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The recent advancements of single-cell RNA sequencing in pre-eclampsia

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Preeclampsia (PE) is a multisystem syndrome that manifests after 20 weeks of gestation, with a global incidence of 2%–8%, and is one of the leading causes of maternal and perinatal mortality. Its etiology is complex, involving multiple mechanisms such as abnormal placentation, immune dysregulation, and angiogenic imbalance, with early-onset PE (EOPE) and late-onset PE (LOPE) exhibiting distinct pathological foundations. In recent years, the application of single-cell RNA sequencing (scRNA-seq) has provided a novel perspective for deciphering the cellular heterogeneity and molecular mechanisms of PE. This review systematically summarizes the latest advances in scRNA-seq applications in PE research, focusing on how this technology reveals: (1) Dysfunction of trophoblast subpopulations and its association with defective spiral artery remodeling; (2) Dynamic changes in the immune microenvironment, including macrophage polarization, functional subsets of uNK cells, and T cell regulatory networks; (3) Cell-specific dysregulation of key signaling pathways; (4) The distinct cytopathological features of early-onset versus late-onset PE. Furthermore, scRNA-seq has facilitated the discovery of multi-gene-based early diagnostic models and potential therapeutic targets. Compared to traditional bulk sequencing, scRNA-seq enables the resolution of cellular heterogeneity, identification of rare cell subpopulations, and elucidation of intercellular communication networks. However, its limitations include difficulties in sample acquisition, high technical costs, complex data analysis, and challenges in capturing multinucleated syncytial structures. In the future, scRNA-seq is expected to provide highly promising therapeutic strategies for PE patients.

KEYWORDS

mechanisms, pre-eclampsia, pregnancy, single-cell RNA sequencing, treatment

1 Introduction

Pre-eclampsia (PE) is a pregnancy-related disorder manifesting after 20 weeks of gestation, with severe ramifications for both the mother and fetus (1). The manifestations of this condition can be categorized into several distinct classifications, including maternal hypertension, proteinuria, and damage to the mother's heart, kidneys, liver, or brain (2, 3). Other manifestations include uteroplacental dysfunction and intrauterine growth

restriction (IUGR) (4). With a global incidence of 2%–8% (5), it poses a significant challenge to obstetricians. The classification of PE is based on the timing of its onset. PE is categorized into two distinct classifications, namely early-onset pre-eclampsia (EOPE) and late-onset pre-eclampsia (LOPE) (6). The former is defined as the onset of the condition before 34 weeks of gestation, while the latter is characterized by the onset of the condition after 34 weeks of gestation. Research indicates that various genetic, angiogenic, structural, and metabolic pathways are implicated in preeclampsia, including spiral artery remodeling, placental oxygenation, redox and immune tolerance at the maternal-fetal interface, and the balance between angiogenic and antiangiogenic factors. The available evidence suggests that EOPE arises from abnormal placental formation, secondary to defective spiral artery remodeling in the uterus (7, 8), the etiology of LOPE is thought to stem from an imbalance between placental aging and maternal susceptibility to cardiovascular and metabolic diseases (9–11).

Single-cell sequencing technology is a technique that utilizes molecular biology to sequence genetic information at the single-cell level. This process involves a series of steps, including single-cell isolation, lysis, amplification, and sequencing (12). In accordance with the objectives of the sequencing process, the following categorizations can be proposed: genome sequencing, transcriptomics sequencing, epigenomics sequencing, proteomics sequencing, and multi-omics integrated analysis (13, 14). Conventional sequencing is conducted at the multicellular level, yielding genetic data that represents the mean of multiple or diverse cell types (14, 15). Single-cell RNA sequencing (scRNA-seq) is a technique that addresses cellular heterogeneity and diversity to elucidate complex biological systems. This methodology facilitates the identification of rare cell types and the detection of molecular dysregulation associated with perturbations or diseases within individual cells. This finding unveils cell-specific regulatory mechanisms, thereby providing novel insights into normal tissue physiology, organ differentiation, and the pathophysiology of various disease processes (16–19).

In recent years, scRNA-seq has emerged as a valuable tool for studying the placenta in preeclampsia, offering a comprehensive approach to address cellular heterogeneity at the maternal-fetal interface. This paper summarizes the application of scRNA-seq in preeclampsia, offering novel insights for its diagnosis and treatment (20–22).

2 Preeclampsia

Hypertensive disorders are prevalent complications of pregnancy, placing women and their fetuses at disproportionate risk of further complications and lifelong sequelae. The severity of gestational hypertensive disorders varies, encompassing chronic hypertension during pregnancy, PE and chronic hypertension complicated by PE (23). Among these, PE carries the highest incidence and mortality rates, contributing to over 70,000 maternal

deaths and 500,000 fetal deaths globally each year (24–26). Symptoms and signs are known to resolve postpartum in most patients, though some may experience persistent or progressive manifestations following delivery (23, 27). New-onset or persistent postpartum PE has emerged as a significant risk factor for perinatal morbidity (28) and is recognized as being associated with substantial cardiovascular disease (CVD) and cerebrovascular disease risk (29).

The etiology and pathogenesis of PE remain incompletely understood. PE is a multifactorial disorder involving multiple mechanisms and pathways, and cannot be explained by a single-factor theory. The predominant theory in this field is the “two-stage” model. Phase 1 is the preclinical stage, characterized by impaired trophoblast remodeling of the spiral arteries, leading to placental ischemia and hypoxia (30, 31). The establishment of uteroplacental circulation is typically completed by 12 weeks of gestation, while the invasion of intravascular and interstitial trophoblast cells is completed between 20 and 22 weeks of gestation, suggesting that this phase occurs between 12 and 22 weeks of gestation (32, 33). Phase 2 involves the secretion of soluble factors such as soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) and soluble endoglin (sEng) by syncytiotrophoblasts, which trigger a systemic maternal inflammatory response, resulting in more extensive and severe clinical manifestations (30, 34).

Trophoblast cells migrate to the spiral arteries of the maternal uterus, forming vascular sinuses at the fetus-maternal interface to supply nutrients to the fetus. In the context of a normal pregnancy, this invasive process extends into the spiral arteries within the uterine myometrium, remodeling maternal arterioles into vessels characterized by high capacity and high flow (7). In placentas that are at risk of PE, trophoblast cells are unable to transition from the proliferative epithelial subtype to the invasive endothelial subtype. This results in incomplete spiral artery remodeling, maternal vascular stenosis, and subsequent relative placental (35).

In addition to uteroplacental insufficiency, inadequate uterine decidualization has also been demonstrated to influence the development of PE (1, 36–39). A plethora of studies have revealed the upregulation of hypoxia-inducible transcription factors (TFs) and hypoxia-related gene expression profiles in the placenta, alongside elevated expression of hypoxia-inducible factor (HIF)-1 α and -2 α in proliferative trophoblast cells and placentas from women with PE. This finding suggests that hypoxia may be a central factor in the pathogenesis of PE (40). Intermittent hypoxia and reoxygenation resulting from inadequate spiral artery invasion have been demonstrated to trigger oxidative stress. Research has identified an imbalance between enzymes that generate reactive oxygen species (ROS) and antioxidants in pre-eclamptic placentas (41, 42). Furthermore, studies indicate that heme oxygenase (HO), a catalyst for heme degradation, plays a crucial role in maternal and fetal vascular function, as well as placental development and function (43, 44).

The contemporary management of PE in developed countries involves a multifaceted approach encompassing pre-pregnancy counseling, perinatal blood pressure monitoring and control, antenatal administration of aspirin for high-risk women, betamethasone for patients less than 34 weeks gestation, intravenous magnesium sulfate, and meticulous postpartum

Abbreviations: PE, pre-eclampsia; IUGR, intrauterine growth restriction; EOPE, early-onset pre-eclampsia; LOPE, late-onset pre-eclampsia; scRNA-seq, single-cell RNA sequencing; CVD, cardiovascular disease; ROS, reactive oxygen species; PCR, polymerase chain reaction; EVT, extravillous trophoblast cells; SMC, smooth muscle cell.

blood pressure monitoring (23). The prompt delivery of the fetus and placenta remains the sole definitive treatment (1). The considerable success achieved with small molecules in treating hypertensive disorders of pregnancy offers novel therapeutic avenues for the treatment of PE.

3 Single-cell RNA sequencing

Tang et al. (45) first reported a sequencing technology capable of achieving high-efficiency, unbiased sequencing of cDNA up to 3,000 base pairs (kb) within a single cell. This technology is regarded as the first relatively complete generation of scRNA-seq technology.

In recent years, single-cell transcriptomics has achieved significant technological breakthroughs, with costs being reduced markedly while automation and throughput have increased substantially. scRNA-seq is a technique that allows for the analysis of transcriptomes at the single-cell level, thereby enabling the study of millions of cells in a single experiment. This methodology facilitates the classification, characterization and distinction of individual cells at the transcriptional level, thereby enabling the identification of rare yet functionally significant cell populations (17, 46, 47).

Single-cell RNA sequencing offers the advantages of high resolution, high sensitivity, and the ability to dynamically monitor transcriptional profiles, making it particularly suitable for analyzing tissues that are difficult to dissociate (48, 49) or samples containing cells with irregular sizes and shapes (50). The workflow of the method is comprised of the following stages: single-cell isolation and capture, reverse transcription and cDNA amplification, establishment of single-cell libraries, high-throughput sequencing, and data analysis (17, 51). The process of cell separation may be achieved through a variety of methodologies, including the isolation of entire cells, cell-specific nuclei, or cell-specific organelles. Alternatively, the isolation of desired cells expressing specific marker proteins can be employed. The ability to swiftly, precisely and efficiently capture individual cells continues to represent a significant challenge in the realm of single-cell sequencing (52, 53). Conventional microscopy techniques have been utilized to capture individual cells from low-abundance samples; however, this approach is laborious and has a low throughput capacity. The methodology employed for the isolation and capture of individual cells varies considerably depending on the organism, the nature of the tissue, or the characteristics of the cells in question (54). Conventional single-cell isolation and capture techniques encompass limiting dilution, fluorescence-activated cell sorting (FACS), and laser microdissection (LCM) (17). Recent technological advancements have resulted in a notable enhancement in the efficiency, scale, and precision of single-cell isolation, primarily through the utilization of cell sorting platforms based on magnetic-activated cell sorting (MACS) and microfluidics. These methodologies are now the preferred approach for single-cell separation on transcriptomic platforms (55–58).

The process of cDNA synthesis and polyadenylation of RNA enrichment is both achieved through reverse transcription. However, it is estimated that only 10%–20% of transcripts undergo

reverse transcription (59). This step is critical, given the highly dependent nature of scRNA-seq efficiency. With the exception of the primer-designed RNA sequencing method DP-Seq (60), all published studies employ poly(T) primer approaches (61–63).

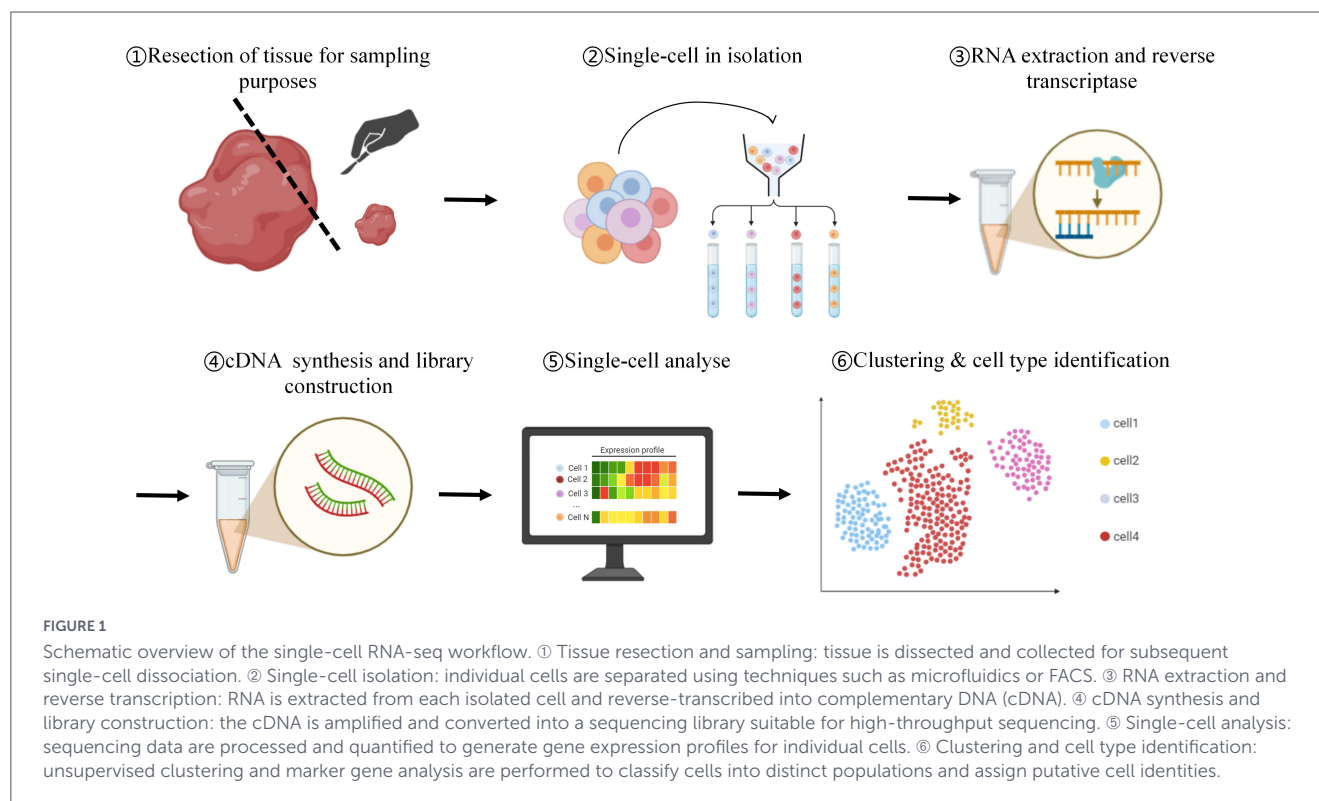
Second-strand synthesis in reverse transcription can be achieved via polyadenylation tailing (64) or template switching mechanisms and STRT-Seq technology (63). The former method is rapid, but it introduces amplification errors, with termination of the reverse transcriptase reaction reducing coverage at the 5' end of transcripts. The latter method's primary benefit is its capacity to obtain full-length transcript coverage, thus reducing 3' end coverage bias caused by incomplete reverse transcription. However, it is important to note that the sensitivity of this method is lower than that of the previously mentioned method (62, 65).

Subsequent to the conversion of RNA into the first strand of cDNA, the resulting cDNA is amplified via polymerase chain reaction (PCR) or *in vitro* transcription (IVT) (66). PCR is a non-linear amplification process, achieved through two techniques. The first of these is SMART technology, the most commonly employed method for cDNA amplification (67). The alternative technique involves the ligation of poly(A) or poly(C) to the 5' end of cDNA, thus constructing universal adapters for PCR reactions (17). IVT is a linear amplification process that necessitates an additional round of reverse transcription on amplified RNA, thereby introducing further 3' coverage bias (17). It is important to note that both of these methods have the potential to induce amplification bias. In order to overcome amplification-related biases, unique molecular identifiers (UMIs) were incorporated during the reverse transcription step, thus enabling the barcoding of individual mRNA molecules within a cell. This enhanced the quantitative nature of scRNA-seq and improved read accuracy by effectively eliminating PCR amplification bias (68).

Once single-cell barcoded cDNA has been generated from a single cell or mononuclear cell, it can be sequenced using numerous deep sequencing platforms. In high-throughput sequencing based on DNA nanoballs (DNBseq), selected DNA fragments are repaired to blunt ends and modified at all three ends to incorporate dATP overhangs. Subsequently, dTTP tailing sequences are ligated to each end of the DNA fragments. Subsequent to this, the ligation products are amplified through several cycles prior to undergoing the next single-strand cycle. A specific strand of the PCR product undergoes reverse complementary ligation with a specific molecule, which is subsequently joined to the single-stranded molecule via DNA ligase, ultimately yielding a single-stranded circular DNA library (17).

The analysis of scRNA-seq data involves a series of procedures, including the alignment of sequencing fragments, the implementation of quality control (QC) measures, quantification, data normalization, the removal of confounding factors, dimensionality reduction, feature selection, clustering analysis, and downstream data interpretation (1) (Figure 1).

As a crucial method for revealing cellular metabolites and expressed genes, scRNA-seq is primarily employed to analyze interactions between cellular subpopulations and gene regulatory processes (69). Its application is currently extensive, encompassing diverse research domains, including embryonic development (70, 71), tissue and organ development (72), and studies of tumors and the immune system (73).



4 scRNA-seq and the mechanisms of PE

Campbell et al. (74) collected villous placental tissue biopsies from placentas shortly after delivery from healthy, full-term, singleton uncomplicated Cesarean sections. After cutting away the basal and chorionic plates and scraping the villous tissue, they performed dissociation to generate single-cell RNA sequencing samples. By integrating these samples with existing data, they established the largest deconvolution reference to date, encompassing 19 fetal and 8 maternal cell types. Their findings revealed that significant placental cellular heterogeneity in preeclampsia contributes to the numerous previously observed gene expression differences, laying the foundation for studies investigating the role of cellular heterogeneity in placental dysfunction and adverse birth outcomes. Muñoz-Blat et al. (75), through scRNA-seq of endometrial samples from patients with severe preeclampsia (sPE), revealed a cellular composition characterized by a stromal mosaic state, featuring proliferating stromal cells (MMP11⁺ and SFRP4⁺) alongside IGFBP1⁺ decidualized cells, concurrent with an epithelial mosaic and an absence of epithelial–stromal transformation associated with decidualization.

Early-onset and late-onset preeclampsia exhibit distinctly different cellular pathological features. Solt et al. (76), through single-cell and single-nucleus RNA sequencing analysis of approximately 90,000 placental cells, found that early-onset preeclampsia (EOPE) is characterized by widespread dysregulation across multiple cell lineages, including disrupted angiogenic signaling pathways in syncytiotrophoblasts and extravillous trophoblasts (upregulation of FLT1, downregulation of PGF), stress responses in stromal and vascular compartments, and

significant upregulation of pro-inflammatory cytokines (e.g., SPP1, CXCL2, CXCL3) in maternal-fetal immune cells. In contrast, late-onset preeclampsia (LOPE) only exhibited focal dysregulation of extracellular matrix remodeling and angiogenesis markers, retaining certain features of placental adaptability.

Immune cell dysfunction is considered one of the mechanisms of PE. Multiple studies point to the roles of macrophages, NK cells, and T cells in the pathogenesis of PE. Fei et al. (77) demonstrated that transferring F4/80⁺CD206⁺ pro-inflammatory monocytes with a Fcrl2⁺Ccl7⁺Ccl8⁺C1qa⁺C1qb⁺C1qc⁺ phenotype from the uteri of PE-like mouse models into normal pregnant mice could promote the generation of memory-like Th17 cells via the IGF1-IGF1R pathway, thereby inducing the occurrence and recurrence of preeclampsia-like symptoms. This study revealed a PE-specific immune cell network regulated by pro-inflammatory monocytes, providing new insights into the pathogenesis of the disease. Uterine natural killer (uNK) cells are thought to regulate trophoblast invasion and spiral artery remodeling (78). Research by Whettlock et al. (79) also revealed that uNK cells can be divided into three functionally distinct subsets, playing key roles in embryo implantation and spiral artery remodeling. Xiao et al. (80), using scRNA-seq and Gene Set Variation Analysis (GSVA) on placental and decidual tissues for differential expression, cell reclustering, and pseudotime analysis, discovered that understanding how T cells differentiate during early pregnancy and how the CD8⁺ T-cell-dominated immune environment affects villous cytotrophoblast (VCT) differentiation is crucial for elucidating the pathogenesis of PE. Rong et al. (81) downloaded raw single-cell sequencing data and gene expression matrices from the Gene Expression Omnibus (GEO) and found that aberrant gene expression affects the efferocytosis function of decidual macrophages, subsequently impacting their

interaction with other immune cells, thereby disrupting the original immune regulatory mechanism and ultimately leading to the occurrence of preeclampsia. Couture et al. (82) have demonstrated that placental CD163⁺ cells and 1st trimester blood pressures can be valuable non-invasive and predictive biomarkers of PPPE with strong clinical application prospects.

Notably, immune cells in early-onset preeclampsia exhibit a more pronounced pro-inflammatory activation state, whereas immune alterations in late-onset preeclampsia are relatively limited (76) (Table 1).

Dysregulation of specific signaling pathways has been validated across multiple independent studies. For example, Solt

TABLE 1 Summary of representative studies applying single-cell RNA sequencing (scRNA-seq) to investigate preeclampsia (PE).

Data set	Sample source	Tissue type	Time	Key findings
Campbell et al. (74)	Healthy, singleton, uncomplicated pregnant women	Placenta	After term Cesarean section	Generated single-cell RNA sequencing samples, integrated with existing data to create the largest deconvolution reference for 19 fetal and 8 maternal cell types.
Muñoz-Blat et al. (75)	Non-pregnant women: with a history of previous severe preeclampsia (sPE) pregnancy and with a history of previous normal pregnancy	Endometrial	Late secretory phase of the menstrual cycle	Revealed DR cell and molecular characteristics associated with former sPE patients.
Liu et al. (84)	PE patients, mouse models	Placenta	/	CD74 plays an important protective role in the pathogenesis of PE by regulating the MAPK signaling pathway, which can be regulated by SIRT1.
He et al. (85)	Pregnant mice (PE-like group, normal control group)	Cell types expressing NEP in the placenta	/	NEP, as a protein cargo in PL-EVs, links the two developmental stages of preeclampsia by disrupting endothelial CNP-dependent VSMC relaxation, thereby contributing to the clinical manifestations of preeclampsia.
Liu et al. (88)	EOPE group, normal control group	Placental and decidual tissue	After term pregnancy Cesarean section	The expression of DAB2 in EVT is crucial for coordinating the phenotypic switch and motility of dVSMCs. These processes may be closely related to the CXCL8/PI3K/AKT pathway, highlighting its core role in complex SPA remodeling.
Botha et al. (86)	EOPE group, normal control group	Maternal plasma	36 weeks of gestation	Cystatin 6 (CST6) and Legumain (LGMN) are potential mediators in the pathogenesis of PE.
		Placenta	Within 30 min after term delivery	
Xiao et al. (80)	PE group, normal control group	Placental and decidual tissue	After term pregnancy Cesarean section	Revealed cellular dysfunction at the maternal-fetal interface in preeclampsia patients.
Rong et al. (81)	PE group, normal control group	Gene Expression Omnibus	/	Dysfunction of decidual macrophages is a potential risk factor in the occurrence of PE.
Fei et al. (77)	PE patients, mouse models	Uterus from pregnant mice and placentas from volunteers	/	Revealed the PE-specific immune cell network, which was regulated by pro-inflam Macs, providing new ideas about the pathogenesis of PE.
Jiang et al. (83)	PE group, normal control group	Placenta	Within 10 min after term pregnancy Cesarean section	Identified JUNB as a key gene in macrophages within PE; interfering with JUNB expression can promote macrophage polarization toward the M2 phenotype. Research indicates JUNB affects PE through the MIIP/PI3K/AKT pathway.
Huang et al. (93)	PE group, normal control group	Peripheral venous blood	11 ⁺ 6–13 weeks of gestation, 4–7 days before delivery	A2M is a potential target for EOPE, providing initial therapy for inhibiting the A2M-low density lipoprotein receptor-related protein 1 (LRP1) combination.

(Continued)

TABLE 1 (Continued)

Data set	Sample source	Tissue type	Time	Key findings
Li et al. (92)	PE group, normal control group	Decidual tissue	6–8 weeks of gestation, late pregnancy	KIR ⁺ CD8 ⁺ T cells increase during human pregnancy, potentially promoting maternal tolerance by inhibiting fetal-specific T cells.
		Human peripheral blood and peripheral blood mononuclear cell (PBMC)	First trimester, second trimester, third trimester	

In this table summarizes key studies cited in this review, detailing data sources, sample sources, tissue types, timing of sample collection, and major findings related to PE pathogenesis, immune dysregulation, and therapeutic targets. EOPE, early-onset preeclampsia; EVT, extravillous trophoblast; dVSMC, decidual vascular smooth muscle cell; NEP, neprilysin; PL-EVs, placental extracellular vesicles; VSMC, vascular smooth muscle cell; SPA, spiral artery; CST6, cystatin 6; LGMN, legumain; A2M, alpha-2-macroglobulin; LRP1, low-density lipoprotein receptor-related protein 1; KIR, killer-cell immunoglobulin-like receptor; PBMC, peripheral blood mononuclear cell; “/” indicates not reported or not applicable.

et al. (76) confirmed the prevalence of FLT1/PGF imbalance in early-onset preeclampsia. Jiang et al. (83) revealed a novel mechanism regulating macrophage polarization through the JUNB-MIIP-PI3K/AKT axis. Liu et al. (84) discovered through scRNA-seq technology that the MAPK signaling pathway, which can be regulated by SIRT1, plays an important protective role in the pathogenesis of PE. He et al. (85) intravenously injected extracellular vesicles (EVs) from normal placentas and those complicated by preeclampsia into pregnant mice and recorded blood pressure throughout gestation. They found that PE placental EVs (PE-PL-EVs) induced preeclampsia-like clinical manifestations in pregnant mice, including hypertension, proteinuria, and fetal growth restriction. Botha et al. (86), through computational analysis of two publicly available single-cell and single-nucleus RNA sequencing datasets, discovered an inverse relationship between CST6 and LGMN in the placenta and the maternal circulation in preeclampsia. They thus proposed that elevated circulating levels of CST6 might be caused by placental hypoxia. Cysteine-rich angiogenic inducer 61 (CYR61), also known as CCN1, is widely distributed throughout the body, particularly in vascular-rich tissues, developing embryonic structures, and tissues requiring repair. Previous studies have reported that CCN1 promotes cell adhesion, proliferation, migration, and angiogenesis. Analysis of public single-cell RNA sequencing datasets revealed that CYR61 expression is significantly downregulated in EVT cells of preeclamptic placentas. The G protein-coupled estrogen receptor (GPER) is expressed in human EVT cells and has been linked to promoting cell invasion. Wang et al. (87) identified a novel GPER-YAP-Snail-CYR61 signaling axis that regulates EVT invasion and suggested that dysregulation of this pathway may contribute to defective trophoblast invasion in PE. Liu et al. (88) performed single-cell RNA sequencing analysis on whole placental tissues from patients with early-onset preeclampsia (EOPE) and their corresponding controls. From genes including DAB2, MMP2, MMP9, α -SMA, SM22 α , Calponin, MYH11, GAPDH, IL20R, CCL20, IL1A, CXCL2, CXCL1, CXCL6, CXCL3, TGFA, and IL21R, they selected DAB2 as the key gene of interest. Through cell models and a placenta-decidual co-culture (PDC) model, they explored the mechanisms of communication between extravillous trophoblasts (EVT) and decidual vascular smooth muscle cells (dVSMC) *in vitro*, discovering the core role of DAB2 in complex spiral artery remodeling.

Notably, several genes closely associated with the pathogenesis of PE are genomically imprinted genes, whose expression is tightly regulated by epigenetic mechanisms such as DNA methylation (89). For example, IGF2 (insulin-like growth factor 2) and H19 are a classic imprinted gene pair that play central roles in placental development and fetal growth. Loss of imprinting of IGF2/H19 in trophoblast cells may lead to overexpression or silencing of IGF2, thereby affecting placental nutrient delivery and vascular remodeling (90). In addition, imprinted genes such as CDKN1C (p57KIP2), PEG10, and PLAGL1 are highly expressed in placental trophoblast cells and participate in cell proliferation, differentiation, and vascular remodeling. Among these, aberrant methylation of CDKN1C may interfere with the decidualization process and contribute to the pathogenesis of PE (91) (Table 1).

5 scRNA-seq and the treatment of PE

Single-cell RNA sequencing has not only deepened the understanding of PE pathogenesis but also provided clues for the discovery of therapeutic targets.

Targeting the polarization state of immune cells may serve as a therapeutic approach for PE. Jiang et al. (83) utilized single-cell RNA sequencing (scRNA-seq) to delineate the cellular landscape of the placenta in PE, identifying 11 distinct cell subsets, among which macrophages played a key role in mediating intercellular communication. They discovered that the transcription factor JUNB is a key gene in macrophages, influencing the interactions between macrophages and epithelial as well as endothelial cells. Interference with JUNB expression promoted macrophage polarization toward the M2 phenotype, thereby facilitating trophoblast invasion, migration, and angiogenesis. The study further demonstrated that targeting JUNB could activate the PI3K/AKT pathway via transcriptional activation of MIIP, thereby promoting M2 polarization and potentially delaying the onset of PE. Similarly, Li et al. (92), through analysis of peripheral blood and peripheral blood mononuclear cells (PBMCs) from pregnant women, found that KIR⁺CD8⁺ T cells may promote maternal tolerance by regulating fetal-specific alloreactive T cell responses, and

that higher frequencies of KIR⁺CD8⁺ T cells are associated with preeclampsia.

Intervening in specific signaling pathways is also a research direction. Huang et al. (93), through scRNA-seq analysis of peripheral venous blood samples, placental, and decidual tissues from pregnant women, found that smooth muscle cell (SMC) α 2-macroglobulin (A2M) promotes the progression of preeclampsia by directly upregulating RhoA-GTPase. Their results also indicated that A2M is a potential target for EOPE, providing initial therapeutic insights for inhibiting the A2M-low density lipoprotein receptor-related protein 1 (LRP1) combination. Research by Huang et al. (94) found that c-Fos silencing inhibits p-AMPK (phosphorylated AMP-activated protein kinase)/deacetylated tubulin pathway, disrupting lipid droplet transport and metabolism, promoting lipid droplet accumulation and altered lipid profiles. c-Fos deficiency induced a preeclampsia-like phenotype in mice, while c-Fos overexpression partially alleviated preeclampsia symptoms. This suggests that c-Fos is a potential therapeutic target for preeclampsia.

Many studies explore novel therapeutic approaches for PE by identifying key genes. Zhong et al. (95) integrated preeclampsia-specific genome-wide association study (GWAS) data, expression and protein quantitative trait loci (eQTL and pQTL) data, as well as single-cell data from peripheral blood mononuclear cells (PBMCs). Using scRNA-seq technology, they identified and highlighted significant cellular differences, determining IFITM3, NINJ1, COTL1, CD69, and YWHAZ as key genes associated with PE pathogenesis. Ruan et al. (96) utilized scRNA-seq to identify shared hub genes and immune pathways in PE, characterized by crosstalk between BIN2, LYN, NEDD9, and PIK3AP1, shedding light on the pathogenesis of PE, which may pave the way for developing effective diagnostic, therapeutic, and management strategies.

Early-onset and late-onset preeclampsia may require specific therapeutic strategies. Based on single-cell atlases, Solt et al. (76) proposed that early-onset preeclampsia could benefit from interventions targeting angiogenic pathways, whereas the management of late-onset preeclampsia should focus on optimizing maternal cardiovascular function. Research by Bernier et al. (97) further confirmed significant differences in immune cell profiles between early-onset and late-onset preeclampsia patients, as well as distinct effects on the vascular endothelium, suggesting that treatment strategies must consider subtype specificity. Zheng et al. (98) discovered that HSD17B1 is a major coordinator of trophoblast-endocrine crosstalk, and that impaired trophoblast crosstalk in placental trophoblasts may contribute to EOPE pathogenesis. This provides a mechanistic basis for developing HSD17B1-targeted interventions that may help simultaneously restore placental capacity and hormonal regulation, thereby improving perinatal outcomes in EOPE patients.

6 scRNA-seq and the diagnosis and risk prediction of PE

Over the past 30 years, the incidence of gestational hypertension and preeclampsia has increased alarmingly, posing a serious threat to the life safety of pregnant women. Therefore,

early prediction of risk and assessment of patient prognosis are of paramount importance (99).

Polygenic combination models are considered a tool for PE diagnosis and risk prediction. Xiong et al. (100), by integrating WGCNA and machine learning algorithms, screened five characteristic genes from 101 metabolism-related genes to construct a predictive model, enhancing predictive efficacy. Four apoptosis-related genes identified by Liu et al. (101)—CD44, MIF, PIK3R1, and TLR4—were also integrated into a diagnostic nomogram, demonstrating good clinical utility. Tarca et al. (102) found that placenta cell-specific mRNAs in maternal peripheral blood, such as H19, FN1, TUBB6, and FPR3, could predict the future development of PE, offering new insights for non-invasive early screening. Sun et al. collected placental and plasma samples from pregnant women to detect TPBG (trophoblast glycoprotein) expression and explored the downstream molecular mechanisms of TPBG using RNA sequencing and single-cell RNA sequencing data. They discovered that TPBG overexpression increases the risk of preeclampsia through its disruptive effects on trophoblast and extravillous trophoblast migration/invasion during spiral artery remodeling; consequently, TPBG levels in maternal blood may serve as a predictor of PE risk (103).

Different PE subtypes possess specific markers. Guo et al. (104) systematically compared the transcriptomic features of early-onset and late-onset PE, identifying subtype-specific biomarkers such as EBI3, IGF2, ORMDL3, GATA2, and KIR2DL4, and validated them at the protein level. Liu et al. (101) also found that CD44, MIF, and PIK3R1 exhibited opposing expression trends in early-onset versus late-onset PE (downregulated in early-onset, upregulated in late-onset), reflecting the placenta's adaptive response to different adverse pregnancy conditions (Table 1).

7 Conclusion and prospect

Recent years have seen a major advancement in our understanding of the cellular composition of complex organs in health and disease through the application of scRNA-seq (105–107). This technological advancement has also facilitated a more profound comprehension of cellular diversity and heterogeneity across diverse tissue (16, 108, 109), including the placenta (22, 110–114). scRNA-seq enables a comprehensive analysis of gene expression, thus enabling the cataloging of cell types and the identification of molecular dysregulation associated with perturbations or disease at the level of individual cells.

In the context of placental and PE research, scRNA-seq offers the following advantages: Firstly, in contrast to batch RNA-seq, scRNA-seq is capable of addressing cellular heterogeneity at the maternal-fetal interface, thus providing a distinct advantage for the study of the placenta in PE. Secondly, scRNA-seq has the capacity to identify alterations in gene expression, including temporal variations, at the single-cell type level (e.g., trophoblasts, endothelial cells, immune cells, and stromal cells). This process enables the determination of their specific contributions to the pathogenesis of PE and the identification of potential pathways for targeted therapies. Thirdly, scRNA-seq has the capacity to detect rare cell populations that are potentially pivotal in

the context of PE pathology, such as aberrant stromal cells or inflammatory cells. Such cell populations may be obscured in batch analyses. Fourthly, scRNA-seq facilitates analysis of immune cell dynamics, trophoblast invasion defects, endothelial dysfunction, and disrupted intercellular communication, thereby aiding exploration of angiogenesis mechanisms.

Single-cell RNA sequencing is also subject to certain limitations, including the accessibility of samples (e.g., the procurement of placental or decidual tissue) and the cost and complexity of the technique itself. Furthermore, scRNA-seq faces challenges in addressing the multinucleated structure of syncytiotrophoblasts, necessitating the employment of single-nucleus RNA-seq technologies to overcome this shortcoming (1, 115–118).

This review systematically elaborates on the significant role of single-cell RNA sequencing (scRNA-seq) in preeclampsia (PE) research and distills the following core perspectives through integrative analysis: First, early-onset and late-onset PE exhibit fundamentally distinct cytopathological features, with the former characterized by multi-lineage placental cell dysfunction and the latter by relatively localized molecular alterations. Second, dysregulation of the immune microenvironment is a common pathway in PE, though its manifestations are subtype-specific. Third, angiogenic imbalance is a mechanism validated across multiple cross-validated studies. Fourth, signaling pathways such as PI3K/AKT and MAPK have been identified as key regulatory nodes with potential as therapeutic targets. Fifth, polygenic combination-based predictive models and liquid biopsy technologies offer new avenues for the early diagnosis and risk prediction of PE.

However, current research still faces numerous challenges, including a lack of unified criteria for defining cell subpopulations, limited overlap in key genes across studies, heterogeneity in results arising from differences in technological platforms and methodologies, and bottlenecks in translating mechanistic discoveries into clinical applications. Most findings lack validation in large-scale prospective cohorts, and functional experiments alongside clinical translational research urgently need to be strengthened. Consequently, further in-depth investigation is required. Looking ahead, the future holds promise for offering highly promising therapeutic strategies for patients with PE.

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