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Research Article

The SARS-CoV-2 receptor, angiotensin-converting enzyme 2, is required for human endometrial stromal cell decidualization[†]

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Abstract

The coronavirus disease 2019 (COVID-19) first appeared in December 2019 and rapidly spread throughout the world. The SARS-CoV-2 virus enters the host cells by binding to the angiotensin-converting enzyme 2 (ACE2). Although much of the focus is on respiratory symptoms, recent reports suggest that SARS-CoV-2 can cause pregnancy complications such as pre-term birth and miscarriages; and women with COVID-19 have had maternal vascular malperfusion and decidual arteriopathy in their placentas. Here, we report that the ACE2 protein is expressed in both endometrial epithelial and stromal cells in the proliferative phase of the menstrual cycle, and the expression increases in stromal cells in the secretory phase. It was observed that the ACE2 mRNA and protein abundance increased during primary human endometrial stromal cell (HESC) decidualization. Furthermore, HESCs transfected with ACE2-targeting siRNA impaired the full decidualization response, as evidenced by a lack of morphology change and lower expression of the decidualization markers PRL and *IGFBP1*. Additionally, in mice during pregnancy, the ACE2 protein was expressed in the uterine epithelial cells, and stromal cells increased through day 6 of pregnancy. Finally, progesterone induced *Ace2* mRNA expression in mouse uteri more than vehicle or estrogen. These data establish a role for ACE2 in endometrial physiology, suggesting that SARS-CoV-2 may be able to enter endometrial stromal cells and elicit pathological manifestations in women with COVID-19, including an increased risk of early pregnancy loss.

Summary sentence

ACE2 protein is highly expressed in human endometrial stromal cells during the secretory phase and is essential for human endometrial stromal cell decidualization.

__Key words:__ SARS-CoV-2, ACE2, endometrium, stromal cells, decidualization.

Introduction

Although much of the focus during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/coronavirus disease 2019 (COVID-19) pandemic has been on respiratory symptoms, some reports suggest that SARS-CoV-2 and the related Middle East Respiratory Syndrome Coronavirus can cause pregnancy complications such as pre-term birth and miscarriages [1]. Additionally, a few reports have noted that pregnant women with COVID-19 had maternal vascular malperfusion and decidual arteriopathy in their placentas [2, 3], and a recent clinical case study reported a second trimester miscarriage in a woman with COVID-19 [4]. However, whether SARS-CoV-2 infects the uterus has not been determined.

It seems likely that SARS-CoV-2 could infect the uterus because its receptor, Angiotensin Converting Enzyme 2 (ACE2), is expressed fairly ubiquitously in human tissues such as the lungs, heart, intestine, kidneys, and placenta [5–7]. Moreover, ACE2 functions by cleaving the vasoconstrictor angiotensin II to the vasodilator angiotensin [1–7]. As a component of the renin–angiotensin system, ACE2 plays an important role in regulating maternal blood pressure during pregnancy. ACE2 is expressed in the rat uterus from mid- to late pregnancy [8, 9]. In addition, ACE2 mRNA expression is noted in the uterus of both rats [10] and humans [11], in which its expression may be higher in the secretory phase than in the proliferative phase of the menstrual cycle [11].

During the secretory phase, the uterine stromal cells prepare for embryo implantation by undergoing a progesterone-mediated differentiation process called decidualization. In this process, the stromal cells divide, change from a fibroblastic to an epithelioid morphology, and change their pattern of gene expression. Decidualization is essential for trophoblast invasion and placentation [12–14], and defects in this process may underlie early pregnancy loss in some women. Given the important function of the uterine stroma and the possibility that SARS-CoV-2 could infect the uterus, our goal here was to determine whether ACE2 is expressed in endometrial stromal cells is regulated by progesterone and required for decidualization.

Results and discussion

We first sought to determine whether ACE2 is expressed in the endometrium and whether its expression differs according to the phase of the menstrual cycle. We, therefore, obtained endometrial biopsies from women during the proliferative or secretory phase of the menstrual cycles and performed immunohistochemistry with an ACE2-specific antibody. In the proliferative phase, ACE2 was more abundant in epithelial cells than in stromal cells (Figure 1A and B). However, in the secretory phase, the ACE2 expression was increased in the stromal cells (Figure 1A and B and Supplementary Figure S2A). To ensure antibody specificity, isotype control was included with the staining procedure (Figure 1C). Furthermore, we carried out the western blotting and found that ACE2 was elevated around 2-fold in the secretory phase endometrium compared to the proliferative phase endometrium (Figure 1D). Interestingly, the TMPRSS2 also expressed in the epithelial cells and stromal cells of proliferative as well as secretory phase endometrium (Supplementary Figures S1A and B and S2B). Given the elevated expression of ACE2 in stroma of the secretory phase endometrium, we wondered whether the ACE2 expression increased during in vitro decidualization of human endometrial

stromal cells (HESCs). We isolated primary HESCs, exposed them to decidualizing conditions, and confirmed that expression of the decidualization markers Prolactin (*PRL*) and Insulin-like growth factor-binding protein-1 (*IGFBP1*) increased over 6 days. ACE2 mRNA also increased over this time (Figure 2A). Consistent with this finding, the ACE2 protein abundance increased during decidualization, as shown by both immunoblotting (Figure 2B) and immunofluorescence (Figure 2C). As expected, ACE2 protein predominantly localized in the cytoplasm and cell membrane of decidualized HESCs.

Next, we wondered whether ACE2 was required for primary HESC decidualization. To answer this question, we transfected the HESCs with control or ACE2-targeting siRNAs and then exposed the cells to decidualization conditions. HESCs transfected with control siRNA changed from fibroblastic to epithelioid morphology (Figure 3A) and had increased expression of the decidualization markers *PRL* and *IGFBP1* (Figure 3B). In contrast, HESCs transfected with ACE2-targeting siRNA did not show a morphology change over 6 days (Figure 3A) and expressed significantly less *PRL*, *IGFBP1*, and ACE2 than the control cells (Figure 3B). Furthermore, quantification of secreted Prolactin (PRL) from cultured media revealed a significant decrease in PRL protein levels on day 6 in the HESCs with ACE2 knockdown (Figure 3C). As expected, siRNA against ACE2 effectively downregulated ACE2 protein levels on day 6 (Figure 3D). These results suggest a functional role for ACE2 in HESC decidualization.

Finally, we examined the expression of ACE2 in the endometrium during early pregnancy in mice. We mated female wild-type mice with males of proven fertility and then stained their uteri with an ACE2-specific antibody on different days during early pregnancy. In days 1–4, ACE2 localized to the cytoplasm and cell surface of epithelial and stromal cells. However, beginning on day 3, strong ACE2 staining was seen in the cytoplasm of stromal cells. This staining was evident at least through day 6, which is when robust decidualization occurs (Figure 4 and Supplementary Figure S3). Given this change in ACE2 abundance during pregnancy, we wondered whether ACE2 expression was regulated by steroid hormones. To test this, we ovariectomized 6-week-old mice, waited 2 weeks, treated the mice with either estrogen or progesterone for 6 h, and then collected the uteri (Figure 5A). Uteri from progesterone-treated mice expressed significantly more ACE2 mRNA than uteri from vehicle-treated mice, which expressed significantly more ACE2 mRNA than uteri from estrogen-treated mice (Figure 5B). Consistent with this, immunofluorescence revealed that uteri from progesterone-treated mice had significantly more ACE2 protein in stromal cells than did uteri from vehicle- or estrogen-treated mice (Figure 5C and Supplementary Figure S4).

Taken together, our findings suggest that ACE2 expression in the endometrial stroma is promoted by progesterone in both humans and mice. Moreover, we show that knockdown of ACE2 impairs the human endometrial stromal decidualization process. Given the high ACE2 expression in the human endometrium, SARS-CoV-2 may be able to enter endometrial stromal cells and elicit pathological manifestations in women with COVID-19. If so, women with COVID-19 may be at an increased risk of early pregnancy loss. As more data become available, epidemiologists and obstetricians should focus on this important issue and determine whether women who intend to get pregnant should undergo additional health screenings during the COVID-19 pandemic.
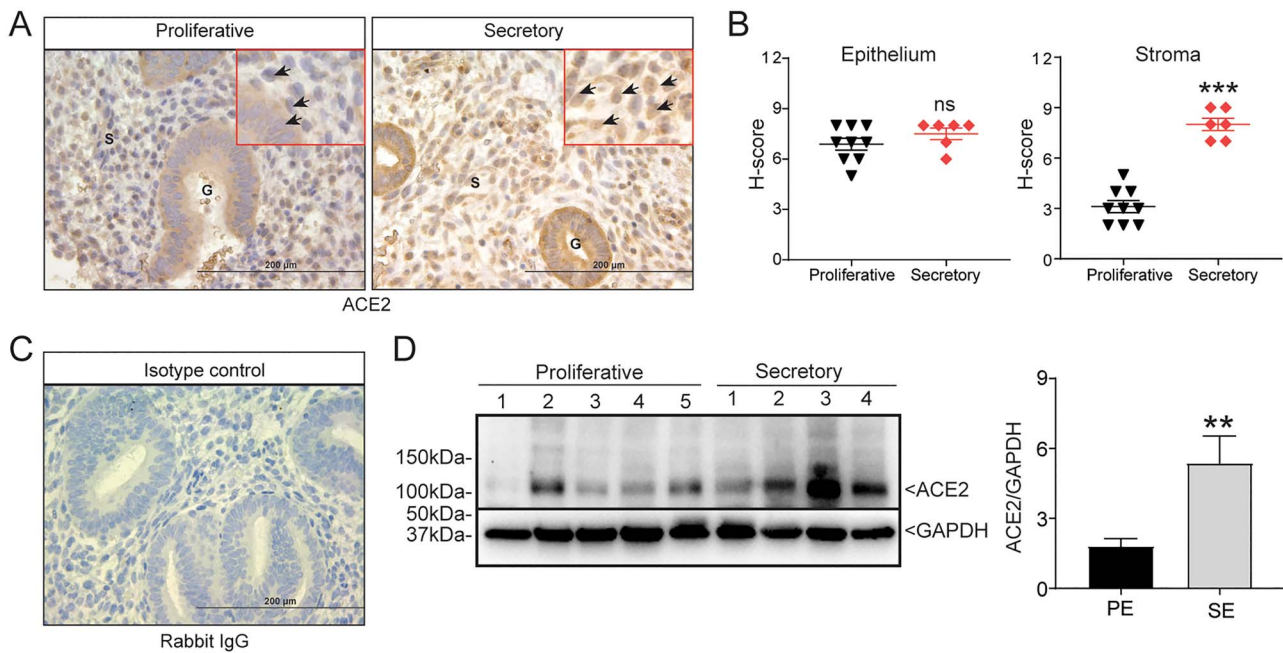
Figure 1. ACE2 protein expression is elevated in stromal cells of the secretory phase human endometrium. (A and B) Representative images showing immunolocalization of ACE2 (brown) counterstained with hematoxylin (blue) (A) in proliferative ($n = 9$) and secretory ($n = 6$) phase endometrium and quantitative analysis (B) scored according to staining intensity and number of positive cells [low (score ≤ 5) or high (score ≥ 5)]. G, gland; S, stroma. Black arrows indicate ACE2-positive cells. (C) Rabbit IgG was used as an isotype control for staining. (D) Western blot and quantification of ACE2 protein isolated from proliferative ($n = 5$) and secretory ($n = 4$) phase of human endometrium; GAPDH was used as an internal loading control. Scale bar: 200 μm; $**P < 0.01$, $***P < 0.001$, and ns, non-significant.

Materials and methods

Human ethical approval and endometrial stromal cell isolation

Informed consent was obtained in accordance with a protocol approved by the Washington University in St. Louis Institutional Review Board (IRB ID #: 201612127). Additionally, all work involving human subjects followed the guidelines of the World Medical Association Declaration of Helsinki. Human endometrial biopsies of healthy women of reproductive age were collected during the proliferative phase ($n = 9$) with the mean age 30 ± 1.47 years and during the secretory phase ($n = 6$) with the mean age of 29.37 ± 2.49 years of the menstrual cycle. HESCs were isolated as described previously [15, 16]. Briefly, proliferative phase endometrial biopsies were minced with sterile scissors and then digested in DMEM/F12 medium containing 2.5-mg/ml collagenase (Sigma-Aldrich, Saint Louis, MO) and 0.5-mg/ml DNase I (Sigma-Aldrich, Saint Louis, MO, USA) for 1.5 h at 37 °C. Then, detached cells were centrifuged at 800 g for 2.5 min, and then collected and layered over a Ficoll-Paque reagent layer and centrifuged for 30 min at 400 g (GE Healthcare Biosciences, Pittsburgh, PA) to remove lymphocytes. The HESC fraction from the top layer was collected and filtered through a 40-μm nylon cell strainer (BD Biosciences, Franklin Lakes, NJ). HESCs collected from the filtrate were suspended in DMEM/F-12 media containing 10% FBS, 100-U/ml penicillin, and 0.1-mg/ml streptomycin at 37 °C with 5% CO_2. Primary HES cells isolated from five ($n = 5$) different subjects were used for in vitro studies.

Transfection and HESC decidualization

HESCs isolated from the proliferative phase of the menstrual cycle were grown in a six-well culture plate at 60–70% confluence

and transfected with 60 pmol of nontargeting siRNA (D-001810-10-05) or siRNAs targeting ACE2 (L-005755-00-0005) (GE Healthcare Dharmacon Inc., Lafayette, CO) in Lipofectamine 2000 reagent (Invitrogen Corporation, Carlsbad, CA), as described previously [15]. After 48 h, HESCs were decidualized by culturing in EPC (Estrogen, Medroxy Progesterone Acetate and cAMP) medium (1x Opti-MEM reduced-serum media containing 2% FBS, 100-nM estradiol [cat. no. E1024, Sigma-Aldrich, Saint Louis, MO, USA, 10-μM Medroxyprogesterone17-acetate [cat. no. M1629, Sigma-Aldrich, Saint Louis, MO, USA], and 50-μM 8-Bromoadenosine 3′,5′-cyclic monophosphate sodium salt [cat. no. B7880, Sigma-Aldrich, Saint Louis, MO, USA]). The EPC medium was changed every 48 h until day 6, when the cells were harvested for RNA isolation with the total RNA isolation kit (Invitrogen/Life Technologies, Grand Island, NY) or for protein isolation.

Quantitative real-time PCR

Total RNA was extracted from uterine tissues or HESCs by using the total RNA isolation kit (Invitrogen/Life Technologies) according to the manufacturer's instructions. RNA was quantified with a Nano-Drop 2000 (Thermo Scientific, Waltham, MA). Then, 1 μg of RNA was reverse-transcribed with the High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific, Waltham, MA, USA). The amplified cDNA was diluted to 10 ng/μl, and quantitative PCR was performed with primers specified in Supplementary Table S1 and Fast Taqman 2X mastermix (Applied Biosystems/Life Technologies, Grand Island, NY) on a 7500 Fast Real-time PCR System (Applied Biosystems/Life Technologies). Ribosomal RNA (18S) was used as an internal control for gene-specific primers [15, 17, 18].
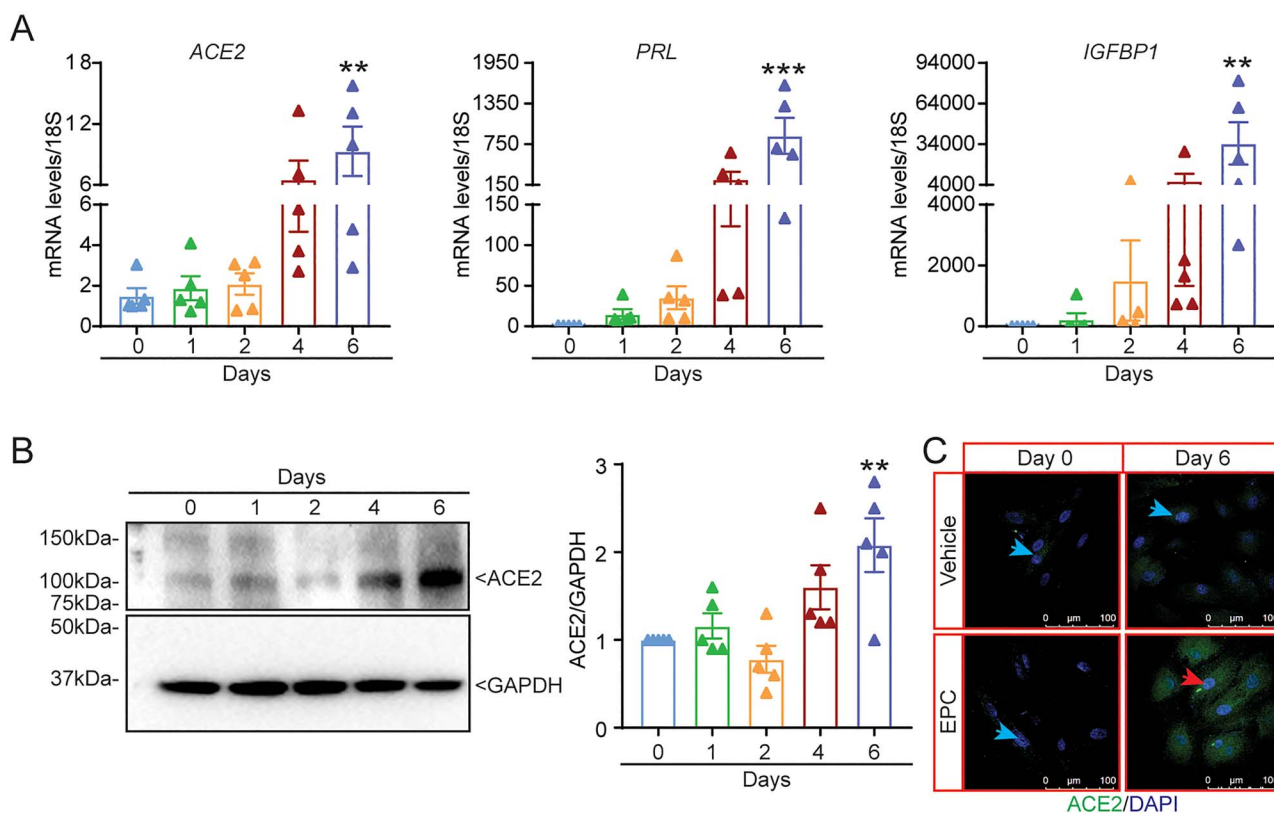
Figure 2. ACE2 is upregulated during in vitro HESC decidualization. (A) Abundances of ACE2, *PRL*, and *IGFBP1* transcripts from HESCs induced to decidualize for the indicated numbers of days. (B) Western blot analysis and quantification of ACE2 from HESCs cultured in decidualization media for the indicated numbers of days; GAPDH was used as an internal loading control. (C) Immunofluorescence detection of ACE2 (green) in HESCs cultured with vehicle or decidualization media (EPC) for the indicated numbers of days. Blue stain is DAPI. Red arrowhead indicates a decidualized cell, and blue arrowheads indicate nondecidualized cells. Representative data from five primary HES cells derived from different subjects ($n = 5$) are shown as mean ± SEM ($n = 5$). The experiments were repeated five times. Scale bar: 100 μm. **$P < 0.01$ and ***$P < 0.001$.

SDS-PAGE and Western blotting

Protein extracts were prepared from human endometrial biopsies or HESCs, as described previously [19]. Briefly, total proteins were extracted by homogenizing cells in RIPA lysis buffer (cat. no. 9806, Cell Signaling Technology, Danvers, MA, USA) and centrifuging at 14 000*g* for 15 min at 4 °C. The supernatants were collected and protein was quantified with the BCA Protein Assay kit according to the manufacturer's instructions (Pierce BCA protein assay kit, cat no. 23227). Lysates containing 40 μg of protein were loaded on a 4–15% SDS-PAGE gel, separated with 1x Tris-Glycine Running Buffer, and transferred to PVDF membranes on a wet electro-blotting system (all from Bio-Rad, Hercules, MA, USA), all according to the manufacturer's directions. The PVDF membranes were washed, blocked for 1 h in 5% non-fat milk in TBS-T (Bio-Rad, Hercules, MA, USA), and incubated with primary antibodies anti-ACE2 (1 μg/1000 μl, ab15348, Abcam, Cambridge, MA, USA) and anti-GAPDH (1 μg/3000 μl, #2118S Cell Signaling Technology, Danvers, MA, USA) in 5% BSA in TBS-T overnight at 4 °C. Then, blots were probed with anti-Rabbit IgG conjugated with horseradish peroxidase (1 μg/5000 μl, #7074, Cell Signaling Technology, Danvers, MA, USA) in 5% BSA in TBS-T for 1 h at room temperature. Signal was detected by using the Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA), and blot images were collected with a Bio-Rad ChemiDoc imaging system [17] and quantification of the blots were conducted by densitometry using Image Lab software.

Immunohistochemistry

The human endometrial biopsy tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and then sectioned (5 μm) with a microtome (Leica Biosystem, Germany). For immunostaining, sections were deparaffinized, incubated in 10 mM sodium citrate buffer, pH 6, for 20 min. for antigen retrieval. Endogenous peroxidase activity was quenched by incubating sections in BLOSALL (SP-6000, Vector Laboratories Inc., CA) for 20 min. Tissue sections were blocked with 2.5% goat serum and then incubated overnight at 4 °C in 2% goat serum containing the following primary antibodies: Rabbit anti-ACE2 (1 μg/200 μl, ab15348, Abcam, Cambridge, MA, USA), anti-TMPRSS2 (1.25 μg/200 μl 14437–1-AP, Proteintech, Rosemont, IL, USA), or normal rabbit IgG (1.25 μg/200 μl, #2729, Cell Signaling Technology, Danvers, MA, USA). Sections were then incubated with biotinylated secondary antibody for 1 h, and then with ABC reagent (PK-4001, Vector Laboratories Inc.) for 1 h. Next, slides were incubated with the 3, 3′-diaminobenzidine peroxidase substrate (SK-4100, Vector Laboratories Inc.) and counter-stained with hematoxylin. Finally, sections were dehydrated and mounted
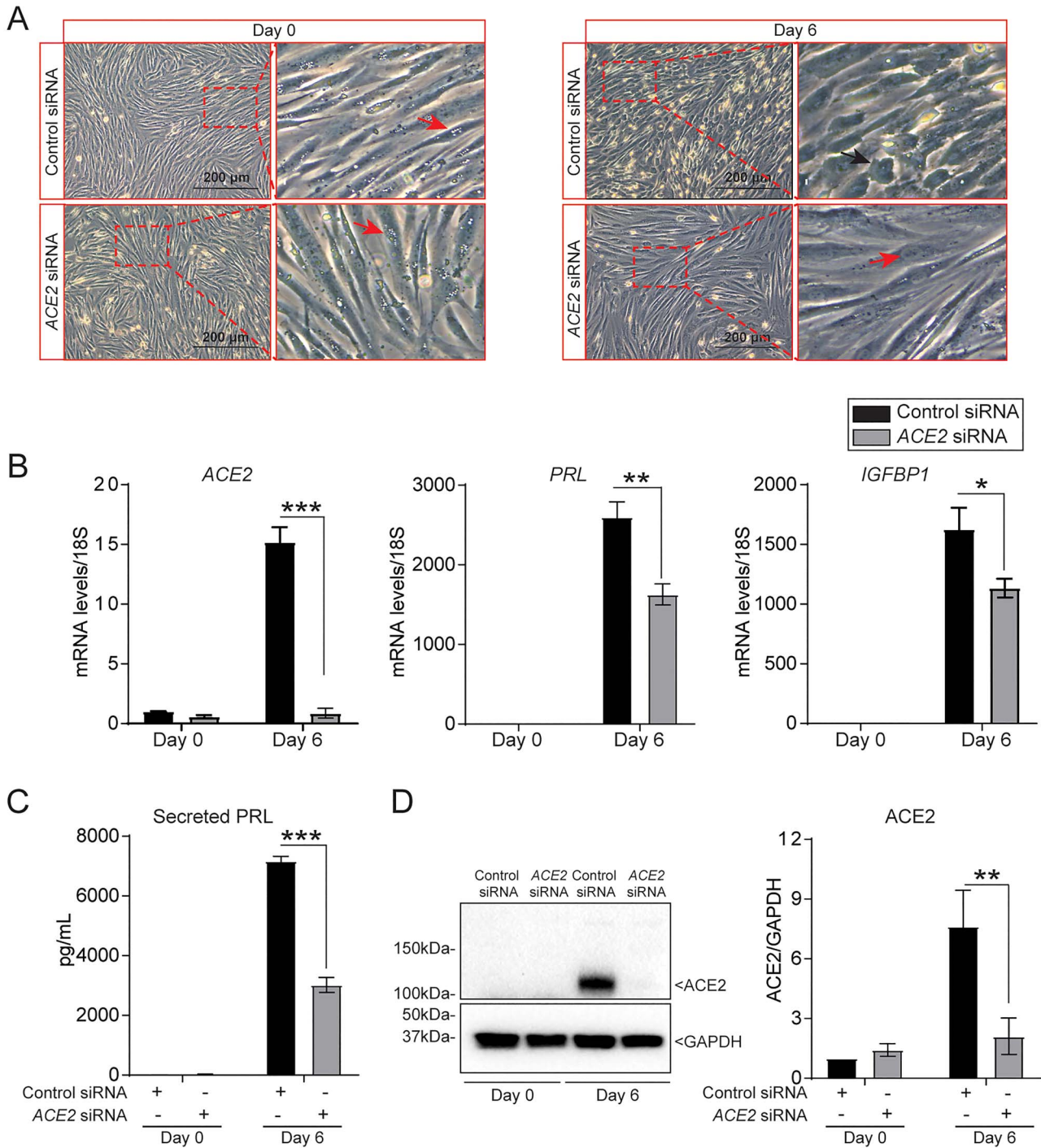
Figure 3. Knockdown of ACE2 impairs HESC decidualization. (A) Morphology of HESCs transfected with control or ACE2 siRNA at day 0 or after 6 days of culture in decidualization conditions. Red arrows indicate nondecidualized cells, and the black arrow indicates a decidualized cell. Scale bar: 200 μm. (B) Abundances of ACE2, *PRL*, and *IGFBP1* transcripts in HESCs transfected with control or ACE2 siRNAs and induced to decidualize for the indicated numbers of days. (C) Levels of secreted Prolactin (PRL) into the media were measured using ELISA-based assay in HESCs transfected with control or ACE2 siRNAs at day 0 or 6 days after EPC treatment. (D) Western blot and quantification of ACE2 protein from HESCs transfected with control or ACE2 siRNA; GAPDH was used as an internal loading control. Representative data from three replicates from one subject sample are shown as mean ± SEM. The experiments were repeated five times; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

in Permount histological mounting medium (Fisher Scientific Inc., Hampton, NH, USA). The H-score for tissue samples was scored according to staining intensity and number of positive cells (low score ≤ 5, high score > 5) by two independent, blinded investigators.
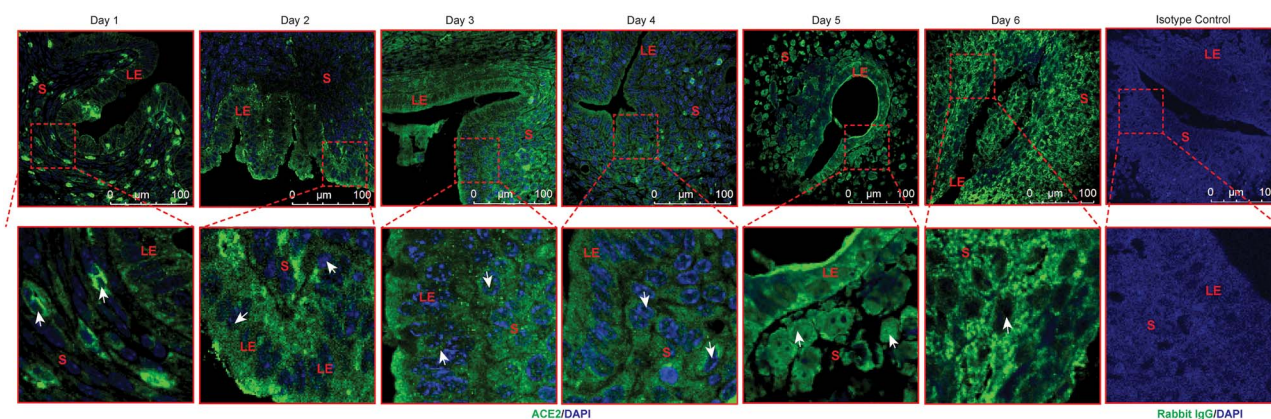
Figure 4. ACE2 protein expression in the mouse uterine stroma increases during early pregnancy. Representative images of the immunocytochemical localization of ACE2 in mouse uteri are shown on the indicated days of pregnancy. LE, luminal epithelium; S, stroma; G, glands. Scale bar: 100 μm. White arrows indicate ACE2-positive cells. Samples from at least five mice were examined. All uteri were collected between 9:00 am and 10:00 am on the indicated days of pregnancy.

Immunofluorescence

Formalin-fixed, paraffin-embedded sections (5 μm) of mouse uteri were deparaffinized in xylene, rehydrated in an ethanol gradient, and then boiled in antigen retrieval citrate buffer (Vector Laboratories Inc., CA). Subsequently, sections were blocked with 2.5% goat serum in PBS (Vector laboratories) for 1 h at room temperature, and then incubated overnight at 4 °C with anti-ACE2 antibody (1 μg/200 μl, ab15348, Abcam, Danvers, MA, USA) or normal rabbit IgG (1 μg/200 μl, #2729, Cell Signaling Technology). Then, sections were washed with PBS, incubated with Alexa Fluor 488-conjugated secondary antibody (Life Technologies, Carlsbad, CA, USA) for 1 h at room temperature, washed three times with PBS, and mounted with ProLong Gold Antifade Mountant with DAPI (cat. no. P36962 Thermo Scientific, Waltham, MA, USA). Immunofluorescence images were captured on a confocal microscope (Leica DMI 4000B).

Immunocytochemistry

HESCs were grown on poly-L-Lysine coated coverslips in 12-well plates and allowed to decidualize for 6 days in EPC media, as described above. Then, cells were fixed with 4% paraformaldehyde (Alfa Aesar, Haverhill, MA, USA) in PBS for 20 min at room temperature, washed with PBS, and permeabilized with 0.2% Triton X-100 (Sigma Aldrich, Saint Louis, MO, USA) in PBS for 20 min at room temperature. Then, cells were washed, blocked with 2.5% normal goat-serum (Vector laboratories) in PBS for 1 h at room temperature, and incubated overnight at 4 °C with anti-ACE2 antibody (ab15348, Abcam, 1 μg/200 μl) in 2.5% normal goat serum. The cells were washed and incubated with Alexa Fluor 488-conjugated secondary antibodies (Life Technologies) for 1 h at room temperature and mounted with ProLong Gold Antifade Mountant with DAPI (Thermo Scientific, Waltham, MA, USA). Images were captured on a confocal microscope (Leica DMI 4000B).

Prolactin ELISA

Post-transfection for 48 h with either control or ACE2 siRNA, the cells were treated with decidualization media, and the media was changed every 48 h until day 6. The cell culture supernatant was collected at day 0 and 6 and stored at −80 °C until use. According to the manufacturer's instructions, the Prolactin ELISA (cat. no. EHIAPRL, Invitrogen) was performed in cell culture media. Briefly, 50 μl of collected media was used to quantify the secreted Prolactin protein.

The concentration of Prolactin was calculated from the standard curve. Each experiment was performed in triplicates and repeated in five primary HES cells derived from different subjects ($n = 5$).

Mice and hormone treatments

All experimental procedures with mice followed a protocol approved by the Washington University in St. Louis Institutional Animal Care and Use Committee (Protocol Number: 20191079). CD1 wild-type mice (Charles River, Saint Louis, MO) were maintained on a 12-h light: 12-h dark cycle. Sexually mature (8-week-old) CD1 females were mated to fertile wild-type males, and copulation was confirmed by the presence of vaginal plug the following morning, designated as 1 day post-coital (dpc). Mice were euthanized, and uteri were collected on 1, 2, 3, 4, 5, and 6 dpc. To determine the uterine estrogen or progesterone responses, 6-week-old CD1 mice ($n = 5$ mice per group) were bilaterally ovariectomized, rested for 2 weeks to allow the endogenous ovarian-derived steroid hormones to dissipate, and then subcutaneously injected with 100-μl sesame oil (vehicle control), 1-mg progesterone, or 100-ng estradiol (Sigma-Aldrich, Saint Louis, MO, USA) in 100-μl sesame oil. Six hours later, the mice were euthanized, uterine tissues were collected and fixed in 4% paraformaldehyde, and RNA was isolated and processed for qRT-PCR [17].

Statistical analyses

A two-tailed paired Student *t*-test was used to analyze experiments with two experimental groups, and analysis of variance by nonparametric alternatives was used for multiple comparisons to analyze experiments containing more than two groups. $P < 0.05$ was considered significant. All data are presented as mean ± SEM. GraphPad Prism 8 software was used for all statistical analyses.

Supplementary data

Supplementary data are available at *BIOLRE* online.
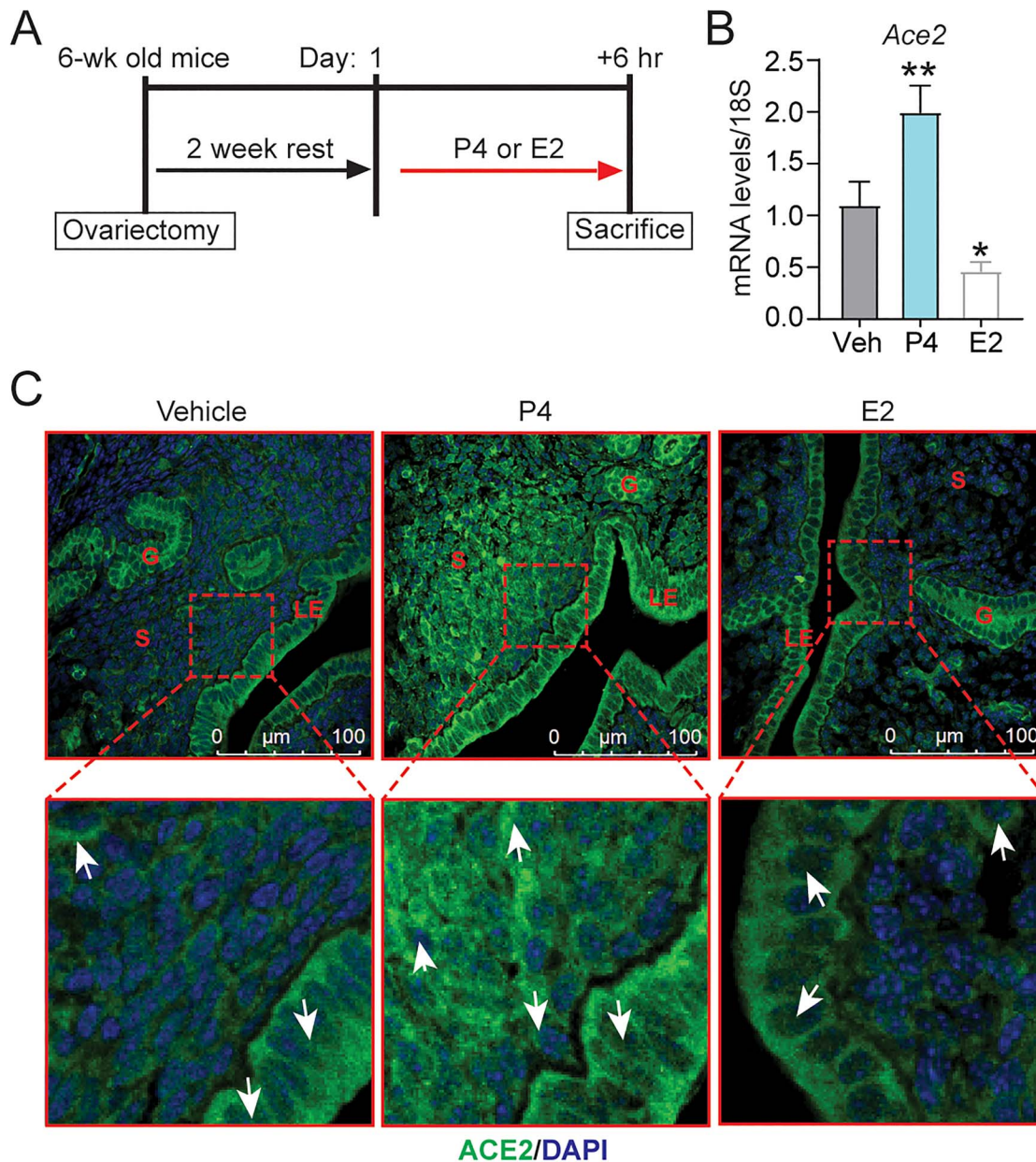
Acknowledgment

Figure 5. ACE2 expression in the mouse uterus is upregulated by progesterone exposure. (A) Experimental protocol and hormone treatment. E2, estrogen; P4, progesterone. (B) Relative ACE2 mRNA abundance after 6 h of estrogen or progesterone treatment. Data are presented as mean ± SEM ($n = 5$ mice per group). *$P < 0.05$ and **$P < 0.01$. (C) Representative cross-sectional images of uteri stained for ACE2 (green) and DNA (blue); LE, luminal epithelium; S, stroma; G, glands, scale bar: 100 μm.

Conflict of interest

Author contributions

Non-standard Abbreviations

SARS-CoV-2	severe acute respiratory syndrome coronavirus
ACE2	Angiotensin converting enzyme 2

WT	Wild Type
HESC	Human Endometrial Stromal Cells
dpc	Days Post Coitum
E2	Estrogen
P4	Progesterone
PRL	Prolactin
TMPRSS2	Transmembrane protease serine 2

References

1. Favre G, Léo Pomar, Didier Musso, David Baud. 2019-nCoV epidemic: What about pregnancies? *Lancet* 2020; 395:e40.

2. Schwartz DA, Dhaliwal A. Infections in Pregnancy with Covid-19 and Other Respiratory Rna Virus Diseases Are Rarely, if ever, Transmitted to the Fetus: Experiences with Coronaviruses, Hpiv, Hmpv Rsv, and Influenza. *Arch Pathol Lab Med* 2020; **144**:920–928.

3. Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental pathology in COVID-19. *medRxiv* 2020.

4. Baud D, Greub G, Favre G, Gengler C, Jaton K, Dubruc E, Pomar L. Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection. *JAMA* 2020; **323**:2198–2200.

5. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**:631–637.

6. Riviere G, Michaud A, Breton C, VanCamp G, Laborie C, Enache M, Lesage J, Deloof S, Corvol P, Vieau D. Angiotensin-converting enzyme 2 (ACE2) and ACE activities display tissue-specific sensitivity to undernutrition-programmed hypertension in the adult rat. *Hypertension* 2005; **46**:1169–1174.

7. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett* 2002; **532**:107–110.

8. Merrill DC, Karoly M, Chen K, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) in normal and preeclamptic pregnancy. *Endocrine* 2002; **18**:239–245.

9. Neves LA, Stovall K, Joyner J, Valdés G, Gallagher PE, Ferrario CM, Merrill DC, Brosnihan KB. ACE2 and ANG-(1-7) in the rat uterus during early and late gestation. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**:R151–R161.

10. Brosnihan KB, Bharadwaj MS, Yamaleyeva LM, Neves LAA. Decidualized pseudopregnant rat uterus shows marked reduction in Ang II and Ang-(1-7) levels. *Placenta* 2012; **33**:17–23.

11. Vaz-Silva J, Carneiro MM, Ferreira MC, Pinheiro SVB, Silva DA, Silva-Filho AL, Witz CA, Reis AM, Santos RA, Reis FM. The vasoactive peptide angiotensin-(1-7), its receptor mas and the angiotensin-converting enzyme type 2 are expressed in the human endometrium. *Reprod Sci* 2009; **16**:247–256.

12. Carson DD, Dharmaraj N. Embryo implantation. *Dev Biol* 2000; **223**:217–237.

13. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med* 2001; **345**(19):1400–1408.

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

15. Camden AJ, Szwarc MM, Chadchan SB, DeMayo FJ, O'Malley BW, Lydon JP, Kommagani R. Growth regulation by estrogen in breast cancer 1 (GREB1) is a novel progesterone-responsive gene required for human endometrial stromal decidualization. *Mol Hum Reprod* 2017; **23**:646–653.

16. Michalski SA, Chadchan SB, Jungheim ES, Kommagani R. Isolation of human endometrial stromal cells for in vitro decidualization. *J Vis Exp* 2018; **139**:57684.

17. 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.

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

19. Oestreich AK, Chadchan SB, Popli P, Medvedeva A, Rowen MN, Stephens CS, Xu R, Lydon JP, Demayo FJ, Jungheim ES, Moley KH, Kommagani R. The autophagy gene Atg16L1 is necessary for endometrial decidualization. *Endocrinology* 2020; **161**:bqz039.