

Can single-cell and spatial omics unravel the pathophysiology of pre-eclampsia?

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ABSTRACT

Pre-eclampsia is a leading cause of maternal and fetal morbidity and mortality. Characterised by the onset of hypertension and proteinuria in the second half of pregnancy, it can lead to maternal end-organ injury such as cerebral ischemia and oedema, pulmonary oedema and renal failure, and potentially fatal outcomes for both mother and fetus. The causes of the different maternal end-organ phenotypes of pre-eclampsia and why some women develop pre-eclampsia condition early in pregnancy have yet to be elucidated. Omics methods include proteomics, genomics, metabolomics, transcriptomics. These omics techniques, previously mostly used on bulk tissue and individually, are increasingly available at a single cellular level and can be combined with each other. Multi-omics techniques on a single-cell or spatial level provide us with a powerful tool to understand the pathophysiology of pre-eclampsia. This review will explore the status of omics methods and how they can and could contribute to understanding the pathophysiology of pre-eclampsia.

1. Introduction

Pre-eclampsia is a pregnancy specific multisystem disorder that develops in the second half of pregnancy. Pre-eclampsia is commonly diagnosed by new-onset hypertension in pregnancy and significant proteinuria (Chappell et al., 2021). The natural course of pre-eclampsia, if untreated, results in maternal end-organ complications and therefore it is one of the major causes for maternal mortality. These maternal

deaths are caused by pulmonary oedema, cerebral complications, acute renal failure, haemolysis elevated liver enzymes low platelet syndrome (HELLP) or eclampsia (generalised seizures) (Babazhanova et al., 2021). Additionally, stillbirth and neonatal deaths, caused by placental abruption, are significantly more common in women with pre-eclampsia.

Pre-eclampsia can be categorised on the timing of disease onset as it can develop at different time points in pregnancy. Classically, the

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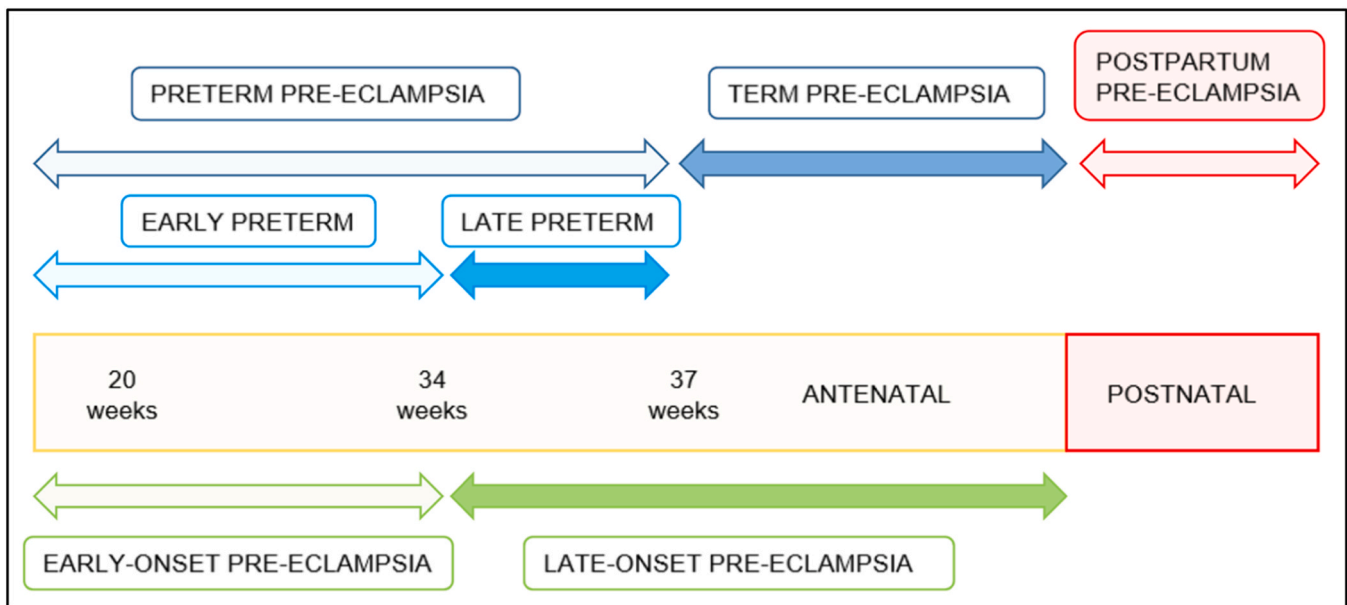


Fig. 1. Timeline of different onsets of pre-eclampsia.

disease has been categorised as term pre-eclampsia (delivery after 37 weeks' gestation) or preterm pre-eclampsia (delivery before 37 weeks). Preterm pre-eclampsia is then further divided into early preterm (delivery before 34 weeks' gestation) and late preterm pre-eclampsia (delivery after 34 weeks but before 37 weeks' gestation) (Duhig et al., 2021). Another classification is early versus late onset pre-eclampsia. Here, early-onset is defined as the occurrence before 34 weeks' gestation and late-onset is defined as the development of pre-eclampsia after 34 weeks' gestation (Fig. 1).

Some severe features of pre-eclampsia have a higher incidence in early-onset disease, specifically heavier proteinuria, eclampsia and HELLP syndrome (Kucukgoz Gulec et al., 2013). Despite this the numbers of complications are relatively greater among women with late-onset disease as early onset disease is rarer (Kucukgoz Gulec et al., 2013). There are also significant differences in the epidemiology of early- and late-onset pre-eclampsia. In most high-income countries late-onset pre-eclampsia represents 80–90% of cases, whilst in some low-and-middle-income countries early-onset pre-eclampsia represents up to 35% of cases (Robillard et al., 2022; Teka et al., 2023). This disparity highlights possible differences in genetics or potential underlying immunity to environmental and infectious pathophysiological processes that have an impact on timing and severity of disease (Aimée, 2022, WHO, 2023).

Women with pre-eclampsia produce greater amounts of anti-angiogenic factors like soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin. sFlt-1 binds to circulating vascular endothelial growth factor and placental growth factor leading to lower concentrations in the bloodstream. This disturbed equilibrium results in endothelial dysfunction with multi-organ involvement (Maynard et al., 2003; Tong et al., 2022). Interestingly, women with eclampsia, HELLP syndrome and severe hypertension have even higher levels of these biomarkers. Women with pre-eclampsia with pulmonary oedema did not show significant changes in sFlt-1 levels (Hastie et al., 2022). Further investigation into why certain women develop severe complications such as HELLP syndrome, pulmonary oedema or eclampsia are needed.

2. Unravelling pre-eclampsia with omics techniques

To investigate the pathogenesis of preeclampsia, a detailed analysis of processes occurring on a cellular level may be important. With a combination of different targeted and untargeted omics methods, crucial

pathways may be discovered. Multi-omics is a state-of-the-art technique that enables the simultaneous analysis of multiple molecular compartments and changes therein at a high resolution. Together, data generated from genomics, epigenomics, transcriptomics, proteomics and metabolomics may be combined. The resulting data has led to a revolution in the field of biology and medicine, allowing an integrative analysis for a deeper understanding of the interplay of molecules and therefore improving the prognosis of diseases finally leading to enhanced treatment and prevention (Hasin et al., 2017). Omics techniques are becoming increasingly feasible on a single-cell level. This high resolution allows for a change in perspective, allowing the discovery of cell-specific pathways and cell interactions. These techniques can now be used to explore underlying pathophysiological mechanisms. Molecular signatures may be identified rather than target molecules.

3. Genomics and epigenomics

Genomics is the study of a person's genetic make-up. Several genomics studies have investigated the maternal and fetal genome. Genomics is widely used to investigate disease and has led to a greater understanding of underlying mechanisms. Evolving technologies have made genomics more affordable and cost-efficient by allowing high-throughput analysis.

In pre-eclampsia, genomic studies have identified the gene *TIMP3* as a potential marker for early-onset disease (Yuen et al., 2010) and a polymorphism in the maternal genome (*HLA-DRB5*) has been associated with the development of pre-eclampsia (Xu et al., 2023). Table 1 details the genomic studies that have been performed on different tissue types to investigate pre-eclampsia.

Genomic methods are ideal to investigate genes and their potentially altered expression in pre-eclampsia but are not able to provide information on functional alterations. Epigenomics can show reversible modifications of the DNA or DNA-associated proteins but have not yet been able to describe pathophysiological pathways in pre-eclampsia.

4. Transcriptomics: single-cell RNA sequencing and spatial transcriptomics

Single-cell transcriptomics allows the detection of heterogeneity in single cells. By analysing transcribed genes in a single cell, the cell state, differentiation potential, cell to cell interaction and the disease related

Table 1
Genomic and transcriptomic studies to investigate pregnancy and pre-eclampsia.

Study type/ method	Tissue	Finding	Reference
DNA methylation profiling	Placenta (early-onset pre-eclampsia)	Hypomethylation of 4 loci, <i>TIMP3</i> potential marker for early-onset pre-eclampsia	Yuen et al., 2010
Genome-wide analysis of expression profiles	Placenta	63 genes are significantly expressed in pre-eclampsia	Trifonova et al., 2022
GWAS	Blood samples	Polymorphism in maternal <i>HLA-DRB5</i> is associated with pre-eclampsia	Xu et al., 2023
GWAS	Fetal genome	SNPs in <i>sFlt1</i> are associated with pre-eclampsia	Mcgininis et al., 2017
GWAS	Maternal genome	No SNPs associated with pre-eclampsia	Zhao et al., 2013
GSEA	Placenta and serum	miR-34a-5p and miR-106a-5p are associated with pre-eclampsia	Zhang et al., 2022
miRNA and RNA expression analysis	Placenta, trophoblasts	miRNAs targeting the TGF- β pathway are altered	Brooks et al., 2016
miRNA expression analysis	Maternal plasma exosomes	Increased miR-885-5p levels in pre-eclampsia	Sandrim et al., 2016
RNA-seq, GSEA	Plasma	cfRNA signature of pre-eclampsia is linked to the pathophysiology	Rasmussen et al., 2022
scRNA-seq	Fetal tissue, decidua and blood samples	Single-cell reconstruction of the maternal-fetal interface	Vento-Tormo et al., 2018
scRNA-seq	Placenta and decidua	Cell-type specific transcription factors	Suryawanshi et al., 2018
scRNA-seq	Placental villi, basal plate, chorionamniotic membrane	Cell-specific transcriptomic differences between pre-term and term delivered placental tissues	Pique-Regi et al., 2019
scRNA-seq	Trophoblasts	Trophoblast subtypes, patterns of differentiation in the placenta	Liu et al., 2018
Transcriptome analysis	Decidua basalis	Apoptosis and cell signalling genes are altered in pre-eclampsia	Yong et al., 2015

cfRNA = circulating free-RNA, GWAS = Genome wide association studies, GSEA = Gene set enrichment analysis, miRNA = micro-RNA, scRNA-seq = single-cell RNA sequencing, SNP = Single nucleotide polymorphism

changes in gene expression can be ascertained (Tang et al., 2019). There are several steps that need to take place when performing single-cell sequencing. Firstly, one may need to perform pre-processing. Here, certain cells of interest may need to be enriched if they are underrepresented (Vento-Tormo et al., 2018). Processing then entails converting RNA into complementary DNA. Polymerase chain reaction amplification is then performed as a single cell only contains 1–50 pg RNA, and 0.1–1 μ g complementary DNA is needed to detect a signal with RNA-seq (Svensson et al., 2018). The single-cell RNA sequencing data is then analysed using computational methods such as unsupervised clustering where cell populations are identified based on their transcriptomic profiles using marker genes (Zhang et al., 2019). This information is then connected with cellular descriptions such as location and morphology to finally create a reference map of the molecular state of cells (Regev et al., 2017).

Single-cell RNA-sequencing has been used to study pregnancy. Examples include studies investigating the decidua of the first trimester. Several subpopulations with enrichment of cell type specific transcription factors and trophoblast subtypes with different patterns of differentiation were found (Suryawanshi et al., 2018; Liu et al., 2018). Others have reconstructed the early maternal-fetal interface to investigate the immunological crosstalk of decidual immune cells (Vento-Tormo et al., 2018).

Transcriptomic changes of genes related to pre-eclampsia have been investigated in global RNA sequencing studies. Identified genes include *SERPIN1*, *PRG2*, *FSTL3*, *FABP4*, *RDH13*, *PLAC1*, *GSTA3* in STBs (Gormley et al., 2017; Trifonova et al., 2022) together with several miRNAs (Yuen et al., 2010; Brooks et al., 2016; Tsai et al., 2011; Sandrim et al., 2016; Zhang et al., 2022). Differentially expressed genes have also been described in the decidua basalis of the placenta (*CD72*, *PER3* or *PDK4*) (Yong et al., 2015). Additionally, a recent large multi-national transcriptomics study showed that plasma cell-free RNA signatures, with a moderate predictive accuracy, could identify women who were at risk of developing pre-eclampsia. Interestingly, transcriptomics was able to track pregnancy progression and define gestational length (Rasmussen et al., 2022) (Table 1).

Spatial transcriptomics connects tissue histology and expression changes based on two approaches, namely targeted and untargeted spatial omics. Targeted methods include fluorescence *in situ* hybridisation (FISH) based methods. Untargeted approaches often use methods where transcripts are directly labelled to tissue sections which enables the atlas mapping of their location after sequencing (Burgess, 2019). A non-spatial approach is the use of Drop-seq beads (Binan et al., 2019). Droplets with barcoded beads encapsulate single cells, labelling the transcripts with specific barcodes. Spatial adaption techniques like Slide-seq (Rodrigues et al., 2019) are then used and the barcoded beads are arrayed on a solid surface. This is then sequenced to identify the position of each barcode. When a tissue section is now placed on the slide and RNA is released through tissue digestion, RNA sequenced libraries can be generated containing the position specific barcodes (Rodrigues et al., 2019). This technique combines the general omics tools, yielding measurements of different biomolecules together with the analysis of a smaller number of molecules within intact tissues or cells obtained by immunostaining or *in situ* hybridisation (Tian et al., 2023).

These spatial transcriptomic methods may be important in understanding the pathophysiology of pre-eclampsia. In a recent study, digital spatial transcriptomic profiles using *in situ* sequencing enabled the visualisation of gene expression within trophoblast structures. In the pre-eclamptic placentas there was an increased senescent expression profile and in the villous wall consisting of trophoblasts there were more senescent areas closer to fetal vessels. This spatial dysregulation may be associated with disrupted transplacental transport (Nonn et al., 2022).

5. Proteomics

Proteomics is the study of the interactions, function, composition, and structures of proteins and their cellular activities (Wilkins et al., 1996). Proteomics can provide a better understanding of the structure and function of the organism than genomics. Pathophysiological changes may be missed on a transcriptomic level making multi-omics with proteomics a better option.

Techniques include mass-spectrometry with targeted and untargeted approaches like matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF), liquid chromatography mass spectrometry (LC-MS) and Olink®, a proximity extension assay screen for small proteins (Carlyle et al., 2022). More advanced methods include MALDI-TOF imaging (MALDI IMS) which enables the spatial visualisation of proteins (Leinweber et al., 2009). Here, enzyme activities are traced by applying marked substrates to tissues and subsequently analysing their conversion (Klein et al., 2020). Further studies using techniques such as SOMAmer, which are aptamer like single stranded

Table 2
Proteomic approaches to study pregnancy and pre-eclampsia.

Method	Tissue	Finding	Reference
LC-MS	Plasma	Apolipoprotein-A1, hemopexin and haptoglobin are differentially expressed in pre-eclampsia	Mary et al., 2017
2D-LC-MS/MS	Serum	Increased fibronectin expression in pre-eclampsia	Rasanen et al., 2010
2D-LC-MS/MS	Trophoblasts	Laminin expression is downregulated in pre-eclampsia	Ma et al., 2014
LC-MS/MS	Serum	RBP4 concentration is lower in pre-eclampsia	Lu et al., 2016
MALDI-TOF	Placenta/chorion	Altered expression of proteins involved in apoptosis and mitochondrial dysfunction in pre-eclampsia	Yang et al., 2015
Olink®	Plasma	CCL20 is a novel predictive and inflammatory marker for pre-eclampsia	Wang et al., 2022
SOMAmer	Plasma	PlGF and MMP-7 are markers for late-onset pre-eclampsia	Erez et al., 2017
SOMAmer	Plasma	VEGF-121, PlGF and activin are predictors for late-onset pre-eclampsia	Tarca et al., 2019
SOMAmer	Plasma	4 distinct clusters are either linked to early-onset pre-eclampsia, anti-fetal rejection, extracellular matrix regulation or angiogenic imbalance	Than et al., 2023
UPLC-UDMS ^E	Urine	Pre-pregnancy BMI is a predictor for pre-eclampsia	Joenväärä et al., 2022

MS = Mass spectrometry, LC = Liquid chromatography, MALDI-TOF = Matrix assisted laser desorption ionisation – time of flight, SOMAmer = Slow Off-rate Modified Aptamers, UPLC-UDMS^E = ultra performance liquid chromatography-ultra definition mass spectrometry

deoxyoligonucleotides which bind specific molecular targets such as proteins, aimed to understand differences in the proteome of women with pre-eclampsia and find molecular subclasses (Than et al., 2023). Deep visual proteomics also has recently been described. Deep visual proteomics combines artificial-intelligence-driven image analysis of cellular phenotypes with automated single-cell or single-nucleus laser microdissection and ultra-high-sensitivity mass spectrometry. One is therefore able to obtain precise information about spatial distribution of proteins in the context of tissues (Mund et al., 2022). Some of the earlier techniques have been modified for high-throughput investigations of thousands of proteins in cells or body fluids (Hein et al., 2013).

Several studies have aimed to understand differences in the proteome of women with pre-eclampsia and are summarised in Table 2. No proteomic studies have been performed for the different severe phenotypes of preeclampsia like eclampsia, pulmonary oedema and HELLP syndrome.

Table 3
Multi-omics approaches to study pregnancy and pre-eclampsia.

Omics study	Tissue	Gestational Age	Finding	Reference
Spatial proteomics, spatial transcriptomics	Placenta, decidua, and vessels	6 – 20 weeks	Spiral artery remodelling progression is preferentially correlated with extravillous trophoblast invasion	Greenbaum et al., 2023
Spatial transcriptomics, spatial proteomics, snRNA-seq	Placenta, decidua, maternal serum	5–10 weeks, 27–33 weeks, > 38 weeks	Trophoblast differentiation causes disturbed placental senescence profile	Nonn et al., 2022
Metabolomics, Transcriptomics	Blood, urine, vaginal swabs	35–36 weeks	Early prediction of pre-eclampsia	Marić et al., 2022
Proteome and lipidome analysis	Plasma	38–39 weeks	Visualisation of the end-stage molecular state in pre-eclampsia	Odenkirk et al., 2020
scRNA-seq, snRNA-seq, spatial multi-omics	Placenta and decidua	4–13 weeks	Trophoblast development in early pregnancy	Arutyunyan et al., 2023

scRNA-seq = single-cell RNA-sequencing, snRNA-seq = single-nucleus RNA-sequencing

6. Metabolomics

Metabolomics can quantify amino acids, fatty acids, carbohydrates, or other by-products of cellular metabolic processes. Metabolite concentrations and ratios represent metabolic activity, and abnormal changes are often a sign of disease. With the development of microfluidic systems, high-throughput single-cell metabolomics has allowed the analysis of the metabolome of a single cell (Côte-Real et al., 2023). This can then be incorporated with spatial proteomic techniques (Ali et al., 2019; Ong et al., 2015). Spatial metabolomics enables one to localise metabolites, lipids and drugs in tissue sections (Alexandrov, 2020).

7. Multi-omics for unravelling subtypes of pre-eclampsia

Only a few studies have used multi-omics in pre-eclampsia (Table 3).

Integrated single-cell RNA sequencing with spatially resolved proteo- and transcriptomics may prove beneficial in understanding pre-eclampsia pathophysiology and subtypes. By combining several omics techniques, an enhanced understanding of cell-cell interaction and cell specific dysregulation can be explored. More research is urgently needed.

In the future, fundamental questions regarding pre-eclampsia research need to be addressed to improve the outcome of the disease. In the context of single-cell and spatial multi-omics approaches it must be investigated, how differences in pre-eclampsia subtypes lead to variations in the onset of pre-eclampsia. Furthermore, it is still unknown why some women are more likely to develop severe complications of pre-eclampsia than others. Lastly, one of the major gaps in knowledge is why pre-eclampsia and its severe forms differ geographically and ethnically. Here, single-cell and spatial multi-omics techniques offer a promising approach to answer those questions.

8. Conclusion

Multi-omics techniques have the potential to help unravel the puzzle of pre-eclampsia. By employing novel single-cell and spatial multi-omics techniques one may be able to better understand the pathophysiology of pre-eclampsia and its different phenotypes. These innovative approaches allow for the comprehensive analysis of multiple biological layers, encompassing genomics, transcriptomics, proteomics, and other molecular facets. By integrating data from various omics disciplines, a deeper probe into underlying molecular mechanisms of pre-eclampsia will be obtained. This will shed light on the complex interplay between genes, proteins, and cellular processes that contribute to the development and progression of the disease. Such multi-omics investigations hold tremendous promise in unravelling the intricate web of molecular events associated with pre-eclampsia, opening new avenues for targeted therapeutic interventions and improved management strategies.

Declaration of Competing Interest

None.

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