Review

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Placental Development and Pregnancy-Associated Diseases

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Abstract

Serving as the interface between the fetal and maternal environments during gestation, the placenta plays critical roles in the protection of the developing fetus and the maintenance of maternal health. The placenta is primarily derived from the embryonic trophectoderm which differentiates into various subtypes of trophoblast cells through villous and extravillous pathways. The interactions among trophoblasts and multiple decidual cells and immune cells at the maternal-fetal interface fundamentally form the functional units of the placenta, which are responsible for blood perfusion and maternal-fetal material exchange, immune tolerance, and the regulation of pregnancy adaptation. Defects in placental development and functional maintenance are in tight association with adverse pregnancy outcomes such as preeclampsia. In this article, we review recent advances on human trophoblast cell differentiation and the construction of placental functional units and discuss the placental and maternal factors that may contribute to the occurrence of preeclampsia.

Keywords: Placenta; Human placenta; Trophoblast cell differentiation; Functional units; Preeclampsia

Introduction

The placenta is a transient organ that plays a critical role in the protection of the developing fetus and the maintenance of maternal health during pregnancy. Increasing evidence is also demonstrating the imprinted influence of the placenta on the long-term health of the offspring and the mother throughout their whole life. Serving as the interface between the fetal and maternal environments during gestation, the placenta is responsible for the exchange of gases, nutrients, and waste products between the mother and the growing fetus. It also acts as an endocrine organ to produce several pregnancy-associated hormones and growth factors and ensures the protection of the fetus from maternal immune attack.[1]

Placental development is coordinated with the process of embryonic development. The trophectoderm (TE) of the blastocyst is the first cell lineage that exhibits a highly differentiated function during embryonic development, which is the origin of multiple subtypes of placental trophoblasts. In humans, the TE gives rise to differentiated trophoblast cells through two general pathways: villous trophoblasts (VTs) and extravillous trophoblasts (EVTs). VTs primarily include mononucleated cytotrophoblasts (CTBs) which can fuse into multinucleated syncytiotrophoblasts (STBs) through, a process of cell-cell fusion, syncytialization. STBs form the syncytial layer that covers the placental villous tree, which is surrounded by maternal blood in intervillous space (IVS). STBs are primarily involved in pregnancy-related hormone production and in the fetal-maternal material exchange. In the extravillous pathway, CTB cells proliferate to form cell column trophoblasts (CCTs) of the anchoring villi. EVTs detach from CCTs and migrate into the decidua, thereby anchoring the fetus to the uterine wall. They also penetrate the uterine spiral arteries to replace maternal endothelial cells, thus remodeling the uterine spiral arteries into low-resistance, high-capacity uteroplacental arteries that provide increasing blood flow toward the placenta to meet the requirements of the growing fetus.[2,3]

The form of placentation varies widely across mammalian species. Human implantation is almost unique among mammals in which trophoblast cells are highly invasive, penetrating the inner-third of the myometrium along with the maternal vasculature, and the conceptus embeds itself completely within the maternal uterine endometrium. Human placentation is also characterized by a remodeling of the spiral arteries. Owing to the appropriately controlled invasion of uterine stroma and spiral arteries by placental trophoblasts, the uteroplacental circulation can be successfully formed. Thus, the human placenta has been demonstrated to be one of the most invasive placenta types.

The well-organized differentiation of placental trophoblasts and the dynamic interactions among trophoblasts and a multitude of maternal cells at the fetal-maternal

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interface form multiple functional units of the placenta, which coordinately guarantee the health of the growing fetus and the mother. The dysregulation of trophoblast activities has been revealed as the key mechanism underlying the development of many pregnancy-associated disorders. Elucidating the regulatory mechanism of placental development is a crucial way to explore the pathogenesis of pregnancy complications and to identify reliable biomarkers that can be used as predictive or therapeutic targets for the disorders.

In this review, we first summarize the current views on human trophoblast cells differentiation and their interactions with various uterine cells. We then concentrate on recent advances regarding the formation of functional units of the placenta. Finally, the mechanisms by which the dysregulation of placental development contributes to the onset or development of preeclampsia (PE) are discussed. This study aims to highlight our latest knowledge on the development and functions of the human placenta and to provide perspectives on the exploration of the mystery of human pregnancy and the translation of bench research into effective clinical treatments for pregnancy complications. However, due to the limited space of the article, the mechanisms regarding placentation and pregnancy maintenance are not described in detail.

Differentiation of human placental trophoblast

Placental development initiates from the differentiation of the TE at the blastocyst stage. On the 6th–7th day post fertilization (dpf) in humans, the polar TE (near inner cell mass (ICM)) forms primitive syncytium and rapidly invades the endometrium to pilot the implantation of the blastocyst. The blastocyst is completely embedded into the endometrium around the 14th dpf. Along with the embryonic implantation, TE-derived CTB cells proliferate rapidly and break through the primitive syncytium. They fuse into multinucleated STB and form the primary villous structure of the placenta. With the continuous proliferation and differentiation of trophoblasts, the villi expand and branch into a tree structure, forming a complete trophoblastic barrier to protect the fetus. From the 3rd-week post fertilization, rapidly growing CTBs break through the covering syncytial layer and gather as CCTs to anchor the villi into uterine wall. EVTs that differentiate from CCTs infiltrate into the decidua and remodel uterine spiral arteries[4] (Fig. 1).

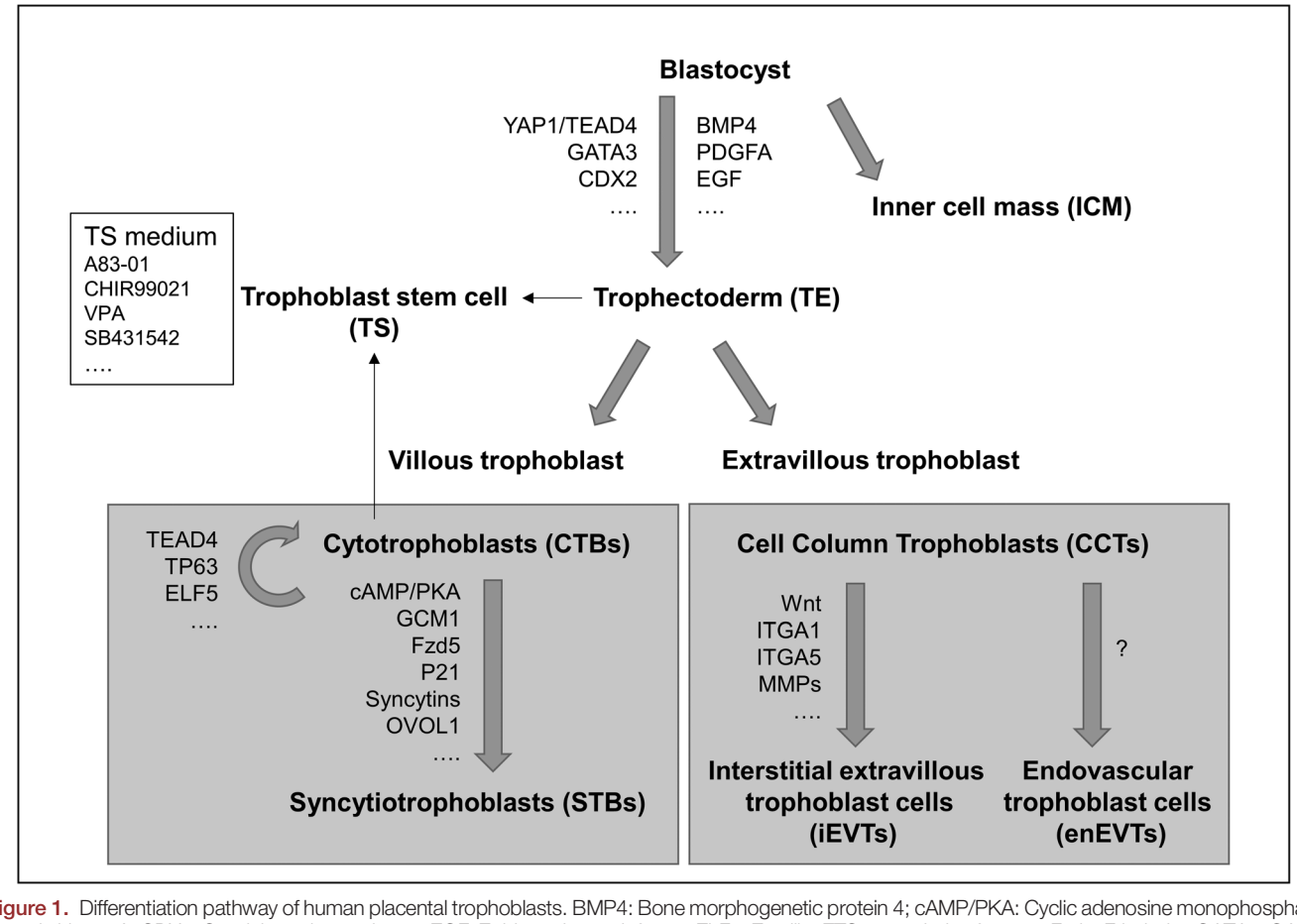

Figure 1. Differentiation pathway of human placental trophoblasts. BMP4: Bone morphogenetic protein 4; cAMP/PKA: Cyclic adenosine monophosphate /protein kinase A; CDX2: Caudal type homeobox 2; EGF: Epidermal growth factor; ELF5: E74 like ETS transcription factor 5; Fzd5: Frizzled 5; GATA3: GATA binding protein 3; GCM1: Glial cells missing-1; ITGA1: Integrin subunit alpha 1; ITGA5: Integrin subunit alpha 5; MMPs: Matrix metallopeptidases; OVOL1: Ovo like transcriptional repressor 1; P21: CDKN1A, cyclin dependent kinase inhibitor 1A; PDGFA: Platelet-derived growth factor subunit A; Syncytins: Syncytin-1 and 2; TEAD4: TEA domain transcription factor 4; TP63: Tumor protein p63; YAP1: Yes1 associated transcriptional regulator; VPA: Valproic acid.

TE – the first cell lineage in pre-implantation embryo

On the 5th dpf, the embryo undergoes compaction and polarization, and TE differentiation initiates. This is the first cell lineage process in pre-implantation embryo. TE and ICM separately develop toward the fetal tissues of the placenta.

Increasing data of single-cell transcriptome of human or rodent embryo have provided in-depth evidence on the differentiation lineage of the early embryo. As the embryo develops to the morula stage, TE-related genes such as GATA binding protein 3 (GATA3) and platelet-derived growth factor subunit A begin to express, while such TE cells are still capable of developing to ICM.[5] Cell lineage markers are specifically expressed, and lineage separation of ICM-TE occurs at the early blastocyst stage, whereas TE and ICM cells at this stage still have pluripotency and retain the ability to trans-differentiate toward each other, indicating the cell fate has not been completely determined.[6] Till late blastocyst stage, yes1 associated transcriptional regulator (YAP1)/TEA domain transcription factor 4 (TEAD4) signal is specifically activated in TE cells, which regulates caudal type homeobox 2 expression and is critical for the differentiation of human embryonic stem cell (ESC) to TE.[7] Data from in vitro experiments have also demonstrated the effect of bone morphogenetic protein 4 to promote the differentiation of ESC to trophoblastic lineage.[8] Additionally, at peri-implantation stage, polar TE adjacent to ICM and mural TE at the distal side of ICM undergo different processes of proliferation and differentiation. By far, a multitude of culture systems to induce trophoblastic lineage differentiation have been reported.[7] However, further investigations are needed to explore the regulatory mechanisms of cell fate determination of the early embryo.

Human trophoblast stem cell (hTSC)

The establishment of mouse trophoblast stem cell lines was first reported in 2004. However, the generation of hTSC lines has been a long and hard way. Till 2018, Okae *et al.*[9] reported the successful derivation of stable hTSC lines from a human early embryo or the first-trimester placental villi. The cultured hTSCs possess stemness property and potentials to differentiate toward various trophoblastic subtypes. Moreover, recent studies have demonstrated that human naïve pluripotent stem cells (PSCs), rather than primed PSCs, can be successfully induced to TE lineage and further differentiate to hTSCs.[10] However, a report from Wei *et al.*[11] indicates the differentiation potential of primed PSCs to hTSCs with similar characteristics as the blastocyst-derived hTSCs. The promoting effect of bone morphogenetic protein 4 in this process is proved.

The long-term culture of genotype-stable placental organoid has been generated from the 3-D culture of hTSCs or isolated villous CTBs, which can be induced to differentiate toward STBs and EVTs.[12] The successful construction of hTSCs and placental organoid provides unprecedented and much-needed research tools for exploring the development and function of the human placenta in normal and pathological processes.

Differentiation of trophoblasts along villous pathway

In the villous pathway, mononucleated CTBs fuse into multinucleated STBs, forming the syncytial layer that covers the placental villous tree. CTBs locates beneath the syncytial layer, with fetal blood vessels and mesenchymal cells being surrounded by these two layers of VTs.[4] STBs are in direct contact with the maternal blood within IVS, thus are intimately involved in the material exchange between the mother and the fetus. STBs also produce a large amount of pregnancy-related hormones including progesterone (P_4), human chorionic gonadotropin (hCG), and human prolactin (hPL), which play critical roles in the maintenance of pregnancy. What's more, STBs exhibit a degree of tolerance to the maternal immune system.

Mononuclear CTBs

Recent single-cell sequencing studies demonstrate three subtypes of CTB at the first trimester,[13] including proliferative CTBs, differentiating CTBs which exit cell cycle and express syncytin-1 and -2, and CTBs with uncertain function which may be the "reserve" cells in G0 phase of cell cycle. With the progress of pregnancy, the stemness and the proportion of CTB gradually decrease.

CTBs are epithelial cells specifically expressing transcription factors including YAP, TEAD4, caudal type homeobox 2, tumor protein p63, E74 like ETS transcription factor 5, GATA3, transcription factor T cell factor 1, TFAP2C, transcription factor AP-2 gamma (TFAP2C), and Myc proto-oncogene protein.[14] Myc proto-oncogene protein regulates multiple miRNAs and inhibits the expression of glial cells missing-1 (GCM-1) and cytochrome P450 family 19 subfamily A member 1, thereby restraining CTB differentiation.[15] Binding of TEAD4 with YAP can up-regulate cell cycle regulators (such as cyclin A and cyclin dependent kinase 6) to maintain the pluripotency of CTBs and to repress the cell fusion progress to form STB.[16]

It has been reported that a hypoxic environment at early pregnancy is conducive to CTB cell proliferation,[14] and increased oxygen concentration will induce epithelial-mesenchymal transition of CTBs, which is in association with their differentiation to EVT.[17]

Multinucleated STBs

Syncytial layer is a huge multinucleated structure with a continuous surface area being 12–14 m^2 at term. The number of nuclei in STB at late gestation is estimated to be >10 billion. The formation of such huge multinucleated STB is generally recognized as a cascade of the fusogenic event which begins with increased cyclic adenosine monophosphate (cAMP) levels via the activation of protein kinase A (PKA). The downstream activation of transcription factors, such as GCM-1, and its target genes, primarily the fusion peptide Syncytins, induces syncytialization and subsequent increase in hCG production.

Syncytialization is a complex process including a series of cell events that are regulated by multiple factors. Importantly, CTBs that are prepared to fusion highly express p21 leading to cell cycle restriction. The binding of p21 with GCM-1 induces the expression of fusogenic genes including Syncytin-2.[18] The expression of GCM-1 in the chorion can be regulated by an amplifying feedback loop of GCM-1-Fzd5 signaling, which also upregulates vascular endothelial growth factor (*Vegf*) expression in the chorion. Such action of canonical Fzd5 signaling determines the initiation of

syncytialization and the vascularization of the primary villi.[19] Other transcription factors that modulate GCM-1 expression include TEAD4/YAP and OVOL.[20]

A large number of hormones and growth factors have been found to affect the fusion process of CTBs, such as hCG, epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor, leukemia inhibition factor, and activin-A.[21] hCG is predominantly produced by STB, which promotes cell fusion in autocrine/paracrine manner. It binds with luteinizing hormone/chorionic gonadotropin receptor and induces intracellular cAMP production to activate PKA signal and phosphorylate the downstream cAMP responsive element binding protein and GCM-1, resulting in the expressions of key fusogenic genes, including hCG, connexin 43, and syncytins.[22] The key fusogens that mediate trophoblast cell fusion are syncytins, the envelope proteins encoded by human endogenous retroviral genes (HERVs) that are nearly exclusively expressed in the placenta. Syncytin-1, encoded by a defective HERV, endogenous retrovirus group W member 1, is the first identified fusion peptide to trigger cell fusion by interacting with human sodium-dependent neutral amino acid transporter type 1 or type 2 receptors.[23] Syncytin-2 is also shown to promote cell fusion, whereas its expression level significantly decreases after STB formation. Several other trophoblast-specific HERVs, such as HERV-Fb1, HERV-H7/F (XA34), and HERV-HML6-c14, have been characterized in human placental tissues, while their exact functions remain unknown. The different localization of syncytins, their receptors, and related proteins in trophoblast cells, as well as the observation that STB is not completely absent in *syncytin*-deficient mice, raise the possibility that syncytins may not be the only fusogens that participate in syncytialization.

Differentiation of trophoblasts along extravillous pathway

The formation of anchoring villi serves to attach the placenta to the uterine wall and to create the placental perfusion that is necessary to sustain the growing fetus. A subpopulation of rapidly proliferating CTBs at the proximal ends of the anchoring sites, naming CCTs, participate in the creation of the cell column bridge between the placental villous tip and the maternal decidua.[22] At the distal ends of the columns, CCTs exit the cell cycle and begin to lose their cell-cell contacts. These CCTs detach from the columns and, as they come into contact with the decidual extracellular matrix (ECM), differentiate into interstitial extravillous trophoblast cells (iEVTs) and endovascular extravillous trophoblast cells (enEVTs), which have distinct roles in maternal decidua.

Interstitial extravillous trophoblast cells

iEVTs are highly invasive trophoblasts, with a distinct expression profile of adhesion molecules, proteases, and histocompatibility antigens. There is a marked down-regulation of E-cadherin, integrin α6β4, and an upregulation of integrin α5β1, integrin α1β1 in the iEVTs that invade into decidua.[24] Consistent with their invasive phenotype, iEVTs secrete several proteases such as urokinase-type plasminogen activator and several matrix metalloproteinases, as well as the inhibitors of these enzymes, plasminogen activator inhibitor 1/2 and tissue inhibitor of metalloproteinase, which control the limited invasiveness of iEVTs into the decidual tissue. Furthermore, unlike the VTs, iEVTs express atypical class I major histocompatibility complex (MHC) antigens, specifically including HLA-E, trophoblast specific HLA-G, and the polymorphic HLA-C.[1]

Accumulating evidence has revealed multiple signaling pathways and transcription factors that regulate EVT differentiation, migration, and invasion. EVTs present up-regulated expressions of migration-related molecules, such as proteoglycan 2, erythroblastic oncogene B2, integrin subunit alpha 1 and integrin subunit alpha 5, multiple matrix metalloproteinases, and proteolytic enzymes.[25] Various transcription factors are involved in the regulation of EVT invasion, including GCM-1, TFAP2A, transcription factor AP-2 alpha, signal transducer and activator of transcription 3 (STAT3), FOS like 1, and classic Wnt-β-catenin signal.[26] In addition, hypoxia is recognized as an important regulator of EVT differentiation.[17]

At the maternal-fetal interface, iEVTs interact with various maternal cells including decidual stromal cells, uterine blood vessel endothelial cells, vascular smooth muscle cells (VSMCs), decidual NK (dNK) cells, macrophages, and T cells. The complex and coordinated interactions among these cells are critical to generate functional units of placental perfusion and maternal-fetal immune tolerance.[22]

iEVTs can invade deeply into the decidua as far as the inner third of the myometrium. They finally differentiate into placental bed giant cells which also can produce hPL and hCG. Furthermore, these giant cells produce protease inhibitors that may be involved in limiting EVT invasion past the myometrium.[27]

Endovascular trophoblast cells

Maternal-placental circulation is established between the 8th week and 12th week, with uterine spiral arteries being fully remodeled by approximately 20th–22nd week of gestation. Endovascular EVTs invade maternal vasculature in the decidua and the inner third portion of the myometrium. In this way, uterine spiral arteries are remodeled from high-resistance, low-flow muscular vessels to low-resistance, and high-flow sac-like vessels. The process involves crosstalk between different cell types, with enEVTs as the key players. The vascular remodeling leads to drastic changes in the spiral arteries and blood flow to ensure maximum maternal blood perfusion into the placenta and to prevent villi from damage.[22]

The invasion of EVTs into the lumen of the arteries and the replacement of the endothelial cells of the maternal vessels are fulfilled through a process that is referred to as pseudovasculogenesis or vascular mimicry,[24] therefore, these EVT cells are named as endovascular EVTs. However, the origin of enEVTs has long been debatable. It is suggested that the switch from iEVTs to enEVTs occurs only in regions of the spiral arteries in the superficial zone of the decidua, while the deeper regions of the arteries are remodeled by enEVT from a second origin, that is, trophoblast plugs. Histological analysis has indicated the existence of trophoblast plugs at the implantation site to block the maternal arterial blood flow at early gestation,

allowing only a small amount of maternal blood flow to the IVS.[1] The trophoblast plugs begin to disappear at 12–13 weeks of gestation, in parallel with the extensive infiltration of the spiral arteries by enEVTs. It is thus deduced that enEVTs may be originated from the plugs and retrogradely travel down the vessel lumen.[22]

The property of enEVTs is, to some extent, similar to endothelial cells primarily due to their switch from an epithelial to an endothelial adhesion molecule phenotype. These cells downregulate the epithelial-type markers E-cadherin and integrin α6β4 and upregulate the expression of the adhesion molecules VE-Cadherin, platelet-endothelial cell adhesion molecule-1, and NCAM (CD56) as well as integrins α5β1, α1β1, and αVβ3.[24] The characteristics of vascular adhesion integrate enEVTs into the vascular endothelial layer, thereby promoting vascular endothelial apoptosis and finally completely replacing vascular endothelium.[28] In addition, in comparison with iEVTs, enEVTs specifically express Jagged1, and EFNB2, which is presumed to promote enEVT migration to maternal vasculature.[29]

Functional units of human placenta

Functional unit of placental endocrine activity

The placenta is a unique and powerful endocrine organ during pregnancy, which produces various hormones, neuropeptides, neurotransmitters, and growth factors, such as hCG, hPL, gonadotropin-releasing hormone (GnRH), and various steroid hormones (P_4, estrogens, androgens, etc), thus forming a hypothalamus-pituitary-gonad-like endocrine axis in the placenta. Hormones secreted by the placenta play vital roles in embryonic implantation, placental cell differentiation, immune adaptation, fetal development, and delivery onset (briefly summarized in Table 1).

Human chorionic gonadotropin

The pre-implantation blastocyst initiates hCG production, which is superseded by STBs. As one of the most important hormones produced by the placenta, hCG stimulates P_4 production and enhances syncytialization.[1] In addition, hCG is a tissue-specific angiogenic factor, influencing the expression of VEGF and its receptors, angiopoietins (Angs) and their receptor Tie-2, basic fibroblast growth factor, or placental growth factor (PlGF) in ovary and testis.[30] Recent findings reveal the stimulatory effect of hCG on the productions of EG-VEGF and its two receptors, PROKR1 and PROKR2, in human placental villous explants and isolated CTBs through cAMP signaling.[31] In vitro and in vivo evidence indicate the stimulation of capillary formation in endothelial cells by hCG.[32] The receptor of hCG is expressed in placental vascular tree and hCG promotes the proliferation of placental microvascular endothelial cell and vessel formation, demonstrating the role of hCG in placental vascular development.[33]

Hyperglycosylated hCG, one of the five isoforms of hCG with multiple N-linked oligosaccharides at residues of its α-subunit, β-subunit, and C-terminal extension, is mainly produced by CTBs and EVTs. Hyperglycosylated hCG can cooperate with hCG to promote uterine artery angiogenesis and facilitate umbilical circulation, facilitate trophoblast cell invasion, and MMP activity, indicating its vital roles for driving hemochorial placentation.[1]

Gonadotropin-releasing hormone

Mammalian Type I GnRH (GnRH I), a decapeptide at the most upstreaming of the hypothalamic-pituitary-gonadal axis, is vital for the regulation of human reproduction.[35] Type II GnRH (GnRH II), being specifically identified in humans, differs from GnRH I by three amino acid residues. Usually, GnRH is produced by neurons of the hypothalamus in a pulsatile fashion, reaching the anterior pituitary where it binds GnRH receptor on the pituitary gonadotrophic cells and initiates downstream signaling to synthesize gonadotropins including follicle-stimulating hormone and luteinizing hormone.[35] GnRH also targets

Table 1

Functions of some placenta-produced hormones during pregnancy.

Hormones	Functions	References
hCG	Stimulates P_4 production and enhances syncytialization	1
	Angiogenic factor	30,31
hPL	Stimulate DNA synthesis	32
	Insulin antagonist	32
	Regulates lipolysis	32
GnRH	Stimulates hCG, prostaglandin, hPL, and hCS production	1,33
	Enhance EVT invasion	1,34
P_4	Stimulates decidualization and facilitates embryo implantation	35,36
	Inhibits uterine myometrium contractility and keeps uterine quiescence	35,36
	Maintains immune tolerance	37
E_2	Participates in endometrial maturation and differentiation	38
	Potent vasodilators	39
	Involved in labor initiation	40
T_0	Modulates insulin secretion and inhibits glucose uptake	42,43
	Regulates blood vessel contraction	42,43
	Promotes cervical remodeling and inhibits uterine muscle contraction	42,43

E_2: 17β-estradiol; EVT: Extravillous trophoblast; GnRH: Gonadotropin-releasing hormone; hCG: Human chorionic gonadotropin; hCS: Human chorionic somatomammotropin; hPL: Human prolactin; P_4: Progesterone; T_0: Testosterone.

extra-pituitary compartments such as the ovary, placenta, uterus, and immune system.

In human placenta, GnRH I is widely expressed in various subpopulations of trophoblast throughout gestation, whereas GnRH II expression is restricted to villous CTBs and EVTs at the first trimester. Trophoblast cells possess GnRH receptor and are thus responsible for GnRH I stimulation to produce hCG, prostaglandin, hPL, and human chorionic somatomammotropin.[1] In addition, both types of GnRH can strongly enhance EVT cell invasion with the upregulation of several invasion-associated proteases, such as MMP-2, MMP-9, MMP-26, tissue inhibitor of metalloproteinase-1, urokinase-type plasminogen activator, and plasminogen activator inhibitor.[1] These effects of GnRH are mediated by the activation of protein kinase C, extracellular signal-regulated kinase 1/2, and c-Jun N-terminal kinase.[36] GnRH II can also elicit its invasion-promoting action by transactivating tyrosine kinase activity of EGF receptor in trophoblast cells.[37] It remains unclear whether the invasion-promoting effect of GnRH in EVTs involves the crosstalk with other hormones such as hCG.

Progesterone

P_4 is an essential steroid hormone for pregnant success. It is synthesized from cholesterol in ovarian corpus luteum during the early weeks of pregnancy, with the tropic stimulation of hCG from the implanted conceptus. After about 6–8 weeks of pregnancy, luteal production of P_4 declines, and the placenta becomes the predominant organ to synthesize P_4. Placental P_4 continues to increase up to term.

STB is the major cell type of P_4 synthesis. In contrast to other steroidogenic organs, the placenta does not express steroidogenic acute regulatory, the protein that transfers cholesterol toward mitochondrial inner membrane, which is a critical and limiting step for P_4 synthesis.[38] Metastatic lymph node 64, a protein that is closely related to steroidogenic acute regulatory, is expressed constitutively in the placenta and may be responsible for intramitochondrial translocation of cholesterol in STBs. Cholesterol is converted to pregnenolone within the inner mitochondrial membrane by cytochrome P450scc (CYP11A1), and is subsequently converted to P_4 by type 1 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase.[38]

During embryonic implantation, P_4 stimulates decidualization of human ESCs through increasing cAMP levels and activating PKA signaling. It facilitates embryo implantation by blocking the proliferation-inducing effect of estrogens in uterine epithelial cells and inducing genes that promote uterine receptivity. P_4 is critical to inhibit uterine myometrium contractility and keep uterine quiescence throughout pregnancy by opposing the labor-inducing effects of estrogen, prostaglandins, and oxytocin. P_4 acts in both genomic and non-genomic ways. The non-genomic way involves membrane-associated progesterone receptors (mPR) and the activation of intracellular cAMP and Ca^{2+} signals, as well as the transactivation of classic nuclear progesterone receptors β, which decreases the expression of contraction-associated genes.[39]

Importantly, P_4 is a key hormone to suppress maternal immune rejection against fetal antigens and keep an immunotolerant environment at the fetal-maternal interface. Specifically, P_4 enhances the production of Th2 cytokine IL-4 and inhibits the production of Th1 cytokines interferon-γ and tumor necrosis factor-α, leading to a shift from Th1 to Th2 bias.[40] It promotes the differentiation of Th2 lymphocytes and suppresses cytolytic activity and perforin exocytosis of uterine NK cells via the mediation by progesterone-inducing binding factor.[41]

Estrogens

Placental estrogens mainly include four hormones such as estrone (E_1), 17β-estradiol (E_2), estriol (E_3), and estetrol (E_4). Among these, E_2 is the most abundant estrogen throughout pregnancy. The synthesizing site of estrogens shifts from corpus luteum to placental STBs, in a similar manner to that of P_4 synthesis. Maternal circulating levels of estrogens increase throughout pregnancy, peaking at term with a concentration of 100–120 mg/24 hour.

The human placenta is lack of 17 alpha-hydroxylase/17, 20-lyase, the key enzyme to convert pregnenolone and P_4 to androgens, the precursors of estrogen synthesis. Therefore, the placenta utilizes fetal and maternal adrenal gland-origin dehydroepiandrosterone sulfate and converts it into androstenedione and testosterone (T_0). In STBs, the activity of CYP450 aromatase acts to convert androstenedione and T_0 to E_1 and E_2, respectively.

Estrogens are multifunctional during gestation, and their effects are classically mediated by their nuclear receptors, ERα and ERβ. The binding of estrogen leads to receptor dimerization and recognition of specific estrogen response elements in the promoter of target genes. Various evidence has demonstrated the critical roles of estrogens in embryonic implantation. E_2 stimulates the growth of endometrial epithelial cells (EECs) and modulates the expression of several genes that participate in endometrial maturation and differentiation. Data from co-cultures of EECs and ESCs indicate that the enhancing effect of E_2 on EECs is mediated through ESC-secreted insulin like growth factor 1.[42]

Estrogens are the potent vasodilators and increase the blood flow to several organs including the uterus. Owing to its vasodilatory actions, a rise in estrogen levels during pregnancy may contribute to maintaining the uteroplacental vascular functions. Although the direct role of estrogen in mediating vasodilation has been identified, it has been proposed that estrogen may also indirectly produce vasodilation through prostacyclin, nitric oxide, or endothelium-derived hyperpolarizing factor.[43]

E_2 is also involved in labor initiation by promoting the formation of connexin 43 gap junctions, inducing the expression of pro-contraction gene oxytocin receptor via an ERα-dependent activation of extracellular signal-regulated kinase 1/2 signaling,[44] and therefore increasing the contraction of myometrial cells.

In human placenta, ERα expression is mainly confined to CTBs, whereas ERβ is predominantly expressed in STBs. Estrogens have been implicated in inducing differentiation of syncytial trophoblast, whereas the functional mechanism remains largely unclear.

Accumulating evidence shows nongenomic actions of estrogens by activating membrane-associated estrogens receptors, thus leading to intracellular Ca^{2+} mobilization, activation of adenylyl cyclase, and consequently increase of cAMP levels

and MAPK activation. A recent report shows that the inactivation of G protein-coupled estrogen receptor 1 in mice leads to fetal demise. Mechanically, G protein-coupled estrogen receptor 1 is a central regulator of IFN signaling during pregnancy that allows dynamic antiviral responses in maternal tissues and preservation of fetal health.[45]

Androgens

To date, placental androgens are the least studied pregnancy-related steroid hormones. In pregnant women, the circulating T_0 level increases from the first trimester and further elevates toward the end of pregnancy, rising up to almost sixfold higher than the unpregnant level. It is estimated that approximately 50% of androgen during pregnancy is synthesized in the placenta.[46] Apart from acting as the precursors of estrogen synthesis, androgens in pregnant women also play roles in modulating insulin secretion from the pancreas, inhibiting glucose uptake in fat and muscle cells, regulating blood vessel contraction, promoting cervical remodeling, and inhibiting uterine muscle contraction to facilitate parturition.[47]

Our recent study demonstrates an autocrine/paracrine function of T_0 in trophoblasts, enhancing the process of syncytialization through androgen receptor (AR) signaling.[47] There has been evidence showing the colocalization of AR and β-hCG in human trophoblasts. In addition, sequence analysis indicates seven potential androgen response elements in the promoter region of *hCGB* gene (encoding β-hCG). Therefore, it is most likely that T_0 may directly regulate the transcription of HCGB via androgen/AR signal. Further studies are needed to clarify the working mechanisms of androgen in the placenta.

Functional unit of maternal-fetal exchange

The human placenta is a type of hemochorial placenta with the placental villi being soaked in the maternal blood pool, and the fetal vessels being embedded in the villous core. The special villous structure ensures continuous delivery of maternal nutrients to the fetus, meanwhile transportation of fetal metabolic wastes to maternal blood. The maternal-fetal metabolic communication involves the two-way exchange of metabolites, endocrine molecules, and other secretory factors between the mother and the fetus.[48] The placental villi thus form a maternal-fetal exchange unit, which is vital to guarantee in utero fetal survival and growth, modulate maternal adaptation to pregnancy, and contribute to the long-term health of the offspring and the mother.

Maternal-fetal nutrient transportation through the placenta

For the in utero growing fetus, the metabolic fuels including carbohydrates, amino acids, and fatty acids are primarily supplied by the mother. As stated, the placental villi are the predominant site to transport nutrients from the mother to the fetus. The extensive microvilli on the surface of STBs substantially increase the surface area to contact maternal blood, efficiently ensure the maternal-fetal exchange of membrane-permeable molecules, particles, and polar molecules, during which a variety of transmembrane transporters are involved.[48]

The most important glucose transporter (GLUT) in the placenta is GLUT. GLUT1 is expressed in the basal membrane of STBs throughout pregnancy, while high expression of GLUT3 is found in CTBs at the first trimester, but not at full-term. GLUT4 is highly expressed in the cytoplasm of STBs at the first trimester, and decreases along gestation.[49] Glycogen storage is another source of glucose transporting from the placenta to the fetus, but clear evidence on its specific transport is lacking.

Amino acids are very important for protein synthesis, biological macromolecule production, and energy production. The concentration of amino acids in human fetal venous blood is higher than that in maternal peripheral blood. Amino acid transporters are divided into three categories according to their transport modes, namely accumulative, exchange, and facilitated transporters.[50] In the placenta, the expression manners of these amino acid transporters on the placental microvillous membrane and basal membrane together determine the amino acid transport to the fetus. Specifically, accumulative amino acid transporters, SLC1, SLC6, and SLC38, mediate the transport of amino acids from maternal blood to trophoblasts against concentration gradient. Exchange transporter, SLC7, flows out the nonessential amino acids from trophoblasts and allows the essential amino acids to enter. Facilitated transporters, SLC16A10 and SLC43, allow amino acids transport along concentration gradient, leading to the transfer of amino acids from trophoblasts to the fetus.[51]

Fatty acids, the important metabolic molecules, are essential for molecular synthesis, energy production, signal transmission, and placental hormone production. The chorionic microvillous membrane of human placental can bind lipoproteins and transport triglycerides and other esterified lipids. Placental fatty acid-binding proteins and fatty acid transport protein (FAT/CD36) facilitate fatty acid transport from high to low concentrations.[52] At term, the concentration of non-esterified fatty acids in maternal blood is three times higher than that in fetal blood, whereas fetal blood exhibits a higher concentration of albumin and lower level of non-esterified fatty acid/albumin ratio. In addition, fatty acid transporters FATP1 (SLC27A1) and FATP4 (SLC27A4), the members of very long-chain acyl-CoA synthetase family, are expressed in the placenta,[52] suggesting a higher transport efficiency for long-chain fatty acids in the placenta.

Adaptation of the placenta to nutrient stress

In mammalian, the nutritional allocation between the mother and fetus critically impacts fetal growth and pregnancy viability, in particular when nutrients are scarce. Disrupted nutrient homeostasis may lead to compromised fetal-maternal health, thus creating a selective pressure to adapt to diminished resources. The placenta constitutes the main interface between mother and fetus and is believed to shape mammalian pregnancy outcomes by sophisticatedly adapt to nutrient environment.

Our recent study discovers that differentiation of trophoblasts toward syncytium triggers an endocytosis strategy, macropinocytosis, to uptake large extracellular molecules as an alternative nutrient source. This unique

machinery of nutrient uptake is strikingly boosted under amino acid shortage conditions via inhibition of mammalian target of rapamycin (mTOR) signaling, which is essential for fetal survival.[53] In pregnancy complicated with FGR, the placentas display notable repression in mTOR activity, augment in trophoblast syncytialization, and macropinocytosis activity. Thus, macropinocytosis in STBs is a physiologically important adaptation of the placenta to achieve a prime goal of nutrient delivery in the context of poor blood flow or other limitations in maternal nutrient supply. In this process, mTOR can act as a sensor to environmental nutrition and thus switches nutrient uptake route. With ample nutrient supply, activated mTOR favors amino acid uptake through specific transporters. However, under nutritional stress such as limited amino acids availability, inhibition of mTOR signaling sufficiently promotes trophoblast syncytialization and activates macropinocytosis to uptake macromolecules. Besides, mTOR is reported to participate in integrating a large number of growth-related signals, including hormones and growth factors such as insulin, IGF-I, EGF, cellular adenosine triphosphate levels, hypoxia, glucose, and fatty acids, to regulate trophoblast mitochondrial respiration, nutrient transport, and protein synthesis, thereby influencing placental and fetal growth.[54] It warrants further investigations on how mTOR pathway orchestrates nutrient microenvironment, trophoblast differentiation, and cell metabolism to maintain functional plasticity of the placenta.

Functional unit of blood perfusion into the maternal-fetal interface

Sufficient blood perfusion from the mother to the fetus is essential for a successful pregnancy, which is primarily guaranteed by the process of uterine spiral artery (SPA) remodeling. The un-remodeled SPA consists of intact endothelial cells and VSMCs interspersed with elastin fibers.[1] By far the physiological process of SPA remodeling during pregnancy remains unclear, whereas it is assumed to divide the process into five stages: (1) decidua-associated early vascular remodeling, (2) iEVT-associated vascular remodeling, (3) enEVT migration, (4) the incorporation of enEVT into the vessel wall, and (5) the re-endothelization and subintimal thickening.[2,3] At the first stage, the decidual stromal cells and leukocytes, specifically dNKs and macrophages, participate in "priming" the maternal vessels in a trophoblast-independent manner. The alterations in uterine spiral arteries mainly include endothelial cell vacuolation, smooth muscle swelling, and VSMC disorganization.[55] The infiltration of iEVTs surrounding the spiral arteries induces the substantial dedifferentiation of VSMCs and thus the disruption of the vascular smooth muscle layer.[55] enEVTs invade the lumen of the arteries and replace the endothelial cells of the maternal vessels in a pseudovasculogenesis manner.[24] The remodeling process results in a low-resistance high-capacity circulation system at the maternal-fetal interface.

Several cell events occur during the process of SPA remodeling, primarily including the disappearance of SPA-VSMC, the replacement of uterine SPA endothelial cells by enEVTs, and the catabolism of perivascular ECM. Complicated and coordinated interactions among various cell types determine the success of SPA remodeling.

Replacement of uterine SPA endothelial cells by enEVTs

As stated above, enEVTs exhibit reduced E-cadherin expression while specific expressions of VE-cadherin, vascular cell adhesion molecule-1, platelet-endothelial cell adhesion molecule-1, and integrin α4 to mimic endothelial cells.[28] It is suggested that these molecules may mediate the interactions between enEVTs and niche cells, especially endothelial cells. Deregulation in these enEVT-expressing factors is associated with severe pregnancy complications such as PE.[28]

The process of enEVT infiltration into SPA has been limitedly described. It is not a homogeneous process, with the density, depth, and degree of cell invasion diminishing toward the placental margin.[3] Blankenship and coworkers have presented a detailed observation of enEVTs in the rhesus monkey.[56] They found that enEVTs asymmetrically adhered to the arterial wall at early gestation, and the basement membrane underlying the endothelium lost along with trophoblast moving outwardly into the tunica media. Subsequently, trophoblast cells appear being sequestered in the vessel wall where they were hypertrophied and surrounded by a capsule containing type IV collagen and laminin. The muscular layer of the artery became discontinuous when trophoblasts were established on the vessel wall.

Several in vitro models have been generated to study the replacement of endothelial cells by enEVTs. Cartwright and coworkers examine the interactions between the invading trophoblast cells and SPA endothelial cells by using a model in which fluorescently labeled trophoblasts are seeded on top of the unmodified (non-placental bed) SPA segments that are embedded in fibrin gels or are perfused into the lumen of arteries mounted on a pressure myograph.[57] Other studies use explant co-culture of placental villi and decidual tissue to build in vitro model mimicking the physiologic conditions of the interactions between EVTs and decidual cells and vessels during early gestation. Three-dimensional co-culture of trophoblastic and endothelial cells in matrigel matrix also provides in vitro system to study cell interactions.[58]

Although there has been evidence revealing the role of multiple factors inducing the differentiation of trophoblasts toward endovascular phenotypes, such as platelet-derived soluble factors, dNK-derived VEGFs (including VEGF-C and PlGF), Ang-1 and Ang-2, transforming growth factor β (TGF-β), etc, the regulatory mechanisms of how uterine artery endothelial cells are replaced by enEVTs have been poorly understood.

Another interesting question is the physiological significance of enEVT-replacement of SPA endothelium. Along with the maternal-placental circulation, the fetal-derived enEVTs in the remodeled SPA and the placenta VTs immersing into maternal blood at IVS directly contact the maternal lymphocytes, while do not cause maternal immune rejection. Our recent study illustrates the stable distribution of regulatory T (Tregs) cells in lumen of the remodeled arteries and IVS. What's more, enEVTs but not

iEVTs or uterine endothelial cells, are identified to specifically express TGF-β1 to induce the differentiation of maternal $CD4^+$ T cells to immune protective Tregs.[59] The findings strongly demonstrate the immune regulatory property of enEVTs, which provides new thoughts on the significance of endothelium replacement by enEVTs in maintaining immune tolerance during pregnancy.

Dedifferentiation of VSMCs around uterine SPA

VSMCs around uterine SPA present a regular and intact arrangement in non-pregnant endometrium. Upon pregnancy, the initiation of decidual SPA remodeling process is accompanied by smooth muscle swelling, separation, and misalignment of VSMCs and the catabolism of ECM and elastin fibers. The histological analysis demonstrates that the morphological change of VSMCs occurs before trophoblast cell invasion, whereas full vascular transformation depends on the presence of EVT invasion. Along the SPA remodeling process, gradual disappearance of VSMCs is in parallel with the replacement of endothelial cells by enEVTs, and the peri-vascular elastic and collagenous ECM are eventually replaced by extracellular fibrinoid deposits.

The fate of SPA-VSMCs during SPA remodeling has long been understudied. Some studies indicate VSMC apoptosis during the remodeling process, which may be due to the stimulations from decidual leukocytes including dNK cells and macrophages, and the invasive trophoblasts that produce soluble FasL and TRAIL.[60] It is also assumed that SPA-VSMCs may migrate away during SPA remodeling. These points have been debatable since there has been evidence showing the existence of apoptotic signals mostly in EVTs, decidual lymphocytes, and vascular endothelial cells, but few in SPA-VSMCs.[61] What's more, the migrating VSMCs have not been traced with any typical SMC markers. More and more evidence is emerging supporting the dedifferentiation of SPA-VSMCs during pregnancy.

The dedifferentiation of VSMCs has been found in blood vessels in association with cardiovascular diseases. Typical characteristics of VSMC dedifferentiation include apparently morphological transformation to round, misalignment or alteration in distribution pattern, and diminished expression of typical VSMC markers such as smooth muscle alpha actin, smooth muscle protein 22-alpha, Calponin, myosin heavy chain 11.[62] SPA-VSMCs have been found to gradually undergo these morphological and molecular changes during SPA remodeling.[55] Our recent study demonstrates the primary role of decidual stromal cells in initiating SPA-VSMC dedifferentiation, which is subsequently boosted by dNK cells and macrophages before EVTs participate. The dramatic dedifferentiation of SPA-VSMCs occurs after EVT invasion, eventually leading to the vanishment of VSMCs in fully remodeled stage.[55]

The physiological events of stroma decidualization, lymphocyte infiltration, EVT invasion, replacement of SPA endothelium, and disappearance of SPA-VSMCs are highly programmed and synergistic. Data of single-cell sequencing analysis in human decidua have provided robust molecular evidence for the complicated interactions among decidual stromal cells, immune cells, EVTs, endothelial cells, and perivascular SMCs. Within the complicated decidual compartment, MMP9, Ang-1, Ang-2, interferon-γ, VEGF-C from dNK cells,[62] IP-10, and CXCL10 from EVTs,[63] and phagocytotic function of macrophages[64] have been indicated to sufficiently disrupt VSMCs integrity and inhibit VSMC marker gene expression.[63] PDGF-BB and cysteine cathepsin 8 from EVTs are also involved in VSMCs dedifferentiation. Trophoblast-produced miR-210 can target Efna3 and Bcl2, which participate in angiogenesis and transformation of VSMCs.[65] On the other hand, several studies demonstrate that VSMCs and endothelium of SPAs express chemokines including CCL4, 14, 16, 21, 22, and CXCL12, which can specifically attract macrophages and NKs, and regulate trophoblast invasion.[60] Therefore, a complex regulatory network among multiple cell types in decidua determines the success of vascular remodeling. It is worthy of further exploring how the multiple cells interact in a spatial-temporally dynamic and harmonized manner to regulate SPA-VSMC dedifferentiation.

To date, the fate of dedifferentiated SPA-VSMCs remains a mystery. There have been varieties of evidence showing the dedifferentiate of smooth muscle cells toward macrophages, osteochondrocytes, or adipocytes in various tissues under pathological conditions.[66] We recently present that a few dNK cells around the remodeling SPA specifically exhibit H3K4dime modification in myosin heavy chain 11 promoter, indicating their potential origin from smooth muscle cells. It is likely that SPA-VSMCs may, at least in part, differentiate into dNK cells during SPA remodeling process.[55]

The degradation of internal elastic lamina and medial elastin fibers involves many elastases and proteases. It has been indicated that MMP-2, MMP-7, MMP-9, and MMP-12 from EVTs, dNK, and decidual macrophages (dMϕ),[60] and SPA-VSMCs[67] contribute to elastin catabolism, establishing a proper microenvironment for SPA remodeling.

Unit of immune adaptation at the maternal-fetal interface

Pregnant success in placental mammals substantially depends on the establishment of maternal immune tolerance to the semi-allogenic fetus, which means the immune response of the mother during pregnancy is dampened against fetal-expressed antigens. Meanwhile, maternal immune response remains intact against microbe-specific antigens, which is responsible for the protection against placental infection. Multiple lines of evidence from clinical analysis or animal model studies have indicated the tight association of failures in maternal immune adaptation with various adverse pregnancy outcomes, such as early pregnancy loss and PE.[68,69]

There are two interfaces of immune tolerance at the maternal-fetal interface. One interface is within the decidua part where the dynamic and well-orchestrated interactions among EVTs and various maternal immune cells construct a local environment in adaptation to pregnancy. The other interface at risk of immune rejection primarily includes the remodeled SPAs in which enEVTs come in direct contact with maternal lymphocytes, and IVS

where fetal VTs immerse into maternal blood that perfuse from SPA.

At early pregnancy, a large number of maternal immune cells accumulate in decidua, which accounts for about 40%–50% of decidual cells. The populated immune cells in uterine mucosa include dNK cells (~50%−70%), macrophages (~20%), T cells (~10%−20%), and a small amount of dendritic cell (DC), Mast cell, and B cell. These immune cells are deeply involved in a variety of events including local immune response, trophoblast differentiation and invasion, and vascular remodeling, etc.[70] Importantly, the phenotype and function of immune cells at the maternal-fetal interface need to be fine-adjusted through interacting with trophoblast cells which are the active builders of local immune tolerance.[70] The dialogues between trophoblasts and these immune cells primarily involve two manners: the direct ligand-receptor recognition and mutual education, and the indirect interactions mediated by growth factors, cytokines, or chemokines.

Direct interactions between trophoblasts and maternal immune cells

Placental trophoblasts are the source of paternal antigens during gestation. It has been well proved that EVTs express a unique library of MHC ligands including HLA-C, HLA-E, and HLA-G, with their specific surface receptors predominantly in dNK cells. Specifically, the activation or inhibition of dNK cells depends on the binding of activating or inhibitory receptors to MHC ligand. Human HLA-C is dimorphism, with two allotypes HLA-C1 and HLA-C2, recognizing killer cell immunoglobulin receptors (KIRs) including inhibitory haplotypes (such as KIR2DL2 and KIR2DL3 specific for HLA-C1 and KIR2DL1 for HLA-C2) and activating haplotypes (such as KIR2DS1 for HLA-C2). HLA-G and HLA-E can directly bind to the inhibitory receptors KIR2DL4, LILRB1, and CD94/NKG2, respectively, on dNK cells.[71] The MHC ligand-receptor recognition between EVTs and dNKs provides an overall inhibitory signal for dNKs to maintain tolerance to the semi-allogeneic fetus.

Besides, HLA-G-mediated immune tolerance can be achieved through a special biological process, phagocytosis, referring to the transfer of membrane proteins between cells. Successful HLA-G phagocytosis confers a transient immunosuppressive phenotype on the recipient cells. The interaction between HLA-G$^+$ EVTs and dNK cells leads to phagocytosis and endocytosis of HLA-G by dNK cells. The degradation of internalized HLA-G occurs after the activation of cytokines and is accompanied by the recovery of cytotoxicity. The HLA-G cycle thus provides a mechanism for dNK tolerance and immunity at the maternal-fetal interface.[72]

The interaction between programmed cell death protein 1 (PD-1) and its ligand PD-L1 also participates in constructing immune tolerance at the maternal-fetal interface. It has been found that PD-L1 is highly expressed in EVTs and villous STB.[73] The PD-L1/PD-1 interactions between trophoblasts and decidual immune cells including macrophages and CD8$^+$ T cells may produce an inhibitory signal, thereby helping to form an M2 phenotype or inhibit the cytotoxicity of CD8$^+$ T cells.[74]

Indirect interactions among trophoblasts and maternal immune cells

Trophoblast cells and decidual immune cells can secrete large amounts of cytokines, chemokines, and growth factors to induce selective trafficking of leukocytes to the maternal-fetal interface, and to affect varieties of cell events in paracrine or autocrine manner.

Various types of leukocytes are dramatically accumulated in decidua upon pregnancy, which are probably recruited from peripheral blood and/or other tissues. It has been evidenced that various CCLs and CXCLs produced by trophoblasts or decidual cells play dominant roles in the recruitment and homing of NKs, macrophages, DCs, and T cells to the maternal-fetal interface via corresponding chemokine receptors on these immune cells. The chemokine-chemokine-receptor interactions at the maternal-fetal interface form a complex and orchestrated regulation network, whereas our understanding on the molecular basis of leukocyte trafficking is still incomplete.

Placenta-derived factors are also crucial in characterizing the functional leukocytes to maintain the immune-adaptive environment during gestation. For instance, trophoblast-derived CXCL16 induces polarization of macrophages to M2, and M2 cells and further promotes the inactivation of NK cells by reducing the expression of IL-15. Macrophage colony stimulating factor (M-CSF, also known as CSF-1) and IL-34 which share the same M-CSF receptor are produced by trophoblasts and support the polarization of steady-state CD14$^+$CD163$^+$ CD206$^+$CD209$^+$ decidual M2 cells and the active production of IL-10 and CCL18.[75] Trophoblast-secreted IL-6 contributes to biased polarization of M2 via STAT-3 signaling.[76] In addition to chemokines and cytokines, trophoblast-produced hyaluronic acid, the most abundant component in ECM, interacts with CD44 and activates downstream PI3K/Akt-STAT-3/STAT-6 signal to promote M2 polarization.[77] Indoleamine 2,3-dioxygenase expressed by trophoblasts and macrophages is involved in catabolizing tryptophan in T cells, leading to cell cycle arrest and apoptosis of activated T cells, thereby inhibiting the activity of T cells and preventing allogeneic rejection.[78]

At the maternal-fetal interface during early pregnancy, about 4% of CD4$^+$ T cells are CD25^{hi}FOXP3$^+$ Treg cells. The proportion of Treg in decidua is higher than in peripheral blood, and many studies have indicated the recruitment and education of Treg cells to confer tolerance to fetal antigens at the maternal-fetal interface. IL-35 secreted by trophoblast cells inhibits T cell proliferation and induces the transformation of naive conventional T cells into IL-35-producing induced Treg cells through STAT1/STAT3 signal.[79] Our recent study reveals that enEVTs actively secrete TGF-β1 to induce the differentiation of maternal CD4$^+$ T cells into immunosuppressive Treg cells in the remodeled spiral arteries, indicating a new mechanism of the education of maternal immune cells along with the maternal-placental circulation.[59]

On the other hand, a variety of immune cell-produced factors are functionally active in modulating trophoblast cell behaviors. dNK cells are highly active in producing a large number of growth factors, angiogenesis factors, and cytokines, which are crucial to modulate trophoblast cell migration and invasion, SMC dedifferentiation, and

enEVT instruction during SPA remodeling. The specific production of growth factors including PTN and OGN through transcription factor PBX1 in dNK cells helps to promote embryonic development.[80] Interestingly, it is recently discovered that a subset dNK cells with high expression of growth factors and immunomodulatory proteins, such as IFN-γ and VEGFA, are highly enriched in multigravid women. This subset is named pregnancy-trained dNK.[81] Recent studies using single-cell transcriptomics have identified three dNK subpopulations in decidua at the first trimester, among which the characteristics of dNK1 are similar to pregnancy-trained dNK. It is likely that dNK1 cells may be "primed" by conception and expanded quickly to promote decidual receptivity in the subsequent pregnancy. dMϕ are cable of producing many factors, such as VEGF and MMP9, to promote angiogenesis and tissue remodeling. dMϕ also play roles in the regulation of adaptive T cell response and innate NK cell response.[82]

Placental dysfunction and PE

Numerous human and animal studies have documented that disrupted placental development and function are tightly associated with adverse pregnancy outcomes and even fetal lethality. In humans, various pregnancy-associated diseases, e.g., PE, recurrent miscarriage, fetal growth restriction, and placenta accreta spectrum, etc., have been suggested as placenta-origin disorders. A recent study with large-scale phenotyping analyses of over 100 mutant mouse lines reveals gene network that bridges placenta defects with fetal developmental defects and embryonic lethality.[83] In humans, the molecular basis that links placental dysfunction and adverse pregnancy outcomes and fetal/maternal health remains to be systematically elucidated. Here in this article, we take PE as an example of pregnancy-associated disease to highlight some recent advances regarding the placental and maternal factors that may contribute to the occurrence of the disorder (Fig. 2).

Overview of PE

PE, affecting approximately 5%–7% of pregnancies worldwide, is characterized by newly onset hypertension accompanying proteinuria or dysfunctions in multiple maternal organs after the 20th week of gestation. It is a leading cause of maternal and perinatal morbidity and

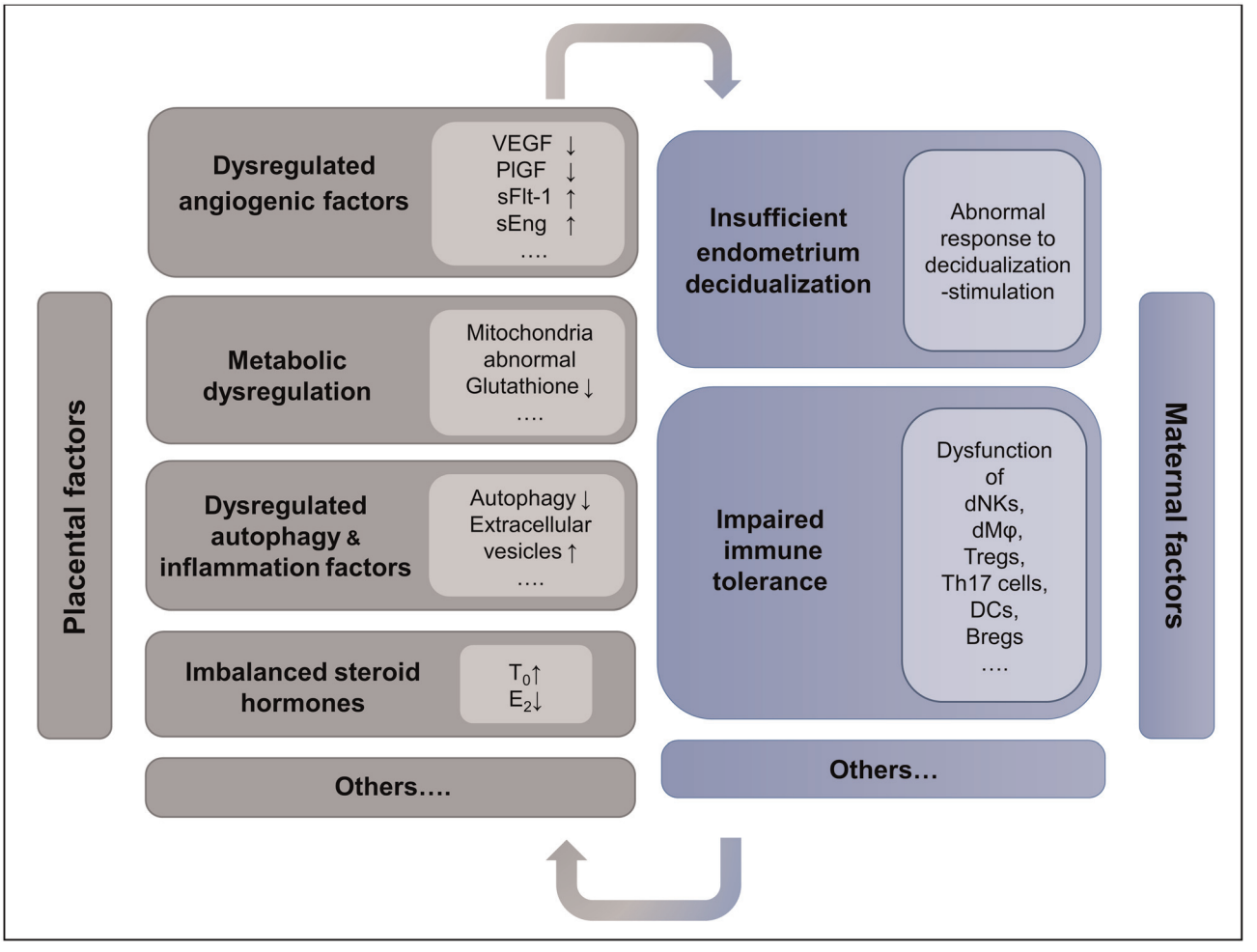

Figure 2. A brief summary of the recently identified maternal factors and placental factors for PE. Bregs: Regulatory B cells; DCs: Dendritic cells; dNKs: Decidual natural killer cells; dMϕ: Decidual macrophages; E_2: 17β-estradiol; PE: Preeclampsia; PlGF: Placental growth factor; sEng: Soluble endoglin; sFlt-1: Soluble fms-like tyrosine kinase-1; T_0: Testosterone; Tregs: Regulatory T cells; Th17 cells: T-helper 17 cells; VEGF: Vascular endothelial growth factor.

mortality. What's more, women with preeclamptic pregnancies and the babies born to preeclamptic mothers demonstrate increased risk for hypertension and cardiovascular disease later in life. According to the onset time of the clinical manifestation, early-onset PE (EOPE) and late-onset PE are diagnosed before or after the 34th week of gestation.[84] Generally, impaired placental development in early pregnancy and subsequent growth restriction is often associated with EOPE, while late-onset PE is usually associated with maternal endothelial dysfunction. Currently, the only treatment available for PE, especially EOPE, is still premature delivery and termination of pregnancy. The major challenge is to effectively early-diagnose the condition and develop preventive and therapeutic strategies that will minimize the burden of PE, all of which will largely depend on the in-depth study of its pathogenesis.[1]

It is generally accepted that the molecular events leading to the onset of PE occur early in pregnancy, specifically the placental deficiencies are the principal pathological origin of the disorder. The pathological changes in PE placenta have been well described. The number, density, and invasiveness of iEVTs are significantly reduced in PE cases than in normal pregnancies. In severe cases, enEVT migration and vascular remodeling are consistently incomplete, and enEVTs are much less in uterine spiral arteries. Poor differentiation and increased apoptosis may account for the shallow invasion of EVTs in PE placenta. Furthermore, an increase in syncytial shedding and a switch from normal apoptotic to necrotic pathway are observed in PE placenta due to insufficient SPA remodeling and thus higher velocity blood flow. An increase in systemic and local inflammation is also demonstrated in PE pregnancies.[1]

Currently, our understanding concerning this frustrating pregnancy-related disease is improving. In addition to poor placentation occurring at early pregnancy, the maternal factors, including uterine microenvironment, genetic, behavioral, and environmental factors, may reciprocally interact with the developing placenta and fetus in multiple ways.[1]

Placental factors that are associated with PE

Angiogenic and anti-angiogenic factors

A variety of studies have demonstrated the predictive value of the circulating angiogenic factors, including VEGF, PlGF, soluble Flt-1 (sFlt-1) which is the antagonizing receptor for VEGF and PlGF, and soluble endoglin (sEng) that neutralizes TGF-β as reliable biomarkers for PE. Decreased levels of VEGF or PlGF, or increased concentration of sFlt-1, sEng, or sFlt/PlGF in maternal serum before the clinical manifestation have been suggested to be correlated with a much greater risk for PE, especially EOPE.[1]

The more remarkable evidence comes from animal models. The clinical features and placental defects of PE can be recapitulated in mouse or rat models with systematic or placenta-specific overexpression of sFlt-1. Simultaneous adenoviral administration of sEng and sFlt-1 in pregnant rats leads to clinical features of HELLP syndrome, which is an extremely severe subtype of PE.[85] In a baboon PE model that is generated by induction of uteroplacental ischemia via ligation of a single uterine artery, specific siRNAs for sFlt-1 suppresses sFlt-1 overexpression and the PE-like signs.[86] The study strongly indicates a therapeutic strategy for patients with preterm PE.

Imbalanced steroid hormones

As stated above, the placenta acts as the main source of steroid hormones from around 8 weeks of gestation. Ours and other studies have demonstrated elevated T_0 and repressed E_2 in maternal circulation of PE patients, especially the EOPE cases. In parallel, upregulated 17β-HSD3 whereas inhibited aromatase expression and activity are observed in the placentas of the EOPE patients.[87,88] A small RNA, miR-22, plays a role to balance the placental production of T_0 and E_2 through responding to T_0 and targeting ERα to control E_2/ERα-induced aromatase expression in trophoblasts. It is likely that the aberrantly higher T_0 in PE placenta may cause the inhibition of E_2 production via miR-22/ERα/aromatase route.[87] By far it remains unclear how 17β-HSD3, the major synthase of T_0 is upregulated in preeclamptic placenta.

The T_0/E_2 balance is crucial because sex hormones are highly pleiotropic and T_0 and E_2 frequently exhibit opposite effects in many physiological events. T_0 increases vascular responses to vasoconstrictor agents such as arachidonic acid and norepinephrine, and studies in the rat model show the induction of blood vessel damage and subsequent hypertension by T_0 through a PKCδ-dependent pathway.[89] Estrogens are potent vasodilators that lead to an increase in blood flow in selected organs, with the greatest response in the uterus. Estrogen and P_4 act as modulators of uterine vessels and decrease the resistance of uterine spiral arteries. In contrast, androgens inhibit the angiogenesis process and reduce blood flow to the fetal-maternal interface. The altered T_0/E_2 in preeclamptic patients may, therefore, cause insufficient vascular dilation and aggravate blood vessel damage in both the fetoplacental unit and the maternal organs.

Physiologically relevant mechanisms can protect against the possible detrimental effects of pregnancy-induced androgen excess. We recently identified a protective mechanism to androgen excess in PE placenta through protein O-GlcNAcylation, a reversible and ubiquitous post-translational modification of intracellular proteins in eukaryotic cells.[90] We identify hundreds of proteins that are dynamically O-GlcNAcylated during trophoblast syncytialization using quantitative proteomics. Interestingly, O-GlcNAcylation of cystathionine g-lyase (CSE) promotes its enzymatic activity to produce H_2S, which in turn represses T_0-enhanced trophoblast syncytialization via inhibiting AR dimerization. The remarkably enhanced CSE O-GlcNAcylation, H_2S production, and restricted trophoblast syncytialization are presented by preeclamptic placentas.[47] The findings reflect the complexity in the regulation of placental cell differentiation and indicate a mechanism whereby the placenta may counteract the possibly excessive syncytialization caused by over-produced T_0 through increasing CSE O-GlcNAcylation and H_2S production.

Compromised metabolic regulation

Proteomic analysis of PE placenta reveals a dysregulated function of mitochondrial, as indicated by the molecules in association with tricarboxylic acid, cycle electron transport chain, fatty acid oxidation, etc. A recent study using HR-MAS MRS technology identifies five metabolites that are unique to PE placenta, which is expected to provide new evidence for PE classification.[91] Abnormal expression of glutathione metabolism-related molecules in PE placenta may lead to increased oxidative stress and inflammatory response. Activation of the classical mitochondrial unfolded protein response pathway reflects the mitochondrial stress in the placenta of EOPE.[92]

Placental autophagy and inflammation factors

A recent study demonstrates the abnormal protein degradation process in PE placenta, whereby the accumulation of harmful protein aggregates may lead to placental dysplasia.[93] Increased extracellular vesicles and excessive platelet activation and coagulation activity are found in PE placenta.[94] What's more, extracellular vesicles from PE patients can specifically activate platelets and induce the activation of inflammatory factors in trophoblasts, leading to the appearance of PE-like symptoms in mouse model.[94] These findings provide new thought on the etiology of PE, and suggest the potential treatment strategies by targeting lysosomal biogenesis, autophagy or extracellular vesicles.

Maternal factors that contribute to PE

Insufficient endometrium decidualization

The normal development and maintenance of decidualization of human uterus provide crucial "soil preparation" for embryo implantation and subsequent placental development. A transcriptome analysis of PE decidua reveals significant downregulation of decidualization-related genes. Interestingly, abnormal response to decidualization-stimulation is observed in the endometrium from the women with PE history.[95] The study strongly suggests that inadequate endometrium decidualization is a key soil factor for PE.

Impaired immune tolerance

Dysfunctions of various immune cells have been reported in PE, including dNKs, dMϕ, Tregs, T-helper 17 (Th17), DCs, and even regulatory B cells.[96] In PE patients, reduced expression of HLA-G in EVTs may lead to diminished inhibition of the cytotoxicity and altered profile of cytokine production in dNKs. As stated above, the direct interaction between dNKs and EVTs through KIR and HLA-C is an important way to generate immune tolerance at the maternal-fetal interface. Genetic study reveals a higher risk for PE in KIR AA genotype mother carrying a HLA-C2 fetus.[97] However, this finding is confirmed in Caucasian populations, but seemingly not supported in East Asian populations.[98] This may be due to the different repertoire of KIR genotype frequencies among various ethnicities.

Polarization of dMϕ is shifted toward the M1 phenotype, and Th response is polarized toward the Th1/Th17 phenotype in PE, leading to a pro-inflammatory microenvironment. M1 cells also secrete sFlt-1 that is associated with impaired angiogenesis in PE. It is proposed that the compromised immune status impairs trophoblast differentiation and causes a higher rate of EVT apoptosis, which may exceed the phagocytic ability of macrophages. Excessive release of cell debris can further trigger inflammatory response, which is worsened by hypoxia and ischemia due to deficient placentation.[99]

In maternal circulation of PE patients, decreased percentages of Tregs are reported compared to that in normal pregnant women.[100] We recently demonstrate the critical function of enEVT to induce Treg differentiation along with the maternal-placental circulation.[59] Remarkable defects in enEVT differentiation and SPA remodeling have been well recognized in PE placenta. It is therefore most likely that the incomplete SPA remodeling and the compromised Treg function in PE are reciprocally affected events.

Summary and perspectives

More and more exciting progress has been made in recent years, ranging from the regulation of trophoblastic cell lineage and placental development to the discovery of novel biomarkers and potential therapeutic targets of pregnancy complications including PE. The dominant roles of the placenta in pregnancy outcomes and the health of the mother and the offspring have been well accepted. Accumulating evidence are supporting the point that pregnant success is determined by the dynamic and sophisticated crosstalk among the "seed" (the embryo/fetus and the placenta) and the "soil" (the uterine microenvironment and the maternal immune and metabolic status), the physiological mechanisms and the pathological relevance of which are briefly highlighted in this article. Investigation on such complicated while delicate pregnancy progress requires the systematic strategy to integrate the multiple cell events and their cascading regulation.

Our understanding on human placenta has been much limited. Due to ethical considerations and technology restrictions, it is hard to analyze the maternal-fetal interface at continuous and exact time points of gestation. Big species difference in terms of placentation leads to challenge in obtaining appropriate animal models mimicking the physiological or pathological conditions of human pregnancy. Recent advance in novel research models including hTSCs and placental organoid, the technologies including single-cell omics and high-resolution imaging, as well as the increasingly tight discipline connections have provided promising platforms to interpret the precise regulation of extra-embryonic cell lineage and the dynamic cell interactions at the maternal-fetal interface, and thus to explore the mystery of human pregnancy and to translate the findings of bench research into effective clinical treatments for pregnancy complications.

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Conflicts of Interest

None.

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