

RESEARCH

Vitamin D stimulates multiple microRNAs to inhibit CRH and other pro-labor genes in human placenta

Bingbing Wang¹, Mayra Cruz Ithier¹, Nataliya Parobchak¹, Stacy M Yadava¹, Jay Schulkin² and Todd Rosen¹¹Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA²Department of Obstetrics, Gynecology, University of Washington, Seattle, Washington, USACorrespondence should be addressed to B Wang or T Rosen: wangbi@rwjms.rutgers.edu or rosentj@rwjms.rutgers.edu

Abstract

Maternal vitamin D deficiency is linked to adverse pregnancy outcomes including spontaneous preterm birth (SPB). Placental corticotropin-releasing hormone (CRH) has been proposed to be part of a clock that governs the length of gestation in humans, with elevated maternal serum levels predicting early delivery. In this study, we test the hypothesis that vitamin D could contribute to the prevention of preterm labor by inhibiting CRH and other pro-labor mediators. The biological activity of vitamin D occurs via two pathways: non-genomic and genomic responses, both of which involve binding of 1,25-dihydroxyvitamin D (1,25(OH)₂D), the active metabolite of vitamin D binding to the vitamin D receptor (VDR). By using chromatin immunoprecipitation followed by sequencing (ChIP-seq), we found that 1,25(OH)₂D stimulates association of VDR with a number of miRNA genes including *MIR181B2* and *MIR26B*, and their mature products miR-181b-5p and miR-26b-5p are predicted to target *CRH* and cyclooxygenase-2 (*COX-2*) mRNA at 3'-untranslated region (UTR), respectively. We performed RT-qPCR analysis to validate that expression of mature miR-181b-5p and miR-26b-5p in term human syncytiotrophoblast increased in response to treatment with 1,25(OH)₂D. miR-181b-5p- or miR-26b-5p-mediated inhibition of CRH or COX-2 was further assessed by the use of miRNA mimics/inhibitors and a luciferase reporter assay. Taken together, this study has identified novel mechanisms by which vitamin D downregulates pro-labor genes and could lower the risk of preterm delivery.

Key Words

- ▶ vitamin D
- ▶ microRNA
- ▶ CRH
- ▶ pro-labor genes
- ▶ placenta

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Introduction

Vitamin D deficiency has been linked to an increased risk of preterm delivery. A cohort study by Bodnar and colleagues using specimens obtained from the US Collaborative Perinatal Project found a link between vitamin D deficiency and preterm infants in nonwhite mothers. Placental histology showed that some of these preterm births were inflammation-mediated while other cases occurred in the absence of detectable placental pathology (1). The same group studied a separate case-cohort study on an entirely different group of

pregnant women with banked serum samples and found that sufficient vitamin D levels protected against spontaneous preterm birth (2). There is moderate evidence that from three randomized controlled trials involving 477 women that vitamin D supplementation reduces preterm birth risk (3). Maternal vitamin D deficiency and elevated CRH have also been linked to a number of adverse pregnancy outcomes including preeclampsia, fetal growth restriction and gestational diabetes mellitus (4).

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During pregnancy, the human placenta produces a large amount of corticotropin-releasing hormone (CRH) (5). Maternal plasma CRH levels increase exponentially as pregnancy advances, peaking at the time of delivery. Women who deliver preterm have increased levels of CRH early in pregnancy, while those who deliver post-term tend to have lower CRH levels (6). The link between increased CRH levels and preterm delivery has been demonstrated by a number of investigators (7, 8, 9). Because of this, CRH has been suggested to be involved in the regulation of a placental clock, acting in concert with other factors to regulate a cascade of physiological events leading to parturition (6).

A hypothesis that has been proposed is that vitamin D might inhibit *CRH* gene expression in placenta tissue and thereby decrease the vulnerability for prematurity in childbirth (10, 11). Indeed, Mohamed and colleagues have suggested that vitamin D and CRH might be linked to preterm labor and birth, because they found a correlation between low 25(OH)D and elevated CRH levels in women who delivered preterm (10).

One of the critical events that occurs in the initiation of parturition is the induction of prostaglandin synthesis in both fetal and maternal tissues. Prostaglandins play a role in the onset of effective uterine contractions, cervical ripening and increasing uterine receptivity to oxytocin (12). It has been previously shown that both *CRH* and *COX-2*, a key gene responsible for prostaglandin synthesis, are regulated similarly in the human placenta (13). As such, we chose to explore the potential mechanisms of vitamin D action on both *CRH* and *COX-2*.

The biological activity of vitamin D occurs via two pathways: non-genomic and genomic responses, both of which involve binding of 1,25-dihydroxyvitamin D (1,25(OH)₂D), the active metabolite of vitamin D and the vitamin D receptor (VDR), a member of the superfamily of nuclear receptor for steroid hormones (14). Prevention of bacterial infection-mediated SPB by vitamin D is likely dependent on its non-genomic response, because 1,25(OH)₂D-simulated VDR can physically interact with IKB kinase β (IKK β) to block lipopolysaccharide (LPS)-induced NF-KB activation, which induces production of a variety of proinflammatory cytokines for initiation of labor (15, 16). In contrast, vitamin D supplementation prevents SPB cases not related to inflammation most likely via the genomic mechanism, because a recent study in the myometrium revealed that vitamin D can inhibit multiple labor-associated genes including connexin 43 and prostaglandin receptors (17), which are not NF-KB target genes.

In the current study, we tested the hypothesis that vitamin D may inhibit pro-labor genes via a genomic response. We worked with primary cultures of human syncytiotrophoblast (STB), the source of maternal serum CRH (6) and one of major sites for *COX-2* production during human pregnancy (18). Our results reveal a novel mechanism underlying vitamin D regulation of *CRH* and *COX-2* genes in the placenta and contribute to our understanding of the potential link between vitamin D deficiency and preterm delivery. We also lay the groundwork for future studies of how vitamin D may regulate other genes potentially involved in the initiation of human labor and preterm labor.

Materials and methods

Placental specimens

We collected the placenta from healthy women with estimated gestational age of 38 and 40 weeks who were delivered by elective Cesarean section (C-section). Women with complications of pregnancies, including diabetes, hypertension, autoimmune diseases, infection, fetal growth restriction and preeclampsia, were excluded from the study.

This study was approved by the Institutional Review Board of Rutgers University (#Pro20150001445). Because specimens used for the study would normally be discarded, there was no risk to the patient or her pregnancy from study procedures, and it was not deemed appropriate to approach patients on Labor and Delivery for consent because potential subjects might be under duress, the IRB granted a waiver of consent for this study.

Purification of cytotrophoblast

Briefly, villous tissue fragments from the entire placenta were subject to enzymatic digestions in a solution containing 0.25% trypsin, 0.2% deoxyribonuclease I, 25 mM HEPES, 2 mM CaCl₂ and 0.8 mM MgSO₄ in 1× Hanks' balanced salt solution at 37°C followed by filtration of 100- μ m sieve. Cells were pelleted and resuspended in DMEM/F-12 with 10% fetal bovine serum (FBS). We used a discontinuous density gradient of Percoll (50/45/35/30%) by centrifuging at 1000g at room temperature for 20 min. Target cells at the interface of fractions of 35/45% were collected and further immunopurified by an approach of negative selection with use of human CD9 and CD45 antibodies (BD Biosciences, San Diego, CA, USA) and

Dynabeads (Invitrogen). Cells in the supernatant that were separated from Dynabeads with contaminated cells were pelleted, resuspended in DMEM/F-12 plus 10% FBS, plated at a density of $2.5\text{--}3 \times 10^6/\text{cm}^2$ and maintained at 37°C and 5% CO_2 at least for 48 h to spontaneously differentiate into STB prior to further analysis.

ChIP-sequencing (ChIP-seq)

A total of approximately 1×10^7 of STB cells were cross-linked with 1% formaldehyde for 5 min at room temperature, and the reaction was stopped by the addition of $1 \times$ glycine. Cells were lysed in ChIP lysis buffer (50 mM HEPES-KCl, pH 7.5; 140 mM NaCl; 1 mM EDTA; 1% Triton X-100; 0.1% sodium deoxycholate; 0.1% SDS) with freshly added $1 \times$ protease inhibitor cocktail (Roche Applied Science) and then sonicated to shear chromatin into 150- to 200-bp fragments. Chromatin was then immunoprecipitated with individual ChIP-grade anti-VDR antibody (ThermoFisher Scientific) at $5 \mu\text{g}/25 \mu\text{g}$ chromatin and incubated with protein G agarose beads (Roche Applied Science). Immunoprecipitates were treated with Proteinase K and DNA fragments were recovered by phenol/chloroform extraction and ethanol precipitation.

Concentrations of DNA were determined by Qubit Fluorometric (Invitrogen) and at least 10 ng/per sample were submitted for ChIP-seq with Illumina HiSeq platform and 1×50 bp configuration (GENEWIZ, NJ, USA). Approximately 12 million paired-end reads/per sample were requested.

Gene silencing

siRNA transfection was performed as previously detailed using transfection reagent Lipofectamine 2000 (Invitrogen) with use of FlexiTube siRNAs target VDR or miRNA inhibitors as indicated (Qiagen) (19, 20). Total RNAs were isolated from cells and analyzed by RT-qPCR. Each experiment was repeated in three individual specimens.

Reverse transcription quantitative PCR (RT-qPCR)

We extracted total RNAs by means of TRIzol (Invitrogen). For assessment of mRNA levels, cDNA synthesis was prepared by the oligo-dT primer method using the Superscript II Reverse Transcription kit (Thermo Fischer Scientific). PCR was performed using a StepOne Plus Real Time PCR System (Applied Biosystems) and power SYBR green PCR master (ThermoFisher Scientific). PCR primers

(forward/reverse) included CRH, 5'-GCAGTTAGCACAGCAAGCTCAC-3'/5'-CAAATGGCATAAGAGCAGCG-3'; COX-2, 5'-TGAGCATCTACGGTTTGCTG-3'/5'-TGCTTGCTGGAACAACACTGC-3' and GAPDH, 5'-CTCCCGCTCGCTCTCTG-3'/5'-CTGGCGACGCAAAGAAG-3'.

We used the miScript PCR System (Qiagen) for quantification of mature miRNAs with a universal primer and miRNA-specific primer included in the kit according to the manufacturer's protocol. U6 snRNA (5'-CTCGCTTCGGCAGCAC-3'/5'-AACGCTTCACGAATTTGCGT-3') served as the internal control.

Western blot

The protein samples were separated on SDS-10% PAGE and transferred onto PVDF membranes (Bio-Rad). Membranes were blocked in 5% nonfat milk powder in PBST (10 mM phosphate buffer, pH 7.2; 150 mM NaCl; and 0.1% Tween-20) for 60 min, washed twice with PBST, and incubated with antibodies as indicated in 1% nonfat milk powder-PBST at 4°C overnight. Membranes were washed three times with PBST, incubated with horseradish peroxidase-conjugated secondary antibodies at 1:5000 in 1% nonfat milk powder-PBST and developed by Immuno-Star HRP Substrate (Bio-Rad). The blots were visualized either by autoradiography or scanned by Chemiluminescence Western Blot Scanner (C-DiGit, LI-COR, NE, USA).

Dual-luciferase assay

All firefly luciferase (FL) reporter constructs were made available by GeneScript (NJ, USA) based on PCD FL6X plasmid (#12566, Addgene, MA, USA). The individual FL reporter construct was co-transfected with a *Renilla* luciferase reporter construct, pRL-CMV (Promega) with use of Lipofectamine 2000 (Invitrogen) as previously described (19). Dual-luciferase assay was read on Infinite M200 Pro microplate reader (TECAN, USA) according to the manufacturer's protocol (Promega).

Statistical analysis

Each experiment was repeated a minimum of three times. Data (bars) are presented as mean \pm standard deviation (s.d.). One-way ANOVA was used to compare among ≥ 3 groups. Student *t* test was used to compare the control with each individual test group. $P < 0.05$ was considered statistically significant.

Table 1 Summary of genomic loci associated with VDR stimulated by 1,25(OH)2D ($P < 0.05$).

	Number of genomic loci	Chromosomes
Non-coding RNA genes	1112	1–22, X, Y
MicroRNAs	542	1–22, X, Y
Others	570	1–22, X, Y
Protein-coding genes	148	1–22
Pseudogenes	326	1–22, X, Y
Total	1586	

Results

Genomic sites targeted by 1,25(OH)2D-simulated VDR in human STB

We first used chromatin immunoprecipitation sequencing (ChIP-seq) to perform a genome-wide profile of DNA-binding sites targeted by 1,25(OH)2D-simulated VDR in human STB. Primary cytotrophoblast were purified from the placenta from normal healthy individuals and cultured for at least 48h to spontaneously differentiate into STB, which in turn were exposed to 1,25(OH)2D at 10 μM for additional 24h. DMSO was used as the vehicle control. Cells were cross-linked with 1% formaldehyde and then sonicated to shear chromatin into 100–200-bp DNA fragments, and ChIP was conducted with use of ChIP-grade VDR antibody. Normal rabbit IgG was used as the input control. Recovered DNAs were used for preparation of DNA libraries and in turn sequenced on an Illumina platform.

Accumulated evidence has implicated a key regulatory function for miRNAs in gene expression. In mammals, miRNAs regulate about 30% of all protein-coding genes, typically in a negative manner, and have been shown to participate in the regulation of almost every cellular process examined so far (21). As shown in Table 1, there were a total of 1586 genomic sites targeted by 1,25(OH)2D-simulated VDR in human STB, which include 1112 sites for non-coding RNA genes and 148 for coding genes.

Table 2 Select microRNA genes stimulated by VDR, their mature RNAs and putative target genes involved in initiation of human labor.

Gene symbol	Mature miRNAs	Putative targets involved in human labor	P value
MIRLET7A1	let-7a-5p	STAT3, PTAFR	0.01
MIRLET7I	let-7i-5p	STAT3, PTAFR	<0.01
MIR26B	miR-26b-5p	PTGS2 (COX-2)	<0.01
MIR29A	miR-29a-3p	OXTR, MMP2	<0.01
MIR29C	miR-29c-3p	MMP2	0.03
MIR302C	miR-302c-3p	IL8	<0.01
MIR130A	miR-130-3p	TNF	<0.01
MIR181B2	miR-181-5p	CRH	<0.01
MIR183	miR-183-5p	MMP9	<0.01

Remarkably, 1,25(OH)2D-simulated VDR was able to bind to a total of 542 miRNA genes (Supplementary Table 1, see section on supplementary data given at the end of this article), strongly suggesting a role of vitamin D-mediated regulation of target miRNA genes.

These data support that 1,25(OH)2D-VDR stimulates transcription of miRNA genes by directly associating with their precursor DNA sequences as previously suggested (22).

Pro-labor genes are negatively regulated by miRNAs that are stimulated by 1,25(OH)2D-VDR

Next, we combined TargetScan (23), a web server that predicts biological targets of miRNAs by searching for the presence of sites that match the seed region of each miRNA, with miRBase (24), a biological database that acts as an archive of microRNA sequences and annotations, to annotate target genes by miRNAs.

In order to identify miRNAs that target CRH or COX-2 gene, we first input CRH or PTGS2, an official gene symbol for COX-2, as the gene to be studied in TargetScan. There were a total of 130 miRNAs putatively targeting CRH, however, only 15 of which were stimulated by 1,25(OH)2D-VDR in human STB (Supplementary Tables 1 and 2). We further assessed these 15 miRNAs against miRBase for confidence of annotation and found that miR-181b-5p was only the one of these 15 miRNAs with a defined annotation confidence validating it as a *bona fide* miRNA in humans. There were four miRNAs including miR-1297, miR-26b-5p, miR-26a-5p and miR-4465 that putatively target COX-2, and only miR-26b-5p was stimulated by 1,25(OH)2D-VDR. A search of miR-26b-5p in miRBase determined it to be a validated human miRNA.

We used a similar approach to determine if any miRNAs might target other pro-labor genes. The results of this analysis may be found in Table 2.

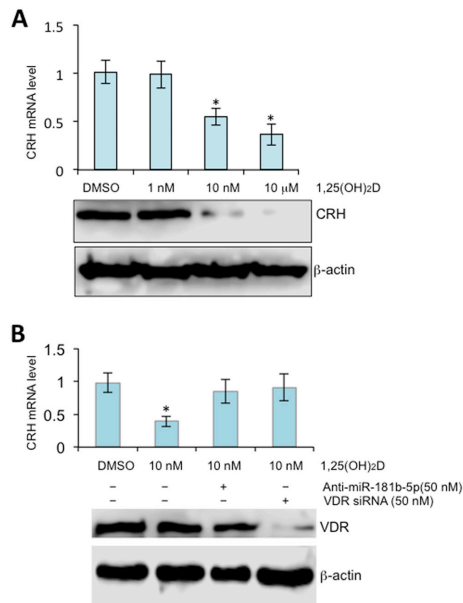


Figure 1
1,25(OH)₂D stimulates miR-181b-5p to inhibit CRH in the human placenta. (A) Term STB were treated with 1,25(OH)₂D at concentrations as indicated for 24h. mRNA and protein levels were assessed by RT-qPCR with normalization to GAPDH (top panel) or Western blot (bottom panel), respectively. Error bars were derived from three independent experiments. (B) Term STB were treated with *miR-181b-5p* antagonists (inhibitors) or VDR siRNA for 24h followed by 1,25(OH)₂D at final concentration of 10nM for additional 24h. mRNA and protein levels were assessed by RT-qPCR (top panel) or Western blot (bottom panel), respectively. Three independent experiments were performed.

Vitamin D inhibits CRH via VDR-miR-181b-5p pathway

The primate placenta is unique in its ability to produce CRH, which is considered to be part of placental clock that controls gestational length and parturition.

To assess whether vitamin D-upregulated *miR-181b-5p* can directly suppress *CRH* mRNA as predicted by the analysis depicted in Table 2, we first incubated 1,25(OH)₂D with human STB and used RT-PCR to assess mRNA level changes. As shown in Fig. 1, 1,25(OH)₂D exposure inhibited CRH in dose-dependent manner, and this repression was reversed when cells were treated with VDR siRNA or *miR-181b-5p* inhibitors. This data suggests that vitamin D downregulates *CRH* by stimulating VDR, and in turn, expression of *miR-181b-5p*.

Vitamin D downregulates COX-2 via the VDR-miR-26b-5p pathway

We incubated 1,25(OH)₂D with human STB and used RT-PCR to assess mRNA level changes of *COX-2*. As shown in Fig. 2, 1,25(OH)₂D treatment led to a significant decrease

of *COX-2*, and such a repression was reversed when cells were treated with VDR siRNA or *miR-26b-5p* inhibitors. This data supports that vitamin D downregulates *COX-2* by stimulating VDR, and in turn, expression of *miR-26b-5p*.

We further employed RT-qPCR to validate VDR-stimulated expression of mature *miR-181b-5p* and *miR-26b-5p* by RT-qPCR with *miR-21-5p* as the negative control (Fig. 3). Collectively, these data suggest that the protective effect of vitamin D against spontaneous preterm delivery is a consequence of genomic response at the *CRH* and *COX-2* gene.

miR-181b-5p and miR-26b-5p directly target CRH and COX-2, respectively

We further assessed the ability of *miR-181b-5p* to bind to the 3'-UTR of *CRH* or *miR-26b-5p* to bind to the 3'-UTR of *COX-2*. To achieve this goal, we performed a luciferase reporter assay and observed a significant decrease in luciferase activity in the presence of *miR-181b-5p* (Fig. 4A and B) or *miR-26b-5p* mimics in term STB compared with the controls

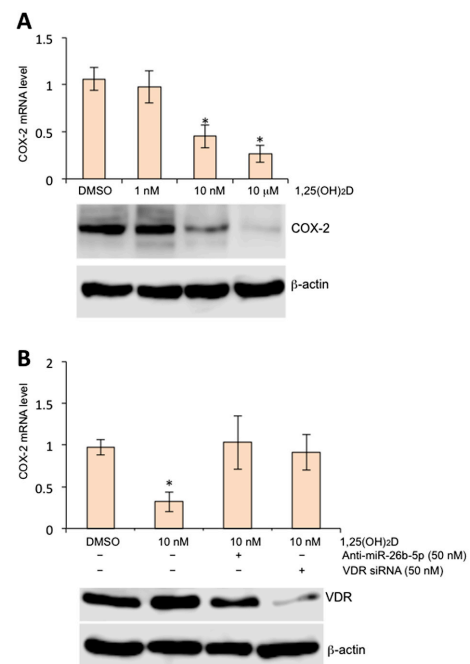


Figure 2
1,25(OH)₂D stimulates miR-26b-5p to inhibit COX-2 in the human placenta. (A) Term STB were treated with 1,25(OH)₂D at concentrations as indicated for 24h. mRNA and protein levels were assessed by RT-qPCR (top panel) or Western blot (bottom panel), respectively. Error bars were derived from three independent experiments. (B) Term STB were treated with *miR-26b* antagonists or VDR siRNA for 24h followed by 1,25(OH)₂D at final concentration of 10nM for additional 24h. mRNA levels were assessed by RT-qPCR. Three independent experiments were performed.

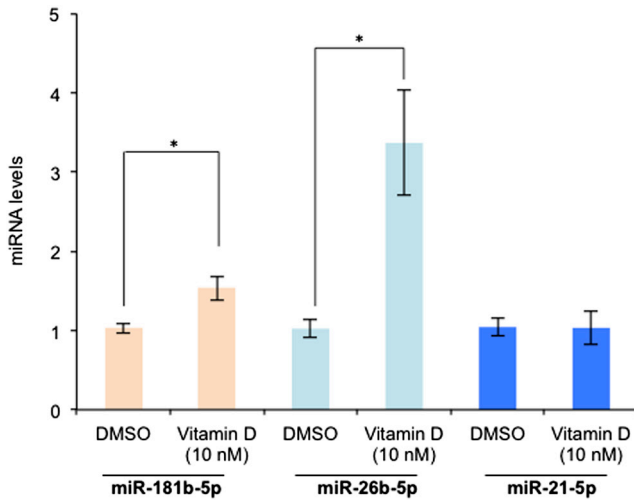


Figure 3
1,25(OH)2D induces transcription of miR-181b-5p and miR-26b-5p in the human placenta. Term STB were exposed to 1,25(OH)2D at 10 nM for 24 h and levels of miR-181b-5p, miR-26b-5p or the negative control miR-21-5p were assessed by RT-qPCR with normalization to the internal control U6 snRNA. Three independent experiments were performed. * $P < 0.01$ (Student t test, 2-tailed).

(Fig. 5A and B). We further mutated the *miR-181b-5p* (Fig. 4C) and *miR-26b-5p*-binding sites in the 3'-UTR of *CRH* or *COX-2* and found a loss of repression (Fig. 5C).

Taken together, the data suggest that *CRH* is directly targeted by *miR-181b-5p* and *COX-2* by *miR-26b-5p*.

Basal occupancy and 1,25(OH)2D-stimulated VDR binding in human STB

In order to further confirm the role of 1,25(OH)2D/VDR-miRNA signaling in regulation of pro-labor mediators in human STB, we compare peaks of *MIR181B2* or *MIR26B* gene in human STB treated with DMSO or 1,25(OH)2D. Wiggle tracks were obtained from UCSC Browser (25). As shown in Fig. 6, peaks were called for either *MIR181B2* or *MIR26B* following 1,25(OH)2D treatment.

Discussion

A better understanding of the molecular mechanisms underlying spontaneous preterm labor is essential for the development of better diagnostic tools and therapeutics to reduce the incidence of preterm deliveries and improve neonatal outcomes. There is strong evidence that *CRH* is a component of the clock that governs the length of pregnancy, and the role of *COX-2* and prostaglandins in the initiation of labor has been extensively documented (26).

Here, we provide evidence that vitamin D-VDR signaling stimulates transcription of the miRNAs including miR-181b-2 and miR-26b, which cause post-translational inhibition of *CRH* and *COX-2*, respectively. These processes may support the observed association between vitamin D deficiency and preterm birth and reduction in preterm birth incidence with vitamin D supplementation. In our previous work, we have demonstrated that both *CRH* and *COX-2* are regulated by the non-canonical NF-KB pathway (19), but vitamin D appears to exert its effects independent of NF-KB here.

Vitamin D-stimulated VDR can inhibit LPS-induced activation of the canonical NF-KB signaling in a direct manner. However, the majority of preterm labor cases are not necessarily a consequence of bacterial infection. As such, we started by seeking to investigate the genome-wide effects by vitamin D-VDR signaling in the human placenta with use of chromatin immunoprecipitation followed by DNA sequencing to study known mediators

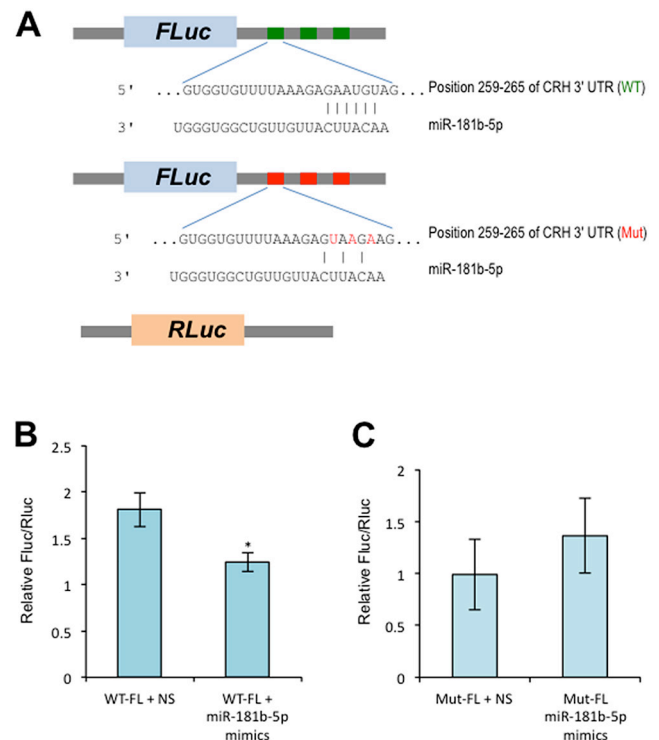


Figure 4
Validation of *CRH* targeted by miR-181b-5p with dual-luciferase assay. (A) Schematic presentation of DNA constructs as used in this assay. (B) Term STB were transiently transfected with WT constructs and *miR-181b-5p* mimics or non-specific miRNA mimics (NS) as indicated for 48 h followed by dual-luciferase assay. (C) Term STB was transiently transfected with mutant (Mut) constructs and *miR-181b-5p* mimics or non-specific miRNA mimics (NS) as indicated for 48 h followed by dual-luciferase assay. Three independent experiments were performed. * $P < 0.01$ (Student t test, 2-tailed).

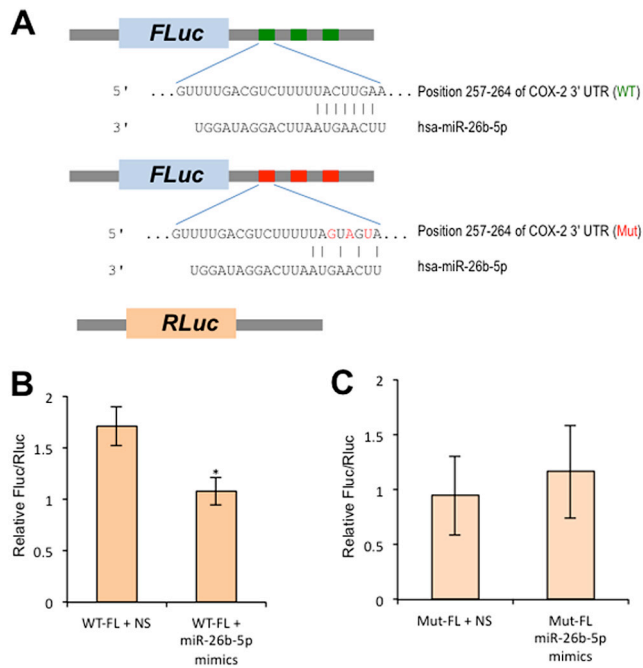


Figure 5
Validation of COX-2 targeted by miR-26b-5p with dual-luciferase assay. (A) Schematic presentation of DNA constructs as used in this assay. (B) Term STB were transiently transfected with WT constructs and *miR-26b-5p* mimics or non-specific miRNA mimics (NS) as indicated for 48h followed by dual-luciferase assay. (C) Term STB was transiently transfected with mutant (Mut) constructs and *miR-26b-5p* mimics or non-specific miRNA mimics (NS) as indicated for 48h followed by dual-luciferase assay. Three independent experiments were performed. * $P < 0.01$ (Student *t* test, 2-tailed).

of normal parturition. Such a genome-wide profiling of VDR-occupied genomic loci could further assist in clarification of mechanisms underlying their molecular action. In addition, our understanding of the biological functions of miRNA relies on the identification of relevant target genes. The majority of the miRNA-target sequence matching is based on imperfect complementarity of the

miRNA with the 3'-UTRs of target mRNAs. As a result, combined computational prediction of miRNA targets and subsequent experimental validation would further justify our conclusions in this study.

Our data further suggest that apart from miR-181b-2 and miR-26, other miRNAs may also be involved in regulation of CRH, COX-2 and other labor-associated mediators. As described in Table 1, vitamin D-VDR also binds to genomic loci of multiple let-7s (-a, -e or -i) and those like miR-29a, miR-29c and miR-183. The predicted targets of let-7s include transcriptional factor STAT3, phospholipid platelet-activating factor (PAF) receptor (PTAFR) and NF- κ B2 (p100/p52), and those of miR-29a, miR-29c and miR-183 include matrix metalloproteinase-2 (MMP-2), MMP-9 and oxytocin receptor (OXTR).

In the human placenta, cortisol promotes interaction of the glucocorticoid receptor with STAT3 to facilitate transcription of COX-2 (27), and we have shown that knockdown of STAT3 led to downregulation of both CRH and COX-2 in a dose-dependent manner (20). In addition, a recent study examining the role of fetal signals in initiation of parturition in the mouse demonstrates that surfactant protein-A (SP-A) and glycerophospholipid PAF secreted by mature fetal lungs led to a fall in progesterone that led to the onset of parturition in the mouse model (28), supporting the earlier observation that developing fetal lung-secreted PAF into amniotic fluid contributes to initiation of term or preterm labor (29). Moreover, extracellular matrix (ECM) remodeling has been implicated in many processes in human term and preterm labor, which include cervical ripening, fetal membrane rupture and placental detachment from the maternal uterus. Most of the ECM and basement membrane components can be degraded by matrix metalloproteinases, a group of structurally related zinc-dependent enzymes (30). These include MMP-2 and MMP-9, which are capable of

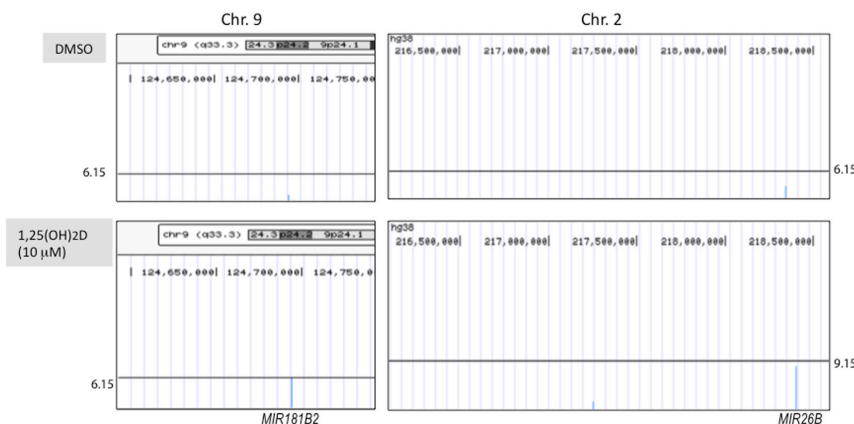


Figure 6
VDR ChIP-seq analysis for *MIR181B2* and *MIR26B*. VDR ChIP-seq data shown for peaks of *MIR181B2* (Chr9: 124693710...124693798) or *MIR26B* (Chr2: 218402637...218402746) in either resting (DMSO, $n = 1$) or after induction with 1,25(OH) $_2$ D for 24h ($n = 1$), which were generated from UCSC Genome Browser tracks by uploaded wiggle files.

digesting collagen IV, a major component of basement membrane. To this end, our future directions could include investigating the role of the miRNAs identified in this study in regulation of pro-labor mediators in the placenta as well as other gestational tissues.

In summary, this study has revealed a novel mechanism, by which vitamin D may contribute to reduced risk of preterm labor by regulation of CRH and other pro-labor mediators. These findings will provide significant insights into harnessing the medical benefits provided by 1,25(OH)₂D-VDR complex in future efforts.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-18-0345>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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