OBSTETRICS

Inflammatory gene networks in term human decidual cells define a potential signature for cytokine-mediated parturition

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BACKGROUND: Inflammation is a proximate mediator of preterm birth and fetal injury. During inflammation several microRNAs (22 nucleotide noncoding ribonucleic acid (RNA) molecules) are up-regulated in response to cytokines such as interleukin-1β. MicroRNAs, in most cases, fine-tune gene expression, including both up-regulation and down-regulation of their target genes. However, the role of pro- and antiinflammatory microRNAs in this process is poorly understood.

OBJECTIVE: The principal goal of the work was to examine the inflammatory genomic profile of human decidual cells challenged with a proinflammatory cytokine known to be present in the setting of preterm parturition. We determined the coding (messenger RNA) and noncoding (microRNA) sequences to construct a network of interacting genes during inflammation using an in vitro model of decidual stromal cells.

STUDY DESIGN: The effects of interleukin-1β exposure on mature microRNA expression were tested in human decidual cell cultures using the multiplexed NanoString platform, whereas the global inflammatory transcriptional response was measured using oligonucleotide microarrays. Differential expression of select transcripts was confirmed by quantitative real time—polymerase chain reaction. Bioinformatics tools were used to infer transcription factor activation and regulatory interactions.

RESULTS: Interleukin-1β elicited up- and down-regulation of 350 and 78 nonredundant transcripts (false discovery rate < 0.1), respectively, including induction of numerous cytokines, chemokines, and other inflammatory mediators. Whereas this transcriptional response included marked changes in several microRNA gene loci, the pool of fully processed, mature microRNA was comparatively stable following a cytokine challenge. Of a total of 6 mature microRNAs identified as being differentially expressed by NanoString profiling, 2 (miR-146a and miR-155) were validated by quantitative real time—polymerase chain reaction. Using complementary bioinformatics approaches, activation of several inflammatory transcription factors could be inferred downstream of interleukin-1β based on the overall transcriptional response. Further analysis revealed that miR-146a and miR-155 both target genes involved in inflammatory signaling, including Toll-like receptor and mitogen-activated protein kinase pathways.

CONCLUSION: Stimulation of decidual cells with interleukin-1β alters the expression of microRNAs that function to temper proinflammatory signaling. In this setting, some microRNAs may be involved in tissue-level inflammation during the bulk of gestation and assist in pregnancy maintenance.

Key words: inflammation, microribonucleic acid, preterm birth, systems biology, transcription factor

The uterine decidua, situated at the interface between the maternal decidua and the fetal compartments, plays a pivotal role in the events leading to both term and preterm parturition in humans. Decidual activation by cytokines and physical stretch drives the molecular cascades that prompt the onset of labor at term,[1-6] and disruption of decidual integrity by infection or bleeding causes localized inflammation that is associated with preterm birth (PTB).[1,7,8] Classic studies by Gustavil and colleagues[9-11] demonstrated a pivotal role of the decidua in the production of prostaglandin $F_2\alpha$ upon the instillation of hypertonic saline into the intraamniotic cavity for termination of pregnancy.

Term and preterm labors are associated with increased expression of proinflammatory cytokines and chemokines at the fetal-maternal interface that summon the infiltration of leukocytes into the decidual microenvironment.[12,13]

Human labor is an inflammatory process, although debate persists over whether term and preterm parturitions are similar or different biological entities.[14,15] Inflammation in the setting of parturition occur systemically and locally.[16,17] High-dimensional profiling indicates that molecular signatures accompanying labor differ, depending on clinical context.[18-20]

Choriodecidual chemokine expression patterns differ in idiopathic (ie, noninfectious etiology) compared with PTB in the presence of histologically verified chorioamnionitis.[21,22] Such results underscore previous findings and foreshadow the evolving concept that PTB is a complex syndrome with distinct phenotypes and underlying pathogenesis that can best be appreciated by considering them in separate contexts.[1,12,17]

Cataloging by gene expression profiling has been the mainstay of molecular methods in research efforts into PTB.[17,18] With the advent of sophisticated bioinformatics tools, it is now possible to use systems biology approaches to study gene networks in complex, physiologically relevant contexts (ie, gene regulatory network [GRN] analysis). GRN analysis is a computational method that identifies relationships among genes up-regulated or down-regulated in response to a given stimulus.

Noncoding ribonucleic acids (RNAs), including microRNAs (miRNAs), provide an important level of molecular fine-tuning of biological and

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pathobiological processes. These miRNAs are small noncoding RNAs (~ 22 nucleotides in length) that, in general, dampen gene expression.[23-25] In addition, some miRNAs target mRNAs whose gene products exert inhibitory activity, and in this case, these miRNAs may actually enhance expression of small subsets of genes. More than 2500 unique mature human miRNAs have been identified, each with the ability to regulate the expression of potentially hundreds of genes.

The miRNAs represent one of the most abundant and conserved classes of regulatory molecules that influence global gene regulation. Mature miRNAs (generated following the post-transcriptional processing of precursor miRNAs) block protein translation or promote mRNA degradation by imperfect Watson-Crick base pairing.[26-28] The miRNAs arise from larger transcripts located within structural genes (intragenic miRNAs) or from independently transcribed units (intergenic miRNAs) that are processed from primary transcripts into functional miRNAs.[29]

A few miRNA profiling studies have used clinical samples in the context of parturition to report on which miRNAs are expressed in gestational tissues in the setting of normal or infection-mediated PTB.[30-32] However, the relationship between miRNAs and the mRNAs they regulate is ill defined and has not been studied in depth in the context of preterm labor. It is crucial that we bridge the gap in our understanding of the role of miRNAs in inflammatory gene regulation to more effectively identify new biomarker and/or therapeutic strategies to combat preterm parturition.

We conducted a profiling investigation of miRNAs and their cognate mRNA targets in human decidual stromal cells to construct gene regulatory networks to begin defining the inflammatory pathways involved in preterm labor. We also considered these mRNA-miRNA relationships in the context of the transcription factors (TFs) that govern both gene expression programs to build a framework for future hypothesis testing by clinical and laboratory experimentation. These studies have important implications for translating gene expression signatures into a more coherent molecular picture of inflammation-mediated pathways involved in term and preterm parturition.

Materials and Methods

Study design

The overarching design of the present study was the evaluation of putative inflammatory gene targets by miRNAs expressed in decidual cells in response to cytokine challenge. In addition, we sought to determine whether progesterone could reverse the proinflammatory effects of cytokines and mRNA and miRNA gene expression.

Decidual stromal cell culture and treatments

All tissues were obtained following written informed consent, and the study was approved by the Institutional Review Boards (IRB) at Yale University and The Ohio State University. Cells were initially isolated at Yale under IRB approval and then transferred to Ohio State, requiring additional local IRB approval.

After written IRB approval and informed consent, fibroblasts were prepared from the decidua parietalis of reflected fetal membranes obtained from 3 uncomplicated pregnancies after elective repeat cesarean delivery as previously described.[33] All specimens were obtained prior to the onset of labor. At confluence, decidual cells were primed for 13 days in medium supplemented with 10^{-8} M 17β-estradiol (Sigma-Aldrich, St Louis, MO) and 10^{-7} M medroxyprogesterone acetate (Sigma-Aldrich) to stimulate in vitro decidualization (see Supplemental Methods for further details).

On day 1, cultures were treated in a defined medium with PBS containing 0.1% bovine serum albumin (vehicle control) or with 1 ng/mL of human recombinant interleukin-1β (IL-1β; R&D Systems, Minneapolis, MN) for 6 hours. IL-1β is a cytokine shown previously to be produced in decidual cells.[34] Cells from 3 different patients were used for the studies.

RNA isolation and quantitative real-time–reverse transcriptase analysis

Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA), and after chloroform and centrifugation, the aqueous phase was precipitated overnight at −20°C with an equal volume of 70% ethanol and then applied to a miRNeasy spin column (QIAGEN, Valencia, CA) and processed according to the manufacturer's protocol. This included on-column deoxyribonuclease digestion using the RNase-Free deoxyribonuclease kit (QIAGEN) to remove contaminating genomic DNA. RNA was quantified by absorbance at 260/280 nm using a NanoDrop spectrophotometer (Thermo Scientific, Hudson, NH). Methodological details of the quantitative real time–polymerase chain reaction (qRT-PCR) studies can be found in the Supplemental Methods.

For all reactions, fold changes were calculated using the comparative cycle threshold method described by Schmittgen and Livak[35] using large ribosomal protein (RPLP0; Applied Biosystems, Foster City, CA) as the internal control gene run in parallel assays. Statistical analysis was performed using the Mann-Whitney statistical test, after determining that gene amplification data did not exhibit a Gaussian distribution.

Microarray profiling and data analysis

Prior to processing, the quality and purity of total RNA were evaluated using the Agilent 2100 Bioanalyzer RNA 6000 Nano Kit and on the ND1000 NanoDrop platform (Agilent Technologies, Palo Alto, CA). Total RNA (250 ng per sample) was processed using the Ambion WT expression kit (Ambion, Austin, TX) and labeled with the Affymetrix GeneChip whole transcript sense target labeling assay (Affymetrix, Santa Clara, CA), followed by hybridization to the Affymetrix Human Gene 2.0 ST array according to the manufacturers' protocols. Hybridized arrays were scanned on an Affymetrix GeneChip Scanner 3000 7G and analyzed using Affymetrix software. Details of the data processing and analysis for gene expression data