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Placental Origins of Preeclampsia: Potential Therapeutic Targets*

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Summary: Preeclampsia (PE) remains a leading cause of maternal and perinatal morbidity and mortality in obstetrics worldwide. No effective treatments to reduce its incidence and severity in clinical practice are currently available. A variety of hypotheses have been generated aiming to explain the origins of PE, notably being the genetic predispositions and placental dysfunction. As regard to placental dysfunction, much progress has been made in basic research and several potential therapeutic targets have been identified. This review will discuss in detail the potential therapeutic targets in PE models including uteroplacental blood flow, oxidative stress, vasoactive factors and inflammation/immune response, and introduce the evolving technologies for placental research nowadays.
Key words: preeclampsia; placental dysfunction; therapeutic targets

Preeclampsia (PE) is a pregnancy-specific syndrome that affects 3%–5% of all pregnancies[1]. Complications such as eclampsia, stroke, liver rupture, pulmonary edema, kidney failure, fetal growth restriction (FGR) and preterm birth can occur in severe PE patients. Management of PE involves stabilisation of the mother and fetus with timely delivery to prevent deterioration of the condition and subsequent morbidity and mortality[2]. Although systemic pathological changes usually resolve following delivery of the placenta, PE still predisposes mothers to cardiovascular disease in the long run[3]. Given its severity and absence of curative treatment, prediction and prevention of PE count. Much efforts are being made to establish a reliable risk prediction model, maily based on high risk factors (previous PE, chronic kidney disease, hypertension, diabetes, autoimmune disorders, etc.), abnormal concentrations of factors in maternal blood[4] (decreased placental growth factor, increased soluble fms-like tyrosine kinase 1, etc.) and uterine artery Doppler ultrasound examination[5] in early pregnancy. It is noteworthy that all these models need further validation in clinical practice as for the sensitivity and specificity. Preventive measures such as low-dose aspirin[6] or heparin, calcium supplementation and lifestyle interventions, show potential but small benefit actually.

The pathophysiology of preeclampsia is complex, generally considered to occur in two stages. The first stage is aberrant development of the placenta, which results in placental hypoxia (with or without reperfusion injury), oxidative stress, inflammation and apoptosis of the placental syncytium. Generation and release of anti-angiogenic factors and inflammatory cytokines from the placenta result in widespread maternal endothelial dysfunction, finally culminating in multiple organ dysfunction. Numerous *in vitro* and *in vivo* PE models have been investigated in basic scientific research, demonstating that targeting uteroplacental blood flow, oxidative stress, vasoactive factors or inflammation/immune response may halt or reverse PE progression.

1 Enhancement of Uteroplacental Blood Flow

During pregnancy, uteroplacental blood flow is considerably increased via a large reduction in vascular resistance, marked enlargement and structural remodeling of the uterine vasculature. Deficits in uteroplacental or fetal blood flow are strongly associated with PE and FGR as evidenced by Doppler ultrasound measures of impedance to blood flow. Therefore, vasodilators or substances that promote angiogenesis

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may increase the uteroplacental blood flow. There is increasing evidence suggesting that endothelium-derived nitric oxide (NO) plays an important role in the modulation of vascular tone in the uterine resistance vasculature[7]. Pregnant mice containing genetic modifications which prevent NO production such as endothelial nitric oxide synthase (eNOS) knockout exhibited reduction of uterine blood flow, spiral artery elongation and placental oxygenation[8]. Hence, supplement of NO precursors[9] (L-arginine or L-citrulline), administration of NO donors[10] (such as glyceryl trinitrate, SE175) or inhibition of the degradation of NO downstream messengers may increase the NO bioavailability. Sildenafil citrate is a phosphodiesterase-5 inhibitor which efficiently inhibits the breakdown of NO second messenger cyclic guanosine monophosphate (cGMP). It is not only used as a treatment for pulmonary hypertension and male erectile dysfunction, but also found to potentiate NO-dependent vasodilatation of myometrial small arteries from women with PE[11]. Since then, lots of animal studies have been done to investigate the effectiveness of sildenafil citrate in treating placental dysfunction and the majority of them showed that it could improve the uteroplacental blood flow and fetal weight in FGR[12]. Nevertheless, evidence in favor of its effectiveness as a treatment for PE remains less robust. Similarly, several studies using sheep and guinea pig models demonstrated that administration of adenovirus vectors encoding the VEGF-A gene (Ad-VEGF) in uterine arteries could increase the uteroplacental blood flow and therefore fetal growth[13, 14]. Some researchers thought that the short-term effects were attributed to the upregulation of eNOS, whereas longer-term effects were most likely associated with increased uterine artery vascularization. One study also showed that Ad-VEGF had no damage to the structural and functional integrity of human placental villous explants and cotyledons when used at a range of doses, which needs further verification[15].

Placental chorionic plate arteries (CPAs) are assumed to be resistance vessels, which may contribute significantly to overall placental vascular resistance and blood flow. CPAs are rarely remodeled by the trophoblast and lacking autonomic innervation. Numerous studies have demonstrated structural and functional changes of CPAs in cases of pathological pregnancy, especially in PE[16]. For example, CPAs constitutively express Ca^{2+}-activated K^+ (Kca) channels, whose reduced expression and activity contributed to the increase of the resistance of CPAs and impaired relaxation responses to NO[17, 18]. Heat shock protein 20 participates in the dilatation of blood vessels and suppression of platelet aggregation, whose abnormal expression on the CPAs may also contribute to the pathogenesis of PE[19]. We can speculate that any factor affecting the function of CPAs can in turn influence the placental blood supply.

2 Reduction of Oxidative Stress

Failed transformation of uterine spiral arteries can result in ischemia-reperfusion injury, then contributing to excessive oxidative stress[20]. Many researchers think that enhanced oxidative stress can lead to apoptosis of the syncytium and subsequent release of inflammatory cytokines and anti-angiogenic factors. In the circulation of patients with PE, the levels of antioxidants such as vitamin C, vitamin E, β-carotene, glutathione and superoxide dismutase (SOD) are significantly lower than normal, while lipid peroxidation higher[21]. Strategies were therefore proposed to target placental mitochondrial dysfunction and oxidative stress to improve placental health. In reduced uterine perfusion pressure (RUPP) rat model, treatment with mitochondrial-specific antioxidants (MitoQ/MitoTEMPO) can improve the electron transport chain activity and attenuate elevated blood pressure with improvement in fetal outcomes[22]. When pregnant rats were exposed to hypoxia (11% O_2) from gestational day 15–21, MitoQ loaded onto nanoparticles could act as an intervention to prevent long-term negative cardiovascular outcomes of the offspring in this suboptimal uterine environment[23]. Melatonin is a powerful endogenous antioxidant, and the decreased expression of melatonin receptor in placental tissues of PE patients has been reported[24]. Researchers found the protective effects of melatonin on lipopolysaccharide-induced intra-uterine fetal death and intra-uterine growth retardation in mice via counteracting the oxidative stress effectively[25]. Resveratrol, a polyphenol found in a number of plants, has also been recognized as an antioxidant. *In vitro* human term placental explants and umbilical vein endothelial cells (HUVECs) experiments indicated that resveratrol could reduce placental oxidative stress and production of anti-angiogenic factors and/or improve endothelial dysfunction, partly via activating gene transcription of a variety of cytoprotective enzymes mediated by nuclear factor erythroid 2-related factor-2 (Nrf2)[26]. *In vivo* experiments showed resveratrol supplementation could increase uterine artery blood flow velocity and pup growth in catechol-O-methyltransferase knockout mice and no evidence of teratogenesis was found[27]. Recently, researchers explored the role of reactive oxygen species (ROS) by genetically modifying the Keap1-Nrf2 pathway, a cellular antioxidant defense system, in a mouse model of renin-angiotensin system-induced PE, and found ROS-mediated signaling was indispensable for maintaining placental angiogenesis[28]. It can be seen that the placenta is a dynamically developing organ,

with oxidative stress taking critical roles at different developmental stages. When exploring the therapeutic effect of antioxidants on PE, appropriate timing of therapy initiation should be taken into account.

3 Balancing of Pro-angiogenic and Anti-angiogenic Factors

Anti-angiogenic factors such as soluble fms like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) are involved in the pathogenesis of PE by antagonizing and reducing bioavailable VEGF and placental growth factor (PlGF)[29]. *In vitro* co-culture experiments of human first-trimester extravillous trophoblasts (TEV-1) and HUVEC showed that under low oxygen condition trophoblast-derived sFlt-1 could impair the endothelial dysfunction, including endothelial monolayer barrier function and NO secretion capacity[30]. Pregnant rats injected with adenovirus vectors encoding the sFlt-1 gene (Ad-sFlt-1) showed decreased circulating levels of free VEGF and PlGF and PE-like syndrome including hypertension, proteinuria and glomerular endotheliosis[29]. Therefore, some researchers explored the therapeutic effect of blocking sFlt-1 in PE mice model. In a rat model induced by TNF-α infusion, poly-amidoamine loaded with siRNA-sFlt1 (siRNA-sFlt1-PAMAM) effectively decreased sFlt1 secretion and enhanced the weight of fetuses and placentas compared to rats treated with TNF-α alone[31]. Administration of purified recombinant human PlGF could also lower blood pressure and prevent the reductions in glomerular filtration rate in RUPP pregnant rats[32]. Statins have been demonstrated to inhibit the placental release of sFlt-1 and sEng, while up-regulate the eNOS expression, but they are currently classified into Category X of the FDA's pregnancy categories[33, 34]. A large number of preclinical experiments are needed to verify their safety and efficacy.

4 Balancing of Vasoactive Factors

Numerous studies have shown that endothelin-1 (ET-1) system is involved in the pathogenesis of PE, evidenced by higher levels of ET-1 in the circulation and placenta of PE patients[35, 36]. ET-1 activates endothelin receptor type-A (ET_AR) and type-B (ETBR). ET_AR is mainly expressed in vascular smooth muscle to induce vasoconstriction, while ET_BR is predominately expressed in endothelial cells to induce vasodilation via the release of vasodilator substances (NO and prostacyclin). ET_AR antagonist could abolish the hypertension in response to TNF-α and reductions in uteroplacental perfusion pressure in the pregnant rats[37]. But several studies reported that disruptions of ET-1 expression in pregnant rats resulted in abnormal fetal development and growth, thus indicating that exploring the best time to use ET_AR antagonist and reducing the fetal drug exposure is necessary[38]. In pregnant RUPP rats, the expression and activity of ET_BR in mesenteric microvessels were downregulated, which might compromise ET_BR-mediated NO signaling pathway[39]. The author speculated that ET_BR expression/activity may be affected by the vasoactive factors released from the ischemic placenta, which deserves further studies.

Recent studies have shown plasma from PE patients contains angiotensin receptor type-1 agonistic antibodies, commonly termed AT1-AA, and its titer was proportional to the level of sFlt-1 and the severity of the disease[40]. AT1-AA binds to and activates AT1 receptors on a variety of cell types (trophoblast cells, endothelial cells, mesangial cells and vascular smooth muscle cells), leading to impaired trophoblast invasion, abnormal contraction of smooth muscle cells and so on[41]. Studies have reported that AT1-AA derived from PE patients caused elevated levels of sFlt-1 and hypertension in pregnant mice[42]. While angiotensin receptor blockers (such as losartan) can prevent the binding of AT1-AA to AT1 receptors, such drugs having a known toxic effect on the fetus. In pregnant RUPP rats, B cells were depleted by rutiximab infusion, as a mechanism to suppress endogenous AT1-AA generation[43]. The results showed that this method decreased AT1-AA concentration, in turn blunted increases in blood pressure and ET-1 activation in response to placental ischemia. However, inhibition of B cells would have a wide range of effects, and its safety and efficacy need further investigation. A newly constructed, modified AT1-AA inhibitory peptide ('n7AAc') could target specifically against the AT1-AA and thereby keep it from binding to AT1 receptor[44]. In pregnant RUPP rats, 'n7AAc' significantly decreased sFlt-1, ET-1, and ROS levels, and improved blood pressure and renal function.

5 Modulation of Inflammation/Immunity

Immune dysfunction is closely related to the occurrence and development of PE[45]. The placenta in early pregnancy contains a large number of immune cells, including natural killer cells and macrophages, as well as a small number of dendritic cells and mast cells[46]. They not only have immune regulatory function, but also participate in the regulation of trophoblast invasion, angiogenesis and spiral artery remodeling[47]. Among them, TNF-α and $CD4^+$ T cells have been widely studied. Previous studies have demonstrated that TNF-α is elevated in PE patients and pregnant rats with RUPP[48]. Administration of a selective TNF-α inhibitor, etanerecept, to pregnant RUPP rats could effectively lower blood pressure and reduce ET-1 concentration, but did not alter pup and

placental weight[49]. Th17 cells and regulatory T cells (Tregs) are subpopulations of $CD4^+$ cells, the former mainly mediates the pro-inflammatory response, while the latter mainly plays an immunosuppressive role. Clinical studies have shown that the imbalance of Th17/Treg cells is implicated in pregnancy complicated by PE[50, 51]. To examine whether or not a reduction of Tregs during early pregnancy would impair uterine artery function, *Foxp3-DTR* mice were injected with diphtheria toxin to induce Tregs depletion[52]. The results showed that acute Tregs depletion in early pregnancy caused increased fetal resorption accompanied by maternal systemic inflammatory response, enhanced uterine artery resistance and ET-1 concentration which were measured at midgestation. One study investigated whether or not supplementation of Tregs derived from normal pregnant rats into RUPP pregnant rats before placental insult could attenuate the pathology associated with placental ischemia[53]. The results showed that restoration of the Tregs lowered blood pressure, normalized tissue oxidative stress, and blunted inflammation and ET-1 expression in response to placental ischemia. Another study demonstrated that supplementation of anti-inflammatory cytokine IL-10 also increased Tregs and decreased hypertension in the RUPP rat model[54].

6 Evolving Technologies for Placental Research

In addition to the genome editing strategies to generate animal models, evolving technologies for separation of/analyzing extracellular vesicles have also attracted the researchers' attention. Placenta appears to release a complex repertoire of extracellular vesicles whose cargo (proteins, DNA, mRNA transcripts, microRNAs, noncoding RNA, and other molecules) may have effects on numerous maternal cells, tissues and organs[55]. Plasma, conditioned medium from placenta explants and perfusate of the placenta are commonly used for vesicles separation. Placenta-derived exosomes and microvesicles from PE patients showed functional eNOS whose activity was reduced compared to normal pregnancies[56]. Recently, one study reported[57] that specific miRNAs cargos (miR-1269b and miR-525-5p) in maternal plasma exosomes obtained at the first trimester from the women who developed PE later were involved in embryo implantation process and inflammatory response, suggesting that changes in functional properties of exosomes may be potential biomarkers for early diagnosis or even therapeutic targets.

To minimize or eliminate placental drug/gene transportation and fetal exposure, several novel methods of drug/gene delivery and targeting during pregnancy have been developed. This may address the concerns about impact of pharmaceutical interventions on developing babies and potential long-term deleterious effects. In addition to various viral vectors[58], several new carrier systems have been developed. Targeting ligands such as antibodies, peptides, or small molecules can be conjugated or adsorbed onto the surface of the vectors (such as nanoparticles and liposomes) to promote the accumulation of the particles in the placenta, thus potentiating the therapeutic effects of drugs or genes and reducing any untoward effects for either the mother or fetus. Recently, a research team synthesized a placental chondroitin sulfate A-binding peptide (plCSA-BP). When conjugated onto lipid-polymer nanoparticles, plCSA-BP specifically and efficiently directed the payloads to the mouse placenta[59]. Another study[60] demonstrated that arginine-glycine-aspartic acid peptide modified PEGylated cationic liposome also efficiently delivered H19x siRNA to the placenta.

7 Conclusion

In order to promote the transformation of basic research results, we elaborate on the potential therapeutic targets for PE. Given the inconsistency of drug specificity, dosage, mode of delivery, timing and length of exposure, and animal models in different studies, conclusive evidence of benefit or no effect is hard to draw. When exploring the treatment for placental insufficiency, minimization of risk to both mother and fetus has to be the priority. Last but not least, it makes more sense to establish a reliable risk prediction model and prevention measure for PE.

Conflict of Interest Statement

The authors declare no conflict of interest.

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