

MicroRNA-200a serves a key role in the decline of progesterone receptor function leading to term and preterm labor

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During pregnancy, uterine quiescence is maintained by increased progesterone receptor (PR) activity, but labor is facilitated by a series of events that impair PR function. Previously, we discovered that miR-200 family members serve as progesterone (P₄)-modulated activators of contraction-associated genes in the pregnant uterus. In this study, we identified a unique role for miR-200a to enhance the local metabolism of P₄ in myometrium and, thus, decrease PR function during the progression toward labor. miR-200a exerts this action by direct repression of STAT5b, a transcriptional repressor of the P₄-metabolizing enzyme 20 α -hydroxysteroid dehydrogenase (20 α -HSD). We observed that miR-200a expression increased and STAT5b expression coordinately decreased in myometrium of mice as they progressed to labor and in laboring myometrium from pregnant women. These changes were associated with a dramatic increase in expression and activity of 20 α -HSD in laboring myometrium from mouse and human. Notably, overexpression of miR-200a in cultured human myometrial cells (hTERT-HM) suppressed STAT5b and increased 20 α -HSD mRNA levels. In uterine tissues of ovariectomized mice injected with P₄, miR-200 expression was significantly decreased, STAT5b expression was up-regulated, and 20 α -HSD mRNA was decreased, but in 15 d postcoitum pregnant mice injected with the PR antagonist RU486, preterm labor was associated with increased miR-200a, decreased STAT5b, and enhanced 20 α -HSD expression. Taken together, these findings implicate miR-200a as an important regulator of increased local P₄ metabolism in the pregnant uterus near term and provide insight into the importance of miR-200s in the decline in PR function leading to labor.

It has long been appreciated that progesterone (P₄) acting through progesterone receptor (PR) plays a critical role in maintaining uterine quiescence throughout most of pregnancy (see refs. 1 and 2 for review). The finding in rodents that circulating maternal P₄ levels decline precipitously near term (3) has led to the concept that labor is associated with P₄ withdrawal. On the other hand, in humans and guinea pigs, circulating P₄ levels remain elevated throughout pregnancy and into labor, as do myometrial levels of PR (4, 5). Nonetheless, treatment with PR antagonists, mifepristone (RU486) or onapristone, can cause increased cervical ripening and spontaneous labor or enhanced sensitivity to labor induction by oxytocin or prostaglandins (6–10). It should be noted that even in mice maternal P₄ levels at term remain well above the K_d for binding to PR. These collective findings have led to the concept that parturition in all species is initiated by a concerted series of biochemical mechanisms that antagonize the ability of the P₄/PR to regulate target genes in the uterus and cervix that maintain myometrial quiescence. These mechanisms may include altered expression of PR coregulators (11–13), antagonistic interaction of PR with the inflammatory transcription factor NF- κ B (14, 15) [which is activated in the myometrium near term (16)], increased expression of inhibitory PR isoforms (17), and enhanced local metabolism of P₄ to inactive products (18, 19). Indeed, increased P₄

metabolism by the pregnant uterus approaching term has been observed in a number of species (19–23). In myometrium of pregnant women at term, there is a dramatic increase in the ratio of 20 α -dihydroprogesterone (20 α -OHP) to P₄ (23). 20 α -OHP is an inactive metabolite of P₄ generated by 20 α -hydroxysteroid dehydrogenase (20 α -HSD), a member of the aldo-ketoreductase (AKR) superfamily (24).

Recently, we have uncovered a role for microRNAs (miRNAs, miRs) in the regulation of genes that influence uterine quiescence/contractility during pregnancy and labor (25). We identified a conserved miRNA family, the miR-200 family, that is highly up-regulated at term in myometrium of mice and humans, as well as two coordinately down-regulated targets of miR-200, the zinc finger E-box binding homeobox proteins ZEB1 and ZEB2 (25). We further demonstrated that during pregnancy ZEB1 is directly up-regulated by P₄/PR. Importantly, ZEB1 and ZEB2 inhibit expression of the contraction-associated genes, oxytocin receptor (OXTR) and connexin-43 (CX43) and block oxytocin-induced myometrial contractility. Near term, the decline in PR function results in decreased expression of ZEB1/2, an induction of miR-200 family expression, and increased transcription of contraction-associated genes leading to labor (25).

Interestingly, one of the members of the miR-200 family, miR-200a, is predicted by TargetScan analysis (<http://www.targetscan.org/>) to target the transcription factor, STAT5b, which serves as a P₄-responsive transcriptional repressor of 20 α -HSD in reproductive tissues (26, 27). Stat5b deficiency in mice resulted in pregnancy loss during midgestation. This finding was correlated with increased expression of ovarian 20 α -HSD and decreased circulating P₄ (27). Furthermore, the abortion rate in Stat5b-deficient mice was partially corrected by combined 20 α -HSD deficiency (27). In the present study we have demonstrated that STAT5b is a bonafide target of miR-200a and observed that up-regulation of miR-200a within the myometrium of laboring mice and humans was associated with decreased STAT5b expression and increased expression and activity of 20 α -HSD. P₄ treatment significantly decreased miR-200a expression, up-regulated STAT5b mRNA and protein, and decreased 20 α -HSD mRNA in mouse uterine tissues. Conversely, RU486 induction of preterm labor in mice was associated with increased miR-200a expression, decreased STAT5b, and induction of 20 α -HSD expression. Taken together, these findings suggest that miR-200a plays a key role in the induction of local P₄ metabolism in the pregnant

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uterus near term and in the decline in PR function leading to labor.

Results

miR-200a Is Up-Regulated During Late Gestation and Labor. In previous microarray analysis of quiescent pregnant mouse myometrium at 15.5 d postcoitum (dpc) vs. contractile myometrium at 18.5 dpc, we observed that the miR-200 family was significantly up-regulated toward term but their targets, ZEB1 and ZEB2, were coordinately down-regulated (25). Quantitative RT-PCR (qRT-PCR) analysis of pregnant mouse myometrium during late gestation previously confirmed increased expression near term of the two miR-200 family members that were most significantly increased on the microarray, miR-200b and miR-429 (25). These miR-200 family members are clustered together with miR-200a on mouse chromosome 4 and on human chromosome 1. Notably, miR-200b/200a/429 are encoded within a 7.5-kb polycistronic primary miRNA transcript that was shown to be repressed by ZEB1 (28), suggesting their coordinate regulation. The 3' UTR of ZEB1 contains binding sites for miR-200b/200a/429, which allow these miRNAs to collectively contribute to inhibition of ZEB1 expression in various tissues (25). Indeed, qRT-PCR of myometrial RNA from pregnant mice at 15.5 dpc to labor established that miR-200a expression increased in a pattern similar to that of miR-200b and miR-429 (Fig. 1A) and in a manner reciprocal to expression of ZEB1 (25).

Transcriptional Repressor STAT5b and Its Predicted Target 20 α -HSD Are Reciprocally Regulated with miR-200a in Late Gestation and Labor.

Using TargetScan prediction software, we identified two putative binding sites for miR-200a in the 3' UTR of STAT5b, a P₄-responsive transcription factor that inhibits transcription of 20 α -HSD (27, 29). In mice, STAT5b deficiency caused pregnancy loss during midgestation; this was correlated with increased expression of ovarian 20 α -HSD and decreased circulating levels of P₄ (27). In contrast, 20 α -HSD^{-/-} mice manifest delayed parturition and increased fetal demise at birth (27, 30). In one of these studies, circulating P₄ levels declined at term in a manner similar to wild-type (30), suggesting that local P₄ metabolism in PR target tissues, such as the uterus, may be critical. In the present study, qRT-PCR and immunoblotting revealed a decline in STAT5b mRNA and protein expression in the mouse myometrium between 15.5 dpc and labor (Fig. 1B and C). This gestational decline in STAT5b expression during late gestation was associated with a dramatic, but transient increase in 20 α -HSD mRNA (Fig. 1D) that resulted in a sustained increase in both 20 α -HSD protein (Fig. 1E) and enzymatic activity (Fig. 1F).

To determine whether this temporal pattern of regulation is conserved from mouse to human, we analyzed expression of miR-200a and STAT5b, as well as 20 α -HSD expression and activity in myometrial biopsies from women at term who were either in labor or not in labor. Similar to our findings in the mouse, myometrial miR-200a was increased (Fig. 1G), but STAT5b mRNA (Fig. 1H) and protein (Fig. 1I) expression were decreased in myometrium from women in labor compared with those not in labor. In contrast, 20 α -HSD expression (Fig. 1J and K) and activity (Fig. 1L) were increased in myometrium from women in labor, compared with women not in labor. These findings corroborate previous observations of increased levels of the 20 α -HSD metabolite, 20 α -OHP, in the myometrium from pregnant women at term (23).

STAT5b Negatively Regulates 20 α -HSD in Myometrium and Is a Direct Target of miR-200a.

Although previous studies indicate that STAT5b negatively regulates 20 α -HSD expression in the ovary (27), this relationship has not been established in the myometrium. To investigate whether STAT5b regulates 20 α -HSD expression in the myometrium, we used an immortalized human myometrial

cell line (hTERT-HM) (31). Transfection of hTERT-HM cells with a CMV-driven expression plasmid for STAT5b augmented STAT5b expression at the mRNA and protein levels and caused a decrease in 20 α -HSD mRNA levels (Fig. 2A–C). Conversely, 20 α -HSD mRNA expression was increased as a consequence of siRNA-mediated knockdown of STAT5b in hTERT-HM (Fig. 2D–F).

Previous studies suggest that STAT5b inhibits 20 α -HSD expression by binding to a putative STAT5b response elements within the proximal 20 α -HSD promoter. In those studies, cotransfection of reporter constructs containing –2,500 bp of the 20 α -HSD 5'-flanking region fused to luciferase together with an expression vector for constitutively active Stat5b caused substantial inhibition of 20 α -HSD promoter activity (32). This genomic region contains putative STAT5b response elements at –155 and –547 bp. To assess gestational changes in binding of endogenous STAT5b to the proximal region of the 20 α -HSD promoter that contains these putative STAT5b response elements, we performed ChIP assays comparing myometrial tissues from pregnant mice at 15.5 dpc to myometrium collected just before labor at 18.5 dpc. Our findings revealed that STAT5b binding to the 20 α -HSD promoter was relatively high at 15.5 dpc and declined markedly just before labor (Fig. 2G). Taken together, these data suggest that during most of pregnancy, STAT5b-mediated inhibition of 20 α -HSD expression within the myometrium helps to maintain high local P₄ levels and sustains uterine quiescence. However, during the transition to labor, STAT5b levels decline within the myometrium allowing for increased expression of 20 α -HSD and enhanced local P₄ metabolism.

As mentioned, the 3' UTR of STAT5b contains two putative binding sites for miR-200a; however, direct binding of miR-200a to the STAT5b 3' UTR and miR-200 inhibition of STAT5b expression have not previously been tested. To determine whether miR-200a inhibits endogenous STAT5b expression in human myometrial cells, hTERT-HM cells were infected with recombinant adenovirus expressing miR-200b/200a/429. Following transduction of miR-200 family members, STAT5b expression was repressed at the mRNA (Fig. 3A) and protein (Fig. 3B) levels, but 20 α -HSD mRNA expression was up-regulated (Fig. 3C). Moreover, transfection of the hTERT-HM cells with miR-200a mimics also significantly decreased STAT5b mRNA expression (Fig. 3D). To assess whether miR-200a directly targets STAT5b, we transfected COS7 cells with miR-200a mimics and a luciferase reporter plasmid comprised of a portion of the STAT5b 3' UTR, containing two putative miR-200a binding sites subcloned downstream of the luciferase gene (Fig. 3E). A miR-1 mimic was cotransfected as a control in these experiments, because miR-1 is not predicted to target STAT5b by TargetScan analysis (<http://www.targetscan.org/>). Enhanced expression of miR-200a significantly repressed luciferase reporter activity, but transfection of miR-1 had no effect (Fig. 3F). In cells transfected with a luciferase reporter construct in which both miR-200a binding sites were mutated, this repression was lost (Fig. 3F).

Progesterone Regulates miR-200a, STAT5b, and 20 α -HSD in the Uterus.

As mentioned, ZEBs and miR-200 family members exist in a double-negative feedback loop, whereby miR-200s suppress expression of ZEB1/2 posttranscriptionally and ZEBs inhibit miR-200 transcription (28, 33). Previously, we observed that P₄ treatment of mice and cultured myometrial cells specifically induced ZEB1, resulting in inhibition of miR-200b/429 expression (25). P₄ was previously reported to repress 20 α -HSD expression in rat corpus luteum (34, 35) and to induce STAT5 expression in human breast cancer cells (26). To test the hypothesis that P₄/PR-mediated repression of miR-200a results in enhanced expression of STAT5b and inhibition of 20 α -HSD in myometrium, we analyzed effects of P₄ injection in uterine tissues of ovariectomized mice after 24 h. We observed that P₄

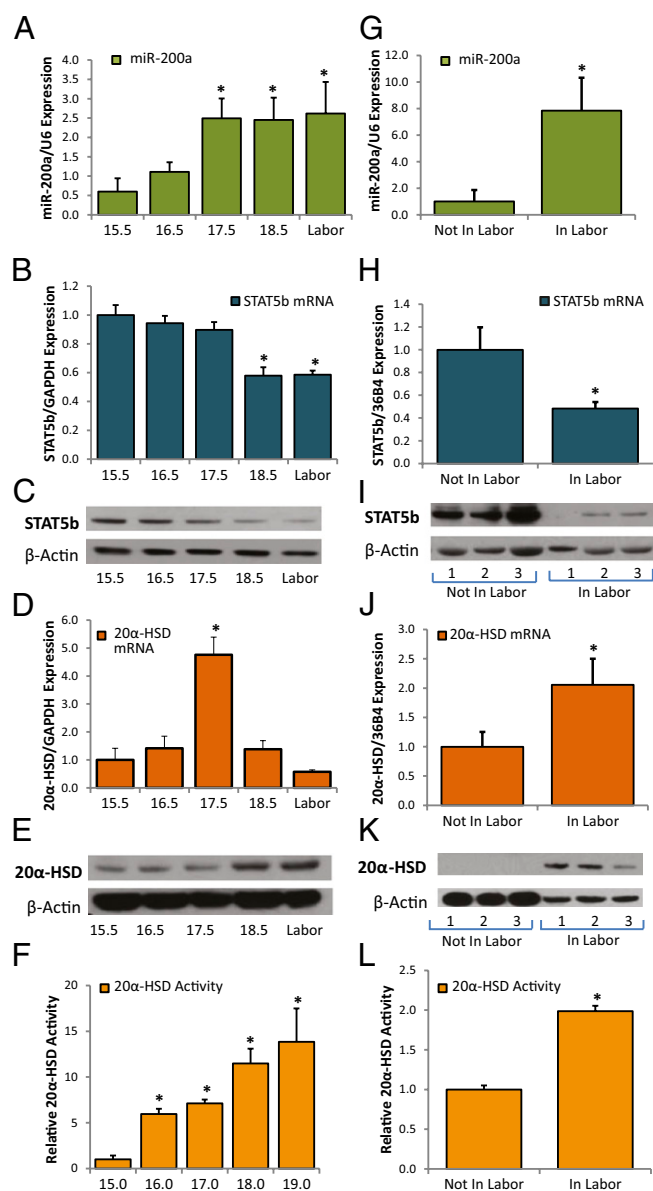


Fig. 1. miR-200a, STAT5b, and 20 α -HSD are coordinately regulated during late gestation and labor in mouse and human myometrium. (A) qRT-PCR revealed that miR-200a expression was significantly increased in myometrium as mice progressed to labor in a pattern similar that previously observed for miR-200b and miR-429 (25). STAT5b mRNA (B) and protein (C) expression were coordinately decreased between 15.5 dpc and labor. 20 α -HSD mRNA levels were markedly increased in pregnant mouse uterus between 15.5 and 17.5 dpc and subsequently declined (D), but 20 α -HSD protein (E) and activity (F) progressively increased in mouse myometrium between 15.5 dpc and labor. qRT-PCR values in A, B, and D are the mean \pm SEM of data from three independent gestational series of mice. Expression of each miR/mRNA was determined by qRT-PCR, normalized to U6/GAPDH, and expressed as arbitrary units. Immunoblots shown in C and E are representative of findings in three gestational series of mice. The 20 α -HSD activity data shown in F are the mean \pm SEM of values from three independent gestational series of mice. *Significantly different from values at 15.5 dpc ($P < 0.05$). (G) qRT-PCR revealed that miR-200a expression was increased in term myometrium of women in labor, compared with tissues from women not in labor. (H) STAT5b mRNA was decreased in myometrium from women in labor, compared with tissues from women not in labor. (I) Immunoblot analysis of STAT5b protein expression in myometrial samples from three women not in labor and three women in labor at term. Endogenous β -actin was analyzed as a loading/transfer control. (J) 20 α -HSD mRNA was increased in term myometrium of women in labor, compared with women not in labor. (K) Immunoblot of 20 α -HSD protein levels in myometrial samples from

treatment inhibited miR-200a expression in myometrium (Fig. 4A). This inhibition was associated with a P₄-mediated induction of STAT5b mRNA (Fig. 4B) and protein (Fig. 4C) and a coordinate repression of 20 α -HSD mRNA (Fig. 4D). In contrast, treatment of 15.5 dpc mice with the PR antagonist RU486 to induce preterm labor significantly increased miR-200a expression (Fig. 4E), inhibited STAT5b mRNA (Fig. 4F) and protein (Fig. 4G), and increased 20 α -HSD expression (Fig. 4H). These findings suggest that P₄/PR-mediated repression of miR-200 expression caused by induction of ZEB1 maintains uterine quiescence, in part, via up-regulation of STAT5b and subsequent repression of 20 α -HSD. Conversely, the increase in myometrial miR-200 expression caused by RU486 treatment inhibits STAT5b and induces 20 α -HSD, resulting in enhanced local metabolism of P₄ and a further decline in PR function leading to preterm labor.

Discussion

The molecular mechanisms that maintain quiescence of the myometrium throughout most of pregnancy and mediate its conversion into a synchronously contractile unit, culminating in parturition, remain incompletely understood. It is clear that P₄ acting through PR is essential for maintaining myometrial quiescence, and an enhanced inflammatory response caused by signals from mother and fetus promotes the progression to labor (1). P₄/PR mediates uterine quiescence, in part by suppressing NF- κ B activation of contraction-associated genes (15). Our recent studies revealed that P₄/PR also contributes to the maintenance of uterine quiescence during pregnancy via up-regulation of the transcription factor, ZEB1, which inhibits expression of the contraction-associated genes, *OXR* and *CX43*, and suppresses the miR-200 family to promote further up-regulation of ZEB1 and ZEB2 (25).

In rodents, labor is heralded by a pronounced decline in P₄ production by the corpus luteum, whereas in humans, circulating P₄ remains elevated throughout pregnancy and into labor because of increased placental P₄ synthesis (4, 5). As mentioned above, even in rodents, levels of circulating P₄ at term remain higher than the K_d for its binding to PR. This finding suggests that a decline in PR function and increased local metabolism of P₄ near term are of paramount importance for the initiation of parturition in all mammals. Notably, in the present study we discovered that the P₄-metabolizing enzyme 20 α -HSD is markedly up-regulated in pregnant mouse and human myometrium near term and elucidated a unique role for the miR-200 family member, miR-200a, in its regulation. Our findings indicate that within myometrium, miR-200a directly targets STAT5b (Fig. 3), a transcription factor known to suppress 20 α -HSD expression (27). As noted above, Stat5b-deficient mice manifest mid-gestation pregnancy loss, correlated with increased ovarian 20 α -HSD expression and decreased circulating P₄ levels (27). Although the mid-gestation abortions in the STAT5b null mice were presumed to be caused by enhanced ovarian 20 α -HSD expression, circulating P₄ levels in these animals remained higher than the K_d for binding to PR (36). This finding suggests the potential importance of STAT5b and 20 α -HSD in the control of local P₄ metabolism in tissues such as myometrium before labor. Notably,

three women not in labor and three women in labor at term. Endogenous β -actin was analyzed as a loading/transfer control. (L) 20 α -HSD activity was increased in homogenates of term myometrium from women in labor compared with homogenates from women not in labor. Data are the mean \pm SEM of values from five women not in labor and five women in labor at term. Data shown in G, H, and J are the mean \pm SEM of values from 12 myometrial samples from women not in labor and 11 myometrial samples from women in labor. *Significantly ($P < 0.05$) different from tissues of women not in labor.

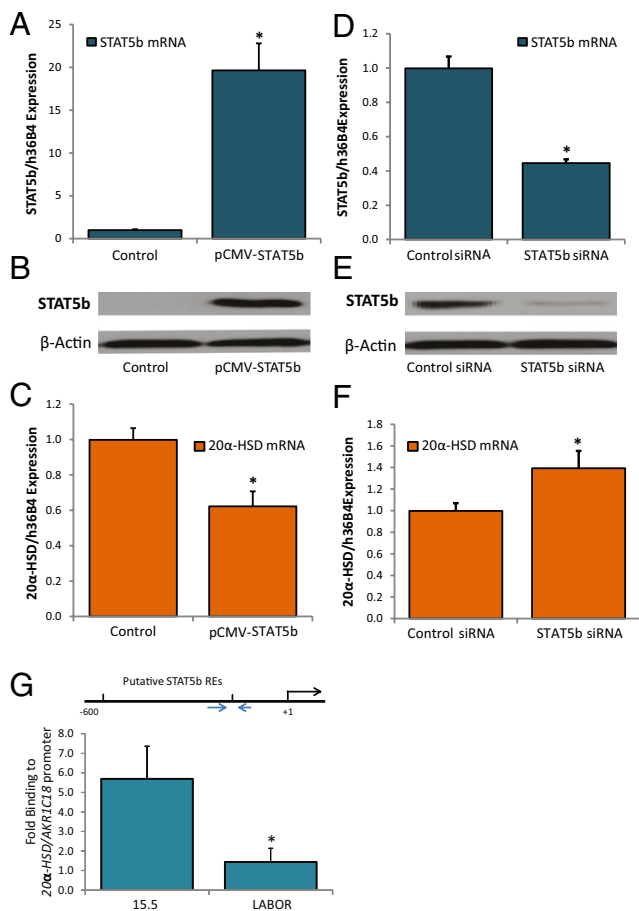


Fig. 2. STAT5b negatively regulates 20 α -HSD in human myometrial cells. Binding of endogenous STAT5b to response elements in the 20 α -HSD/*AKR1C18* promoter declines in pregnant mouse myometrium at term. (A–C) hTERT-HM cells were transfected with a pCMV-STAT5b expression plasmid or with an empty vector (control). Levels of STAT5b mRNA (A) and protein (B) were increased after 24 h. Up-regulation of STAT5b was associated with a significant suppression of 20 α -HSD mRNA levels (C). (D–F) hTERT-HM cells were transfected with STAT5b siRNA or with a scrambled siRNA (control). Levels of STAT5b mRNA (D) and protein (E) were significantly reduced after 72 h. (F) The decline in STAT5b expression was associated with a significant induction of 20 α -HSD mRNA levels. Data shown in A, C, D, and F are the mean \pm SD of values from three independent experiments, each conducted in triplicate. *Significantly ($P < 0.05$) different from control. (G) Binding of endogenous STAT5b to the *AKR1C18* promoter is significantly decreased in pregnant mouse myometrium between 15.5 dpc and just before labor. ChIP using antibodies to STAT5b was used to assess binding of endogenous STAT5b to the region of the *AKR1C18* promoter that contains putative STAT5b response elements. The STAT5b binding was quantified by PCR, normalized to input and expressed as fold-increase over binding using nonimmune IgG. Data are the mean \pm SEM (* $P < 0.05$; $n = 5$ mice per group).

STAT5b expression declined in mouse and human myometrium during the progression to labor in concert with the up-regulation of miR-200a and induction of 20 α -HSD expression and activity (Fig. 1). Identical findings were obtained upon induction of preterm labor in mice using RU486 (Fig. 4 E–H) and in an inflammatory preterm labor model using intra-amniotic injection of LPS (Fig. S1). Because the seed sequence of miR-200a is nearly identical to that of miR-200b and miR-429, which are encoded within the same transcript, it is likely that other members of the miR-200 family similarly target myometrial STAT5b and regulate 20 α -HSD. Furthermore, miR-200a also directly

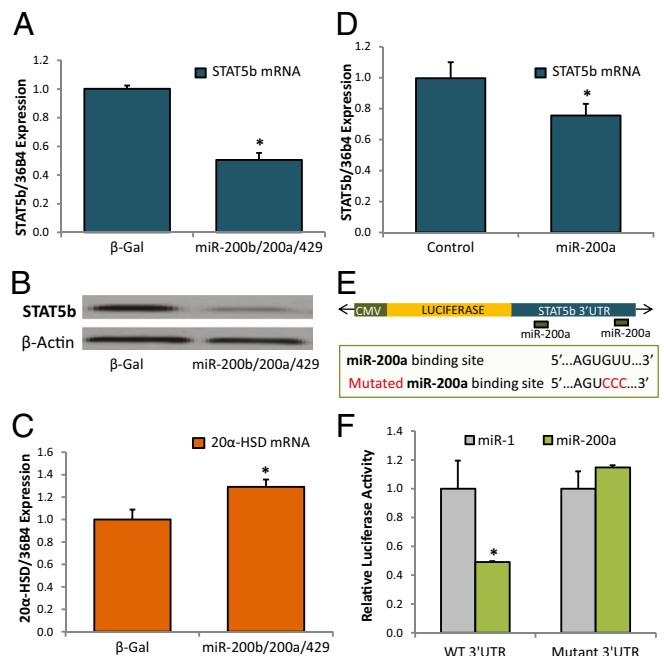


Fig. 3. miR-200s directly target STAT5b and inhibit 20 α -HSD expression in human myometrial cells. (A–C) hTERT-HM cells were infected overnight with recombinant adenoviruses expressing the miR-200b-200a-429 cluster. After 72 h, miR-200 family overexpression inhibited STAT5b mRNA (A) and protein (B) and increased 20 α -HSD mRNA (C) levels. (D) Transfection of hTERTs with miR-200a mimics also caused a significant reduction in STAT5b mRNA expression. Data shown in A, C, and D are the mean \pm SD of values from three independent experiments, each conducted in triplicate. *Significantly ($P < 0.05$) different from control or β -Gal. (E) Schematic diagram of *Luciferase* reporter containing the subcloned 3' UTR of STAT5b with miR-200a binding sites. Wild-type and mutated binding site sequences are also shown. (F) Luciferase assays were conducted in COS7 cells cotransfected with either miR-200a or miR-1 (as control) mimics and the *luciferase:STAT5b-3' UTR* reporter containing WT or mutant miR-200 binding sites. Luciferase activity in cells cotransfected with miR-200a are plotted relative to miR-1, which had no effect on reporter activity and was assigned an arbitrary value of "1." Values are the mean \pm SD of three independent experiments, each conducted in triplicate. *Significantly ($P < 0.05$) different from cells cotransfected with miR-1. Cotransfection data indicate that miR-200a directly targets the STAT5b 3' UTR. Repression was lost when the putative miR-200a binding sites were mutated.

targets ZEB1 and ZEB2 (37), suggesting that near term, increasing levels of miR-200a act cooperatively with miR-200b/429 to inhibit ZEB expression, allowing for derepression of contraction-associated genes.

Increased P₄ metabolism by the pregnant uterus approaching term has been observed in a number of species, including humans. In rats (20), mice (19), sheep (21), and guinea pigs (22), there is a significant decline in local P₄ levels in uterine tissues near term. In myometrium of pregnant women at term, there is a dramatic decrease in the ratio of P₄ to 20 α -OHP, an inactive metabolite of P₄ generated by 20 α -HSD, compared with women in early pregnancy (23). Indeed, we observed a significant increase in 20 α -HSD mRNA and protein expression and activity in myometrium from women in labor, compared with those not in labor (Fig. 1 J–L). In pregnant mice, we found that 20 α -HSD mRNA expression increased dramatically in the myometrium at 17.5 dpc and then declined (Fig. 1D). On the other hand, 20 α -HSD protein expression and enzyme activity continued to increase to term and into labor (Fig. 1 E and F), suggesting that this enzyme serves an important role in local P₄ metabolism during the transition to labor.

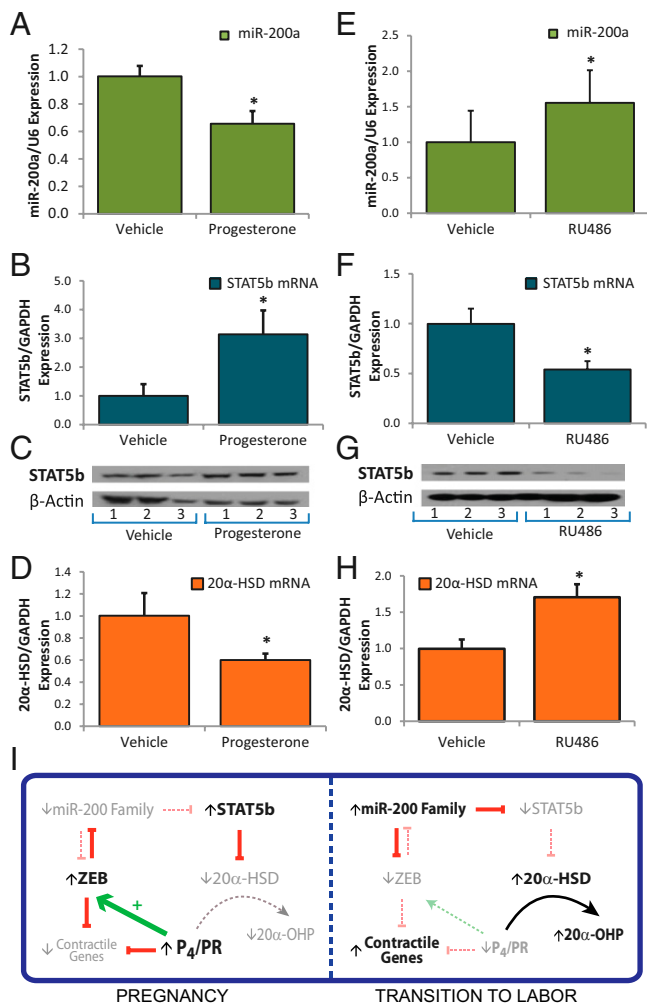


Fig. 4. P₄/PR regulates 20α-HSD in the uterus via modulation of the miR-200-STAT5b axis. (A–D) Ovariectomized mice were injected with P₄ (1 mg) or with vehicle; uterine tissues were harvested 24 h later. P₄ treatment inhibited miR-200a expression (A) induced STAT5b mRNA (B) and protein (C) and inhibited 20α-HSD mRNA (D). (E–H) Timed pregnant 15.5 dpc mice were injected subcutaneously with the PR antagonist RU486 (200 μg) or with vehicle. RU486 injected mice were killed upon the preterm birth of one pup; a vehicle-injected control was killed immediately afterward and myometrial tissues were isolated. RU486 treatment increased miR-200a (E), inhibited STAT5b mRNA (F) and protein (G), and induced 20α HSD mRNA (H) expression. Expression of each miRNA/mRNA was determined by qRT-PCR, normalized to U6/GAPDH, and expressed as fold-change over vehicle-treated controls. Mean ± SEM values are shown. Student *t* test, **P* < 0.05, *n* = 5 mice per treatment group. (I) Schematic diagram of the regulation of PR function during pregnancy and labor via the miR-200/STAT5b/20α-HSD axis.

As mentioned, 20α-HSD is a member of the AKR superfamily of NADPH-dependent oxidoreductases, comprising >100 proteins, which catalyze the reduction of a wide variety of substrates, including steroid hormones, prostaglandins, carbohydrates, and xenobiotics (38) (<http://www.med.upenn.edu/akr/index.html>). 20α-HSD belongs to the steroid-metabolizing HSD subgroup of the AKR1 family. In humans, there are four clustered HSD genes on chromosome 10, corresponding to 20α-HSD (AKR1C1), 3α-HSD type III (AKR1C2), 17β-HSD type V (AKR1C3), and 3α-HSD type I (AKR1C4). In mice, there are four distinct HSD genes that correspond to their human gene counterparts in the order listed above (AKR1C18, AKR1C6, AKR1C12, and AKR1C13), as well as four novel HSDs, all clustered on chromosome 13 (39). The AKR family members in human and mouse that metabolize

P₄ with the highest activity are AKR1C1 and AKR1C18, respectively (40).

In rats and mice, 20α-HSD is expressed at relatively high levels in the corpus luteum of the ovary, and increases in 20α-HSD expression during the estrous cycle and at the end of pregnancy have been associated with functional luteolysis (30, 41). The correlation of the increase in corpus luteum 20α-HSD expression and the fall in circulating P₄ levels at the end of pregnancy in rodents has led to the view that this enzyme plays a crucial role in the decline in ovarian P₄ secretion leading to parturition. Consequently, mice carrying targeted deletions in the 20α-HSD gene manifest a delay in parturition of several days (27, 30). Although in one of these studies, circulating P₄ levels in the 20α-HSD null mice were sustained at levels three- to four-times higher than those of wild-type mice at term (27), in the other, P₄ levels were only marginally elevated in the knockouts, compared with wild-type (30). These findings suggest that the decline in ovarian production of P₄ at term may not be sufficient and that actions of 20α-HSD to catalyze local metabolism of P₄ also may be critical for the decline in PR function leading to labor. This paradigm is exemplified in women, where circulating P₄ levels remain elevated into labor. Importantly, mice deficient in the P₄ metabolizing enzyme 5α-reductase type I fail to deliver because of a lack of cervical ripening (19). This parturition defect, which is manifest despite a decline in circulating P₄, is caused by impaired local metabolism of P₄ within the cervix. Based on these collective findings, we suggest that increased local metabolism of P₄ in the myometrium near term by 20α-HSD may contribute to the loss of PR function leading to labor.

As depicted in Fig. 4I, our collective findings suggest that miR-200a serves a critical role in the regulation of P₄ metabolism in the myometrium during pregnancy and labor. During pregnancy, high levels of P₄/PR function increase expression of transcription factor ZEB1, which inhibits expression of miR-200a and other members of the miR-200 family (25), as described above. The decreased levels of miR-200 enhance expression of STAT5b, which represses 20α-HSD to sustain elevated P₄ levels within the myometrium. During the transition to term and preterm labor, an increased inflammatory response promotes a decline in PR function in myometrium (11, 14, 17, 42), resulting in decreased levels of ZEB, which releases repression of the miR-200 family. The increased levels of miR-200a inhibit STAT5b expression, releasing repression of 20α-HSD. The increase in 20α-HSD, in turn, catalyzes metabolism of P₄ to reduce local P₄ levels in the myometrium and further promote the progression of labor. The decline in PR function may act in a positive feed-forward manner to further increase miR-200 expression, suppress STAT5b, and induce 20α-HSD. Taken together, these findings highlight another important function for the miR-200 family in the timing of parturition and emphasize its potential importance as a therapeutic target for prevention of preterm labor.

Materials and Methods

Mouse and Human Myometrial Preparations. Myometrial tissues from timed-pregnant mice and pregnant women undergoing cesarean section before and during the onset of active labor were collected and prepared as described previously (25) and in *SI Materials and Methods*.

Mouse Model of Preterm Labor Induced by Pharmacological P₄/PR Withdrawal. Induction of preterm labor using RU486 was implemented as described previously (10, 25) and in *SI Materials and Methods*.

P₄ Treatment Studies. Ovariectomized mice were injected with P₄ or with sesame oil (vehicle) and uterine tissues collected, as described in *SI Materials and Methods*.

Transfection and Transduction of Human Myometrial Cells. Immortalized human myometrial cells (hTERT-HM) (31) were cultured, transduced with recombinant adenoviruses, or transfected, as described in *SI Materials and Methods*.

qRT-PCR. RNA was DNase treated (Invitrogen) and reverse-transcribed using the SuperScript III-RT kit (Invitrogen) or TaqMan microRNA reverse transcription kit (Applied Biosystems). Gene expression analysis was conducted using SYBR Green (Applied Biosystems) or TaqMan Universal PCR Master Mix (Applied Biosystems). Relative gene expression was calculated using the $\Delta\Delta C_t$ method. Primer sets are listed in *SI Materials and Methods*.

Immunoblot Analysis. Cytoplasmic and nuclear proteins were isolated and analyzed for STAT5b and 20 α -HSD proteins by immunoblotting using specific antibodies as described in *SI Materials and Methods*.

Luciferase Reporter Assays. The pMIR-REPORT system (Ambion) was used to validate miR-200a binding sites in STAT5b 3'-UTR, as described in *SI Materials and Methods*.

20 α -HSD Enzyme Activity. Uterine tissue homogenates from pregnant Institute for Cancer Research mice from 15.0 to 19.0 dpc and from human myometrial biopsies were analyzed for 20 α -HSD enzymatic activity using [^{14}C] P₄ and TLC, as described in *SI Materials and Methods*.

ChIP. Quantitative ChIP was performed using a ChIP Assay Kit (catalog no. 17–295; Millipore) to assess binding of endogenous STAT5b to the 20 α -HSD/*AKR1C18* promoter in myometrial tissues from timed-pregnant mice at 15.5 and 18.5 dpc, as described in *SI Materials and Methods*.

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.

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