Supporting Information

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SI Materials and Methods

Mouse and Human Myometrial Preparations. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center. Eight-week-old timed pregnant Institute for Cancer Research (ICR)/CD1 mice were obtained from Harlan Laboratories. Myometrial tissues were isolated at 15.5–18.5 days postcoitum (dpc) and following the delivery of the first pup (in labor), as described previously (1). All human studies and consent forms were approved by the Institutional Review Board of University of Texas Southwestern Medical Center in accordance with the Donors Anatomical Gift Act of the State of Texas. Informed consent was obtained in writing from each woman before surgery. Myometrial biopsies were obtained from the lower uterine segment of pregnant women undergoing cesarean section before and during the onset of active labor. After the tissues were collected, they were flash-frozen and stored at −80° for subsequent protein and mRNA analysis.

Mouse Model of Preterm Labor Induced by Pharmacological P_4/Progesterone Receptor Withdrawal. Induction of preterm labor using RU486 was implemented as described previously (1, 2). Briefly, 200 μg of RU486 (Sigma) or 5% ethanol (vol/vol) (vehicle) were subcutaneously injected into the right flank of 15.5 dpc pregnant ICR mice. Preterm labor occurred in the RU486-injected mice within 12 h, but none of the vehicle-injected mice went into labor. The RU486 injected mice were killed upon the birth of one pup; gestation-matched controls were killed immediately afterward.

Mouse Model of Preterm Labor by LPS-Induced Inflammation. Induction of preterm labor using LPS was implemented as described in detail previously (1). Briefly, 15.5 dpc pregnant ICR/CD1 mice underwent laparotomy to expose the uterus after anesthesia. LPS (1.5 μg in 50 μL PBS) or sterile PBS (vehicle) were injected into each amniotic sac and then the uterus was carefully reinserted into the abdominal cavity, the abdominal muscle wall and skin were sutured, and the mouse was allowed to recuperate. There was a high rate of preterm labor in the LPS-injected mice and in none of the vehicle-injected mice. The LPS-injected mice were killed upon the birth of one pup; gestation-matched controls were killed immediately afterward.

P_4 Treatment Studies. Six- to 8-wk-old ovariectomized ICR mice were obtained from Harlan Laboratories. Two weeks after ovariectomy, the mice were subcutaneously injected with progesterone (P_4) (1 mg in 0.25 mL sesame oil) or with sesame oil (vehicle). Uterine tissues were collected 24 h postinjection and flash frozen for subsequent protein and mRNA analysis.

Transfection and Transduction of Human Myometrial Cells. Immortalized human myometrial cells (hTERT-HM) (3) were cultured in DMEM-F12 medium (Gibco) with 10% FBS (vol/vol). In the miR-200 overexpression experiments, hTERT-HM cells were infected overnight with recombinant adenoviruses expressing miR-200b/200a/429 or β-galactosidase (control) at a multiplicity of infection of 500. The cells were harvested 72 h after infection. β-Gal staining (β-Gal Staining Kit; Invitrogen) was performed to assess transduction efficiency. Other dishes of cells were transfected with miR-200a mimics. Briefly, hTERT-HM cells were transfected with miR-200a or scramble mimics (20 nM; Qiagen) using HiPerFect Transfection Reagent (Qiagen). Cells were harvested after 24 h for mRNA and protein analysis. In the STAT5b knockdown studies, hTERT cells were transfected either with 20 nM STAT5b siRNA or scramble siRNA and harvested 72 h later. For STAT5b overexpression experiments, hTERT-HM cells were transfected with 100 ng of a CMV-driven expression plasmid expressing STAT5b or an empty CMV plasmid and harvested 72 h after transfection. The hTERT-HM cells were at ∼80% confluency at the time of these experiments.

Quantitative RT-PCR. For gene expression analysis, total RNA was DNase-treated (Invitrogen), reverse-transcribed using SuperScript III-RT kit (Invitrogen) and subsequently amplified using SYBR Green (Applied Biosystems) and specific primers with either GAPDH or human 36B4 as an internal standard. The primer sequences for analysis were as follows: mouse GAPDH (Forward: 5′ AGG TCG GTG TGA ACG GAT TTG 3′; Reverse: 5′ TGT AGA CCA TGT AGT TGA GGT CA 3′), mouse STAT5b (Forward: 5′ CAC CCG CAA TGA TTA CAG CG 3′; Reverse: 5′ CTC TTG ATT CGT TTC AGG GAC A 3′), mouse 20α-hydroxysteroid dehydrogenase (20α-HSD)/aldo-ketoreductase (AKR)1C18 (Forward: 5′ GGA GGC CAT GGA GAA GTG TA 3′; Reverse: 5′ ATG GCA TTC TAC CTG GTT CG 3′), human 36B4 (Forward: 5′ TGC ATC AGT ACC CCA TTC TAT CA 3′; Reverse: 5′ AAG GTG TAA TCC GTC TCC ACA GA 3′), human STAT5b (Forward: 5′ GAA CAC CCG CAA TGA TTA CAG T 3′; Reverse: 5′ ACG GTC TGA CCT AAT TCG T 3′) and human 20α-HSD/AKR1C1 (Forward: 5′ CAG CCA GGC TAG TGA CAG AA 3′; Reverse: 5′ ATT GCC AAT TTG GTG GC 3′). For microRNA analysis, RT using a TaqMan Reverse Transcription kit (Applied Biosystems) was performed, followed by qPCR using miRNA-specific Taqman primers and TaqMan Universal PCR Master Mix (Applied Biosystems). miRNA expression was normalized to U6 snRNA using the comparative cycle threshold (ΔΔCt) method.

Immunoblot Analysis. Cytoplasmic and nuclear proteins were isolated from cell and tissue samples using a NE-PER Extraction Reagent Kit (Pierce). The expression of proteins of interest was determined using primary polyclonal antibodies against STAT5b (Abcam) and 20α-HSD (generously provided by Geula Gibori, University of Illinois, Chicago, IL) at 1:1,000 and 1:5,000 dilutions, respectively. β-Actin (Abcam) was analyzed as a loading control.

Luciferase Reporter Assays. TargetScan software was used to identify putative miR-200a binding sites in STAT5b. STAT5b 3′ UTR containing potential miR-200a binding sites was amplified from human genomic DNA using the following primers: forward: 5′-GGGACTAGTCTTCAGCTTCTTCATCTTCACCAGAGGAAT-3′; reverse: 5′-GGGAAGCTTTTTAAAAAAGTCATCTTCCAATAAATAATT-3′. The 530-bp fragment was then cloned 3′ of luciferase in pMIR-REPORT (Invitrogen); sequence of the recombinant plasmid was confirmed before transfection. For mutation analysis, QuikChange II Site-Directed Mutagenesis Kit (Stratagene) was used to mutate three nucleotides in both putative miR-200a binding sites (AGUGUU to AGUCCC). COS-7 cells were cotransfected with these reporter plasmids and 20 nM miR-200a or miR-1 (control) and then assayed for relative luciferase activity 48 h later. To normalize for transfection efficiency, cells were cotransfected with a β-galactosidase expression plasmid.

20α-HSD Enzyme Activity. Uteri were isolated from pregnant ICR mice from 15.0 to 19.0 dpc. Tissues were homogenized in 10 mM

potassium phosphate, 150 mM KCl, and 1 mM EDTA. Protein concentrations were determined using a Bio-Rad Protein Assay Kit. P_4 metabolism was assessed by incubating tissue homogenates (5 μg protein) in 0.1 M Tris-citrate buffer, pH 7.0, containing 5 μM [^{14}C]P_4 (New England Nuclear) and 5 mM NADPH (Sigma) in a total volume of 0.5 mL for 1 h at 37 C. Steroids were extracted into 5 mL methylene chloride and dried under a stream of nitrogen. Steroids were dissolved in 20 μL chloroform-methanol (2:1, vol/vol), spotted onto Silica Gel 150 TLC plates (4855-821; Whatman), and resolved by development in chloroform-ethyl acetate (3:1, vol/vol). Radio-labeled steroids were visualized on a Bioscan imaging detector.

ChIP. ChIP was performed using a ChIP Assay Kit (catalog no. 17–295; Millipore) to assess binding of endogenous STAT5b to the *20α-HSD/AKR1C18* promoter in myometrial tissues from timed-pregnant mice at 15.5 and 18.5 dpc. Briefly, mouse myometrial tissues were isolated, homogenized, cross-linked with formaldehyde (1%; vol/vol), and sonicated to produce sheared soluble chromatin, as described in detail previously (1). Precleared chromatin was incubated with STAT5b antibody (ab7969; Abcam) or nonimmune IgG, as control, at 4 °C overnight. Immune complexes were collected on Protein A agarose beads using a ChIP Assay Kit (catalog no. 17–295; Millipore). Chromatin complexes were eluted from the beads and cross-linking was reversed. DNA purified from the samples and input controls was analyzed for *akr1c18* promoter sequences containing putative STAT5b response elements using qPCR and the following primers: forward: 5′-GCTTCCTCACTGGGTGGAGTTGG-3′; reverse: 5′-GGGGCACAAAACCTCAGGAGCC-3′.

Statistical Analysis. Excel (Microsoft) was used to perform statistical analyses. The two-tailed Student *t* test was used to calculate statistical significance. We considered $P > 0.05$ to be significant.

1. Renthal NE, et al. (2010) miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. *Proc Natl Acad Sci USA* 107: 20828–20833.
2. Dudley DJ, Branch DW, Edwin SS, Mitchell MD (1996) Induction of preterm birth in mice by RU486. *Biol Reprod* 55:992–995.
3. Condon J, et al. (2002) Telomerase immortalization of human myometrial cells. *Biol Reprod* 67:506–514.

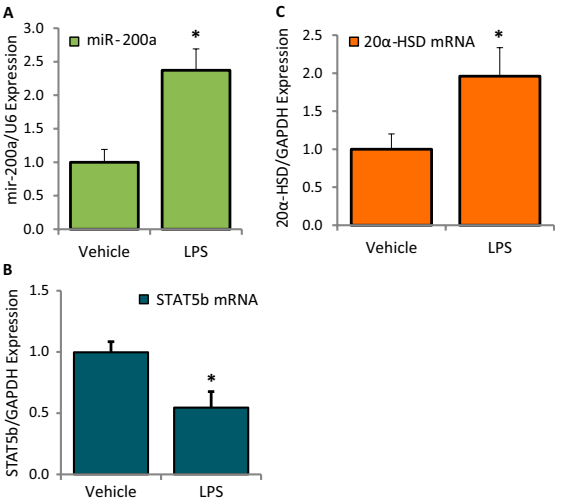

Fig. S1. miR-200a is up-regulated, STAT5b is down-regulated and 20α-HSD is up-regulated with LPS induction of preterm labor in mice. Timed pregnant mice (15.5 dpc) were injected intra-amniotically with LPS (1.5 μg in 50 μL) or vehicle into each amniotic sac to induce preterm labor, as described previously (1). Upon preterm birth of the first pup, each LPS injected mouse was killed together with a vehicle-injected control and myometrial tissues were harvested. Using quantitatitve RT-PCR, we observed that expression of miR-200a was significantly increased (*A*) and its target, STAT5b, was reciprocally decreased (*B*) in myometrial tissues of LPS injected mice, compared with vehicle-injected controls. The LPS-induced decline in STAT5b was associated with a significant increase in 20α-HSD expression, compared with controls (*C*). This mirrors that observed for miR-200a, STAT5b, and 20α-HSD expression in pregnant mice injected subcutaneously with RU486, as shown in Fig. 4 *E–H*. (*$P < 0.05$; $n = 5$ mice per group).