Molecular Determinants of Microvascular Dysfunction in Hypertensive Pregnancy and Preeclampsia

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Abstract

Preeclampsia is a pregnancy-related disorder characterized by hypertension and often fetal intrauterine growth restriction, but the underlying mechanisms are unclear. Defective placentation and apoptosis of invasive cytotrophoblasts cause inadequate remodeling of spiral arteries, placental ischemia and reduced uterine perfusion pressure (RUPP). RUPP causes imbalance between the anti-angiogenic factors soluble fms-like tyrosine kinase-1 and soluble endoglin and the pro-angiogenic vascular endothelial growth factor and placental growth factor, and stimulates the release of proinflammatory cytokines, hypoxia-inducible factor, reactive oxygen species, and angiotensin AT_1 receptor agonistic autoantibodies. These circulating factors target the vascular endothelium, smooth muscle and various components of the extracellular matrix. Generalized endotheliosis in systemic, renal, cerebral and hepatic vessels causes decreases in endothelium-derived vasodilators such as nitric oxide, prostacyclin and hyperpolarization factor, and increases in vasoconstrictors such as endothelin-1 and thromboxane A2. Enhanced mechanisms of vascular smooth muscle contraction such as intracellular Ca^{2+}, protein kinase C and Rho-kinase cause further increases in vasoconstriction. Changes in matrix metalloproteinases and extracellular matrix cause inadequate vascular remodeling and increased arterial stiffening, leading to further increases in vascular resistance and hypertension. Therapeutic options are currently limited, but understanding the molecular determinants of microvascular dysfunction could help in the design of new approaches for the prediction and management of preeclampsia.

Keywords

Cytokines; Endothelium; Extracellular Matrix; Growth Factors; Hypertension; Microvessels; Placental Ischemia; Vascular Smooth Muscle

INTRODUCTION

Normal pregnancy is associated with several uteroplacental and hemodynamic changes in order to meet the metabolic demands of the growing fetus. Placental remodeling and trophoblast invasion of spiral arteries maintain adequate uteroplacental perfusion.[1] Also, the

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CONFLICT OF INTEREST

None

increases in maternal plasma volume and cardiac output are counterbalanced by systemic vasodilation and decreased vascular resistance, leading to slight decrease in blood pressure (BP).[2,3] These pregnancy-related changes involve marked uteroplacental and vascular remodeling and redistribution of blood flow in different maternal tissues.[4] Matrix metalloproteinases (MMPs) are major regulators of tissue remodeling, and play an important role in the uteroplacental and vascular remodeling during pregnancy.

In 5 to 8% of pregnancies, women may have hypertension in pregnancy (HTN-Preg) in one of four forms: chronic HTN that predates pregnancy, preeclampsia (PE)-eclampsia, chronic HTN with superimposed PE, and nonproteinuric gestational HTN.[5] PE is diagnosed after the 20th week of pregnancy by new onset HTN, and occasional proteinuria, edema, increased platelet aggregation, and fetal intrauterine growth restriction (IUGR).[5] PE may be a part of hemolysis elevated liver enzymes low platelets (HELLP) syndrome. If not treated, PE may progress to eclampsia with severe HTN and convulsions, and could culminate into coma and death, causing an estimated 14% of pregnancy-related maternal deaths.[6]

Although PE is a major cause of maternal and fetal morbidity and mortality, its etiology and pathophysiology are not fully understood. Certain genetic and environmental risk factors are believed to cause inadequate placentation and reduction in uteroplacental perfusion pressure (RUPP), and the resulting placental ischemia/hypoxia causes the release of circulating factors that target the blood vessels (Fig. 1). Because of the difficulty to perform mechanistic studies in pregnant women, animal models of HTN-Preg have been developed. RUPP during late pregnancy in sheep, dog, rabbit and rat has been shown to induce a hypertensive state that closely resembles PE.[7,8] BP is increased, and the litter size and pup weight are decreased in RUPP versus normal pregnant rats.[9,10] Studies have also shown changes in circulating levels of pro-angiogenic and anti-angiogenic factors, proinflammatory cytokines, hypoxia-inducible factor, reactive oxygen species and angiotensin II (AngII) type 1 receptor (AT_1R) agonistic autoantibodies (AT_1-AA) in PE. These circulating factors are believed to target endothelium-derived relaxing and contracting factors and the mechanisms of vascular smooth muscle (VSM) contraction leading to increased vasoconstriction. Also, abnormalities in MMPs could affect extracellular matrix (ECM) and lead to inadequate vascular remodeling. Changes in systemic vessels cause generalized vascular dysfunction and HTN, while changes in renal glomeruli cause glomerular endotheliosis, increased glomerular permeability and proteinuria. Also, changes in cerebral vessels cause cerebral edema and seizures, while changes in hepatic vessels could lead to HELLP syndrome.[5,11]

In this review, we will discuss the possible initiating events leading to RUPP, and the various circulating factors released in response to placental ischemia. We will then discuss the different molecular targets in the vascular endothelium, VSM and ECM that could lead to microvascular dysfunction, decreased vascular relaxation, increased vasoconstriction, aberrant vascular remodeling, and HTN. Throughout the review we will briefly define the factor involved, and describe the levels during normal pregnancy followed by the changes in human PE and experimental HTN-Preg. We will then discuss how identifying the molecular determinant of microvascular dysfunction could help design new approaches in the prediction and management of PE.

Defective Placentation and Uteroplacental Ischemia in PE

During early pregnancy, the placenta is developed as a maternal-fetal interface through several biological processes including vasculogenesis, angiogenesis, and trophoblast invasion and remodeling of spiral arteries. Vasculogenesis is the development of *de novo* blood vessels from endothelial progenitor cells and occurs ~18–35 days after conception in humans. Angiogenesis is the sprouting of new blood vessels from preexisting vessels and is regulated by the coordinated actions of pro-angiogenic factors and the invasive capability of trophoblast cells.[12] Healthy pregnancy requires adequate placental vascularization. During the first trimester, the placental extravillous trophoblasts invade deep into the maternal decidua up to one-third of the myometrium, progressively invading the spiral arteries, replacing endothelial cells and VSM, and substituting the elastic tissue with fibrinoid material.[13] This causes gradual dilation and transformation of the spiral arteries from low-capacity high-resistance to high-capacity low-resistance vessels, thus ensuring sufficient blood and nutrient supply to the developing fetus.

The symptoms of PE remit after delivery of the baby and placenta, implicating the placenta as a central culprit in the disorder. Defective placentation, RUPP and placental ischemia/hypoxia are important initiating events in PE.[8,14] Predisposing genetic, demographic and environmental factors could affect placental development. Defective placentation could be caused by abnormal immune responses and accumulation of natural killer (NK) cells and macrophages, apoptosis of cytotrophoblasts, and abnormal expression of integrins and MMPs, leading to shallow trophoblast invasion and poor remodeling of spiral arteries.

Predisposing genetic, demographic and environmental factors in PE

Mutations in placental genes have been associated with PE, and 31 out of 36 placental genes are downregulated in PE.[15] Susceptibility genes include *ACVR2A* gene on chromosome 2q22 and *STOX1* gene on chromosome 10q22. *STOX1* Y153H polymorphism has been linked to inadequate trophoblast invasion and IUGR, and was detected in families with several generations of women who developed early and severe PE.[16] Also, wild-type female mice crossed with transgenic male mice overexpressing human *STOX1* show PE features including HTN and proteinuria.[17] *FOXP3* is another gene that plays a role in the activation of regulatory T cells (Tregs) and controls the immune response and maternal tolerance during pregnancy. Downregulation or polymorphism in *FOXP3* gene could alter the maternal immune response, reduce maternal tolerance and predispose to PE.[18,19]

Ethnic background, age, maternal lifestyle, pre-pregnancy weight, previous and family history of PE, primiparity, and multiple pregnancy could be risk factors for PE.[20] The rate of PE is higher among African-American (5.2%) than Asian women (3.5%).[21] Very young <16 years or older women >40 years are more prone to PE, and studies in Finland and India support that older women are at higher risk of developing PE.[22,23] The incidence of PE is ~3% in women with normal body mass index (BMI, 18.5–24.9), but increases to 7% in overweight women with BMI 30–34.9 and to 13% in obese women with BMI around 50.[24] Preexisting medical condition such as heart disease, chronic respiratory disorders, diabetes, renal disorders, systemic lupus erythematosus, mental stress, reproductive tract surgery and history of antepartum hemorrhage also increase the risk for PE.[20] The relationship between

age, ethnic background, obesity and other predisposing factors and placental development in the setting of PE should be an important area of investigation.

Immune Responses and Defective Placentation in PE—Pregnancy is a physiological process that poses a challenge to maternal tolerance and the immune response. For healthy pregnancy, the maternal systems must tolerate the semi-allogenic fetus, and likewise, the fetus needs to be protected from rejection by the maternal immune response.[25] PE is associated with augmented immune response and increased pro-inflammatory cytokines TNFα and IL-6. In support, HIV-positive women, who often have suppressed immune response, show lower incidence of hypertensive disorders and PE.[26]

During normal pregnancy, cytotrophoblasts express the major histocompatibility complex molecules HLA-C, HLA-E and HLA-G which interact with their respective inhibitory receptors KIR, CD 94/NKGs and ILT-2 on NK cells. These interactions reduce the activity of NK cells and prevent them from attacking normal placental and fetal tissues.[27] A decrease in HLA-C/KIR interaction would lead to increased activity of NK cells, which attack placental and fetal tissues and lead to PE.[28] Also, healthy pregnancy is associated with moderate activation of the complement system. Increased complement activation products Bb, C3a and C5a have been associated with PE.[29] Also, small subcutaneous vessels from PE women show more neutrophils adherent to the endothelium, which may contribute to endothelial dysfunction.[30] Inhibition of complement activation or depletion of neutrophils decreases BP in RUPP rat model of placental ischemia, supporting a role of complement activation and innate immune response in HTN-Preg.[29,31]

Integrins and Reduced Trophoblast Invasion of Spiral Arteries—Trophoblast invasion and remodeling of the spiral arteries is in part regulated by integrins and other adhesion molecules. Cytotrophoblasts initially express epithelial cell-type adhesion molecules such as integrins α_6/β_4 and α_6/β_1, and E-cadherin. During normal pregnancy cytotrophoblasts become more invasive, and the epithelial cell-type adhesion molecules are replaced by the endothelial-type integrins α_1/β_1 and α_V/β_3; a process known as vascular mimicry or pseudovasculogenesis.[32] These phenotypic changes in integrins may be impaired during placental hypoxia and PE. Hypoxia increases expression of integrin α5 and fibronectin and decreases expression of integrin α1.[33] Abnormal expression of epithelial cell-type adhesion molecules and apoptosis of cytotrophoblasts cause limited invasion of spiral arteries, placental ischemia and RUPP.[32,34] Ezrin, an integrin involved in cell adhesion, organization and migration, is downregulated in syncytiotrophoblast microvesicles from PE women, resulting in reduced invasiveness of cytotrophoblasts, shallow placentation and decreased vascularization of the placenta.[35] The decreased trophoblast invasion and replacement of vascular cells also leads to retention of VSM cells in the spiral arteries, causing more vasoconstriction and placental ischemia.[36]

Endothelial intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) promote leukocyte migration and adhesion to the endothelium. Also, soluble ICAM-1 (sICAM-1) in the plasma could function as a regulatory molecule of ICAM-1/2-integrin interaction. Endothelial ICAM-1 and VCAM-1 are downregulated during normal pregnancy, thus minimizing leukocyte adhesion to endothelial cells, and

maintaining patency and blood flow in the spiral arteries. In contrast, plasma levels of sICAM-1 and soluble VCAM-1 (sVCAM-1) are increased in PE, indicating endothelial cell dysfunction and increased endothelial ICAM-1 and VCAM-1, which would increase leukocyte adhesion to endothelial cells and restrict blood flow in the spiral arteries.[37,38]

PE is associated with increased placental expression of microRNA miRNA-125b-1–3p which reduces the expression of S1PR1, a G-protein coupled receptor that facilitates invasion of human trophoblasts.[39] PE is also associated with increased expression of placental miRNA-517a/b and miRNA-517c, which may contribute to the decreased trophoblast invasion of extravillous trophoblasts under hypoxic conditions.[40]

MMPs, Abnormal Placentation, and Placental Ischemia

MMPs are zinc-dependent proteases that play a role in tissue remodeling.[41,42] MMPs include collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other MMPs.[43] MMPs play a role in endometrial tissue remodeling during the estrous cycle and menstrual cycle, and the uterine and vascular remodeling during pregnancy.[44,45] Trophoblast invasion into the decidual stroma requires degradation of ECM proteins by MMPs. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are abundantly expressed in invading extravillous trophoblasts.[46,47] Also, epidermal growth factor (EGF)–mediated trophoblast invasion is associated with increased expression of MMP-2 and MMP-9.[48] MMP-2 is the main MMP in the umbilical cord[5], and serum MMP-9 level is elevated in normal pregnancy.[41] The pregnancy-related increase in MMPs may be caused by estrogen and progesterone. Estrogen enhances the release of MMP-2 from human VSM cells.[49] Also, MMP-2 and MMP-9 are increased in the uterus and aorta of pregnant rats, and estrogen and progesterone enhance MMP-2 and MMP-9 expression in the aorta of virgin rats.[50]

The role of MMPs in trophoblast invasion of spiral arteries is supported by the observation that in first trimester trophoblasts, suppression of MMP-9 inhibits the invasive capability of trophoblasts.[51] Also, MMP-9 ablation in MMP-9 knockout mice shows a phenotype that mimics PE possibly due to impaired trophoblast invasion.[52] Genetic polymorphisms in MMP-2 and MMP-9 transcription have been described in PE, and decreased MMP-9 levels have been observed in PE compared with normal placenta.[53] In PE, increased expression of miRNA-519d-3p and miRNA-204 could target MMP-2 and MMP-9 and decrease trophoblast invasion of spiral arteries.[51,54] These observations suggest a relationship between decreased MMP-2 and MMP-9 and impaired trophoblast invasion in PE. However, measurements of plasma MMP levels have not been consistent in PE, with some studies showing an increase in MMP-2 and MMP-9,[55] while other studies showing a decrease in MMP-9,[41] making it important to further measure MMPs in the plasma, placenta and other tissues of PE women and animal models of HTN-Preg.

We have examined whether alteration of MMP expression/activity is a potential mechanism in the uteroplacental and vascular remodeling in animal models of HTN-Preg. We examined the specific changes in three important tissues during pregnancy; the uterus which undergoes remodeling to accommodate the growing fetus, the placenta which provides nutrient supply to the developing fetus, and the aorta for the vascular changes in the maternal circulation. The uterus, placenta, and aortic tissue weight was reduced in RUPP versus normal pregnant

rats. Also, histological morphometry showed reduction in uterine, placental and aortic cross-sectional area in RUPP versus normal pregnant rats, supporting growth-restrictive remodeling in RUPP rats.[42] Western blots, gelatin zymography and immunohistochemistry revealed abundant MMP-2 and MMP-9 in tissues of normal pregnant rats.[50] MMPs immunostaining was prominent in the aortic media, consistent with reports that VSMCs are a major source of MMPs.[56,57] The levels of MMP-2 and MMP-9 were reduced in uterus, placenta and aorta of RUPP versus normal pregnant rats. The decreases in MMP levels, in parallel with the decreases in uterine, placental, and aortic tissue weight and cross-sectional area suggest a role for reduced MMP-2 and MMP-9 in growth-restrictive remodeling in tissues of RUPP rats.[42]

Other MMPs may also be involved in uterine and vascular remodeling in PE. The collagenase MMP-1 is expressed in cytotrophoblasts and syncytiotrophoblasts of the placenta and decidua and may play a role in trophoblast invasion. Some studies have shown low levels of MMP-1 in umbilical cord blood, placenta and decidua of PE versus normal pregnant women.[58] Other studies suggest a role of MMP-1 in the pathogenesis of PE.[59] Also, the matrilysin MMP-7 could play a role in endometrial tissue remodeling during the menstrual cycle and pregnancy.[60] Cytotrophoblasts and VSM also release MMP-12, which could mediate elastolysis and remodeling of spiral arteries.[61] Also, some MMPs cleave other pro-MMPs, and MT1-MMP is a key activator of proMMP-2.[62] MMP activity could also be influenced by tissue inhibitors of metalloproteases (TIMPs) and other MMP modulators.[62] For instance, TIMP-2 or specific MMP-2 blocking antibody inhibits cytotrophoblast invasion *in vitro*.[47] Also extracellular MMP inducer (EMMPRIN, CD147, Basigin) is a widely expressed membrane protein of the immunoglobulin superfamily,[63] that has been implicated in tissue remodeling, cancer, and heart failure.[50] EMMPRIN stimulates the production of MMP-1, MMP-2, MMP-3, and MMP-9, and regulates MMPs in endothelial cells and tumors.[64] We have shown that EMMPRIN expression is increased in the aorta of pregnant compared with virgin rats as well as in the aorta of virgin rats treated with estrogen and progesterone. Also, the sex hormone-induced increases in aortic MMP-2 and MMP-9 were blocked by EMMPRIN neutralizing antibody, supporting a role of EMMPRIN in the increases in vascular MMPs during pregnancy.[50]

Circulating Bioactive Factors in PE

Placental hypoxia/ischemia is believed to trigger the release of several bioactive factors including the antiangiogenic factors sFlt-1 and sEng, pro-inflammatory cytokines such as TNFα and IL-6, HIF, ROS and AT_1-AA (Fig. 1).[65–68] These factors could target uteroplacental and vascular MMPs causing further vasoconstriction of spiral arteries and placental ischemia, as well as the vascular endothelium, VSM and ECM in the systemic circulation leading to generalized vasoconstriction and HTN-Preg.[11]

Pro-angiogenic and Anti-angiogenic Factors in PE

Vascular Endothelial Growth Factor (VEGF)—The *VEGF* gene is located on chromosome 6p21.3, and consists of 8 exons involved in the expression of a family of growth factors including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF.[69] VEGF-A, VEGF-B and PlGF bind to tyrosine kinase receptor Flt-1 (VEGFR-1). VEGF-A binds to

VEGFR-2 (Flk-1 or KDR) to promote the development of the placental vasculature.[69] VEGF regulates endothelial cell proliferation, angiogenesis and vascular permeability.[69] In endothelial cells, VEGF increases $[Ca^{2+}]_i$, Ca^{2+}/calmodulin, endothelial nitric oxide synthase (eNOS) activity, and prostacyclin (PGI_2).[70,71] VEGF also stimulates Ca^{2+}-independent generation of NO by promoting Akt activation and eNOS Ser^{1177} phosphorylation in human umbilical vein endothelial cells (HUVECs).[72]

Some studies show an increase in circulating VEGF in PE.[73–75] Also, villous explants from PE women produce greater amounts of VEGF than those form normal pregnant women.[76] It is likely that the severe vasoconstriction in PE would increase vascular shear-stress, and in turn increase circulating VEGF.[20] Other studies have shown a decrease or unchanged serum levels of VEGF in PE.[77,78] Women with the T allele of VEGF 936C/T have lower levels of VEGF and a higher risk of PE than women with VEGF 936C/C.[79] Plasma VEGF levels are decreased in RUPP rat model of HTN-Preg,[8] although as with human villous explants, placenta from RUPP rats show greater VEGF production.[80] The differences in the results may be related to the method of VEGF measurement.

A decrease in VEGF may also play a role in the glomerular endotheliosis and proteinuria in PE. VEGF is synthesized constitutively by podocytes in the glomerulus where it maintains endothelial cell health and induces the formation of fenestrae. Endotheliosis and loss of fenestrae have been detected in genetic glomerular VEGF deficiency.[81] Also, in clinical cancer trials the use of VEGF neutralizing antibodies is associated with proteinuria.[82] In mice, infusion of VEGF antibodies leads to glomerular endotheliosis and proteinuria.[83] Also, mice lacking one VEGF allele in renal podocytes develop a renal pathology similar to that in PE. Importantly, infusion of VEGF ameliorates the renal lesions, glomerulonephritis and thrombotic microangiopathy in RUPP rats, suggesting potential benefits of pro-angiogenic factors in the glomerular endotheliosis associated with HTN-Preg.[84,85]

MMPs induce the release of growth factors by cleaving the growth factor-binding proteins or matrix molecules. MMPs may also be regulated by growth factors.[86] MMPs may mediate the angiogenic effects of VEGF by virtue of their proteolytic activity and other mechanisms including helping to detach pericytes from the vessels undergoing angiogenesis, releasing ECM-bound angiogenic growth factors, exposing cryptic pro-angiogenic integrin binding sites in ECM, generating pro-migratory ECM component fragments, and cleaving endothelial cell-cell adhesions.[87] Interestingly, VEGF increases the expression of MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, and MMP-19 in HUVECs, and induces MMP-10 expression via PI_3K and MAPK pathways.[88] The interaction between MMPs and VEGF in the setting of uteroplacental and vascular remodeling in normal pregnancy and PE should be further examined.

Placental Growth Factor (PlGF)—PlGF is a pro-angiogenic factor that binds to VEGFR-1 and enhances the angiogenic effects of VEGF.[89] PlGF has only 1/10th the affinity of VEGF for VEGFR-1, but its levels are ~40 times higher than those of VEGF during normal pregnancy. PIGF promotes endothelial cell growth, placental vasculogenesis, and vasodilation of uterine vessels.[11]

Plasma PlGF levels are low in non-pregnant women (~44 pg/mL), and markedly increase during normal pregnancy.[89] PlGF levels are ~353 pg/mL during gestational weeks 21 and 22, rising steadily to ~574 pg/mL after gestational weeks 29 and 30.[90] Circulating PlGF levels decrease in PE,[73,91] and the decrease is more apparent in early than late PE.[92] PlGF has four alternatively spliced mRNA forms (PlGF 1–4), and its predominant isoform PlGF-1 is downregulated in PE.[93] Circulating levels of PlGF are also decreased in RUPP and deoxycorticosterone acetate (DOCA)-salt hypertensive rats.[8,94]

In addition to its growth promoting effects, PlGF promotes vasodilation via VEGFR-1 and endothelium-derived hyperpolarizing factor (EDHF)-mediated activation of small conductance Ca^{2+}-activated K^+ channels (SK_{Ca}).[95,96] In small mesenteric arteries of pregnant rats treated with L-NAME and indomethacin, a second exposure to PlGF produces greater vasodilation and greater reduction in VSM $[Ca^{2+}]_i$ than the first PlGF application. VEGF and PlGF may promote VEGFR-1 dimerization, and the initial exposure to PlGF may facilitate the formation of receptor homodimers and their submembrane signaling, leading to augmented vasodilator responses to repeated PlGF stimulation.[96] A decrease in the levels of PlGF may be partly responsible for the decreased vasodilator responses in PE.

Soluble fms-like Tyrosine Kinase-1 (sFlt-1)—sFlt-1 (sVEGFR-1) is an anti-angiogenic factor expressed as an alternatively spliced variant of VEGFR-1 that lacks both the transmembrane and cytoplasmic domains. sFlt-1 binds VEGF and PlGF and blocks their angiogenic effects on VEGFR. sFlt-1 may also form a heterodimer with the surface membrane VEGFR-1 and inhibit its post-receptor signaling actions.[97] Trophoblasts express sFlt-1 mRNA. sFlt-1 levels are ~0.15 ng/mL in non-pregnant women and increase to ~1.5 ng/mL in normal pregnant women.[11] sFlt-1 levels are largely stable in normal pregnant women, and show an increase after gestational week 36. Throughout the third trimester, an increase in sFlt-1 is associated with some reduction in VEGF and PlGF levels. PE women show imbalance between sFlt-1, VEGF and PlGF.[77,92] The *sFlt-1* gene has a gene locus on chromosome 13q12. In women with trisomy 13, an extra copy of the *sFlt-1* gene is associated with increased circulating sFlt-1, reduced PlGF and increased risk of PE.[98] Studies have shown higher circulating levels of sFlt-1 in early and late PE.[91,92] Serum sFlt-1 is also higher in women with previous PE (~0.5 ng/mL) than in women with previous normal pregnancy (~0.3 ng/mL), and the increases can be detected even 6 months after delivery.[99] sFlt-1 levels are also greater in villous explants from PE compared with normal pregnant women.[76]

Placental ischemia/hypoxia may trigger the production of sFlt-1. During placental hypoxia, HIF-1 may bind to the promoter region of *flt-1* gene leading to upregulation of sFlt-1.[76,77] In extravillous trophoblasts, overexpression of miR-517a/b and miR-517c increase the expression of TNFSF15, a cytokine that promotes Flt-1 splicing, and increases the production of sFlt-1.[40] sFlt-1 e15a, a splice variant of sFlt-1 and the most abundant form released by the placenta, binds VEGF and in turn decreases endothelial cell migration, invasion, and tube formation. sFlt-1 e15a is expressed in syncytiotrophoblasts and its serum levels are 10-fold higher in PE than normal pregnant women.[100]

Because of the increased levels of sFlt-1, a 53% decrease in VEGF/sFlt-1 ratio and a 70% decrease in PlGF/sFlt-1 ratio have been observed in PE placenta.[76] The circulating sFlt-1/PlGF ratio is higher in PE than normal pregnant women from second trimester onwards and may serve as a predictor of the onset of PE,[92] However, some studies suggest that the circulating sFlt-1/PlGF ratio could be lower in late versus early PE.[92,101] Circulating sFlt-1 levels and sFlt-1/PlGF ratio are higher in twin than singleton pregnancies, and the difference is likely related to the greater placental mass in twin pregnancies.[102,103] The proportionate increases in sFlt-1 and sFlt-1/PlGF ratio in twin versus singleton pregnancies support the concept that the placenta is a major source of these factors. Angiogenic imbalance may affect endothelin-1 (ET-1) levels. PE women with sFlt-1/PlGF ratio >85 have higher levels of ET-1 than women with sFlt-1/PlGF ratio <85.[104] Importantly, extracorporeal removal of circulating sFlt-1 from PE patients decreases sFlt-1/PlGF ratio, improves symptoms and prolongs pregnancy,[105] further supporting a role of sFlt-1 in PE.

RUPP rats show increases in plasma and placental levels of sFlt-1 and plasma sFlt-1/PlGF ratio.[8,106] Other animal models of HTN-Preg show either increased or little change in circulating sFlt-1 levels.[94,107–111] Importantly, Infusion of exogenous sFlt-1 or adenoviral overexpression of sFlt-1 in pregnant rats causes increases in BP, decreased plasma VEGF, proteinuria, and glomerular endotheliosis with occlusion of renal capillaries and focal fibrin deposition in glomerular cells.[77,112] Also, mice treated with sFlt-1 show increased vascular response to ET-1.[113] Treatment of endothelial cells with plasma of PE patients decreases angiogenesis, and removal of sFlt-1 or treatment with VEGF or a sFlt-1 antibody restores endothelial cell angiogenesis.[76]

Of note, VEGF through an action on VEGFR-2 stimulates the production of sFlt-1 in human placental explants.[114] This feedback modulation of VEGF by sFlt-1 may represent a local protective mechanism at the maternal-fetal interface to control VEGF levels and prevent damage to the placenta or fetus by excess VEGF during normal pregnancy,[114] and this VEGF-sFlt-1 feedback mechanism may be altered in PE.

Some studies suggest that sFlt-1-induced inhibition of VEGFR-2 could decrease endothelial VEGF production and MMP-2 and MMP-9 expression/activity.[115] In mouse model of abdominal aortic aneurysm treatment with sFlt-1 reduces aneurysm size and attenuates MMP-2 and MMP-9 activity in peri-aortic tissue.[116] Also, our recent studies have supported a role of sFlt-1 as a potential upstream mechanism to decrease MMPs in HTN-Preg.[42] We found that sFlt-1 reduced MMPs in uterine, placental and vascular tissues of normal pregnant rats, while VEGF reversed the sFlt-1 induced decreases in MMPs in tissues of normal pregnant rats and increased MMPs levels in tissues of RUPP rats to levels similar to those in normal pregnant rats. These observations are consistent with reports that infusion of VEGF reduces BP in RUPP rats.[117]

Soluble Endoglin (sEng)—Transforming growth factor-β1 (TGF-β1) binds to TGF receptor and induces proliferation and migration of endothelial cells.[69] Endoglin (Eng) is a co-receptor for TGF-β1 and TGF-β3 that is highly expressed on cell membrane of endothelial cells and syncytiotrophoblasts, where it mediates proliferation of angiogenic endothelial cells and trophoblasts.[118] Mutations in *Eng* gene are associated with loss of

capillaries, arterio-venous malformations, and hereditary hemorrhagic telangiectasia.[119] sEng is an anti-angiogenic protein that binds TGF-β1 and prevents it from binding to its natural angiogenic receptor, and thereby inhibits TGF-β1-induced eNOS activation and vasodilation.[69] Hypoxia induces the release of sEng. In placental extracts, exposure to hypoxia increases the expression of sEng.[120]

Serum levels of sEng are barely detectable in non-pregnant women and are much lower in normal pregnant women.[121] The levels of sEng are 3-, 5- and 10-fold higher in women with mild PE, severe PE and HELLP syndrome, respectively, compared with gestational age–matched control pregnant women.[121] Serum levels of sEng may be increased in early and late PE.[122,123] However, one study showed an increase in sEng levels at gestational weeks 10–17 in women who developed early PE, but not in those who developed late PE.[101]

In RUPP rat model of HTN-Preg, sEng levels are increased in the serum and placenta, and serum TGF-β levels are decreased.[67] However, sEng levels did not show detectable change in DOCA-salt or L-NAME treated rat models of HTN-Preg.[94,107] It is likely that sEng acts in concert with sFlt-1 to aggravate vascular permeability, proteinuria, IUGR and HTN.[121] In support, pregnant rats infused with both sEng and sFlt-1 show HELLP syndrome-like characteristics.[124] In cultured HUVECs, sEng impairs endothelial formation.[121] Whether sEng targets MMPs and affects uteroplacental and vascular remodeling in HTN-Preg is unclear. Of note, MMP-14 cleaves Eng, the TGF-β co-receptor, and inhibits its angiogenic effects,[125] and these effects may play a role in PE.

Cytokines, TNFα, and Interleukins

In PE, defective trophoblast invasion and decreased uteroplacental blood flow result in periods of ischemia/reperfusion. During placental reperfusion injury, reestablished blood flow causes the release of the pro-inflammatory cytokines TNFα and interleukins (ILs).[5,11,25,126] The circulating levels of TNFα are greater in PE than normal pregnant women,[127,128] although the placental levels of TNFα may not be different in PE versus normal pregnancy.[129] LIGHT, or TNF superfamily member 14, is also increased in PE and may contribute to placental ischemia.[130] The plasma levels and CD4+T cell production of TNFα are increased in RUPP versus normal pregnant rats.[126,131,132] Infusion of TNFα causes HTN and proteinuria in late pregnant mice, rats, and baboons.[110,111,133] Similarly, infusion of the TNF superfamily member LIGHT in pregnant mice increases BP, proteinuria, and the expression of ET-1 and sFlt-1.[130] TNFα may work in concert with IL-6 to increase ET-1 levels and cause HTN in RUPP rats.[126] TNFα may also function in synergy with sFlt-1 to promote a pro-inflammatory and antiangiogenic state. Treatment of HUVECs with both TNFα and sFlt-1 causes an increase in the adhesion molecules ICAM and VCAM and promotes the release of markers of endothelial dysfunction such as ET-1 and von Willebrand factor.[72] In support of a role of TNFα in HTN-Preg, blockade of TNFα with the TNFα decoy receptor etanercept reduces BP in RUPP rats. Also, treatment of HUVECs with serum from RUPP rats treated with the TNFα blocker etanercept produces less ET-1 than serum from nontreated RUPP rats.[126]

TNFα modulates the immune response. TNF-α increases vascular permeability, fibroblast proliferation and lymphocyte activation, and promotes the production of IL-6 and IL-8.

TNFα downregulates eNOS and mitochondrial biogenesis, leading to mitochondrial dysfunction, oxidative stress and increased ROS production.[134] TNFα also alters the expression of adhesion molecules in placental vessels.[126] and MMP production in PE.[135]

IL-6 is another cytokine that is elevated in PE.[123,127] RUPP rats show increased plasma levels and higher CD4+T cell production of IL-6.[131,132] Chronic infusion of IL-6 in pregnant rats causes HTN, proteinuria,[136] enhanced vascular contraction and reduced endothelium-dependent relaxation.[65] IL-6 promotes dimerization of the surface receptor GP-130 on endothelial cells leading to abnormal cell signaling and vascular dysfunction. IL-6 also increases vascular permeability by disrupting the tight junctions in endothelial cells.[137]

IL-1β could also promote the inflammatory response and disrupt endothelial function in PE. Monocyte production of IL-1β is greater in PE than normal pregnant women.[138]

IL-10 is an anti-inflammatory cytokine whose levels are reduced in the plasma and placenta of PE women and plasma of RUPP rats.[128,129,132] Also, in placental trophoblasts, exposure to hypoxia increases pro-inflammatory cytokines and decreases IL-10.[139]

The source of pro-inflammatory cytokine in PE is mostly in the maternal circulation. Monocytes and macrophages are the main reservoirs of cytokines and are the first cells to be activated in nonspecific immune response.[140] Monocytes produce more TNFα and IL-6 when treated with plasma from PE than normal pregnant women.[140] IL-10 may regulate the monocytes and the inflammatory response during normal pregnancy by controlling TNFα and IL-1β gene expression,[138] and the IL-10-mediated regulatory effects appear to be lost in PE. Interestingly, uric acid stimulates monocytes to release cytokines, and hyperuricemia is often observed in PE patients. Also, monocytes from PE patients with high levels of uric acid produce more TNFα and IL-1β than monocytes from normal pregnant women.[138] MMPs may also promote the release of cytokines in PE.[141]

Hypoxia-Inducible Factor (HIF)

HIF is a transcriptional factor that plays a role in the physiologic responses to hypoxia. HIF-1 is a heterodimer consisting of an oxygen-regulated HIF-1α and HIF-2α subunits and a constitutively expressed HIF-1β subunit. While hypoxia is an important inducer of HIF, *de novo* synthesis of HIF-1α may occur in response to non-hypoxic stimuli such as pro-inflammatory factors. TNFα upregulates HIF-1α mRNA expression.[111] Also, a large number of genes are regulated by HIF-1 including VEGF, leptin, TGF-β3, and NOS. DNA microarray analysis in arterial endothelial cells have shown that more than 2% of human genes are regulated directly or indirectly by HIF-1.[5]

HIF expression increases during pregnancy, likely due to increased estrogen and progesterone. Estrogen stimulates uterine HIF-2α, and progesterone upregulates uterine HIF-1α expression.[142] HIF shows further increase in PE.[143] Circulating HIF-1α levels are increased in PE compared with normal pregnant women.[144] HIF-1α may contribute to the pathogenesis of PE by upregulating the anti-angiogenic factors sFlt-1 and sEng, binding to ET-1 gene and changing ET-1 mRNA expression, reducing the trophoblast invasion capability, and inducing AngII-converting enzyme (ACE) expression in the lungs and kidney

and AngII production.[5,145] In support, HIF-1α increases sFlt-1 in human villous trophoblasts.[146] Placental levels of HIF-1α are elevated in RUPP rat model of HTN-Preg.[67] Also, downregulation of HIF-1α mRNA using siRNA reverses the increases in BP, proteinuria, renal damage and serum levels of sFlt-1 in mice models of HTN-Preg.[146]

In addition to the role of HIF in oxygen homeostasis and its regulation by oxygen,[147] cytokines, hormones, metallic ions and mechanical stretch induce HIF expression.[147,148] Prolonged mechanical stretch increases HIF-1α and HIF-2α mRNA expression and protein levels in skeletal muscle fibers.[149,150] Also, upregulation of HIF-1α has been observed in rat cardiac myocytes, aortic VSM cells and fibroblasts exposed to mechanical stretch.[151–153] HIF-1α mRNA is upregulated in VSM cells subjected to cyclic stretch for 4 hours.[151] Also, HIF-1α protein is increased in fibroblasts subjected to cyclic stretch for 24 hours.[153] The mechanisms via which mechanical stretch upregulate HIF may involve PI_3K and MAPK. [148,149,151] Studies have suggested that HIF regulates the expression of MMP-2 and MMP-9.[154,155] Also, we have shown that mechanical stretch increases HIF-1α expression, and that HIF could increase MMP expression in rat inferior vena cava.[156] Whether HIF functions as a transducing signaling mechanism between vascular mechanical stretch and the expression of MMPs during pregnancy needs to be examined.

Reactive Oxygen Species (ROS)

ROS such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl ion (OH^-) contain highly reactive O_2. Normal pregnancy represents a state of oxidative stress caused by increased maternal metabolism and metabolic activity of the placenta. Although generation of ROS is increased during pregnancy,[157] placental production of ROS is normally counterbalanced by antioxidants.[5] In contrast, in PE, defective placentation and decreased uteroplacental vascularization result in periods of placental ischemia/reperfusion and a hypoxic environment that favors oxidative stress.[134] In PE, the levels of antioxidants may be too low to counterbalance the increased ROS production.[158]

Hemeoxygenase (HO) is the rate-limiting enzyme responsible for degradation of heme to biliverdin, free iron and carbon monoxide (CO) in the endoplasmic reticulum. Biliverdin is rapidly reduced to bilirubin, an antioxidant, by the cytosolic enzyme biliverdin reductase. CO is a potent vasodilator with anti-apoptotic properties. HO has two main isoforms, HO-1 and HO-2. HO-2 is a 36 kDa protein constitutively expressed at high levels in the brain, testis and vascular endothelium. HO-1 is an inducible 32 kDa protein that is widely distributed in the body, with high levels in the liver and spleen. HO-1 is induced by its substrate heme and by heavy metals. Stimuli that cause oxidative stress, such as peroxynitrite, modified lipids, hypoxia, hyperoxia, ischemia/reperfusion, hyperthermia and endotoxic shock, up-regulate HO-1 expression.[159] HO-1 via its products inhibits oxidative stress, inflammation and apoptosis.[160] HO-1 deficiency results in endothelial damage as indicated by elevation of thrombomodulin and von Willebrand factor.[161]

Expression of antioxidant enzymes such as HO-1, HO-2, copper/zinc superoxide dismutase, glutathione peroxidase and catalase is decreased in PE. Also, the total antioxidant capacity is lower in serum of PE than normal pregnant women.[162] ROS/antioxidants imbalance leads to lipid peroxidation, increased thromboxane A2 (TXA_2) and loss of glutathione peroxidase

activity in the placenta.[163] Antioxidant levels were reduced in women who were later diagnosed with early PE,[164] supporting a role of oxidative stress in the pathogenesis of PE. In PE women, reduced brachial artery flow-mediated dilation is associated with decreased plasma levels of the antioxidant ascorbate, and administration of ascorbic acid improves flow-mediated dilation, supporting a relation between oxidative stress and endothelial dysfunction in PE.[165] Also, placental levels of HO-1 are reduced in RUPP compared with normal pregnant rats,[67] supporting a role of ROS in HTN-Preg.

Neutrophils and monocytes are major sources of ROS in PE. Monocytes from PE women produce more H_2O_2 and $O_2^{\bullet-}$ and cause more endothelial cell damage than monocytes from normal pregnant women.[166,167] Neutrophils also produce NO, which can protect cells from the damaging effects of $O_2^{\bullet-}$ during normal pregnancy. However, in PE, excess $O_2^{\bullet-}$ scavenge the NO produced by neutrophils to form peroxynitrite ($ONOO^-$), thus reducing NO bioavailability and causing endothelial cell damage.[167] NADPH oxidase is a membrane-bound enzyme that catalyzes the one-electron reduction of oxygen to $O_2^{\bullet-}$ via NADPH. NADPH oxidase isoform NOX1 is overexpressed in the placenta of PE women.[168] In HUVECs, treatment with serum from PE women increases mRNA expression of the NADPH oxidase subunit gp91^{phox}, and augments $O_2^{\bullet-}$ production.[169] Treatment of HUVECs with PE serum also causes overexpression of iNOS,[169] which produces excess NO and in turn increase ROS and promotes endothelial cell injury. In RUPP rat model of HTN-Preg treatment with iNOS inhibitors decreases BP, aortic levels of ROS and NADPH-dependent production of ROS.[170] Biopterin (BH_4) promotes eNOS dimerization and activity. Hypoxia reduces BH_4 causing eNOS uncoupling, increased ROS production, and decreased NO bioavailability.[171] In DOCA-salt hypertensive rats, supplementation with a BH_4 such as sepiapterin decreases production of $ONOO^-$ and $O_2^{\bullet-}$ and increases NO production.[171]

Other markers of lipid peroxidation and oxidative stress such as malondialdehyde and prostaglandin $F_{2\alpha}$ are increased in serum of PE women at gestational weeks 10–14. This may cause gradual oxidative damage in the placenta, even before overt symptoms of PE.[172] Plasma levels of the oxidative stress marker 8-isoprostane, and total aortic and placental levels of ROS are higher in RUPP than normal pregnant rats.[132,170] In first-trimester villous trophoblasts, excessive oxidative stress affects the expression of miRNAs involved in angiogenesis, apoptosis, immune response and inflammation, and this could be a potential mechanism in PE.[173] MMPs may also contribute to the increases in ROS in PE.[141]

AngII and AT$_1$ Receptor Agonistic Autoantibodies (AT$_1$-AA)

The renin-angiotensin-system (RAS) is involved in the regulation of salt, water, and BP. Normal pregnancy is associated with increased blood volume with little change in BP, raising the possibility that RAS plays a role in PE. In a study to investigate the role of RAS and plasma progesterone levels in HTN-Preg, sequential measurements were made throughout pregnancy in normotensive subjects, HTN-Preg patients in whom HTN became manifest only during pregnancy (PE), and patients with chronic HTN antedating pregnancy. Among the normotensive subjects, plasma renin activity and substrate, and plasma aldosterone and progesterone levels were elevated as early as gestational week 6. Progressive increases were noted in renin substrate, aldosterone and progesterone levels

during pregnancy, but plasma renin activity did not continue to rise. In HTN-Preg groups, plasma levels of renin substrate and progesterone were not different from those in normotensive pregnancy, but plasma renin activity and aldosterone levels were suppressed during the last trimester, likely through secondary effects. RAS suppression did not appear to be due to a decrease in renin substrate levels and a hypothesized deficiency of plasma progesterone was not observed in the hypertensive subjects suggesting that it may not play a permissive role in the development of HTN-Preg.[174] Another prospective longitudinal study explored the relationship between RAS and the development of superimposed PE. In pregnant women with chronic HTN in whom PE did not develop, BP decreased and RAS was stimulated, beginning in the first trimester and continuing throughout pregnancy as in normotensive pregnant women. Plasma estradiol and progesterone levels also increased progressively. In women with chronic HTN in whom PE developed, BP decreased and RAS was stimulated in the first trimester as in the other groups. However, later in pregnancy BP began to rise in the second trimester. Initially RAS remained stimulated, but in the early third trimester, when PE was diagnosed, plasma renin activity and urine aldosterone excretion decreased, and atrial natriuretic factor increased,[175] supporting that RAS is modulated in HTN-Preg and PE.

AngII is an important regulator of water and electrolyte homeostasis and BP. AngII activation of vascular AT_1R promotes vascular growth, inflammation, and vasoconstriction and increases $[Ca^{2+}]_i$ and Rho-kinase activity in VSM. AngII activation of endothelial AT_2R increases eNOS activity and NO production, PGI_2, and vasodilation, and thereby counteracts AngII-induced vasoconstriction. Therefore, while normal pregnancy is associated with increased plasma levels of renin and AngII, the pressor effects of AngII are decreased due to decreased AT_1R and/or increased AT_2R. On the other hand, the dose of AngII required to elicit a 20 mmHg pressor response in the diastolic BP in women at gestational weeks 23–26 was lower in women who subsequently developed PE compared with normal pregnant women who remained normotensive,[176] suggesting an increase in the pressor response to AngII long before overt PE.

AngII levels and AT_1R mRNA expression are increased in chorionic villi and placenta of PE versus normal pregnant women.[177,178] Plasma hemopexin activity increases during normal gestation from week 10 onward, and active hemopexin downregulates AT_1R in human monocytes and endothelial cells, and decreases functional AT_1R and AngII-induced contraction in rat aortic rings. In PE, hemopexin activity may be inhibited resulting in enhanced AT_1R expression and increased vasoconstriction.[179]

AT_1-AA is a bioactive factor that promotes vasoconstriction and VSM growth via AT_1R. Serum levels of AT_1-AA are elevated in PE than normal pregnant women,[109,180] and are further elevated in severe PE and in early versus late PE.[181] AT_1-AA has been linked to increased BP, reduced trophoblast invasion, increased sFlt-1, ROS and cellular Ca^{2+}, activation of coagulation tissue factor and thrombosis, vascular damage in the adrenal glands, and reduced aldosterone secretion in PE.[109,182] AT_1-AA also promotes collagen-induced platelet aggregation, which may contribute to the hypercoagulability in PE.[180] In cultured trophoblasts, stimulation of AT_1R with IgG isolated from PE women causes increases in sFlt-1 levels.[183] In HUVECs, treatment with AT_1-AA isolated from PE women

induces the release of the cell death and necrosis marker lactate dehydrogenase,[184] suggesting that AT_1-AA causes endothelial cell damage and necrosis. Also, in HUVECs, AT_1-AA induces the activity of caspase-3 and caspase-8, suggesting that it promotes endothelial cell apoptosis.[184] Circulating levels of AT_1-AA are also increased in RUPP compared with normal pregnant rats.[185,186] Infusion of AT_1-AA in pregnant mice causes some of the manifestations of PE including increased BP, proteinuria and plasma sFlt-1 levels.[109] Also, infusion of AT_1-AA in pregnant rats increases ET-1 levels 4-fold in the placenta and 11-fold in the renal cortex.[187] Endothelium-dependent acetylcholine (ACh)-induced vasodilation is reduced in the renal interlobar arteries of pregnant rats infused with AT1-AA, suggesting a link between AT1-AA and renal endothelial dysfunction in HTN-Preg. The impaired ACh-induced vasodilation in AT1-AA infused pregnant rats is reversed by an ET_AR antagonist, suggesting an interplay between AngII and ET-1 in the setting of endothelial dysfunction and HTN-Preg.[188] While the mechanisms causing the release of AT_1-AA in PE are not understood, plasma levels of AT_1-AA are increased in pregnant rats infused with TNFα, suggesting cytokine-mediated pathways.[186]

Extracellular Vesicles (EVs) in PE

Extracellular vesicles (EVs) are lipid-bilayer structures that are released from cells into the extracellular environment. They contain proteins, miRNA, growth and apoptotic factors, and other regulatory components to induce cell-to-cell communication and signaling throughout the body. EVs are released under normal and pathological conditions, and multiple EV types can be produced from different cells, including red blood cells, fibroblasts, endothelial cells, and trophoblasts. After secretion from cells, EVs modify the activity of adjacent cells or travel to regions distal to the site of release in several body fluids. In PE, impaired placental function with placental apoptosis and necrosis causes increased release of microvesicles and nanovesicles. These exosomes contain proteins, miRNA, DNA, RNA; as well the lipids comprising the vesicular wall. EVs (including exosomes) originating from placental explant and cells promote pro-inflammatory cytokines production and endothelial dysfunction, and may be involved in different stages of PE.[189]

Vascular Dysfunction in HTN-Preg and PE

Normal pregnancy is associated with vasodilation of the maternal uterine, renal and systemic vessels,[190] and reduction in the mechanisms of vascular contraction, likely due to increased plasma levels of estrogen and progesterone.[191,192] Estrogen causes relaxation of VSM of the rat aorta and uterine artery.[193,194] Also, progesterone inhibits contraction of rat blood vessels.[193] PE is associated with endothelial dysfunction, increased mechanisms of VSM contraction and inadequate remodeling of ECM.

Endothelial Dysfunction in PE

Normal and functional endothelium ensures healthy gestation and a favorable prognosis for the mother and fetus.[195] Brachial artery diameter and flow-mediated dilation increase as gestation progresses.[196] Also, endothelium-dependent bradykinin-induced relaxation is increased in small subcutaneous arteries from pregnant compared with non-pregnant women.[197] ATP also causes periodic bursts in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) that are more frequent in uterine artery endothelial cells from pregnant compared with non-

pregnant ewes,[198] leading to increased vasodilation, decreased uterine artery myogenic tone, and adequate uterine blood flow during pregnancy.[199]

In addition to their effects on the uterus, gonadal hormones contribute to the vascular changes during pregnancy. Estrogen promotes endothelium-dependent vascular relaxation by increasing the release of NO, PGI$_2$ and EDHF.[200] Estrogen also causes relaxation of endothelium-denuded vessels by inhibiting the mechanisms of VSM contraction including $[Ca^{2+}]_c$ and protein kinase C.[201,202] Estrogen may have additional effects on the vascular cytoskeleton, ECM, lipid profile and inflammatory response.[200] Progesterone also causes vasodilation by mechanisms similar to estrogen.[202,203] Some of the vascular effect of estrogen could involve MMPs. In cultured human coronary artery and umbilical artery VSM cells, estrogen causes dose-dependent increases in MMP-2 levels in culture media.[49]

In contrast with normal pregnancy, women with PE show systemic endothelial cell dysfunction and HTN.[5] Brachial artery flow-mediated dilation is less in PE than normal pregnant women.[195,204] PE women also show less vasodilation in the radial artery when compared to normal pregnant women.[205] Bradykinin-induced relaxation is decreased in small subcutaneous arteries of PE compared with normal pregnant women.[197] Circulating endothelial cells and other markers of endothelial activation/injury such as soluble VCAM-1, E-selectin and endocan are increased in PE compared with normal pregnant women.[99,206–208] On the other hand, circulating endothelial progenitor cells are decreased and may serve as a marker of endothelial damage in PE women.[209]

The RUPP rat shows some of the characteristics of PE including high BP, proteinuria, decreased glomerular filtration rate and renal plasma flow, and IUGR, and therefore has been used to study the vascular mechanisms of HTN-Preg.[210–212] ACh is less potent in inducing relaxation in the aorta and mesenteric microvessels of RUPP than normal pregnant rats, suggesting endothelial damage in RUPP rats.[7,212] Endothelial cells release various vasodilator substances including nitric oxide (NO), prostacyclin (PGI$_2$) and endothelium-derived hyperpolarizing factor (EDHF) as well as contracting factors as endothelin-1 (ET-1) and thromboxane A2 (TXA$_2$). Endothelial dysfunction is associated with abnormal release of endothelium-derived vasodilator and vasoconstrictor factors.

Changes in Nitric Oxide (NO) in PE—NO is a potent vasodilator and relaxant of VSM. NO diffuses into VSM and increases cyclic guanosine monophosphate (cGMP), which promotes Ca^{2+} efflux, decreases VSM $[Ca^{2+}]_c$ and causes VSM relaxation. Nitrites are important metabolites of NO that are increased in serum of normal pregnant compared with non-pregnant women.[213] Plasma levels and urinary excretion of cGMP, a second messenger of NO, are also increased in normal pregnancy. NOS expression/activity increase in human uterine artery and in the placenta with gestational age.[214,215]. Also, urinary nitrite levels, mRNA expression of eNOS, iNOS and nNOS, and protein level of activated phospho-eNOS are increased in normal pregnant compared with virgin rats,[216] supporting pregnancy-related increase in NO.

Polymorphisms in *eNOS* gene could be a risk factor for PE. The VNTRa and 894T alleles of *eNOS* gene are associated with early and late severe PE, respectively. For the *eNOS*

VNTRb/a polymorphism, plasma NO metabolites are lower in subjects homozygous for the "a" allele. Also, the eNOS 894T allele is prone to selective proteolytic cleavage in endothelial cells and vascular tissues, thus accounting for the reduced NO production in subjects homozygous for this variant.[217] The T786C allele is also increased in PE compared with normal pregnant women.[218,219] Also, normal pregnant women with the TT phenotype for the T-786C allele have lower plasma nitrite levels than those with the CC phenotype,[220] and the TT phenotype has been proposed as a risk factor for PE in Tunisian women.[218]

Endothelial dysfunction is often associated with decreased NO due to decreased synthesis or bioavailability.[221] Clinical studies have shown increased[222] or decreased[223–225] plasma nitrite levels in PE compared with normal pregnant women. Also, urinary nitrite levels may not differ in PE versus normal pregnant women,[223] and this may not be solely related to dietary nitrate intake since a study that carefully controlled dietary nitrate/nitrite intake did not show decreased NO production in PE women.[226]

The lack of change in whole-body NO despite the increase in BP and the renal damage in PE suggest tissue-specific changes in NOS expression and NO bioavailability.[5] Studies have shown a decrease in nitrites in placentae from PE women.[225] Also, eNOS expression is decreased in umbilical cord of PE compared with normal pregnant women,[227] and the decrease is greater in women with severe PE.[228,229] However, some studies showed an increase in eNOS mRNA expression in placenta of PE women.[230] Also, while the levels of cGMP are increased during normal pregnancy, the plasma and urinary cGMP levels are not different in PE versus normal pregnant women.[223]

The role of NO has also been examined in animal models of HTN-Preg. In mid- to late pregnant rats, NOS blockade with N_ω-nitro-L-arginine methyl ester (L-NAME) causes PE-like manifestations including increased BP, renal vasoconstriction, proteinuria, thrombocytopenia and IUGR.[231] However, similar to the observation in humans, measurements of NO in HTN-Preg animals have not been consistent. Studies showed no difference in nitrite levels in L-NAME treated versus nontreated pregnant rats.[107] Also, while plasma nitrite levels were lower in RUPP than normal pregnant rats,[210] no differences were observed in urinary nitrite levels.[211,232] Also, consistent with the studies in human, no changes in circulating levels of the NOS substrate L-arginine were observed in RUPP versus normal pregnant rats.[233] Vascular function studies have shown increased aortic vascular reactivity to phenylephrine in L-NAME treated pregnant rats.[231] Also, ACh-induced relaxation, eNOS expression, and NO production are reduced in mesenteric artery and aorta of RUPP versus normal pregnant rats, supporting reduced NO synthesis in the vasculature.[7,212] In DOCA-salt rat model of HTN-Preg, NO-dependent relaxation was reduced in mesenteric vessels despite elevation of eNOS mRNA expression.[171]

We should note that NO has diverse functions that extend beyond vasodilation/VSM relaxation. NO plays a role in regulation of renal tubular function, immune modulation, and as an antioxidant, and dysregulation of these NO functions may be important mechanisms underlying HTN-Preg and PE.

Changes in Prostacyclin (PGI$_2$) in PE

PGI$_2$ is produced from the metabolism of arachidonic acid by cyclooxygenase 2 (COX)-2 and COX-1, and is a potent vasodilator and inhibitor of platelet aggregation. During normal pregnancy, the synthesis of 6-keto-PGF1α (a stable metabolite of PGI$_2$) is increased in fetoplacental tissues, suggesting a role in the regulation of the maternal and fetal circulation. [234,235] Plasma and urinary levels of PGF1α are decreased in severe PE, suggesting that the overall PGI$_2$ synthesis is diminished. Endothelial PGI$_2$ production may also decrease in PE. [236] While the release of PGI$_2$ may not be different in apical and basal trophoblasts of PE compared with normal pregnant women, the release of TXA$_2$, another COX product, from basal trophoblast cells is increased in PE and may contribute to increased placental vasoconstriction.[237]

Hydrogen Sulfide (H$_2$S)—In some arteries, relaxation responses cannot be fully explained by NO and PGI$_2$, and a possible role of H$_2$S has been suggested. Reduction in plasma levels of H$_2$S has been reported in PE pregnancies. Also, cystathionine-γ-lyase (CSE), the primary H$_2$S-synthesizing enzyme in the vasculature, is reduced in PE.[238]

Endothelium-Derived Hyperpolarizing Factor (EDHF) in PE—EDHF is a relaxing factor with specialized role in the control of small resistance vessels, local organ blood flow, peripheral vascular resistance and BP. Although the nature of EDHF is unclear, it often presents as K$^+$ efflux from endothelial cells through intermediate and small conductance Ca^{2+}-activated K$^+$ channels (IK$_{Ca}$ and SK$_{Ca}$, respectively) causing hyperpolarization of endothelial cells. Endothelial cell hyperpolarization then spreads via myoendothelial gap junctions (MEGJs) and connexins to cause VSM hyperpolarization, reduction of Ca^{2+} influx via voltage-dependent Ca^{2+} channels and suppression of the activity of phospolipase C, an enzyme involved in signal transduction in VSM. The opening of endothelial cell IK$_{Ca}$ and SK$_{Ca}$ could also cause some accumulation of K$^+$ ion in the myoendothelial interface which could induce VSM hyperpolarization by activating the inwardly rectifying K$^+$ (K$_{IR}$) channels and the Na$^+$/K$^+$-ATPase.[239] EDHF relaxation may also be caused by diffusible factors released from endothelial cells. EDHF may be a product of cytochrome P450 (CYP450), such as epoxyeicosatrienoic acid (EET), which activate large conductance K$_{Ca}$ (BK$_{Ca}$) and cause hyperpolarization of VSM. In some vessels, H$_2$O$_2$ may mimic EDHF-mediated responses by mechanisms involving K$_{Ca}$ activation.[240] Thus multiple EDHFs may exist and the identity of EDHF could vary depending on the vascular bed and animal species studied.[241]

In small subcutaneous and myometrial arteries of normal pregnant women, EDHF is responsible for ~50% of bradykinin-induced relaxation, acting together with NO to maintain proper vascular tonus.[242,243] The gap junction proteins connexins 37, 40 and 43 are partly involved in EDHF-mediated vascular response during normal pregnancy.[244] An increase in endothelial cell [Ca^{2+}]$_c$ activates IK$_{Ca}$ and SK$_{Ca}$ and EDHF-mediated dilation in uterine radial arteries of pregnant rats.[245] The delayed rectifier type of voltage-sensitive K$^+$ channels (K$_v$) may also play a role in EDHF-mediated dilation in uterine artery of pregnant rats.[246]

Studies in subcutaneous arteries from normal pregnant women have shown that MEGJs alone are the main pathway of EDHF-mediated relaxation, while in women with PE MEGJs alone or in combination with H_2O_2 or CYP450 epoxygenase metabolites of arachidonic acid could mediate EDHF-induced vasodilation. The changes in the role of MEGJs may be caused by morphological changes within the vascular wall during PE.[247] Small myometrial arteries from PE women also showed reduced vasodilatory responses that were attributed to decreased contribution of EDHF due to physical disruption of MEGJs.[248] Studies in mice have shown pregnancy-associated adaptations in the form of decreased sensitivity to phenylephrine and enhanced bradykinin-induced vasodilation in normal pregnant wild-type mice, but not in knockout mice lacking pregnane X receptor, a nuclear receptor that induces the expression of CYP450. Also, treatment with CYP450 inhibitor changed the vasodilatory response to bradykinin in wild-type but not the knockout mice, supporting that metabolites of CYP450 such as EET may play a role in the vascular adaptations during pregnancy.[249] As EET is one of the possible factors involved in EDHF-mediated relaxation, it is plausible to suggest that alterations in EDHF may lead to impaired vascular function and HTN-Preg. Although studies in mesenteric microvessels have suggested that the EDHF relaxation may not be compromised in RUPP versus normal pregnant rats,[212] decreased EDHF-mediated relaxation contributes to the vasoconstriction observed in HTN and diabetes, and its role in PE needs to be further examined.

Endothelin-1 (ET-1) in PE—ET-1 is a major endothelium-derived vasoconstrictor that could play a role in PE.[250] ET-1 synthesis is initiated from the long 203 amino acid preproET, which is cleaved by furin-like protease to biologically inactive 37 to 41 amino acid big-ET. Big-ET is cleaved by endothelin converting enzymes, members of the metalloprotease family, to produce active 21 amino acid ET-1. Circulating factors in PE such as cytokines, hypoxia and AT_1-AA may stimulate endothelial cells to produce ET-1.[250] In support, serum from PE women causes HUVECs to produce greater amounts of ET-1 than normal pregnant serum.[251] Some studies suggest that plasma ET-1 levels are elevated in PE. [252] ET-1 levels are higher during later stages of PE and return to normal levels within 48 hours after delivery,[253] suggesting that ET-1 may be involved in the progression rather than the initiation of PE. However, in most studies serum ET-1 levels do not differ in PE versus normal pregnant women, and higher levels of ET-1 are observed mainly in HELLP syndrome.[74,254,255] Of note, ET-1 is released in a paracrine fashion from endothelial cells directly toward VSMCs, and the increases in ET-1 levels in PE may be localized in tissues. Studies have shown a 4- to 8-fold increase in ET-1 levels in umbilical cord cells and in renal tissues during later stages of PE.[253,256] In perfused placentas under hypoxia, both the maternal and fetal side produce increased levels of ET-1.[257] In RUPP rats, preproET levels show a 45% increase in renal cortex and 22% increase in renal medulla.[258] Thus, circulating ET-1 levels may not always reflect local ET-1 levels in tissues. It is possible that in severe PE and in HELLP syndrome ET-1 production is so augmented such that it "loses" its paracrine directionality and leads to increased circulating ET-1 levels. In support, rat models that mimic severe PE and HELLP syndrome show increased plasma levels of ET-1.[124,259]

ET-1 may play a role in the pathogenesis of PE by inducing apoptosis of trophoblast cells and increasing oxidant and anti-angiogenic substances.[250,260] ET-1 activates endothelin

receptor type A (ET_AR) and type B (ET_BR).[261–263] ET-1 activation of VSM ET_AR stimulates Ca^{2+} release from the intracellular stores and Ca^{2+} entry through Ca^{2+} channels, and causes protein kinase C-dependent inhibition of K^+ channels leading to increased $[Ca^{2+}]_c$ and VSM contraction.[261] ET-1-induced vasoconstriction is reduced in mesenteric vessels of normal pregnant compared with non-pregnant rats,[264] and VSM ET_AR is reduced in aortic media and VSMCs of late-pregnant rat.[265] Also, treatment with ET_AR antagonist reduces BP in RUPP rat and other animal models of HTN-Preg.[258,266,267] Of note, among Brazilian women with PE, 52% of the patients with severe PE exhibited increases in ET_AR agonistic autoantibodies (ET_A-AA) which targets ET_AR and increases vasoconstriction.[268] ET-1 also activates ET_BR in endothelial cells and stimulates the release of NO, PGI_2, and EDHF which in turn reduce myogenic vascular tone, promote vasodilation of renal arteries and hyperfiltration in pregnant rats.[261,269] Downregulation of ET_BR may impair trophoblast invasion in PE and decrease microvascular dilation in pregnant rats. ET_BR expression is reduced in endothelial and renal cells of RUPP rats. Also, ET_BR-mediated NO production is less in the aorta and mesenteric artery of RUPP versus normal pregnant rats, supporting that downregulation of endothelial ET_BR could play a role in HTN-Preg.[212]

Thromboxane A_2 (TXA_2) in PE—TXA_2 is a potent stimulator of platelet aggregation, vasoconstriction, and VSM cell proliferation and mitogenesis. PE is associated with decreased vascular production of PGI_2 and increased production of TXA_2, and this may explain HTN and increased platelet aggregation in PE. Imbalance between urinary TXB_2 metabolites, markers of TXA_2 synthesis, and PGI_2 predates clinical symptoms of PE.[236] This led to the suggestion that antiplatelet agents such as low-dose aspirin and other thromboxane modulators might prevent or ameliorate PE. In clinical trials, women at high risk for PE showed no benefit of low dose aspirin as a preventive measure.[270] However, ozagrel, a thromboxane modulator, was found to reduce the occurrence of HTN-Preg and proteinuria,[271] making it important to further examine the role of TXA_2 in PE.

Vascular Smooth Muscle (VSM) Dysfunction in PE

VSM Ca^{2+} in PE—Ca^{2+} is a major determinant of VSM contraction and growth. Ca^{2+} release from the intracellular stores and Ca^{2+} entry from the extracellular space increase $[Ca^{2+}]_c$ in VSM. Ca^{2+} binds calmodulin to form Ca^{2+}-calmodulin complex which activates myosin light chain kinase, and causes myosin phosphorylation, actin-myosin interaction and VSM contraction. Decreased Ca^{2+} in VSM activates myosin phosphatase which dephosphorylates myosin light chain and leads to dissociation of the Ca^{2+}-calmodulin complex. Endothelium-derived relaxing factors act on VSM to decrease $[Ca^{2+}]_c$. During normal pregnancy, increased K_{Ca} channel activity decreases uterine artery tonicity and increases uteroplacental blood supply. In PE, K_{Ca} channel activity is suppressed leading to increased uterine artery $[Ca^{2+}]_c$, vasoconstriction and reduced fetal blood supply.[272] Myometrial vessels may show similar vasoconstriction responses to high KCl, phenylephrine and AngII in normal pregnancy and PE.[273] However, basal and agonist-stimulated $[Ca^{2+}]_c$ are reduced in renal arterial VSM of normal pregnant rats, and increased in pregnant rats treated with L-NAME.[274] AT_1R activation increases $[Ca^{2+}]_c$ in platelets, erythrocytes, and lymphocytes of PE women, and these effects subside 6 weeks after delivery.[182] AngII- and caffeine-induced contraction and $[Ca^{2+}]_c$ in Ca^{2+}-free solution are

similar in VSM of normal pregnant and RUPP rats, while KCl-induced maintained $[Ca^{2+}]_c$ in a Ca^{2+}-containing medium is greater in VSM of RUPP than normal pregnant rats, suggesting that it is not Ca^{2+} release from the intracellular stores, but Ca^{2+} entry from the extracellular space that increases vasoconstriction in HTN-Preg.[275]

Protein Kinase C (PKC) in PE—PKC is an important mediator of VSM contraction. PKC phosphorylates CPI-17 which inhibits myosin phosphatase and in turn increases myosin light chain phosphorylation and VSM contraction. PKC also phosphorylates calponin, an actin binding protein that inhibits myosin ATPase, leading to more actin-myosin interaction and VSM contraction. Phorbol esters activate PKC to cause VSM contraction with no detectable change in $[Ca^{2+}]_c$, suggesting that PKC increases Ca^{2+} sensitivity of the contractile proteins. PKC activity and contraction are reduced in uterine artery of late pregnant ewes and gilts and the aorta of late pregnant rats.[276–278] Also, the expression and subcellular redistribution of Ca^{2+}-dependent α-PKC and Ca^{2+}-independent δ- and ζ-PKC are reduced in aortic VSM of late pregnant rats, but are increased in L-NAME treated pregnant rat.[277,279] PKC may increase the production of AT_1-AA which stimulates AT_1R. In cultured rat cardiomyocytes treatment with IgG obtained from PE women enhances AT_1R-mediated response which is ameliorated with the PKC inhibitor calphostin C.[280] Increased BK_{Ca} channel activity inhibits PKC-mediated contraction in ovine uterine arteries during pregnancy, and gestational hypoxia may upregulate PKC and inhibit BK_{Ca}.[272] PKC inhibitors decrease TXA_2 mediated contraction in uterine and mesenteric arteries of virgin rats and in mesenteric artery of pregnant rats, supporting a role of PKC in mediating VSM contraction during pregnancy.[281] Blocking PKC can prevent PKC-mediated vasoconstriction in PE. Cicletanine is an anti-hypertensive drug that prevents the increase in PKC and lowers BP in HTN-Preg rats. MMP-2 may reduce vascular contraction by degrading the actin-binding protein and PKC substrate calponin,[282] and a decrease in MMP-2 would spare calponin and affect VSM contraction in HTN-Preg.

Mitogen-activated protein kinase (MAPK) is a serine/threonine protein kinase that regulates cellular activities such as gene expression, mitosis, differentiation, and VSM contraction. During VSM contraction, PKC may phosphorylate MAPK kinase which in turn phosphorylates and activates MAPK. Activated MAPK phosphorylates the actin-binding protein caldesmon thus preventing its inhibition of ATPase and increases actin-myosin interaction and VSM contraction. Changes in PKC activity in VSM during normal pregnancy and HTN-Preg could affect MAPK/caldesmon phosphorylation and VSM contraction.[283] In support, inhibition of p38 MAPK reduces TXA_2-induced contraction in uterine and mesenteric arteries of virgin and pregnant rats.[281]

VSM Rho-Kinase (ROCK) in PE—Rho is a family of small GTP-binding proteins that are involved in cell migration, cytoskeletal reorganization and VSM contraction. RhoA binding to GTP activates Rho-kinase (ROCK), and activated ROCK is inactivated by hydrolyzing GTP to GDP. ROCK has two isoforms, ROCK-1 (ROCK-I, ROKβ) and ROCK-2 (ROCK-II, ROKα), which contribute to the formation of microvilli structures during pregnancy. In PE, placental villi show abnormal expression of ROCK-II and apoptosis of the syncytium.[284] ROCK increases Ca^{2+} sensitivity of the contractile proteins in

subcutaneous resistance arteries of PE women.[285] Also, AngII via AT_1R induces RhoA/ROCK activity in L-NAME treated hypertensive rats.[286] ROCK may stimulate IL-17 to phosphorylate the inhibitory eNOS Thr495 residue thus decreasing NO production in PE.[287] ROCK Inhibition reduces TXA_2-induced contraction in uterine vessels of non-pregnant rats.[281] However, some studies show decreased ROCK mRNA expression in umbilical arteries of PE women,[270,288] making it important to further examine the role of ROCK in the vascular changes in PE.

Extracellular Matrix and Vascular Remodeling in PE

The extracellular matrix (ECM) is an integral component of the vascular wall. Pregnancy-associated changes in MMPs play a role in ECM and vascular remodeling, angiogenesis, and structural changes in blood vessels.[87] MMPs degrade different substrates including collagen, gelatin, and other proteins.[43,62] We have investigated the changes in MMPs substrates in the RUPP rat model of HTN-Preg. Picro-Sirius Red staining revealed an increase in collagen content in uterus, placenta and aorta of RUPP versus normal pregnant rats.[42] Because MMPs facilitate cell growth and migration by promoting proteolysis of ECM, the decreased MMP-2 and MMP-9 and increased collagen deposition in RUPP tissues could impede cell growth, proliferation and migration, and thus interfere with uteroplacental tissue invasion and uterine and placental growth. Also, while the aortic collagen content increased, there was a decrease in aortic tissue weight and thickness in RUPP rats, likely because the decreased MMPs and increased collagen content would interfere with VSMC growth and migration. The decreased MMP activity and increased vascular collagen content could also increase the blood vessel rigidity and decrease its plasticity and thus contribute to increased vascular resistance and HTN. This is consistent with reports that MMP-1, MMP-2, and MMP-9 activity is decreased and collagen deposition is increased in internal mammary artery from hypertensive compared with normotensive patients undergoing coronary artery bypass surgery.[289] It is important to note that collagen has 18 types and different subtypes.[290] MMP-2 can degrade collagen I, II, III, IV, V, VII, X, and XI while MMP-9 can degrade collagen IV, V, VII, X, XIV.[43,62,291] Studies should measure the changes in various collagen subtypes in HTN-Preg. Also, RT-PCR experiments should determine whether any increases in a collagen subtype are due to decreased degradation or increased *de novo* collagen mRNA expression and protein biosynthesis.

While MMPs are largely known for their proteolytic effects on ECM, we and others have identified novel MMP-induced downstream pathways that could affect membrane receptors, cell signaling and vascular function.[59,292–294] Prolonged increases in intravascular pressure and wall tension cause increases in MMP-2 and MMP-9 expression.[293–295] Also, MMP-2 and MMP-9 cause relaxation of phenylephrine precontracted rat aorta[294] and inferior vena cava.[293,295] Thus during normal pregnancy, plasma volume expansion could lead to increased MMP-2 and MMP-9, vasodilation and decreased BP. The decrease in vascular MMP-2 and MMP-9 is expected to hinder vasorelaxation in RUPP rats, consistent with the observed decrease in ACh-induced relaxation in blood vessels of RUPP versus pregnant rats.[7,264]

MMPs break down big-ET-1 into different endothelins with different affinities for ET_AR and ET_BR. MMPs degrade big-ET into ET-1, which largely stimulates ET_AR and promotes vasoconstriction.[296] Studies have suggested a role of ET-1 and ET_AR in some forms of HTN including HTN-Preg.[258,266,297–299] In omental vessels of pregnant women, MMP-1 causes vasoconstriction and enhances reactivity to AngII via an endothelium-dependent protease-activated receptor (PAR) and ET-1 pathway.[59] ET-1 in turn can stimulate VSM contraction mechanisms including $[Ca^{2+}]_c$, PKC, and ROCK.[300–302]

MMP-2 and MMP-9 could degrade big-ET to ET_{1-32} which preferentially stimulates endothelial ET_BR and promotes relaxation. MMP-2 and MMP-9 cause vascular relaxation by decreasing Ca^{2+} influx into VSM,[294] and a decrease in these MMPs could lead to increased Ca^{2+} influx, vasoconstriction and HTN-Preg. We have shown increases in MMP-2, MMP-9 and ET_BR in normal pregnant rats.[50,264,292] ET_BR is downregulated in RUPP rats, and infusion of the ET_BR antagonist BQ788 increases BP in pregnant rats.[212] Also, MMP-2 induces vascular relaxation partly via hyperpolarization and activation of K^+ channels.[293,295] Thus, the decrease in MMP-2 and MMP-9 mediated relaxation may contribute to the enhanced vascular contraction and increased BP in RUPP rat model of HTN-preg.[7,106,212]

Prediction and Management of PE

PE has a relatively long preclinical phase before manifesting in late gestation, and the identification of women at risk, early diagnosis using biomarkers, and prompt management could improve the maternal and perinatal outcome.[5] Thrombocytopenia is common and progresses with severity of PE.[303] Longitudinal studies have shown that women who develop PE have higher mean platelet volume 4.6 weeks prior to the appearance of symptoms.[270] Doppler screening at 23 weeks of pregnancy and detection of early diastolic bilateral uterine artery notching in the waveform could predict PE. PE women with uterine artery notching have altered levels of fibrinolytic activators and inhibitors such as tissue-type plasminogen activator (t-PA), PAI-1, PAI-2, plasmin-α2-antiplasmin (PAP) and D-dimers. Increased t-PA levels in PE women appears to be related to endothelial cell activation/dysfunction.[304] Also, a decrease in brachial artery flow-mediated vasodilation is an early indicator of endothelial dysfunction between the 24th and 28th gestational weeks and before clinical diagnosis of PE. The sensitivity of flow-mediated vasodilation is 87.5% and 95.5% for the prediction of early and late PE, respectively.[195]

Biomarkers allow early assessment in asymptomatic pregnant women at increased risk of PE based on their clinical history of PE or HTN in a previous pregnancy, or pre-pregnancy state e.g. HTN, obesity, or autoimmune disease. Angiogenic imbalance is an important feature in PE. Measurements of plasma VEGF, PlGF, sFlt-1 and sEng may help early detection in asymptomatic women at high risk for PE.[5] In PE, circulating levels of sFlt-1 are increased more than one month before the onset of clinical symptoms, and PlGF is decreased in women who subsequently develop PE from the end of the first trimester.[305] The sFlt-1/PlGF ratio is increased in both early and late PE.[92] A meta-analysis of 20 different studies suggest that the overall diagnostic accuracy of sFlt-1/PlGF ratio for PE is relatively high, and is higher in early than late PE.[306] However, patients with late onset PE could show lower levels of sFlt1/PlGF ratio compared to early onset PE,[92] suggesting that other factors may be

involved and could serve as markers of PE. sFlt1–14 is human-specific splicing variant of VEGFR-1 produced by nonendothelial cells and a potent inhibitor of VEGF. The expression of sFlt1–14 is elevated in placenta of PE women, specifically in abnormal clusters of degenerative syncytiotrophoblasts known as syncytial knots.[307]

Abnormal maternal immunological response could help in predicting PE in early pregnancy, and is often presented as a change in monocytes and natural killer (NK) cells, increased pro-inflammatory cytokines, increased AT_1-AA, and activation of AT_1R.[5,308] The alternate complement pathway is upregulated in PE and plasma levels of factor B-derived Bb fragment are higher in PE than normal pregnant women.[270] TNFα levels could be an early predictor of PE. Plasma obtained at gestational weeks 11–13 showed high TNFα levels in women who later developed PE.[309] Elevated plasma TNFα levels in association with changes in uterine artery Doppler at 11–13th gestational weeks have a 100% sensitivity in predicting PE.[310] Also, plasma levels of sTNF-R1 and sTNF-R2 are elevated in PE.[311] Other reports suggest that plasma TNFα levels may be useful in predicting PE in the early third trimester, but not the first or second trimesters.[312]

Uric acid is a marker of oxidative stress, tissue injury and renal dysfunction. During normal pregnancy, uric acid levels decrease initially, but then gradually increase over gestational time. Hypoxia and ischemia of the placenta and cytokines such as interferon induce expression of xanthine oxidase which increases the production of uric acid and ROS. During PE, hyperuricemia may develop as early as 10th week of gestation. Increased circulating uric acid may attenuate trophoblast invasion and spiral artery remodeling, stimulate monocytes to produce TNFα, IL-6 and IL-1β, and contribute to endothelial dysfunction and reduced NO production.[313] Plasma levels of malondialdehyde, a highly reactive compound and a marker for oxidative stress are also higher in PE than normal pregnant women, and positively associated with maternal plasma sFlt-1 levels.[314]

Elevated serum level of placental glycoprotein pregnancy-associated plasma protein-A (PAPP-A) and of placental protein 13 (PP13), together with abnormal Doppler ultrasound of the uterine artery, have a high predictive value in PE.[315] Also, serum levels of fetal hemoglobin, α1-microglobulin, activin A, and inhibin A are altered in PE.[314,316]

Amniocentesis and amniotic fluid analysis in the second trimester may be useful in predicting PE. Insulin-like factor 3 (INSL3) is a member of the insulin/relaxin family of peptide hormones made by the fetal testis and is responsible for the first trans-abdominal phase of testicular descent. In the presence of a male fetus, INSL3 is elevated in amniotic fluid samples of women who subsequently develop PE.[270] The amniotic fluid levels of inhibin A, a glycoprotein produced by syncytiotrophoblast, are higher in pregnant women who subsequently develop severe PE than in normal pregnant women[317]. Also, the level of sFlt-1 is higher in the amniotic fluid of PE than normal pregnant women.[318]

Microarray analysis could be used to screen the placental transcriptome for upregulated and downregulated genes in PE. The mRNA levels of plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA), VEGFR-1 (Flt-1), endoglin, placenta-specific protein 1 and P-selectin were increased in plasma from pregnant women who later

developed PE. Flt-1 had the highest detection rate while placenta-specific protein 1 had the lowest detection rate, with the best multivariable model obtained by the combination of all markers.[319] miRNA-206 interacts with several genes involved in PE and is elevated at gestational week 28 in plasma and placenta from women who subsequently develop PE,[320] although other studies show little predictive value of miRNAs.[321]

Measurements of MMPs have not been consistent in PE, with some studies showing an increase in MMP-2 and MMP-9,[55] while other studies showing a decrease in MMP-9.[41] However, plasma MMPs represents global changes in MMPs in different tissues, and localized changes in uteroplacental tissues and fluids may carry more predictive value.

In search for other PE biomarkers, studies have shown that pregnant mice deficient in catechol-O-methyltransferase (COMT) show a PE-like phenotype. COMT deficiency leads to an absence or decrease of 2-methoxyestradiol (2-ME), a natural metabolite of estradiol, which is normally elevated during the third trimester of normal pregnancy. Administration of 2-ME ameliorated several of the PE-like features in the $Comt^{-/-}$ pregnant mice, and suppressed placental hypoxia, HIF-1α expression, and sFlt-1 elevation. Plasma levels of COMT and 2-ME are decreased in PE women, and may be used as biomarkers for PE.[322] Despite the existence of several markers for PE, their predictive value needs to be further assessed in order to identify the best marker combinations for use in clinical settings.

Currently, inducing labor and delivery of the fetus and placenta are the most effective measures for PE. Prenatal care is most important in management of PE and includes bed rest and anti-HTN drugs such as oral nifedipine or intravenous hydralazine or diazoxide, depending on severity of HTN. International guidelines recommend one antihypertensive agent e.g. methyldopa, labetalol, another beta-blocker (acebutolol, metoprolol, pindolol, propranolol), or Ca^{2+} channel blocker (nifedipine).[323] Angiotensin receptor blockers (ARBs) and angiotensin converting enzyme (ACE) inhibitors should not be used due to their teratogenic effects, and atenolol and prazosin are not recommended prior to delivery. If PE worsens to eclampsia, airway patency should be maintained to prevent fluid aspiration, and anticonvulsants are given, with Mg^{2+} sulfate infusion being the drug of choice.[324]

Sildenafil may be useful in women with severe early-onset IUGR, as it could promote fetal growth with no maternal side effects.[325] Sildenafil citrate dilates myometrial artery and restores endothelial cell integrity in placental vessels of L-NAME treated mouse model of HTN-Preg.[326] Eculizumab, an anti-C5 antibody, normalized laboratory values and prolonged pregnancy by 17 days in a woman with PE/HELLP syndrome, suggesting the benefits of manipulating the complement system during PE. However, complement inhibitors could increase susceptibility to infection and their long-term use requires close monitoring.[327]

Correcting the angiogenic imbalance may be beneficial in PE. In trophoblast cells and HUVECs treated with cobalt chloride to simulate hypoxic conditions, the free radical scavenger edaravone inhibits sFlt-1 expression in trophoblast cells and protects against the decrease in vascular development and tube formation in HUVECs.[328] VEGF could improve the angiogenic imbalance, but may impair bradykinin-induced vascular relaxation and enhance basal tone and vascular permeability in PE.[13] Modulators of PlGF could be more

promising in PE, and low molecular weight heparin increases circulating PlGF levels during the third trimester.[329] Infusion of PlGF or VEGF reduces BP in RUPP rats.[117,330]

TNFα antagonists such as etanercept decrease BP, increase eNOS expression and decrease ET-1 levels in RUPP rats.[126,331] IL-17 soluble receptor C inhibits IL-17, prevents the recruitment of host defense cells, suppresses the inflammatory response, decreases AT$_1$-AA and ROS, and ameliorates HTN and pup and placental weight in RUPP rats.[332] Infusion of anti-inflammatory IL-10 decreases BP in DOCA/salt rat model of HTN-Preg.[333]

Another approach is to aim at downstream targets affected by cytoactive factors. If MMPs are a central target in HTN-Preg, then modulating MMP expression/activity should promote vasodilation and reduce BP. Doxycycline is an MMP inhibitor that could alleviate HTN and vascular dysfunction, but may decrease placenta weight and cause IUGR in normal pregnant rats, and reduce trophoblast invasion and placental perfusion in HTN-Preg rats.[135] Novel approaches to indirectly or directly correct mediators of microvascular dysfunction should provide new strategies in the management of HTN-Preg and PE.

Perspective

Normal pregnancy is associated with uteroplacental and vascular remodeling in order to adapt for the growing fetus. Genetic and environmental factors cause defective placentation, altered maternal immune response, and abnormal expression of integrins, inflammatory cytokines and MMPs, leading to apoptosis of trophoblast cells, shallow trophoblastic invasion and inadequate spiral artery remodeling, RUPP, and placental ischemia/hypoxia. Ischemic/hypoxic placenta causes the release of bioactive factors such as sFlt-1, sEng, TNFα, IL-6, HIF, ROS and AT$_1$-AA. Bioactive factors could affect endothelial cells, cause endothelial dysfunction, decrease vasodilators or increase ET-1, or target MMPs in ECM leading to increased vasoconstriction, altered uteroplacental and vascular remodeling, increased uteroplacental and vascular collagen, growth-restrictive remodeling, and HTN-Preg.[156,334] These bioactive factors and vascular mediators may be different with respect to timing, severity and mechanisms in early versus late-onset PE. Further understanding of the interaction between bioactive factors, vascular mediators and molecular mechanisms should help design more efficient measures for early detection and management of PE.

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List Of Abbreviations:

2-ME	2-methoxy estradiol
ACh	acetylcholine

AngII	angiotensin II
ARB	angiotensin receptor blocker
AT$_1$R	AngII type 1 receptor
AT$_1$-AA	AngII AT$_1$R agonistic autoantibodies
BP	blood pressure
$[Ca^{2+}]_c$	cytosolic free Ca^{2+} concentration
cGMP	cyclic guanosine monophosphate
COX	cyclooxygenase
DOCA	deoxycorticosterone acetate
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor
eNOS	endothelial nitric oxide synthase
EMMPRIN	extracellular MMP inducer
EGF	epidermal growth factor
ET-1	endothelin-1
HO	hemeoxygenase
H$_2$O$_2$	hydrogen peroxide
HIF	hypoxia-inducible factor
HELLP	hemolysis elevated liver enzymes low platelets
HTN-Preg	hypertension in pregnancy
HUVECs	human umbilical vein endothelial cells
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IUGR	intrauterine growth restriction
L-NAME	N$_\omega$-nitro-L-arginine methyl ester
MAPK	mitogen-activated protein kinase
MEGJ	myoendothelial gap junction
MMP	matrix metalloproteinase
NO	nitric oxide

NOS	nitric oxide synthase
O2•-	superoxide anion
PE	preeclampsia
PGI$_2$	prostacyclin
PlGF	placental growth factor
PKC	protein kinase C
RAS	renin-angiotensin-system
ROCK	Rho-kinase
ROS	reactive oxygen species
RUPP	reduced uterine perfusion pressure
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase-1
TGF-β	transforming growth factor-β
TIMP	tissue inhibitor of metalloproteinases
TNFα	tumor necrosis factor-α
TXA$_2$	thromboxane A2
VEGF	vascular endothelial growth factor
VCAM-1	vascular cell adhesion molecule-1
VSM	vascular smooth muscle

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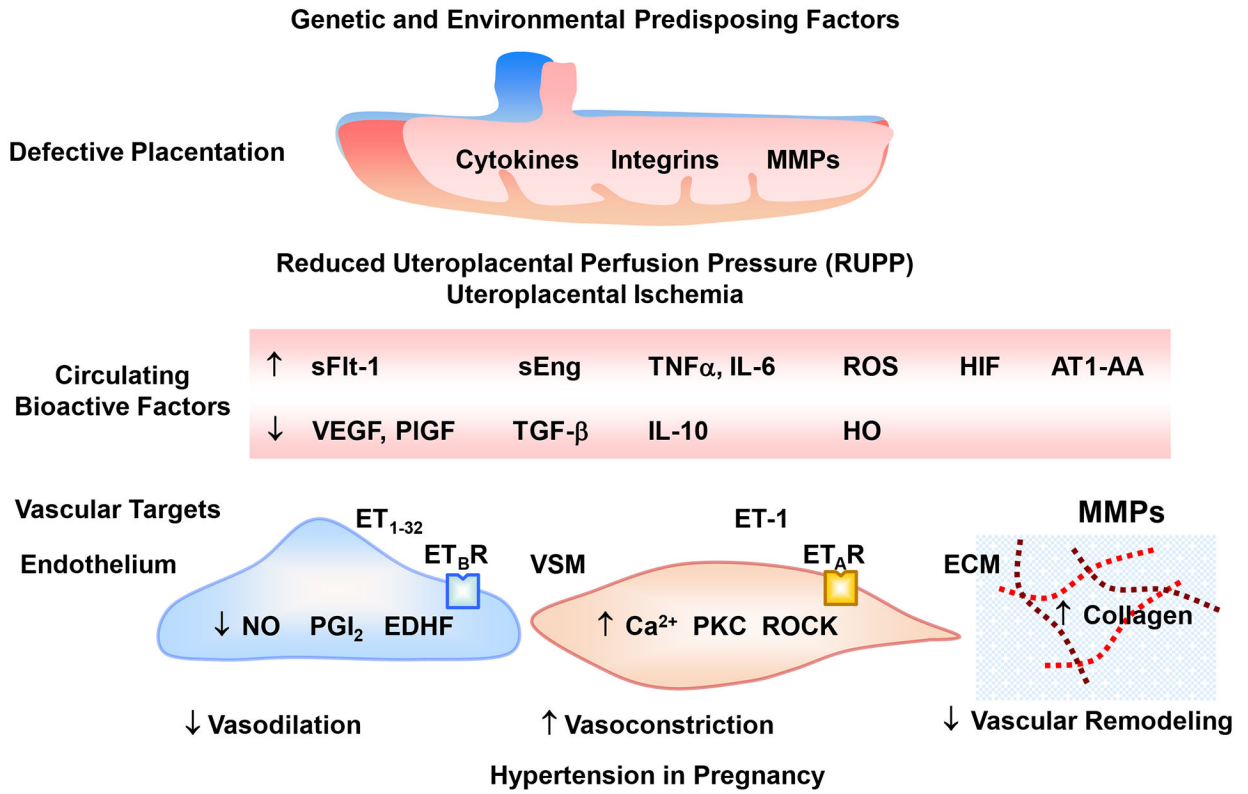
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Genetic and Environmental Predisposing Factors

Defective Placentation

Cytokines Integrins MMPs

Reduced Uteroplacental Perfusion Pressure (RUPP)
Uteroplacental Ischemia

Circulating Bioactive Factors

| ↑ | sFlt-1 | sEng | TNFα, IL-6 | ROS | HIF | AT1-AA |
| ↓ | VEGF, PlGF | TGF-β | IL-10 | HO | | |

Vascular Targets

Endothelium

ET$_{1-32}$
ET$_B$R
VSM
ET-1
ET$_A$R
MMPs
ECM

↓ NO PGI$_2$ EDHF

↑ Ca^{2+} PKC ROCK

↑ Collagen

↓ Vasodilation   ↑ Vasoconstriction   ↓ Vascular Remodeling

Hypertension in Pregnancy

Fig. 1.

Mechanisms of microvascular dysfunction in HTN-Preg. Initial reduction of uteroplacental perfusion pressure (RUPP) and uteroplacental ischemia causes the release of bioactive and circulating factors, which target blood vessels leading to decreased endothelium-dependent vascular relaxation pathways, increased endothelin-1 (ET-1) and mechanisms of VSM contraction, and abnormalities in MMPs expression/activity and increased collagen deposition in extracellular matrix (ECM), resulting in increased vascular resistance and HTN-Preg.

AT$_1$-AA, AngII AT$_1$R agonistic autoantibodies; EDHF, endothelium-derived hyperpolarizing factor; HIF, hypoxia-inducible factor; HO, hemeoxygenase, IL-6, interleukin-6; NO, nitric oxide; PKC, protein kinase C; PlGF, placental growth factor; ROS, reactive oxygen species; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1; TNFα, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; VSM, vascular smooth muscle