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REVIEW ARTICLE

Single-cell and spatial transcriptomics: Discovery of human placental development and disease

Mi Tang[1] | **Liling Xiong**[2] | **Jianghui Cai**[3] | **Li Fan**[1] | **Cheng Huang**[4] | **Shimao Zhang**[2] | **Ying Jin**[2] | **Er-dan Luo**[1] | **ShaSha Xing**[1] | **Xiao Yang**[2]

[1]Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China

[2]Obstetrics department, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China

[3]Department of Pharmacy, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China

[4]Clinical laboratory, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China

Correspondence
Shasha Xing, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, 611731, China.
Email: xingshasha1230@126.com

Xiao Yang, Obstetrics department, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 611731, China.
Email: wcchgcptg@163.com

Abstract

The human placenta is a vital organ, encompassing many distinct cell types, that maintains the growth and development of the fetus and is essential for substance exchange, defense, synthesis, and immunity. Abnormalities in placental cells can lead to various pregnancy complications, but the mechanisms remain largely unclear. Single-cell and spatial transcriptomics technologies have been developed in recent years to demonstrate placental cell heterogeneity and spatial molecular localization. Here, we review and summarize the current literature, demonstrating these technologies and showing the heterogeneity of various placenta cells and cell–cell communication of normal human placenta, as well as placenta-related diseases, such as preeclampsia, gestational diabetes mellitus, advanced maternal age, recurrent pregnancy loss, and placenta accreta spectrum disorders. Meanwhile, current weaknesses and future direction were discussed.

KEYWORDS

cell, placenta, placenta-related diseases, sc-RNA seq, spatial transcriptomics

Mi Tang, Liling Xiong and Jianghui Cai contributed equally to this work.

1 | INTRODUCTION

The placenta consists of the amnion and chorion frondosum of the fetal part and decidua basalis of the maternal part.[1] It is an important organ, encompassing many distinct cell types, to maintain the growth and development of the fetus and is essential for material synthesis, gas and nutrient exchange, defense function, and maternal tolerance of the fetal allograft.[2–4]

The villus is the functional unit of the fetal part of the placenta and consists of a trophoblast layer and an inner core derived from the trophectoderm and the extraembryonic mesoderm, respectively. Trophectoderm is located in the outer layer of the blastocyst. Day 5–12 postfertilization, blastocyst imbed to the endometrium, polar trophectoderm first adhere to the endometrium, trophoblast becomes thick and differentiate into villous cytotrophoblast (VCT/CTB) and multinucleated syncytiotrophoblast (STB). Trophoblastic lacunae, which finally become the intervillous space in the future, first appear within the STB and be filled with maternal blood.

After implantation, CTB proliferates and penetrates the STB to form primary villi. In the third week, extraembryonic mesoderm begins to invade the primary villi and transform them into the secondary villi. The extraembryonic mesoderm in the stem villus then differentiates into connective tissue and blood vessels, called tertiary villus. (Figure 1).

As the blastocyst implants into the decidualized endometrium, a specialized population of placental trophoblasts, extravillous trophoblasts (EVTs),[5] are involved in remodeling maternal spiral arteries. EVTs can be divided into two subtypes: interstitial extravillous trophoblast (iEVT) and endovascular EVT (eEVT). iEVT cells migrate through maternal decidua, preparing for the invasion of eEVT. eEVT cells then replace the vascular endothelium and convert the narrow spiral artery into a dilated, low-resistance uteroplacental vessel.

Beyond the outer trophoblast layer, there is an inner core consisting of fetal endothelial cells (ECs), fibroblasts (FBs), and Hofbauer cells (HCs) which are fetal macrophages. (Figure 2).

2 | SINGLE-CELL TRANSCRIPTOMICS

As the placenta is such a complex and heterogeneous organ consisting of multiple cell types, single-cell RNA sequencing (scRNA-seq) is a breakthrough technology to sequence the transcriptome of the placenta at the single-cell level, solving the problem of cellular heterogeneity and discovering rare cells by tissue sample sequencing. The process of scRNA-seq study includes sample collection, single cell isolation and capture, cDNA amplification, high-throughput sequencing, and data analysis. (Figure 3).

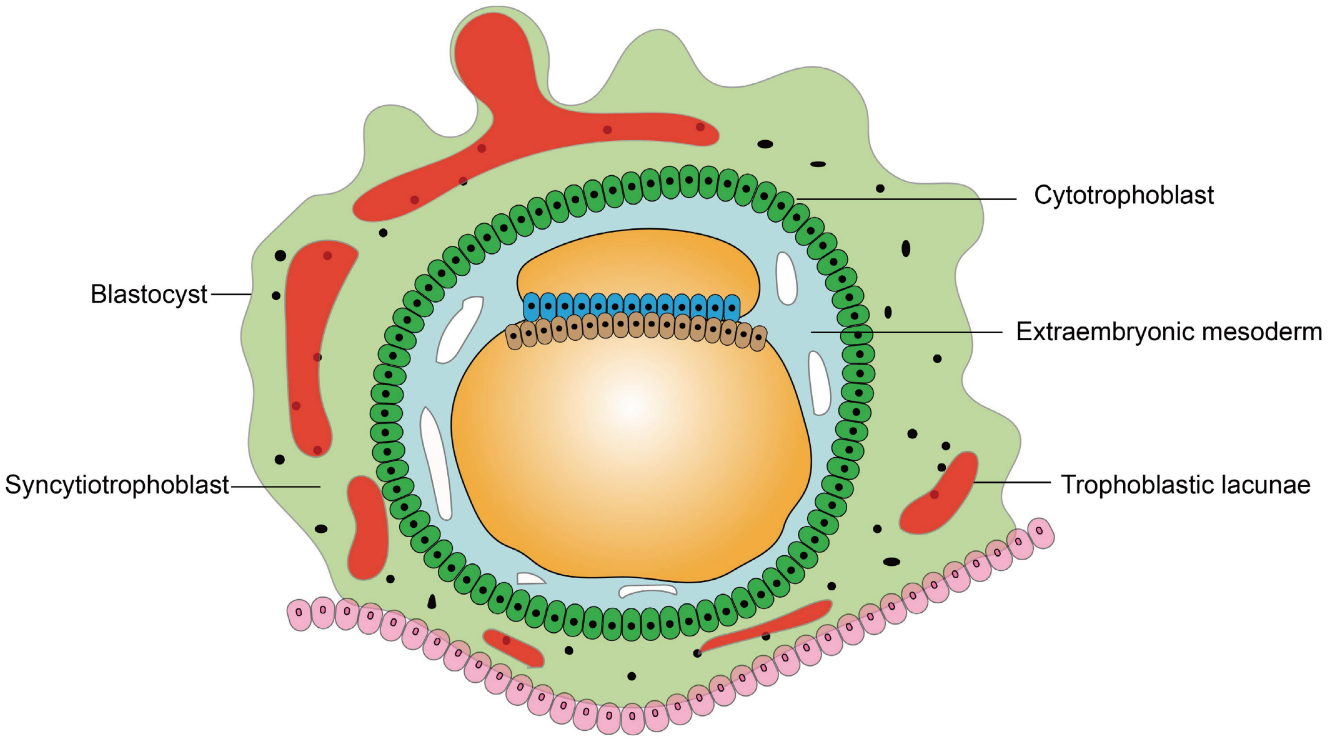

FIGURE 1 Implantation of blastocyst into the endometrium (day 12 postfertilization).

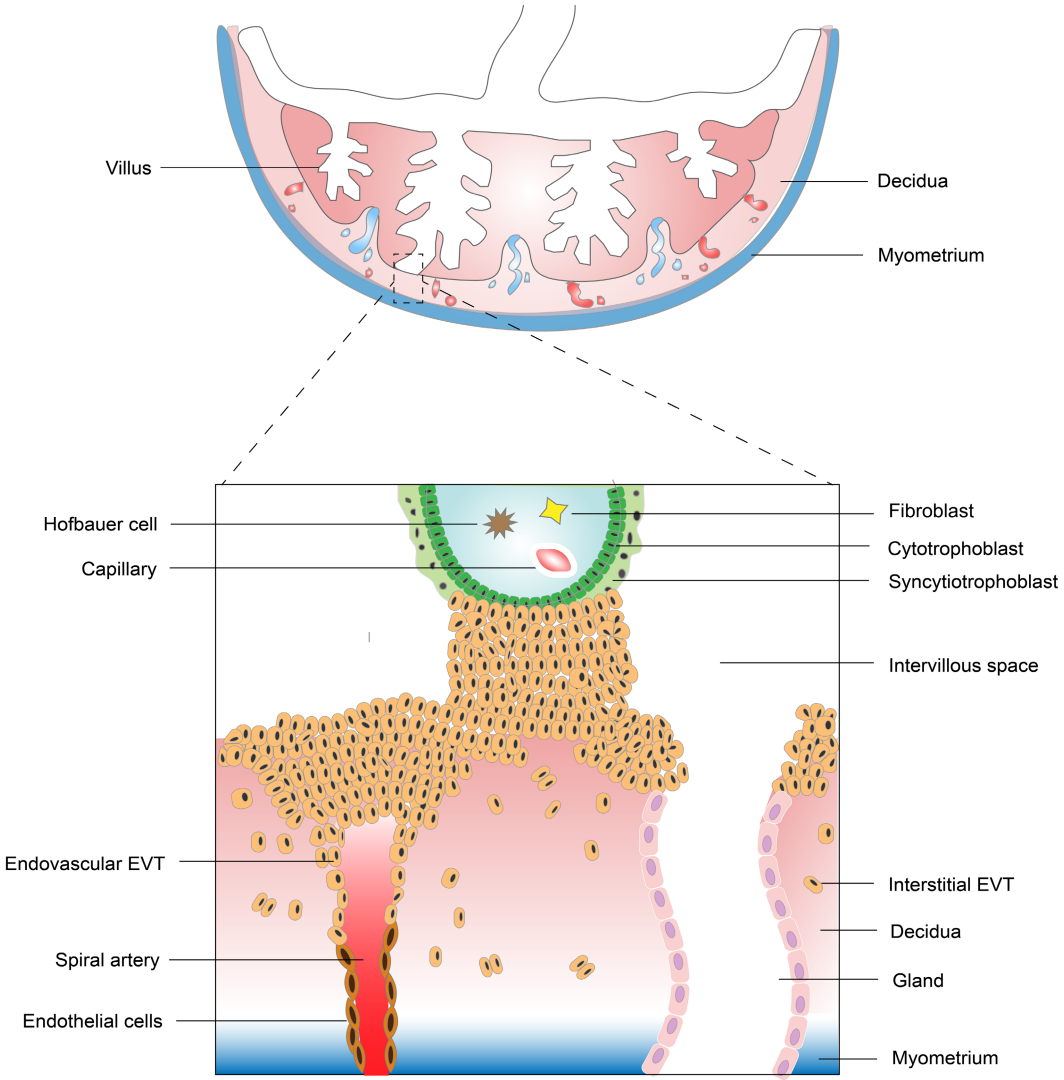

FIGURE 2 Constitution of placental tissue and cells.

Commonly used single-cell isolation techniques can be classified as low-throughput methods, including fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), limiting dilution, micromanipulation, laser-capture microdissection, and high-throughput methods, including droplets, microfluidics, and microwells.

FACS uses fluorescein to label different molecules and distinguishes target cells from nontarget cells by fluorescence, giving a great advantage in sorting a variety of labeled cells. MACS is an affinity-based technique that uses antibodies with magnetic beads to label target cells and then separates cells by a magnetic field. Although this procedure is simple, sorted cell types are limited.

FACS-based Smart-seq2 is a modified version of SMART-seq[6] designed to improve sensitivity, accuracy, and full transcript coverage. Smart-seq2 relies on template switching and preamplification, and allows the generation of full-length cDNA and sequencing libraries by using standard reagents.[7,8]

10x genomics chromium, usually based on droplets, enables large-scale scRNA-seq studies. This technology can mix single cells with gel beads, which contain barcode, unique molecular identifiers (UMIs), and primers to achieve large-scale single-cell isolation and single-cell library construction. The transcript coverage contains 3′ and 5′-end sequencing.

Microfluidic single-cell transcriptomic technology can classify a group of diverse cells at the transcriptome level and present the heterogeneity of the cell population. First, cells pass through an 8-channel microfluidic chip[9] and combine with gel beads to form gel beads in emulsion (GEM). Each gel bead is functionalized with barcoded oligonucleotides, allowing each cell to be specifically labeled. After passing through the microfluidic chip, the GEM is subjected to cell lysis and reverse transcription. Finally, the cDNA in each droplet was recovered for

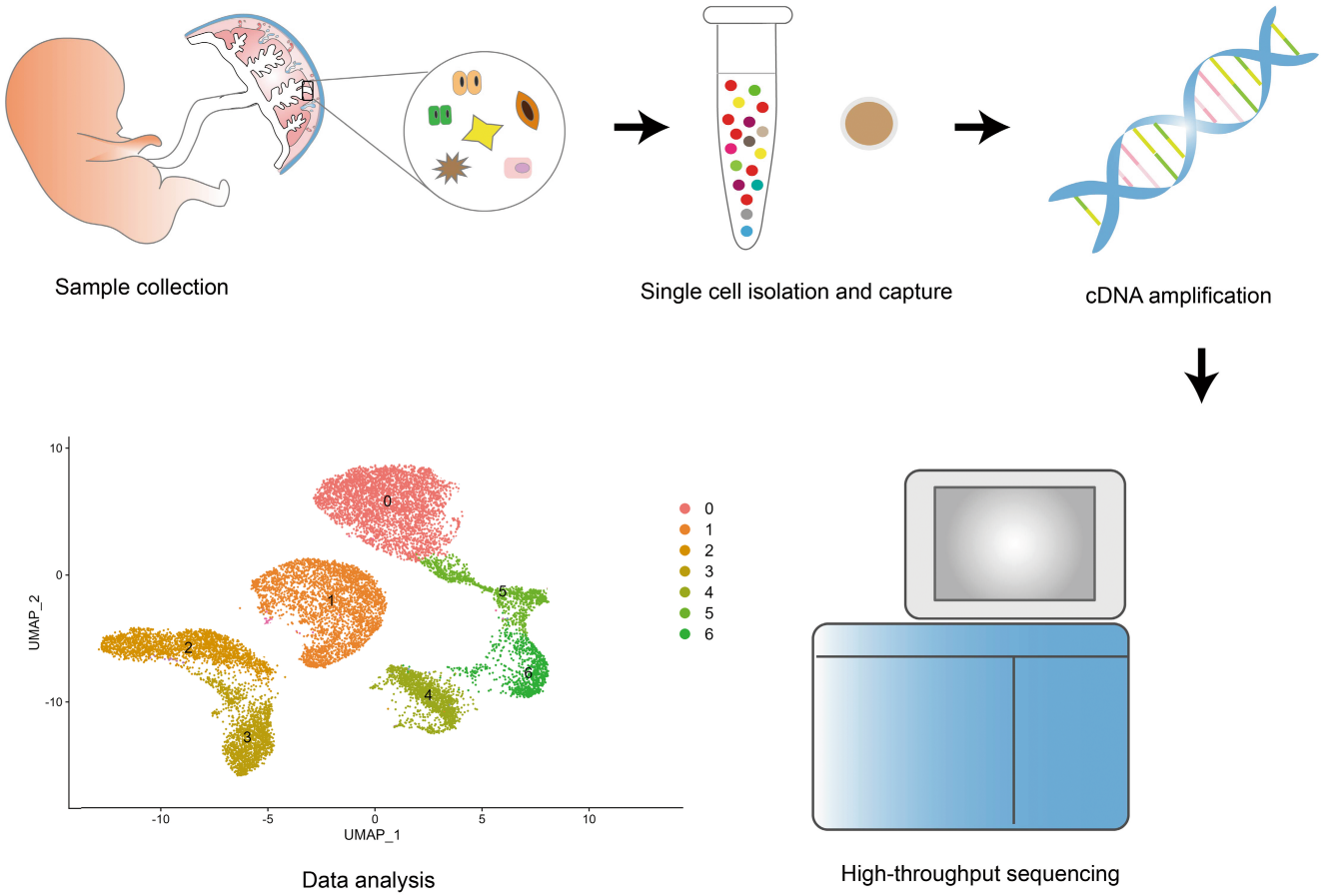

FIGURE 3 The progress of single cell RNA-sequencing on placenta cells.

amplification, library construction, sequencing, and subsequent analysis.[9]

However, because the proportions of the different types of known placental cells are so different in the placenta, an unbiased selection strategy would probably miss the potentially important but rare populations. We chose not to use fluorescence-activated cell sorting (FACS) technology because the primary cells, especially placenta-derived trophoblast cells, are very vulnerable to damage, and the high speeds experienced by cells during FACS can be detrimental. In contrast, magnetic-activated cell sorting (MACS) technology is beneficial for maintaining better cell vitality. Unsupervised clustering analysis can overcome the potential purity issue associated with this technology.

3 | SPATIAL TRANSCRIPTOMICS

The main problem with single-cell transcriptomics is that cell location information is lost during library construction. Meanwhile, spatial structure plays a key role in determining different functions and cell types. Spatial transcriptomics solves this deficiency of single-cell transcriptomics. Spatial transcriptomics includes[10] direct measurement using laser capture microdissection (LCM) coupled with next-generation sequencing (NGS) protocols, image-based detection, and in situ capturing technologies with NGS.[10–12]

LCM is a powerful cutting system incorporating UV light as a contact- and contamination-free knife to cut out tissue regions identified under a microscope and collect cells of interest in different containers to minimize spatial information loss.[13] Subsequently, direct RNA-seq or a multiplexed procedure with spatial barcodes can be chosen to profile these cells.[10]

In the field of image-based detection, in situ hybridization (ISH) technique can hybridize fluorescently labeled probes to predefined RNA targets in order to visualize gene expression in fixed tissue.[12] Then, single-molecule fluorescence in situ hybridization (smFISH) was formed through continuous evolution. smFISH uses multiple short oligonucleotide probes to target different regions of the same mRNA transcript, and it can measure one to several mRNAs simultaneously at subcellular resolution.[10] Although smFISH has high sensitivity, only a few genes are targeted at a time due to the limitation of spectral overlapping. Since then, seqFISH, MERFISH, osmFISH,

and other in situ hybridization techniques have been developed.

In situ capturing technologies with NGS contain 10x Genomics Visium, Nanostring GeoMx Digital Spatial Profiler (DSP), Slide-seq, Stereo-seq, DBiT-seq, et al. 10x Genomics technology uses the method of oligonucleotide-based spatial barcoding followed by NGS. Spatial barcodes, poly(T) oligonucleotides and UMIs on commercial microarray slides can capture polyadenylated transcripts. As a result, spatial locations of transcripts can be preserved from tissue sections on in situ arrays.[14] The Visium assay from 10X Genomics has improved resolution (55 μm in diameter and smaller distances between barcode regions) as well as runtime. This approach captures twice as many transcripts as LCM-seq. Unlike Visium, which can apply whole-mRNA analysis, DSP manually selects regions of interest (ROIs) via microscopy of varying sizes (10–600 μm in diameter). UV light then excites the ROIs and triggers the release of RNA target probe coupled barcoded tags. DSP has a high level of automation and reduced background noise; however, when using smaller ROIs, DSP has low sensitivity. Stereo-seq uses rolling circle amplification (RCA) to locally amplify unique barcode sequences. In this technology, the random barcode-labeled DNA nanoball is generated in stereo chip and each colony barcode is sequenced, then a poly(T) capture sequence can be ligated onto the nanoball to enable capture of released mRNA.[11] (Figure 4).

4 | TROPHOBLAST CELLS

4.1 | VCT/CTB

At 8 weeks of gestation, CTB is divided into three subtypes according to a scRNA-seq study of human placental villi at 8 weeks, including CTB_8W_1, CTB_8W_2, and CTB_8W_3.[15] Not all, but only CTBs exiting the cell cycle were found capable of fusion.[16,17] Although both CTB_8W_1 and CTB_8W_2 were nonproliferative subtypes exiting the cell cycle, only CTB_8W_1 had ERVFRD-1 (Syncytin-2), which was proved to be a prerequisite for the fusion of CTB into STB. Thus, CTB_8W_1 may be the progenitor cells of the STB. Besides, the CTB_8W_3 cells showed the highest proliferative activity.

Bryan Marsh et al.[18] applied 10× Genomics scRNA-seq to villous chorion and smooth chorion (SC) regions from second-trimester human placentas to better understand the differences in the cell types and functions of these regions. CTB was divided into four populations, CTB 1–4. CTB 4, also termed SC-CTBs, was a novel subcluster of CTBs unique to the SC and marked by a specific cytokeratin (KRT6) expression. The SC-CTBs form a stratified epithelium above a basal layer of progenitor CTBs. Extracellular matrix (ECM) components in SC-CTBs were upregulated, and several potential regulators which could translate a common villous CTB-like cell to SC-CTBs were identified, such as epidermal transcription factors KLF4 and YAP1. SC-CTBs were demonstrated to be building blocks and critical regulators of the SC barrier, playing a role in protection against pathogen infection and physical forces. Moreover, this study also supported a paracrine signaling mechanism by which SC-CTBs inhibit EVT invasion in the SC, as numerous interactions between EVTs and SC-CTBs, including both modifiers of the ECM (FN1 and THBS1) and canonical cell signaling pathways (TNFa, PGF, TGFB1, FGF1, and PDGFB).

While at the first and second trimesters (6–16 gestational weeks), scRNA-seq was performed to profile the transcriptomics of human placental villous tissues, finally five subtypes of VCT (VCT-1, -2, -3, -4, and -5) were identified.[19] Syncytin-related gene (ERVW-1 and its receptor gene ASCT1 and ASCT2, ERVFRD-1 and its receptor gene MFSD2A) selectively expressed in SCT and VCT-5, suggesting the fusion capacity of VCT-5. In addition to VCT-5 exhibiting a proliferative inactivation state, there is another proliferative inactivation subtype, VCT-1, which was not involved in the process of trophoblast differentiation and may be an in-depth supplement to CTB_8W_2 mentioned above. VCT-2, VCT-3, and VCT-4 expressed the cell cycle-related genes and had higher proportion of G2/M stage cells than the other two subtypes. VCT-2 exhibited the highest proliferative activity. Furthermore, VCT-3 was marked by TAGLN and proved as trophoblast EVT progenitors with the potential to form EVT outgrowth.[19]

A spatial multiomics study in early pregnancy has carried on the further detail to VCT subsets and divided them into VCT, VCT-proliferative (VCT-P), VCT-fusing, and VCT-cytotrophoblast cell columns (CCCs).[20] VCT-P was likely to be similar to CTB_8W_3 and VCT-2 mentioned above. This study indicated that VCT-fusing and VCT-CCC gave rise to SCT and EVTs, respectively. VCT-fusing may be the same cluster of cytotrophoblast cells as CTB_8W_1, SC-CTBs, and VCT-5 mentioned above. As VCT committed into VCT-fusing, they downregulate Wnt (WLS, TNIK, and LGR5) and BMP signals (BMP7) and upregulate the endogenous retroviral genes (ERVW-1, ERVFRD-1, and ERVV-1). As VCT or VCT-p committed to become VCT-CCCs, they downregulate the Wnt pathway, upregulate NOTCH1, undergo an integrin shift, and modulate epithelial–mesenchymal transition. Activation of the FOXM1-NOTCH1 axis may lead to the differentiation of VCTs into VCT-CCCs.[20]

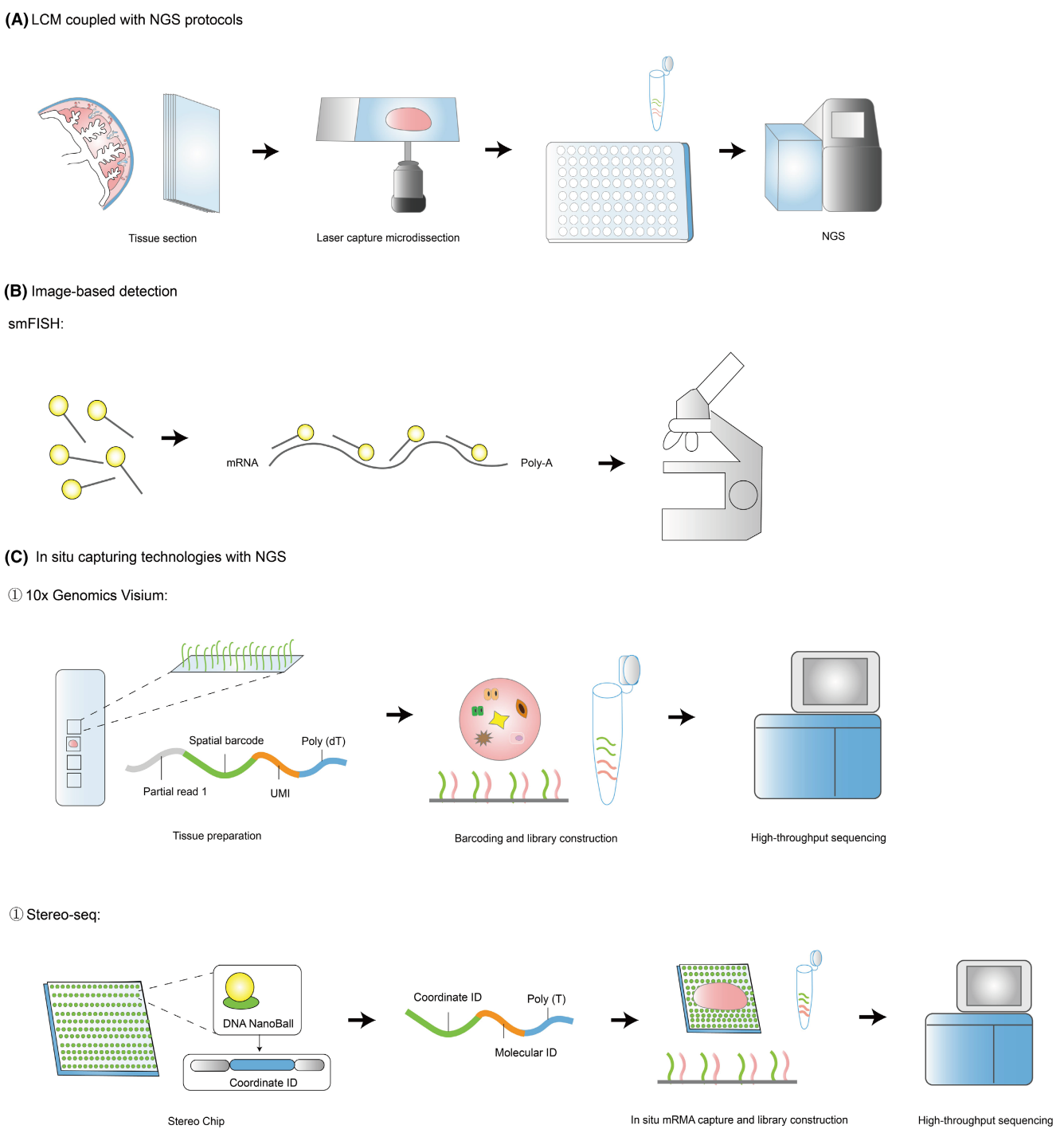

FIGURE 4 The progress of spatial transcriptomics on placenta cells.

Besides, Shannon MJ et al. established scRNA-seq dataset from first trimester placentas and connected with human trophoblast stem cell lines (hTSCs) organoid single cell data. Cell adhesion molecule BCAM was explored as a primitive progenitor marker identifying and defining CTB progenitors.[21] In another study of scRNA-seq analyses in first-trimester human placenta, along with mechanistic analyses in human trophoblast stem cells, Hippo signaling cofactor WW domain containing transcription regulator 1 (WWTR1) was found to promote CTB self-renewal and EVT differentiation and prevent induction of the STB fate in undifferentiated CTBs. Furthermore, the analyses of placentae from pathological pregnancies show that extreme preterm births were often associated with loss of WWTR1 expression in CTBs.[22]

Characteristics of cytotrophoblast subsets in human placentas are demonstrated in Table 1.

TABLE 1 Characteristic of cytotrophoblast subsets in human placentas.

Reference	Sample site	Sample time	Cytotrophoblast subsets					
			Villous cytotrophoblast	SCT progenitors	EVT progenitors	Proliferative activity	Non-proliferative	Undefined
Yawei Liu et al. (2018)[15]	Villi and decidua	First trimester		CTB_8W_1		CTB_8W_3	CTB_8W_2	
Bryan Marsh et al. (2022)[18]	Villous chorion and smooth chorion	Second trimester	CTB 1	CTB4				CTB2; CTB3
Han Li et al. (2022)[19]	Placental chorionic villi	First and second trimesters		VCT-5	VCT-3	VCT-2	VCT-1	VCT-4
Arutyunyan, Anna et al. (2023)[20]	Decidual and placental samples	First and second trimesters	VCT	VCT-fusing	VCT-CCCs	VCT-P		

4.2 | STB

Pavličev M et al. collected STB from a fresh-frozen single term placenta, processing by LCM coupled with the conventional RNA-seq.[23] STB highly expressed hormones (CSH2, CSHL1, and GH2), CGA, and pregnancy-specific glycoproteins (PSG-family).

4.3 | EVT

EVT_8W and EVT_24W cells were obtained in a scRNA-seq study on human placental villi at 8 weeks and decidua at 24 weeks of gestation.[15] EVT_8W cells were associated with control organic anion transport and epithelial cell proliferation, while EVT_24W cells were related to ECM organization and cellular component movement due to their invasive property. The EVT_8W cells scattered into EVT_8W_1, EVT_8W_2, and EVT_8W_3. EVT_8W_1 was likely localized at the proximal end of the cell column and had proliferative potential. EVT_8W_3 cells were associated with receptor activity regulation and the immune response and displayed a strong similarity with EVT_24W cells in terms of gene expression. EVT_24W is divided into EVT_24W_1 and EVT_24W_2 cells. EVT_24W_1 cells were associated with the response to wounding, digestion, and the negative regulation of the immune system in the subtype, whereas EVT_24W_2 cells were associated with growth regulation, gonadotropin secretion, and pregnancy. Besides, SNAI1, STAT1, ASCL2, and ID1 played an essential role in EVT cell differentiation and the maintenance of EVT cell characteristics.[15] EVT cell subtypes expressed many polypeptide hormone genes such as CSH1, FSTL1, PAPPA2, TAC3, and PSG genes.[15]

In another study, which performed scRNA-seq to profile the transcriptomics of human placental villous tissues in the first and second trimesters,[19] Han Li et al. divided EVT into EVT-1 and EVT-2.

EVT-1 represented a mature stage characterized by the regulation of locomotion, adhesion, and invasion. Meanwhile, EVT-2 could be marked by C1QA and might be in an early differentiation stage characterized by anti-inflammatory and immunomodulation, low expression of the EVT signature gene, and the presence of epithelial to mesenchymal transition (EMT) signature. Besides, integrin-mediated signaling pathway and transforming growth factor beta (TGF-β) receptor signaling pathway were enriched in EVT-1 and EVT-2.[19]

10x Genomics Visium technology and smFISH were used in combination with scRNA-seq and snRNA-seq in a recent study, depicting EVTs into EVTs-1, EVTs-2, iEVTs, and eEVTs.[20] EVTs-1 were proliferative, while EVTs-2 did not proliferate and located at the distal end of the

anchoring villi. EVTs-2 could transition either into iEVTs that invaded through decidual stroma, or into eEVTs that were present inside spiral arteries. iEVTs, which were marked by TGFβ upregulation and Wnt inhibition, eventually fused to form placental bed giant cells (GCs) deeper in the decidua and myometrium. Whereas eEVTs, which were characterized by strong upregulation of Notch signaling and downregulation of TGFβ signaling, formed plugs inside the maternal arteries. Overall, GCs and eEVTs were defined as the final cell states of trophoblast invasion.

5 | FETAL NON-TROPHOBLAST CELLS

5.1 | Stromal cells

ScRNA-seq study of human placental villi at 8 weeks of gestation revealed that the CD90, ENG, and CD74 tripositive mesenchymal stromal cells were clustered into two subtypes (Mes_1 and Mes_2). Mes_1 and Mes_2 cells were distinguished by low and high expression of DLK1, respectively. Mes_1 cells were implicated in the regulation of cell adhesion and migration, whereas Mes_2 cells indicated involvement in the development of blood vessels, the mesenchyme, and the tube.[15] The stromal cells expressed many hormone genes such as ANGPTL1, ANGPTL2, ANGPTL4, CTGF, and ACTN1.[15]

5.2 | Hofbauer cells

The CD68-positive Hofbauer cells in first-trimester human placentas clustered into two subtypes (Macro_1 and Macro_2).[15] Macro_1 and Macro_2 marked by CD74 and MRC1, respectively. Macro_1 cells highly expressed genes encoding different chains of the HLA class II histocompatibility antigen. Compared with Macro_2 cells, Macro_1 cells seemed to be in an activated state and participated in removing dead cells or cellular debris during the early development of the placenta.[15]

5.3 | Endothelial cells

Han Li et al. identified three major EC clusters (Endo-1, -2, and -3) in a scRNA-seq study of human placental villous tissues in the first and second trimesters.[19] Endo-2 was a population of endothelial progenitor cells, and most genes involved in glycolysis were upregulated in Endo-2. Endo-1 was marked by CTHRC1 and might be involved in forming immature intervillous vascular beds in early pregnancy, while Endo-3 was marked by VEGFC and participated in active placental angiogenesis after the first trimester. Furthermore, PFKFB3, a gene encoding glycolysis rate-limiting enzyme, was upregulated in Endo-3 and might be associated with the proangiogenic environment and signal stimulation in the placenta.[19]

6 | MATERNAL CELLS

6.1 | Decidualized stromal cells (DSCs)

Suryawanshi H et al. used 10x Genomics and drop-seq platforms to analyze first-trimester placental villous and decidual tissues.[24] Cell types of decidua included DSCs and two populations of decidual fibroblast cells (FB1 and FB2). Two trajectories were described to address stromal cell differentiation, originating from the FB1 towards DSCs and FB2 population with a gradual increase in expression of PRL and GEM, respectively.

Vento-Tormo R et al. found three clusters of stromal cells (dS1, dS2, and dS3) in first-trimester deciduas, all of which express the WNT inhibitor DKK1.[25] Furthermore, dS1 shares the expression of ACTA2 and TAGLN, and lacks the classical decidual markers prolactin (PRL) and IGFBP1. According to a spatial transcriptomic technique, smFISH, dS1 cells were found between glands in the decidua spongiosa. By contrast, dS2 and dS3 express IGFBP1, IGFBP2, and IGFBP6. The dS3 subset expresses PRL as well as genes involved in steroid biosynthesis (for example, CYP11A1). dS2 and dS3 cells were found located in decidua compacta.[25]

6.2 | Perivascular cells (PVs)

In a scRNA-seq study of first-trimester placentas and deciduas, two clusters of perivascular cells (PV1 and PV2) were identified according to the expression of smooth muscle marker (MGP) and distinguished by different levels of MCAM, which is higher in PV1, and MMP11, which is higher in PV2. These cells were confirmed to be present in the smooth muscle media of the spiral arteries through smFISH.[25] PV1 was further divided into PV1-AOC3 (AOC3-high, MYH11-high, FNDC1-high, and NTRK2-high) and PV1-STEAP4 (STEAP4-high, EPHB6-high, and LZTS1-high) according to the combination of scRNA-seq and smFISH.[20]

6.3 | Dendritic cells (DCs)

According to the single-cell transcriptomes of villous tissue of two human term placentas, placental cells were divided

into intravillous CTB, EVT, and dendritic cells (DCs), characterized by MHC II subunits (HLA-DRA in particular).[23] Suryawanshi H et al. divided the dendritic cells of the first-trimester decidua into DC1 and DC2, which were CLEC9A+ and CD1C+ subtypes, respectively.[24]

6.4 | NK cells

Suryawanshi H et al. differentiated NK cells of the first-trimester decidua into a resting (NK1) and a proliferating (NK2) subpopulation based on the expression of MKI67 and TOP2A.[24]

Vento-Tormo R et al. demonstrated three subtypes of dNK cells (dNK1, dNK2, and dNK3) in the early maternal-fetal interface, and predicted the likely function of dNK cells was to mediate the extent of trophoblast invasion and coordinate multiple immunomodulatory pathways that involve T cells, myeloid cells, and stromal cells. The defining marker of dNK1 cells was CD39, while the marker of dNK2 cells was ITGB2. dNK1 increased expression of glycolytic enzymes, expressed higher levels of KIRs (KIR2DS1, KIR2DS4, KIR2DL1, KIR2DL2, and KIR2DL3), the receptors for trophoblast HLA-C molecules and expressed LILRB1, the receptor for trophoblast HLA-G molecules. Both dNK1 and dNK2 expressed receptors for HLA-E molecules (NKG2C, NKG2E, and NKG2A). These results predicted that dNK1 could be connected with the recognition and response to EVT. dNK3 highly expressed CD103 and CCL5. CCR1, the receptor for CCL5, was expressed by EVT, which suggests a role for dNK3 in regulating EVT invasion.[25]

6.5 | Decidual macrophages (dMs)

dM1 (EREG+ and IL1B+) and dM2 (FOLR2+ and CD14high) were defined in a spatial multiomics study during early pregnancy, and both of them expressed the chemokine genes CXCL16 and CCL3. This study found CXCR6 + HLA-G+ EVTs and CXCL16 + CD14+ dMs were in close proximity in the implantation site, confirming that CXCL16 played a role in promoting trophoblast motility and function.[20]

6.6 | Lymphatic endothelial decidual cells (LEDs)

Pique-Regi R et al. used scRNA-seq to profile the placental villous tree, basal plate, and chorioamniotic membrane (CAM) of women with or without labor at term or preterm labor.[26] This study identified a new cell type, LEDs in the CAM. LEDs formed a distinct transcriptional cluster, which was of maternal origin, separated from other endothelial cell types. LEDs had a likely function to mediate the influx of immune cells into the CAM, as LEDs were involved in cell–cell and cell–surface interactions at the vascular wall, ECM organization, tight junction, and focal adhesion.

Markers of human placental villus and deciduas are demonstrated in Table 2.

7 | CELL COMMUNICATION

The cellular communication network at the maternal-fetal interface of the human term placenta has been characterized for the first time by Mihaela Pavličev et al.[23] In this study, the highly cell type-specific expression of G protein-coupled receptors implied that ligand-receptor profiling may be useful for cell type identification. Most upregulated receptors and ligands during decidualization had their counterparts in trophoblast cells. Along the transmitting signals, immune signals and growth factors occupied a dominant position.

Hemant Suryawanshi et al.[24] described the interactome of the most abundantly expressed ligands and receptors within and between decidua and villi cells, indicated that CCL21 was uniquely expressed by decidual LEDs, and the receptor CCR7 was expressed by CTBs and STBs, the interaction of them showed a likely role of trophoblast migration in decidua.

A repository of ligand-receptor complexes and a new database of the curated complexes (www.CellPhoneDB.org) were developed by Roser Vento-tormo et al. to systematically study the interactions between fetal and maternal cells. CellPhoneDB was used to identify the expression of cytokines and chemokines by dNKs and to predict molecular interactions with other cells at the maternal-fetal interface in the first trimester, such as EVT, DC, EC, stromal cells, and macrophages. For example, dNK interacted with EVT cells through multiple ligand-receptor pairs, including CSF1R-CSF1, CCR1-CCL5, PVR-CD96, and PVR-TIGIT.[25] This study showed that in the decidua, all damaging maternal T or NK cell responses to fetal trophoblast cells were prevented, and in the immune microenvironment of the decidua, inflammatory responses were prevented, which had the potential to be triggered by trophoblast invasion and destruction of the spiral arteries by trophoblast.

Stereo-seq was first used in the human placenta in a recent study. Liu Z et al. used chorionic villi at 8 weeks gestational age from two healthy pregnant women for spatial transcriptome sequencing, and adopted published scRNA-seq data from a previous study (Vento-Tormo et al., 2018) for the spatial data deconvolution.[27] This study demonstrated the usefulness of spatial transcriptome in studying

TABLE 2 The markers of human placental villus and deciduas.

Villus						Deciduas
Trophectoderm			Extraembryonic mesoderm			
Cytotrophoblast	Syncytiotrophoblast	Extravillous trophoblast	Fibroblasts / mesenchymal stromal cells	Hofbauer cells	Vascular endothelial cells	Decidualized stromal cells
KRT8, GCM1, ERVW-1, FLT1, KRT7, CYP19A1, SEPP1, and ERVFRD1	KRT8, GCM1, ERVW-1, FLT1, KRT7, CSH2, CSHL1, GH2, CGA, and PSG-family	KRT8, GCM1, ERVW-1, FLT1, KRT7, ADAM12, PRG2, DIO2, TAC3, HLA-G, PSG2, ITGA5, PSG5, and MCAM				
CDH1 and EGFR	CGB and CSH1	HLA-G, MMP2, and CDH1	THY1 and CD90	CD14 and CD68		
KRT7, PERP, PARP1, PAGE4, and PEG10	KRT7, PERP, and ERVFRD-1	KRT7, PERP, HLA-G, DIO2, and LAIR2	VIM, COL1A1, COL1A2, COL3A1, ACTA2, DLK1, and EGFL6	CD163, CD14, CSF1R, CD68; CCL4, CXCL8; AIF1	CDH5 and PECAM1	PRL, IGFBP1; APOA1, CHI3L2, SERPINA3, IL1B, and PROK1
GATA3, GLUT1, EGFR, NRP2, and MET	GATA3 and GLUT1	GATA3, GLUT1, HLA-G, HLA-E, and HLA-C I	PGF and HGF	HBEGF		PRL, IGFBP1; DKK1
TEAD4, ELF5, and EGFR	ERVFRD-1 and CGA	HLA-G and ITGA1	VIM and ACTA2		CD34 and VCAM1	Not mentioned
KRT7; PAGE4, PEG10	CGA, CYP19A1, CSH1, CSH2	KRT7, HLA-G, DIO2	VIM			
EGFR, ITGA6, and LRP5	CGA, PSG1, and CSH1	MMP2, FN1, and HLA-C	COL1A1, COL1A2, and PITX2	MTF, MAF, and POU2F2	ZEB1, ZBTB20, and PECAM1	
KRT7 and PARP1	ERVFRD-1, LGALS16, GDF15	HLA-G and ASCL2	COL1A1	LYVE1, VSIG4, MRC1, HPGDS, and CD14	CDH5 and PECAM1	
TP63, CDH1, EGFR, YAP1, and BCAM	TFAP2A, CYP19A1, and MFSD2A	ITGA1, PLAC8, HLA-G; CSH1, FBLN1, TIMP3, CD81, and EBI3				

ligand-receptor pairs, not only confirming the previously found ligand-receptor pairs, including DLK-NOTCH3 and PGF-FLT1, but also identifying new pairs, such as IGF2-IGF2R, WNT-LDLR, and WNT-FRZB pairs. Moreover, the location of various ligand receptors in the villi was shown and demonstrated that proximity was the important determining factor in cell-to-cell interactions. For paracrine or membrane-bond ligands, the interaction could exist between neighboring cells.

10x Genomics Visium technology was also applied in the spatial multiomics map of the maternal-fetal interface in early pregnancy. Cell–cell communication was predicted to contribute to trophoblast invasion and placental bed giant cell formation.[20] CSF1R-CSF1 interaction was found enriched near the trophoblast shell, confirming the findings of Vento-Tormo et al. EVTs interacted with decidual maternal macrophages and dNK cells through multiple ligand-receptor pairs, just as macrophages expressed the chemokine genes CXCL16 and CCL3, and the receptor genes CXCR639 and CCR1 were upregulated in invading EVT.[20] Ligand-receptor pairs were also detected in iEVT and PV subsets. Expression of EFNB1 by iEVTs could induce their tropism towards the arteries as PVs express the cognate receptor gene, EPHB6. Moreover, iEVTs upregulated specific cell signaling molecules (PTPRS and NTN4) whose cognate receptor genes (NTRK2 and NTRK3) were upregulated in PV1-AOC3. As a result, interactions between PV1-AOC3 and iEVT might drive iEVT tropism towards the arterial wall and lead to medial destruction.[20] eEVTs had a specific ECM that could allow them to form the plugs; they expressed both ITGB1 and ITGA2 (forming the integrin α2β1) and its cognate collagen ligands (COL6A1, COL19A1, COL26A1, and COL21A1). Besides, upregulation of ligand (JAG1 and JAG2) and receptor (NOTCH2 and NOTCH3) genes suggested active Notch signaling. The expression of ECM component (COL21A1–ITGA2)

Perivascular cells	Decidual fibroblasts	Smooth muscle cells	Endometrial epithelial cells	Natural killer cells	Maternal macrophages	Dendritic cells	T cells	Lymphatic endothelial cells	Reference
						ITGAX, CD14, CD4, CD83, and CD86			Mihaela Pavličev et al. (2017)[23]
									Yawei Liu et al. (2018)[15]
	ARC, GEM, BDKRB1, and PLIN2	MYH11, ACTA2, RGS5; PI15; and NDUFA4L2	PAEP; pithelial markers such as KRT8 and CDH1	NKG7 and NCAM1	CD74; HLA-DRA, HLA-DPA1; HLA-DRB1, and HLA-DPB1		CD3D and CD69	LYVE1	Hemant Suryawanshi et al. (2018)[24]
MGP, MCAM, and MMP11			CD34 (endothelial cells)	CD49A (ITGA1) and CD9					Roser Vento-Tormo et al. (2018)[25]
Not mentioned				PTPRC, encoding the CD45 protein					Matthew J Shannon et al. (2022)[21]
				CD45					Bryan Marsh et al. (2022)[18]
									Liu Z et al. (2022)[27]
					FCGR3A and HLA-DRA				Han Li et al. (2022)[19]
MCAM, AOC3, STEAP4, and MMP11				NCAM1	EREG, IL1B, FOLR2, and CD14				Arutyunyan, Anna et al. (2023)[20]

and Notch (NOTCH2–JAG1) interactions was visualized in the arterial plug. eEVTs also had specific interactions with endothelial cells, allowing them to adhere to endothelial cells. EPHA1, CXCL12, FLT4, and ANGPT4 could mediate the interaction of eEVTs in the vasculature, with their interacting partners EFNA1, EFNA5, VEGFC, and TEK expressed by endothelial cells.[20]

Single-cell transcriptomes and spatial transcriptomes in the normal human placenta are illustrated in Table 3.

8 | PLACENTA-RELATED DISEASES

8.1 | Preeclampsia (PE)

PE[28] is a hypertensive disorder of pregnancy, leading to the presence of proteinuria, multiorgan system involvement, and uteroplacental dysfunction after 20 weeks of gestation. Tsang JCH et al.[29] established a large-scale cellular transcriptomic atlas of normal-term and early preeclamptic placentas using microfluidic single-cell transcriptomic technology. This study found that EVT in early preeclamptic placentas released free RNA associated with apoptosis into maternal peripheral blood, an "alarm" of the unhealthy placenta. Rong M et al.[30] used published single cell data of the decidua parietalis of severe PE, derived from GSE94643 in the GEO database. After comparing the differentially expressed genes (DEGs) in the PE and ligand-receptor pairs of immune cells, finally two downregulated DEGs (ICAM1 and CXCL3) and six upregulated DEGs (NRP1, IGF1, LRP6, CXCL12, PDGFD, and PDGFRB) were found. Most of these genes were involved in the interaction between decidual macrophages and other decidual immune cells. By further analyzing the expression

TABLE 3 Single-cell transcriptomes and spatial transcriptomes in normal human placenta.

Single-cell transcriptomes								Spatial transcriptomes			
Sample type	**Sampling time**	**Sample number**	**Method**	**Number of cells**	**Single cell isolation**	**Average reads length (bp)**	**Platform**	**Sample type**	**Sample number**	**Method**	**Reference**
Villous tissue	Term placentas	2	Single-cell transcriptomes	87	Enzyme	75	Illumina HiSeq2500	Syncytiotrophoblast was collected from a single-term placenta	2	LCM and analyzed by the conventional RNA-seq pipeline	Mihaela Pavličev et al. (2017)[23]
Villi and decidua	8 weeks and 24 weeks of gestation	8	Modified smart-seq2 method	1567	MACS	150	Illumina Hiseq4000	NA	NA	NA	Yawei Liu et al. (2018)[15]
Villi and decidua	6–11 weeks of gestation	8 (villi), 6 (decidua)	10x Genomics; Drop-seq platforms	14,341 (villi), 6754 (decidua)	NA	26 (Read1) and 98 (Read2); 20 (Read1) and 60 (Read2)	Illumina HiSeq 2500; Illumina NextSeq 500	NA	NA	NA	Hemant Suryawanshi et al. (2018)[24]
Villi and decidua	6–14 weeks of gestation	5 (villi), 11 (decidua)	10x Genomics; Smart-seq2	18,547 (villi), 40,512 (decidua)	FACS	NA; 75	Illumina HiSeq 4000; Illumina HiSeq 2000	Decidua parietalis	2	smFISH	Roser Vento-tormo et al. (2018)[25]
Placentas	6–7 weeks and 9–12 weeks of gestation	7	10x Genomics	12,794	FACS	NA	Illumina Nextseq 500	NA	NA	NA	Matthew J Shannon et al. (2022)[21]
Villous chorion and smooth chorion	18–24 weeks of gestation	4	10× Genomics	50,496	NA	NA	NovaSeq 6000 S4	NA	NA	NA	Bryan Marsh et al. (2022)[18]
NA	NA	NA	NA	NA	NA	NA	NA	Chorionic villi at 8 weeks gestational age	2	Stereo-seq	Liu Z et al. (2022)[27]
Placental chorionic villi	6–16 weeks of gestation	11	10× Genomics	52,179	MACS	150	Illumina NovaSeq 6000	NA	NA	NA	Han Li et al. (2022)[19]
Decidual and placental samples	5–13 weeks of gestation	5 (decidua); 3 (placenta)	10x Genomics	NA	NA	NA	Illumina NovaSeq 6000	Human implantation sites (6–9 postconceptional weeks)	3 (10x Genomics)	10x Genomics Visium; smFISH	Arutyunyan, Anna et al. (2023)[20]

profile of the decidua of three healthy term deliveries derived from GSE130560, they demonstrated that decidual macrophages could affect the functions of other immune cells through export. As a result, dysfunction of decidual macrophages might be a risk factor in the occurrence of PE. Zhou W et al.[31] used placental samples of two severe PE and two normal pregnant women. This study used SCENIC software to identify associated TFs and their potential target genes in EVTs. CEBPB and GTF2B were found for further functional verification through the knockout experiment of these two molecules in human chorionic trophoblast cells HTR-8/SVneo; they indicated that these molecules participated in trophoblast dysfunction in PE.

Fang Guo et al.[32] collected maternal peripheral blood from early- (EOPE) and late-onset preeclampsia (LOPE) and combined the published transcriptome data of placenta and maternal peripheral blood, as well as the placental droplet-based single-cell transcriptomic datasets for bioinformatics analysis. This study finally proposed that EOPE and LOPE definitely should be treated as placental- and maternal-origin diseases, as the EOPE had the abnormal function of EVT and FBs in the maternal-fetal interface, and LOPE showed gene expression alteration in peripheral blood. Several classical biomarkers and novel biomarkers of preeclampsia were identified; both the classical diagnostic markers (such as PAPPA2, LEP, FLT1, and ENG) and novel biomarkers (such as FLT4, EBI3, GPC4, and LOXL1) expressed high in the EOPE, but not LOPE. EBI3 was further validated as a very sensitive biomarker of EOPE.[32] Moreover, according to protein–protein interaction analysis, FLT1 may facilitate preeclamptic symptoms through other unrevealed mechanisms; lycoprotein neuropilin 1 (NRP1) was identified as a target of FLT1. In LOPE, insulin-like growth factor 2 (IGF2), and regulator of G protein signaling 2 (RGS2) were reduced and might serve as the key regulators of LOPE pathology. Wang H et al. used scRNA-seq data of placentas in healthy pregnancy and EOPE to predict PE risk.[33] This analysis revealed that DCs were closely associated with EOPE, and C1QB and C1QC may be involved in driving early-onset PE by mediating inflammation.[33] Meanwhile, another study performed scRNA-seq on the placenta and the decidual from healthy pregnancy and LOPE. In the placenta, there may be defects in overall trophoblast development, impaired invasion of EVT characterized by the downregulation of the EMT process, and increased maternal immune rejection and inflammation. In the decidua, there was likely insufficient decidualization of DSC manifested by the upregulation of the EMT process, increased inflammation, and suppressed regulatory functions of decidual immune cells.[34]

8.2 | Gestational diabetes mellitus (GDM)

GDM is the most common metabolic disturbance in pregnant women, defined as diabetes diagnosed during pregnancy. Yuqi Yang et al.[35] collected a small piece of placental tissue nearby the umbilical cord from two GDM and two control groups to perform scRNA sequencing. This study identified several potential novel markers, such as SLC1A2 expressed in STB, SLC1A6 expressed in EVT, and ADRB1 in CTB. The preliminary results indicated that NK cytotoxicity might increase in the placenta of the GDM group. Although the percentage of macrophages was not significantly different between GDM and control samples, there was a trend towards enhanced M2 (CD206+) polarization and attenuated M1 (CD80+) polarization. Potential ligand-receptor interactions were discovered, including SPP1-CD44 ligand-receptor complex between trophoblasts and macrophages, and RPS19-C5AR1 interactions between EVT and T/NK cells.

8.3 | Advanced maternal age (AMA)

AMA is defined as pregnancy delivered at 35 years of age or older. Bin Zhang et al.[36] collected placentas from two AMA and two normal pregnant women for scRNA-seq and found that SERPINE1 was highly expressed in EVT. Transfecting a lentiviral vector of hSERPINE1 into HTR8-S/Vneo trophoblast cells reduced invading cells. After obtaining trophoblast cells from pregnant women and conducting transwell assay, it further revealed that reduced cell invasion ability might be an important mechanism of placental defect in AMA women.[36]

8.4 | Recurrent pregnancy loss (RPL)

RPL, also known as recurrent spontaneous abortion,[37] refers to two or more pregnancy losses before 24 weeks of gestation. Chuang Guo et al.[38] isolated 18,646 human decidual immune cells from 9 RPL patients and 15 healthy controls. Three known dNK cell subsets were identified: CD39+ CD18-CD103 -(dNK1), CD18+ CD103-CD39 -(dNK2), and CD18+ CD103+ CD39-(dNK3), as well as a group of proliferating NK cells. The normal angiogenic function of dNK1 cells is diminished, accompanied by the enhancement of dNK2 and dNK3 cells, which play proinflammatory functions. It was also inferred that impaired CD39-CD18-dNK cell accumulation may lead to a decrease in dNK1 cells and, thus, insufficient support for fetal growth in RPL patients. Besides, a disease-specific

interaction network among major cell types in the decidual immune microenvironment was constructed.

8.5 | Placenta accreta spectrum disorders (PAS)

With a single-cell atlas of an invasive PAS placenta, Ma J et al. identified two new CTB cell types with LAMB4+ and KRT6A+ expression and revealed the intermediate states during the differentiation pathway from primitive CTBs to EVTs. Moreover, in the absence of the decidua, ADIRF + and DES + maternal stromal cells located in deep muscle still had the ability to control invasive trophoblasts. The hypervascularity in PAS might be associated with the enhanced crosstalk of trophoblasts, vascular endothelial cells, and stromal cells.[39]

Afshar Y et al. further used 10X Chromium and NanoString GeoMX Digital Spatial Profiler for single-cell and spatially resolved transcriptomes to profile endothelial and decidual cell types in PAS. The contributions of these two cells were demonstrated through alterations in the ECM, growth factors, and angiogenesis. The etiologic explanation transferred from "invasive trophoblast" to "loss of boundary limits" in the decidua. Among the gene expression of all cells, the endothelial-stromal populations exhibited the greatest variation, driven by changes in COL3A1, EGFL6, HGF, DLK1, and PECAM1. Intraplacental tropism was driven by differences in endothelial-stromal cells, with notable differences in BMP5 and SPP1 between the adherent and nonadherent sites of PAS.[40]

9 | CONCLUSION

Recent studies in single-cell transcriptomics and spatial transcriptomics have significantly improved the knowledge of the human placenta. In this review, we have highlighted the advances in the placenta, from the initial mapping of normal placental cells to the recent exploration of gestational complications. Although there are certain challenges in this field, for example, ethical concerns create difficulties in obtaining a normal human placenta before delivery; the limitation of sample size, sample number, and sampling site; temporal, spatial, and individual variability; compared with the progress of spatial transcriptomics technology such as 10x Genomics Visium in other fields such as cardiovascular,[6] liver,[41] and embryo research,[42] the results in placenta are scarce. Besides, the limitation of scRNA-seq in capturing multinucleated cells may cause the loss of important trophoblast cells STB, which may cause certain bias in the study results. Moreover, in contrast to scRNA-seq, which requires the preparation of living single cell suspension from fresh tissue, snRNA-seq can use frozen tissue, effectively utilize archived clinical samples, and reduce batch effects. New approaches, such as multiomics studies of scRNA-seq, snRNA-seq, and spatial transcription, combined use of public database, advances in computational analysis tools, utilization of hTSCs and primary trophoblast organoids to further validate the omics results, will get an insight into the spatiotemporal distribution of placental function and development, as well as placenta-related disease mechanism and resolution in the future.

AUTHOR CONTRIBUTIONS

Mi Tang was involved in methodology, data curation, and writing-original draft preparation. Liling Xiong and Jianghui Cai were involved in methodology and data curation. Li Fan and Cheng Huang were involved in data curation. Shimao Zhang, Ying Jin, and Er-dan Luo were involved in validation. ShaSha Xing and Xiao Yang were involved in conceptualization and writing-reviewing and editing. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to report.

DATA AVAILABILITY STATEMENT

No new data.

ORCID

Mi Tang https://orcid.org/0000-0001-7284-6097
Liling Xiong https://orcid.org/0000-0002-5692-6457
Jianghui Cai https://orcid.org/0000-0003-2510-3236
Li Fan https://orcid.org/0000-0003-4361-905X
Cheng Huang https://orcid.org/0009-0008-1057-4573

Shimao Zhang https://orcid.org/0000-0003-4789-5190
Ying Jin https://orcid.org/0000-0003-3929-810X
Er-dan Luo https://orcid.org/0000-0001-9342-4547
ShaSha Xing https://orcid.org/0000-0002-8245-0683
Xiao Yang https://orcid.org/0000-0001-9805-221X

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