OBSTETRICS

Exogenous oxytocin modulates human myometrial microRNAs

Joanna R. Cook, MRCOG; David A. MacIntyre, PhD; Eleni Samara, MSc; Sung Hye Kim, PhD; Natasha Singh, MRCOG; Mark R. Johnson, MRCOG, PhD; Phillip R. Bennett, FRCOG, PhD; Vasso Terzidou, MRCOG, PhD

OBJECTIVE: MicroRNAs (miRNAs) play a modulatory role in pathways that lead to labor onset, although oxytocin is known to modulate gene expression within the myometrium. We aimed to identify miRNAs whose expression is regulated by oxytocin in pregnant human myometrium.

STUDY DESIGN: Myometrial miRNA expression profiles were compared between samples collected from women at term before the onset of labor (no labor; n = 8) and after labor onset after early exogenous oxytocin treatment (n = 8). Multivariate modelling was used to assess differences in miRNA profiles. Biologic validation was undertaken on 3 independent patient cohorts (no labor, n = 10; labor induced with oxytocin, n = 8; and spontaneous labor with no oxytocin treatment, n = 10). In vitro studies that used primary myocytes were undertaken to assess target miRNA expression after oxytocin treatment. Target genes of candidate miRNAs were identified in silico and cross-referenced with genes that are known to be associated with labor or expressed in myometrium.

RESULTS: In total, 1309 miRNAs were analyzed by microarray, of which 494 were detected in human myometrium. Multivariate modeling identified 12 target miRNAs the differential expression of which was most responsible for the observed separation of the 2 patient populations in the primary discovery cohorts. Biologic validation in the independent secondary sample cohorts showed that oxytocin independently regulated 5 miRNAs (hsa-miR-146b-3p, hsa-miR-196b-3p, hsa-miR-223-3p, hsa-miR-873-5p, and hsa-miR-876-5p). Additionally, hsa-miR-146b-3p was increased both in labor that was induced with oxytocin and in myometrium from spontaneous labor with no oxytocin treatment compared with no labor samples. Four of the validated miRNAs (hsa-miR-146a-5p, hsa-miR-146b-3p, hsa-miR-196b-3p, and hsa-miR-876-5p) were expressed in primary human myocytes; oxytocin treatment of these cells replicated the directional changes that were observed in vivo.

CONCLUSION: Oxytocin alters the expression of a unique set of myometrial miRNAs. These results suggest a further role for oxytocin as a signaling molecule that is involved in the regulation of gene expression during parturition.

Key words: induction, labor, microRNA, myometrium, oxytocin

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The mechanisms that control the length of human pregnancy remain poorly understood. Although most women labor spontaneously from 37-42 weeks, approximately 10% deliver preterm and approximately another 10% fail to labor spontaneously at all.[1] Both preterm and postmature births are associated with serious fetal and neonatal morbidity and death.[2,3] Understanding the pathways of parturition is a critical component of developing effective future therapeutic strategies that will be designed to predict and prevent preterm birth and induce labor more effectively in the case of prolonged pregnancies.

Tightly regulated changes in gene expression in myometrium and fetal membranes are central to the onset of labor. Increasing evidence implicates microRNAs (miRNAs), which are small, noncoding, single-stranded 19-25 nucleotide molecules, as regulators of key gene expression pathways that are involved in parturition. It is thought that approximately one-third of the human genome is regulated by miRNAs,[4] primarily at the posttranscriptional level.[5] MiRNAs bind

From the Imperial College Parturition Research Group, Division of the Institute of Reproductive and Developmental Biology, Imperial College London (all authors), and Academic Department of Obstetrics and Gynaecology, Chelsea and Westminster Hospital (Drs Singh, Johnson, and Terzidou), London, England, UK.

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Corresponding author: Vasso Terzidou, MRCOG, PhD. v.terzidou@imperial.ac.uk

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to the 3'untranslated region of specific target messenger RNAs (mRNAs) to inhibit their translation to protein via imperfect complementary base pairing.[6] A number of recent studies have examined miRNA expression during labor onset in reproductive tissues that have included the placenta,[7] cervix,[8] and fetal membranes.[9] Renthal et al[10] reported that the miR-200 family of miRNAs are up-regulated in laboring murine and human myometrium at term. In mouse myometrium, ZEB1 and ZEB2 were identified as 2 targets that act as transcriptional repressors of the contraction-associated genes, which include the oxytocin receptor and connexin-43, through a mechanism that involves the repression of STAT5b, a transcriptional repressor of the P4 metabolizing enzyme 20α-hydroxysteroid dehydrogenase.[11]

The oxytocin/oxytocin receptor system plays a major role in promoting myometrial contractility in term and preterm labor. Although circulating oxytocin levels do not rise with labor onset, the term pregnant human uterus displays increased sensitivity to oxytocin, mediated via marked up-regulation in oxytocin receptor mRNA and protein expression.[12] Oxytocin is a potent uterotonin that is used for induction of labor and in the augmentation of dysfunctional labor.[13] Conversely, oxytocin receptor antagonists are used as tocolytics in the management of acute preterm labor.[14] In addition to direct effects on contractions via calcium signaling, oxytocin has been shown to function as a signaling molecule that participates in the inflammatory cascade that is associated with labor that can induce uterine activation via prostaglandin and inflammatory cytokine production.[15]

Given the known wide range of oxytocin actions, in this study we have set out to determine whether oxytocin may affect the miRNA profile of pregnant human myometrium at term. To do this, we initially compared miRNA expression using microarray analysis in primary discovery cohorts of myometrium in samples taken from women at term who had not been in labor (NL) with samples from women whose labor was induced or augmented with oxytocin (LOxy). This identified a series of candidate miRNAs the expression of which might be effected by oxytocin. We then used quantitative real-time polymerase chain reaction (qRT-PCR) to examine the expression of these candidates in 3 independent, biologic replication sample cohorts that were taken from women at term who had not been in labor, who were in spontaneous labor but had not had oxytocin treatment, or whose labor had been induced or augmented with oxytocin. Finally, we examined the expression of those miRNAs that remained candidates after this secondary biologic validation in isolated human myocytes in vitro and demonstrated that their expression was modulated by exogenous oxytocin.

Materials and Methods

Study design

To examine miRNA expression patterns, myometrial biopsy specimens were obtained initially from women in the following groups: (1) normal term pregnancy, delivered by elective cesarean section before labor onset (n = 8 women) and (2) laboring at term after early exogenous oxytocin treatment, delivered by emergency caesarean (n = 8 women). A second, independent population of women was recruited as a biologic validation cohort (NL, 10 women; LOxy, 8 women; and term spontaneous labor without any oxytocin [LSpon], 10 women). Samples in the initial cohort were collected from women at Queen Charlotte's and Chelsea Hospital, London; the validation samples were taken from women at Chelsea and Westminster Hospital, London.

Normal pregnancy was defined by the absence of medical, surgical, or obstetric complications. *Term* was defined as delivery at >37 completed weeks gestation. *Labor* was defined as regular, painful contractions with cervical dilation >3 cm. *Early exogenous oxytocin treatment* was defined as women who received intravenous oxytocin when cervical dilation was <4 cm and when the duration of treatment was at least for 6 hours. Women with premature rupture of membranes, multiple gestations, stillbirth, or fetal anomalies were excluded. Myometrial biopsy specimens were taken from the upper margin of lower segment incisions during cesarean delivery. Samples were washed in phosphate-buffered saline solution to remove excess blood, immediately snap frozen, and stored at −80°C. All women provided written informed consent for the collection of clinical data and tissue samples. Ethical approval for the collection of myometrial biopsy specimens was granted by the local research ethics committee (REC 3357).

RNA isolation

Total RNA was extracted from myometrial tissue (30 mg; for the discovery and validation groups) or cultured cells (approximately 1×10^7; for the in vitro model) using the NucleoSpin column kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. RNA concentration and integrity were assessed with a nanodrop spectrophotometer and Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA). RNA that was isolated from myometrial tissue was used for miRNA microarray profiling and qRT-PCR validation. RNA from myometrial cells was used for specific miRNA measurement by qRT-PCR.

In vitro modeling: cell culture and cell treatments

Myometrial tissue was washed in phosphate-buffered saline solution and mechanically dissected with 2 sterile blades; the cells were isolated by incubation with 1 mg/mL of collagenase 1A, 1 mg/mL of collagenase X, and 20 mg of bovine serum albumin in 40 mL of Dulbecco's modified Eagle's medium for 45 minutes at 37°C. The suspension was filtered through a cell strainer and centrifuged at 400*g* for 5 minutes. The cells were resuspended and cultured in Dulbecco's modified Eagle's medium that contained 10% fetal calf serum, 2 mmol/L L-glutamine, 100 μg/mL penicillin, and 100 μg/mL of streptomycin and grown in T25 flasks at 5% CO_2. Myocytes were cultured until confluent and treated with 100 nmol/L oxytocin (Alliance Pharmaceuticals, Chippenham, UK) for 1, 2, and 4 hours or 1 μg/mL

interleukin 1β for 2 hours (or alcohol vehicle control).

Microarray

Microarray expression analysis of 1309 miRNAs was performed with the miRCURY LNA miRNA microarray (version 8.0; Exiqon, Vedbaek, Denmark). Samples were labeled with miRCURY LNA miRNA Hi-Power Labeling Kit, Hy3/Hy5, and were hybridized on the array. To minimize nonbiologic experimental variation, samples were randomized for the array and spike-in oligo nucleotide controls were included. The quantified signals (background corrected) were normalized with the global locally weighted scatterplot smoothing regression algorithm.[16] The positive effect of normalization was confirmed by assessment of log ratio/log mean-intensity plots.

Real-time qRT-PCR for miRNA

QRT-PCR was used to quantify the expression of candidate miRNAs with the miRCURY LNA kit (Exiqon) with 0.05 ng complementary DNA. LNA assays were custom designed (Exiqon) for miRNAs of interest: hsa-miR-21-5p (204230), hsa-miR-29a-3p (204698), hsa-miR-29a-5p (204430), hsa-miR-29b-3p (204679), hsa-miR-29c-3p (204729), hsa-miR-29c-5p (204132), hsa-miR-141-3p (204504), hsa-miR-146a-5p (204688), hsa-miR-146b-3p (204374), hsa-miR-196b-3p (204619), hsa-miR-223-3p (204256), hsa-miR-873-5p (204175), and hsa-miR-876-5p (204527). MiRNA expression data were normalized to 5S ribosomal RNA (203906).[17]

Statistical analysis

Background signal was defined as expression 1.2 times the 25th percentile of overall signal intensity and subtracted from the array dataset. MiRNAs that were detected above background in $\geq$6 samples in any phenotypic group, and therefore expressed in most of those samples, were included in the analysis. Array data were subjected to unsupervised principal components analysis (PCA) and supervised partial least squares–discriminatory analysis (PLS-DA) with the use of SIMCA-P (Soft independent modelling of class analogies-P; version 13.0.2; Umetrics, Umeå, Sweden). Data were scaled to unit variance by the division of each variable by 1/(S*k*), where S*k* represents the standard deviation value of the variable to ensure that all variables retained equal importance during the generation of the model. Goodness-of-fit and predictive ability parameters were calculated (the latter by a 7-round internal cross-validation of the goodness-of-fit parameter) and used to assess model fit.[18,19] Although PCA permits an overall examination of covariance in the data sets, PLS-DA was used to examine specific differences in the miRNA profiles that were associated with patient groups. PLS-DA analysis differs from PCA in that the algorithm is provided with class membership of clinical sample groups and components are projected and rotated around the axis to obtain maximum separation between these classes, thus permitting the best description of class-related variability. MiRNAs that differed most between classes were identified by examination of their calculated variable importance for the projection (VIP) score, which was calculated in SIMCA-P with a proprietary algorithm. The VIP score describes the relative contribution of each miRNA (X variables) to patient class variation (Y variables) accounted for by each component in the PLS-DA model. A VIP score >1 is considered to indicate an important contributor to the separation of patient classes that are observed in the PLS-DA model.[18] In this study, miRNAs that exhibit VIP scores >1.5 were defined as significant and selected for further investigation.

For qRT-PCR, relative expression of individual miRNAs in tissue and cell samples was calculated by the $\Delta\Delta$CT method. Statistical significance of the data was inferred with a 2-tailed Mann-Whitney test for tissue samples and a 1-way analysis of variance for treated cells. A probability value of $< .05$ was considered statistically significant.

TABLE 1
Demographics of original sample cohorts

Characteristic	Term: Not in labor (n = 8)	Term: In labor receiving oxytocin (n = 8)	*P* value
Median maternal age, y (range)	37 (24–44)	31 (27–36)	.08[a]
Median parity, n (range)	1 (0–2)	0 (0–1)	.06[a]
Black ethnicity, n (%)	1 (11)	1 (13)	1.0[b]
Median gestational age at delivery, wk (range)	39 (38–39)	40 (38–41)	.06[a]

The maternal age, parity, ethnicity, and gestation of the original cohort participants are described. There were no significant differences in age, ethnicity, parity, or race between the 2 clinical groups.

[a] Continuous variables compared by Mann-Whitney tests; [b] Discrete variables compared by χ^2 test.

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Identification of potential target genes

Target genes of the candidate differentially expressed myometrial miRNAs were sought with the use of the miRTarBase platform, which describes experimentally validated miRNA targets in the current literature (http://mirtarbase.mbc.nctu.edu.tw).[20] These candidate genes were cross-referenced with genes that previously were reported in gene microarray studies of pregnant human myometrium[21,22] and those known to be implicated in parturition. This method produced a focused short-list of validated potential gene targets.

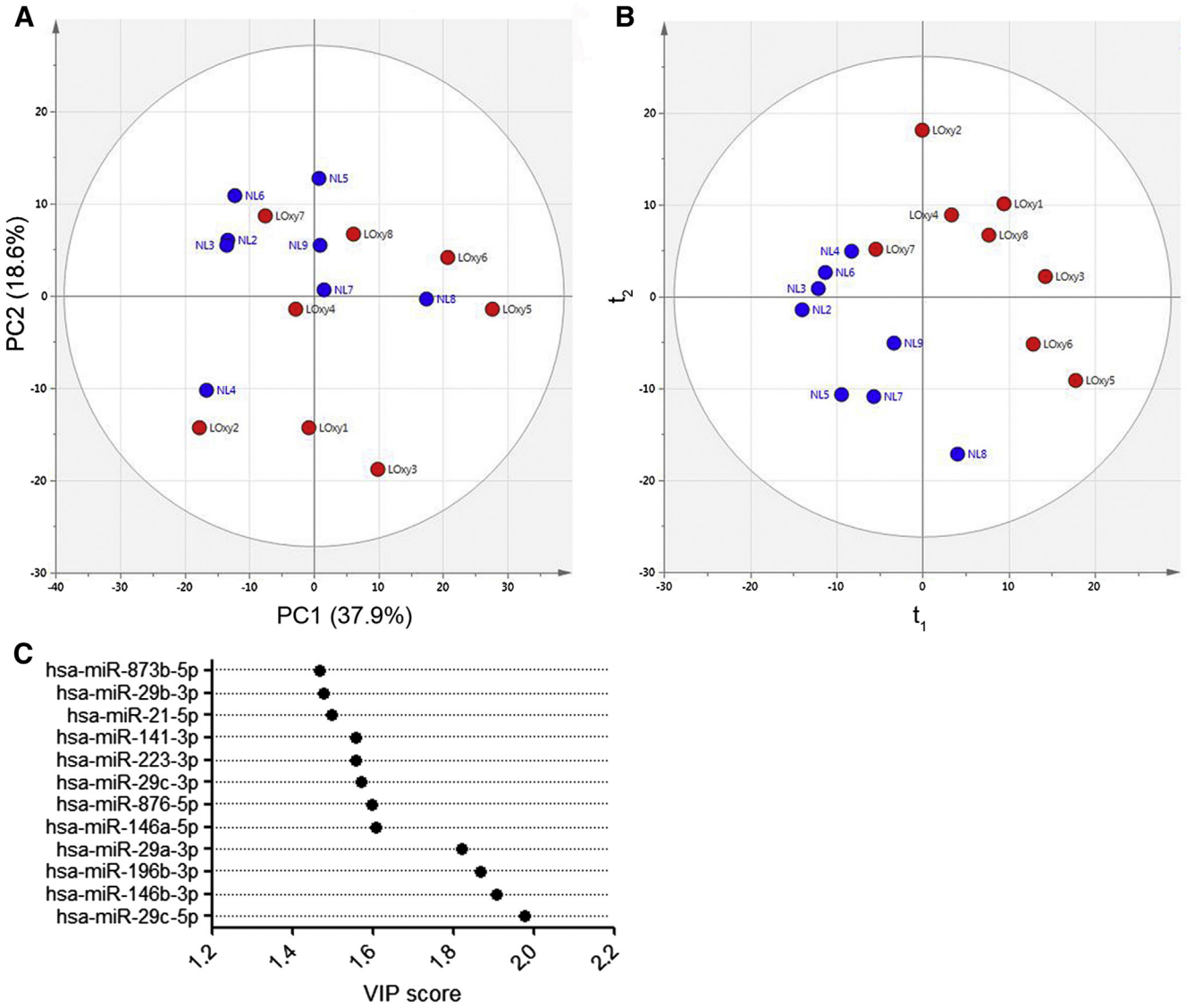
RESULTS

Exogenous oxytocin modulates the expression of a subset of miRNAs in human myometrium

Analysis of patient demographic characteristics revealed no significant differences in age, ethnicity, parity, or gestation between those women who provided

FIGURE 1

Multivariate modeling of microRNA expression in myometrium of women laboring with oxytocin and those women not in labor



A, Unsupervised principal components analysis of myometrial microRNA expression did not reveal any clear clustering of patients not in labor (*blue*) or patients laboring with oxytocin (*red*), which indicates that most variance in the data was independent of oxytocin treatment. **B,** Supervised partial least squares—discriminatory analysis of microRNA expression profiles facilitated separation of the patient classes. **C,** Variable importance plot scores show which individual microRNA variance contributes to the separation of phenotype (ie, VIP, ≥1.5).

LOxy, labor with oxytocin; *NL*, not in labor; *PC1*, first principal component; *PC2*, second principal component; *VIP*, variable importance plot.

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myometrial biopsy specimens that were taken before labor onset (n = 8) and those who received exogenous oxytocin (n = 8; Table 1). A total of 494 miRNAs were detected above background expression levels and were used for multivariate modeling of LOxy and NL myometrium profiles (Figure 1). Unsupervised PCA was first used to assess overall variation of the data set. As seen in Figure 1, A, no obvious clustering of the 2 patient populations was detected, which indicates that most miRNA variation in the sample population was independent of oxytocin-induced/augmented labor. To focus on oxytocin-specific–induced variation in the miRNA profiles, supervised PLS-DA was used (Figure 1, B). Analysis of the calculated VIP values (Figure 1, C) permitted selection of a panel of 12 miRNAs that were most responsible for the observed separation of the 2 patient populations. Those miRNAs with a VIP score

TABLE 2
Demographic data from the biologic validation cohorts

Characteristics	Term				
	Not in labor (n = 10)	In labor, no exogenous oxytocin (n = 8)	*P* value	Term, in labor, receiving oxytocin (n = 9)	*P* value
Median maternal age, y (range)	33 (19–41)	30 (19–37)	.7[a]	36 (26–41)	.1[a]
Median parity, n (range)	1 (0–2)	0.5 (0–2)	.56[a]	0	.01[a]
Black ethnicity, n (%)	1 (10)	0	1.0[b]	1 (10)	1.0[b]
Median gestational age at delivery, wk (range)	39 (37–41)	39 (37–41)	.32[a]	40 (38–42)	.08[a]

The maternal age, parity, ethnicity, and gestation of the validation cohort participants are described. There were no significant differences in age, ethnicity, or gestation between the nonlaboring and laboring with exogenous oxytocin groups, however, women in the laboring with exogenous oxytocin groups were more likely to be primiparous ($P = .01$). There were no significant differences in age, parity, ethnicity, or gestation between the nonlaboring and spontaneous laboring groups.

[a] Continuous variables compared by Mann-Whitney tests; [b] Discrete variables compared by $\chi 2$ test.

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≥1.5 were defined as significantly contributing to the separation in the model. These comprised hsa-miR-21-5p, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-29c-3p, hsa-miR-29c-5p, hsa-miR-141-3p, hsa-miR-146a-5p, hsa-miR-146b-3p, hsa-miR-196b-3p, hsa-miR-223-3p, hsa-miR-873-5p, and hsa-miR-876-5p.

The laboring myometrial miRNA profile of women who were treated with oxytocin differs from those in spontaneous labor

To validate our findings, qRT-PCR was used to examine the expression of the 12 selected target miRNAs in myometrial samples that were isolated from secondary, independent validation cohorts of women at term not in labor (n = 10), at term in labor after early oxytocin treatment (n = 8) and additionally women in spontaneous labor not receiving oxytocin (n = 10). Demographic characteristics of the validation cohort are provided in Table 2. No significant differences in age, ethnicity, or gestation between the patient groups were observed, although all women who received oxytocin were nulliparous, which is consistent with the common clinical use of exogenous oxytocin. To examine whether parity impacts on the expression of our target miRNAs, we undertook logistic regression on available PCR data from 6 miRNAs that were analyzed in our discovery set. When adjusted for parity, relative expression of miRNAs was not affected (Table 3). Similar analyses were not performed in the validation cohort because all women in the LOxy group were primiparous and most women in the NL group were multiparous. Differential expression of 6 of the 12 candidate miRNAs (hsa-miR-146a-5p, hsa-miR-146b-3p, hsa-miR-196b-3p, hsa-miR-873-5p, hsa-miR-876-5p, and hsa-miR-223-3p) was confirmed in the myometrium of women who received oxytocin in labor (LOxy vs NL; Figure 2). Expression of hsa-146a-5p, hsa-miR-196b-3p, hsa-miR-873-5p, and hsa-miR-876-5p was decreased in LOxy myometrium (−4 fold change, $P = .02$; −3.3 fold change, $P = .04$; −5 fold change, $P = .01$, and −10 fold change, $P = .02$, respectively), whereas hsa-miR-146b-3p and hsa-miR-223-3p levels were increased in the myometrium of oxytocin-treated women (2.7 fold change, $P = .0055$; 9 fold change, $P = .0025$, respectively). Only hsa-miR-146b-3p was found to be up-regulated in both LSpon (3.3 fold change, $P = .04$) and LOxy groups (Figure 2).

Oxytocin treatment regulates miRNA expression in human myocytes

To investigate whether the observed miRNA differences represent a signature of dysfunctional labor that require

TABLE 3
Expression of microRNAs in original cohort using polymerase chain reaction

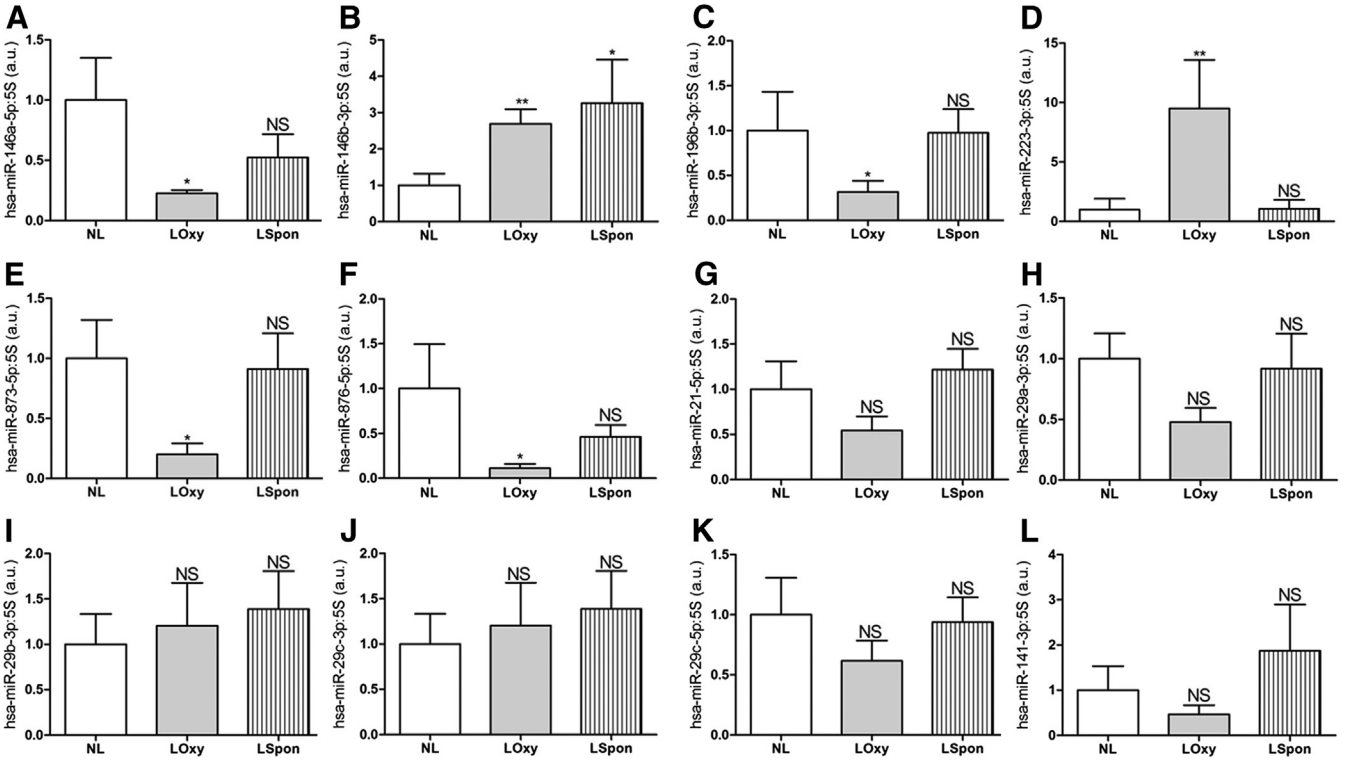
MicroRNA	Women in labor who received exogenous oxytocin compared with women not in labor, *P* value	
	Unadjusted	Adjusted for parity
hsa-miR-200c-3p	.25	.15
hsa-miR-29c-3p	.023	.012
hsa-miR-29b-3p	.022	.034
hsa-miR-223-3p	.012	.0038
hsa-miR-873-5p	.0007	.0040
hsa-miR-876-5p	.0009	.0042

Univariate logistic regression modeling was used to compare expression of a selection of individual myometrial microRNAs that were measured with real-time polymerase chain reaction in the same samples as those analyzed by microarray. Samples were taken from women in labor who received oxytocin and women not in labor. The findings reproduce those of the microarray. Multivariate logistic regression was also used to adjust for parity, which although not significantly different in the 2 groups, was slightly dissimilar ($P = .06$). The probability values remained consistent, and we conclude that small variations in parity did not introduce bias to our results. Analysis was carried out using STATA software (StataCorp LP, College Station, TX).

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FIGURE 2

Biologic validation with polymerase chain reaction in a second cohort



Expression of **A-F,** 6 microRNAs (hsa-miR-146a-5p, hsa-miR-146b-3p, hsa-miR-196b-3p, hsa-miR-223-3p, hsa-miR-873-5p and hsa-miR-876-5p) remained significantly different in nonlaboring women compared with those women laboring with oxytocin. However, when expression levels of microRNAs were compared between the nonlaboring group and those laboring spontaneously without oxytocin, only hsa-miR-146b-3p was expressed differentially. **G-L,** Hsa-miR-21-5p, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-29c-3p, hsa-miR-29c-5p, and hsa-miR-141-3p did not alter in myometrium from women who received oxytocin or in those laboring spontaneously in our validation population. The *single asterisk* indicates $P < .05$; the *double asterisks* indicate $P < .01$.

LOxy, women laboring with oxytocin; *LSpon*, laboring spontaneously without oxytocin; *NL*, nonlaboring women; *NS*, not significant.

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augmentation with oxytocin or whether they represent a direct effect of oxytocin on myometrial miRNA expression, we used an in vitro model of primary cultured myocytes. Human myocytes that were taken from women before the onset of labor (n = 5) were treated with oxytocin, and levels of the 6 miRNAs found to be differentially expressed in vivo after oxytocin treatment were examined. Treatment of these cells with oxytocin resulted in significant down-regulation of hsa-miR-146a-5p, hsa-miR-196b-3p, and hsa-miR-876-5p (n = 5; 1-way analysis of variance; p = .008, .02, and .02, respectively), consistent with in vivo observations (Figure 3). Conversely, hsa-miR-223-3p and has-miR-873-5p were not expressed at detectable levels in cultured myocytes, and the expression of hsa-miR-146b-3p was not effected by oxytocin treatment. Our findings provide good evidence that oxytocin regulates hsa-miR-146a-5p, hsa-miR-196b-3p, and hsa-miR-876-5p, whereas hsa-miR-146b-3p is not induced by oxytocin but increases in response to the process of labor itself.

Potential targets of oxytocin-regulated miRNAs

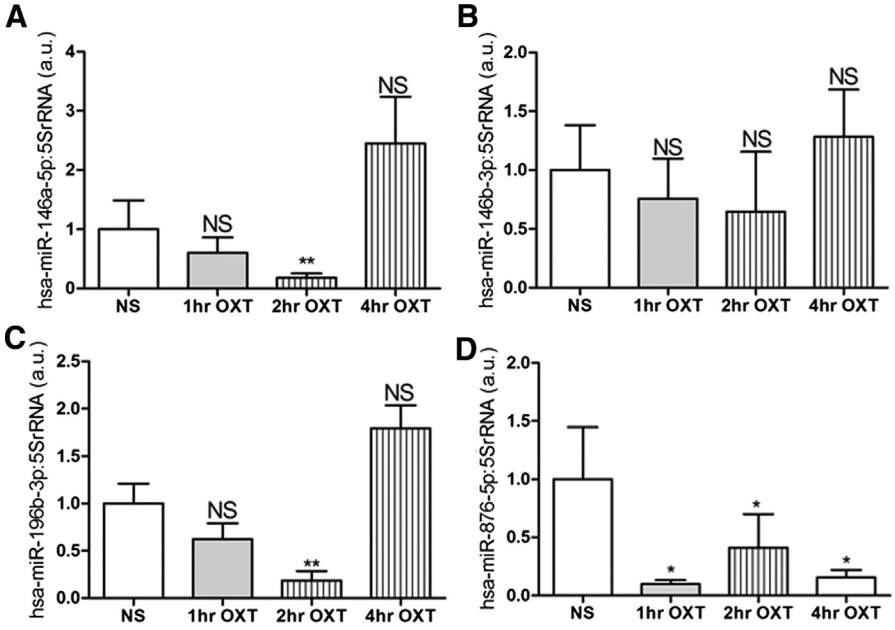
Potential target genes of oxytocin-regulated miRNAs were sought with the use of the miRTarBase platform and were cross-referenced with genes that are known to be expressed in pregnant human myometrium or implicated in parturition (Table 4). Hsa-miR-146a-5p, hsa-miR-146b-3p, and hsa-miR-223-3p have been linked with functional studies to potential target genes within the myometrium, whereas hsa-miR-196b-3p, hsa-miR-873-5p, and hsa-miR-876-5p, so far, have not been associated with the regulation of genes within the myometrium.

Comment

At the end of pregnancy, oxytocin acts as a potent stimulator of uterine contractions and accordingly is used extensively for induction of labor, whereas oxytocin receptor antagonists are used widely to delay preterm birth. However, the effectiveness of these treatment strategies is variable at best. Understanding how

FIGURE 3

Myocyte expression of hsa-miR-146a-5p, hsa-miR-146b-3p, hsa-miR-196b-3p, and hsa-miR-876-5p after oxytocin exposure



A, Hsa-miR-146a-5p expression in myocytes was reduced significantly after exposure to oxytocin (100 nmol/L) for 2 hours with no change in expression seen at 1 and 4 hours. **B,** Hsa-miR-146b-3p expression in myocytes was not altered by the addition of oxytocin. **C,** Hsa-miR-196b-3p expression in myocytes was reduced significantly after exposure to oxytocin for 2 hours with no change in expression seen at 1 and 4 hours. **D,** Hsa-miR-876-5p expression in myocytes was reduced significantly after exposure to oxytocin for 1, 2, and 4 hours. Values are expressed as mean ± SEM (n = 4). The *double asterisk* indicates a probability value of $< .01$; the *single asterisk* indicates a probability value of $< .05$.

NS, non-stimulated; *OXT*, oxytocin.

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oxytocin modulates pathways of parturition is critical for enabling the development of more effective therapeutic strategies to predict and prevent preterm birth or, alternatively, for induction of labor in prolonged pregnancies. This study reports that oxytocin affects the miRNA profile of human myometrium at term. We describe a miRNA "fingerprint" that is expressed differentially in myometrium after oxytocin administration that is distinct from the miRNA profile that was observed in the samples that were obtained during spontaneous labor. Our results highlight a role for oxytocin in the regulation of specific myometrial miRNAs both in vivo and in vitro.

Our qRT-PCR analysis of selected myometrial miRNAs in samples that were derived from women in spontaneous labor or after oxytocin administration indicates that oxytocin drives the labor phenotype through a distinct mechanism to that observed in spontaneous labor. This observation is consistent with previous proteomic studies of myometrial biopsy specimens that were obtained from women in spontaneous and oxytocin-induced labor.[23] However, it is feasible that changes in the myometrial miRNA profile after exogenous oxytocin simply may reflect underlying molecular differences in women who experience dysfunctional labor and who subsequently require clinical intervention and induction with oxytocin. To examine whether observed differences in miRNA expression represent a signature of dysfunctional labor or reflect a direct effect of oxytocin on the myometrium, we used an established in vitro model of cultured primary myocytes.[24,25] Treatment of these cells with oxytocin led to the transient suppression of hsa-miR-146a-5p, hsa-miR-196b-3p, and hsa-miR-876-5p that were consistent with in vivo observations. In contrast, hsa-miR-146b-3p expression was not altered in myocytes that were treated with oxytocin, although its levels were increased in both spontaneous and oxytocin-treated laboring myometrium samples. This finding highlights hsa-miR-146a-5p, hsa-miR-196b-3p, and hsa-miR-876-5p as oxytocin responsive miRNAs, whereas hsa-miR-146b-3p likely represents a miRNA that is modulated as a consequence of the inflammatory process of labor, which itself is stimulated up-stream by oxytocin.

Although further work is required to define the biologic significance of these changes, interrogation of the miRTarBase platform enabled the identification of putative gene targets of these miRNAs. Of particular interest, hsa-miR-146a-5p, which was reduced in myometrial samples from oxytocin-treated women and also in myometrial cells treated with oxytocin, is a known suppressor of nuclear factor kappa B (NF-κB) activity in human breast,[26] pancreatic,[27] and gastric[28] cancer cells, which results in the suppression of NF-κB target genes that include the key labor-associated genes interleukin-8, interleukin-6, and matrix metalloproteinase-9. Decreased levels of hsa-miR-146a-5p that were observed in oxytocin-treated myometrial tissue and cells may reflect withdrawal of NF-κB inhibitory activity and promote a contractile phenotype. Consistent with this, hsa-miR-146a-5p has also been shown to attenuate the expression of α-smooth muscle actin, which is the major protein constituent of uterine smooth muscle and essential component of the contractile machinery, in human dermal fibroblasts.[29] We recently have shown a proinflammatory role for oxytocin in human gestational tissues whereby specific stimulation of oxytocin by its ligand drives the sequential activation of MAPKinases and NF-κB and subsequent prostaglandin and proinflammatory

TABLE 4
Potential gene targets of oxytocin regulated microRNAs

MicroRNA	Direction of expression change with oxytocin	Experimentally validated gene targets that are known to be expressed in myometrium[a] or implicated in parturition[b]
hsa-miR-146a-5p	Decrease	Toll-like receptor 2[b31]
		Interleukin 8[a26,27,36]
		Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1[b26,27,37]
		Toll-like receptor 4[b38]
		BCL2-related protein A1[a39]
hsa-miR-146b-3p	Increase	Platelet-derived growth factor beta polypeptide[b24]
hsa-miR-223-3p	Increase	Leukemia inhibitory factor[a40]
hsa-miR-196b-3p	Decrease	No matches
hsa-miR-876-5p	Decrease	No validated targets[c]
hsa-miR-873-5p	Decrease	No validated targets[c]

Potential target genes of oxytocin-regulated microRNAs were sought with the use of the miRTarBase platform and cross-referenced with genes that are known to be expressed in pregnant human myometrium or implicated in parturition.[21,22]

[a] Experimentally validated gene targets of microRNAs were cross-referenced with genes that are known to be expressed in pregnant human myometrium; [b] Reported target known to be involved in parturition; [c] As per miRTarBase, August 2014.

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cytokine and chemokine production.[30] This suggests an additional and hitherto unrecognized role for oxytocin in the onset of human labor via modulation of the inflammatory pathways that lead to NF-κB activation. Our finding that oxytocin can reduce hsa-miR-146a-5p expression may provide a further mechanistic link between miRNA expression and NF-kB activation. Further, hsa-miR-146a-5p has also been shown to interfere with TLR2 downstream signaling and cytokine production,[31] which suggests that oxytocin-induced suppression of hsa-miR-146a-5p could result in enhanced TLR2-dependent NF-κB signaling that is analogous to the activation of this pathway by lipopolysaccharide because occurs in infection-induced preterm labor.

Currently, miRTarBase does not reveal putative experimentally validated gene targets for hsa-miR-876-5p and hsa-miR-873-5p. However, hsa-miR-146b-3p, which was increased significantly in myometrial samples from both spontaneous and oxytocin-induced labors, is linked functionally to the regulation of platelet-derived growth factor beta polypeptide, which is an inflammatory cytokine that recently has been reported as being released by uterine myocytes in response to stretch.[24] Hsa-miR-146b-3p has also been implicated recently in regulating mRNA levels of TRAF6 and IRAKI, which are proteins involved in the modulation of NF-κB activation.[32] In this context, hsa-miR-146b-3p is thought to function in a negative feedback loop, whereby its induction by both interleukin-6 and signal transducer and activator of transcription 3 result in the inhibition of both NF-κB-dependent IL6 expression and downstream activation of signal transducer and activator of transcription 3.[33] Interleukin-6 is a key inflammatory mediator in parturition and stimulates cervical and fetal membrane remodeling. Thus, increased expression of hsa-miR-146b-3p in response to labor may represent a homeostatic response to the raised inflammatory mediators accompanying labor, such as interleukin-6.

Both hsa-miR-223-3p and hsa-miR-873-5p were found to be expressed differentially in laboring myometrium that was obtained from women who received exogenous oxytocin; however, we were unable to detect their expression in primary human myocyte cultures. We hypothesize that their presence in myometrial tissue may be the result of the inflammatory cell infiltrate that remains present in whole tissue extracts but would not persist in cell culture. Regardless of their cell of origin, miRNAs may act in a paracrine fashion by exerting an effect on adjacent and distant cell types.[34] This represents an additional challenge to establish mechanistic links between such miRNAs and their functional gene targets. Recent studies have also identified a labor-associated increase of hsa-miR-223-3p in human cervical tissue[8] and in chorioamniotic membranes in the presence of inflammation.[9] Hsa-miR-223-3p has been found to target IKKα during monocyte-macrophage differentiation, which suggests a possible role in the noncanonical and canonical NF-κB pathways,[35] which are important in both term and preterm labor.

It has been reported that labor is associated with increased myometrial expression of members of the miR-200 family (miR-200a/141 and miR-200b/c/429).[10,11] In our microarray, hsa-miR-200a/b/c and hsa-miR-429 were not expressed above background; although hsa-miR-141 was detected, it was not expressed differentially in those women who received oxytocin. This potentially supports the hypothesis that women who require oxytocin during labor exhibit a dysfunctional miRNA expression profile; however, we acknowledge that the authors of these studies do not explicitly state that their study participants had not received oxytocin.

In conclusion, our findings provide a novel insight into how oxytocin contributes to normal human parturition and modulates human myometrial miRNAs. Although further research is required to understand the role of these miRNAs in myometrial contractility and function, our results implicate oxytocin as a signaling molecule in their regulation. ■

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