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Uterine transcriptomes of bacteria-induced and ovariectomy-induced preterm labor in mice are characterized by differential expression of arachidonate metabolism genes

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Objective: The purpose of this study was to identify changes in gene expression that are associated with preterm labor induced by either bacteria or ovariectomy.
Study design: Pregnant mice (14.5 days of gestation) were allocated to: (1) intrauterine injection of heat-inactivated *Escherichia coli*; (2) media alone; (3) ovariectomy; or (4) sham operation. The uterine transcriptome was studied with photolithographic, very short oligonucleotide-based microarrays, and arachidonate metabolism genes were assayed with quantitative reverse transcriptase–polymerase chain reaction. Significance was determined by analysis of variance.
Results: Microarray-based gene expression changes in the arachidonate metabolism pathway are associated globally with bacteria-induced preterm labor ($P \leq .0031$) and ovariectomy-induced preterm labor ($P \leq .00036$). Quantitative real-time reverse transcriptase–polymerase chain reaction measurements demonstrated that bacteria-induced preterm labor substantially increased the expression of genes involved in prostaglandin synthesis. In contrast, ovariectomy-induced preterm labor increased the expression of genes involved in lipoxin, leukotriene, and hydroxyeicosatetraenoic acid synthesis.
Conclusion: Bacteria-induced and ovariectomy-induced preterm labor each express a different balance of genes that are required for the synthesis of prostaglandins, lipoxins, leukotrienes, and hydroxyeicosatetraenoic acids.

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Preterm labor and delivery accounts, which accounts for 12% of births in the United States, has multiple etiologies.[1] In this study, we have focused on two etiologies. Intrauterine infection is a well-defined cause and is responsible for 25% to 40% of premature births.[2] Suspension of progesterone action also has been implicated in preterm labor and delivery. The strongest evidence for progesterone involvement in preterm birth is based on the reduction in the frequency of recurrent preterm birth by treatment with 17 hydroxy-progesterone caproate[3] or natural progesterone.[4] In addition, termination of pregnancy can be precipitated by progesterone receptor antagonists such as mifepristone. These two lines of evidence are consistent with the requirement for progesterone for pregnancy maintenance.

To investigate the role of intrauterine infection and progesterone in human preterm labor (PTL), we performed transcriptome analysis using two previously established mouse models. The first model induces PTL by the introduction of heat-killed *Escherichia coli* into the uterus.[5,6] The second mouse model, which induces PTL by ovariectomy, has been proposed as a progesterone withdrawal model, because mean serum progesterone concentrations fall 60% by 1 hour and 81% after 7.5 hours.[6]

Several investigators have used genomic approaches to study gene expression changes in uterine tissue obtained from both term and preterm gestations of humans, mice, and rats.[7-10] To date, however, only one study has used transcriptional profiling to investigate changes in uterine gene expression after the induction of PTL in mice.[9] This study analyzed uterine tissue samples obtained within 3 to 5 hours after administering the treatment that results in PTL, which may prevent the detection of additional essential genes that are relevant for the process of PTL.[9]

We undertook this study to identify the biological processes that underlie uterine gene expression changes after the experimental induction of PTL. In this report, we describe the identification of a known biochemical pathway that exhibits differential expression dependent on the method used to induce PTL.

Material and methods

Detailed methods are available as supplementary material on the Journal's web site. The experimental design is depicted in Figure 1, **A**. PTL was induced in mice by either the injection of heat-killed *E coli* (1×10^{10} colony-forming units in 100 μL) or by ovariectomy. Microarrays were analyzed with open source Bioconductor software (www.bioconductor.org), gene ontology (GO) annotations (www.geneontology.org), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (www.genome.jp/kegg/). Quantitative real-time reverse transcriptase–polymerase chain reaction (qRT-PCR) assays were performed as described (Table I).[11]

Results

Principal components analysis suggests bacteria-induced and ovariectomy-induced PTL exhibit fundamentally different patterns of gene expression

Principal components analysis was used to garner a global view of the data. Principal components 2 and 3 demonstrated the clearest separation of all experimental groups (Figure 1, **B**). The control samples for both the bacteria-induced and ovariectomy-induced PTL are similar, and the PTL samples are distinct from each other (Figure 1, **B**). Because all control samples were similar to PCA and all pair-wise comparisons (unpublished data) detected fewer than 5 genes with significantly different expression, the controls were grouped together as a single control group. We focused on gene expression changes in PTL relative to controls.

Gene expression changes in bacteria-induced PTL are associated with inflammation, fatty acid metabolism, and carboxylic acid metabolism

Discriminant analysis demonstrated that 249 genes were differentially expressed between the ovariectomy-induced PTL and the control samples. Nine hundred thirty-five genes were expressed differentially between the bacteria-induced PTL and the control group (unpublished data). To gain biological insight from the differentially expressed genes, GO annotation was applied (www.geneontology.org). No "Biological Process" categories were enriched significantly in the ovariectomy-induced PTL gene list. After removing the expected categories that related to inflammation, it is noteworthy that bacteria-induced PTL exhibited a modest enrichment for genes in the GO "Biological Process" category of "angiogenesis." We also found that genes involved in "fatty acid metabolism" and "carboxylic acid metabolism" were enriched significantly, which include genes required for arachidonic acid metabolism (Table II; Figure 2, **A**).

Pathway analysis indicates a significant association of the prostaglandin and leukotriene metabolism pathway with both bacteria-induced and ovariectomy-induced PTL

To extend this result, the prostaglandin and leukotriene metabolism pathways were investigated (Figure 2, **A**). Both bacteria-induced and ovariectomy-induced PTL showed highly significant association ($P \leq .0031$ and $\leq .00036$, respectively). Hierarchical clustering verified