OBSTETRICS

Distinct cervical microRNA profiles are present in women destined to have a preterm birth

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OBJECTIVE: Although premature cervical remodeling is involved in preterm birth (PTB), the molecular pathways that are involved have not been elucidated fully. MicroRNAs (miRNAs) that are highly conserved single-stranded noncoding RNAs that play a crucial role in gene regulation have now been identified as important players in disease states. The objective of this study was to determine whether miRNA profiles in cervical cells are different in women who are destined to have a PTB compared with a term birth.

STUDY DESIGN: A nested case-control study was performed. With the use of a noninvasive method, cervical cells were obtained at 2 time points in pregnancy. The cervical cell miRNA expression profiles were compared between women who ultimately had a PTB (n = 10) compared with a term birth (n = 10). MiRNA expression profiles were created with the Affymetrix GeneChip miRNA Array. The data were analyzed with the Significance of Analysis of Microarrays and Principle Components Analyses. A false-discovery rate of 20% was used to determine the most differentially expressed miRNAs. Validation was performed with quantitative polymerase chain reaction. In vitro studies were performed to confirm expression and regulation of select miRNAs.

RESULTS: With a false-discovery rate of 20% of the 5640 miRNAs that were analyzed on the array, 99 miRNAs differed between those with a PTB vs a term birth. Qualitative polymerase chain reaction validated the array findings. In vitro studies confirmed expression of select miRNAs in cervical cells.

CONCLUSION: MiRNA profiles in cervical cells may distinguish women who are at risk for PTB months before the outcome. With the large downstream effects of miRNAs on gene expression, these studies provide a new understanding of the processes that are involved in premature cervical remodeling and allow for the discovery of new therapeutic targets.

Key words: cervical remodeling, ectocervical cells, microRNA, preterm birth

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Preterm birth remains the leading cause of childhood morbidity and death. Although there have been some clinical trials that have demonstrated a reduction in the preterm birth rate by targeting women who are at high risk,[1-4] preventative or interventional strategies that significantly reduce the national and international preterm birth rate have not yet been realized. The major impediment to more impactful interventional strategies has been our lack of understanding of the essential mechanisms that are involved in the pathogenesis of preterm birth.

★ EDITORS' CHOICE ★

Clinical data demonstrate that a short cervix is one of the most consistent predictors of preterm birth.[5,6] However, what the molecular equivalent to a sonographic short cervix is remains unknown. Indeed, although a sonographic short cervix is believed to be a surrogate for "premature cervical remodeling," there is a lack of evidence revealing what actually is occurring (ie, molecularly, biochemically) in cervical tissue at the time when a sonographic short cervix becomes evident. This information is critical if we are to discover more effective prevention strategies for preterm birth. It is known that remodeling of the cervix must occur at any gestational age to allow the passage of the fetus. Hence, cervical remodeling, at term or preterm, is an obligatory molecular event in the process of parturition. Animal studies have revealed some key insights regarding the pathways that are involved in the process of cervical remodeling.[7-11] However, whether these revealed processes in animal models correlate to what occurs in

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human pregnancy is assumed but not proved. Human studies are very limited in regards to assessing the molecular events in the pregnant cervix. Two studies have been performed that involved the biopsy of the cervix in women at term.[12,13] These studies demonstrated how gene expression patterns differ in the cervix in laboring vs nonlaboring women as well as between those women with and without a ripe cervix.[12,13] Although these studies provide some insight to the process of cervical ripening at term, they involve a more invasive method to assess the molecular phenotype of the pregnant cervix. Notably, there are no studies to date that provide information regarding the molecular events that occur in the cervix that might be associated with premature cervical remodeling and preterm parturition.

Although animal studies and 2 human studies suggest some changes in gene expression in the cervix in preparation of parturition,[7,8,12,14,15] most of these studies have been limited to investigations of messenger RNA (mRNA) expression. Although these studies provide molecular insight, recent emergence of the role of microRNAs (miRNAs) in gene regulation support the concept that investigation of miRNAs in normal and pathologic processes of cervical remodeling might provide greater information.

In the last decade, miRNA biology has emerged as an important player in both physiologic and pathophysiologic responses.[16-19] MiRNAs are small (approximately 22 nucleotides in length) highly conserved single-stranded RNA molecules that play a critical role in posttranscriptional gene regulation by interacting with the 3′ untranslated regions of specific mRNA targets to affect mRNA stability and translation negatively. In most organisms, there are a limited number of miRNAs (estimated at approximately 1000 in human genome) compared with the number of mRNAs (approximately 30,000). However, 1 miRNA may regulate hundreds of mRNAs and consequently have significant and wide-ranging effects on gene expression networks. A primary role of miRNAs appears to be specific regulation or "fine-tuning" of gene expression to control development and tissue homeostasis. However, under certain conditions (eg, stress, inflammation, hypoxia), the functions of miRNAs become pronounced, which suggests an essential role of miRNAs in disease states.[20-22] MiRNAs are now implicated in varied disease states that include cancer and cardiovascular disease and are considered as important therapeutic targets.[23-27] Despite the growing importance of miRNAs to pathogenic states, there is no existing study that has explored the role of miRNAs in cervical remodeling and their possible involvement in preterm birth.

Our objective for this study was to determine whether there was a distinct miRNA profile in cervical cells that were collected from women who were destined to have a preterm birth compared with a term birth. For this study, we performed a nested case-control study within a prospective cohort of women who were at high risk for preterm birth. MiRNA array analysis was performed and followed by validation by quantitative polymerase chain reaction (QPCR). Exploration of the regulation of discovered miRNAs by inflammation was performed with in vitro model systems.

Methods

Nested case-control within a prospective cohort

This study used biospecimens that were collected as part of a prospective cohort study that was called "PREDICT."[28] This study was approved by the Institutional Review Board at the University of Pennsylvania and was performed at a single, urban tertiary care center between August 2011 and November 2012. The cohort consisted of women who were at high risk for preterm birth because of history of previous spontaneous preterm birth or second-trimester loss, previous cervical surgery without a subsequent full-term birth, and/or uterine anomaly with a singleton pregnancy. Women who used systemic steroids or immunosuppressive therapy, who had HIV, lupus, pregestational diabetes mellitus, rheumatoid arthritis, Crohn's disease, ulcerative colitis, or who had cancer or an organ transplant were excluded. Additionally, women who had anything in the vagina within the last 24 hours (including sexual intercourse, vaginal probe, digital cervical examination, lubricants), had active vaginal bleeding, had evidence of rupture of membranes, and/or had cervical dilation ≥3 cm at the time of enrollment were excluded. In this study, clinical and biospecimen data were collected at 2 time points: (1) visit 1 occurred at 20 weeks to 23 weeks 6 day gestation (V1) and (2) visit 2 occurred at 24 weeks to 27 weeks 6 days gestation (V2). For the study presented here, 10 women with a spontaneous preterm birth at <37 weeks of gestation and 10 women with a term delivery (>37 weeks of gestation) were identified. No matching by race or parity was performed. All cases of preterm birth were reviewed by 1 of the investigators (J.B.) to confirm that the preterm delivery was a spontaneous delivery.

Biospecimen collection

To ascertain what might be occurring molecularly in the cervix during pregnancy, we created a "RNA PAP" technique. This technique involves the collection of cervical cells in a method similar to the performance of a Papanicolaou (PAP) smear. A cytobrush is used to collect cells from the ectocervix. The collected cells are placed in QIAzol lysis reagent (Qiagen, Valencia, CA), and the specimen is immediately placed in liquid nitrogen. The frozen sample is stored at −80°C until future use. The method for RNA extraction from these samples is noted later. We have confirmed that using this technique, in any trimester of pregnancy, provides adequate RNA to perform expression analyses (unpublished data). Based on existing data from studies that have assessed cell populations from PAP smears, we assumed that the RNA PAP contains mostly epithelial cells (ectocervical and endocervical cells) and some leukocytes. Our ability to harvest adequate RNA for gene expression studies is consistent with what has been reported with the use of nonpregnant PAP specimens.[29] For this study, an RNA PAP was performed at each study visit.