Placenta at single-cell resolution in early and late preeclampsia: insights and clinical implications

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Preeclampsia, one of the great obstetrical syndromes, manifests through diverse maternal and fetal complications and remains a leading contributor to adverse perinatal outcomes. In this review, we describe our work on single-cell and single-nuclei RNA sequencing to elucidate the molecular mechanisms that underlie early- and late-onset preeclampsia. Analysis of 46 cell types, encompassing approximately 90,000 cells from placental tissues collected after delivery, demonstrated cellular dysregulation in early-onset preeclampsia, whereas late-onset preeclampsia showed comparatively subtle changes. These findings were observed in all cell lines, including all types of trophoblast, lymphoid, myeloid, stromal, and endothelial cells. Key findings in early-onset preeclampsia included disrupted syncytiotrophoblast and extravillous trophoblast angiogenic signaling, characterized by an up-regulation of *FLT1* and down-regulation of *PGF*, consistent with an angiogenic imbalance. The stromal and vascular compartments exhibited stress-induced transcriptomic shifts. Both endothelial cells and pericytes showed evidence of stress, including up-regulation of heat shock proteins and markers of apoptosis. In addition, the inflammation- and stress-responsive states were more abundant in early-onset preeclampsia than in matched controls. Inflammatory pathways were markedly up-regulated in both the maternal and fetal immune cells; for example, we observed a marked increase in pro-inflammatory cytokines, including secreted phosphoprotein 1 and C-X-C motif chemokine ligand 2 and 3. Conversely, late-onset preeclampsia retained adaptive placental features with localized dysregulation of extracellular matrix remodeling and angiogenic markers, underscoring its possible maternal cardiovascular etiology.

Single-cell and single-nuclei RNA sequencing investigations of placental tissues support the proposed classification of preeclampsia into a placental dysfunction type, primarily presenting early in pregnancy, and a maternal cardiovascular maladaptation type, primarily presenting later in pregnancy, each with distinct biomarkers, risk factors, and therapeutic targets. The early-onset preeclampsia findings advocate for interventions that target angiogenic pathways, such as RNA-based therapies that target specific cells of the placenta, to modulate soluble fms-like tyrosine kinase-1 levels. In contrast, late-onset preeclampsia management may benefit from maternal cardiovascular optimization, including individualized antihypertensive and metabolic treatments. These results underscore the heterogeneity of preeclampsia, emphasizing the need for individualized diagnostic and therapeutic strategies.

This molecular atlas of preeclampsia advances our understanding of the complex interplay among elements of the maternal-placental-fetal array, thereby bridging clinical phenotypes and cellular mechanisms. Future research should focus on integrating these insights into longitudinal studies to develop precision medicine approaches for preeclampsia to enhance outcomes for mothers and neonates.

Key words: early-onset preeclampsia, endothelial cells, immune cells, late-onset preeclampsia, maternal cardiovascular adaptation, placenta, PlGF, preeclampsia, sFlt-1, single-cell RNA sequencing, single nuclei RNA sequencing, stromal cells, trophoblast

Introduction

Preeclampsia represents a significant and multifaceted challenge in obstetrical care and is characterized by a spectrum of maternal and fetal effects, ranging from maternal hypertension and proteinuria to potential organ-specific injury in the maternal heart, kidneys, liver, or brain and intrauterine growth restriction (IUGR).[1–4] This great obstetrical syndrome affects 2% to 8% of pregnancies, underscoring the critical need for deeper insight into the pathophysiological mechanisms that underpin the disorder. Historically, research efforts have concentrated on the role of placental dysfunction and highlighted inadequate trophoblast invasion and spiral artery remodeling as hallmarks of

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We encourage sharing our data. We have built an online resource of our RNA-seq data available at: https://zeisellab.org/preeclampsia/.

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preeclampsia.[5,6] However, recent paradigms propose that the origins of preeclampsia may also lie within maladaptive maternal cardiovascular physiology.[7,8] This dual perspective emphasizes the interplay between maternal and placental factors in the etiology of the disease.

A healthy pregnancy demands complex coordination between the maternal cardiovascular system, the developing placenta, and the growing fetus.[7,9,10] This maternal-placental-fetal array involves dynamic crosstalk and requires precise cardiovascular adaptations to sustain uteroplacental perfusion and to meet the metabolic demands of both the mother and fetus. Failure of this integrated system can manifest as preeclampsia, and its heterogeneity reflects variations in the contribution of the placenta and the maternal cardiovascular system. Early-onset preeclampsia is often linked to defective placentation, whereas late-onset preeclampsia frequently correlates with maternal cardiovascular strain, which may also be exacerbated by preexisting conditions, such as chronic hypertension, diabetes, chronic kidney disease, or obesity.[7,8,11] Some investigators posit[11] that the developing maternal syndromes of early- and late-onset preeclampsia share syncytiotrophoblast stress as the convergent feature that sets off the preeclampsia cascade that is the hallmark of the disease.[11] As the syndrome progresses, the syncytiotrophoblast shows increasing markers of stress, including apoptosis, pyroptosis, autophagy, syncytial knots, and necrosis, with syncytiotrophoblast stress signals visible in the maternal circulation, such as soluble Fms-like tyrosine kinase-1 (sFlt-1), a specific biomarker for preeclampsia.[11,12] Our work shows that this syncytiotrophoblast stress is substantially more marked in early-onset preeclampsia than in late-onset preeclampsia.[13] Some late-onset preeclampsia cases may display features of early-onset disease.[14,15]

The placenta vs heart debate[7,9,10,16] has profound implications in understanding preeclampsia. Evidence that supports the placental model includes altered angiogenic and antiangiogenic factor balances, exemplified by elevated sFlt-1 and reduced placental-derived growth factor (PlGF) in preeclampsia.[12,17,18] Conversely, the maternal cardiovascular model draws parallels between preeclampsia and chronic cardiovascular pathologies, positing that impaired cardiac remodeling and hemodynamic maladaptations significantly contribute to disease progression.[10,19]

Recognizing preeclampsia as a disorder of the maternal-placental-fetal array with at least 2 phenotypes opens avenues for novel diagnostic and therapeutic approaches.[7,20] For instance, integrating maternal hemodynamic assessments with biomarkers, such as PlGF and sFlt-1, could refine risk stratification and early detection.[21,22] Furthermore, therapeutic strategies that target angiogenic imbalances, such as RNA interference-based interventions, are emerging as potential treatments.[23,24]

The clinical manifestations of early- and late-onset preeclampsia and their implications for maternal and fetal health differ significantly.[15,25] We proposed a classification system based on the key characteristics of each phenotype, including risk factors, screening modalities, and treatment strategies (Figure 1).[7,20] Our classification system resembles the classification of diabetes mellitus (DM) into type I (formerly juvenile diabetes) and type II (adult onset) DM. Both types of DM have similar presenting signs and symptoms, namely hyperglycemia, glucosuria, polydipsia, polyuria, fatigue, blurred vision, and repeat infections, and similar screening tests. However, they have distinct pathophysiologies, particularly in the production and use of insulin, which require disparate treatment approaches. In type 1 DM, the immune system attacks and destroys insulin-producing beta cells in the pancreas, leading to little or no insulin production and subsequently glucosuria. Type 2 DM is characterized by insulin resistance, which leads to an unmet need with the pancreas unable to produce sufficient insulin to overcome this resistance, and this also leads to glucosuria.

Although preeclampsia is diagnosed based on new-onset hypertension and proteinuria and other organ effects, the pathophysiology of the early and late types differs in many respects. It is necessary to differentiate between the 2 types to provide antihypertensive treatment that aims to normalize the underlying hemodynamic abnormalities to achieve blood pressure control to mitigate the maternal and fetal risks.[25–27] Early-onset preeclampsia is characterized by an increased sFlt/PlGF ratio, decreased cardiac output, and increased peripheral vascular resistance. Calcium channel blockers and nitric oxide donors are appropriate to enable relaxation of vascular smooth muscle; vasodilation reduces peripheral vascular resistance, and fluid support is aimed at vasodilation, which leads to a reduction in arterial blood pressure.[26]

In contrast, late-onset preeclampsia is usually associated with increased cardiac output and reduced peripheral vascular resistance; the sFlt-1/PlGF ratio may be slightly elevated. This phenotype is better treated with beta-blockers to modulate cardiac output,[26] fluid control, and tailored surveillance to mitigate risks associated with fetal macrosomia or, conversely, late-onset IUGR.

Understanding the heterogeneity of preeclampsia opens avenues for personalized prevention, diagnosis, and management strategies.[26,28,29] By aligning treatment with pathophysiological mechanisms, we aim to improve maternal and fetal outcomes while laying the foundation for future research into targeted therapeutic interventions.

The advantages of single-cell RNA sequencing

In recent years, single-cell RNA sequencing (RNA-seq) has revolutionized our understanding of the cellular building blocks across complex organs in health and disease.[28,30,31] In fact, single-cell RNA-seq has revolutionized our understanding of the cellular diversity and heterogeneity across many tissues,[32–34] including the placenta.[30,31,35–40] The method builds on the detailed profiling of gene expression, thereby enabling cell type cataloging and identification of the molecular dysregulation associated with perturbations or disease in individual cells. Single-cell RNA-seq for molecular distinction of the

FIGURE 1
Proposed classification of PE types I and II

CO, cardiac output; *EFW*, estimated fetal weight; *HTN*, hypertension; *IUGR*, intrauterine growth restriction; *KD*, kidney disease; *MAP*, mean arterial pressure; *PE*, preeclampsia; *PlGF*, placental growth factor; *PVR*, peripheral vascular resistance; *sFlt-1*, soluble Fms-like tyrosine kinase-1; *UtA*, uterine artery.

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different cell types in developing organs, tumors, or complex organs, like the brain or kidneys, has provided significant new understanding of the normal tissue physiology and organ differentiation and the pathophysiology of different disease processes.[41–43]

Unlike bulk RNA-seq, which averages gene expression across cells, single-cell RNA-seq clarifies complex biologic systems by resolving cellular heterogeneity, thereby enabling the identification of rare cell types, such as stem cells or progenitor cells, and uncovering cell-specific regulatory mechanisms.[33] For example, in cancer, single-cell RNA-seq differentiates between tumor microenvironment cell subpopulations, including cancer stem cells, thereby offering insights into precision therapies.[32,34] Its integration with spatial transcriptomics links gene expression with specific anatomic locations and thus enhances the study of cellular interactions, developmental trajectories, and disease processes.

In the context of placental and preeclampsia research, single-cell RNA-seq provides advantages in studying the placenta in preeclampsia by resolving the cellular heterogeneity of the maternal-fetal interface, which bulk RNA-seq cannot achieve. This technology enables the identification of gene expression changes at the level of individual cell types, such as trophoblasts, endothelial cells, immune cells, and stromal cells, thereby ascertaining their specific contributions to preeclampsia pathophysiology. It enables the detection of rare cell populations, such as abnormal stromal or inflammatory cells, that may play critical roles in the pathology and that would be masked in bulk analysis. In addition, single-cell RNA-seq facilitates the analysis of immune cell dynamics, defective trophoblast invasion, endothelial dysfunction, and disrupted cell-cell communication, such as impaired vascular endothelial growth factor or PIGF signaling, which is critical for angiogenesis. It captures temporal changes in gene expression and provides insights into ligand-receptor interactions and cell-specific biomarkers, offering potential pathways for targeted therapies.

Single-cell RNA-seq offers significant advantages but also presents challenges in preeclampsia research, such as sample accessibility (eg, obtaining placental or decidual tissues) and the cost and complexity of the technology. In addition, the multinucleated structure of the syncytium has made it challenging for cell dissociation-based methods, prompting work in single-nuclei RNA-seq.[44–47] Although single-cell RNA-seq captures both cytoplasmic and nuclear transcripts, single-nuclei RNA-seq measures solely nuclear transcripts, which account for only 10% to 15% of the total RNA.[48] The multinucleated syncytium presents challenges in this regard. The prefusion cytotrophoblast, a precursor of the fused syncytiotrophoblast, provides a window for investigation, because it has a similar gene expression

profile as the syncytiotrophoblast but it remains single-nucleated.

Several groups have performed single-cell RNA-seq on first- and second-trimester normal human placentas and early-onset preeclamptic placentas and found new subtypes of known placental cells, regulatory interactions that prevent harmful innate or adaptive immune responses, and interactions that are critical for placentation and reproductive success.[30,31,49] Tsang et al,[35] in their seminal study, focused on cell-free RNA in maternal circulation as a noninvasive way to uncover the cellular dysfunction in early-onset preeclamptic placentas. The researchers validated their findings at the tissue level and performed single-cell RNA-seq on 4 placentas from early preeclamptic and 4 control placentas. They found that the transcriptional heterogeneity of 15 genes involved in cell migration, cell death, and proliferation was significantly more variable in early preeclamptic placentas, whereas DNA damage repair and antigen presentation ontology were more variable in term placentas. They also demonstrated that genes annotated in cell death showed not only higher expression variability but also higher levels of gene expression in early preeclamptic extravillous trophoblast cells. However, in our analysis of their data (kindly shared by the authors), we found variability in sampling between the control and preeclampsia groups; for example, most of the extravillous trophoblast cells examined were derived from 1 sample, which had very few stromal cells when compared with the other control samples.

FIGURE 2
Anatomical organization of the human placental villus

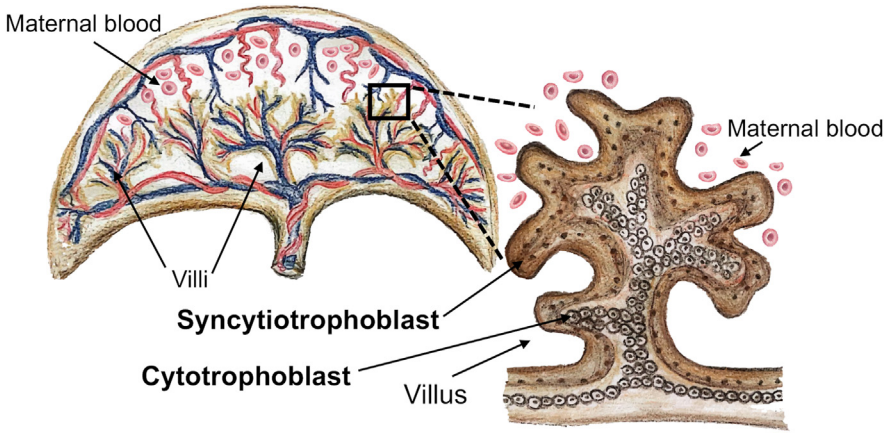

Illustration showing the maternal-fetal interface with key cellular components including syncytiotrophoblast layer in direct contact with maternal blood and underlying cytotrophoblasts.

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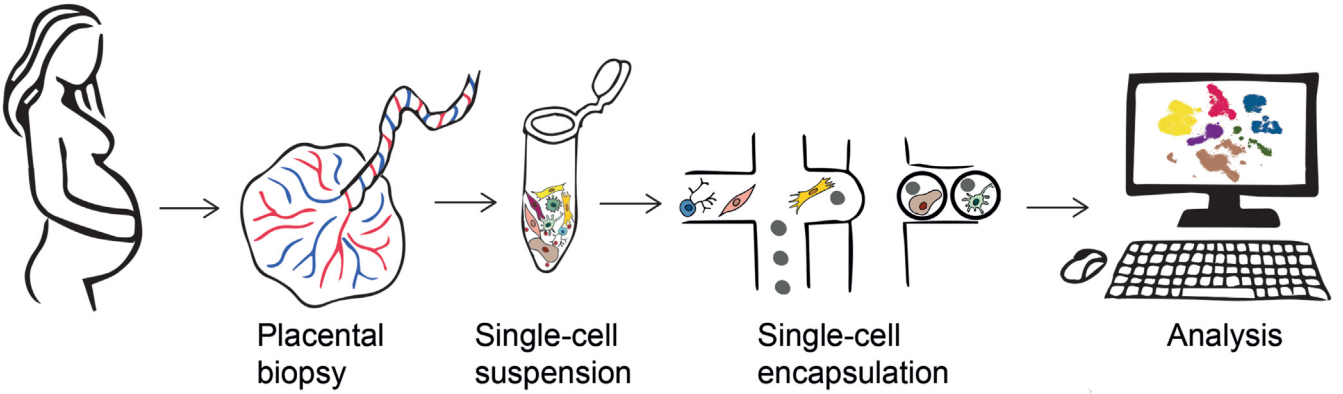
Yang et al[40] performed single-cell RNA-seq on placental and decidual tissues from patients with late-onset preeclampsia and compared it with those of normal pregnancies. Their analysis revealed global developmental deficiencies in trophoblasts, impaired extravillous trophoblast invasion, increased maternal immune rejection, and heightened inflammation in the placenta. In addition, they observed insufficient decidualization of decidual stromal cells and suppressed regulatory functions in decidual immune cells in late-onset preeclampsia cases.[40]

These earlier studies did not compare early- and late-onset preeclampsia cases

FIGURE 3
Experimental workflow for single-cell transcriptomic analysis



Sequential steps from placental biopsy to data analysis enable comprehensive profiling of individual placental cells.

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with controls. Therefore, we collected placental cells from 10 early- and 7 late-onset preeclampsia cases and 3 early and 6 late nonpreeclamptic gestational age–matched controls using single-cell and single-nucleus RNA-seq[13] (Figure 2). Our survey revealed cell type–resolved differences in the transcriptional states and biologic processes specific to early- and late-onset preeclampsia across all major placental cell populations.[13] This approach allowed us to map the cellular and molecular heterogeneity across the placenta's diverse cell populations (Figure 3).

Our single-cell RNA-seq data set comprised about 90,000 cells, representing trophoblasts and stromal, vascular, and immune cells. We validated our findings through single-nucleus RNA-seq and captured additional insights into multinucleated syncytiotrophoblast. By integrating data from both approaches, we created a comprehensive cell atlas, including 46 distinct cell types and states. This allowed us to delineate molecular relationships and functional pathways across the placenta's cellular taxonomy (Figures 4–5).

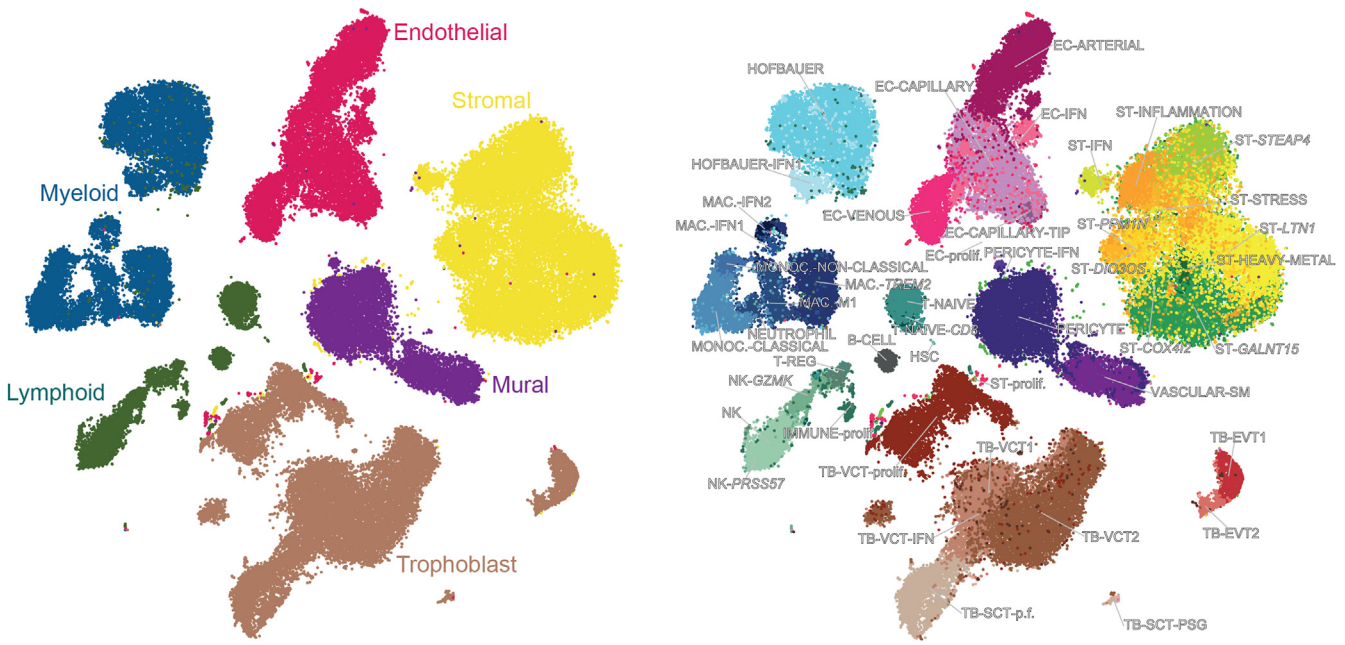
The two faces of preeclampsia

Our comprehensive molecular survey revealed widespread dysregulation in early-onset preeclampsia, particularly in syncytiotrophoblast, stromal cells, and vascular tissue, such as endothelial cells.[12,13,17] Conversely, late-onset preeclampsia demonstrated minimal cellular impact, emphasizing the dramatic differences between the preeclampsia subtypes. The insights gained from these findings will guide future investigations into targeted biomarkers and therapeutic strategies to address this complex disorder.

Early-onset preeclampsia as a disease of profound placental dysregulation

Early-onset preeclampsia emerges from a fundamentally dysregulated placenta, and transcriptomic analyses have revealed significant perturbations across all major cell types. Among these, trophoblast cells—the fetal interface with maternal tissues—displayed the most striking changes (Figure 6). Syncytiotrophoblast and extravillous trophoblasts, in particular, exhibited cell-autonomous dysregulation (ie, in the same cell) along the *FLT1/PGF* axis, a hallmark of angiogenic imbalance (Figure 7).[12,13,17] *FLT1* (encoding the vascular endothelial growth factor receptor-1) was upregulated, whereas its ligand, *PGF* (placental growth factor), was markedly down-regulated.[21,22] This phenomenon, validated by fluorescence in

FIGURE 4
t-SNE demonstrating placental cell taxonomy revealed by single-cell RNA sequencing



Each cell is represented as a single dot, has full gene expression profile, and similar gene expression profiles are modelled by nearby points while dissimilar gene expression profiles are modelled by distant points. Left: Major cell classes identified among about 90.000 cells.
Right showing a detailed map of 46 distinct cell types showing molecular relationships.

EC, endothelial cells; *EVT*, extravillous trophoblast; *HSC*, hematopoietic stem cells; *IFN*, interferon; *MAC*, macrophages; *NK*, natural killer; *PSG*, pregnancy-specific glycoproteins; *SCT*, syncytiotrophoblast; *ST*, stromal; *TB*, trophoblast; *t-SNE*, t-distributed stochastic neighbor embedding; *VCT*, villous cytotrophoblast.

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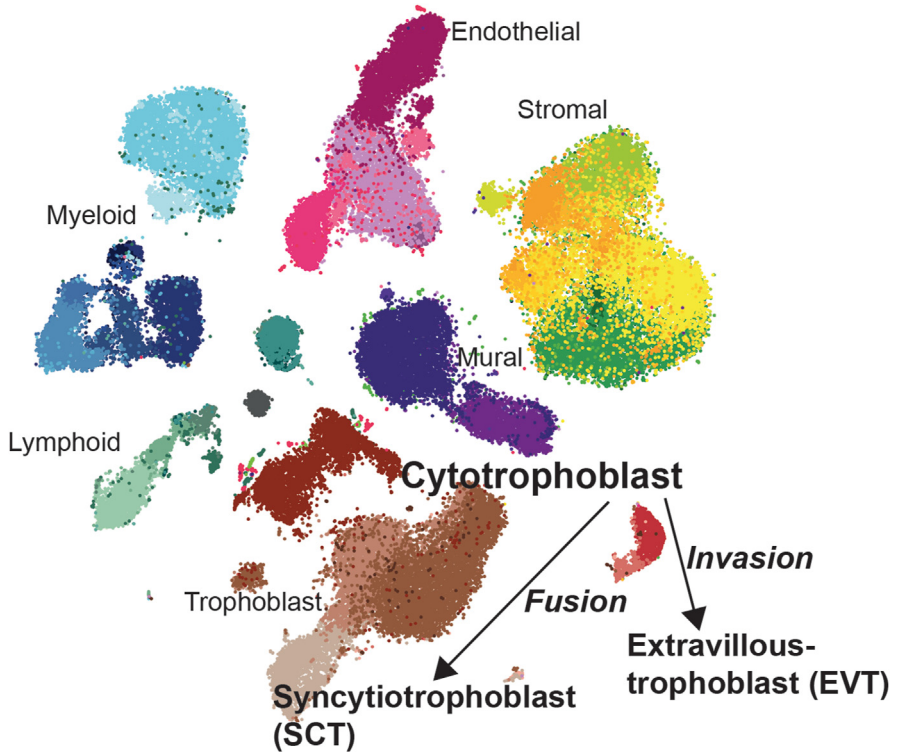
FIGURE 5
t-SNE algorithm of cytotrophoblast differentiation pathways



Single-cell analysis reveals distinct trophoblast populations and their differentiation trajectories toward syncytiotrophoblast or extravillous trophoblast.

t-SNE, t-distributed stochastic neighbor embedding.

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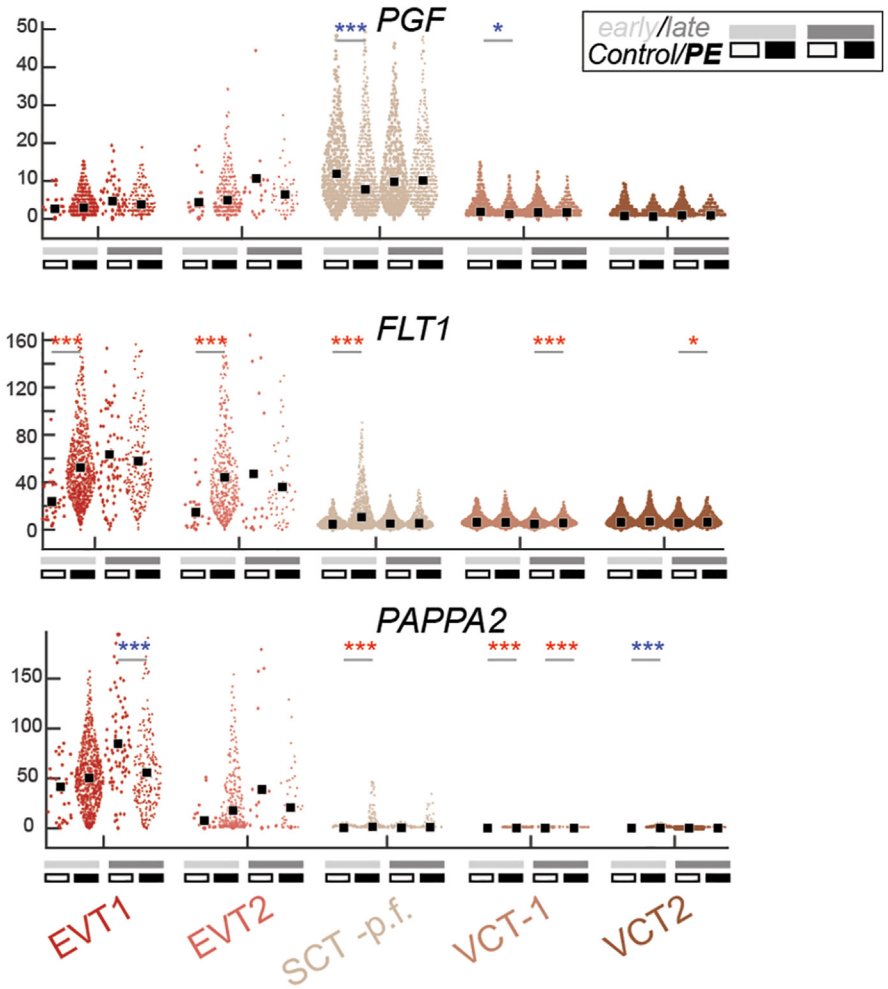
situ hybridization, was exclusive to early-onset preeclampsia, underscoring the pivotal role of placental angiogenic dysfunction in early disease. This cell-level resolution sheds new light on the involvement of various systems in the phenotypes of preeclampsia. Extravillous trophoblast cells, in addition to syncytiotrophoblasts, have increased expression levels of *FLT-1* and *PAPP-A* and reduced *PGF* levels. These are important findings as we strive to devise precision medicine approaches for the treatment of preeclampsia.

The immune landscape of the early-onset preeclamptic placenta is equally distinctive.[50,51] The placental immune niche sets the tone for inflammation in early, but not late, preeclampsia. Triggering receptor expressed on myeloid cells 2 (TREM2) is expressed on macrophages and is known to be overexpressed in many tumor types in which it plays a role in modulating anti-inflammatory responses. Fetal-origin Hofbauer and maternal-origin TREM2 macrophages were found to be involved in these processes, whereas local cells of the adaptive immune system were largely unaffected. Secreted phosphoprotein 1 (SPP1), or osteopontin, a multifunctional glycoprotein involved in bone remodeling, immune regulation, and inflammation, was markedly elevated in early-onset, but not late-onset, preeclampsia.

The marked increase in pro-inflammatory cytokines, including SPP1, C-X-C motif chemokine ligand 2 (CXCL2), and CXCL3, and the altered abundance of immune cell subtypes underscore the role of immune dysregulation in early-onset preeclampsia pathogenesis. Importantly, these findings provide a cellular and molecular basis for the systemic inflammatory state observed in clinical manifestations of early-onset preeclampsia (Figure 8).

Stromal cells, which form the connective tissue scaffold of the placenta, also reflect the profound pathologic environment of early-onset preeclampsia.[49,52] Unique to the stromal compartment, we found that, instead of distinct subtypes, stromal cell clusters resembled cell states that were marked by a handful of more or less distinct genes, some of which were greatly enriched or depleted in preeclampsia. For example, 3 subtypes were marked by cassettes of gene expression related to specific known biologic processes, namely inflammation (*CXCL2, TNFAIP3*, and *BDKRB1*), stress

FIGURE 6
Violin plots of selected key marker genes



Violin plots of selected key marker genes of single-cell origin per EVT1 and EVT2, SCT-p.f., VCT1, and VCT2 (*left*) and all single nucleus—origin trophoblast subtypes (*right*). *Dots* represent single cells. *Red asterisks* indicate statistically significant up-regulation; *blue asterisks* indicate statistically significant down-regulation. ***$P<.0001$, **$P<.001$, *$P<.01$; *black squares* indicate the mean. Adapted from Admati et al (reference 13), with permission from Elsevier.

- early; - late.

- preeclampsia; - control.

EVT, extravillous trophoblast; *Flt-1*, Fms-like tyrosine kinase-1; *PE*, preeclampsia; *PGF*, placental growth factor; *SCT-pf*, syncytiotrophoblast prefused; *VCT*, villous cytotrophoblast.

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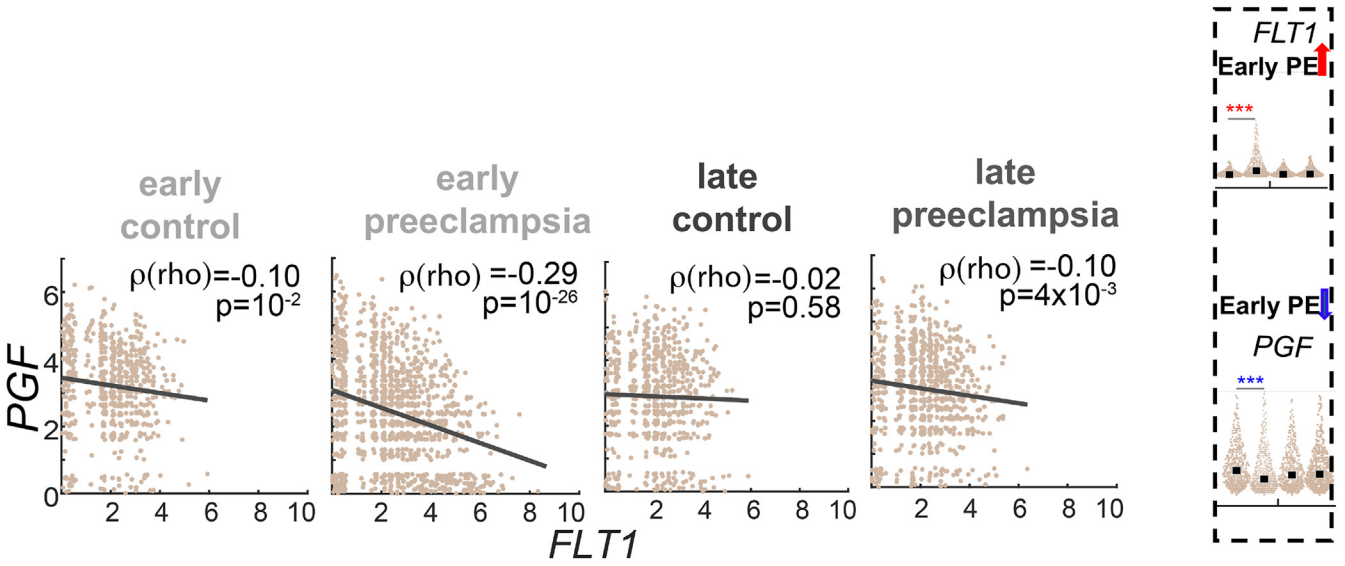
response (*HSPA1A*, *HSPA1B*, and *DNAJB1*, heat shock proteins), or heavy metals response (*MT1A*, *MT1G*, *MT1M*, *MT1E*, and *MT1X*, genes that are part of the metallothionein family, which is critical for heavy metal detoxification, oxidative stress mitigation, and maintaining cellular metal homeostasis). Altered expression of these genes may reflect a compensatory response to systemic inflammation and placental dysfunction. Between patient groups, the inflammation- and stress-responsive states were more abundant in early-onset preeclampsia than in matched controls with considerable patient variability, but there were similar tendencies in late-onset cases.[13]

Aside from the distinct relative abundance of reactive cell states, we subsequently analyzed the differential gene expression between preeclampsia cases and controls in preeclampsia-related processes. We noticed that, in early-onset preeclampsia, the down-regulated genes were related to the ECM and cytoskeleton, whereas stress-related genes were up-regulated. Genes linked to the negative regulation of growth (eg, *CDKN1C* and *LGALS1*) were up-regulated, whereas growth- and proliferation-promoting factors (eg, *EGFL6*, *HGF*, and insulin growth factors) were down-regulated in early-onset disease. Globally, this trend persisted; the most strongly and significantly regulated biologic processes were related to early-onset preeclampsia. We found that processes linked to apoptosis were up-regulated, whereas angiogenesis and blood vessel formation were reduced. These findings are in line with the known pathology of preeclampsia, and our data revealed that stromal cells are prime players.[13]

The transcriptional signatures of these cells were enriched in genes related to inflammation, stress response, and ECM remodeling. Specific gene clusters, including those involved in metallothionein-mediated stress responses and inflammatory signaling pathways, were markedly up-regulated, suggesting a response to chronic oxidative and inflammatory stress. Figure 9 shows a heatmap of genes related to the ECM, cytoskeleton, growth regulation, and stress for each stromal cell type in early- and late-onset preeclampsia in comparison with matched controls. Conversely, genes associated with normal growth and proliferation, such as *IGF*, which is essential in fetal development, tissue repair, and maintaining metabolic homeostasis, were down-regulated, indicating a shift away from homeostasis.

FIGURE 7
Cell-autonomous dysregulation of FLT1/PGF in early PE



Single-cell correlation analysis shows a strong negative correlation between FLT1 and PGF expression specifically in early PE syncytiotrophoblasts, but not in late PE or controls. Right panels show validation of differential expression. This pattern correlates with the sFLT1/PlGF ratio test used by clinicians for PE prediction in patients before the onset of overt signs and symptoms of PE. A cutoff value of 38 to 85 or 38 to 110 shows patients at high risk for developing clinical PE within 4 weeks. (Adapted from Admati et al[13], with permission from Elsevier.)

Flt-1, Fms-like tyrosine kinase-1; *PE*, preeclampsia; *PGF*, placental growth factor; *sFlt-1*, soluble Fms-like tyrosine kinase-1.

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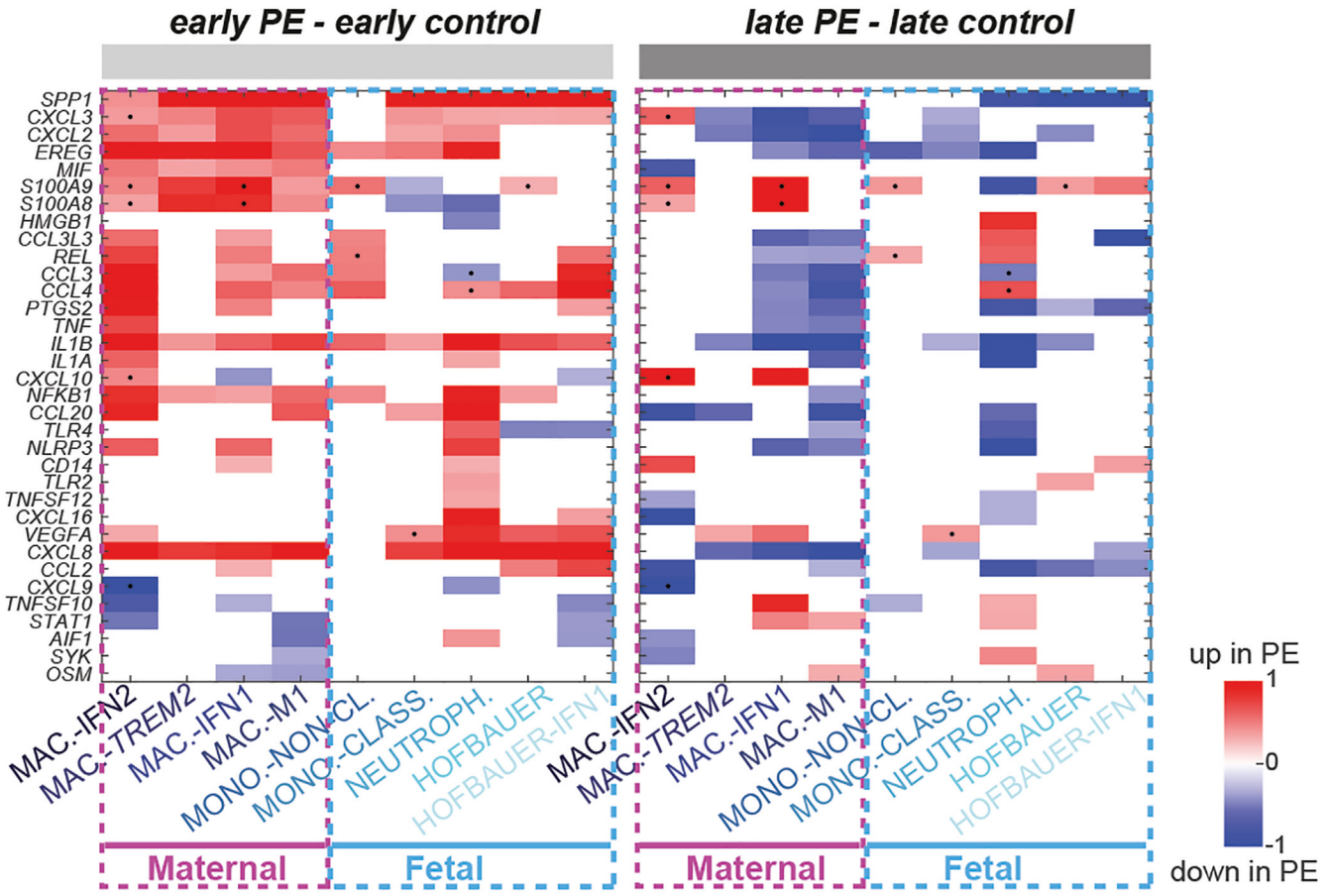
The vascular compartment of the early-onset preeclamptic placentas mirrored these inflammatory changes.[53,54] Both the endothelial cells and pericytes showed evidence of stress, including the up-regulation of genes that encode heat shock proteins and markers of apoptosis (Figure 10). Intriguingly, endothelial tip cells, which are leading cells at the forefront of a growing blood vessel during angiogenesis, highly responsive to guidance cues, critical for proper vascular network formation, and normally associated with active angiogenesis, were paradoxically more abundant in early-onset preeclamptic placentas. However, their angiogenic function seemed to be impaired as evidenced by the dysregulated expression of key genes, such as *FLT1* and *CYR61*, both of which are critical for capillary branching, a hallmark of angiogenesis, and vascular integrity.

Because we analyzed placentas from pregnancies using single-cell and single-nuclei RNA-seq, we compared and validated[55] the 2 methods in 45,836 cells and 27,078 nuclei from 10 and 7 early-onset preeclampsia cases, respectively, and 3 and 2 gestational age—matched controls, respectively, comparing the methods' sensitivities, cell type detection, differential gene expression in preeclampsia, and performing histologic validations. We used in situ validation to estimate the relative abundance of stromal cells in relation to syncytiotrophoblast, which was quantified by 210 counting nuclei in the outer layer of the placental villi and stromal cells (total counted, 211,618 cells) in comparison with their relative abundances detected after cell dissociation and nuclei extraction.[55]

Late-onset preeclampsia: subtle placental contributions

In contrast with early-onset preeclampsia, late-onset preeclampsia demonstrates minimal molecular dysregulation in the placenta with most changes confined to specific cell types and pathways.[13,15] Trophoblasts in late-onset preeclampsia exhibit transcriptional trends that often oppose those seen in early-onset preeclampsia. For example, genes involved in ECM remodeling, such as *PEG10* (a retrotransposon-derived protein crucial for placental development, implicated in promoting trophoblast growth and syncytialization) and *TIMP3* (tissue inhibitor of metalloproteinases 3, a matrix metalloproteinase inhibitor that regulates ECM homeostasis and modulates inflammation and plays a role in tissue remodeling, angiogenesis, and maintaining vascular integrity), are down-regulated in early-onset preeclampsia but up-regulated in late-onset preeclampsia. This suggests that trophoblasts in late-onset preeclampsia may retain some adaptive capacity, potentially mitigating the severity of placental dysfunction.

FIGURE 8
Distinct inflammatory signatures in early vs late PE



Immune cell heatmaps of genes related to inflammatory stress for each immune cell type, in early and late PE in maternal and fetal cells, in comparison with matched controls. Blue, low; red, high; black dots, same direction of change in early and late PE.

IFN, interferon; *MAC*, macrophages; *MONO*, monocytes; *NEUTROPH*, neutrophils; *NON-CL*, nonclassical monocytes; *PE*, preeclampsia.

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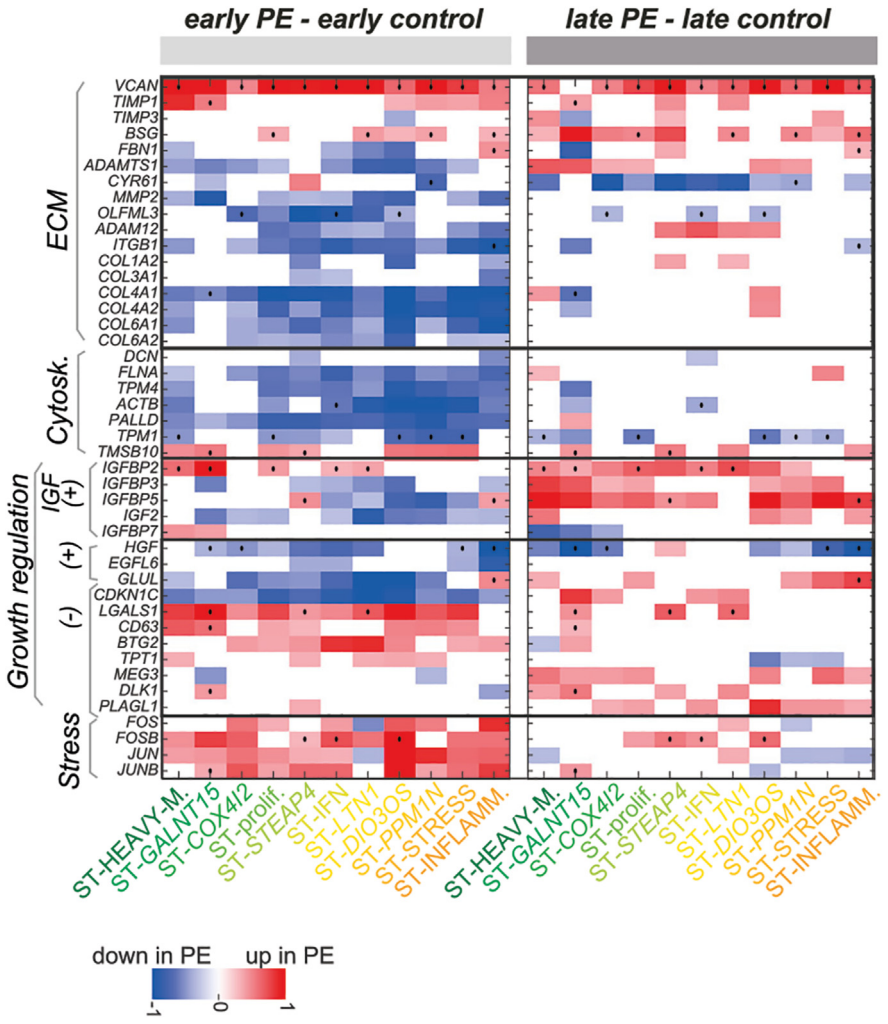
The immune landscape in late-onset preeclamptic placentas also differed significantly from that in early-onset preeclampsia.[50,51] Although pro-inflammatory markers were generally up-regulated in early-onset preeclampsia, late-onset preeclamptic placentas showed either stable or reduced expression of these cytokines. Notably, TREM2 tumor-associated macrophages, which play anti-inflammatory roles, were less abundant in early-onset preeclampsia but preserved in late-onset preeclampsia. This preservation of immune regulatory mechanisms may explain the milder clinical course of late-onset disease (Figure 8).

A unique finding in late-onset preeclampsia was the consistent down-regulation of *CYR61*, an angiogenic factor expressed in vascular cells. Because cysteine-rich angiogenic inducer (CYR61) has a role in promoting vascular adhesion and growth, this suggests that reduced CYR61 may contribute to the subtle placental insufficiency seen in late-onset preeclampsia. However, because widespread vascular or stromal stress markers are absent in late-onset preeclampsia, maternal cardiovascular factors more likely play a role in the pathogenesis of late-onset disease.

Implications for diagnosis and therapy

The molecular divergence between early- and late-onset preeclampsia has far-reaching implications for clinical management. Early-onset preeclampsia, rooted in profound placental dysfunction, represents a primary placental pathology. Therapeutic strategies targeting the angiogenic imbalance, such as interventions that modulate the *FLT1/PGF*

FIGURE 9
Inflammation and stress states override stromal cells' normal function in early-onset PE



Heatmap of genes related to the extracellular matrix (ECM), cytoskeleton (cytosk.), growth regulation, and stress for each stromal cell type, in early- and late-onset preeclampsia in comparison with matched controls. *Blue*, low; *red*, high; *black dots*, same direction of change in early- and late-onset preeclampsia.

Cytosk, cytoskeleton; *ECM*, extracellular matrix; *IFN*, interferon; *IGF*, insulin-like growth factor; *inflamm*, inflammation; *PE*, preeclampsia; *prolif*, proliferation; *ST*, stromal.

Solt. Placenta as single-cell resolution in early and late preeclampsia. Am J Obstet Gynecol 2025.

axis, hold significant promise.[23,24] For example, RNA-based therapies that reduce the sFLT1 levels or recombinant PlGF administration could potentially restore the angiogenic balance and improve maternal and fetal outcomes.

Conversely, the subtler placental involvement in late-onset preeclampsia suggests that therapeutic efforts should focus on maternal cardiovascular health. Interventions aimed at improving vascular compliance, reducing hypertension, and managing metabolic comorbidities could mitigate the progression of late-onset preeclampsia and its associated risks. In addition, the distinct molecular signatures of *CYR61* and other late-preeclampsia–specific markers may aid in identifying patients who would benefit most from such tailored approaches.

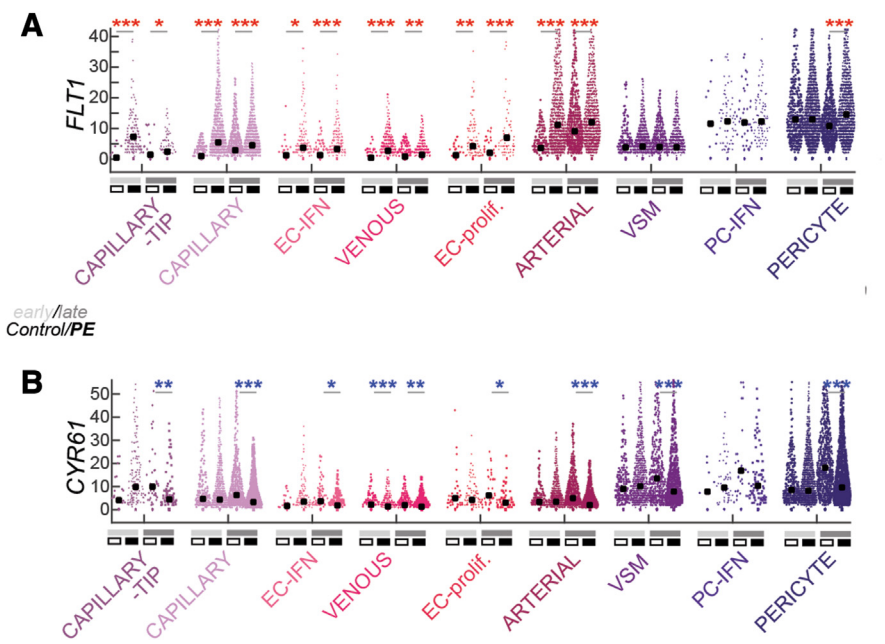
These findings also highlight the potential of transcriptomic biomarkers in the early diagnosis and risk stratification of preeclampsia. The cell-specific dysregulation of *FLT1* and *PGF*, the inflammatory signatures of Hofbauer and TREM2 macrophages, and the stromal stress responses in early-onset preeclampsia could inform the development of diagnostic tools. Similarly, the identification of late-preeclampsia–specific markers, like *CYR61*, could refine the differentiation between preeclampsia subtypes, enabling personalized care strategies.

Discussion and conclusion

The distinction between early- and late-onset preeclampsia, rooted in placental dysfunction and maternal cardiovascular maladaptation, respectively, has emerged as a cornerstone in understanding this complex syndrome.[7,27,56] Our investigation using single-cell and single-nuclei RNA-seq of the placenta was designed to elucidate the differences between early- and late-onset preeclampsia on a molecular, transcriptomic level. This molecular evidence across all cell types—trophoblast, stromal, immune, and endothelial cells——demonstrated the distinct pathophysiology of the 2 types of the disease, bridging clinical research with innovative molecular approaches.[13]

We suggest revisiting the definition of these 2 disease entities as early and late preeclampsia. Similar to DM, which was formerly dubbed juvenile and adult-onset diabetes but was revised to type I and type II DM based on the disparate mechanisms and pathophysiology of the disease and the fact that juvenile diabetes can appear in adulthood and adult-onset diabetes can present in children, we propose that the classification of preeclampsia be revised. An integrated model of type I and type II preeclampsia that

FIGURE 10
Early-onset PE—stressed vasculature exits the functional state



A, FLT1 expression in vascular cell types visualized as a violin plots; *dots* represent single cells. ***$P<.0001$,**$P<.001$,*$P<.01$. *Red*, up-regulation; *black squares*, mean. **B,** CYR61 expression in vascular cell types visualized as a violin plots; *dots* represent single cells. ***$P<.0001$, **$P<.001$,*$P<.01$. *Blue*, down-regulation; *black squares*, mean.

EC, endothelial cells; *IFN*, interferon; *PC*, pericyte; *prolif*, proliferation; *PE*, preeclampsia; *VSM*, vascular smooth muscle.

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highlights the distinctions between early-onset and late-onset preeclampsia beyond the gestational age of presentation and that focuses on the differing angiogenic-antiangiogenic imbalance, maternal hemodynamics, fetal effects, and the distinct placental molecular signatures, should be considered.[7,20,27]

Type I preeclampsia, which in most (but not all) cases presents early, is characterized by profound placental dysfunction, as evidenced by the dysregulation of angiogenic factors, the pro-inflammatory cytokine profiles, and the stromal stress responses.[12,17,18] The *FLT1/PGF* axis plays a pivotal role with sFlt-1 overexpression driving angiogenic imbalance and downstream vascular and immune dysfunction.[21,22,57] These findings implicate the placenta in instigating early-onset disease and highlight its association with severe maternal and fetal outcomes, including IUGR.[30,31]

In contrast, we propose that type II preeclampsia, which most often presents late in pregnancy, usually reflects a failure in the maternal cardiovascular adaptation and other processes and subtler placental involvement.[10,19] This subtype underscores the need to focus on maternal health parameters. The reduced expression of *CYR61* and the preservation of immune regulatory mechanisms highlight the distinct pathophysiology that underlie this phenotype.[50,54] Preeclampsia at term can vary widely in disease severity. Chaiworapongsa et al[14] recently demonstrated that late-onset cases differ in their angiogenic profile and plasma profiles of cytokines and chemokines, essentially showing 2 clusters of disease, with one being characterized by an antiangiogenic state coupled with an excessive inflammatory processes and the other having neither of these features.[14] This heterogeneity in term preeclampsia in terms of its angiogenic and inflammatory profiles deepens our understanding of the spectrum of disease course and clinical outcomes and supports the notion that type I preeclampsia can also develop later in pregnancy.

In addition, we have shown that single-cell RNA-seq has important advantages over single-nuclei RNA-seq for placental transcriptomics. Still, to address the dilemma of an underrepresented syncytium, future studies will benefit from complementary nuclei extraction.[55]

The integration of basic and clinical research provides a robust platform for advancing precision medicine in preeclampsia.[23,58] Molecular insights into the placental and cardiovascular contributions and other processes could enable the development of targeted diagnostic and therapeutic approaches. Identification of subtype-specific biomarkers can refine screening and stratification, whereas interventions that address the angiogenic imbalance or cardiovascular maladaptation may hold promise for tailored treatment strategies.[24,59–63]

Future research should prioritize longitudinal studies to evaluate the effectiveness of these molecularly informed interventions.[26,61] In addition, exploring the interplay between maternal cardiovascular health and placental function across the spectrum of preeclampsia may uncover new preventive and therapeutic avenues.[7,20,25,27]

In summary, the integration of molecular insights into the clinical framework of preeclampsia represents a paradigm shift by transforming our understanding and management of this multifaceted syndrome, well defined as type I and type II preeclampsia. By aligning basic science with clinical needs, this approach offers a potential path forward for improving maternal and fetal outcomes in this enduring obstetrical challenge. ■

GLOSSARY

***BDKRB1* (bradykinin receptor B1):** Encodes a G protein-coupled receptor activated by bradykinin-related peptides and implicated in chronic inflammatory diseases, hypertension, and vascular dysfunction.
CXCL2 and CXCL3 (C-X-C motif chemokine ligand 2 and 3): These chemokines belong to the CXC family and are primarily produced by activated immune cells, including macrophages, neutrophils, and epithelial cells in response to pro-inflammatory stimuli. CXCL3 contributes to tissue repair, angiogenesis, and modulation of extracellular matrix dynamics during wound healing. In preeclampsia, they may drive excessive neutrophil recruitment and activation, thereby contributing to systemic inflammation, endothelial dysfunction, and impaired placental development.
CYR61 (cysteine-rich angiogenic inducer 61): A protein involved in vascular growth and adhesion, with altered expression in late-onset preeclampsia.
DM: diabetes mellitus
Endothelial tip cells: The leading cells at the forefront of a growing blood vessel during angiogenesis, extending filopodia to sense and navigate the extracellular environment, guiding the direction of vessel sprouting, while adjacent stalk cells follow to form the vessel lumen. Tip cells are highly responsive to guidance cues, such as vascular endothelial growth factor (VEGF), and are critical for proper vascular network formation.
Extravillous trophoblast (EVT): A subtype of trophoblast involved in invading the maternal decidua and remodeling spiral arteries to ensure adequate uteroplacental blood flow.
Extracellular matrix (ECM) remodeling: The process by which cells modify their surrounding matrix, with dysregulation implicated in both early- and late-onset preeclampsia.
***FLT1* (fms-like tyrosine kinase-1):** A gene encoding a receptor for vascular endothelial growth factor, elevated in preeclampsia, and associated with antiangiogenic effects. Please see sFlt-1, below.
***HSPA1A, HSPA1B*, and *DNAJB1* (heat shock protein family of genes):** These genes are primarily involved in maintaining protein homeostasis and protecting cells under stress conditions with implications in diseases involving protein misfolding or cellular damage.
Hofbauer cells: Macrophage cells of fetal origin found in the villi, involved in immune modulation, and are particularly significant in the pathophysiology of preeclampsia.
Insulin-like growth factors (IGFs): Peptide hormones structurally similar to insulin. IGF-1 and IGF-2 are vital for placental development and vascular adaptation during pregnancy. IGF-2 is essential for the invasive capacity of trophoblasts; reduced IGF-2 expression leads to shallow trophoblast invasion and inadequate arterial remodeling. In preeclampsia, disruptions in IGF signaling contribute to poor placentation, placental hypoxia, and systemic endothelial dysfunction. IGFs synergize with VEGFs in promoting placental angiogenesis. Reduced IGF-1 and IGF-2 activity exacerbates angiogenic imbalance, compounding the effects of anti-angiogenic factors, such as sFlt-1 (please see below).
KDR (kinase insert domain receptor): A tyrosine kinase receptor, also known as VEGFR-2 (vascular endothelial growth factor receptor 2), primarily involved in mediating the effects of VEGFs. KDR is a key regulator of angiogenesis, vascular permeability, and endothelial cell proliferation, survival, and migration. sFlt-1 (please see below) competes with KDR for VEGF binding, reducing VEGFR-2 activation and impairing angiogenesis.
Maternal-placental-fetal array: The commensal physiological system that encompass the mother, placenta, and fetus and the dynamic crosstalk among them and whose dysfunction can lead to disorders such as preeclampsia.
***MT1A, MT1G, MT1M, MT1E*, and *MT1X* (metallothionein genes):** These genes are part of the metallothionein family, which is critical for heavy metal detoxification, oxidative stress mitigation, and maintaining cellular metal homeostasis. Altered expression of these genes may reflect a compensatory response to systemic inflammation and placental dysfunction.
Osteopontin (OPN), also known as SPP1 (secreted phosphoprotein 1): A multifunctional glycoprotein involved in bone remodeling, immune regulation, and inflammation. It plays a key role in cell signaling, adhesion, and survival with implications in conditions such as cancer, cardiovascular diseases, and autoimmune disorders. Its expression significantly differs between preeclampsia subtypes.
***PEG10* (paternally expressed gene 10)** is a retrotransposon-derived protein crucial for placental development, cellular proliferation, and apoptosis regulation. It exhibits protease and structural functions and is implicated in promoting trophoblast growth and syncytialization. PEG10 is also involved in tumorigenesis and extracellular matrix remodeling, with roles in hemostasis and cancer metastasis.
PlGF: placental-derived growth factor
RNA interference: A therapeutic approach to silence specific gene expression, for example, for to manage sFLT1 levels in preeclampsia.
sFlt-1 (soluble Fms-like tyrosine kinase-1): A circulating anti-angiogenic protein that acts as a soluble receptor for placental growth factor (PlGF) and VEGF, thereby inhibiting their pro-angiogenic activity. Elevated levels of sFlt-1 are implicated in the pathophysiology of preeclampsia and other disorders involving abnormal angiogenesis.
Single-cell RNA sequencing: A technology used to profile gene expression at the single-cell level, enabling detailed exploration of cellular heterogeneity.
Single-nuclei RNA sequencing: Similar to **single-cell RNA sequencing** but focuses on nuclei and is often used for tissues in which the isolation of whole cells is challenging.
TIMP3 (tissue inhibitor of metalloproteinases 3): Protein that regulates extracellular matrix (ECM) homeostasis by inhibiting matrix metalloproteinases (MMPs) and related enzymes like ADAMs and ADAMTS (families of zinc-dependent proteolytic enzymes). It prevents excessive ECM degradation, modulates inflammation, and plays a key role in tissue remodeling, angiogenesis, and maintaining vascular integrity. TIMP3 is unique among TIMPs because it is tightly bound to the ECM.
***TNFAIP3* (tumor necrosis factor alpha-induced protein 3):** Inhibits NF-κB signaling and limits excessive inflammation by terminating pro-inflammatory cytokine signaling. Dysregulation of TNFAIP3 is associated with autoimmune diseases and chronic inflammatory conditions.
TRAM2 (translocation-associated membrane protein 2): A membrane protein localized to the endoplasmic reticulum (ER) that is involved in the translocation of nascent polypeptides across the ER membrane.
TREM2 macrophages: A subset of macrophages that express TREM2 (triggering receptor expressed on myeloid cells 2) with roles in immune surveillance and inflammation modulation, aiding in the recognition and clearance of damaged cells, debris, and pathogens, and in tissue repair and response to injury or infection.

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