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Progress in deciphering trophoblast cell differentiation during human placentation

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Abstract

The maintenance of gestational well-being requires the proper development of both the embryo and the placenta. Placental trophoblast cells are the major building blocks of the developing placenta. Abnormal trophoblast differentiation underpins placental-based pregnancy complications. However, the mechanisms that govern trophoblast differentiation remain largely unclear. Recent studies shed light on several proteins and regulators that are involved in governing trophoblast differentiation. The advancement of new tools and novel technologies, such as the human trophoblast stem cell culture system, 3D placental organoids and single-cell multi-omics, has brought incredible insights to the field. Here we review the current literature, paying particular attention to articles published between 2017 and 2019 that have promoted our understanding of human trophoblast cell differentiation and its roles in pregnancy and its complications. At the same time, we address challenges and questions arising in the field of human placental development and disease.

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Introduction

The human placenta, a vivipara-associated endocrine organ, plays a vital role in the physiological exchange between the foetus and mother and ensures the lifelong well-being of both [1–3]. It serves as the most essential yet least understood foetal organ for the successful continuation of pregnancy. In 2018, Perez-Garcia et al. [4] reported that among 103 gene knockout mice they investigated, almost 70% of embryonic-lethal mice showed disordered placental development. This study strongly emphasized the importance of placentation in supporting embryonic development and brought placental biology to the cutting edge of research. Together with the successful establishment of human trophoblast stem cells [5], 3D placental organoids [6,7] and advancements in methods such as single-cell multi-omics [8–12], research in the last two years has greatly accelerated our understanding of placental development and its pathologies.

The human placenta is mainly made up of several distinct trophoblast cell subtypes with different anatomical locations and functional features [13]. The human placenta differentiates from the extraembryonic trophectoderm, and its development occurs shortly after the first binary decision in mammalian life (the decision to become either a trophoblast or an inner cell mass). Outer cells form the wall of the blastocyst and commit to the restricted trophoblast cell lineage [14]. After implantation, trophoblast cells undergo self-proliferation and simultaneously differentiate into either villous or extravillous trophoblast cells (Figure 1). In the human placenta, there are three major trophoblast subpopulations: the cytotrophoblast (CTB), extravillous cytotrophoblast (EVT), and syncytiotrophoblast (STB) [15]. During trophoblast differentiation, CTB cells can function as stem cell–like progenitor cells that either fuse to a continuous layer of multinucleated STB cells or undergo epithelial–mesenchymal transition to form EVT cells. The STB is the outer lining of the placental villus. It serves as the

main site of gas and nutrient exchange between the mother and the foetus. It secretes hormones and proteins and protects the foetus from pathogens [16,17]. EVT cells are migratory and invasive trophoblast cells that are essential for placental embedment and foetal development. Invasive EVT cells can be further divided into two subtypes based on their distinct functions: those that invade the maternal decidua are named interstitial extravillous trophoblast (iEVT) cells, whereas those that remodel maternal spiral vessels are named endovascular EVT cells [18–20].

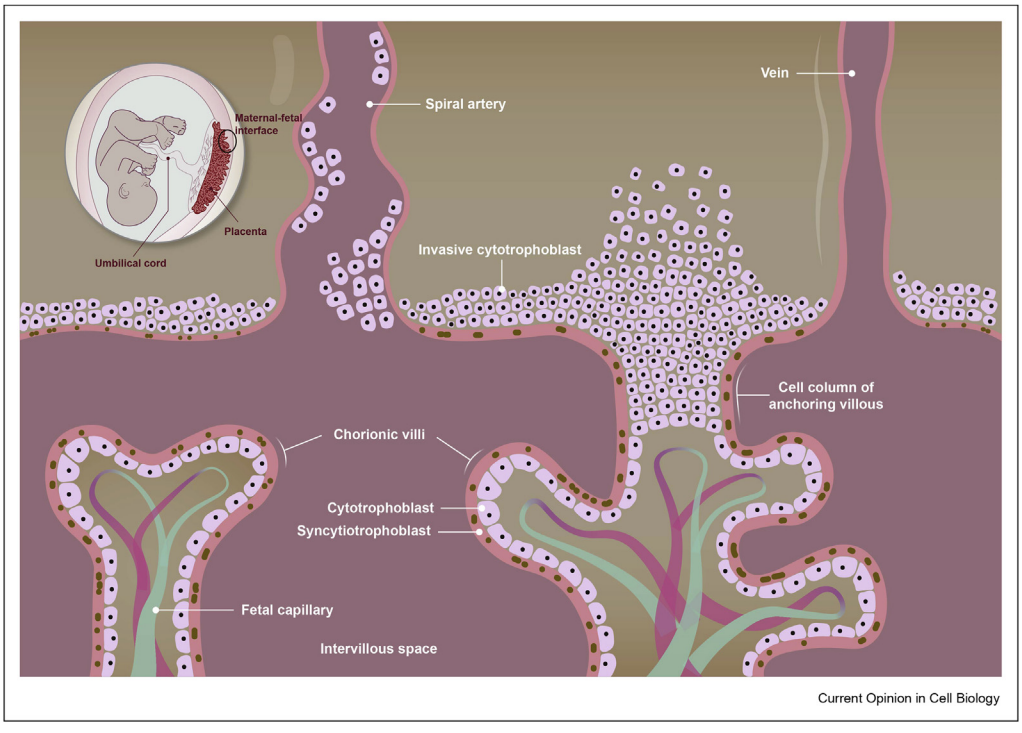
The composition of these cell populations in the placenta may change throughout pregnancy to better accommodate the growing demands of the developing foetus [21]. Defective placentation harms gestational well-being and results in many pregnancy pathologies, including miscarriage, preeclampsia (PE) and intrauterine growth restriction (IUGR) [22–24]. Placental development depends on the well-coordinated proliferation and differentiation of trophoblast cells [25,26]. Defined subtypes of trophoblast cells and their functions are crucial in placental biology. Sophisticated regulation is also needed to support the normal development of the placenta. However, because of the dearth of very early human placenta samples (due to technical and ethical constraints) and the differences in placental types among species, the mechanisms underlying how the human placenta develops are not well understood.

In this review, we summarize our current understanding of the mechanisms regulating trophoblast differentiation and how such processes can go awry in diverse gestation-related diseases. We will also discuss new techniques that have largely broadened our view on trophoblast differentiation, which could support further investigations of possible therapeutic solutions to improve pregnancy outcomes.

Novel players govern trophoblast cell differentiation

A key question in placental biology is what governs trophoblast cell differentiation. A plethora of factors are reported to be essential in regulating trophoblast development, indicating that their expression levels in the placenta could be potential biomarkers in the prediction of gestational diseases such as PE and IUGR [27–29]. Type I interferon alpha/beta receptor signalling in the conceptus leads to abnormal placental development and impaired foetal endothelial development at the maternal–foetal barrier after ZIKV infection [30]. The interferon-induced transmembrane protein (IFITM) family protects cells from viral infection; however, Buchrieser et al. [31] showed that

Figure 1



A schematic representation of the human placenta intervillous space. Schematic depiction of the basal plate of the human placenta, highlighting the cellular composition of placenta villi. A multinucleated-cell layer of STB covers a single-cell layer of CTB. At the tips of the placental villi sit columns of CTB cells that are proliferative, putting out invasive extravillous cytotrophoblast (EVT) that helps anchor the placenta to the uterus and interact with maternal uterine arterioles and veins.

interferon stimulation-induced IFITM expression in trophoblast cells blocks trophoblast cell fusion and disrupts normal placental function. Thus, there may be a well-coordinated balance between the anti-fusion effect and anti-virus effect of IFITMs during placentation. Moreover, single-nucleotide polymorphisms of IFITM3 exist in humans from different regions, such as Western Mexican, East Asia, Europe and American [32], which could serve as important markers for the noninvasive prediction of pregnancy infections. Other factors released from the placenta into the systemic circulation are thought to result in pregnancy-related pathologies; for example, the expression of the soluble fms-like tyrosine kinase-1 (sFLT-1) variant sFLT-1 e15a is upregulated in the placenta of women with PE [33], while the tissue factor pathway inhibitor 2 (TFPI-2) promoter is hypomethylated in PE placenta [34].

Placental syncytialization is maintained during pregnancy by the fusion of the underlying CTB cells. Accumulating evidence suggests that CTB syncytialization could have a crucial role in supporting successful pregnancy [35,36]. Wang et al. [37] indicated that detyrosination of α-tubulin might be one of the underlying mechanisms regulating STB formation and maintenance. Insufficient tubulin detyrosination could be the potential cause of PE. This latter study has shed light on the robust correlation between post-translational modification and trophoblast cell fusion. In the future, mechanistic studies could focus more on the role that post-translational modification plays during placentation. Senescence cellular pathways are also activated following trophoblast fusion. A study reported that placentas from pregnant women with IUGR showed dysfunctional cellular senescence. The study indicated that cellular senescence networks involving p16/pRb and p53/p21 are downregulated in STB of IUGR placentas [38]. It is known that high oxygen level and inflammation can also disrupt the function and development of the placenta, leading to pregnancy-related complications such as IUGR, as demonstrated in humans and animal models [39,40].

Recently, increasing evidence has emphasized the major roles of cell cycle regulators in regulating trophoblast cell division and differentiation. Several cell cycle regulators are expressed in human placenta, with distinct and dynamic expression levels [41]. Lu et al. [42] focussed on the relationship between the cell cycle and trophoblast cell syncytialization and found that in human placenta, trophoblast cells must exit the cell cycle to differentiate and fuse to form multinucleate STB. The interaction of p21 and GCM1 with the binding site of the Syncytin-2 gene promoter is a prerequisite for cell cycle exit and trophoblast fusion. Findings from this study provide a likely explanation for the observation of Syncytin-2 expression in proliferative cells in the placentae of patients with spontaneous abortions.

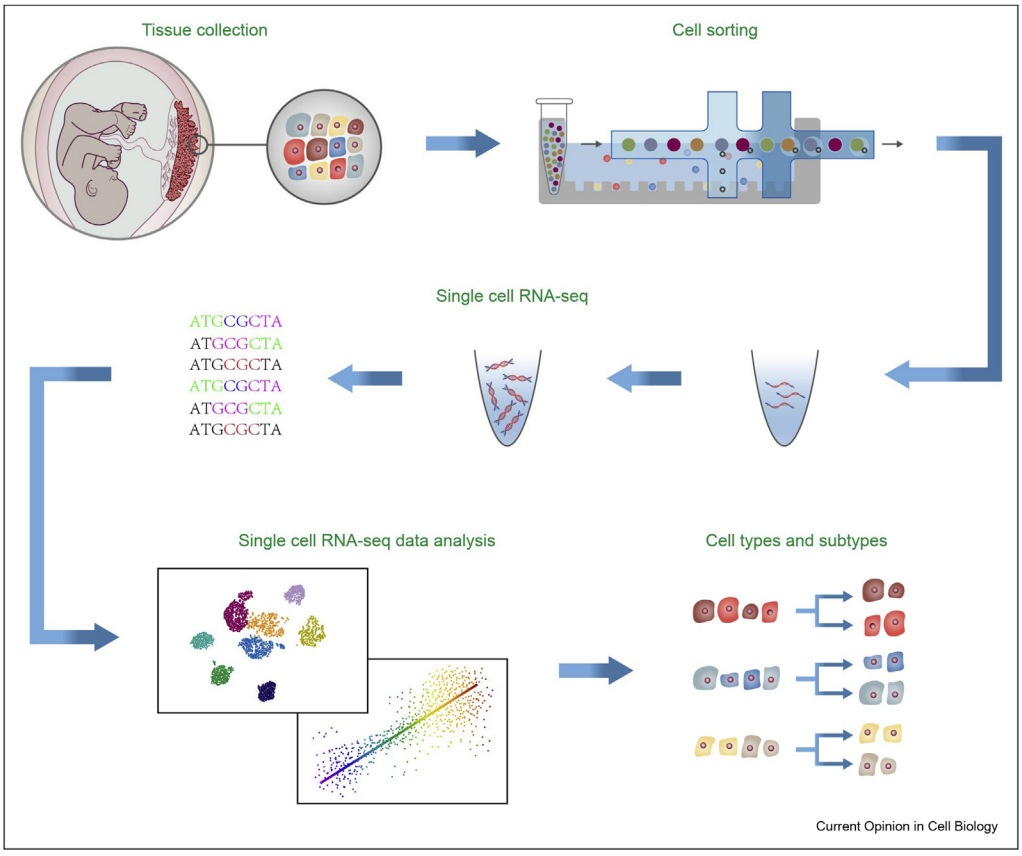
PLAC8 has recently been identified as a novel marker for iEVT cells, a subtype of EVT cells that are involved in the invasion of the maternal uterine wall and anchoring the placenta [43]. PLAC8 plays a vital role in sensing oxygen tension and directing the differentiation of CTB cells into iEVT cells during pregnancy. Overexpression of PLAC8 has been found in preeclamptic placentae. The p21 is also expressed in distinct cell types of primary trophoblasts linked to invasion. Indeed, suppression of p21 compromises the motility and invasion capacity of diverse trophoblastic cell lines [44]. Trophoblasts must escape from the cell cycle before invading, which requires the activation of p21. The p21 is downregulated in trophoblast cells of preeclamptic placentae. Furthermore, E2F8, a transcription factor that is known to promote murine placental growth and development, was recently proven to be expressed in the human placenta and inhibited the invasiveness of human EVT [45].

Single-cell multi-omics has revealed many new subtypes of trophoblast cells

Recently, advances in single-cell transcriptomics/multi-omics technologies have allowed the profiling of thousands of human trophoblast cells at unprecedented resolution, the precise distinction of trophoblast subtypes, and the mapping of their underlying molecular regulation network (Figure 2). The successful delineation of the trophoblast lineage relationship provides an unbiased view of placental complexity and invaluable information not only on normal placentation but also on placental pathologies. In this section, we review studies of single-cell multi-omics to investigate human trophoblast cell differentiation.

A pioneering paper published in 2017 analyzed the gene transcription patterns of 87 single cells from term intervillous and extravillous trophoblasts of two human placentas. This early single-cell transcriptome study offered potential ways of characterizing cells at the foetal–maternal interface from their G-protein coupled receptor expression levels, which could be developed into reliable tools for cell type identification [11]. One year later, a large-scale analysis of the placental gene expression atlas was performed by Suryawanshi et al. [9].They focussed on transcriptional signatures at the human foetal–maternal interface during pregnancy and obtained 14,341 and 6754 high-quality scRNA-seq profiles from villi and decidua samples, respectively, in the first trimester of human pregnancy [9]. They captured 449 high-quality STB expressing novel marker genes. To predict cell type–specific ligand–receptor complexes, Vento-Tormo et al. [8] combined droplet-based encapsulation with plate-based smart seq2 (manually picked cells) profiles and performed high-throughput scRNA-seq on 70,000

Figure 2



A schematic of the basic workflow for single-cell RNA sequencing of the human placenta. First the human placenta is dissociated to make a single-cell suspension. Single cells are then captured using different strategies (10× genomics or smart seq2). The single cells are then lysed to generate cDNA libraries for single-cell RNA sequencing (scRNA-seq) data analysis to define cell types.

single cells from first-trimester placentas. A detailed molecular and cellular map of the human decidual–placental interface and the first comprehensive single-cell transcriptomics atlas of the maternal–foetal interface were drawn based on their results. They further proposed that ligand–receptor interactions regulate trophoblast cell differentiation into either villous STB cells or EVT cells [8]. Then, Yawei et al. isolated human villous stromal cells (STRs), CTB cells, STB cells, and EVT cells from first- and second-trimester placenta and monitored the transcriptome dynamics of 1567 cells at single-cell resolution. Fourteen subtypes of placental cells were characterized based on their functions. A total of 102 polypeptide hormone genes expressed by various subtypes of placental cells were identified. These pioneering single-cell transcriptomics studies together built a strong foundation for understanding how the human placenta develops and functions to maintain a healthy pregnancy [10]. *In vitro*–cultured human blastocysts also enabled the definition of the transcriptomic landscape of the human trophoblast [46,47]. All studies supported a bifurcating model with CTB cells differentiating bidirectionally into STB cells and EVT cells and indicated potential differences between the primitive STB cells and villous STB cells, altogether shedding light on the regulatory mechanisms underlying lineage development.

Analyses of trophoblast cell lineage also have resulted in new insights into the mechanism of human pregnancy complications such as preeclampsia and IUGR. Previous maternal plasma transcriptomic analysesrevealed expression changes of trophoblast transcripts under pathological states such as PE. To reveal the trophoblastic and nontrophoblastic components of the placenta during gestation, Tsang et al. [12] analyzed more than 24,000 unmarked placental cells from healthy and early PE placenta. Their results led to a reconstruction of the trophoblastic differentiation trajectory and confirmed the feasibility of integrating single-cell transcriptomic analysis with plasma-circulating RNAs to noninvasively study placental pathology [12]. These may lead to improved prognosis and selection of appropriate therapies. Future bioinformatics tools can be developed to exploit the cellular transcriptomic signatures established in this

placental atlas to normalize the cellular-composition heterogeneity in whole-tissue profiles.

Human trophoblast stem cell lines and placental organoids provide novel models to study the placenta at *in vivo*–like levels

Recent advancements in the derivation of organoid cultures of the endometrium and placental trophoblast have created new opportunities for systematic research. Human trophoblast stem cells (hTSCs) were successfully established in 2018 by Takahiro Arima's lab [5]. Later, two studies reported three-dimensional organoid systems established from early placental tissues. Turco et al. [6] generated long-term, genetically stable organoid cultures that can differentiate into both the STB and EVTs, and the villous-like structures secrete placental-specific peptides and hormones. Haider et al. [7] established organoid cultures from purified first-trimester CTB cells, in which the organoids express markers of trophoblast stemness and proliferation and are highly similar to primary CTB cells in global gene expression [7]. These studies together open a path for detailed molecular investigations and provide a new platform for research on the molecular regulatory network of placentation and the mechanisms of early embryogenesis with relevance to human gestational diseases that arise during early development. In the future, more analyses of extraembryonic tissue development will be needed.

Conclusion

Although many elements remain to be discovered, thanks to great technological advancement, research over the last two to three years has significantly deepened our knowledge of the placenta and enabled us to categorize the complex processes leading to placental pathogenesis. In this review, we have highlighted the recent advances in trophoblast differentiation, a subject that is now dominated by studies that use single-cell omics strategies. Advances have been made in our understanding of the pathophysiology and clinical management of gestational complications, but several research questions remain to be resolved. We anticipate that new approaches such as single-cell multi-omics will provide insights into the spatiotemporal distribution of regulators of placental function and development within the next few years. Moreover, with the help of hTSCs and placental organoids (3D hTSCs), it is likely that researchers will illuminate the current 'black box' state of early human post-implantation biology and understand the mechanisms of human placentation and related diseases.

Conflict of interest statement

Nothing declared

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The authors screened 103 embryonic lethal and sub-viable mouse knockout lines, 68% of them that are lethal at or after mid-gestation exhibited placental dysmorphologies. Early lethalith is most always associated with severe placental malformations. Placental defects correlate strongly with abnormal brain, heart and vascular development.

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The long-term culture of human villous cytotrophoblast cells need activation of Wnt and EGF and inhibition of TGF-β, histone deacetylase, and Rho-associated protein kinase. The resulting cell lines have the capacity to give rise to three major trophoblast lineages.

6. ** Turco MY, *et al.*: **Trophoblast organoids as a model for maternal-fetal interactions during human placentation**. *Nature* 2018, **564**:263–267.

A pair of papers (see also reference [7**] established long-term, genetically stable organoid cultures of CTBs. The first one detected the secretion of placental-specific peptides and hormones, including hCG, GDF15 and PSG, the second one found the organoids express markers of trophoblast stemness and proliferation and are highly similar to primary CTBs at the level of global gene expression.

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