**Molecular Human Reproduction, Vol.23, No.9 pp. 646–653, 2017**
Advanced Access publication on August 17, 2017 doi:10.1093/molehr/gax045

molecular human reproduction

ORIGINAL ARTICLE

# Growth regulation by estrogen in breast cancer 1 (GREB1) is a novel progesterone-responsive gene required for human endometrial stromal decidualization

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*Submitted on March 31, 2017; resubmitted on July 9, 2017; editorial decision on August 2, 2017; accepted on August 3, 2017*

**STUDY QUESTION:** Is Growth Regulation by Estrogen in Breast Cancer 1 (GREB1) required for progesterone-driven endometrial stromal cell decidualization?

**SUMMARY ANSWER:** GREB1 is a novel progesterone-responsive gene required for progesterone-driven human endometrial stromal cell (HESC) decidualization.

**WHAT IS KNOWN ALREADY:** Successful establishment of pregnancy requires HESCs to transform from fibroblastic to epithelioid cells in a process called decidualization. This process depends on the hormone progesterone, but the molecular mechanisms by which it occurs have not been determined.

**STUDY DESIGN, SIZE, DURATION:** Primary and transformed HESCs in which GREB1 expression was knocked down were decidualized in culture for up to 6 days. Wild-type and progesterone receptor (PR) knockout mice were treated with progesterone, and their uteri were assessed for levels of GREB1 expression.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Analysis of previous data included data mining of expression profile data sets and *in silico* transcription factor-binding analysis. Endometrial biopsies obtained from healthy women of reproductive age during the proliferative phase (Days 8–12) of their menstrual cycle were used for isolating HESCs. Experiments were carried out with early passage (no more than four passages) HESCs isolated from at least three subjects. Transcript levels of decidualization markers prolactin (*PRL*) and insulin-like growth factor-binding protein-1 (*IGFBP-1*) were detected by quantitative RT-PCR as readouts for HESC decidualization. Cells were also imaged by phase-contrast microscopy. To assess the requirement for *GREB1*, PR and *SRC-2*, cells were transfected with specifically targeted small interfering RNAs. Results are shown as mean and SE from three replicates of one representative patient-derived primary endometrial cell line. Experiments were also conducted with transformed HESCs.

**MAIN RESULTS AND THE ROLE OF CHANCE:** Progesterone treatment of mice and transformed HESCs led to an ~5-fold ($5.6 \pm 0.81$, $P < 0.05$, and $5.2 \pm 0.26$, $P < 0.01$, respectively) increase in *GREB1* transcript levels. This increase was significantly reduced in the uteri of PR knock-out mice ($P < 0.01$), in HESCs treated with the PR antagonist RU486 ($P < 0.01$), or in HESCs in which PR expression was knocked down ($P < 0.05$). When *GREB1* expression was knocked down, progesterone-driven decidualization markers in both immortalized

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and primary HESCs was significantly reduced ($P < 0.05$ and $P < 0.01$). Finally, GREB1 knock down signficantly reduced expression of the PR target genes *WNT4* and *FOXOA1* ($P < 0.05$ and $P < 0.01$, respectively).

**LARGE SCALE DATA:** This study used the Nuclear Receptor Signaling Atlas.

**LIMITATIONS, REASONS FOR CAUTION:** Although *in vitro* cell culture studies indicate that GREB1 is required for endoemtrial decidualization, the *in vivo* role of GREB1 in endometrial function and dysfunction should be assessed by using knock-out mouse models.

**WIDER IMPLICATIONS OF THE FINDINGS:** Identification and functional analysis of GREB1 as a key molecular mediator of decidualization may lead to improved diagnosis and clinical management of women with peri-implantation loss due to inadequate endometrial decidualization.

**STUDY FUNDING AND COMPETING INTEREST(S):** This research was funded in part by: a National Institutes of Health (NIH)/National Institute of Child Health and Human Development (NICHD) grant (R00 HD080742) and Washington University School of Medicine start-up funds to R.K., an NIH/NICHD grant (RO1 HD-07857) to B.W.O.M., and a NIH/NICHD grant (R01 HD-042311) to J.P.L. The authors declare no conflicts of interests.

**Key words:** progesterone / endometrium / decidualization / gene transcription / differentiation

# Introduction

In early pregnancy, the uterus must become receptive to embryo invasion and implantation and then permit placentation to establish the maternal-fetal interface (Wilcox *et al.*, 1999; Carson *et al.*, 2000). These processes both require the fibroblast-like endometrial stromal cells lining the uterus to differentiate, or decidualize, into epithelial-like cells (Gellersen *et al.*, 2007; Cha *et al.*, 2012). Inadequate decidualization can lead to implantation failure or early embryo miscarriage of even a healthy blastocyst. In fact, miscarriage after implantation, which is likely due to decidualization defects, occurs in 30% of pregnancies (Salker *et al.*, 2010, 2012; Teklenburg *et al.*, 2010; Koot *et al.*, 2012). Therefore, diagnosis and treatment of recurrent pregnancy loss will require a better understanding of this cellular transformation process.

Decidualization and establishment of a successful pregnancy rely on the steroid hormones estrogen and progesterone produced by the ovary (Wang and Dey, 2006). Estrogen supports embryo implantation by preparing the endometrium to be receptive to embryo invasion, whereas progesterone directs endometrial decidualization (Finn and Martin, 1970, 1972; Ma *et al.*, 2003). The physiological actions of progesterone are mediated by the progesterone receptor (PR), a transcription factor that regulates gene expression through the ligand-dependent recruitment of accessory proteins known as coregulators (Lonard and O'Malley, 2012). Although studies with controlled hormone treatments of mice have revealed many of the cellular events by which progesterone controls initiation and progression of decidualization (Finn, 1982; Wang and Dey, 2006), the underlying mechanisms that direct these changes are unclear. Recent microarray-based transcriptional profiling on uterine tissues from ovariectomized mice acutely treated with progesterone revealed genes that are regulated by progesterone in the endometrium (Wetendorf and DeMayo, 2014). Herein, we sought to determine the role of one such gene, Growth Regulation by Estrogen in Breast Cancer 1 (GREB1), in the progesterone-dependent responses in the endometrium.

GREB1 was initially identified as an early estrogen-responsive gene in MCF-7 breast cancer cells (Ghosh *et al.*, 2000), and its expression is strongly correlated with that of estrogen receptor in primary luminal-A breast cancers (Dunbier *et al.*, 2010; Hnatyszyn *et al.*, 2010). Additionally, GREB1 promotes estrogen-dependent proliferation of breast cancer cells (Rae *et al.*, 2005, 2006), and overexpression of GREB1 stimulates colony formation and tamoxifen resistance in MCF-7 cells (Mohammed *et al.*, 2013). GREB1 is also an androgen-responsive gene in LNCaP prostate cancer cells (Ghosh *et al.*, 2000), and it promotes androgen-dependent proliferation of prostate cancer cells (Rae *et al.*, 2005, 2006). Finally, GREB1 is upregulated in response to estrogen in ovarian cancer cells (Laviolette *et al.*, 2014). Although these data suggest a pan-estrogen response in females, whether GREB1 responds to other closely related steroid hormones and their receptors is as yet unknown.

In this study, we define GREB1 as a novel progesterone-responsive gene in the uterus and demonstrate that it plays an important role in progesterone-driven human endometrial stromal cell (HESC) decidualization.

# Materials and Methods

## Ethical approval

Before endometrial tissue biopsy collection, volunteers provided written informed consent in accordance with an Institutional Review Board protocol from Baylor College of Medicine and the guidelines of the Declaration of Helsinki ('WMA Declaration of Helsinki Serves as Guide to Physicians' 1966).

## HESC isolation

Endometrial biopsies were obtained from healthy women of reproductive age during the proliferative phase (Days 8–12) of their menstrual cycle. HESCs were isolated as described previously (Kommagani *et al.*, 2013), cultured, and propagated in DMEM/F-12 media containing 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Experiments were carried out using early passage (no more than four passages) HESCs isolated from at least three subjects, all of which decidualized under decidualization conditions. Data in figures are from cells from one representative patient, which showed an intermediate level of decidualization. Results are shown as mean ± SE from three biological replicates of one representative patient-derived primary endometrial cell line. Transformed HESCs were obtained from ATCC (ATCC CRL-4003) (Krikun *et al.*, 2004). Transformed HESCs were maintained in phenol-red-free DMEM/F-12 medium with 3.1 g/l glucose and 1 mM sodium pyruvate supplemented with 10% charcoal/dextran-treated FBS, 1% ITS (insulin,

transferrin, sodium selenite + Premix, 1.5 g/l sodium bicarbonate, and 500 ng/ml puromycin [all from Life Technologies, Carlsbad, USA]).

## HESC siRNA Transfection and Decidualization

For transfections, HESCs were plated in 1× Opti-MEM I reduced-serum media (Invitrogen Corporation, Carlsbad, USA) in six-well culture plates and treated in triplicate with Lipofectamine 2000 reagent (Invitrogen Corporation, Carlsbad, USA) and 60 pmol of the following siRNAs: non-targeting siRNA (D-001810-10-05) or siRNAs targeting PR (L-006763-00-0005), SRC-2 (NCOA-2 [L-020159-00-0005]), or GREB1 (L-008187-01-0005) (GE Healthcare Dharmacon Inc., Lafayette, CO). After 48 h, decidualization was initiated by treating HESCs with 100 nM estrogen, 10 μM Medroxyprogesterone acetate (MPA) (Sigma-Aldrich), and 50 μM cyclic adenosine monophosphate (Sigma-Aldrich) in 1× in Opti-MEM I reduced-serum media containing 2% FBS. Decidualization medium was changed every two days until Day 3 or 6, depending on the experiment. Cells were then harvested, and the RNeasy total RNA isolation kit (Qiagen Inc., Valencia, CA) was used to isolate RNA as described previously (Kommagani *et al.*, 2016). Quantitative PCR analyses were performed by using appropriate primers (Supplementary Data Table S2) and TaqMan 2× master mix (Applied Biosystems/Life Technologies, Grand Island, NY). Ribosomal RNA (18 S) was used as an internal control. Transcript levels of decidualization markers *PRL* and *IGFBP-1* were used as an indication of HESC decidualization (Brosens *et al.*, 1999).

## Mice and hormone treatments

CD1 wild-type mice were housed at the mouse facility and maintained on a 12-h light: 12-h dark cycle. Mice were treated humanely, and surgical procedures were performed according to an animal protocol approved by the Institutional Animal Care and Use Committee. PR knockout (PRKO) mice and isogenic wild-type siblings were described previously (Lydon *et al.*, 1995). To assess uterine progesterone responses, six-week-old mice were ovariectomized, rested for two weeks to allow endogenous levels of ovarian-derived steroid hormones to diminish, and then subcutaneously injected with 100 μl sesame oil (vehicle control) or 1 mg progesterone (Sigma-Aldrich, St. Louis, MO) in 100 μl sesame oil as indicated in figure legends. At the indicated times after injections, mice were euthanized, whole uterine tissues were collected, and RNA was isolated with the RNeasy total RNA isolation kit.

## Statistical analysis

A two-tailed paired student *t*-test was used for statistical significance testing. Results were considered statistically significant with a *P*-value $< 0.05$. Asterisks represent the level of significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$.

# Results

Although GREB1 responds to estrogen and androgen in breast and prostate cancer cells, respectively (Rae *et al.*, 2005, 2006), its regulation by other steroid hormones is unknown. To assess the range of hormone pathways by which GREB1 expression might be regulated, we searched for it in the Nuclear Receptor Signaling Atlas (Ochsner *et al.*, 2012). We used the Transcriptomine tool to identify data points from existing expression profile datasets, revealing that *GREB1* expression is affected by numerous hormones/nuclear receptors, including progesterone (Supplementary Data Fig. S1). In-depth mining of this tool revealed that acute (4 or 6 h) treatment with progesterone induced multiple *Greb1* probes in two different microarray datasets from mouse uteri (Fig. 1A and Supplementary Data Table S1).

To validate the *in silico* finding, we ovariectomized wild-type mice, treated them with progesterone, and then examined *Greb1* transcript levels in the uterus. We found that *Greb1* expression was elevated ~5-fold (5.6 ± 0.81) at 6 h after treatment and was back down to control levels at 12 and 24 h (Fig. 1B). Similarly, treatment of transformed HESCs with MPA led to a significant induction (5.2 ± 0.26) in *GREB1* transcript levels within 4 h (Fig. 1C). As anticipated, expression of the

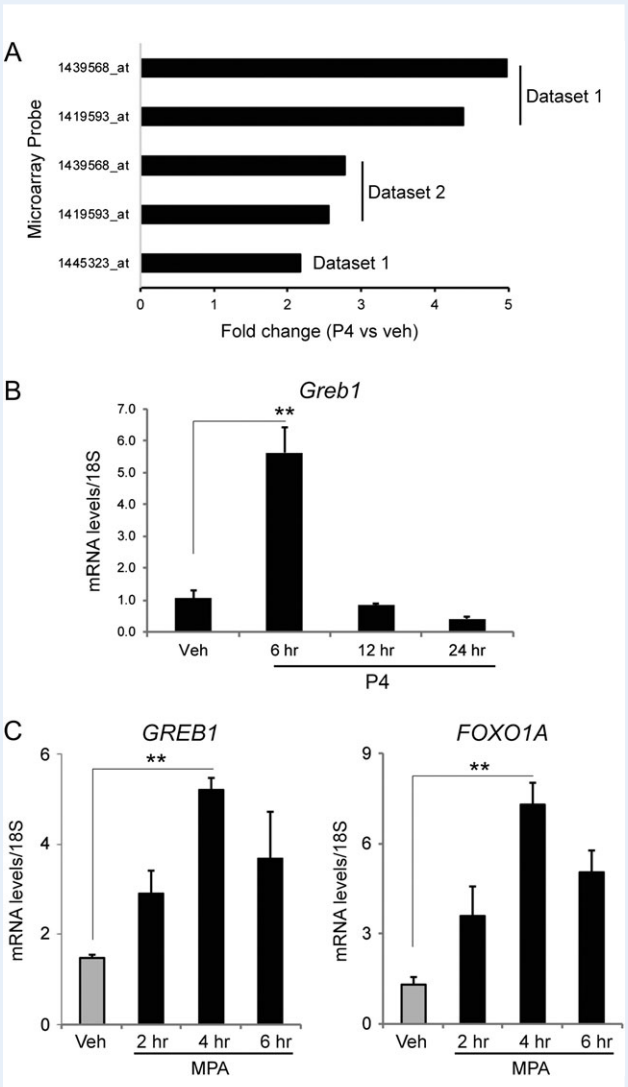


**Figure 1** Greb1 is rapidly induced by progesterone in the mouse and human endometrium**.** (**A**) Graph depicting the *Greb1* probes found to be induced by progesterone in expression profiling data sets in the Nuclear Receptor Signaling Atlas Transcriptomine database. (**B**) *Greb1* transcript levels in uteri from ovariectomized mice treated with vehicle or progesterone (P4) (1 mg) for 6, 12 or 24 h. Results represent the mean ± SE; $n = 3$ mice/group. (**C**) Relative levels of *GREB1* and *FOXO1A* (positive control) transcripts from transformed HESCs treated with vehicle (control) or MPA (1 μM) for indicated time points. Results represent the mean ± SE from three biological replicates; $**P < 0.01$.

well-established progesterone-responsive gene *Forkhead Box Protein O1A (FOXO1A)* was also increased in MPA-treated cells (Fig. 1C). These results suggest that *GREB1* is an early progesterone-responsive gene in the human and mouse endometrium.

Because the PR is required for progesterone-driven transcription, we next wondered whether PR was required for *Greb1* induction by progesterone. To test this, we ovariectomized control and PR knockout (PRKO) mice, treated them with progesterone for 6 h, and then measured *Greb1* transcript levels in the uterus. As shown in Fig. 2A, progesterone significantly induced (6.0 ± 0.25) *Greb1* transcript levels in uteri from control mice but not in those from PRKO mice. As expected, uterine expression of the PR-responsive gene *Amphiregulin* (*Areg*) was also increased upon progesterone treatment in control but not in PRKO mice. Further, *in silico* analysis of a PR chromatin immunoprecipitation-sequencing data set (Rubel *et al.*, 2012) revealed multiple PR-binding sites in the *Greb1* locus (Fig. 2B).

To determine whether PR was required for MPA-mediated induction of GREB1 in HESCs, we performed two assays. First, we demonstrated that whereas MPA treatment led to induction (2.5 ± 0.04) of *GREB1* transcript levels by 4 h, co-administration of RU468 (a PR antagonist) significantly attenuated this induction (Fig. 2C). Second, we used siRNAs to knock down PR expression, resulting in attenuated (2.0 ± 0.10) MPA-induced *GREB1* expression (Fig. 2D). Collectively, these results strongly indicate that PR is required for rapid induction of *Greb1* transcript levels by progesterone in the murine uterus and human endometrial stromal cells.

Next, to determine whether PR was required for *GREB1* expression during *in vitro* human endometrial decidualization, we examined

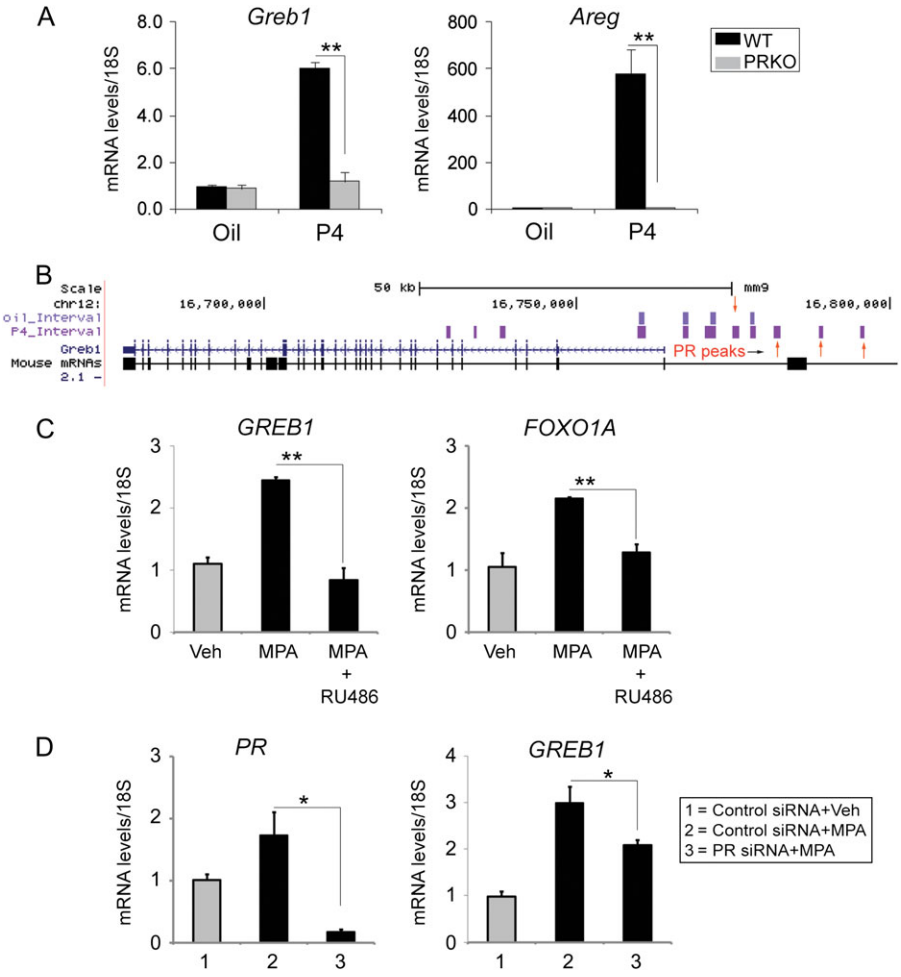


**Figure 2** Greb1 is induced by progesterone and PR in the mouse uterus and HESCs. (**A**) Relative levels of *Greb1* and *Areg* (positive control) transcripts in uteri from ovariectomized wild-type (WT) and PR knockout (PRKO) mice treated for 6 h with P4 (1 mg). Results represent the mean ± SE; $n = 3$ mice/group. (**B**) Distribution of binding sites for PR (orange arrows) on *Greb1* locus from murine uterine tissues treated for 6 h with P4 (1 mg) or vehicle (oil). (**C**) Relative transcript levels of *GREB1* and *FOXO1A* from transformed HESCs (three biological replicates) treated with vehicle (control), MPA or MPA plus RU486 (1 μM) for 4 h. Note: The RU486 was added 1 h before MPA. (**D**) Relative transcript levels of *GREB1* and *PR* from transformed HESCs (three biological replicates) transfected with control siRNA or *PGR* siRNA treated with vehicle or MPA (1 μM) for 4 h. Results represent the mean ± SE; $^{*}P < 0.05$ and $^{**}P < 0.01$. HESC, human endometrial stromal cell; PR, progesterone receptor.

*GREB1* transcript levels in decidualizing primary HESCs and decidualizing HESCs in which PR was knocked down. *GREB1* transcript levels were significantly induced by over 150-fold during HESC decidualization (Fig. 3A), but levels of *GREB1* transcripts were significantly reduced in HESCs transfected with PR siRNA (Fig. 3A). Recently, SRC-2 was identified as an upstream transcriptional coactivator of PR in mediating HESC decidualization (Kommagani *et al.*, 2013). Consistent with this, *GREB1* induction was significantly lower in decidualizing HESCs in which SRC-2 was knocked down (Fig. 3B). Together, these results suggest that a progesterone-PR-SRC-2 signaling axis activates *GREB1* transcription in decidualizing HESCs.

We next asked whether *GREB1* was required for progesterone-driven human endometrial decidualization. As a proof-of-principle, we first used telomerase-transformed HESCs as a model for *in vitro* decidualization. As shown in Fig. 4A, *GREB1* transcript levels were significantly induced in a time-dependent manner as the decidualization program advanced (Fig. 4A). Induction of the well-established decidual marker prolactin (*PRL*) confirmed that the transformed HESCs effectively decidualized (Fig. 4A). When transformed HESCs were transfected with siRNAs targeting *GREB1* before initiating decidualization, PRL expression was significantly reduced (2.0 ± 0.06) (Fig. 4B). Consistent with this finding, the level of PRL secreted into the culture media was significantly reduced by GREB1 knockdown (Supplementary Fig. 2).

To confirm that *GREB1* was essential for hormone-driven endometrial decidualization, we knocked down *GREB1* in primary HESCs and then initiated *in vitro* decidualization (Fig. 5). Whereas control cells transformed from a fibroblastic to an epithelioid cell morphology, HESCs in which *GREB1* was knocked down failed to undergo this cellular transformation process (Fig. 5A). Consistent with this finding, induction of the decidual markers insulin-like growth factor-binding protein-1 (*IGFBP-1*) and *PRL* was abrogated by GREB1 knockdown (Fig. 5B). Together, these results indicate that GREB1 is indispensable for progesterone-dependent HESC decidualization.

Finally, to determine the mechanism by which GREB1 contributed to decidualization, we assessed expression of the PR target genes *FOXO1A* and *WNT4*, which promote endometrial decidualization (Li *et al.*, 2013; Vasquez *et al.*, 2015). As shown in Fig. 6, induction of *FOXO1A* and *WNT4* was significantly reduced in *GREB1*-knockdown HESCs than in control HESCs during decidualization. In contrast, levels of PR transcript were not affected by GREB1 knockdown. These results suggest that GREB1 modulates PR-mediated transcription during endometrial decidualization. In conclusion, we demonstrate that GREB1 is a novel progesterone-responsive gene in the mouse uterus and HESCs and reveal GREB1 as a critical mediator of the progesterone-dependent endometrial stromal cell decidualization program.

## Discussion

Herein, our results demonstrate that GREB1 is a progesterone target gene that functions to control decidualization in human endometrial stromal cells. Rapid induction of GREB1 by progesterone depends on the PR coactivator SRC-2, which we recently showed is essential for

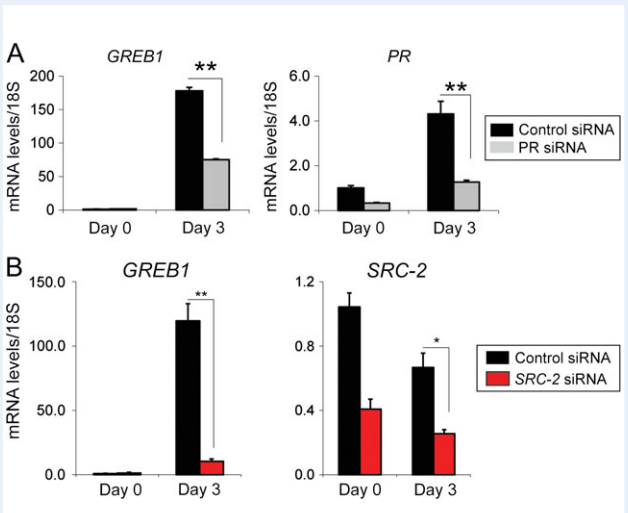


**Figure 3** PR and its coactivator SRC-2 regulate *GREB1* expression in decidualizing HESCs. (**A**) Levels of *GREB1* and *PR* transcripts in primary HESCs (three biological replicates) transfected with control or PR siRNAs and induced to decidualize for the indicated lengths of time. (**B**) Levels of *GREB1* and *SRC-2* transcripts in primary HESCs transfected with control or SRC-2 siRNAs and induced to decidualize for the indicated lengths of time. Representative data from three replicates from one patient sample are shown as mean ± SE; $^{*}P < 0.05$ and $^{**}P < 0.01$.

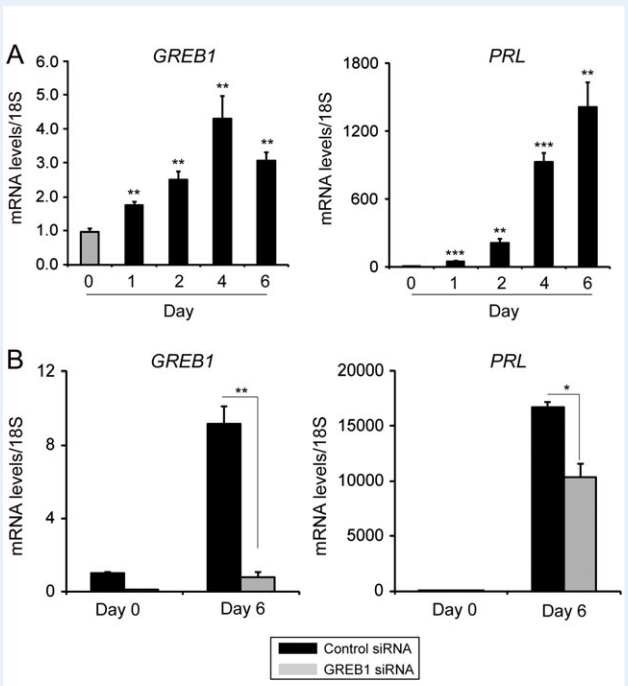


**Figure 4** *GREB1* is required for decidualization of immortalized human endometrial stromal cells. (**A**) Levels of *GREB1* and *PRL* transcripts from immortalized human endometrial stromal cells induced to decidualize for the indicated lengths of time. (**B**) Levels of *GREB1* and *PRL* transcripts in immortalized human endometrial stromal cells transfected with control or *GREB1* siRNAs treated and induced to decidualize for the indicated lengths of time. Results stated as the mean ± SE from three biological replicates from a representative experiment (experiment repeated three times); $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

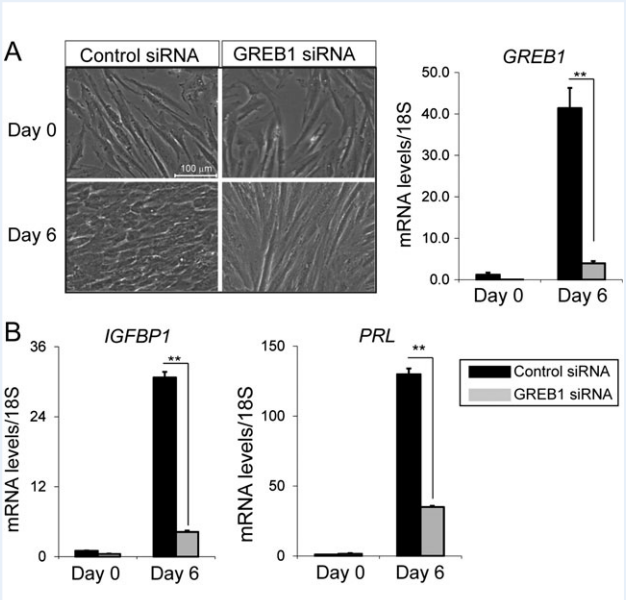


**Figure 5** *GREB1* is essential for primary human endometrial stromal cell decidualization. HESCs were transfected with negative control siRNA or *GREB1* siRNA as indicated. (**A**) Left, morphology of HESCs transfected with control or *GREB1* siRNA after zero or six days of culture in decidualization conditions. Right, levels of *GREB1* transcripts confirm the effective knockdown of *GREB1* in HESCs. (**B**) Levels of *IGFBP-1* and *PRL* transcripts in HESCs transfected with control or *GREB1* siRNAs and induced to decidualize for the indicated lengths of time. from three replicates from one patient sample are shown as mean ± SE; $^{**}P < 0.01$.

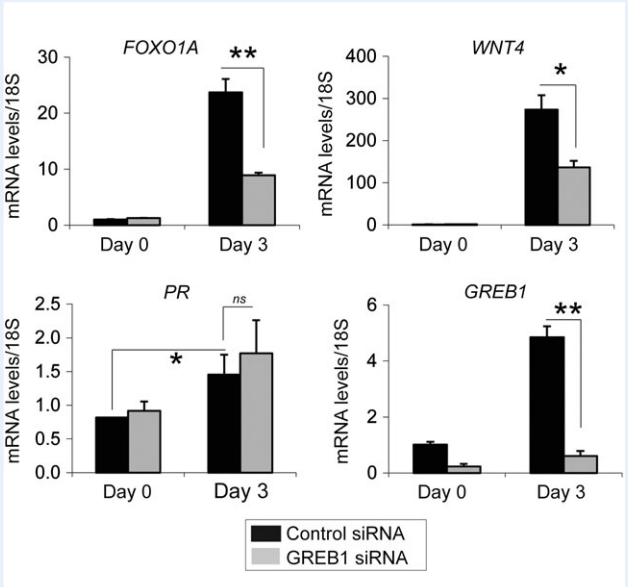


**Figure 6** GREB1 is required for the induction of key decidual genes in decidualizing HESCs. HESC cells were transfected with negative control siRNA or *GREB1* siRNA as indicated. Transcript levels of FOXO1A, WNT4 (P4 targets) and PR (estrogen target) in HESCs transfected with control or *GREB1* siRNAs and induced to decidualize for the indicated lengths of time. Representative data from three replicates from one patient sample are shown as mean ± SE; $^{*}P < 0.05$ and $^{**}P < 0.01$.

progesterone-dependent endometrial stromal cell decidualization (Kommagani *et al.*, 2013). In addition, GREB1 mediates transcription of the PR targets WNT4 and FOXOA1 and thus is a key molecular signal controlling cell fate in progesterone-dependent endometrial decidualization. Collectively, our results demonstrate that GREB1 is induced rapidly by progesterone in endometrial stromal cells and that GREB1 in turn acts as a downstream mediator of progesterone-dependent transcriptional responses that are essential for decidualization. This conceptual advance places GREB1 in a tightly controlled feed-forward regulatory loop that is essential for HESC decidualization.

In addition to progesterone, other hormones also participate in endometrial decidualization. For example, MPA can act as an agonist for the androgen receptor, which plays a role in human endometrial stromal cell decidualization (Bentel *et al.*, 1999; Gibson *et al.*, 2016; Walters *et al.*, 2016). Additionally, locally produced estrogen supports murine endometrial decidualization (Das *et al.*, 2009), and human endometrial decidualization results in increased estrogen biosynthesis (Gibson *et al.*, 2013). Androgen, estrogen, and progesterone all exert their biological actions through their cognate nuclear receptors belonging to nuclear receptor subfamily 3 (Mangelsdorf *et al.*, 1995). Given the observation that GREB1 is required for estrogen- and androgen-mediated proliferation of breast and prostate cancer cells, respectively (Ghosh *et al.*, 2000), GREB1 might function as a common downstream mediator of this nuclear receptor subclass in human endometrial decidualization. Future investigation should be directed at defining the mechanisms by which androgen, estrogen and progesterone differentially regulate GREB1 expression to mediate a wide array of physiological responses such as endometrial decidualization.

Although GREB1 was shown to modulate cell cycle progression in multiple cancer cell lines, our findings are the first to reveal a role for GREB1 in a normal physiological process regulated by a steroid hormone. Because endometrial decidualization is associated with cell cycle arrest initially at the G0/G1 phase, and later at the G2/M phase (Logan *et al.*, 2012), it will be intriguing to test whether GREB1 governs cell cycle progression in this process. Our finding that GREB1 knockdown led to decreased levels of the PR targets FOXO1A and WNT4 suggests that GREB1 acts as a PR transcriptional cofactor in the endometrium, similar to its reported action as an estrogen receptor cofactor mediating estrogen responses in breast cancer cells (Mohammed *et al.*, 2013).

Our findings provide important information for addressing infertility. Whereas the probability of establishing a successful pregnancy within one menstrual cycle (termed the monthly fecundity rate) is very high in other mammals, the rate is only 20–30% in humans (Foote and Carney, 1988; Chard, 1991; Stevens, 1997). Despite recent advances, only ~30% of women using assisted reproductive technologies in the United States have a live birth (Centers for Disease Control and Prevention (http://www.cdc.gov/art/reports/index.html)). Approximately 50% of embryos implant, and up to half of these embryos are lost soon after implantation (Rinehart, 2007). This high attrition rate can be attributed to poor embryo quality, endometrial dysfunction (Macklon and Brosens, 2014) and inadequate endometrial decidualization (Brosens *et al.*, 2014). Identification of key molecular mediators of decidualization such as GREB1 will hopefully lead to improved reproductive outcomes in women with peri-implantation loss.

Our results may also have implications for treatment of endometriosis, as GREB1 expression is elevated in both epithelial and stromal cells in human endometriotic lesions (Pellegrini *et al.*, 2012). Given the results presented here and the established role of GREB1 in estrogen responses, we speculate that GREB1 enables progesterone-dependent proliferation and differentiation of HESCs into decidual cells, thereby generating a receptive endometrium for embryo implantation. However, dysregulation of GREB1 accelerates the estrogen-driven cellular proliferation that leads to ectopic endometriotic lesion expansion. These normal and pathological roles of GREB1 are clinically linked in that a non-receptive endometrium and endometriosis both result from derailment of normal endometrial responses to estrogen and progesterone, which in turn can lead to subfertility or infertility in reproductive-age women. Future work on GREB1 should thus improve our ability to diagnose and manage fertility-associated disorders such as recurrent pregnancy loss and endometriosis.

## Supplementary data

Supplementary data are available at *Molecular Human Reproduction* online.

## Acknowledgement

We thank Deborah J. Frank in the Department of Obstetrics and Gynecology, Washington University School of Medicine, for assistance with manuscript editing.

## Authors' roles

A.J.C., M.M.S., S.B.C. and R.K. conducted most of the studies and wrote the manuscript draft. M.M.S. and R.K. collected human stromal cells. F.J.D. provided reagents and reviewed the final draft of the manuscript. B.W.O. and J.P.L. discussed experimental design and provided resources and funding. R.K. discussed experimental design and provided resources and funding.

## Funding

National Institutes of Health (NIH)/National Institute of Child Health and Human Development (NICHD) grant (R00 HD080742) and Washington University School of Medicine start-up funds to R.K., an NIH/NICHD grant (RO1 HD-07857) to B.W.O., and a NIH/NICHD grant (R01 HD-042311) to J.P.L.

## Conflict of interest

None declared.

## References

Bentel JM, Birrell SN, Pickering MA, Holds DJ, Horsfall DJ, Tilley WD. Androgen receptor agonist activity of the synthetic progestin, medroxyprogesterone acetate, in human breast cancer cells. *Mol Cell Endocrinol* 1999;**154**:11–20.

Brosens JJ, Hayashi N, White JO. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology* 1999;**140**:4809–4820.

Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Lucas ES, Steel JH, Christian M, Chan YW, Boomsma CM *et al*. Uterine selection of human embryos at implantation. *Sci Rep* 2014;**4**:3894.

Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K. Embryo implantation. *Dev Biol* 2000;**223**:217–237.

Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. *Nat Med* 2012;**18**:1754–1767.

Chard T. Frequency of implantation and early pregnancy loss in natural cycles. *Baillieres Clin Obstet Gynaecol* 1991;**5**:179–189.

Das A, Mantena SR, Kannan A, Evans DB, Bagchi MK, Bagchi IC. De novo synthesis of estrogen in pregnant uterus is critical for stromal decidualization and angiogenesis. *Proc Natl Acad Sci USA* 2009;**106**:12542–12547.

Dunbier AK, Anderson H, Ghazoui Z, Folkerd EJ, A'Hern R, Crowder RJ, Hoog J, Smith IE, Osin P, Nerurkar A *et al*. Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women. *J Clin Oncol* 2010;**28**:1161–1167.

Finn CA. Cellular changes in the uterus during the establishment of pregnancy in rodents. *J Reprod Fertil Suppl* 1982;**31**:105–111.

Finn CA, Martin L. The role of the oestrogen secreted before oestrus in the preparation of the uterus for implantation in the mouse. *J Endocrinol* 1970;**47**:431–438.

Finn CA, Martin L. Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biol Reprod* 1972;**7**:82–86.

Foote RH, Carney EW. Factors limiting reproductive efficiency in selected laboratory animals. *Ann N Y Acad Sci* 1988;**541**:683–696.

Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med* 2007;**25**:445–453.

Ghosh MG, Thompson DA, Weigel RJ. PDZK1 and GREB1 are estrogen-regulated genes expressed in hormone-responsive breast cancer. *Cancer Res* 2000;**60**:6367–6375.

Gibson DA, McInnes KJ, Critchley HO, Saunders PT. Endometrial Intracrinology–generation of an estrogen-dominated microenvironment in the secretory phase of women. *J Clin Endocrinol Metab* 2013;**98**:E1802–E1806.

Gibson DA, Simitsidellis I, Saunders PT. Regulation of androgen action during establishment of pregnancy. *J Mol Endocrinol* 2016;**57**:R35–R47.

Hnatyszyn HJ, Liu M, Hilger A, Herbert L, Gomez-Fernandez CR, Jorda M, Thomas D, Rae JM, El-Ashry D, Lippman ME. Correlation of GREB1 mRNA with protein expression in breast cancer: validation of a novel GREB1 monoclonal antibody. *Breast Cancer Res Treat* 2010;**122**:371–380.

Kommagani R, Szwarc MM, Kovanci E, Gibbons WE, Putluri N, Maity S, Creighton CJ, Sreekumar A, DeMayo FJ, Lydon JP *et al*. Acceleration of the glycolytic flux by steroid receptor coactivator-2 is essential for endometrial decidualization. *PLoS Genet* 2013;**9**:e1003900.

Kommagani R, Szwarc MM, Vasquez YM, Peavey MC, Mazur EC, Gibbons WE, Lanz RB, DeMayo FJ, Lydon JP. The Promyelocytic Leukemia Zinc Finger Transcription Factor Is Critical for Human Endometrial Stromal Cell Decidualization. *PLoS Genet* 2016;**12**:e1005937.

Koot YE, Teklenburg G, Salker MS, Brosens JJ, Macklon NS. Molecular aspects of implantation failure. *Biochim Biophys Acta* 2012;**1822**:1943–1950.

Krikun G, Mor G, Alvero A, Guller S, Schatz F, Sapi E, Rahman M, Caze R, Qumsiyeh M, Lockwood CJ. A novel immortalized human endometrial stromal cell line with normal progestational response. *Endocrinology* 2004;**145**:2291–2296.

Laviolette LA, Hodgkinson KM, Minhas N, Perez-Iratxeta C, Vanderhyden BC. 17beta-estradiol upregulates GREB1 and accelerates ovarian tumor progression in vivo. *Int J Cancer* 2014;**135**:1072–1084.

Li Q, Kannan A, Das A, Demayo FJ, Hornsby PJ, Young SL, Taylor RN, Bagchi MK, Bagchi IC. WNT4 acts downstream of BMP2 and functions via beta-catenin signaling pathway to regulate human endometrial stromal cell differentiation. *Endocrinology* 2013;**154**:446–457.

Logan PC, Steiner M, Ponnampalam AP, Mitchell MD. Cell cycle regulation of human endometrial stromal cells during decidualization. *Reprod Sci* 2012;**19**:883–894.

Lonard DM, O'Malley BW. Nuclear receptor coregulators: modulators of pathology and therapeutic targets. *Nat Rev Endocrinol* 2012;**8**: 598–604.

Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM, O'Malley BW. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 1995;**9**:2266–2278.

Ma WG, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proc Natl Acad Sci USA* 2003;**100**:2963–2968.

Macklon NS, Brosens JJ. The human endometrium as a sensor of embryo quality. *Biol Reprod* 2014;**91**:98.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P *et al*. The nuclear receptor superfamily: the second decade. *Cell* 1995;**83**:835–839.

Mohammed H, D'Santos C, Serandour AA, Ali HR, Brown GD, Atkins A, Rueda OM, Holmes KA, Theodorou V, Robinson JL *et al*. Endogenous purification reveals GREB1 as a key estrogen receptor regulatory factor. *Cell Rep* 2013;**3**:342–349.

Ochsner SA, Watkins CM, McOwiti A, Xu X, Darlington YF, Dehart MD, Cooney AJ, Steffen DL, Becnel LB, McKenna NJ. Transcriptomine, a web resource for nuclear receptor signaling transcriptomes. *Physiol Genomics* 2012;**44**:853–863.

Pellegrini C, Gori I, Achtari C, Hornung D, Chardonnens E, Wunder D, Fiche M, Canny GO. The expression of estrogen receptors as well as GREB1, c-MYC, and cyclin D1, estrogen-regulated genes implicated in proliferation, is increased in peritoneal endometriosis. *Fertil Steril* 2012; **98**:1200–1208.

Rae JM, Johnson MD, Cordero KE, Scheys JO, Larios JM, Gottardis MM, Pienta KJ, Lippman ME. GREB1 is a novel androgen-regulated gene required for prostate cancer growth. *Prostate* 2006;**66**:886–894.

Rae JM, Johnson MD, Scheys JO, Cordero KE, Larios JM, Lippman ME. GREB 1 is a critical regulator of hormone dependent breast cancer growth. *Breast Cancer Res Treat* 2005;**92**:141–149.

Rinehart J. Recurrent implantation failure: definition. *J Assist Reprod Genet* 2007;**24**:284–287.

Rubel CA, Lanz RB, Kommagani R, Franco HL, Lydon JP, DeMayo FJ. Research resource: Genome-wide profiling of progesterone receptor binding in the mouse uterus. *Mol Endocrinol* 2012;**26**:1428–1442.

Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C *et al*. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PloS one* 2010;**5**:e10287.

Salker MS, Nautiyal J, Steel JH, Webster Z, Sucurovic S, Nicou M, Singh Y, Lucas ES, Murakami K, Chan YW *et al*. Disordered IL-33/ST2 activation in decidualizing stromal cells prolongs uterine receptivity in women with recurrent pregnancy loss. *PloS one* 2012;**7**:e52252.

Stevens VC. Some reproductive studies in the baboon. *Hum Reprod Update* 1997;**3**:533–540.

Teklenburg G, Salker M, Heijnen C, Macklon NS, Brosens JJ. The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. *Mol Hum Reprod* 2010;**16**:886–895.

Vasquez YM, Mazur EC, Li X, Kommagani R, Jiang L, Chen R, Lanz RB, Kovanci E, Gibbons WE, DeMayo FJ. FOXO1 is required for binding of PR on IRF4, novel transcriptional regulator of endometrial stromal decidualization. *Mol Endocrinol* 2015;**29**:421–433.

Walters KA, Simanainen U, Gibson DA. Androgen action in female reproductive physiology. *Curr Opin Endocrinol Diabetes Obes* 2016;**23**:291–296.

Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet* 2006;**7**:185–199.

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.

Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med* 1999;**340**:1796–1799.