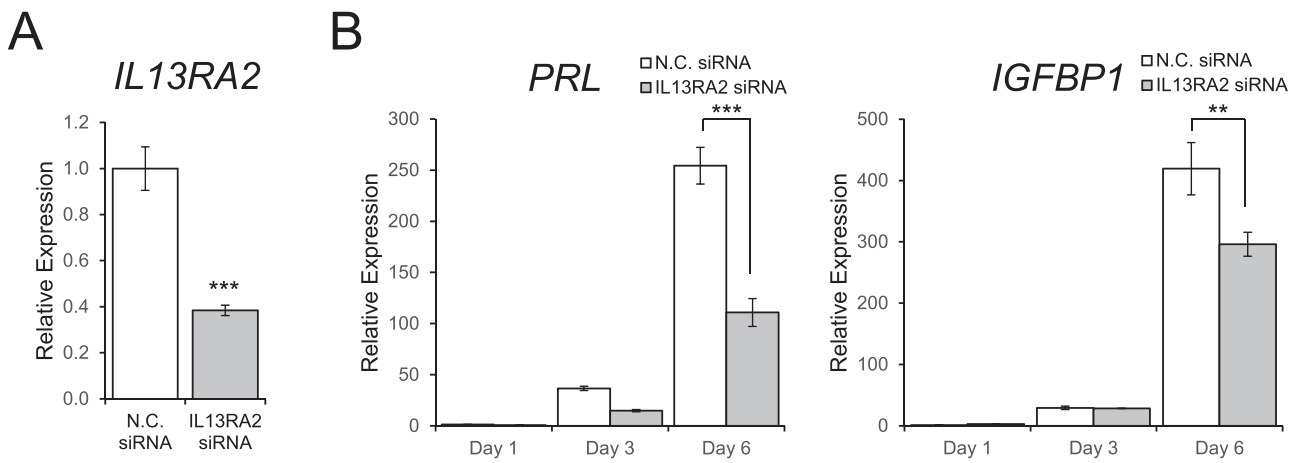

Figure 5. Effect of *Il13ra2* knockdown on in vitro decidualization of primary human endometrial stromal cells. (A) RT-qPCR showing knockdown efficiency of *Il13ra2* mRNA expression after transfection with *IL13RA2* siRNA compared to nontargeting control siRNA. The graph represents the mean ± SEM ($n = 3$; *** $P < 0.001$). (B) RT-qPCR showing the expression of the decidualization marker genes *PRL* and *IGFBP1* on days 1, 3, and 6 of in vitro decidualization induction after transfection with *IL13RA2* siRNA or nontargeting control siRNA. Graphs represent the mean ± SEM ($n = 3$; ** $P < 0.01$, *** $P < 0.001$).

consistent with a previous report showing the induction of *Il13ra2* expression by E2 and P4 treatment is abrogated in SRC-1$^{-/-}$ mice [36]. However, we found that a low level of *Il13ra2* induction still occurs in P4-treated SRC-1$^{-/-}$ mice, which may be due to compensatory or redundant action of SRC-2 and/or SRC-3. Indeed, previous studies have shown that uterine deletion of both SRC-1 and SRC-2 in mice causes uterine defects beyond those seen in mice with individual deletions of SRC-1 or SRC-2 [14, 18, 19]. P4 and PGR action in uterine stromal cells during early pregnancy has been repeatedly shown to be necessary for implantation and decidualization success, and both critical events are in progress at GD 4.5 and GD 5.5 in mice [56]. Our finding that *Il13ra2* expression is strongest at GD 4.5 suggests that it may be involved in the regulation of the early stages of decidualization in mice. However, strong expression in I-IS regions but not IS regions at postimplantation time points indicates *Il13ra2* is not involved throughout the decidualization process but only the early priming of stromal cells. Rather, *Il13ra2* may be downregulated directly at the IS by factors involved in the later stages of implantation.

Though we found that *Il13ra2* expression is induced moderately by short-term P4 treatment alone, it is induced much more strongly by combined E2 and P4 treatment over a longer period. The temporal and spatial interplay between E2 and P4 signaling during early pregnancy is very intricate and complex, but both hormones are necessary in proper proportion [6, 7]. One possible explanation for our observation is that E2 drives PGR expression in the stroma, but P4 downregulates PGR overall through negative feedback [57]. Since we showed PGR expression to be necessary for induction of *Il13ra2* in the stroma using PRKO mice, the addition of E2 to the P4 treatment may have increased *Il13ra2* expression by maintaining stronger PGR activity in the stroma.

Our findings that *IL13RA2* expression is significantly increased in hESCs in vitro decidualization conditions and that knockdown of *IL13RA2* by siRNA transfection before treatment partially

compromises the decidualization response reveal a new steroid hormone-regulated gene involved in decidualization. This finding parallels the uterine *Il13ra2* expression pattern during early pregnancy in mice but not perfectly, since GD 4.5 marks the beginning of the decidualization response [4]. *Il13ra2* appears to then be downregulated as decidual cells mature in mice, whereas it is slightly upregulated in decidualizing hESCs later in the process. This discrepancy could be due to species differences or to in vivo versus in vitro conditions. Interestingly, SRC-1 was previously shown to be necessary for a full decidualization response [18], and our finding that *Il13ra2* is downstream of SRC-1 implicates it as an effector of SRC-1's role in decidualization. Additional support for *Il13ra2* as an important gene in decidualization comes from the known role of *Il13ra2* in facilitating ERK-mediated proliferation through phosphorylation of ERK1/2 [29]. ERK1/2 activation by phosphorylation is necessary for decidualization, and pERK1/2 is upregulated in the subepithelial stroma of mice at GD 4.5, mirroring the *Il13ra2* expression profile that we report here [39]. Thus, IL13RA2 may function in decidualization upstream of ERK1/2 activation. The fact that decidualization was only moderately affected by knockdown of *IL13RA2* may imply a more complex regulation of decidualization by IL13RA2 possibly involving IL-13 and a compensatory or competing role for IL13RA1/IL4R. It is known that IL-13 secretion by cultured stromal cells increases during decidualization, but no functional role has been established for IL-13 in decidual cells [58]. Thus, more detailed functional studies will be necessary to investigate these possibilities.

Notably, IL-13 levels decrease in normal endometrium from fertile women during the mid-secretory phase, which contains the implantation window, but they are significantly increased in women with recurrent spontaneous abortion at this time, leading to the speculation that appropriate levels of IL-13 and its downstream signaling consequences may be important to govern the immune and inflammatory responses necessary for endometrial function and pregnancy establishment [22]. Additionally, both epithelial and stromal cells of the endometrium secrete heightened levels of IL-13 in response to treatment with E2, P4, or E2 + P4 in culture [23]. In the context of our study, this may implicate IL13RA2 as a hormone-responsive modulator of IL-13 signaling during implantation, either to sequester IL-13 or to activate ERK/AP-1 or another signaling pathway. At this point, however, more experiments are necessary to determine if IL13RA2 plays a role modulating IL-13 signaling during implantation and if it is important for decidualization or immune regulation. Overall, our study indicates that *Il13ra2* is induced synergistically by E2 and P4 through PGR and SRC-1 during the early stages of decidualization in the mouse and is functionally involved in complete decidualization of human stromal cells.

Author contributions

K.L., F.J.D., and J.W.J designed experiments; R.M.M., T.H.K., and J.W.J performed experiments; R.M.M., K.L., F.J.D., and J.W.J analyzed and interpreted data; R.M.M., T.H.K., B.L., and J.W.J wrote the manuscript.

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