BASIC SCIENCE: OBSTETRICS

Human effector/initiator gene sets that regulate myometrial contractility during term and preterm labor

Carl P. Weiner, MD, MBA; Clifford W. Mason, PhD; Yafeng Dong, PhD;
Irina A. Buhimschi, MD; Peter W. Swaan, PhD; Catalin S. Buhimschi, MD

OBJECTIVE: Distinct processes govern transition from quiescence to activation during term (TL) and preterm labor (PTL). We sought gene sets that are responsible for TL and PTL, along with the effector genes that are necessary for labor independent of gestation and underlying trigger.

STUDY DESIGN: Expression was analyzed in term and preterm with or without labor (n = 6 subjects/group). Gene sets were generated with logic operations.

RESULTS: Thirty-four genes were expressed similarly in PTL/TL but were absent from nonlabor samples (effector set); 49 genes were specific to PTL (preterm initiator set), and 174 genes were specific to TL (term initiator set). The gene ontogeny processes that comprise term initiator and effector sets were diverse, although inflammation was represented in 4 of the top 10; inflammation dominated the preterm initiator set.

CONCLUSION: TL and PTL differ dramatically in initiator profiles. Although inflammation is part of the term initiator and the effector sets, it is an overwhelming part of PTL that is associated with intraamniotic inflammation.

Key words: effector, initiator, labor, myometrium, preterm birth

Successful parturition requires the synchronization of uterine events (myometrial activation, myometrial contraction, cervical ripening, cervical dilation, and rupture of the fetal membranes).[1] The myometrium specifically must undergo a series of molecular and biochemical changes that transition it from the phase of quiescence, which is characterized by a loss of responsiveness to contractile agents, to the phase of activation with subsequent onset of labor.[2,3] Many investigators have assumed the triggers of parturition are similar, regardless of the gestational age (GA),[2,4-9]

From the Departments of Obstetrics and Gynecology (Drs Weiner, Mason, and Dong) and Molecular and Integrative Physiology (Dr Weiner), University of Kansas School of Medicine, Kansas City, KS; the Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD (Dr Swaan); and the Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT (Drs I. A. Buhimschi and C. S. Buhimschi).

and have focused on the control of term labor as a surrogate for preterm labor. This approach has not met with great success.

Genomics helps expand and characterize the number of molecular pathways that potentially define an underlying condition. Since our initial report in 2000,[10] oligonucleotide/complementary DNA microarrays have been used by many groups for the study of biologic processes that are involved in normal term labor.[11-18] The results of such studies with the use of myometrium that is obtained from term laboring women

suggest that contractile stimulators of labor include, but are not limited to, hormone receptors, cell adhesion molecules, interleukins (ILs), prostaglandins, and gap junctions.[10,11,13-15,17] Other investigators have attempted to correlate human myometrial transcriptional levels at term with samples from idiopathic, spontaneous preterm labor and have concluded that the mechanisms underlying parturition at all stages of pregnancy are related.[1,13,17] This is surprising because there are several well accepted causes of spontaneous preterm birth.[19,20] Although molecules within these groups may well influence parturition during normal labor, there has been little study of myometrial gene expression in women with either preterm or dysfunctional term/preterm labor. Array type investigations have also been used to explore myometrial gene expression in the pregnant rodent.[11,18,21] However, the resulting conclusions should be interpreted cautiously because the gestational and hormonal patterns of these species are not homologous to the human. Although these studies provide insight, they tend as a group to be biased by the limited number of genes that are included on the array and the number of

genes that are selected for confirmatory study.

Rather than compare term labor to 1 of several models for preterm birth, we investigated human pregnancy and hypothesized that there should be a core set of genes the expression of which are necessary for the process of labor independent of GA and the underlying trigger for the labor. We further hypothesized this effector gene set should relate to myometrial contractility and the cellular activities necessary to sustain it. We also hypothesized there must be initiator gene sets that are responsible for the transition of the myometrium from quiescence to activation. But unlike the effector set that would be unaffected by the underlying labor stimulus, there should be separate initiator gene sets for term and preterm labor that reflect the underlying mechanism of labor. The potential result of identifying these gene sets could be the development of alternative treatment options that can be targeted at preterm and term or dysfunctional labor. The purpose of this investigation was to test our hypotheses in a series of women who were undergoing cesarean delivery at term or preterm in labor or absent labor.

MATERIALS AND METHODS
Study design
Myometrium was obtained from the upper pole of the transverse lower uterine segment incision of 4 groups of women (n = 6 per group) at the time their primary cesarean section at Yale University: (1) preterm not in labor and no inflammation (PTNL; mean GA, 28.8 weeks; range, 25.4–32.5 weeks), (2) preterm in labor with inflammation (PTL; mean GA, 29.7 weeks; range, 25.1–32.6 weeks), (3) term not in labor (TNL; mean GA, 39.3 weeks; range, 38.4–41.0 weeks), and (4) term labor (TL; mean GA, 40.2 weeks; range, 39.0–41.2 weeks). The Yale University Institutional Review Board approved the protocol for sample collection, and all women provided informed written consent. Labor was defined by the presence of regular uterine contractions accompanied by progressive cervical dilation. The diagnosis of

intraamniotic inflammation was based on an amniotic fluid mass restricted score of 3 or 4 plus >100 white blood cells/μL^3 in the context of a positive amniotic fluid culture in a sample that was obtained by transabdominal amniocentesis.[22-25] These tests provided the most accurate tools currently available to maximize the likelihood of sample homogeneity. The mass restricted score provides qualitative information regarding the presence or absence of intraamniotic inflammation. Briefly, the score ranges from 0-4, depending on the presence (assigned a value of 1) or absence (assigned a value of 0) of each of 4 protein biomarkers.[25] A score of 3-4 indicates inflammation, whereas a score of 0-2 excludes it. This biomarker pattern is predictive of preterm birth, histologic chorioamnionitis, and adverse neonatal outcome. A detailed description of the mass restricted method has been published previously.[22-25] Indications for cesarean delivery in the PTNL group were all related to preeclampsia and in the TNL were all related to breech presentation. The indication for cesarean delivery in the TL group was an arrest of cervical dilation at ≥6 cm. No normal laboring patient at term underwent cesarean section delivery in this sample. Clinical data were retrieved from the medical records, and statistical analysis of patient demographics was performed using 1-way analysis of variance, followed by Newman Keuls Post Hoc test. All laboratory studies were performed at either the University of Kansas School of Medicine or the University of Maryland Baltimore School of Pharmacy.

Isolation of RNA
Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol. The purity and integrity of each RNA sample was assessed by spectroscopy and formaldehyde-agarose gel electrophoresis.

Microarray preparation
Myometrial gene profiling was performed on each individual RNA sample with the Affymetrix GeneChip Human Genome U133 Plus 2.0 microarray (Affymetrix, Santa Clara, CA) that con-

tained 38,500 human genes. RNA quality was reassessed before spotting with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Once the quality was confirmed, biotin-labeled complementary RNA was generated, and 20 μg of the sample was hybridized to the microarrays, using the manufacturer's standard conditions. Image processing used an Affymetrix GeneArray 3000 scanner.

Microarray data processing and statistical analysis
Oligonucleotide microarrays were analyzed with the Affymetrix Expression Console. Gene expression levels were normalized by the *R* Statistical package and software that is available through the Bioconductor Project (www.bioconductor.org). The process normalizes gene expression using a background adjustment procedure and a sequence-specific expression method as described by Wu et al.[26] Normalized microarray data were then subject to further discriminative analysis. A detection probability value was used to make a reliable call of gene expression (present, marginal, or absent). Genes that were present in <4 of the 6 patient samples were classified as absent overall and not considered further.

To identify the effector and initiator gene sets, we conducted a series of logic operations based on gene presence (detection probability value) and illustrated by the Venn diagram in Figure 1. We first generated 4 groups of genes: group A = (PTL − PTNL); group C = (TL − TNL); group B = (group A − TNL); group D = (group C − PTNL).

The *effector gene set* was defined as those genes common to groups B and D. The effector set was further filtered to exclude genes whose expression during PTL changed ≥2-fold in either direction compared with TL, reasoning there was something unique to the disease state that influenced gene expression. The *preterm initiator gene set* was defined as those genes in group A exclusive to PTL (minus group C). Last, the *term initiator gene set* was defined as those genes in group C exclusive of TL (minus group A).