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Research Article

Transcriptome changes in maternal peripheral blood during term parturition mimic perturbations preceding spontaneous preterm birth†

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Abstract

The complex physiologic process of parturition includes the onset of labor, which requires the orchestrated stimulation of a common pathway involving uterine contractility, cervical ripening, and chorioamniotic membrane activation. However, the labor-specific processes taking place in these tissues have limited use as predictive biomarkers unless they can be probed in non-invasive samples, such as the peripheral blood. Herein, we utilized a transcriptomic dataset to assess labor-specific changes in the peripheral blood of women who delivered at term. We identified a set of

genes that were differentially expressed with labor and enriched for immunological processes, and these gene expression changes were strongly correlated with results from prior studies, providing in silico validation of our findings. We then identified significant correlations between labor-specific transcriptomic changes in the maternal circulation and those detected in the chorioamniotic membranes, myometrium, and cervix of women at term, demonstrating that tissue-specific labor signatures are partly mirrored in the peripheral blood. Finally, we demonstrated a significant overlap between the peripheral blood transcriptomic changes in term parturition and those observed in asymptomatic women, prior to the diagnosis of preterm prelabor rupture of the membranes, who ultimately delivered preterm. Collectively, we provide evidence that the normal process of labor at term is characterized by a unique immunological expression signature, which may serve as a useful tool for assessing labor status and for potentially identifying women at risk for preterm birth.

Summary sentence: The maternal peripheral blood transcriptome undergoes labor-specific changes that correlate with those in the chorioamniotic membranes, myometrium, and cervix and partially overlap with those observed in asymptomatic women prior to preterm birth.

Key words: cervix, chorioamniotic membranes, fetal membranes, immune response, inflammation, myometrium, parturition, placenta, preterm labor, uterus.

Introduction

Parturition is a complex physiologic process that allows the fetus to be delivered once it has matured enough to face extra-uterine life [1–3]. This process includes the onset of labor whose active phase leads to the vaginal delivery of the fetus [2–8]. Therefore, labor is a multi-stage process that requires the orchestrated stimulation of a common pathway involving uterine contractility, cervical ripening, and decidual/chorioamniotic membrane activation [2–7, 9]. Labor is also considered as a physiologic, sterile inflammatory process since most term-laboring women do not experience microbial invasion of the amniotic cavity [10, 11]. In line with such a concept, multiple investigations have demonstrated an increase in cellular and soluble immune mediators in the myometrium [12–24], cervix [12, 14–16, 25–34], decidua [14, 15, 35–44], and chorioamniotic membranes [14, 15, 36, 37, 39, 45–49] during spontaneous labor at term. However, the labor-specific mechanisms taking place in the reproductive and gestational tissues have limited use as predictive biomarkers, given the difficulty of obtaining biopsies from women with an ongoing pregnancy. Thus, there is a need to further explore labor-specific changes detectable in minimally invasive samples such as the peripheral blood.

In the last decade, high-dimensional techniques, such as transcriptomics, proteomics, and metabolomics, have been used to explore the complex and dynamic processes that are modulated in the maternal circulation prior to and during labor [50–52]. Single- and multi-omics studies have revealed distinct signatures that are closely associated with gestational age, including those related to immune cell types of interest, such as T cells, [53, 54] as well as those specific to the placenta and fetal liver [55]. Indeed, our recent study suggested that women who are in labor at term display a different RNA profile than that of non-laboring gestational age-matched controls, featuring an increased expression of T-cell-specific RNAs, among others [56]. Furthermore, integration of the maternal metabolome, proteome, and immunome has been utilized to predict the onset of active labor [57], and we recently assessed maternal transcriptomic and proteomic data for predicting gestational age as well as spontaneous preterm birth, using the Dialogue for Reverse Engineering Assessments and Methods (DREAM) crowdsourcing framework [58]. In this DREAM challenge, the prediction of gestational age was undertaken by using samples collected throughout gestation; thus, samples from women in labor at term were also included. However, the latter study did not assess the changes that occur in women who undergo spontaneous labor at term compared to those of gestational age-matched controls. Moreover, the question of how closely these systemic signatures mirror changes taking place in the reproductive and gestational tissues where the common pathway of labor occurs, such as the chorioamniotic membranes (including the decidua parietalis), myometrium, and cervix, remains unanswered.

Herein, we utilized the transcriptomic dataset featured in the DREAM Preterm Birth Prediction Challenge [58] to assess the changes in the maternal systemic transcriptome occurring in spontaneous labor at term. We have also investigated the extent to which maternal blood RNA changes that occur in term labor reflect those of human parturition in the chorioamniotic membranes [59], myometrium [60], and cervix [61]. Finally, we evaluated the overlap between the expression changes in spontaneous term labor and those observed in asymptomatic women, prior to the diagnosis of preterm prelabor rupture of the membranes (PPROM), who ultimately delivered preterm. The results herein may have implications for the development of non-invasive biomarkers to assess the progress of physiologic labor at term. Furthermore, the understanding of these changes may shed further light on pathological processes such as spontaneous preterm birth.

Materials and methods

Ethics

The collection of biological specimens and the use of clinical data were approved by the Institutional Review Boards of Wayne State University and NICHD. All participants provided written informed consent prior to the collection of samples.

Study population

Women who provided peripheral blood samples included in the transcriptomic analysis were enrolled in a prospective longitudinal study at the Center for Advanced Obstetrical Care and Research of the Perinatology Research Branch, NICHD/NIH/DHHS, the Detroit Medical Center, and the Wayne State University School of Medicine. Blood samples were collected longitudinally from the first or early second trimester until delivery, yet only data for the last available sample before delivery in women with spontaneous term labor as well as gestational age-matched samples from women not in labor

were retained for the analysis reported in this study. Labor was defined by the presence of regular uterine contractions at a frequency of at least two contractions every 10 min with cervical changes resulting in delivery. Women with a multiple gestation or those who had a fetus affected with chromosomal and/or sonographic abnormalities were also excluded from this study. The first ultrasound scan during pregnancy was used to establish gestational age if this estimate was >7 days from the last menstrual period-based gestational age.

Maternal whole blood transcriptomics

Briefly, RNA was isolated from PAXgene Blood RNA collection tubes and was hybridized to Human Transcriptome Arrays 2.0, as previously described [62]. Exon-level gene expression data were generated from array images and were summarized for each ENTREZ gene ID with Robust Multi-array Average [63] implemented in the oligo package [64] by using a chip definition file from http://brainarray.mbni.med.umich.edu. Correction for potential batch effects was performed with the removeBatchEffect function of the limma [65] package in Bioconductor [66]. The transcriptomic dataset was previously deposited by the authors in the Gene Expression Omnibus super-series GSE149440. For each of the two groups included in the GEO series (TL: term in labor and TNL: term not in labor), only the last available samples from each patient were included in the analysis.

Differential expression analyses

Differences in gene expression between the TL and TNL groups were assessed based on linear models implemented in the limma package [67] in Bioconductor. Significant changes were based on a false discovery rate-adjusted p-value (q-value) < 0.25, provided that the fold change (FC) was at least 1.25-fold. Note that given the compression of the dynamic range of microarray-based intensity, even FCs <1.25 observed with this platform were previously validated [56]. Downstream analyses of the differentially expressed genes (DEGs) involved enrichment analysis via a hypergeometric test implemented in the GOstats package [68] to determine the over-representation of gene ontology [69] biological processes among the significant genes. Additional enrichment analyses were based on a hypergeometric test with pathway definitions extracted from the C2 collection of the MSigDB database. The C2 collection in MsigDB includes pathways from the Pathway Interaction Database [70], Kyoto Encyclopedia of Genes and Genomes [71], Reactome database [72], and WikiPathways [73], among other sources. The background list in the enrichment analyses featured all genes profiled on the microarray platform. A network representation of DEGs was performed by using the stringApp version (1.5.0) [74] in Cytoscape (version 3.7.2) [75]. The most interconnected sub-networks were displayed and nodes were annotated to significantly enriched biological processes. A false discovery rate-adjusted p-value (q-value) < 0.1 was applied in all enrichment analyses to infer significance and to allow direct comparisons to previous findings in preterm birth [58].

In silico validation of blood RNA changes in spontaneous labor at term

To assess the overlap of expression changes with term labor obtained in this study and the report of Tarca et al. [56], in which the blood transcriptomes of eight TL and eight TNL cases were contrasted, we calculated the Pearson correlation of $\log_2$FC (TL vs. TNL) between studies for all genes deemed significant in this study.

Correlation of whole blood RNA changes in spontaneous labor at term with previous reports on changes in gestational and reproductive tissues

The correlation between expression changes in fetal membranes with term labor (TL vs. TNL) reported in Haddad et al. [59] and those found in the current study was based on genes detected on both platforms regardless of their significance in the current study (n = 189, Table 2 of original publication). Similarly, we determined the extent to which term parturition, blood transcriptomic changes found herein reflected those reported in the human myometrium (462 genes) [60] and cervix (833 genes) [61]. For the two latter studies, the full lists of DEGs and FCs were obtained from the authors, and we retained only genes represented on microarray platform used in this study. Finally, to determine the relationship between the effects of term parturition on the maternal blood transcriptome and changes preceding a diagnosis of PPROM leading to preterm birth, we have correlated our data with the FCs of 402 RNAs dysregulated in asymptomatic patients at 17–33 weeks of gestation in two diverse populations [58]. Finally, the blood RNA changes preceding a diagnosis of PPROM (regardless of statistical significance) were correlated with those of DEGs with labor in term tissues, as described above for the blood term-labor signature. For each comparison, the proportion of RNAs with matching direction of change between the two studies was also reported. A Pearson correlation of $p < 0.05$ was deemed to be significant.

Identification of genes for which expression changes with term labor are different from those preceding preterm birth

Gene expression differences between the 21 TL and 28 TNL patients were contrasted with those observed between 30 patients sampled at 17–33 weeks of gestation, who subsequently were diagnosed with PPROM and delivered preterm, and gestational age-matched controls (n = 45, derived from the same 49 normal pregnancies used for the TL vs. TNL comparison). Moderated t-tests were used to assess the significance of the interaction. Significant interactions were based on a false discovery rate-adjusted p-value (q-value) < 0.25, provided that the FC was at least 1.25-fold.

Results

Labor involves immune inflammatory processes in the maternal circulation

The onset of labor requires the coordinated activation of different local and systemic pathways that will result in the delivery of the neonate [2–7, 9]. Thus, we first compared the transcriptomes in the peripheral blood between pregnant women at term with and without spontaneous labor by using microarrays. The clinical and demographic characteristics of the patients are shown in Table 1. Among the 49 women included in the study, the first ultrasound exam available to establish gestational age was performed before 14 weeks in 38 women and between 14 and 20 weeks in the remainder of 11 patients. There were no differences in maternal characteristics, gestational age at sampling, and birthweight percentiles between the two groups; yet, women in the TNL group delivered on average 5 days later (0.7 weeks) than those who were in labor ($p < 0.001$).

After comparing the expression profiles of 32 830 genes, we identified 103 DEGs, with 51 upregulated and 52 downregulated in women at TL compared to those without labor ($q < 0.25$, FC >1.25 for all DEGs) (Figure 1A and Table 2). The RNAs with differential

Table 1. Clinical and demographic characteristics of women at term with or without labor

	TNL ($n = 28$)	TL ($n = 21$)	p
Maternal age [years; median (IQR)][a]	25 (21–28.2)	24 (22–28)	0.97
Body mass index [kg/m^2; median (IQR)][a]	26.9 (24.1–34)	24.8 (21.1–27.5)	0.06
Nulliparous[b]	8/28 (28.6%)	8/21 (38.1%)	0.55
Race/ethnicity			
African-American[b]	27/28 (96.4%)	18/21 (85.7%)	0.3
Fetal sex (female)[b]	16/28 (57.1%)	11/21 (52.4%)	0.78
Gestational age at delivery [weeks; median (IQR)][a]	39.6 (39–40.6)	38.9 (38.1–39.3)	<0.001
Birthweight [g; median (IQR)][a]	3452.5 (3153.8–3596.2)	3175 (2930–3380)	0.004
Birthweight percentile [%; median (IQR)][a]	62.4 (39.1–77.4)	52.7 (29.2–71.3)	0.29
Gestational age at sampling [weeks; median (IQR)][a]	38.5 (38.0–39.0)	38.9 (38.1–39.3)	0.4

Data are given as medians (interquartile range, IQR) and percentages (n/N).
[a]Welch's t-test.
[b]Fisher's exact test.

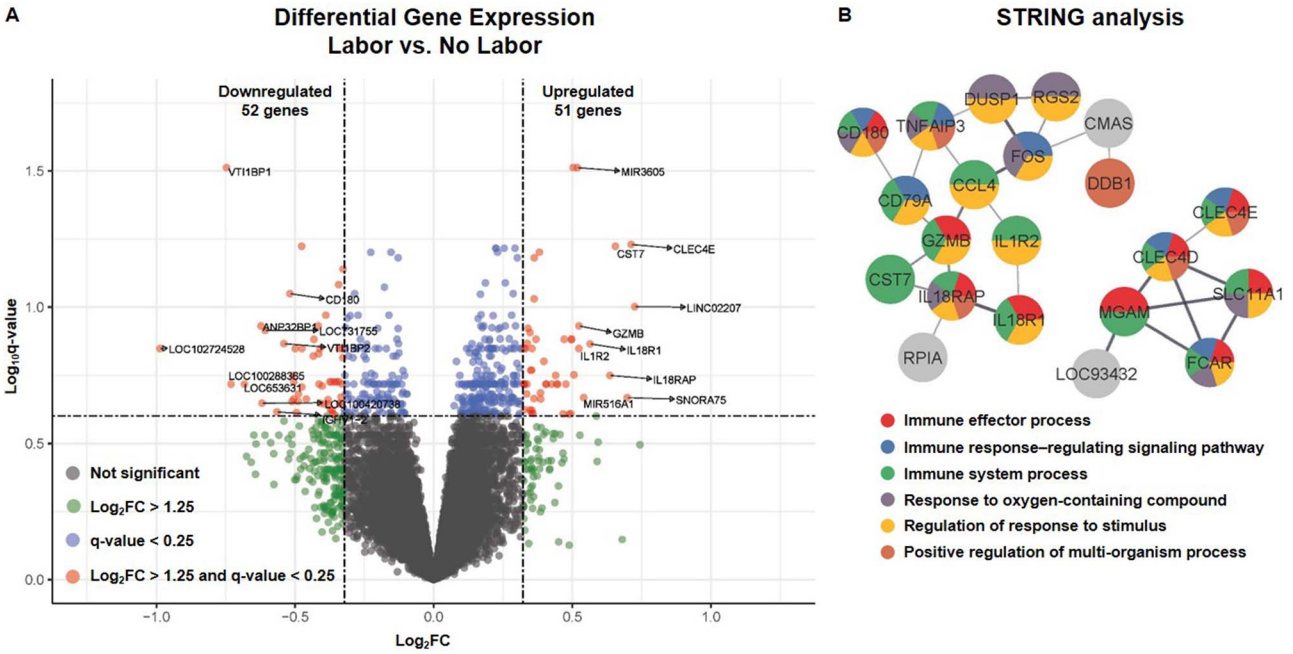

Figure 1. Differential gene expression and pathway enrichment in term labor. (A) Volcano plot showing differential gene expression in peripheral blood from women who underwent term labor compared to that of women who delivered at term without labor, where the Y-axis shows the $-\log_{10}(q\text{-value})$ and the X-axis shows the $\log_2(FC)$. Differentially expressed genes (DEGs) were considered those with $q < 0.25$ and FC > 1.25 (red dots). Blue dots indicate genes with a low q-value but low FC, green dots indicate genes with a high FC but high q-value, and gray dots indicate all other genes tested. (B) STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis of the most interconnected protein–protein interaction sub-networks corresponding to DEGs, with nodes annotated to significantly enriched biological processes.

expression in labor at term included not only mRNAs but also miRNAs and other non-coding genes (Figure 1A and Table 2). STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis of DEGs revealed interconnected protein–protein interaction sub-networks, as displayed in Figure 1B. These genes corresponded to immunologic processes that were found to be enriched with labor at term ($q < 0.1$), namely, "immune effector process," "immune response-regulating signaling pathway," and "immune system process," among others (Figure 1B and Table 3).

To assess the reliability of labor-related transcriptomic changes identified herein, we performed a comparative analysis between our current microarray results and a report of an independent, smaller, microarray-based study from the same population [56]. The changes in expression of the 103 DEGs determined herein were highly correlated with those derived from the available dataset ($r = 0.83$, $p < 0.0001$, 95% agreement in direction of change) (Figure 2), providing an in silico validation of the current findings. Together, these results support the inflammatory nature of the physiologic process of labor at term.

Labor-specific signatures in gestational and reproductive tissues implicated in the common pathway of labor correlate with those in peripheral blood

Labor is a complex process that requires the orchestrated stimulation of a common pathway involving uterine contractility, cervical ripening, and decidual/chorioamniotic membrane activation [2–7, 9]. Therefore, we next utilized previously published datasets to evaluate whether labor-specific signatures in the chorioamniotic membranes,

Table 2. Genes differentially expressed in the peripheral blood in term labor compared to term without labor

SYMBOL	ENTREZ	log_2FC	*p*	Adj. *p*
DUSP1	1843	0.50	1.6E-06	0.031
MIR3605	100500853	0.52	2.2E-06	0.031
VTI1BP1	391559	−0.75	2.8E-06	0.031
CLEC4E	26253	0.71	7.2E-06	0.059
CST7	8530	0.66	1.1E-05	0.060
RPL36P12	100271012	−0.48	1.1E-05	0.060
APMAP	57136	0.38	2.6E-05	0.063
TMCC3	57458	0.36	3.4E-05	0.066
HSP90AB3P	3327	−0.33	4.0E-05	0.073
SNORD101	594,837	−0.34	0.0001	0.083
CD180	4064	−0.52	0.0001	0.089
SLC37A3	84255	0.36	0.0001	0.093
LINC02207	101927153	0.73	0.0001	0.100
LOC100420707	100420707	−0.39	0.0001	0.107
ANP32BP1	646791	−0.62	0.0002	0.117
YBX3P1	440359	−0.42	0.0002	0.117
GZMB	3002	0.52	0.0002	0.117
SLC2A3P4	399495	0.34	0.0002	0.120
LOC731755	731755	−0.61	0.0002	0.122
H2BC2P	100288742	0.35	0.0002	0.124
YBX3	8531	−0.43	0.0002	0.131
CCL4	6351	0.47	0.0002	0.131
FOS	2353	0.50	0.0002	0.131
TNFAIP3	7128	0.37	0.0003	0.131
RGS2	5997	0.50	0.0003	0.131
TPST1	8460	0.34	0.0003	0.135
VTI1BP2	389246	−0.54	0.0003	0.136
IL18R1	8809	0.56	0.0003	0.136
RLIMP1	100533695	−0.48	0.0003	0.142
IGKJ5	28946	−0.50	0.0004	0.142
CAPRIN2	65981	−0.41	0.0004	0.142
ABCB10	23,456	−0.34	0.0004	0.142
REPS2	9185	0.33	0.0004	0.142
IL1R2	7850	0.52	0.0004	0.142
LOC102724528	102724528	−0.99	0.0005	0.142
C3orf86	102724231	0.33	0.0005	0.142
LOC102723340	102723340	0.35	0.0005	0.142
CDC16	8881	−0.33	0.0005	0.142
KAT2B	8850	−0.42	0.0005	0.148
NAP1L4P1	728589	−0.43	0.0006	0.151
METTL7A	25840	−0.33	0.0006	0.153
CRISPLD2	83716	0.40	0.0006	0.158
SIGLEC5	8778	0.35	0.0007	0.170
PTTG2	10744	0.35	0.0007	0.170
IRS2	8660	0.51	0.0008	0.177
HMGB2P1	729119	0.44	0.0008	0.178
IL18RAP	8807	0.64	0.0008	0.178
IGKV1–17	28937	−0.51	0.0008	0.178
PIP5K1B	8395	−0.34	0.0010	0.187
DNAJA4	55466	−0.35	0.0010	0.188
DDB1	1642	−0.36	0.0010	0.188
QRSL1P3	100422374	−0.50	0.0010	0.188
DCK	1633	−0.37	0.0010	0.188
LSM12P1	653122	−0.37	0.0011	0.188
KCNJ15	3772	0.40	0.0011	0.191
SAMSN1	64092	0.45	0.0012	0.191
SNORA2C	677815	0.43	0.0012	0.191
MGAM2	93432	0.48	0.0012	0.191

(Continued)

Table 2. Continued

SYMBOL	ENTREZ	log_2FC	*p*	Adj. *p*
LOC653631	653631	−0.68	0.0012	0.191
GPR141	353345	0.33	0.0014	0.191
NUTM2A-AS1	728190	−0.33	0.0014	0.191
MPZL3	196264	0.33	0.0014	0.191
CYP4F3	4051	0.32	0.0014	0.191
TPGS2	25941	−0.41	0.0015	0.191
F5	2153	0.34	0.0015	0.191
MGAM	8972	0.44	0.0015	0.191
SLC26A8	116369	0.41	0.0015	0.191
LOC100288365	100288365	−0.73	0.0015	0.191
RPIA	22934	−0.40	0.0015	0.194
NTAN1P2	100420307	−0.48	0.0016	0.196
LOC101927851	101927851	0.38	0.0017	0.206
SLC6A8	6535	−0.49	0.0018	0.208
LSMEM1	286006	0.33	0.0018	0.208
ALDH5A1	7915	−0.33	0.0019	0.213
SNORA75	654321	0.70	0.0020	0.214
MIR516A1	574498	0.54	0.0020	0.214
VNN3	55350	0.36	0.0021	0.216
NSRP1P1	100420557	−0.50	0.0021	0.217
LOC101927759	101927759	0.39	0.0021	0.217
C9orf78	51759	−0.46	0.0022	0.217
YBX1P2	646531	−0.38	0.0022	0.218
LOC107986698	107986698	−0.49	0.0023	0.222
STRADBP1	389599	−0.51	0.0023	0.222
LOC100420738	100420738	−0.62	0.0025	0.225
CD79A	973	−0.41	0.0025	0.225
TRAJ23	28732	−0.34	0.0026	0.225
IGHV5–51	28388	−0.34	0.0026	0.226
RPLP2P3	643949	−0.40	0.0026	0.226
MARCHF8	220972	−0.38	0.0026	0.226
FCAR	2204	0.33	0.0029	0.237
SESN3	143686	−0.37	0.0029	0.238
SLC11A1	6556	0.35	0.0030	0.238
CMAS	55907	−0.37	0.0030	0.239
GTF2H2C	728340	0.35	0.0030	0.239
IGHV1-2	28474	−0.56	0.0031	0.242
LOC101928044	101928044	−0.49	0.0032	0.243
PFKFB2	5208	0.35	0.0033	0.244
MIR223	407008	0.36	0.0033	0.245
RNF10	9921	−0.36	0.0034	0.245
CLEC4D	338339	0.49	0.0035	0.245
SCARNA10	692148	0.47	0.0035	0.246
SNORA1	677792	0.49	0.0035	0.247
GUCD1	83606	−0.35	0.0036	0.249

myometrium, and cervix correlated with those in the peripheral blood of women with term labor. The differential expression of 189 genes reported as modulated during labor in the chorioamniotic membranes were correlated to those based on maternal peripheral transcriptomic data ($r = 0.34$, $p < 0.0001$, direction of change agreement 63%) (Figure 3A). Moreover, the differential expression of 462 genes reported as modulated during labor in the myometrium also correlated to those derived from the maternal circulation ($r = 0.30$, $p < 0.0001$, direction of change agreement 58%) (Figure 3B). Finally, a modest but significant correlation was observed between transcriptomic labor-related changes in the peripheral blood and those reported in the cervix ($r = 0.2$, $p < 0.0001$, direction of change agreement 58%) (Figure 3C). Collectively, these results suggest that some of the labor-related transcriptomic changes in the gestational and reproductive tissues are mirrored in the maternal circulation. These findings may have implications for the development of non-invasive biomarkers to monitor the physiologic process of labor at term.

Correlation between gene expression changes in term labor and those occurring prior to preterm birth

The understanding of the mechanisms implicated in the physiologic process of labor at term may provide insights into the pathologic

Table 3. Biological processes enriched in the peripheral blood in term labor compared to term without labor

Biological process	Count	Size	Odds ratio	*q*	*p*	Significant in PPROM[a]
Leukocyte activation	17	1288	4.8	0.001	1.44E-06	1
Immune response	22	2187	3.9	0.001	2.68E-06	1
Cell activation	17	1447	4.2	0.001	7.00E-06	1
Immune system process	26	3156	3.3	0.001	9.69E-06	1
Immune effector process	15	1253	4.2	0.002	2.21E-05	1
Regulation of immune system process	17	1580	3.8	0.002	2.22E-05	1
Leukocyte mediated immunity	12	850	4.8	0.003	3.49E-05	1
Adaptive immune response	10	621	5.3	0.004	5.74E-05	1
Positive regulation of cellular carbohydrate metabolic process	4	62	20.8	0.004	6.67E-05	0
Inflammatory response	11	787	4.6	0.005	8.46E-05	0
Leukocyte activation involved in immune response	10	709	4.6	0.009	0.000171	1
Cell activation involved in immune response	10	713	4.6	0.009	0.000179	1
Positive regulation of carbohydrate metabolic process	4	84	15	0.01	0.000218	0
Lymphocyte activation	10	737	4.4	0.01	0.000234	0
Neutrophil activation	8	497	5.2	0.013	0.000339	1
Granulocyte activation	8	504	5.1	0.014	0.000372	1
Positive regulation of glucose metabolic process	3	42	22.8	0.014	0.000432	0
Myeloid leukocyte activation	9	656	4.4	0.014	0.000457	1
T cell cytokine production	3	43	22.2	0.014	0.000464	0
Lymphocyte activation involved in immune response	5	185	8.4	0.014	0.000484	0
T-helper 1 type immune response	3	44	21.7	0.014	0.000496	1
Regulation of immune response	11	971	3.7	0.014	0.000522	1
T cell activation involved in immune response	4	108	11.6	0.014	0.000568	0
B cell activation	6	305	6.2	0.017	0.000701	0
Regulation of glucose metabolic process	4	120	10.4	0.02	0.000843	0
Defense response	15	1752	2.9	0.02	0.000907	0
Lymphocyte mediated immunity	6	328	5.7	0.022	0.001022	1
Positive regulation of immune system process	11	1058	3.4	0.022	0.001061	1
Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	6	331	5.7	0.022	0.001071	1
Response to reactive oxygen species	5	225	6.9	0.023	0.001167	0
Regulation of mitotic sister chromatid separation	3	61	15.3	0.024	0.001293	0
Mitotic sister chromatid separation	3	64	14.6	0.027	0.001485	0
Neutrophil degranulation	7	483	4.6	0.027	0.001499	1
Neutrophil activation involved in immune response	7	486	4.5	0.027	0.001553	1
Regulation of chromosome separation	3	66	14.1	0.027	0.001623	0
Negative regulation of protein serine/threonine kinase activity	4	144	8.6	0.027	0.001656	0
Neutrophil mediated immunity	7	497	4.4	0.028	0.001764	1
Regulation of cellular carbohydrate metabolic process	4	147	8.4	0.028	0.001786	0
T cell differentiation involved in immune response	3	70	13.3	0.028	0.001922	0
Positive regulation of small molecule metabolic process	4	150	8.2	0.028	0.001923	0
Regulation of mitotic sister chromatid segregation	3	72	12.9	0.03	0.002084	0
Negative regulation of kinase activity	5	264	5.8	0.033	0.002353	0
Leukocyte degranulation	7	533	4.1	0.036	0.002614	1
Regulation of adaptive immune response	4	166	7.4	0.037	0.002777	0
Myeloid cell activation involved in immune response	7	544	4	0.038	0.002929	1
Myeloid leukocyte mediated immunity	7	551	4	0.039	0.003144	1
Response to bacterium	8	704	3.6	0.039	0.00316	0
Regulation of B cell activation	4	173	7.1	0.039	0.003221	0
Regulation of sister chromatid segregation	3	84	11	0.039	0.003231	0
Negative regulation of transferase activity	5	291	5.3	0.041	0.003574	0
Cellular carbohydrate metabolic process	5	292	5.3	0.041	0.003627	0
Positive regulation of immune response	8	721	3.5	0.041	0.003653	1
Monosaccharide metabolic process	5	295	5.2	0.042	0.003788	0
Regulation of response to external stimulus	10	1081	2.9	0.046	0.004264	0

(Continued)

Table 3. Continued

Biological process	Count	Size	Odds ratio	*q*	*p*	Significant in PPROM[a]
Regulation of response to stimulus	25	4308	2.1	0.046	0.004322	0
Chromosome separation	3	94	9.7	0.046	0.004434	0
Negative regulation of inflammatory response	4	190	6.4	0.046	0.004497	0
Defense response to bacterium	5	310	5	0.046	0.004671	0
Leukocyte proliferation	5	310	5	0.046	0.004671	0
B cell proliferation	3	97	9.4	0.047	0.004841	0
Regulation of carbohydrate biosynthetic process	3	98	9.3	0.048	0.004981	0
Innate immune response	9	943	3	0.052	0.005515	0
T cell mediated immunity	3	103	8.9	0.053	0.00572	1
Cytokine production involved in immune response	3	104	8.8	0.053	0.005875	0
Immune response-regulating cell surface receptor signaling pathway	6	468	4	0.053	0.005976	0
Regulation of chromosome segregation	3	105	8.7	0.053	0.006033	0
Immune response-regulating signaling pathway	6	471	3.9	0.053	0.006161	0
Regulation of carbohydrate metabolic process	4	208	5.9	0.053	0.00618	0
Negative regulation of phosphorylation	6	473	3.9	0.053	0.006286	0
Regulated exocytosis	8	793	3.2	0.054	0.006439	0
Glucose metabolic process	4	213	5.7	0.055	0.006713	0
Defense response to other organism	10	1154	2.7	0.055	0.006716	0
Interferon-gamma production	3	113	8.1	0.059	0.007387	0
Fc receptor signaling pathway	4	220	5.5	0.06	0.007509	0
Carboxylic acid transport	5	349	4.4	0.06	0.007639	0
Organic acid transport	5	352	4.3	0.061	0.007911	0
Response to stress	23	4047	2	0.066	0.008709	0
Response to cytokine	10	1203	2.6	0.066	0.008903	0
Fc-gamma receptor signaling pathway	3	121	7.5	0.066	0.008907	0
Regulation of leukocyte chemotaxis	3	124	7.3	0.07	0.009521	0
Secretion by cell	11	1420	2.5	0.073	0.010082	0
Negative regulation of protein kinase activity	4	242	5	0.075	0.010409	0
Positive regulation of response to biotic stimulus	4	248	4.9	0.079	0.01131	0
Leukocyte differentiation	6	537	3.4	0.079	0.01136	0
Negative regulation of intracellular signal transduction	6	543	3.4	0.083	0.011952	0
Hexose metabolic process	4	253	4.8	0.083	0.012098	0
Response to hydrogen peroxide	3	138	6.6	0.085	0.012708	0
Monocarboxylic acid catabolic process	3	138	6.6	0.085	0.012708	0
Export from cell	11	1471	2.4	0.085	0.012923	0
Negative regulation of defense response	4	261	4.6	0.088	0.013431	0
Regulation of response to biotic stimulus	5	404	3.8	0.089	0.013767	0
Exocytosis	8	910	2.7	0.09	0.014096	0
Regulation of defense response	7	735	2.9	0.091	0.014416	0
Cellular response to cytokine stimulus	9	1113	2.5	0.095	0.015419	0
Carbohydrate transport	3	149	6.1	0.095	0.015592	0
Carboxylic acid transmembrane transport	3	149	6.1	0.095	0.015592	0
Regulation of leukocyte activation	6	578	3.2	0.095	0.015842	0
Organic acid transmembrane transport	3	150	6	0.095	0.015871	0
Negative regulation of cellular protein metabolic process	9	1121	2.5	0.095	0.01609	0
Regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	3	151	6	0.095	0.016153	0
Response to other organism	11	1524	2.3	0.095	0.016491	0
Response to external biotic stimulus	11	1526	2.3	0.095	0.016639	0
Negative regulation of response to external stimulus	5	424	3.6	0.095	0.016641	0
Production of molecular mediator of immune response	4	279	4.3	0.095	0.016764	0
Lymphocyte proliferation	4	280	4.3	0.095	0.016963	0
Positive regulation of response to stimulus	15	2392	2	0.095	0.01711	0
Negative regulation of phosphate metabolic process	6	591	3.1	0.095	0.017489	0
Mononuclear cell proliferation	4	283	4.3	0.095	0.017569	0

(Continued)

Table 3. Continued

Biological process	Count	Size	Odds ratio	*q*	*p*	Significant in PPROM[a]
Negative regulation of phosphorus metabolic process	6	592	3.1	0.095	0.01762	0
Import across plasma membrane	3	157	5.7	0.095	0.017903	0
Negative regulation of protein phosphorylation	5	433	3.5	0.095	0.018056	0
Immune response-activating cell surface receptor signaling pathway	5	436	3.5	0.096	0.018545	0
Immune response-activating signal transduction	5	436	3.5	0.096	0.018545	0
Secretion	11	1557	2.2	0.097	0.019064	0
Mitotic sister chromatid segregation	3	161	5.6	0.097	0.019126	0
Response to biotic stimulus	11	1558	2.2	0.097	0.019146	0
Cellular response to reactive oxygen species	3	162	5.6	0.097	0.019439	0
Cell surface receptor signaling pathway	18	3122	1.9	0.097	0.019617	0
Response to oxidative stress	5	443	3.4	0.097	0.019719	0
Regulation of mitotic nuclear division	3	163	5.5	0.097	0.019755	0
Cytokine-mediated signaling pathway	7	787	2.7	0.098	0.020221	0

[a]0, not significant; 1, significant.

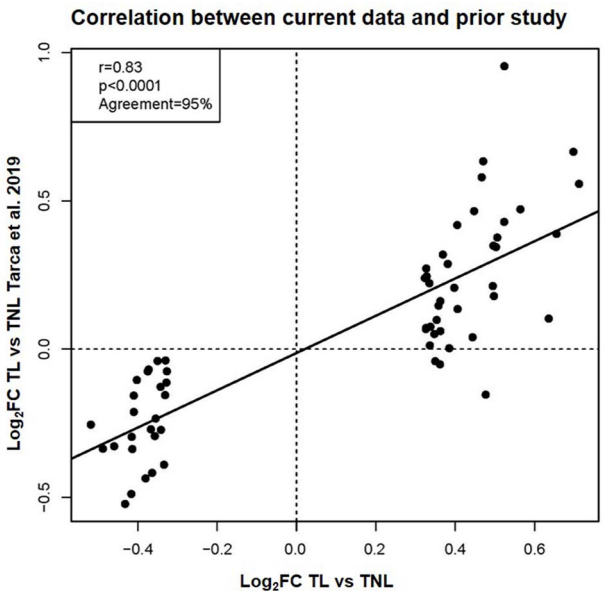

Figure 2. Correlation between labor-specific gene expression in the peripheral blood from the current study and a previous report. Scatterplot showing the correlation between the $\log_2$FC reported in this study and that from a previous report [56]. The Pearson correlation coefficient, *p*-value, and percentage of agreement on direction of gene expression FC are shown.

processes that lead to preterm birth. During normal labor, the activation of the chorioamniotic membranes culminates with rupture of these tissues to allow delivery of the fetus [7]. The premature occurrence of this process is termed PPROM, which accounts for one-third of preterm birth cases [76]. Thus, we evaluated the overlap between the transcriptomic changes observed in the maternal circulation during labor at term and those observed in the peripheral blood of asymptomatic women, prior to the diagnosis of PPROM, who ultimately delivered preterm. This analysis was performed with samples collected at 17–22 weeks and at 27–33 weeks from cases and gestational age-matched controls. The transcriptomic changes of 402 RNAs preceding the diagnosis of PPROM (hereafter referred to as "PPROM signature") were significantly correlated with those derived from the comparison between TL and TNL groups herein ($r = 0.71$, $p < 0.0001$, 86% agreement in direction of change) (Figure 4). Of note, among the 51 biological processes enriched in the PPROM signature [58], 26 were also significant in the spontaneous term parturition-derived signature (Table 3). Yet, 25 of these processes were not significantly enriched in labor at term, highlighting the fact that the sub-clinical processes leading to preterm birth are not merely the premature initiation of normal labor, but instead represent the occurrence of distinct pathological events culminating in the activation of one or more of the components of the common pathway of labor [7]. In addition, four of the five Reactome pathways we found herein to be associated with term parturition (innate immune system, neutrophil degranulation, signaling by interleukins, cytokine signaling in immune system, $q < 0.05$, and enrichment odds ratios >2.5 for all) were previously reported as enriched in the PPROM signature [58]. Taken together, these results suggest that there is a substantial overlap between the transcriptomic changes taking place in the circulation of women who ultimately undergo spontaneous preterm birth and of those observed during spontaneous labor at term.

To fully appreciate the differential gene expression changes between the normal process of labor at term and those occurring prior to preterm birth, we performed a direct assessment of the transcriptomic changes that are specific to term labor but were not observed in asymptomatic women, prior to the diagnosis of PPROM, who ultimately delivered preterm using interaction tests (Supplementary Table S1). The majority of genes with a significant interaction test were dysregulated in term labor but not prior to preterm birth, including *DDIT4* (DNA damage inducible transcript 4), *DUSP1* (dual specificity phosphatase 1), *TPST1* (tyrosylprotein sulfotransferase 1), *FOS* (Fos proto-oncogene, AP-1 transcription factor subunit), *TNFAIP3* (TNF alpha induced protein 3), *CCL4* (C-C motif chemokine ligand 4), and *GZMB* (granzyme B) (Supplementary Table S1). Pathway analysis revealed that these genes are involved in "cytokine signaling in immune system" and "innate immune system" as well as in multiple immune processes (enrichment $q < 0.1$, Supplementary Table S2). By contrast, transcripts that were decreased in term labor but not prior to preterm birth included *RPL36P12* (ribosomal protein L36 pseudogene 12), *HSP90AB3P* (heat shock protein 90 alpha family class B member

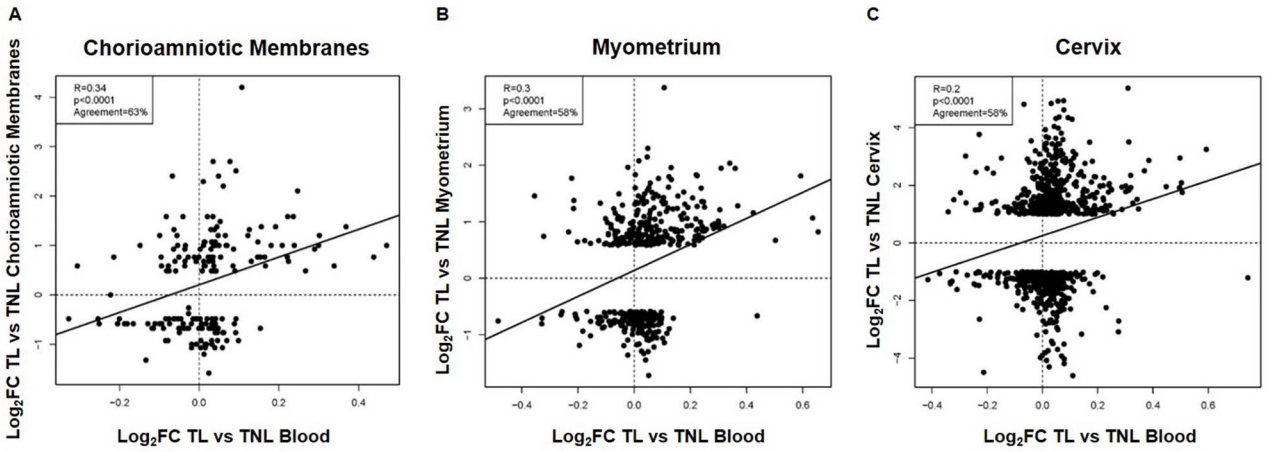

Figure 3. Correlation between labor-specific gene expression in the peripheral blood and that in the chorioamniotic membranes, myometrium, or cervix. Scatterplots showing the correlation between gene expression (log_2FC) reported in this study and that in (A) the chorioamniotic membranes [59], (B) the myometrium [60], or (C) the cervix [61]. The Pearson correlation coefficients, *p*-values, and percentages of agreement on direction of gene expression FC are shown for each comparison.

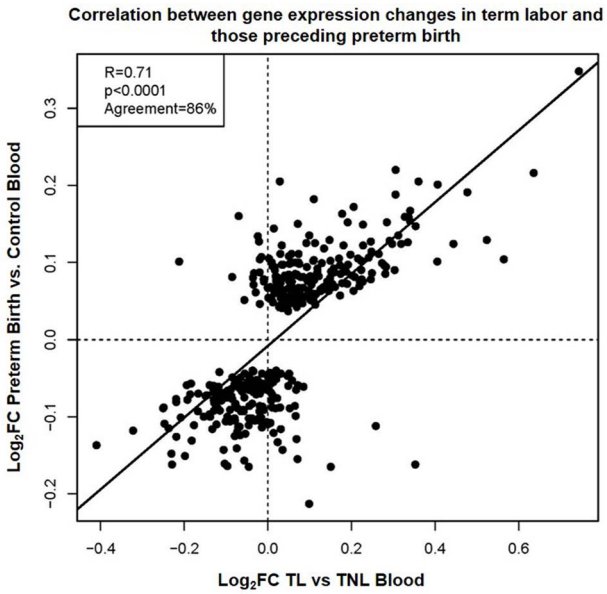

Figure 4. Correlation between gene expression changes in term labor and those preceding preterm birth. Scatterplot showing the correlation between gene expression (log_2FC) derived from the comparison between women at term with and without labor and those from a previous report of asymptomatic women who subsequently underwent preterm birth associated with PPROM [58]. The Pearson correlation coefficient, *p*-value, and percentage of agreement on direction of gene expression FC are shown.

3, pseudogene), *CSNK2A3* (casein kinase 2 alpha 3), *VTI1BP1* (vesicle transport through interaction with t-SNAREs 1B pseudogene 1), and *CD180* (CD180 molecule), among others (Supplementary Table S1). A notable exception was *KLRD1* (killer cell lectin like receptor D1), which displayed a slight increase ($log_2FC = 0.35$) in term labor but a slight decrease ($log_2FC = -0.26$) prior to preterm birth (Supplementary Table S1). These data highlight the differences between the transcriptional activity that accompanies the physiologic process of parturition at term and that preceding preterm birth.

Finally, we determined the correlation between gene expression changes preceding preterm birth in the maternal blood and those previously reported in tissues collected at term. Significant but modest correlations were also noted between the whole blood transcriptomic changes preceding the diagnosis of PPROM in women who ultimately delivered preterm [58] and those reported in the chorioamniotic membranes ($r = 0.23$, $p = 0.002$), myometrium ($r = 0.19$, $p < 0.001$), and cervix ($r = 0.12$, $p < 0.001$) from women with labor at term (Supplementary Figure S1). Of note, the changes derived from samples collected from women who ultimately delivered preterm are not necessarily specific to preterm birth since they are also observed in women with labor at term [as shown in Figure 4 and in the interaction analyses presented herein (Supplementary Tables S1 and S2)]. Yet, these results indicate that the whole blood, labor-derived transcriptomic signature reported herein has potential use as a "liquid biopsy" to identify women who are at risk for preterm birth.

Discussion

Herein, we utilized a large, previously generated, longitudinal dataset to assess labor-specific changes in the peripheral blood transcriptome of women at term. We identified a set of genes that were differentially expressed with labor and enriched for immunological processes. Further, we have shown a strong correlation between the reported expression changes and those reported in a prior study, providing an in silico validation of our findings. Notably, we also identified significant correlations between labor-specific transcriptomic changes in the maternal circulation and DEGs determined in the chorioamniotic membranes, myometrium, and cervix of women at term, demonstrating that the tissue-specific signatures are mirrored with those in the peripheral blood. Finally, we demonstrated a significant degree of overlap between the peripheral blood transcriptomic changes derived from women with labor at term and those obtained from asymptomatic women who subsequently were diagnosed with PPROM and delivered preterm. Therefore, the normal process of labor at term is characterized by an immunological expression signature, which is partially shared with that observed in women who ultimately underwent spontaneous preterm birth, and may potentially serve as a useful tool for assessing labor status and for potentially identifying women at risk for preterm birth.

Labor is a well-characterized state of system-wide physiologic inflammation, including not only the gestational and reproductive tissues but also extending to the maternal circulation. This systemic response includes the dynamic and intricate modulation of peripheral immune cells and their activation status [16, 77–80] as well as fluctuations in the soluble fraction [81–83], which includes cell-free fetal DNA [84–86]. Consistently, we have previously utilized maternal whole blood transcriptomics to identify immune signatures that are modified both throughout gestation and with labor [56, 62]. Notably, gene signatures corresponding to T cells, B cells, and CD71+ erythroid cells, among others, were found to follow unique expression patterns during normal pregnancy, with the T-cell and erythroid signatures increasing in late gestation [62]. In addition, a pilot study of women with or without labor at term revealed that a T-cell signature was overexpressed with labor [56], further emphasizing that this inflammatory process is reflected in the maternal peripheral blood transcriptome. Herein, we utilized exon-level resolution gene expression data to demonstrate labor-specific differences in the maternal blood transcriptome that are characterized by enrichment of immune-related processes, which is in line with previous findings. Importantly, our current data were strongly correlated with our prior pilot study [56], demonstrating agreement in terms of labor-specific gene expression changes. Together, these data may be useful for the development of tools to detect and monitor labor.

In the current study, we observed significant correlations between the labor-specific transcriptomic changes taking place in the maternal circulation and those previously reported in the chorioamniotic membranes [59], myometrium [60], and cervix [61]. Activation of the chorioamniotic membranes/decidua is a component of the common pathway of parturition [2–7, 9, 87], which is characterized by the influx of maternal immune cells that promote the physiologic inflammation required for labor [15, 39, 42, 47, 88]. A prior whole tissue microarray analysis of the chorioamniotic membranes revealed upregulated expression of genes that were enriched for processes such as "taxis" and "chemokine activity, cytokine activity or cytokine binding," demonstrating an acute inflammatory signature specific to the process of labor [59]. These observations are consistent with a more recent microarray-based investigation of choriodecidual leukocytes from women with labor at term, which revealed that infiltrating immune cells displayed upregulated expression of gene networks associated with cell migration and chemotaxis, among others [89]. Given that the process of labor has been proposed to involve the recruitment of circulating immune cells to the gestational and reproductive tissues [15, 39, 42, 47, 88, 90], the above evidence provides a plausible explanation for the observed correlation between the chorioamniotic membrane transcriptome and that of the maternal peripheral blood reported herein.

The uterus, which includes the myometrium, is a contractile organ and key participant in the process of labor [2, 5–7]. Given its important functional role in delivery of the fetus, multiple investigations have explored the changes in gene expression that occur in this tissue using microarrays [60, 91–94], RNA-seq [95, 96], and epigenetic surveys [97]. Similar to the transcriptomic signatures observed in gestational tissues (e.g., the chorioamniotic membranes), enrichment of processes related to immune and inflammatory responses have been reported in the myometrium, such as cytokine and chemokine signaling pathways [60, 94, 95]. Multiple meta-analyses have further established the inflammatory nature of labor-specific changes in the myometrium [94, 98, 99]. Indeed, the latter study demonstrated high-confidence overlap of myometrial transcriptomic changes identified in multiple study populations, providing a labor-specific signature that included known components, such as cytokines as well as novel transcripts related to apoptosis and cell proliferation/differentiation [99]. Consistently, in this study, we identified a significant correlation between previously reported labor-specific gene expression changes in the myometrium and those found in the maternal blood herein, which included immune- and inflammation-related processes. Thus, given the impracticality of sampling the myometrium during pregnancy, the maternal peripheral blood transcriptome may represent a potential "liquid biopsy" to evaluate the changes occurring in the myometrium and other critical gestational and reproductive tissues.

The uterine cervix is the gatekeeper of pregnancy [100] and plays a key role in the maintenance of the conceptus in utero. The cervix evolves from a rigid and closed to a soft and dilated structure through the process of cervical remodeling [100–109]. Such a process occurs throughout pregnancy and culminates in cervical dilation during labor, thereby permitting the delivery of the neonate [100–107, 109]. During labor, multiple cellular and molecular mechanisms take place in the cervix [12, 14, 26, 30, 33, 34, 100, 104, 110], which include inflammatory processes as indicated by the upregulation of immune- and inflammation-related transcripts in this tissue [61, 111]. Notably, given that the transcripts upregulated in the ripened cervices of women at term without labor do not involve inflammatory processes [112], it is tempting to suggest that the process of labor itself shifts the cervical transcriptome from a non-inflammatory to an inflammatory status. This concept is consistent with the observed correlation between the labor-specific transcriptomic changes taking place in the cervix and those detected in the maternal circulation, as reported herein. Therefore, these findings highlight the potential use of transcriptomic signatures monitored in the peripheral blood as biomarkers to evaluate changes occurring in reproductive tissues such as the cervix.

When studying the mechanisms of parturition, it is tempting to hypothesize that preterm labor is merely the premature initiation of normal labor and that these two processes are otherwise similar. However, a growing body of evidence has demonstrated that preterm labor is a syndrome of multiple etiologies involving pathological processes that culminate in the activation of one or more components of the common pathway of labor [4, 6, 7]. In the current study, we found that 86% of the 402 RNAs dysregulated prior to diagnosis with PPROM in asymptomatic women also change in the same direction as it occurs in physiologic term labor. This result is even more striking when considering the fact that the PPROM signature was derived from two diverse cohorts (Detroit, MI, USA, and Calgary, Alberta, Canada), while the changes with term labor reported herein were derived from the same Detroit cohort. Moreover, one-half of the biological processes associated with gene expression changes in the peripheral blood of women with spontaneous labor at term were also significant in the PPROM signature. Differences in the enrichment analysis results for the PPROM signature (402 genes derived from two cohorts including the one in the current study) and the term-labor signature (103 genes derived from this study cohort) are driven by differences between phenotypes, unavoidable cohort differences, and the higher power for enrichment of the larger PPROM signature. Indeed, when interaction tests were used to assess if differences with term labor were different from those detected prior to diagnosis with PPROM using only data from the cohort in this study, we found term labor-specific changes but almost no PPROM-specific changes.

Our findings are consistent with a previous microarray study showing different tissue-specific transcriptomic profiles in women who delivered term or preterm in the presence or absence of labor

[113]. The authors of the study suggested that pregnancy is maintained by the downregulation of chemokines at the maternal-fetal interface and that withdrawal of this downregulation leads to term birth, whereas the pathological activation of multiple inflammation-related pathways culminates in some cases of preterm birth [113], of which the latter concept has also been demonstrated in mice [114, 115]. In line with this concept, we recently utilized scRNA-seq to decipher the cellular landscape of human parturition in the chorioamniotic membranes and placental tissues and demonstrated that parturition-associated single-cell signatures can be monitored in the peripheral blood [116]. Notably, while specific single-cell signatures enriched in the peripheral blood of women with term labor were also observed in women with preterm labor (e.g., T-cell activation), other signatures were only enriched in either preterm labor (e.g., monocytes and macrophages) or term labor (NK cells), further emphasizing the differences between these two processes.

Collectively, these results indicate that labor is characterized by a transcriptomic signature in the maternal peripheral blood, which correlates with term labor-specific changes taking place in the chorioamniotic membranes, myometrium, and cervix. Importantly, the transcriptomic activity of several term labor-specific processes and pathways was also enriched in women who ultimately were diagnosed with PPROM and delivered preterm, demonstrating further the potential utility of these signatures as non-invasive disease biomarkers. Yet, further investigation is required to clinically validate such an approach.

Supplementary material

Supplementary material is available at *BIOLRE* online.

Data availability

The transcriptomic dataset utilized in this study was previously deposited by the authors in the Gene Expression Omnibus superseries GSE149440.

Authors' contributions

A.L.T., R.R., and N.G-L. conceived and designed the study. N.G-L., A.L.T., R.R., J.G., D.M., and M.F-J. wrote the manuscript. A.L.T., G.B., B.D., C.G., and R.P-R. analyzed the data. K.M., E.J., S.S.H., and T.C. provided intellectual input. All authors approved the final version of the manuscript.

Conflict of interest

The authors have declared that no conflict of interest exists.

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