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Single-cell transcriptomics of pathological pregnancies

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Abstract

Pregnancy is a complicated process involving various anatomical and physiological changes to provide a suitable environment for fetal development, to meet the increased metabolic demands, and to prepare for labor. While most pregnancies and births are uneventful, all pregnancies are at risk of complications. No less than 15% of all pregnant women develop a potentially life-threatening complication or may also experience an unpleasant outcome. Conventional sequencing has been widely used in this field for diagnoses and treatment planning. However, it lacks the precise resolution to identify transcriptomic variation between the nearby cell types. Single-cell RNA sequencing is a novel approach that through single-cell isolation, transcript capturing, and development and sequencing of expression libraries allows the assessments of fundamental biological properties of cell populations and biological systems at unprecedented resolution. The single-cell sequencing technology is a powerful tool for investigating cell distribution and cell-to-cell relationships in human reproduction. Since 2016, the technique has contributed to the discovery of many transcriptomic atlases of human embryos, placenta, decidua, and endometrium and the identification of various crucial regulatory pathways in fetal development. Here, we briefly describe the application of single-cell RNA sequencing in the studies of physiological processes and pathological mechanisms of pregnancy.

Key words: single-cell sequencing, transcriptomics, pregnancy loss, preeclampsia, placenta, decidua, trophoblast

INTRODUCTION

Women's reproductive organs undergo a variety of physiological and immunological adaptations during pregnancy to support the development of the fetoplacental unit. Any unexpected changes may result in pathological pregnancies. There are mainly two groups of pathological pregnancies: one is common pregnancy complications, such as gestational diabetes and preeclampsia (PE), and the other is adverse pregnancy outcomes, including preterm labor, low birth weight, pregnancy loss, and idiopathic fetal growth restriction (IFGR). In the United States, about 3.6 million births occur every year, and more than 20% of women were associated with pathological pregnancy.[1]

PREGNANCY PROCESS AND THE LIMITATIONS OF CONVENTIONAL SEQUENCING

Theoretically, biological pregnancy begins from the implantation of the embryo in the uterine wall.[2] The first cell lineage division occurs when the cells of the totipotent morula develop into a blastocyst. The cells split to form either the inner cell mass (ICM) or the trophectoderm. Placental villous development begins at around eight days' post-conception, during which the primitive syncytium

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protrudes into the decidual tissue. Then the proliferation of the primitive cytotrophoblasts (CTBs) generates the first villous structures. Placental villi continue to mature as pregnancy proceeds, as does the vascular network within the placental villi.

The success of all pregnancies is based upon the proper formation of the branching villous structure of the placenta. Disruption of the placental maternal-fetal interface is one of the leading causes of pathogenic pregnancy and could result in adverse pregnancy outcomes such as preterm birth and IFGR.[3,4] The placenta is a complex heterogeneous tissue comprising cells originating from both mother and fetus. Elucidating the perturbations in the maternal-fetal interface requires accurate dissection of the transcriptional activity at the cell type level. However, knowledge of the placenta's cell type composition, transcriptional activity, and compartments during physiologic and pathologic parturitions is limited. In recent years, application of novel cell sorting and droplet-based next-generation sequencing approaches has enabled exploring gene expression at a single-cell resolution. As its name indicates, single-cell RNA sequencing (scRNA-seq) can generate transcriptome data at a single-cell level. In contrast, conventional sequencing only has the resolution of tissue; for this reason, it is also called the bulk sequencing technique. More scientifically, single-cell technologies have the advantage of detecting heterogeneity among individual cells.[5]

ADVANTAGES AND LIMITATIONS OF SINGLE-CELL RNA SEQUENCING

The single-cell sequencing technique combines the single-cell cDNA amplification method with the high-throughput RNA sequencing method, first reported in 2009.[6] The preparation of single cells is the initial and most crucial step in various scRNA-seq procedures. The use of traditional single-cell isolation strategies, including micromanipulation, laser capture microdissection, and fluorescence-activated cell sorting, is limited due to high time and labor consumption. The novel approaches are based on voltage-driven microfluidics, enabling high-throughput, low-cost single-cell expression research. Combining microfluidic and barcode labelling single-cell identification technologies has led to rapid developments in scRNA-seq. Microfluidics is a technique for precisely manipulating minute amounts of liquids in a micro-electronic controlled chip and is conducive for processing single-cell samples. The setup volume of the microfluidic chip is only at the nanoliter level, which improves reaction accuracy and efficiency and reduces reagent consumption and cost. The commercially available and most widely used Chromium Single Cell Gene Expression System developed by 10x Genomics can complete isolation and amplification of 80,000 cells simultaneously in minutes.

Since its introduction, scRNA-seq technology has been continually developed and improved, providing a technical approach for the construction of a complete cell map and greatly promoting the single-cell research process. scRNA-seq is an ideal tool to investigate the high degree of heterogeneity in tumorigenesis and metastasis. Along with reducing costs and setup requirements, scRNA-seq methods have widely spread applications in microbiology, neurology, and immunology.[6] The possible application of scRNA-seq to prenatal diagnosis and assisted reproduction has also been investigated. Selection of female polar cells or embryonic cells by scRNA-seq for a healthy embryo can reduce the birth rate of newborns with congenital genetic diseases.[7] Using a modified version of scRNA-seq, Liu *et al.* obtained the genome-wide epigenetic landscape of human embryos.[8] The result revealed complete DNA methylation and chromatin accessibility data of the embryo at the stage of preimplantation. The distinct difference in the transcriptome profile has been shown between the rescued *in vitro* maturation oocytes and the *in vivo* oocytes. The GATA-1/CREB1/WNT signaling pathway was repressed in rescued *in vitro* matured oocytes compared with *in vivo* oocytes and was further down-regulated in low-quality rescued *in vitro* mature oocytes.[9] However, this review will mainly focus on investigation of pathological pregnancies by means of scRNA-seq methods.

Despite the remarkable progression made by single-cell transcriptomics in life sciences, investigations are limited by the availability of the samples and the technique itself. Sample preservation procedures may require various protocols and produce in cell collections. The current dissociation, encapsulation, or other preparation procedures of scRNA-seq are not compatible with certain types of cells, such as neutrophils, epithelial cells, and neurons. Similar but alternative technique such as single-nucleus RNA-seq may be suited for these cell types.[10] Furthermore, the commonly used poly-A selection technique may limit the ability to examine non-polyadenylated transcripts, such as small nuclear RNA, histone mRNAs, long noncoding RNAs and pre-mRNAs, which may involve in multiple regulatory roles during reproduction of human.[11] In addition, the integration of specifically optimized algorithms and powerful computational pipelines are demanded in the analysis of the genetic/epigenetic heterogeneity, which are present in reproductive organs and cancer tissues.[12,13] In some cases, cellular deconvolution spatial transcriptomics technology will be needed for investigating the complex spatial patterns of cell types as well as cellular heterogeneities.[14]

SINGLE-CELL RNA SEQUENCING IN PLACENTA DEVELOPMENT AND FETAL ADAPTION

The multicellular bi-layered tissue of endometrium is essential to harboring the conceptus. Abnormalities in the endometrium can lead to infertility, adverse pregnancy

outcomes, and substantial health issues for women. The endometrium undergoes remodeling, shedding, and regeneration in the menstrual cycle. These processes are driven by the regulation of gene expression in the underlying cellular hierarchy. The window of implantation opens with an abrupt and discontinuous transcriptomic activation in the epithelia, accompanied by a widespread decidualization feature in the stromal fibroblasts. Decidualization is the cytoskeleton-driven conformational transformation of stromal fibroblasts. They change from elongated, fibroblast-like cells into enlarged round cells, playing essential roles in embryo invasion and pregnancy development.[15]

The first trial of scRNA-seq in pregnancy-related research was published in early 2016. Nelson *et al.* provided the first comprehensive analysis of gene expression patterns in individual placental cell types (Table 1). The single-cell method enabled the identification of Blimp1-dependent transcripts enriched in spiral artery trophoblasts giant cells (SpA-TGCs) and clearly demonstrated that SpA-TGCs are well equipped to suppress the harmful consequences of vascular remodeling. SpA-TGCs strongly express both Entpd1 and Procr, which are known to prevent thrombosis and inflammation.[16] Since then, scRNA-seq has been widely applied to investigate gene expression in human placentas. Another study illustrated the cell-cell interactome of fetal placental trophoblast cells and maternal endometrial stromal cells.[22] A highly cell-type-specific expression of G-protein-coupled receptors was found. It also showed that decidualization enhances the ability to communicate with the fetus. New subtypes of cells from the known CTBs, extravillous trophoblast (EVTs), Hofbauer cells (the fetal macrophages), and mesenchymal stromal macrophages were identified, and cell-type–specific gene signatures were defined.[8]

The human maternal-fetal interface contains subsets of perivascular and stromal cells located in distinct decidual layers. A scRNA-seq reading–based cell-cell network database was developed during a study of first-trimester placentas with matched maternal blood and decidual cells (DCs).[28] Receptors involved in immunomodulation, cellular adhesion, and invasion were found to be up-regulated during EVT differentiation.[14] In addition, three major subsets of decidual natural killer (dNK) cells with distinctive immunomodulatory and chemokine profiles were found, suggesting that the immune microenvironment of the decidua suppresses inflammatory responses that may potentially be driven by trophoblast invasion and destruction of the smooth muscle media of the spiral arteries by trophoblasts.

Alignment of scRNA-seq data with overlapping single-nucleotide polymorphisms obtained from fetal and maternal genomic DNA showed that placental samples contained mostly fetal cells with some maternal macrophages. In contrast, most cells from decidual samples except a few EVTs were of maternal origin.[17] Also, from the study of the first-trimester placenta sample, five major cell types (trophoblasts, stromal cells, Hofbauer cells, antigen-presenting cells, and endothelial cells) were identified with sex differences. Gene ontology (GO) enrichment analysis showed that the top enriched processes include cell proliferation and adhesion in trophoblasts, cell differentiation and signal transduction in stromal fibroblasts, inflammatory responses in Hofbauer cells, and cell migration and angiogenesis in endothelial cells. The hormonal upstream regulators of sexually dimorphic genes were estradiol and transforming growth factor beta 1, whose expression is also up-regulated in the male placenta.[19] Syncytiotrophoblasts (STBs) and EVTs were major sources for polypeptide hormone gene expression, suggesting that both of these cells can also facilitate crosstalk between placental and DCs.[8]

SINGLE-CELL RNA SEQUENCING IN PREGNANCY COMPLICATIONS

Preeclampsia

PE is a severe complication that usually starts in the third trimester of pregnancy, during labor, or in the early period after delivery[29] and is characterized by hypertension, abnormal amounts of protein in the urine, and other systemic disturbances. Changes in the vessels supplying the intervillous space are generally thought to be the critical feature in the genesis of PE.[30] However, what changes occur in gene expression and in cell distribution in the placenta under the abnormal condition of pregnancy-induced are unknown. The blood pressure of humans is regulated by the renin-angiotensin system (RAS).[31] From very early gestation onwards, the human placenta starts to produce its own RAS and angiotensin (Ang) IV receptor, leucinyl aminopeptidase (LNPEP), which can only be detected in STB cells. The Ang IV could suppress respiration of basal mitochondrial in trophoblasts and then may lead to recovering placental metabolism by increasing leptin, followed by down-regulating LNPEP expression. This process suggests a negative feedback loop regulation of the Ang IV/leptin/LNPEP cascade. A study also demonstrated that LNPEP was involved in vasopressin clearance and antigen cross-presentation and was down-regulated in the maternal-fetal interface during early- and late-onset PE. Similar results can also be obtained in first-trimester placenta with a higher risk for developing PE based on a high uterine artery resistance index.[32]

Endoplasmic reticulum function was found to be active in the state of PE, as gene expression of endoplasmic reticulum signaling pathways in STB was up-regulated in the placenta of PE patients. This upregulation was thought to be due to the intermittent hypoxia of the placenta caused by narrow spiral arteries. GO enrichment analysis

Table 1: List of single-cell investigations in pathogenic pregnancy and their key findings

Author	Condition	Type of tissue	Findings	Cell types identified and gene makers
Nelson *et al.* (2016)[16]	Normal	Placenta & decidua	Results suggested that spiral artery trophoblast giant cells were well equipped to suppress harmful consquences of vascular remodelling. Study also reveals a number of cell type specific markers.	● Decidual stroma (*PRL8A2*$^+$, *CRYAB*$^+$, *ADM*$^+$, *WNT4*$^+$,*ANGPT2*$^+$, *ANGPT4*$^+$), ● NK (*EOMES*$^+$, *CD244*$^+$, *HAVCR2*$^+$), ● Spiral artery trophoblast giant cells, (*PRL7B1*$^+$, *RGS5*$^+$, *KDR*$^+$, *CTS7*$^+$, *TPBPA*$^+$, *PAK1*$^+$, *NOS1*$^+$, *AGRP*$^+$, *LGALS9*$^+$, *C5AR2*$^+$, *ACKR2*$^+$, *PLXND1*$^+$, *FLT1*$^+$, *P4HA2*$^+$, *LGFBP7*$^+$, *GPC1*$^+$) ● Novel Prdm1+trophoblast giant cells cells (*ADM*$^+$, *KDR*$^+$, *CD34*$^+$, *CYP11A1*$^+$), ● Progenitor trophoblasts (*GJB3*$^+$, *GJB5*$^+$, *HSD17B2*$^+$, *PHLDA2*$^+$, *UTF1*$^+$, *ELF5*$^+$, *HAND1*$^+$, *LDOC1*$^+$, *LAD1*$^+$), ● Fetal endothelial cells (*TIE1*$^+$, *TEK*$^+$, *PDGFB*$^+$)
Liu *et al.* (2018)[8]	Normal	Placenta & villi	New subtypes of cells of the known CTBs, extravillous trophoblast cells EVTs, Hofbauer cells, and mesenchymal stromal cells were identified and cell-type-specific gene signatures were defined.	● CTB (*CDH1*$^+$, *EGFR*$^+$, *HLA-G*$^-$, *DNMT*$^+$, *NF124*$^-$, *SOX4*$^-$, *ASCL2*$^-$, *ZNF555*$^-$, *SNAI1*$^-$, *STAT1*$^-$, *MYCN*$^-$, *ID1*$^-$, *MLLT1*$^+$, *NFE2L3*$^+$) ■ CTB 8 weeks subtype 1, fusion-competent cells (*ERVFRD-1*$^+$, *RRM2*$^-$, *CCNB1*$^-$, *CDK1*$^-$) ■ CTB 8 weeks subtype 2 (*ERVFRD-1*$^-$, *RRM2*$^-$, *CCNB1*$^-$, *CDK1*$^-$) ■ CTB 8 weeks subtype 3 (*ERVFRD-1*$^-$, *RRM2*$^+$, *CCNB1*$^+$, *CDK1*$^+$) ● EVT (*HLA-G*$^+$, *MMP2*$^+$, *ZNF124*$^+$, *SOX4*$^+$, *ASCL2*$^+$, *ZNF555*$^+$, *SNAI1*$^+$, *STAT*$^+$, *MYCN*$^+$, *ID1*$^+$, *MLLT1*$^-$, *NFE2L3*$^-$) ■ EVT 8 weeks subtype 1, (*RPM2*$^+$, *CCNB1*$^+$, *TAC*$^-$, *SERPINE1*$^-$, *JAM2*$^-$, *LAIR2*$^+$) ■ EVT 8 weeks subtype 2, (*RPM2*$^-$, *CCNB1*$^+$, *TAC*$^+$, *SERPINE1*$^+$, *JAM2*$^-$, *LAIR2*$^+$) ■ EVT 8 weeks subtype 3, (*RPM2*$^-$, *CCNB1*$^-$, *TAC3*$^+$, *SERPINE1*$^+$, *JAM2*$^+$, *LAIR2*$^+$) ■ EVT 24 weeks subtype 1 (*RPM2*$^-$, *CCNB1*$^-$, *TAC3*$^+$, *SERPINE1*$^+$, *JAM2*$^+$, *LAIR2*$^-$, *CGA*$^-$) ■ EVT 24 weeks subtype 2 (*RPM2*$^-$, *CCNB1, TAC3*$^-$, *SERPINE1*$^+$, *JAM2*$^+$, *LAIR2*$^-$, *CGA*$^-$) ● STB 8 weeks (*SMYD2*$^-$, *RPS6KA5*$^+$, *HLA-G*$^-$, *CSH1*$^+$, *DNMT*$^-$) ● Fetal epithelial cells (*CDH1*$^+$) ● Mesenchymal cells (*CD90*$^+$, *ENG*$^+$, *CD74*$^+$) ■ Mesenchymal subtype 1 (*C1R*$^+$, *IGFBP7*$^+$, *DLK1*$^-$, *COL1A1*$^-$ subtypes), ■ Mesenchymal subtype 2 (*C1R*$^-$, *IGFBP7, DLK1*$^+$, *COL1A1*$^+$) ● Microphages (*CD68*$^+$) ■ Microphage subtype 1 (*LYVE1*$^-$, *VSIG4*$^-$, *MRC1*$^-$, *HLA-DRA*$^+$, *CD74*$^+$) ■ Microphage subtype 2 (*LYVE1*$^-$, *VSIG4*$^-$, *MRC1*$^-$, *HLA-DRA*$^+$, *CD74*$^+$)
Vento-Tormo (2018)[17]	Normal	Blood, placenta, decidua	Identifed regulatory interactions involved in the prevention of harmful innate or adaptive immune responses in early maternal-fetal interface.	● dNK (*CD49A*$^+$, *CD9*) ■ dNK1 (*CD39, CYP26A1, B4GALNT1*), ■ dNK2 (*ANXA1*$^+$, *ITGB2*$^+$) ■ dNK3 (*CD160*$^+$, *KLRB1*$^+$, *CD103*$^+$) ● Maternal macrophages (*CD14*$^+$, *S100A9*$^+$, *CD163*$^+$, *CD68*$^+$, *CSF1R*$^+$) ● Perivascular cells (*MGP*$^+$) ■ Perivascular cells subtype 1 (*MMP11*$^-$, *CHI3L1*$^-$, *ACTA2*$^+$) ■ Perivascular cells subtype 2 (*MMP11*$^+$, *CHI3L1*$^+$, *ACTA2*$^-$) ● Decidual stromal cells (*DKK1*$^+$) ● Decidual stromal cells, subtype 1 (*ID2*$^+$, *TAGLN*$^+$, *IL1RL1*$^-$, *PRL*$^-$) ● Decidual stromal cells, subtype 2 (*ID2*$^-$, *TAGLN*$^+$, *IL1RL1*$^-$, *PRL*$^-$) ● Decidual stromal cells, subtype 3 (*ID2*$^-$, *TAGLN*$^-$, *IL1RL1*$^-$, *PRL*$^+$)
Koh *et al.* (2019)[18]	Normal	Cervical & myometrium uterine	The authors generated a framework of cell type expression signatures that can be used to distinguish the various cell types in the biopsied tissues.	● Endothelial cells (*SELP*$^+$, *THBD*$^+$, *PECAM1*$^+$, *CD93*$^+$, *FLT1*$^+$) ● Leukocytes (*LCP1*$^+$, *CCL3*$^+$, *CCL4*$^+$, *LYZ*$^+$, *IL1B*$^+$, *EREG*$^+$, *ITGAX*$^+$) ● Epithelial cells (*SLPI*$^+$, *WFDC2*$^+$) ● Smooth muscle (*ACTG2*$^+$, *MYH11*$^+$, *ACTA2*$^+$) ● Stromal cells (*DCN*$^+$, *LUM*$^+$, *VCAN*$^+$, *CLIP*$^+$)
Sun *et al.* (2020)[19]	Normal	Placenta, decidua	Identified 91 sexually dimorphic receptor-ligand pairs across the maternal-fetal interface. Maternal-fetal crosstalk exhibits sexual dimorphism during placentation early in gestation.	● Trophoblasts (*KRT7*$^+$, *KRT8*$^+$, *CGA*$^+$, *EGFR*$^+$); ■ Male (*RPS4Y1*$^+$, *EIF1AY*$^+$, *DDX3Y*$^+$, *XIST*$^-$, *MAGEA4*$^-$, *TMSB4X*$^-$) ■ Female (*RPS4Y1*$^-$, *EIF1AY*$^-$, *DDX3Y*$^-$, *XIST*$^+$, *MAGEA4*$^+$, *TMSB4X*$^+$) ● Stromal fibroblast cells (*COL3A1*$^+$, *PDGFRA*$^+$, *THY1*$^+$), ■ Male (*RPS4Y1*$^+$, *DDX3Y*$^+$, *EIF1AY*$^+$, *XIST*$^-$) ■ Female (*RPS4Y1*$^-$, *DDX3Y*$^-$, *EIF1AY*$^-$, *XIST*$^+$) ● Hofbauer cells, (*CD14*$^+$, *CD163*$^+$, *CSF1R*$^+$) ■ Male (*RPS4Y1*$^+$, *DDX3Y*$^+$, *EIF1AY*$^+$, *XIST*$^-$) ■ Female (*RPS4Y1*$^-$, *DDX3Y*$^-$, *EIF1AY*$^-$, *XIST*$^+$) ● Antigen presenting cells (*HLA-DRA*$^+$, *HLA-DPB1*$^+$, *CD52*$^+$) ■ Male (*RPS4Y1*$^+$) ■ Female (*RPS4Y1*$^-$) ● Endothelial cells (*PECAM1, CD93*) ■ Male (*RPS4Y1*$^+$, *EIF1AY*$^+$, *RPL36A*$^+$, *XIST*$^-$) ■ Female (*RPS4Y1*$^+$, *EIF1AY*$^+$, *RPL36A*$^+$, *XIST*$^-$)

(Continued...)

Author	Condition	Type of tissue	Findings	Cell types identified and gene makers
Guo *et al.* (2021)[20]	RPL	Decidua	The authors discovered dramatic differential distributions of immune cell subsets in RPL patients compared with the normal decidual immune microenvironment.	● Decidual NK (*NKG7*$^+$, *CD68*$^-$, *CD3D*$^-$ *FGFBP2*$^-$, *CD79A*$^-$, *CLEC9A*$^-$, *KIT*$^-$) ■ dNK1 (*CD39*$^+$, *CD18*$^-$, *CD103*$^-$) ■ dNK2 (*CD18*$^+$, *CD103*$^-$, *CD39*$^-$) ■ dNK3 (*CD18*$^+$, *CD103*$^+$, *CD39*$^-$) ● Macrophages (*NKG7*$^+$, *CD68*$^+$, *CD3D*$^-$ *FGFBP2*$^-$, *CD79A*$^-$, *CLEC9A*$^-$, *KIT*$^-$) ● B cells (*NKG7*$^+$, *CD68*$^-$, *CD3D*$^-$, *FGFBP2*$^-$, *CD79A*$^+$, *CLEC9A*$^-$, *KIT*$^-$) ● T cells (*NKG7*$^+$, *CD68*$^-$, *CD3D*$^+$, *FGFBP2*$^-$, *CD79A*$^-$, *CLEC9A*$^-$, *KIT*$^-$) ● NK T (*NKG7*$^+$, *CD68*$^-$, *CD3D*$^-$ *FGFBP2*$^+$, *CD79A*$^+$, *CLEC9A*$^-$, *KIT*$^-$), ● dendritic cells (*NKG7*$^+$, *CD68*$^-$, *CD3D*$^-$ *FGFBP2*$^-$, *CD79A*$^-$, *CLEC9A*$^+$, *KIT*$^-$), ● progenitor cells (*NKG7*$^+$, *CD68*$^+$, *CD3D*$^-$ *FGFBP2*$^-$, *CD79A*$^-$, *CLEC9A*$^-$, *KIT*$^+$)
Wang *et al.* (2021)[21]	RPL	Blood, decidua	Subset of NK cells were differentially expressed in RPL patients.	● B cells (*CD79A*$^+$, *CD3D*$^-$, *CCR7*$^-$, *S100A4*$^-$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^-$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^-$) ● CD4T naïve (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^-$, *S100A4*$^-$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^-$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^-$) ● CD4T memory (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^+$, *S100A4*$^+$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^-$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^-$) ● mucosal-associated invariant T cells (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^-$, *S100A4*$^+$, *ZBTB16*$^+$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^-$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^-$) ● CD8T naïve (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^+$, *S100A4*$^-$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD8A*$^+$) ● CD8T effector (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^-$, *S100A4*$^+$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD160*$^+$, *CD8*$^+$) ● NK dim (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *CD160*$^+$, *FGFBP2*$^+$, *FCGR3A*$^+$, *FOXP3*$^-$, *ENTPD1*$^-$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^-$) ● NK bright (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *SELL*$^+$, *S100A4*$^-$, *ZBTB16*$^+$, *KLRB1*$^+$, *CD160*$^+$) ● CD14 monocytes (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *S100A4*$^+$, *ZBTB16*$^-$, *CD14*$^+$, *LYZ*$^+$, *CD8A*$^-$), ● CD16 monocytes (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *S100A4*$^+$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^-$, *FCGR3A*$^+$, *CD14*$^-$, *LYZ*$^+$, *CD8A*$^-$) ● Decidual CD8T cells (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^-$, *S100A4*$^-$, *ZBTB16*$^-$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^-$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^+$) ● Decidual CD4T cells (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^+$, *S100A4*$^+$, *ZBTB16*$^-$, *KLRB1*$^-$, *ENTPD1*$^-$) ● Decidual regulatory T cells (*CD79A*$^-$, *CD3D*$^+$, *CCR7*$^-$, *S100A4*$^+$, *ZBTB16*$^+$, *KLRB1*$^-$, *CD160*$^-$, *FOXP3*$^-$, *ENTPD1*$^+$, *CD14*$^-$, *LYZ*$^-$, *CD8A*$^-$) ● Decidual NK ■ dNK1 (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *NCAM1*$^+$, *ENTPD1*$^+$) ■ dNK2 (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *KLRB1*$^+$, *NCAM1*$^+$, *ENTPD1*$^-$) ■ dNK3 (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *ZBTB16*$^+$, *KLRB1*$^+$, *NCAM1*$^-$, *ENTPD1*$^-$, *CD160*$^+$) ■ dNK4 (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *ZBTB16*$^-$, *KLRB1*$^-$, *FGFBP2*$^+$, *FCGR3A*$^+$, *NCAM1*$^-$, *ENTPD1*$^-$, *CD160*$^+$) ● Decidual macrophage (*CD79A*$^-$, *CD3D*$^-$, *CCR7*$^-$, *ZBTB16*$^-$, *CD14*$^+$, *LYZ*$^+$, *FCER1A*$^+$), ● Group 3 innate lymphoid cells (*CD79A*$^-$, *CD3D*$^-$, *ZBTB16*$^+$, *KLRB1*$^+$, *CD160*$^+$, *LYZ*$^-$), ● Dendritic cells (*CD79A*$^-$, *CD3D*$^-$ *S100A4*$^+$, *KIT*$^+$, *FCER1A*$^+$, *TPSAB1*$^+$)
Pavlicev *et al.* (2017)[22]	Preterm birth	Placenta & decidua	Most of the receptors and ligands up-regulated during decidualization have their counterpart expressed in trophoblast cells. Growth factors and immune signals were found to be dominate the various transduction pathways.	● CTB (*ADRB1, PUF60, SNORD3A, XAGE3, XAGE2*) ● EVT (*TAC3, AOC1, B3GNT7, COL17A1*) ● STB (*SEMA3B, CSH2, CSHL1, FCGR2A*) ● Dendritic cells (*AIF1, C1QB, CD11c, CD14*) ● Decidual cells (*ABI3BP, ADRA2C, C1QTNF1, PRL*) ● Endometrial stromal fibroblast (*TRIP13, TOP2A, TACC3, SSTR1*)
Pique-Regi *et al.* (2019)[23]	Preterm birth	Placenta	The author identified two new cell types, and provide a catalogue of cell types and transcriptional profiles in the human placenta.	● T cell-resting (*CRIP1*$^+$, *CREM*$^+$, *PPP1CB*$^+$, *IL7R*$^+$) ● T cell-activated (*DNAJB*$^+$, *HSPA1B*$^+$, *HSPA5*$^+$) ● Stromal (*LGALS1*$^+$, *SERPINF1*$^+$, *CXCL4*$^+$, *EGFL6*$^+$, *MT2A*$^+$) ● NK (*KLRC1*$^+$, *CMC1*$^+$, *CTSW*$^+$, *GZMB*$^+$) ● Monocyte (*S100A8*$^+$, *SOD2*$^+$) ● Fibroblast (*TAGLN*$^+$, *ACTA2*$^+$) ● EVT (*FSTL3*$^+$, *TPM1*$^+$, *PRG2*$^+$, *FLT1*$^+$, *EBI3*$^+$, *KRT19*$^+$)

(Continued...)

Author	Condition	Type of tissue	Findings	Cell types identified and gene makers
				● Macrophage (*NPC2*$^+$, *C1QB*$^+$) ■ Subtype 1 (*HLA-DQB1*$^+$, *HLA-DRB1*$^+$) ■ Subtype 2 (*PFTP*$^+$, *LGMN*$^+$) ● Decidual cells (*RBP1*$^+$, *IGFBP4*$^+$, *PTGDS*$^+$) ● CTB (*DDX3X*$^+$, *EIF1AX*$^+$, *PAGE4*$^+$, *XIST*$^+$) ■ Non proliferative interstitial CTB (*DDX3X*$^+$, *EIF1AX*$^+$, *PAGE4*$^+$, *XIST*$^-$) ● B cells (*CD79A*$^+$, *HLA-DQB1*$^+$) ● Lymphoid endothelial decidual cells (*CD34*$^+$, *CDH5*$^+$, *LYVE1*$^+$, *PDPN*$^+$)
Rong *et al.* (2021)[24]	Preeclampsia	Decidua	Abnormal gene expression affects the export function of decidual macrophages, which in turn affects the interaction of decidual macrophages with other immune cells. Furthermore it leads to the suppression of original immune regulation mechanism, and ultimately result into the occurrence of preeclampsia	● Innate lymphoid cells3 (*LST1*$^+$, *IL7R*$^+$) ● B cells (*IGLC2*$^+$, *CD79A*$^+$) ● Mast cells (*TPSB2*$^+$, *TPSAB1*$^+$) ● Decidual NK ■ dNK-a (*TRDC*$^+$, *XCL2*$^+$) ■ dNK-b (*COTL1*$^+$, *TNFRSF18*$^+$) ■ dNK-c (*ENTPD1*$^+$, *GNLY*$^+$) ■ dNK-d (*ITM2C*$^+$) ■ dNK-e (*FGFBP2*$^+$, *MYOM2*$^+$) ● CD4 T cells (*LTB*$^+$, *JUN*$^+$, *TRAC*$^+$, *CD3D*$^+$) ● Decidual macrophages (*IL1B*$^+$, *G0S2*$^+$) ● CD8 T cells (*LTB*$^+$, *JUN*$^+$, *TRAC*$^-$, *CD3D*$^-$) ● Dendritic cells (*CPVL*$^+$, *C1orf54*$^+$)
Tsang *et al.* (2017)[25]	Preeclampsia	Blood, placenta	Established a transcriptomic atlas of the normal and early preeclamptic placentas and proofed the power of single-cell transcriptomics and plasma cell-free RNA to reconstruct cellular information from plasma	● Decidual cells (*DKK1*$^+$, *IGFBP1*$^+$) ● Endothelial cells (*CDH5*$^+$, *CD34*$^+$, *PLVAP*$^+$) ● Vascular smooth muscle cells (*CNN1*$^+$, *MYH11*$^+$) ● Stromal cells (*COL1A1*$^+$, *ECM1*$^+$) ● Dendritic cells (*CD52*$^+$, *AIF1*$^+$) ● Hofbauer cells (*CD14, CD163*) ● T cells (*CD247*$^+$, *CD2*$^+$, *GZMA*$^+$) ● Erythrocytes (*HBB*$^+$, *HBA1*$^+$, *HBG1*$^+$) ● EVT (*PAPPA2*$^+$, *HLA-G*$^+$, *MMP1*$^+$) ● STB (*CGA*$^+$, *CYP19A1*$^+$) ● CTB (*PARP1*$^+$)
Zhang *et al.* (2021)[26]	Preeclampsia	Trophoblast cells	Endoplasmic reticulum signalling pathwaygene were over exrpressed in syncytiotrophoblast of PE patients.	● Endothelial cells (*PECAM1*$^+$) ● Proliferating macrophages (*TOP2A*$^+$) ● Erythroblasts (*HBB*$^+$, *HBA1*$^+$) ● Fibroblasts (*APOE*$^+$, *MRC1*$^+$) ● Myofibroblasts (*ACTA2*$^+$) ● Plasma cells (*IGKC*$^+$, *JCHAIN*$^+$) ● NK cells (*NKG7*$^+$) ● T cells (*CD37*$^+$) ● Monocytes (*FCN1*$^+$, *VCAN*$^+$) ● Macrophages (*CD68*$^+$, *APOE*$^+$, *SPP1*$^+$) ● Trophoblasts (*PARP1*$^+$, *CGA*$^+$, *CYP19A1*$^+$, *HLA- G*$^+$, *PAPPA2*$^+$) ■ STB (*CGA*$^+$) ■ EVT (*HLA-G*$^+$) ■ VCT (*PARP1*$^+$)
Yang *et al.* (2021)[27]	GDM	Placenta	Presence of immuno-cells in the placenta. DEGs related to agiogenesis were found in placenta ECT of GDM patient	● VCT (*CDH1*$^+$, *MET*$^+$, *CCNB2*$^+$, *NRP2*$^+$, *PARP1*$^+$, *INSL4*$^+$) ● EVT (*HLA-G*$^+$, *PAPPA2*$^+$, *MMP2*$^+$, *TGFB1*$^+$, *CXCR6*$^+$, *MMP11*$^+$) ● STB (*CYP19A1*$^+$, *CGA*$^+$, *ERVFRD-1*$^+$, *LGALS13*$^+$, *EGFR*$^+$) ● NK cells (*CD3G*$^+$, *CD3D*$^+$, *GZMA*$^+$, *TRBC2*$^+$, *GIMAP2*$^+$, *XCL2*$^+$, *GZMK*$^+$, *IFNG*$^+$, *FCER2*$^+$) ● Monocytes (*CD14*$^+$, *CD300E*$^+$, *CD244*$^+$, *HLA-DRA*$^+$, *CLEC12A*$^+$, *FCN1*$^+$) ● Macrophages (*CD14*$^+$, *CD68*$^+$, *AIF1*$^+$, *CD163*$^+$, *CD209*$^+$, *CSFIR*$^+$) ● Granulocytes (*FCGR3B*$^+$, *CXCL8*$^+$, *MNDA*$^+$, *SELL*$^+$) ● Myelocyte (*TCN1*$^+$, *CEACAM8*$^+$, *S100A8*$^+$, *MMP8*$^+$, *DEFA4*$^+$, *CAMP*$^+$)

CTB: cytotrophoblasts; EVT: extravillous trophoblast; STB: syncytiotrophoblasts; dNK: decidual natural killer; VCT: villous cytotrophoblast; ECT: extravillous trophoblast; GDM: gestational diabetes mellitus; PE: preeclampsia; RPL: recurrent pregnancy loss.

of villous cytotrophoblasts (VCTs) and EVTs illustrated that the altered cellular function of the PE group focused mainly on the immune response. Within the 2nd subtype of VCT, proteasomes, spliceosomes, ribosomes, and mitochondria-related genes were up-regulated with the disease state, confirming the presence of endoplasmic reticulum stress and mitochondrial interruption in the pathogenesis of PE.[26]

During pregnancy, the maternal immune system is altered to protect allogeneic fetal tissues against premature rejection. Dysregulated maternal immune system participated in various pregnancy-related pathologies, such as PE and other complications.[33,34] Reconfiguration of the peripheral immune cell phenotype during pregnancy was identified by scRNA-seq of peripheral blood mononulcear cells from 136 pregnant women with gestation between

6 to 40 weeks. Monocytes increased significantly during pregnancy, beginning at the first trimester. $CD4^+$ naïve T cells showed a slight peak in the first trimester of pregnancy. Furthermore, proliferative T cells increased significantly during pregnancy compared to non-pregnancy. Plasmablasts increased progressively in the first and second trimester. The percentage of plasmablasts in the third trimester was higher than that during non-pregnancy. The expression scores of the two immune responses increased significantly during pregnancy and tended to be higher with pregnancy progression, suggesting that enhanced responsiveness to interferon and virus is the result of global enhancement rather than limited to specific genes. 20 interferon-stimulated genes were significantly positively correlated with gestational weeks. The study also developed a model to predict gestational age with an accuracy of days, by using five types of immune related cells ($CD8^+$ cytotoxic T, $CD8^+$ naïve T, $CD4^+$ naïve T, dnT and NK cells).[35] Decidual macrophages were shown to be associated with PE. Abnormal gene expression affects the export function of decidual macrophages, which in turn interrupt the interaction of decidual macrophages with other immune cells, destroying the original immune regulation mechanism, ultimately leading to symptomatic PE.[24]

Marker genes were found to be sexually dimorphic. In female trophoblasts, X-linked MAGEA4 is up-regulated, wherease in male trophoblasts, autosomal MUC15 and NOTUM are up-regulated. MAGEA4 is also the most significantly placenta up-regulated gene than decidua.[19] NOTUM is a polarity determinant that affects stem cell migration, and MUC15 is essential for cell adhesion to the extracellular matrix.[36] These genes may play significant roles in sexually dimorphic trophoblast migration and invasion, providing insight into sexually dimorphic placental dysfunction and disease, such as PE.[37]

The decidual gene transcriptomic profile was altered in severe PE. Two hundred ninety-three key PE-related genes were found to be associated with severe PE. These genes were involved in 19 differentially regulated pathways relevant for the pathogenesis of PE. Mainly, *ENO2, PGK1*, and *HK2* belong to glycolysis/gluconeogenesis and HIF-1 signaling pathway, which are all highly related to tumorigenesis and are severely inhibited in the decidua of PE placentas. Among these PE-associated genes, 20 were core regulatory genes, including the newly identified pseudogenes *BNIP3P1, HK2P1*, and *PGK1P1* that encode long non-coding RNA; interestingly, *BNIP3*/*BNIP3P1*, *HK2*/*HK2P1*, and *PGK1*/*PGK1P1* appear in pairs in core genes.[38]

Although the clinical signs of PE appear late, its initiation is early, and hence early detection is feasible even in the first trimester. The PE-induced increase of EVT-specific mRNA expression was measured in maternal plasma.[25] The study of cellular transcriptome revealed that single-cell placental expression signatures are increased at the time of and before the diagnosis of early PE. A combination of three maternal circulating mRNAs originating from EVT cells (*MMP11*, *SLC6A2,* and *IL18BP*) was able to predict early PE at 11–17 weeks of gestation with a sensitivity of 0.83 (0.52–0.98) and specificity of 0.94 (0.79–0.99).[39]

Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is a status of hyperglycemia that develops during pregnancy. It carries a small but potentially significant risk of adverse perinatal outcomes and a long-term risk of obesity and glucose intolerance in offspring.[40] Only one study has investigated GDM with the scRNA-seq technique. In this study, which constructed a comprehensive cell atlas with affected placental tissues and matched controls, 235 differentially expressed genes (DEGs) were found in trophoblast cells between the GDM and normal control groups, of which 136 were up-regulated, and 99 were down-regulated. Functional analysis implies that the estrogen signaling pathway, antigen processing and presentation, and IL-17 signaling pathway were involved in the pathogenesis of GDM.[27] There are abundant ligand-receptor interactions between trophoblasts and immune cells in the maternal-fetal interface microenvironment related to GDM, including VEGFB-FLT1, MIF-EGFR, ADGRE5-CD55, CCL3-CCR1, CCL5-ACKR2. In addition, the RPS19-C5AR1 ligand-receptor complex was nearly absent in GDM placental samples. Similarly, SPP1-PTGER1 and SPP1-CD44 pairs exhibited a reduction in GDM placenta. Some potential marker genes of specific cell types were newly identified: *SLC1A2* was specifically expressed in STBs, whereas SLC1A6 was strongly expressed in EVT, and ADRB1 was only expressed for VCTs.[27]

INVESTIGATIONS OF ADVERSE PREGNANCY OUTCOMES

Recurrent pregnancy loss

Recurrent pregnancy loss (RPL), defined as loss of two or more consecutive pregnancies, affects up to 5% of women trying to conceive.[41] During the implantation window, decidualization disturbance may result in a breakdown of the fetal-maternal interface and pregnancy loss. Single-cell analysis found that RPL is associated with "excessive decidual senescence" but not "decidualization failure". It also confirmed that decidualization is a progressive process that originates from the acute stress response, followed by the synchronized transition of cells through intermediate transcriptional states, before emerging mainly as DCs and some senescent DCs. An *in vitro* scRNA-seq study suggests that the default trajectory of decidualizing endometrial stromal cells is cellular senescence, which can only be avoided by rapidly eliminating senescent DCs by uterine NK cells. This study also illustrated that senescent DCs rapidly perpetuate the senescent phenotype across the culture, leading to increased expression of

extracellular matrix constituents, proteases, and other senescence-associated secretory phenotype components and concluding with chronic senescence.[42] There has been evidence of significant interruptions of cellular interactions and immune cell subsets in RPL patients compared with the normal decidual immune microenvironment, especially increased dNK 2 and dNK 3 cell subsets with cytokine-secreting functions in RPL.[20] Increase of $CD8^+$ effector T, NK, and mucosal-associated invariant T cells, and decrease in the proportion of $CD4^+$ naïve T, $CD8^+$ naïve T, and $CD4^+$ memory T cells were identified in peripheral blood of RPL patients. In addition, the expression of *CXCL8*, *TNF*, *IFIT2*, *JUN*, and *JUNB* were up-regulated in decidual macrophage cells of women with RPL.[21] Marker genes were also identified in trophoblasts and macrophages of early pregnancy loss placental samples. These studies together provide us with a comprehensive tissue-specific atlas of the immune status of RPL and revealed that the over-activation of immunity at the maternal-fetal interface caused by the increase in pro-inflammatory immune cell subsets may be responsible for harmful effects on fetal survival.

Aneuploidy

Aneuploidy is a condition in which the conceptus has an incorrect number of chromosomes. Aneuploidies are a common cause of pregnancy loss, especially in early pregnancy.[43] No more than 50% of human zygotes survive to birth, mainly due to aneuploidies of meiotic or mitotic origin. scRNA-seq of human embryos revealed widespread mosaic aneuploidies, with 80% of embryos harboring at least one putative aneuploid cell. Immune response genes were up-regulated, and proliferation, metabolism, and protein processing genes were found to be down-regulated in aneuploid embryos.[44] Trisomy 21, trisomy 18, and trisomy 13 are the most common human aneuploidies and have been used extensively as a model to study trisomies. Phenotypes observed in trisomies in massive conventional RNA-seq studies have been attributed to gene dosage imbalance, typically 1.5-fold higher for trisomic genes than diploid genes.[45] However, the difference between mechanisms for altered gene expression and phenotypes in aneuploidies remains to be discovered. At the single-cell level, the scenario becomes more complicated. Reduced or no dosage effect was detected in scRNA-seq analysis of fibroblasts from trisomy 21, trisomy 18 and trisomy 13 patients. The study also revealed that dosage-sensitive genes in the bulk have a significantly lower-fold change in expression in single cells. Evidence indicates that the fraction of expressing cells is the main component of gene dosage imbalance for such genes.[46]

Preterm labor

Preterm labor affects 15 million neonates every year.[47] Massive genomic studies of the cervix, myometrium, and chorioamnionitis membranes identified genes and pathways associated with preterm labor. Understanding changes on a cellular level is essential to tackle the challenge of prematurity.

Maternal and fetal transcriptional signatures derived from placental scRNA-seq are modulated with advancing gestation and are markedly perturbed with term and preterm labor in both biopsy tissues and maternal circulation.[23] The number of EVT cells were found to be significantly reduced in chorioamnionitis membranes. In contrast, these cells were more abundant in the basal plate and placental villous of preterm women than in term groups. Within immune cell lines, the chorioamnionitc membranes primarily contained maternal lymphoid and myeloid cells, including T cells (mostly in a resting state), NK cells, and macrophages; the basal plate included immune cells of both maternal and fetal origin; and the placental villi contained more fetal than maternal immune cells. In preterm samples, the proportion of macrophages of maternal origin was abnormally reduced. In addition, a highly chorioamniotic membrane–specific cell type, lymphoid endothelial DC, was found to be enriched in both preterm and term no-labor groups.[23]

Adherent placenta

Koh *et al.* analyzed cervical and myometrium uterine biopsies from women who underwent a cesarean hysterectomy in the third trimester because of morbidly adherent placenta.[18] They identified the coexpression of cell-type-specific genes and inflammatory activation genes. This finding allows for a more detailed annotation of cell types. Furthermore, it supports the view that groups of chemokines and cytokines can be expressed in unique combinations by the various cell types under different physiological contexts during the process of parturition.

Exposure to paracetamal neurotoxity

Exposure to chemical medications during pregnancy may potentially increase the teratogenic risks in the development of the fetus. Spildrejorde *et al.* investigated the genetic dysregulation in human embryonic stem cells induced by paracetamol exposure, which was considered safe for use as the first line option of analgesic and antipyretic agent to pregnancy women. Shown by the scRNA seq of stem cells, dose-dependent changes in gene expression were linked to paracetamol exposure. The top involved functional pathways were cell-cycle transition (*MKI67*, *PCNA*, *TP53*, *CDK1*, *MYBL2* and *GJA1*), neurite outgrowth and cortical neurogenesis (*GBA2*, *ASPM*), neuronal maturation (*CDKN1C*, *POU2F1, POU3F1*, *ROBO1*, *STMN2* and *STMN4*) and transcription factors associated with brain development (*NKX2.1*, *OTX2*, *FOXG1*, *ASCL1*, *ISL1*, *EMX2* and *HOXA1*).[48]

Congenital heart disease

During the 1st trimester of pregnancy, the placenta and fetal heart develop concurrently, and share key developmental pathways. 328 commonly expressed genes and two major

pathways (vasculature development and angiogenesis) between fetal heart and placenta endothelial cells have been identified. Then the comparison of 1st trimester cadiomycytes and placenta cytotrophoblasts demonstrated 53 co-regulated genes, which belong cellular respiration, ion transport, oxidation-reduction process. Any disruption to these genes may contribute to the placenta related adverse outcome or congenital heart disease.[49]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

In the past three years of coronavirus disease 2019 (COVID-19) pandemic, thousands of pregnant women haven infected by SARS-CoV-2. American team performed scRNA seq investigation on cord blood mononulcear cells from infants, whose mothers were confirmed SARS-CoV-2 positive in the 3rd trimester of pregnancy. Although none of the infants were infected, study identified widespread genes transcriptional changes in cord blood mononuclear cells in SARS-CoV-2 positive cases, including upregulation in interferon-stimulated gens and major histocompatibility complex genes in $CD14^+$ monocytes, in comparasion of that of the uninfected control group. It suggests maternal SARS-CoV-2 infection can lead to the activation of plasmacytoid dendtritic cells, and exhaustion of NK cells and $CD8^+$ T-cells, in the absence of vertical transmission.[47]

CONCLUSION

A human cell atlas program is in the process of being designed to complete the mapping of 37 trillion cells in the human body.[50] To approach this goal, technologies with single-cell sequencing can accurately genotype individual cells and will be an important driving force. Development of a reproductive cell atlas on the basis of scRNA-seq will help in distinguishing cell types and understanding cell-to-cell relationships during fetal development. Furthermore, physiological processes and pathological mechanisms of pregnancy at the single-cell level would be uncovered to promote new diagnostic markers or new therapeutic targets. This will provide a practical basis for improving the diagnosis and treatment of the various types of pathogenic pregnancy.

DECLARATIONS

Author contributions

Zhao X, Tian G, Badillo AC wrote the main manuscript text. Zhao X, Ju W, Zhong N prepared the table. Zhao X, Zhong N checked the grammar and reviewed the manuscript. All authors read and approved the final manuscript.

Conflict of interest

Nanbert Zhong is the Editor-in-Chief of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this member and his research group.

REFERENCE

1. Association BCBS. Trends in Pregnancy and Childbirth Complications in the U.S. Accessed March 22, 2023. https://www.bcbs.com/the-health-of-america/reports/trends-in-pregnancy-and-childbirth-complications-in-the-us
2. American College of Obstetricians and Gynecologists Committee on Terminology, Hughes EC. *Obstetric-gynecologic terminology, with section on neonatology and glossary of congenital anomalies*. F. A. Davis Co.; 1972.
3. Salafia CM, Charles AK, Maas EM. Placenta and fetal growth restriction. *Clin Obstet Gynecol*. 2006;49(2):236–256.
4. Faye-Petersen OM. The placenta in preterm birth. *J Clin Pathol*. 2008;61(12):1261–1275.
5. Wen L, Tang F. Boosting the power of single-cell analysis. *Nat Biotechnol*. 2018;36(5):408–409.
6. Tang X, Huang Y, Lei J, Luo H, Zhu X. The single-cell sequencing: new developments and medical applications. *Cell Biosci*. 2019;9(1):53.
7. Li W, Ma Y, Yu S, *et al*. The mutation-free embryo for in vitro fertilization selected by MALBAC-PGD resulted in a healthy live birth from a family carrying PKD 1 mutation. *J Assist Reprod Genet*. 2017;34(12):1653–1658.
8. Liu Y, Fan X, Wang R, *et al*. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of differentiation in the human placenta. *Cell Res*. 2018;28(8):819–832.
9. Lee AWT, Ng JKW, Liao J, *et al*. Single-cell RNA sequencing identifies molecular targets associated with poor in vitro maturation performance of oocytes collected from ovarian stimulation. *Hum Reprod*. 2021;36(7):1907–1921.
10. Grindberg RV, Yee-Greenbaum JL, McConnell MJ, *et al*. RNA-sequencing from single nuclei. *Proc Natl Acad Sci U S A*. 2013;110(49):19802–19807.
11. He C, Wang K, Gao Y, *et al*. Roles of noncoding RNA in reproduction. *Front Genet*. 2021;12:777510.
12. Welch JD, Kozareva V, Ferreira A, Vanderburg C, Martin C, Macosko EZ. Single-cell multi-omic integration compares and contrasts features of brain cell identity. *Cell*. 2019;177(7):1873–1887.e17.
13. Cha J, Lee I. Single-cell network biology for resolving cellular heterogeneity in human diseases. *Exp Mol Med*. 2020;52(11):1798–1808.
14. Li H, Zhou J, Li Z, *et al*. A comprehensive benchmarking with practical guidelines for cellular deconvolution of spatial transcriptomics. *Nat Commun*. 2023;14(1):1548.
15. Wang W, Vilella F, Alama P, *et al*. Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. *Nat Med*. 2020;26(10):1644–1653.
16. Nelson AC, Mould AW, Bikoff EK, Robertson EJ. Single-cell RNA-seq reveals cell type-specific transcriptional signatures at the maternal-foetal interface during pregnancy. *Nat Commun*. 2016;7:11414.
17. Vento-Tormo R, Efremova M, Botting RA, *et al*. Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature*. 2018;563(7731):347–353.
18. Koh W, Wu A, Penland L, *et al*. Single cell transcriptomes derived from human cervical and uterine tissue during pregnancy. *Adv Biosyst*. 2019;3(11):e1800336.
19. Sun T, Gonzalez TL, Deng N, *et al*. Sexually dimorphic crosstalk at the maternal-fetal interface. *J Clin Endocrinol Metab*. 2020;105(12):dgaa503.
20. Guo C, Cai P, Jin L, *et al*. Single-cell profiling of the human decidual immune microenvironment in patients with recurrent pregnancy loss. *Cell Discov*. 2021;7:1.
21. Wang F, Jia W, Fan M, *et al*. Single-cell immune landscape of human recurrent miscarriage. *Genomics Proteomics Bioinformatics*. 2021;19(2):208–222.
22. Pavličev M, Wagner GP, Chavan AR, *et al*. Single-cell transcriptomics of the human placenta: inferring the cell communication network of the maternal-fetal interface. *Genome Res*. 2017;27(3):349–361.
23. Pique-Regi R, Romero R, Tarca AL, *et al*. Single cell transcriptional

signatures of the human placenta in term and preterm parturition. *Elife*. 2019;8:e52004.
24. Rong M, Yan X, Zhang H, Zhou C, Zhang C. Dysfunction of decidual macrophages is a potential risk factor in the occurrence of preeclampsia. *Front Immunol*. 2021;12:655655.
25. Tsang JCH, Vong JSL, Ji L, *et al*. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. *Proc Natl Acad Sci U S A*. 2017;114(37):E7786–E7795.
26. Zhang T, Bian Q, Chen Y, *et al*. Dissecting human trophoblast cell transcriptional heterogeneity in preeclampsia using single-cell RNA sequencing. *Mol Genet Genomic Med*. 2021;9(8):e1730.
27. Yang Y, Guo F, Peng Y, *et al*. Transcriptomic profiling of human placenta in gestational diabetes mellitus at the single-cell level. *Front Endocrinol (Lausanne)*. 2021;12:679582.
28. Efremova M, Vento-Tormo M, Teichmann SA, Vento-Tormo R. CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes. *Nat Protoc*. 2020;15(4):1484–1506.
29. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science*. 2005;308(5728):1592–1594.
30. Redman CW. Current topic: pre-eclampsia and the placenta. *Placenta*. 1991;12(4):301–308.
31. Nakagawa P, Gomez J, Grobe JL, Sigmund CD. The renin-angiotensin system in the central nervous system and its role in blood pressure regulation. *Curr Hypertens Rep*. 2020;22(1):7.
32. Nonn O, Fischer C, Geisberger S, *et al*. Maternal angiotensin increases placental leptin in early gestation via an alternative renin-angiotensin system pathway: suggesting a link to preeclampsia. *Hypertension*. 2021;77(5):1723–1736.
33. Nair S, Salomon C. Extracellular vesicles and their immunomodulatory functions in pregnancy. *Semin Immunopathol*. 2018;40(5):425–437.
34. Deshmukh H, Way SS. Immunological basis for recurrent fetal loss and pregnancy complications. *Annu Rev Pathol*. 2019;14:185–210.
35. Chen D, Wang W, Wu L, *et al*. Single-cell atlas of peripheral blood mononuclear cells from pregnant women. *Clin Transl Med*. 2022;12(5):e821.
36. Abnave P, Aboukhatwa E, Kosaka N, Thompson J, Hill MA, Aboobaker AA. Epithelial-mesenchymal transition transcription factors control pluripotent adult stem cell migration in vivo in planarians. *Development*. 2017;144(19):3440–3453.
37. Founds SA, Conley YP, Lyons-Weiler JF, Jeyabalan A, Hogge WA, Conrad KP. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. *Placenta*. 2009;30(1):15–24.
38. Tong J, Zhao W, Lv H, Li WP, Chen ZJ, Zhang C. Transcriptomic profiling in human decidua of severe preeclampsia detected by RNA sequencing. *J Cell Biochem*. 2018;119(1):607–615.
39. Tarca AL, Romero R, Erez O, *et al*. Maternal whole blood mRNA signatures identify women at risk of early preeclampsia: a longitudinal study. *J Matern Fetal Neonatal Med*. 2021;34(21):3463–3474.
40. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. *Nat Rev Endocrinol*. 2012;8(11):639–649.
41. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. *Fertil Steril*. 2008;89(6):1603.
42. Lucas ES, Vrljicak P, Muter J, *et al*. Recurrent pregnancy loss is associated with a pro-senescent decidual response during the peri-implantation window. *Commun Biol*. 2020;3:37.
43. Hassold T, Abruzzo M, Adkins K, *et al*. Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen*. 1996;28(3):167–175.
44. Starostik MR, Sosina OA, McCoy RC. Single-cell analysis of human embryos reveals diverse patterns of aneuploidy and mosaicism. *Genome Res*. 2020;30(6):814–825.
45. Rachidi M, Lopes C. Mental retardation in Down syndrome: from gene dosage imbalance to molecular and cellular mechanisms. *Neurosci Res*. 2007;59(4):349–369.
46. Stamoulis G, Garieri M, Makrythanasis P, *et al*. Single cell transcriptome in aneuploidies reveals mechanisms of gene dosage imbalance. *Nat Commun*. 2019;10(1):4495.
47. Muglia LJ, Katz M. The enigma of spontaneous preterm birth. *N Engl J Med*. 2010;362(6):529–535.
48. Spildrejorde M, Samara A, Sharma A, *et al*. Multi-omics analysis of paracetamol exposure identifies dysregulated genes involved in neurotoxicity and neuronal differentiation of human embryonic stem cells. Updated December 10, 2022. Accessed March 13, 2023. https://www.biorxiv.org/content/10.1101/2022.12.08.519620v1.full
49. Wilson RL, Yuan V, Courtney JA, Tipler A, Cnota JF, Jones HN. Analysis of commonly expressed genes between first trimester fetal heart and placenta cell types in the context of congenital heart disease. *Sci Rep*. 2022;12(1):10756.
50. Bianconi E, Piovesan A, Facchin F, *et al*. An estimation of the number of cells in the human body. *Ann Hum Biol*. 2013;40(6):463–471.