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Evidence for shared molecular pathways of dysregulated decidualization in preeclampsia and endometrial disorders revealed by microarray data integration

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ABSTRACT: Microarray data of chorionic villous samples (CVSs) obtained from women of ~11.5 gestational weeks who developed preeclampsia with severe features (sPE; PE-CVS) revealed a molecular signature of impaired endometrial maturation (decidualization) before and during early pregnancy. Because endometrial disorders are also associated with aberrant decidualization, we asked whether they share molecular features with sPE. We employed microarray data integration to compare the molecular pathologies of PE-CVS and endometrial disorders, as well as decidua obtained postpartum from women with sPE. Eight public databases were reanalyzed with R software to determine differentially expressed genes (DEGs) in pathologic tissues relative to normal controls. DEGs were then compared to explore overlap. Shared DEGs were examined for enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Principal component and network analyses were subsequently applied to selected DEGs. There was significant overlap of DEGs changing in the same direction for PE-CVS and endometrial disorders, suggesting common molecular pathways. Shared DEGs were enriched for cytokine-cytokine receptor interaction. Genes in this pathway revealed expression patterns forming 2 distinct clusters, one for normal and the other pathologic endometrium. The most affected hub genes were related to decidualization and NK cell function. Few DEGs were shared by PE-CVS, and PE decidua obtained postpartum. sPE may be part of a biologic continuum of "endometrial spectrum disorders."—Rabaglino, M. B., Conrad, K. P. Evidence for shared molecular pathways of dysregulated decidualization in preeclampsia and endometrial disorders revealed by microarray data integration. FASEB J. 33, 11682–11695 (2019). www.fasebj.org

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Preeclampsia (PE), a hypertensive syndrome that afflicts 3–5% of all pregnant women, is a leading cause of maternal and perinatal morbidity and mortality (1–3). Disease pathogenesis involves placental ischemia and ischemia-reperfusion injury, which contributes to the release of factors into the maternal circulation that damage endothelium producing the clinical manifestations of hypertension and proteinuria, as well as maternal organ hypoperfusion and injury (4). This placental pathology is widely believed to stem from insufficient extravillous trophoblast (EVT) invasion of the uterine decidua and inner myometrium during early gestation that, in turn, results in deficient physiologic transformation of the spiral arteries (5, 6). However, mechanisms underlying dysfunctional EVT

ABBREVIATIONS: BMP7, bone morphogenetic protein 7; BMPR1A, bone morphogenetic protein receptor type 1A; BMPR1B, bone morphogenetic protein receptor type 1B; CVS, chorionic villous sample; DEC, confluent decidualized endometrium; DEG, differentially expressed gene; dNK, decidual NK; ESC, endometrial stromal cell; EVT, extravillous trophoblast; FC, fertile control; IF, recurrent implantation failure; IL-10RA, IL-10 receptor subunit α; IL-10RB, IL-10 receptor subunit β; KEGG, Kyoto Encyclopedia of Genes and Genomes; LSE, late secretory endometrium; non-DEC, nondecidualized endometrium; NP-BP, normal pregnancy-basal plate; NP-CVS, normal pregnancy-chorionic villous sampling; NP-DB, normal pregnancy-decidua basalis; NP-DEC, normal pregnancy-decidua; OSIS, endometriosis; PCA, principal component analysis; PE, preeclampsia; PE-BP, preeclampsia-basal plate; PE-CVS, preeclampsia-chorionic villous sampling; PE-DB, preeclampsia-decidua basalis; PE-DEC, preeclampsia-decidua; PrE, proliferative endometrium; PRL, prolactin; PRLR, prolactin receptor; RM, recurrent miscarriage; sPE, preeclampsia with severe features; TGFBR1, TGF-β receptor 1; TGFBR2, TGF-β receptor 2

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have not been fully elucidated (7). In light of the intimate intermingling of decidual, myometrial, immune, and invading EVT cells, a logical deduction is that impaired EVT invasion could arise from inadequate or defective decidual remodeling of the uterus, which both antedates and coincides with EVT invasion (8). Indeed, endometrial decidualization is initiated during the secretory phase of the menstrual cycle (predecidualization) (9) and by itself is thought to initiate spiral artery remodeling before trophoblast invasion (10, 11). Thus, suboptimal placentation in PE may have endometrial antecedents even before conception.

Previously, we used a bioinformatic approach to reveal differentially expressed genes (DEGs) in chorionic villous samples (CVSs) obtained from women at ~11.5 wk of gestation who developed severe PE (sPE) (PE-CVSs) when compared to CVSs from women who experienced normal pregnancy (NP-CVSs). A large number (or 40%) of these DEGs were related to various stages of endometrial decidualization, and of these, 73% changed in the opposite direction to DEGs associated with the normal biologic process of (pre)decidualization (12). These results supported the idea that insufficient or defective maturation of endometrium during the secretory phase and early pregnancy may precede the development of sPE. As a logical extension, we hypothesized that the transcriptomics of other endometrial disorders associated with aberrant decidualization may significantly overlap with PE-CVSs suggesting shared molecular etiologies. Therefore, the objective of this study was to employ data integration, applying 2 approaches that have been reported in the literature—meta-analysis and data merging (13, 14)—in the analysis of microarray data from 8 published studies, in order to compare the transcriptomics of PE-CVSs with normal endometrial maturation and the endometrial disorders—recurrent implantation failure (IF), recurrent miscarriage (RM), and endometriosis (OSIS). A second aim was to reanalyze the microarray data of endometrial stromal cells (ESCs) isolated from midsecretory biopsies of women who previously experienced sPE or normal pregnancy that were subsequently placed into culture and decidualized *in vitro* (15), in the context of the other aforementioned microarray data sets. A third goal was to compare the transcriptomes of PE-CVSs with decidua procured after delivery from women who experienced sPE. Finally, the cytokine-cytokine receptor pathway, which proved to be prominent in the various samples containing normal control and pathologic endometrium, was investigated using principal component analysis (PCA) and Network Analysis.

MATERIALS AND METHODS

Microarray data sets

Microarray data sets were downloaded from public functional genomic data repositories: Gene Expression Omnibus from the National Center for Biotechnology Information (NCBI; *http://www.ncbi.nlm.nih.gov/geo/*) or the European Bioinformatics Institute from the European Molecular Biology Laboratory (EMBL-EBI; *http://www.ebi.ac.uk/arrayexpress/*). The selected microarray data sets, patient cohorts, and tissues used in this study are presented below by accession number. For our analysis, the number of samples per cohort corresponded to the number of samples collected in the original study that generated each microarray data set except where indicated.

Microarray data sets of transcriptomics in women who developed sPE

- GSE12767 (16): CVSs were obtained from women who developed sPE all >34 wk gestation (PE-CVSs; $n = 4$) or experienced normal pregnancies (NP-CVSs; $n = 8$). Samples were hybridized to the Affymetrix Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific, Waltham, MA, USA). A secondary analysis of this data set and the list of DEGs were previously reported by Rabaglino *et al.* (12).
- GSE91077 (15): ESCs isolated from midsecretory endometrial biopsies of women with previous early onset sPE [PE–confluent decidualized endometrium (DEC); $n = 5$] or normal pregnancy (NP-DEC; $n = 7$) were placed into culture and decidualized *in vitro* with medroxyprogesterone acetate and adenosine 3′,5′-cyclic monophosphate for 5 d. The endometrial biopsies were obtained from women with regular menstrual cycles who had been pregnant between 1 and 5 yr earlier with no underlying endometrial pathology. Samples were hybridized to the Agilent-026652 Whole Human Genome Microarray v.2 (*https://gemma.msl.ubc.ca/arrays/showArrayDesign.html?id=603*).

Microarray data sets of (pre)decidual transcriptomics in healthy women

- GSE4888 (17): Twenty-seven endometrial biopsies were obtained from women with normal ovulatory cycles and hybridized to the Affymetrix Human Genome U133 Plus 2.0 Array. The samples from proliferative endometrium (PrE; $n = 4$) and late secretory endometrium (LSE; $n = 6$) were included in the present analysis.
- E-MTAB-680 (18): Twenty-four endometrial samples were obtained at ~59 d of gestation and hybridized to the Affymetrix Human Genome U133 Plus 2.0 Array. The samples used in the current study were classified as intrauterine confluent-decidualization (DEC; $n = 7$) or without decidualization changes [nondecidualized endometrium (non-DEC); $n = 5$] by Duncan *et al.* (18) as determined by morphology on hematoxylin and eosin stained sections.

Microarray data sets of transcriptomics in different endometrial disorders

- GSE26787 (19): Fifteen endometrial biopsies were procured in the midsecretory phase of a nonconceptive cycle from women with a history of recurrent implantation failure following transfer of at least 10 high-quality embryos after *in vitro* fertilization/intracytoplasmic sperm injection ($n = 5$). Additional midsecretory endometrial biopsies were obtained from women with at least 3 unexplained RMs ($n = 5$), and women who gave birth after 1 or 2 attempts of intrauterine insemination or *in vitro* fertilization/intracytoplasmic sperm injection related to male-factor infertility as fertile controls (FCs; $n = 5$). Samples were hybridized to the Affymetrix Human Genome U133 Plus 2.0 Array.

- Dyson (20): The corresponding author generously provided this microarray data set upon request. This data set was based on an *in vitro* study in which ESCs derived from normal endometrial tissues (n = 5) and ovarian OSIS (n = 6) were placed into culture and then decidualized *in vitro* using medroxyprogesterone acetate, E_2, and 8-bromoadenosine 3′,5′-cyclic monophosphate over a 6-d period. Samples were hybridized to the Illumina HT-12v4 expression beadchips (Illumina, San Diego, CA, USA).

Microarray data sets of decidual transcriptomics at the end of gestation in women who developed sPE

- GSE94643 (15): Decidua basalis was microdissected from placental bed biopsies obtained immediately after delivery in women with early onset sPE (PE-DB; n = 4) and normal pregnancies (NP-DB; n = 4). Samples were hybridized to Affymetrix Human Gene 2.0 ST Array.
- GSE14722 (21): Basal plate decidua dissected from delivered placentas obtained from women affected by sPE (PE-BP; n = 4) and women who experienced normal pregnancies (NP-BP; n = 4). Samples were hybridized to Affymetrix Human Genome U133A Array. Only samples procured after 34 wk of gestation were considered for this analysis (late-onset PE) to match with PE-CVSs.

Data analysis

A flowchart of the bioinformatic analyses employed in this work is shown in **Fig. 1**.

Data input

The R software platform (*http://www.r-project.org*) was employed for all the analyses. The raw data obtained from samples hybridized to the Affymetrix, Agilent, or Illumina platform were processed with the gcRMA (22), limma (23), and Lumi packages (24), respectively. These packages were employed to import the raw data into R, perform background correction, and then transform and normalize the data using the quantile normalization method. Next, rows of each data set were collapsed in order to retain the microarray probe with the highest mean value (maximal mean) from the group of the genes with the same Entrez ID. The function applied was the "collapseRows" from the Weighted Gene Correlation Network Analysis (WGCNA) package (25). The purpose of row collapsing is to obtain unique identifiers for each gene in the working data set.

Statistical analysis

For each study, the DEGs between groups, *i.e.*, LSE *vs.* PrE; DEC *vs.* non-DEC; IF *vs.* FC; RM *vs.* FC; OSIS *vs.* ESCs; PE-DEC *vs.* NP-DEC; PE-CVSs *vs.* NP-CVSs; PE-DB *vs.* NP-DB; and PE-BP *vs.* NP-BP were determined with moderated t-statistics, a variation of the classic Student's t test, developed by Smyth (26). This algorithm has robust behavior even for small numbers of arrays. Also, compared to classic tests, the method results in more stable inference when the number of arrays is limited. Significant DEGs were defined as those with a value of $P < 0.05$ and a fold change >2.

Data comparison

The resulting DEGs were arranged in a list of unique Entrez ID in order to allow comparison between the different data sets. For example, if 5 Entrez ID represented a probe, they were listed as 5 genes. Duplicates were then removed to obtain the final lists of up- or down-regulated DEGs for each comparison. The up- and down-regulated DEGs in PE-CVSs *vs.* NP-CVSs were compared to DEGs in: LSE *vs.* PrE; DEC *vs.* non-DEC; IF and RM *vs.* FC; OSIS *vs.* ESCs; PE-DB *vs.* NP-DB; and PE-BP *vs.* NP-BP. The same comparisons were made for the up- and down-regulated DEGs in PE-DEC *vs.* NP-DEC. We also compared PE-CVS vs NP-CVS with PE-DEC vs NP-DEC. Statistical comparisons were made by the test of independence (Pearson's χ^2 test) to determine the relatedness of up- and down-regulated DEGs observed in PE-CVSs *vs.* NP-CVSs or PE-DEC *vs.* NP-DEC with up- and down-regulated DEGs in LSE, DEC, IF, RM, and OSIS *vs.* their respective controls groups.

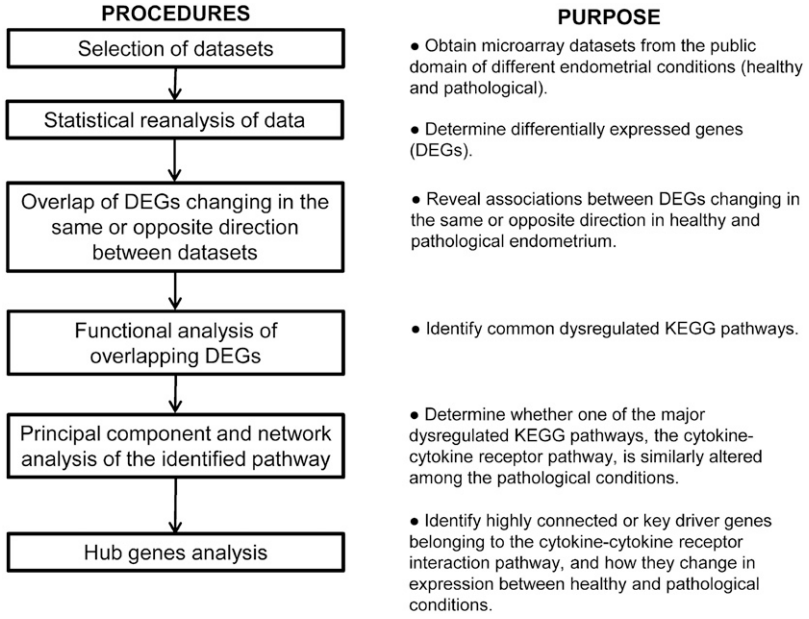

Figure 1. Overview of the methodology. The flowchart presents an overview of the major procedures employed in this study, and their purpose.

Functional analysis

In order to determine the gene pathways involved in the normal decidualization process that may be impaired in sPE and endometrial disorders, overlapping DEGs were interrogated for significant KEGG pathways with Database for Annotation, Visualization and Integrated Discovery (DAVID software (*https://david.ncifcrf.gov/*;) (27). The interrogated DEGs were:

- Overlapping DEGs between LSE *vs.* PrE and DEC *vs.* non-DEC to identify Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in common between normal predecidualization and decidualization.
- Overlapping DEGs changing in the opposite direction between (PE-CVSs *vs.* NP-CVSs + PE-DEC *vs.* NP-DEC) and (LSE *vs.* PrE + DEC *vs.* non-DEC) to identify KEGG pathways that are impaired in the (pre)decidualization process of women who developed sPE.
- Overlapping DEGs changing in the same direction between (PE-CVSs *vs.* NP-CVSs + PE-DEC *vs.* NP-DEC) and the endometrial disorders to identify common KEGG pathways impaired in the (pre)decidualization process of women who developed sPE and in women with endometrial disorders.
- Overlapping DEGs between (PE-CVSs *vs.* NP-CVSs + PE-DEC *vs.* NP-DEC) and (PE-DB *vs.* NP-DB + PE-BP *vs.* NP-BP) to identify common KEGG pathways impaired in the (pre)decidualization process of women who developed sPE and in the decidua at the end of gestation in women with sPE.

PCA

Correlations between samples for genes involved in the cytokine-cytokine receptor interaction pathway (selected based on the functional analysis explained above) were evaluated by PCA. First, batch effects between studies were removed with the ComBat function of the surrogate variable analysis package (28). Then, the principal components from the data were calculated with the function prcomp from the stats package for R (29). The first and second principal components were plotted in a 2-dimensional plot using the plot function from the graphics package for R based on the expression of the genes in the cytokine-cytokine receptor interaction pathway. The 36 samples corresponding to physiologic conditions (LSE, DEC, FCs, ESCs, NP-CVSs, NP-DEC) were depicted in different shades of green according to the group. Samples from non-DEC ($n = 5$) and PrE ($n = 4$) were portrayed in shades of blue. Samples of endometrium from women obtained at delivery with PE (PE-BP and PE-DB) were presented in shades of pink ($n = 8$), whereas those from normal pregnancies (NP-BP and NP-DB) in shades of yellow ($n = 8$) according to the cohort. Finally, the 25 samples belonging to women with pathologic endometrium (PE-CVSs, PE-DEC, IF, RM, OSIS) were illustrated in different shades of red also according to the cohort.

Global test analysis

The "global test" reported by Goeman *et al.* (30) was employed in order to determine whether a prespecified group of genes (in our case, the genes in the cytokine-cytokine receptor interaction pathway) was differentially expressed between each condition and corresponding control. First, we used the normalized expression of all the genes in the pathway for input into the global test. Second, we cast a wide net to select the genes in the cytokine-cyokine receptor pathway that comprised each condition and its corresponding control (*e.g.*, LSE *vs.* PrE, PE-CVSs *vs.* NP-CVSs, *etc.*). That is, we applied a value of $P < 0.2$ to the normalized expression in order to select the genes in each condition and corresponding control for input into the global test. The method was applied with the "globaltest" package for R.

Network analysis

First, a gene network was constructed by using the CytoScape software (31) v.3.6.0 through the GeneMania plugin (32), which was used to infer network data. The set of functional association data between genes was downloaded from the Homo sapiens database. The 294 genes belonging to the cytokine-cytokine receptor interaction pathway (again, selected based on the functional analysis explained above) were imported into the GeneMania plugin to retrieve the corresponding association network. The association data employed was genetic or physical interaction (*i.e.*, 2 genes are functionally associated if the effects of perturbing 1 gene were found to be modified by perturbations to a second gene, or if their products were found to interact in a protein-protein interaction study). From this first network, new networks were generated for each data set by selecting those nodes corresponding to genes with a value of $P < 0.2$ for each of the 9 comparisons. Nodes in the resulting networks were colored according to whether they were up-regulated (green) or down-regulated (red). The proportions of up-regulated genes for each network were statistically compared by Logistic Regression analysis using the Logistic procedure of SAS v.9.4 (SAS Institute, Cary, NC, USA). The proportion of up-regulated genes in the LSE *vs.* PrE group was used as reference.

Determination of hub genes

Highly connected hub genes in each network were identified using the plugin CytoNCA (33) by running the 8 typical centralities parameters for the weighted networks (netweeness, closeness, degree, eigenvector, local average connectivity–based method, network, subgraph, and information centralities). The top 20 hub genes for each comparison were employed, in order to identify those hub genes that were repeated in at least 2 comparisons. These common hub genes were used to construct a single network that was colored according to the fold-change for each of the 9 comparisons.

RESULTS

All the samples that were selected for comparison of microarray data contained endometrium whether from nonpregnant women (endometrium *per se*), women during early pregnancy (CVS), or immediately postpartum (decidual basalis from placental bed or basal plate of delivered placenta). The transcriptomic raw data of these samples containing endometrium were all available in the public domain for download and subsequent bioinformatic analysis. The overall approach was identical to that we previously published (12). Thus, even though the tissues investigated were diverse, we focused on the endometrial component they all had in common, targeting those genes that we identified to be associated with endometrial maturation [*i.e.*, (pre)decidualization].

We initially identified DEGs related to the normal biology of predecidualization [endometrial maturation in the late secretory phase before implantation (LSE) relative to proliferative endometrium (PrE)], decidualization (endometrial maturation after implantation (DEC) relative to

nondecidualized endometrium (non-DEC)], and the overlap as predecidualization and decidualization are a biologic continuum. With these cohorts of DEGs in hand representing the molecular signature of normal endometrial maturation, we then explored the differential expression of the same genes in pathologic endometrium associated with PE—PE-CVSs or PE-DEC, OSIS, IF, and RM.

Data comparison

We first tested whether there was significant overlap in the DEGs changing in the opposite direction between the normal and pathologic (preeclamptic) samples containing endometrium. **Figure 2** depicts the Venn diagrams for the comparisons of up- or down-regulated DEGs in PE-CVSs relative to CVSs from women who experienced normal pregnancy (NP-CVSs) (Fig. 2*A*) and in PE-DEC relative to ESCs isolated from endometrial biopsies of women with normal pregnancies, placed into culture and then decidualized *in vitro* (NP-DEC) (Fig. 2*B*) with DEGs from normal (pre)decidualized endometrium, *i.e.*, LSE compared to PrE and decidualized endometrium (DEC) compared to non-DEC. There was significant overlap of DEGs changing in opposite direction for PE-CVSs and PE-DEC compared to the healthy endometrium LSE and DEC (P values ranging from 10^{-3} to 10^{-148}). In contrast, no significant overlap was observed for DEGs changing in the same direction for PE-CVSs and PE-DEC compared to the healthy endometrium. Of note, PE-CVSs and PE-DEC showed overlap of DEGs changing in the same direction, suggesting a significant degree of shared molecular pathology (Fig. 2*C*). In summary, these new bioinformatical analyses revealed dysregulated endometrial maturation in PE-CVSs, thus reinforcing our earlier work (12), and in PE-DEC.

Our next goal was to test whether or not, in our hands, dysregulated predecidualization is a pathologic component of IF, OSIS, and RM as previously reported (34–36). The Venn diagrams presented in Supplemental Fig. S1 strongly support this concept because the overlap of DEGs changing in the opposite direction between the normal and pathologic endometrial samples was highly significant, with P values ranging from 10^{-6} to 10^{-229}. In the case of OSIS, ESCs were isolated from biopsies and cultured and decidualized *in vitro*.

With this confirmation of dysregulated predecidualization in IF, OSIS, and RM, we next set out to determine whether there was significant overlap in DEGs changing in the same direction between these endometrial disorders and PE, suggesting shared molecular pathways of dysregulated predecidualization. **Figure 3** portrays the Venn diagrams for the comparisons of up- or down-regulated DEGs in PE-CVSs relative to NP-CVSs (Fig. 3*A*) and in PE-DEC relative to NP-DEC (Fig. 3*B*), with DEGs from IF and RM compared to FC endometrium, and OSIS compared to ESCs isolated from biopsies

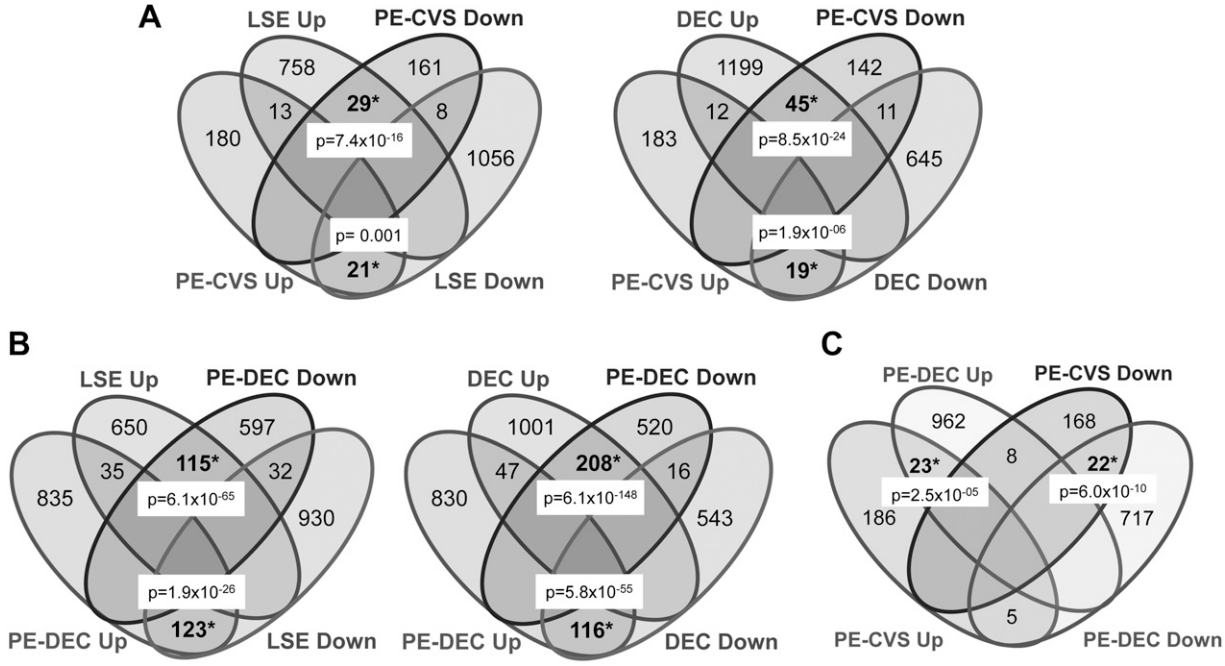

Figure 2. DEGs between PE-CVSs relative to NP-CVSs (*A*), PE-DEC relative to NP-DEC (*B*), compared to DEGs associated with normal (pre)decidualization and PE-CVSs relative to NP-CVSs compared to PE-DEC relative to NP-DEC (*C*). The Venn diagrams show significant overlap between up-regulated DEGs in PE-CVSs (*A*) and PE-DEC (*B*) and down-regulated DEGs in LSE (relative to PrE) and ~9 wk gestational endometrium with DEC (relative to non-DEC). The opposite is also observed. Taken together, many DEGs observed between PE-CVSs and NP-CVSs and between PE-DEC and NP-DEC changed in the opposite direction to DEGs associated with normal endometrial maturation. The Venn diagram shows significant overlap between DEGs changing in the same direction in PE-CVSs relative to NP-CVSs and PE-DEC relative to NP-DEC (*C*). $^*P < 0.05$ (Pearson's χ^2 test).

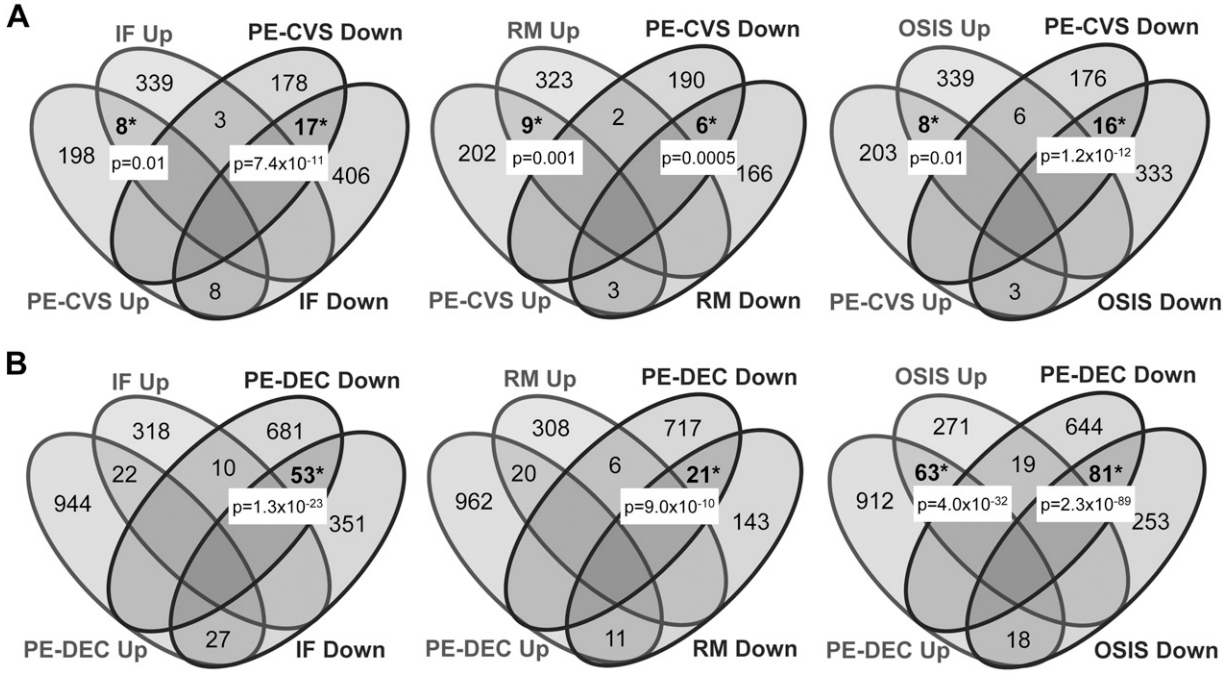

Figure 3. DEGs between PE-CVSs relative to NP-CVSs (*A*) and PE-DEC relative to NP-DEC (*B*) compared to DEGs associated with endometrial disorders relative to healthy endometrium. The Venn diagrams show significant overlap between up-regulated or down-regulated DEGs in PE-CVSs relative to NP-CVSs (*A*) and PE-DEC relative to NP-DEC (*B*), with DEGs up-regulated or down-regulated in secretory endometrium from women with recurrent IF and secretory endometrium from women with RM—both relative to FCs, and ESCs isolated from ovarian OSIS that were cultured and decidualized *in vitro* in comparison to ESCs from normal endometrial tissues. Taken together, many DEGs observed between PE-CVSs and NP-CVSs and between PE-DEC and NP-DEC changed in the same direction as DEGs associated with endometrial pathologies. For other abbreviations, see Fig. 1. $^*P < 0.05$ (Pearson's χ^2 test).

of normal endometrium, cultured, and then decidualized *in vitro*. There was significant overlap between DEGs changing in the same direction for PE-CVSs and PE-DEC compared to the pathologic endometrium (*i.e.*, IF, RM, and OSIS), with *P* values ranging from 10^{-2} to 10^{-89}. In contrast, no significant overlap was observed for DEGs changing in the opposite direction for PE-CVSs and PE-DEC compared to the pathologic endometrium. These results suggest that the molecular pathology of PE-CVSs and PE-DEC overlaps at least in part with endometrial disorders.

A final objective was to assess whether the molecular signature of dysregulated (pre)decidualization observed in PE-CVSs and PE-DEC was also observed in decidual basalis or basal plate after delivery from women who experienced sPE. **Figure 4** shows the Venn diagrams for up- and down-regulated DEGs in PE-CVSs relative to NP-CVSs (Fig. 4*A*) and in PE-DEC relative to NP-DEC (Fig. 4*B*) compared to up- and down-regulated DEGs in postpartum decidual tissue from women who experienced sPE [*i.e.*, decidua basalis dissected from placental bed biopsies obtained immediately after delivery in women with sPE (PE-DB) *vs.* normal pregnancy (NP-DB) and basal plate tissue from delivered placentas obtained from women affected by sPE (PE-BP) *vs.* normal pregnacy (NP-BP)]. Remarkably, little if any overlap was observed between PE-CVSs or PE-DEC and PE-DB or PE-BP, implying that the molecular pathology of decidual tissues procured after delivery from women who suffered sPE diverged markedly from early gestation in women who developed sPE and from ESCs isolated from midsecretory endometrium of women with a history of sPE that were cultured and decidualized *in vitro.*

Functional analysis

There were 607 DEGs in common between LSE *vs.* PrE and DEC *vs.* non-DEC [*i.e.*, genes changing in expression during the (pre)decidualization process]. Top enriched KEGG pathways within these 607 DEGs were NK cell-mediated cytotoxicity ($P = 7 \times 10^{-14}$), antigen processing and presentation ($P = 3 \times 10^{-09}$), and cytokine-cytokine receptor interaction ($P = 2.1 \times 10^{-04}$). DEGs changing in opposite direction between PE-CVSs and PE-DEC combined *vs.* normal (pre)decidualization (Fig. 2*A, B*) were significantly enriched for the KEGG pathways PI3K-Akt signaling ($P = 2.8 \times 10^{-05}$), Janus kinases–signal transducer and activator of transcription proteins signaling ($P = 9.3 \times 10^{-05}$), and cytokine-cytokine receptor interaction ($P = 1.5 \times 10^{-04}$). DEGs changing in the same direction among PE-CVSs and PE-DEC combined *vs.* endometrial disorders (Fig. 3) were significantly enriched for the KEGG pathways PI3K-Akt signaling ($P = 0.001$), TGF-β signaling ($P = 0.008$), Janus kinases–signal transducer and activator of transcription proteins signaling ($P = 0.03$), and cytokine-cytokine receptor interaction ($P = 0.006$). Finally, there were no significantly enriched KEGG pathways among the few overlapping DEGs

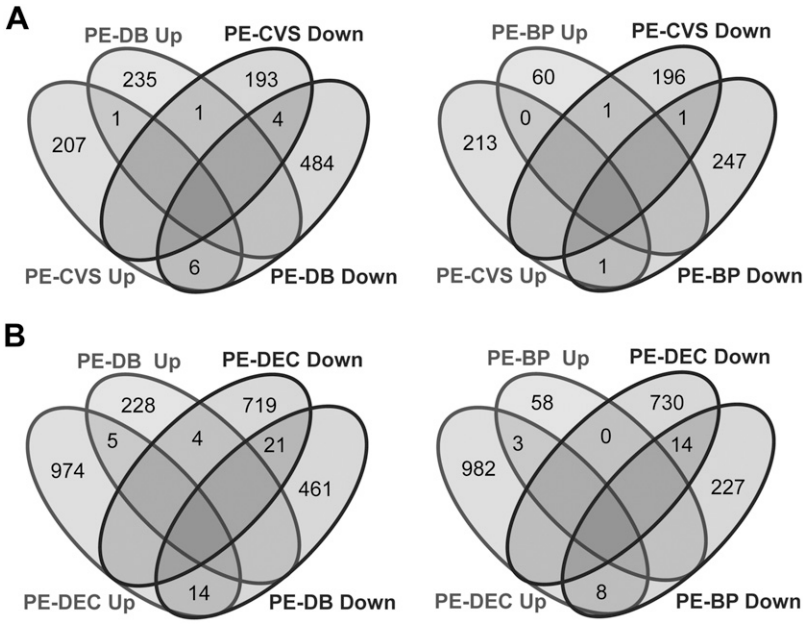

Figure 4. DEGs between PE-CVSs relative to NP-CVSs (*A*) and PE-DEC relative to NP-DEC (*B*) compared to DEGs associated with late gestation decidua in women who developed sPE relative to normal pregnancy. The Venn diagrams show no significant overlap between up-regulated or down-regulated DEGs in PE-CVSs relative to NP-CVSs (*A*) and PE-DEC relative to NP-DEC (*B*), with DEGs up-regulated or down-regulated in decidua basalis or basal plate obtained after delivery from women who experienced either sPE or normal pregnancy. PE- and NP-DB tissues were obtained by placental bed biopsy after cesarean section. PE- and NP-BP tissues were harvested from delivered placentas. For other abbreviations, see Fig. 1.

between PE-CVSs and PE-DEC with PE-DB and PE-BP (Fig. 4).

Because the cytokine-cytokine receptor interaction pathway was significantly enriched in all of the comparisons presented above, we further explored this KEGG pathway database (hsa04060).

PCA

The cytokine-cytokine receptor interaction pathway gene list downloaded from the KEGG pathway database contained 294 genes (*https://www.genome.jp/dbget-bin/get_linkdb?-t+9+path:hsa04060*). Several of these genes were not represented in the platforms employed for the data sets reanalyzed in the current study. After filtering out these genes, a total of 264 remained for PCA. After plotting the first and second principal components of the expression levels of the DEGs in the cytokine-cytokine receptor interaction pathway as revealed in the functional analyses above, samples from healthy endometrium formed 1 distinct cluster, whereas samples from pathologic endometrium including PE-CVSs and PE-DEC formed another distinct cluster, but with several notable exceptions (**Fig. 5**). Specifically, PrE and non-DEC segregated with pathologic endometrial samples, whereas PE-DB and PE-BP clustered with normal endometrial samples. These paradoxical findings, however, do not signify that PrE and non-DEC are pathologic or PE-DB and PE-BP are normal tissues. Rather, they reflect the expression pattern of the 264 genes involved in the cytokine-cytokine receptor interaction pathway, which in the case of PrE and non-DEC was more similar to pathologic endometrium, and for PE-DB and PE-BP, healthy endometrium. Nevertheless, in the decidual tissues procured postpartum, there were a few significantly dysregulated genes in the cytokine-cytokine receptor pathway (Supplemental Table S1), and others outside of this pathway that were related to immunologic processes (Supplemental Fig. S2). As detailed below, the relatively few dysregulated genes in the cytokine-cytokine receptor pathway was also consistent with the network analysis in which there were also very few nodes (Supplemental Fig. S3*I, J*). Thus, the PCA analysis suggests that the cytokine-cytokine receptor interaction pathway was similarly affected in endometrium from PE-CVSs, PE-DEC, IF, RM, and OSIS, but not in endometrium obtained after delivery in women who suffered sPE.

Global test analysis

We employed this test (30) as another approach to demonstrate that the expression pattern for the group of genes belonging to the cytokine-cytokine receptor interaction pathway was significantly different between each pathologic condition and its respective control (P values ranging from 0.047 to 2.2×10^{-8}), with the exceptions of PE-BP and PE-DP, which did not differ from their NP controls (P = 0.45 and P = 0.79, respectively). These results reinforce the findings of the PCA. When the group of genes in this pathway was limited to those with a value of $P < 0.2$, the P values in the global test, for all the conditions, ranged from 0.02 to 1.2×10^{-8}.

Network analysis

The network constructed with the 294 genes belonging to the cytokine-cytokine receptor interaction pathway contained 277 nodes that were connected according to genetic or physical interaction between genes. This network is depicted in Supplemental Fig. S3*A*, whereas Supplemental Fig. S3*B–J* show subnetworks of Supplemental Fig. S3*A*, each corresponding to one of the 9 comparisons as shown in Figs. 2–4, including LSE *vs.* PrE and DEC *vs.* non-DEC. Genes corresponding to the nodes in each of these

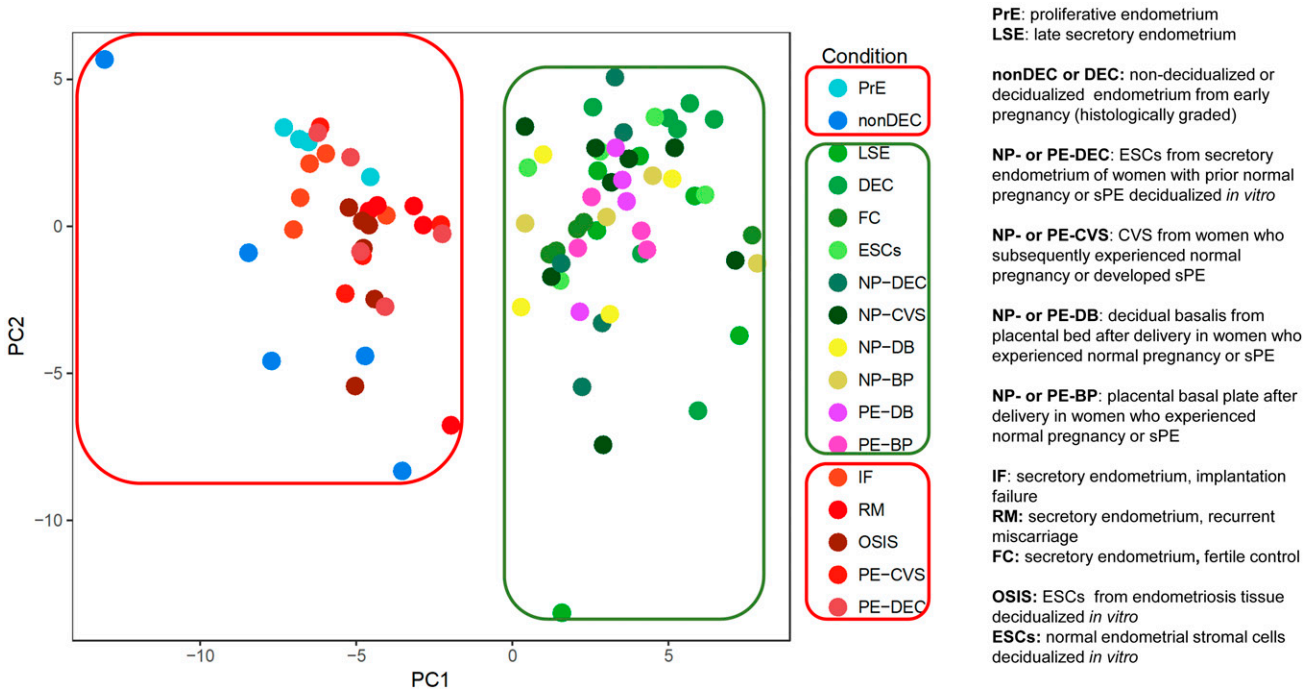

Figure 5. PCA of endometrial samples using the expression of genes belonging to the cytokine-cytokine receptor interaction pathway. Principal component plots show that normal endometrial samples obtained from healthy women (shades of green, $n = 36$) and late gestation endometrium from women with PE (shades of pink, $n = 8$) or normal pregnancies (shades of yellow, $n = 8$) formed a distinct cluster, whereas endometrial samples from women with pathologic endometrium (shades of red, $n = 25$) and samples from non-DEC or PrE (shades of blue, $n = 9$) formed another distinct cluster. The analysis was applied to 264 genes belonging to the cytokine-cytokine receptor interaction pathway. PE- and NP-DB tissues were obtained by placental bed biopsy after cesarean section. PE- and NP-BP tissues were harvested from delivered placentas.

networks (Supplemental Fig. S3*B–J*) were differentially expressed with a value of $P < 0.2$ for each condition relative to its control. A value of $P < 0.2$ was used, in order to cast a wide net, and because these groups of genes were significantly different between each condition and its respective control according to the global test (30).

A majority of genes in the cytokine-cytokine receptor interaction pathway were up-regulated in LSE and DEC when compared to their respective controls, PrE and non-DEC (green symbols in Supplemental Fig. S3*B, C*). LSE showed the highest percentage of up-regulated genes (78%). Compared to this percentage, the proportion of up-regulated genes in DEC was not statistically different (67%, $P = 0.072$). In other words, the expression of the majority of genes in the cytokine-cytokine interaction receptor pathway was increased during the (pre)decidualization process. However, the situation was reversed in the endometrial disorders because genes in this pathway were mostly down-regulated in IF and RM relative to the endometrium from FCs (red symbols in Supplemental Fig. S3*D, E*), with the percentage of up-regulated genes being only 20.3 and 36.6%, respectively (both $P < 0.0001$ when compared to 78% of up-regulated genes in LSE). OSIS, PE-CVSs, and PE-DEC showed intermediate responses in the expression of these genes relative to their controls ESCs, NP-CVSs, and NP-DEC (Supplemental Fig. S3*F–H*)—54.2, 52.3, and 40.4%, respectively. However, these percentages of up-regulated genes were still significantly lower than proportion of genes up-regulated in LSE (all $P < 0.007$). There were few genes in the cytokine-cytokine receptor interaction pathway that were differentially expressed between PE-DB *vs.* NP-DB and PE-BP *vs.* NP-BP, but the majority of these were up-regulated and not different from the proportion in LSE and thus more comparable to physiologic rather than pathologic endometrium [Supplemental Fig. S3*I, J*, percentage of up-regulated genes: 64.1% ($P = 0.1$) and 70% ($P = 0.45$), respectively].

Determination of hub genes

In order to further explore the cytokine-cytokine receptor interaction pathway in the different conditions, hub genes for each network in Supplemental Fig. S3 were determined, and the top 20 for each condition, according to connectivity parameters, are listed in Supplemental Table S1. Hub genes shared by at least 2 of the endometrial conditions were used to construct another subnetwork, the nodes for which were colored according to the fold change for each condition when compared to its respective control. The resulting colored networks for each condition are shown in Supplemental Fig. S4, whereas **Fig. 6*A*** lists all these genes with their respective fold change for each condition compared with its control.

Cohorts of genes whose direction of changing expression diverged between normal and pathologic endometrium in more than 1 endometrial disorder are highlighted by different colors in Fig. 6*A, B*. There were several genes

A

Symbol	LSE vs PrE	DEC vs NonDEC	IF vs FC	RM vs FC	OSIS vs ESCs	PE-CVS vs NP-CVS	PE_DEC vs NP-DEC	PE-DB vs NP-DB	PE-BP vs NP-BP
ACVR1	1.21	**-1.29**	-1.16	-1.19	-1.20	-1.01	-1.07	1.00	-1.13
ACVR2A	1.26	**1.31**	-1.12	-1.09	**1.07**	1.20	1.76	-1.30	1.02
ACVR2B	-1.20	**-1.66**	1.04	**1.12**	-1.02	-1.02	-1.03	-1.06	1.08
BMP2	3.33	**-2.75**	-1.21	-1.50	**-1.39**	-1.02	1.20	-1.04	-1.25
BMP4	1.08	**2.36**	-1.29	1.24	**1.52**	**1.14**	**4.54**	1.02	1.00
BMP7	**-1.61**	**-1.13**	**1.15**	1.08	1.04	1.05	1.41	-1.09	-1.06
BMPR1A	-1.43	**-1.64**	1.22	1.14	1.16	1.12	1.16	-1.05	-1.06
BMPR1B	**-4.38**	**-4.31**	1.67	1.63	1.11	1.16	2.21	-1.07	1.00
BMPR2	**1.59**	-1.11	**-1.23**	**-1.27**	1.05	**1.08**	1.90	-1.19	-1.09
CCL13	1.04	**2.02**	-1.05	-1.02	**-1.38**	**1.21**	1.31	-1.33	-1.30
CCL4	**13.80**	**2.31**	**-2.49**	-1.18	1.03	1.24	-2.63	-1.28	-1.49
CCR1	**4.57**	**5.95**	**-1.89**	**-1.37**		1.19	2.30	-1.37	-1.04
CCR2	1.00	1.00	-1.08	**-1.25**		**1.04**	-1.85	1.35	1.00
CCR5	1.72	1.38	-1.64	**-1.70**	**-1.04**	-1.04	-1.37	1.17	1.11
CD27	1.11	1.15	1.14	1.23	1.04	1.03	-1.26	1.09	1.03
CD40	1.05	1.17	**-1.20**	**-1.20**	**-1.07**	**-1.18**	-3.01	1.15	-1.24
CSF3R	**2.12**	1.54	**-1.14**	-1.01	**-1.59**	-1.11	**-3.49**	1.65	-1.03
CXCL12	-1.97	-1.32	**-2.83**	-2.05	**1.89**	1.10	1.26	1.03	1.00
CXCL13	-1.00	1.22	**-5.15**	**-3.64**	1.00	**-1.05**	**-6.28**	1.16	1.18
CXCR4	**8.32**	-1.29	-1.57	-1.72	**-1.28**	-1.17	**-2.48**	1.07	**-2.18**
CXCR5	-1.01	**-1.05**	1.00	1.00	1.01	-1.05	-1.50	1.07	1.00
EDA	**-1.11**	**-1.06**	1.17	**1.09**	1.00	1.01	1.03	1.13	1.00
FAS	2.15	**1.33**	**-1.30**	**-1.32**	-1.01	-1.13	-1.07	-1.11	1.02
GDF11	**-2.17**	**-2.10**	**1.28**	1.35	1.04	**1.04**	**2.36**	-1.09	1.05
IFNAR1	**1.65**	**1.52**	**-1.25**	-1.09	-1.10	-1.03	-1.26	-1.19	1.00
IL10RB	1.25	**1.88**	1.27	1.22	**-1.18**	-1.29	**-1.81**	1.07	-1.10
IL15	**13.40**	**12.48**	**-2.68**	**-1.69**	**-3.05**	**-2.62**	**-11.75**	-1.02	-1.74
IL15RA	**3.57**	**3.04**	**-2.51**	**-1.97**	**-1.13**	-1.17	**-3.12**	-1.01	1.00
IL16	1.21	**1.49**	-1.06	-1.03	-1.01	-1.03	-1.49	1.05	1.13
IL1A	**2.06**	**2.30**	1.00	1.08	**-1.47**	-1.12	**-6.32**	-1.24	1.10
IL1RAP	1.09	**1.65**	**1.15**	1.07	**-1.06**	**-1.69**	-1.30	-1.27	-1.21
IL1RN	1.37	-1.53	1.17	**1.58**	**-1.04**	-1.02	**-3.65**	-1.39	-1.06
IL2RB	**12.28**	**4.96**	**-2.55**	-1.44		**-3.01**	**-2.71**	1.10	-1.05
IL2RG	**2.80**	**3.18**	**-1.91**	-1.46	-1.02	-1.12	-1.29	1.05	1.13
IL34	-1.25	-1.09	1.04	1.06	**-1.04**	-1.15	-1.51	**-1.54**	
IL4R	**1.26**	1.25	-1.52	-1.42	**1.76**	**1.05**	1.01	1.04	1.21
IL6ST	1.80	1.19	**-1.93**	-1.77	-1.02	-1.05	-1.79	**-1.42**	-1.26
INHBA	**15.44**	**-5.49**	-1.01	-1.01	-1.01	-1.56	-1.51	-1.23	1.25
LIFR	-1.02	1.30	**1.92**	1.30	1.03	**-1.37**	**-4.77**	**-1.55**	1.44
OSMR	**4.14**	**2.78**	**-3.03**	**-2.32**	**-1.34**	-1.15	**-2.21**	1.03	-1.17
PRL	**6.28**	**1078.73**	**-1.54**	-1.52	**-20.13**	**-2.94**	**-7.66**	-1.07	1.14
PRLR	**5.98**	**15.09**	**-1.63**	-1.23	**-4.09**	**-1.23**	**-4.70**	-1.22	-1.25
TGFB2	**6.86**	**-2.91**	**-2.80**	-1.91	**1.12**	-1.08	**4.07**	-1.09	-1.13
TGFBR1	**1.56**	**4.23**	-1.12	-1.16	-1.02	-1.07	-2.73	-1.12	-1.02
TGFBR2	1.28	**1.85**	**-2.19**	**-1.76**	**-1.49**	-1.16	-1.38	-1.05	-1.20
TNFRSF1A	1.52	1.16	**-1.73**	-1.34	**2.10**	1.07	-1.13	1.27	1.16
TNFRSF21	**2.05**	**2.10**	-1.52	1.28	-1.28	1.02	**1.72**	1.43	-1.04
TNFSF18	1.11	1.00	1.00	1.00	**-1.04**	-1.06	1.35	**-1.53**	1.07

B

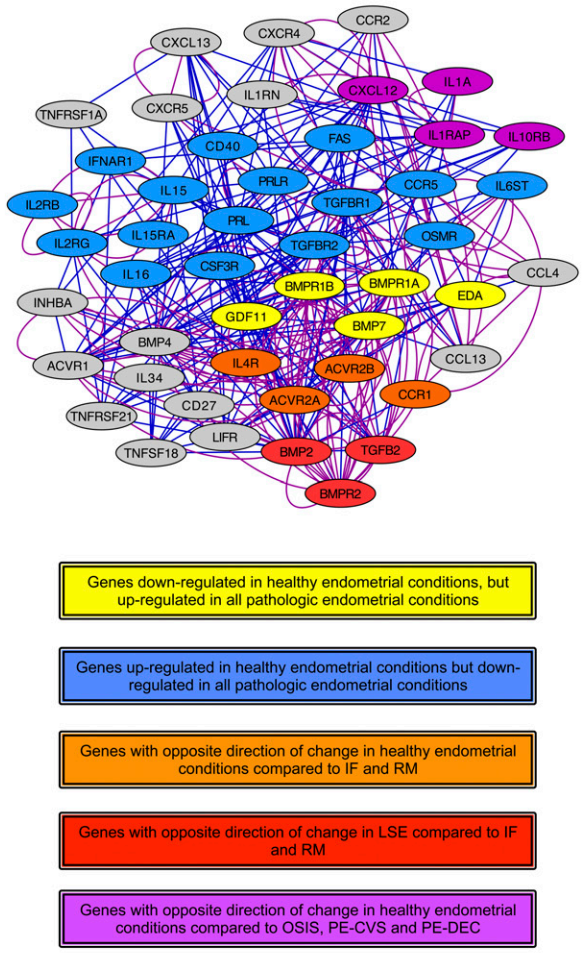

Figure 6. Genes that are part of the 20 top hub genes from each of the networks shown in Supplemental Fig. S3, appearing in at least 2 conditions, presented as list (*A*) or network (*B*). Genes are classified according to the direction of change in healthy or pathologic endometrial conditions compared to their respective controls regardless of the statistical significance. Genes highlighted in yellow are those whose direction of change is down-regulated in the healthy endometrial conditions (LSE and DEC) but up-regulated in pathologic endometrial conditions (IF, RM, OSIS, PE-CVSs, and PE-DEC). Genes highlighted in blue are those that are up-regulated in the healthy endometrial conditions but down-regulated in the pathologic endometrial conditions. Genes highlighted in orange are those changing in opposite direction between LSE/DEC and IF/RM, whereas those in purple are changing in opposite direction between LSE/DEC and OSIS/PE-CVSs/PE-DEC. Genes highlighted in red are those changing in opposite directions between LSE and IF/RM. Gray genes are those that couldn't be classified in the categories above. *A*) The first column lists the gene symbols, whereas the remaining columns (one for each comparison) show the corresponding fold change *vs.* respective control fold changes greater or <1 are shaded in green and red, respectively. Bold-typed numbers indicate that the fold change is significant at $P < 0.1$. Cells in black indicate that the gene was not represented in the array. *B*) Genes are visualized as nodes in a network. Nodes (genes) were associated according to whether they had genetic (blue edges) or physical interactions (purple edges). ESCs were subsequently isolated, cultured and decidualized *in vitro*. PE- and NP-DB tissues were obtained by placental bed biopsy after cesarean section. PE- and NP-BP tissues were harvested from delivered placentas.

(highlighted in yellow) that were down-regulated in normal (pre)decidualization (LSE *vs.* PrE and DEC *vs.* non-DEC) but up-regulated in all pathologic endometrium relative to their respective controls (IF, RM, OSIS, PE-CVSs and PE-DEC): bone morphogenetic protein 7 (BMP7), its receptors type 1A (BMPR1A) and type 1B (BMPR1B), ectodysplasin A, and growth differentiation factor 11. Conversely, there were several genes (blue) that increased expression in normal (pre)decidualization but decreased expression in all pathologic endometrium. These genes were C-C motif chemokine receptor 5, CD40, colony stimulating factor 3 receptor, Fas cell surface death receptor, interferon α and β receptor subunit 1, IL-15 and IL-16, IL-2 receptor subunit β and subunit γ, IL-15 receptor subunit α, IL-6 signal transducer, oncostatin M receptor, prolactin (PRL) and its receptor (PRLR), and the TGF-β receptors 1 (TGFBR1) and 2 (TGFBR2). The direction of change of several genes (orange) in the endometrial disorders, IF and RM, was opposite to normal (pre)decidualization: activin A receptor type 2A and type 2B, C-C motif chemokine receptor 1, IL-34 and IL-4 receptor; whereas the direction of change in other genes (purple) in OSIS, PE-CVSs, and PE-DEC was also opposite to normal (pre)decidualization: C-X-C motif chemokine

ligand 1, IL-10 receptor subunit β (IL-10RB), IL-1A, and IL-1 receptor accessory protein. Finally, genes highlighted in red were those up-regulated in LSE *vs.* PrE but down-regulated in IF and RM: bone morphogenetic protein 2 and its receptor, and TGF-β2. (See Discussion for potential functional significance of several of these DEGs.)

DISCUSSION

We hypothesized that sPE is antedated by dysregulated endometrial maturation; that is, insufficient or defective decidualization both before and during early pregnancy contributes to the genesis sPE (8, 12, 16). The decidualization process entails changes in morphology and function of the endometrial luminal and glandular epithelial, as well as stromal cells, which begin in the secretory phase of the menstrual cycle (predecidualization) and continue after implantation (decidualization) (37), thereby optimizing the uterine milieu for implantation and placentation. Additionally, the initial stages of spiral arteries remodeling occur prior to trophoblast invasion during the decidualization process (10, 11). Thus, insufficient or defective endometrial maturation could be 1 etiological factor in the development of sPE. If the concept of endometrial antecedents is valid, then one might predict some degree of shared molecular features with other diseases affecting the endometrium.

In the present study, we reanalyzed and integrated the data from 8 different microarray data sets available in the public domain to test this prediction. A significant proportion of DEGs that were either up- or down-regulated in chorionic villous samples obtained from women at ~11.5 wk of gestation who developed sPE (PE-CVSs) and in ESCs isolated from midsecretory endometrial biopsies of women with previous early onset sPE, placed into culture, and then decidualized *in vitro* (PE-DEC) demonstrated an opposite directional change in the LSE and DEC (Fig. 2*A*, *B*). This finding was consistent with dysregulated endometrial maturation in the secretory phase and during early pregnancy in women who developed sPE. There was also significant overlap of up- and down-regulated genes changing in the same direction in the PE-DEC and PE-CVSs data sets (Fig. 2*C*). These new bioinformatics analyses reinforced our previous work supporting the idea of insufficient or defective endometrial maturation in women who developed sPE (12).

A significant proportion of DEGs that were either up- or down-regulated in PE-CVSs and PE-DEC demonstrated the same directional change in IF, RM, and OSIS consistent with the prediction of partial overlapping molecular etiologies among these different pathologies (Fig. 3). In fact, the number of DEGs either up- or down-regulated in PE-DEC demonstrating the same directional change as in the other endometrial disorders was greater and more significant than for PE-CVSs. The more robust results for PE-DEC might reflect the tissue homogeneity of PE-DEC (cultured ESCs decidualized *in vitro*) relative to PE-CVSs (admixture of maternal decidual and fetal trophoblast cells) and the possibility that *in vitro* decidualization may maximize deficiencies or defects in decidualization potential.

In contrast, we observed that there was little if any overlap of DEGs in PE-CVSs and PE-DEC with decidua basalis or basal plate derived from women who experienced sPE (PE-DB and PE-BP), indicating that decidual transcriptomics after delivery are dissimilar from those of ESCs isolated from midsecretory endometrial biopsies obtained 1–5 yr after sPE that were then cultured and decidualized *in vitro* (PE-DEC) or from CVSs tissue obtained during early pregnancy in women who developed sPE (PE-CVSs; Fig. 4). These discordances may not be unexpected given the timing of the sample procurement—*i.e.*, PE-DB (decidua basalis obtained from placental bed biopsy) and PE-BP (basal plate decidua obtained from delivered placentas) were procured during clinically active disease that by itself seems likely to perturb decidual gene expression, thus perhaps reflecting consequence rather than cause of disease. In the same vein, recent studies demonstrated impaired *in vitro* decidualization of ESCs isolated from delivered placentas consistent with the concept of endometrial antecedents of sPE (15, 38). However, delivered tissues are not temporally related to predecidualization, decidualization, and trophoblast invasion in early pregnancy, and as underscored by the bioinformatics gene expressions analyses herein, the molecular pathologies are dissimilar. Thus, designing corrective strategies based on the molecular pathology of delivered tissues may be misguided and ineffectual.

To provide additional evidence for a potential endometrial contribution to sPE, we performed a functional analysis followed by a pathway driven approach, which revealed cytokine-cytokine receptor interaction to be one of the major molecular pathways in normal (pre)decidualization that was dysregulated in sPE and the endometrial disorders IF, RM, and OSIS. The prominence of the cytokine-cytokine receptor interaction KEGG pathway is not unexpected because changes in endometrial immune function is part and parcel of (pre)decidualization [reviewed in (8)]. When the expression of genes in the cytokine-cytokine receptor interaction pathway was employed to determine relatedness among samples, PCA revealed a clear separation between normal and pathologic endometrium (Fig. 5). Whereas PE-CVSs and PE-DEC clustered with the endometrial disorders, the decidual basalis obtained by placental bed biopsy (PE-DB) or from delivered placentas (PE-BP) in women who were affected by sPE segregated with normal endometrial conditions in the PCA. Samples from PrE and non-DEC associated with the endometrial disorders. As mentioned previously, these findings do not signify that PrE and non-DEC are pathologic or PE-DB and PE-BP are normal tissues. Rather, they reflect the expression pattern of the 264 genes involved in the cytokine-cytokine receptor interaction pathway, which for PrE and non-DEC was more similar to pathologic endometrium, and PE-DB and PE-BP more comparable to healthy endometrium.

We further performed network analyses based on genes in the cytokine-cytokine receptor interaction pathway. First, we cast a wide net for genes in this pathway that were differentially expressed with a value of $P < 0.2$ for

each condition relative to its control, in order to create 1 network for each condition (Supplemental Fig. S3). Second, we determined the top 20 hub genes (*i.e.*, highly connected genes) in each network (Supplemental Table S1) and focused on those that were top hub genes in at least 2 of the disorders (Fig. 6). Third, we created a new subnetwork of these 20 hub genes and their connecting genes in order to compare their direction and magnitude of change in each disease relative to its control (Fig. 6*A* and Supplemental Fig. S4). Finally, several genes were classified according to the direction of change in the healthy or pathologic conditions compared to their respective controls (Fig. 6*A, B*). Caveats of the current work are that the bioinformatics approach taken revealed significant associations among genes, but not causal linkages, nor was the functional relevance of these genes elucidated or proven. In order to partly address these limitations, we consulted the available literature and next discuss the potential functional relevance of several genes that we identified from this analysis in the context of (pre)decidualization.

Genes highlighted with yellow in Fig. 6 were down-regulated in LSE and DEC but up-regulated in all pathologic endometrial conditions. They included BMP7 and its receptors, BMPR1A and BMPR1B. In mice, BMP7 conditional knockout led to defective decidualization and a nonreceptive endometrium suggesting an essential role for endometrial BMP7 in implantation (39). However, Kodama *et al.* (40) reported that endometrial BMP7 gene expression decreased after the midsecretory phase in women, and BMP7 inhibited decidualization and proliferation of ESCs in culture. These researchers also observed that progesterone suppressed the gene expression of BMP7 in cultured ESCs. Our findings are more consistent with Kodama *et al.*, insofar as BMP7 including its receptors, BMPR1A and BMPR1B, were down-regulated during normal (pre)decidualization but up-regulated in the endometrium of women with pathologic obstetric outcomes consistent with dysregulated (pre)decidualization.

Conversely, genes highlighted with blue in Fig. 6 were those up-regulated during normal (pre)decidualization but down-regulated in the pathologic conditions. Among these genes, perhaps the most noteworthy were PRL and its receptor PRLR; IL-15 and the 3 chains of its receptor IL-15 receptor subunit α, IL-2 receptor subunit β, IL-2 receptor subunit γ; and TGBR1 and TGFBR2.

PRL is a widely recognized biomarker of decidualized endometrial cells (41). Expression of PRLR in the endometrium is temporally regulated throughout the menstrual cycle being minimal in the proliferative phase and maximal during the mid-to-late secretory phases (42). Down-regulation of PRL and PRLR could lead to suboptimal (pre)decidualization, and consequently, disruption of placentation and adverse pregnancy outcomes (43). Although not in the cytokine-cytokine receptor interaction pathway, another widely recognized biomarker of decidualization, IGFBP1, was also significantly up-regulated in LSE (FC: 36.9, $P = 5.8 \times 10^{-08}$) and DEC (FC: 9.6, $P = 0.0005$) but down-regulated in OSIS (FC: −4.9, $P = 8.6 \times 10^{-06}$), PE-CVSs (FC: −2.3, $P = 0.01$), and PE-DEC (FC: −88.2, $P = 4.6 \times 10^{-05}$).

IL-15 is linked to activation of decidual NK (dNK) cells, the predominant immune cell at the maternal-fetal interface (44). Implantation sites of mice with global knockout of IL-15 were devoid of dNK cells with unmodified spiral arteries and impaired decidual integrity compared to wild-type mice (45). Furthermore, dysregulation of dNK may be a contributing factor to the genesis of PE (8, 12, 46). Expression of TGFBR1 was reported to be involved in recruitment of dNK cells and spiral artery remodeling in mice, and conditional ablation of TGFBR1 in the female reproductive system led to down-regulation of genes related to the cytokine-cytokine receptor interaction and NK cell-mediated cytotoxicity pathways (47). TGFBR2 forms a heterodimeric complex with TGFBR1, and thus dysregulation of 1 receptor subunit or the other would likely compromise receptor function. These findings support the concept of dNK cell dysregulation in all of the pathologic endometrial conditions analyzed in this study. Interestingly, several of the genes mentioned above related to dNK cell function (IL-15, IL2RB, and IL2RG) and decidualization (PRL and PRLR) were more dysregulated as suggested by larger fold changes of expression in OSIS, PE-CVSs, and PE-DEC than in IF and RM. This observation suggests that these genes may not be essential for embryo implantation and survival but may be critical for optimal placentation.

We found other genes, highlighted with purple in Fig. 6 that were uniquely down-regulated in OSIS, PE-CVSs, and PE-DEC including IL-10RB. The proteins encoded by IL-10RB and IL-10 receptor subunit α (IL-10RA) form the IL-10 receptor complex, which is required for IL-10–mediated signal transduction. The IL-10 receptor complex was reported to be expressed in decidua stromal cells, macrophages, and uterine NK cells (48). Although IL-10RA is not shown in Fig. 6 because it was not a hub gene, IL-10RA was strongly and significantly up-regulated in both LSE *vs.* PrE and DEC *vs.* non-DEC (each >3 FC, $P < 0.05$), and significantly down-regulated in both PE-CVSs *vs.* NP-CVSs and PE-DEC *vs.* NP-DEC (FC: −1.4 and −4.8, respectively, both $P < 0.05$). This gene, however, was not dysregulated in the other pathologic endometrial conditions. Thus, the reduced expression of these receptors in sPE could have impaired IL-10 signaling. Several studies demonstrated decreased levels of IL-10 in placentas delivered from women affected by PE (49), but this cytokine was not expressed in the endometrial samples reanalyzed in this study possibly because trophoblast is the major source (50). In summary, IL-10 signaling is a key anti-inflammatory pathway that may contribute to fetoplacental protection from the maternal immune system (51).

Finally, another gene highlighted with purple in Fig. 6 that was uniquely down-regulated in OSIS, PE-CVSs, and PE-DEC was IL-1A. Several studies have observed polymorphisms of IL-1A gene in women with PE in Sinhalese (52), Brazilian (53), and Chinese (54) populations, perhaps suggesting a genotypic component that might impair the expression of IL-1A in the endometrium of women afflicted by PE, but studies showing the functional significance of these polymorphisms are lacking.

CONCLUSIONS

In summary, using data integration and a systems biology approach, we presented evidence for overlap in the molecular pathologies of dysregulated (pre)decidualization, including cytokine-cytokine receptor interaction in sPE, IF, RM, and OSIS. As outlined above, many of these cytokines and their receptors were reported by other investigators to be critical for normal (pre)decidualization. We previously provided evidence for dysregulation of endometrial maturation and NK cell number or function both before and after implantation in women who developed sPE, but a potential limitation of this study was that it was based on bioinformatics analyses of transcriptomic data derived from CVSs, which contain both decidual and trophoblast tissues (12). In the present study, we reanalyzed transcriptomic data available in the public domain based upon ESCs isolated from midsecretory biopsies of women between 1 and 5 yr after sPE or uncomplicated pregnancy, which were subsequently cultured and decidualized *in vitro* (15). These transcriptomic data also revealed a strong molecular signature consistent with impaired endometrial maturation that significantly overlapped with PE-CVSs. Therefore, the overall concept of the endometrial genesis of sPE is now based on results from 2 independent laboratories each using different methodological approaches, and they are mutually reinforcing both supporting the idea (12, 15).

Perspectives

It is well accepted that sPE is a complex, polygenic syndrome with multiple etiologies. We suggest that sPE affecting at least some women may be part of a biologic continuum of "endometrial spectrum disorders" that includes IF, RM, and OSIS. Because impaired trophoblast invasion and spiral artery remodeling can also underlie second trimester abortion, preterm delivery, abruptio placentae, normotensive intrauterine growth restriction, and possibly stillbirth at least in some cases, these disorders may also fall along the biologic continuum of endometrial dysregulation (10, 55). Viewing sPE in this context could at least partly explain why women with OSIS who become pregnant may have increased PE risk as documented in some (56–62), but not all (63–66), reports. In the same vein, RM has also been linked to increased PE risk (67–69). One potential limitation of the current work was that a bioinformatics approach was taken, in which associations among genes and not causal linkages were described. Moreover, the functional significance of the genes was not revealed, although evidence from the literature illustrated the critical role of many in the normal biologic process of (pre)decidualization. Nevertheless, this approach may provide new and valuable insights into the molecular regulation of the endometrium in women affected by sPE in the context of other endometrial disorders, thereby informing future laboratory investigation, biomarker discovery, and the development of novel therapeutics. In fact, normal endometrial maturation is highly regulated by epigenetic mechanisms, which dysregulation may therefore be a prime candidate for the endometrial etiology of sPE (8).

Précis

New findings

Bioinformatics analyses of 8 microarray databases from normal and pathologic endometrium or decidua revealed significant overlap of molecular pathology in sPE and recurrent IF, RM, and OSIS.

Little or no overlap of molecular pathology was observed either in early placentas containing decidua from women who developed sPE (CVSs) or in *in vitro* decidualized ESCs derived from midsecretory biopsies of women who previously experienced sPE with decidua obtained from placental bed biopsies or basal plate of delivered placentas from women with sPE.

Relevance

Although the genesis of sPE is uncertain, inadequate placental development during early pregnancy is widely believed to contribute. Previous work proposed endometrial dysregulation as 1 potential initiating factor. The current study reinforces this concept by demonstrating molecular similarities in the decidua of women with sPE and endometrial disorders.

The molecular pathology of placenta or decidua procured after delivery in women affected by sPE may result from the disease and may not be informative of molecular etiology during the first trimester or before pregnancy in secretory endometrium.

Summary

sPE may be in a continuum of endometrial disorders with varying severity of molecular dysregulation that affects implantation, placentation, or both. FJ

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AUTHOR CONTRIBUTIONS

M. B. Rabaglino and K. P. Conrad contributed to research design, data interpretation, and drafting the manuscript; and M. B. Rabaglino contributed to bioinformatical analyses.

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