



Published in final edited form as:

Prog Mol Biol Transl Sci. 2017 ; 148: 87–165. doi:10.1016/bs.pmbts.2017.04.001.

Matrix Metalloproteinases in Normal Pregnancy and Preeclampsia

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Abstract

Normal pregnancy is associated with marked hemodynamic and uterine changes that allow adequate uteroplacental blood flow and uterine expansion for the growing fetus. These pregnancy-associated changes involve significant uteroplacental and vascular remodeling. Matrix metalloproteinases (MMPs) are important regulators of vascular and uterine remodeling. Increases in MMP-2 and MMP-9 have been implicated in vasodilation, placentation and uterine expansion during normal pregnancy. The increases in MMPs could be induced by the increased production of estrogen and progesterone during pregnancy. MMP expression/activity may be altered during complications of pregnancy. Decreased vascular MMP-2 and MMP-9 may lead to decreased vasodilation, increased vasoconstriction, hypertensive pregnancy and preeclampsia. Abnormal expression of uteroplacental integrins, cytokines and MMPs may lead to decreased maternal tolerance, apoptosis of invasive trophoblast cells, inadequate remodeling of spiral arteries, and reduced uterine perfusion pressure (RUPP). RUPP may cause 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, or stimulate the release of inflammatory cytokines, hypoxia-inducible factor, reactive oxygen species, and angiotensin AT₁ receptor agonistic autoantibodies. These circulating factors could target MMPs in the extracellular matrix as well as endothelial and vascular smooth muscle cells, causing generalized vascular dysfunction, increased vasoconstriction and hypertension in pregnancy. MMP activity can also be altered by endogenous tissue inhibitors of metalloproteinases (TIMPs) and changes in the MMP/TIMP ratio. In addition to their vascular effects, decreases in expression/activity of MMP-2 and MMP-9 in the uterus could impede uterine growth and expansion and lead to premature labor. Understanding the role of MMPs in uteroplacental and vascular remodeling and function could help design new approaches for prediction and management of preeclampsia and premature labor.

Keywords

Blood Vessels; Contraction; Cytokines; Estrogen; Growth Factors; Hypertension; Hypoxia; Placental Ischemia; Progesterone; Uterus

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CONFLICT OF INTEREST

None

1. INTRODUCTION

Normal pregnancy is associated with several uteroplacental and hemodynamic changes in order to meet the growing and metabolic demands of the developing fetus. The pregnant uterus undergoes hypertrophy and distension in order to provide sufficient space for the growing fetus. Placental remodeling and cytotrophoblast invasion of spiral arteries maintain adequate blood supply to the developing fetus.^{1,2} Also, the increases in maternal blood volume and cardiac output are counterbalanced by systemic vasodilation and decreased vascular resistance, leading to only slight change in blood pressure.^{3,4} These uterine and vascular changes involve marked uteroplacental and vascular remodeling and redistribution of blood flow in different maternal tissues and organs.^{5,6}

Matrix metalloproteinases (MMPs) are zinc-dependent proteases that play a role in tissue remodeling.^{7,8} MMPs include collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other MMPs with different tissue expression, distribution and substrate specificity.⁹ MMPs degrade various proteins in the extracellular matrix (ECM) including collagen and elastin.⁹ MMPs are produced as pro-MMPs which are cleaved by other MMPs or proteases into active MMPs.^{9,10} MMPs play a role in endometrial tissue remodeling during the estrous cycle and menstrual cycle, and may be involved in the uterine and vascular tissue remodeling during normal pregnancy.⁸

In 5 to 8% of pregnancies, women may have hypertension in pregnancy manifested in one of four forms: chronic hypertension that predates pregnancy, preeclampsia-eclampsia, chronic hypertension with superimposed preeclampsia, and nonproteinuric gestational hypertension.¹¹ Preeclampsia is diagnosed after the 20th week of pregnancy by new onset hypertension (systolic pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg), often proteinuria and may be associated with edema and increased platelet aggregation.¹² Preeclampsia may also be a part of hemolysis elevated liver enzymes low platelets (HELLP) syndrome. If not treated, preeclampsia may progress to eclampsia, characterized by severe hypertension and convulsions, which could culminate into coma and death, causing an estimated 14% of pregnancy-related maternal deaths.¹³

Premature labor is another major complication of pregnancy that could lead to prematurity at birth and neonatal death. Preterm delivery complicates 10% to 15% of all pregnancies, and is a leading cause of perinatal morbidity and death.¹⁴ Preeclampsia may be associated with intrauterine growth restriction (IUGR), a condition that could cause premature birth.¹⁵ Of the spontaneous births, 3.2% of preterm births and 2.2% of very preterm births are associated with preeclampsia.¹⁶ These complications of pregnancy are particularly important in developing countries where the incidence of preeclampsia is greater and the rates of maternal mortality and preterm births are higher than those in developed countries.¹⁷

Although preeclampsia and premature labor are major causes of maternal and fetal morbidity and constitute a significant burden on the healthcare system, their etiology and pathophysiology are not fully understood. Certain genetic and environmental risk factors may trigger reduction in uteroplacental perfusion pressure (RUPP) and the resulting placental ischemia/hypoxia could cause the release of bioactive factors that could target the

blood vessels and the uterus (Fig. 1). Because of the difficulty to perform mechanistic studies in pregnant women, and in order to further understand the pathophysiological mechanisms of preeclampsia, animal models of hypertension in pregnancy have been developed. Studies in animal models support that placental ischemia could be an initiating event, and RUPP in pregnant rats shows some of the characteristics of preeclampsia including hypertension in pregnancy and IUGR.^{18–20} Studies have also shown changes in the local and circulating levels of pro-angiogenic vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), the anti-angiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), hypoxia-inducible factor (HIF), reactive oxygen species (ROS) and angiotensin II (AngII) type 1 receptor (AT₁R) agonistic autoantibodies (AT₁-AA). These bioactive factors could target systemic vessels causing generalized vascular dysfunction and hypertension, renal glomeruli causing glomerular endotheliosis and increased glomerular permeability and proteinuria, cerebral vessels causing cerebral edema and seizures,^{11,21} and possibly the uterus leading to premature labor.

Because normal pregnancy involves significant uterine and vascular remodeling, and because MMPs are major regulators of tissue remodeling, MMPs may be involved in the uteroplacental and vascular remodeling during normal pregnancy. Changes in MMP expression/activity could also cause uterine and vascular dysfunction and in turn contribute to the pathogenesis of preeclampsia and premature birth. These observations have raised interest in the hormonal and growth factors that could affect MMP expression/activity during normal pregnancy, and the bioactive factors that could target uteroplacental and vascular MMPs in the setting of preeclampsia and premature labor.

In this chapter, we will discuss MMP expression/activity in blood vessels during pregnancy and how changes in vascular MMP could lead to severe vasoconstriction and increased blood pressure in human preeclampsia and animal models of hypertension in pregnancy. We will describe the potential upstream mechanisms that could cause changes in MMPs including the genetic and demographic risk factors, RUPP and placental ischemia, and discuss how the release of bioactive factors could target MMPs in the systemic vessels. We will also describe MMP expression/activity in the uterus during normal pregnancy and how changes in uterine MMP could lead to excessive uterine contraction and premature labor. Because of the ambiguity of the etiology and pathogenesis of preeclampsia and premature labor, the available remedies are currently limited. We will discuss how the advances in our knowledge of the role of MMPs and the potential predisposing factors and circulating bioactive factors could help design new biomarkers for diagnosis, and novel approaches for management of preeclampsia and premature labor.

2. VASCULAR AND PLACENTAL MMPs DURING PREGNANCY

Normal pregnancy is associated with significant hemodynamic, vascular and uteroplacental changes to ensure adequate placentation of the embryo, and sufficient blood and nutrient supply to the developing fetus. Normal pregnancy is associated with marked vasodilation of the maternal uterine, renal and systemic vessels,²² and reduction in the mechanisms of vascular contraction.^{23,24} The pregnancy-associated vasodilation and reduction in vascular

contraction could be related to increased plasma levels of the female sex hormones estrogen and progesterone.²⁵ For instance, estrogen causes relaxation of vascular smooth muscle (VSM) of the rat aorta and uterine artery.^{26,27} Also, progesterone inhibits contraction of rat blood vessels.²⁶ Adequate placentation is also critical during normal pregnancy. Extravillous trophoblasts invade the maternal decidua and remodels spiral arteries to achieve maximal vasodilation and adequate nutrient supply to the embryo. Trophoblast invasion into the decidual stroma may require degradation of ECM proteins by proteolytic enzymes such as MMPs. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) play a role in endometrial tissue remodeling during the menstrual cycle and pregnancy^{28–30}. MMP-2 and MMP-9 are abundantly expressed in invading extravillous trophoblast cells, and the expression of these two gelatinases is highly related to trophoblast cell invasiveness.^{31–34} Also, factors that promote trophoblast invasion appear to have regulatory effects on MMP-2 and/or MMP-9 activity. For instance, epidermal growth factor (EGF)-mediated induction of trophoblast invasion is associated with increased expression/activity of MMP-2 and MMP-9.^{35,36} MMP-2 is the main MMP in the umbilical cord¹¹, and serum MMP-9 level is elevated in normal pregnant women.⁷ The pregnancy-associated increase in expression/activity of vascular MMPs may be partly induced by the female sex hormones estrogen and progesterone. We have shown that the expression and activity of MMP-2 and -9 are increased in the aorta during pregnancy in rats.³⁷ Also, consistent with the report that estrogen enhances the release of MMP-2 from human VSM cells,³⁸ we found that estrogen +progesterone enhanced MMP-2 and MMP-9 expression/activity in the aorta of virgin rats. The pregnancy-associated increases in vascular MMPs could play a role in vascular remodeling, angiogenesis, and the systemic changes in blood vessels.³⁹ In addition to their proteolytic effects, MMPs may affect membrane receptors and cell signaling. For instance, MMP-2 and MMP-9 cause relaxation of precontracted rat aorta⁴⁰ and inferior vena cava.^{41,42} The increased expression/activity of MMPs in the aorta together with reported effects of MMPs effects on vascular remodeling and contraction mechanisms are consistent with a role of MMPs in the reduced vascular contraction and enhanced systemic vasodilation during pregnancy.

Another factor that could affect MMP expression/activity is extracellular MMP inducer (EMMPRIN, CD147, Basigin, BSG). EMMPRIN is a widely expressed membrane protein of the immunoglobulin superfamily,⁴³ that has been implicated in tissue remodeling⁴⁴ and various pathological conditions including cancer,⁴³ rheumatoid arthritis⁴⁵, heart failure,⁴⁶ and atherosclerosis.⁴⁷ EMMPRIN stimulates the production of MMP-1, MMP-2, MMP-3, and MMP-9,⁴⁸ and may regulate MMPs in endothelial cells and tumors.⁴⁹ We have shown that EMMPRIN expression is increased in the aorta of late-pregnant compared with virgin and mid-pregnant rats as well as in the aorta of virgin rats pretreated with estrogen +progesterone. The sex hormone-induced increases in aortic MMP-2 and MMP-9 expression/activity were blocked by EMMPRIN neutralizing antibody, supporting a role of EMMPRIN in the increases in vascular MMPs.³⁷ These observations are consistent with the contention that the pregnancy-associated increase in expression/activity of vascular MMP-2 and MMP-9 are mediated by EMMPRIN and induced by the female sex hormones estrogen and progesterone.

In contrast with the pregnancy-associated increases in MMPs in the aorta, MMP-2 and MMP-9 expression did not increase in the placenta of late pregnant rats. In effect, a decrease in the expression/activity of placental MMP-2 and MMP-9 was observed in late compared with mid-pregnancy in rats.³⁷ Some studies have shown an increase in MMP-2 and MMP-9 in the placenta of diabetic rats at mid-gestation,⁵⁰ but did not follow the changes in MMPs during late-gestation. Other studies have suggested that MMPs are involved in placental remodeling during pregnancy.⁵⁰ Serum MMP-2 and MMP-9 activity are increased in pregnant bitches.⁵¹ Also, the expression of MMP-2, MMP-14 and EMMPRIN is increased in the bovine placenta during late gestation.⁵² The differences in the expression of MMPs in the rat versus canine or bovine placenta could be related to species differences in the MMP regulation mechanisms or differences in the role of MMPs in early, mid, and late pregnancy. It is possible that the placenta as a potential source of MMPs may have finite capacity particularly during the late stages of pregnancy. Also, most of the placental remodeling takes place during the peri-implantation period and during fetal and organ development in early and mid-gestation, and further placental remodeling may not be needed during late pregnancy. Interestingly, we observed that the pregnancy-associated decrease in placental MMPs expression was associated with a decrease in the expression of placental EMMPRIN in late-pregnant compared with mid-pregnant rats, supporting a role of EMMPRIN as a critical inducer of MMPs during pregnancy.³⁷ The decreased MMP-2 and MMP-9 and reduced EMMPRIN expression in the placenta of late-pregnant rats suggest reduced role of these MMPs in the fetoplacental circulation during late pregnancy. While the observed changes in MMP-2 and MMP-9 highlight their role in vascular remodeling during pregnancy, that should not minimize possible involvement of other members of the MMP family.

3. UTEROPLACENTAL AND VASCULAR CHANGES IN PREECLAMPSIA

Preeclampsia is a major complication of pregnancy characterized by hypertension in pregnancy and often proteinuria,^{11,21} and is a major cause maternal and fetal morbidity and mortality, IUGR, fetal programming of cardiovascular and metabolic disease, and predisposition to adulthood hypertension and diabetes.^{53,54} Observational studies in preeclamptic women and mechanistic studies in animal models of hypertension in pregnancy have helped to uncover some of the pathogenic mechanisms.^{19,55–58} RUPP during late pregnancy in sheep, dog, rabbit and rat has been shown to induce a hypertensive state that closely resembles preeclampsia.^{19,56} Also, certain risk factors have been associated with placental ischemia as a potential initiating pathogenic event.^{18,23} Placental ischemia has also been associated with increased release of bioactive factors,^{20,59–62} which could target various vascular mediators and MMPs in the vascular ECM, endothelial cells and VSM leading to increased vasoconstriction and hypertension in pregnancy (Fig. 1).

4. RISK FACTORS IN PREECLAMPSIA

Predisposing genetic, demographic and environmental factors could affect placental development. Mutations in placental genes have been associated with preeclampsia, and 31 out of 36 placental genes are downregulated in preeclampsia.⁶³ Mutations in placental mitochondrial genes could interfere with oxygen reduction leading to accumulation of ROS

and oxidative stress in the uteroplacental circulation.⁶⁴ 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 preeclampsia.⁶⁵ Also, wild-type female mice crossed with transgenic male mice overexpressing human *STOX1* show preeclamptic features including hypertension and proteinuria.⁶⁶ *FOXP3* is another gene that plays a role in the activation of regulatory T cells (Tregs) and thereby controls the immune response and maternal tolerance during normal pregnancy. Downregulation or polymorphism in the *FOXP3* gene could alter the maternal immune response, reduce maternal tolerance and predispose to preeclampsia.^{67,68} The role of paternal genes in preeclampsia has been the subject of debate. Although some studies showed a 2.7% risk of preeclampsia associated with men whose mothers developed preeclampsia compared with men whose mothers had normal pregnancy,⁶⁹ other studies showed a limited association between paternal genes and preeclampsia.⁷⁰

Ethnic background, age, maternal lifestyle, pre-pregnancy weight, previous and family history of preeclampsia, primiparity, and multiple pregnancy could be risk factors for preeclampsia.⁶ The rate of preeclampsia is higher among African-American (5.2%) than Asian women (3.5%).⁷¹ Very young <16 years or older women >40 years are more prone to preeclampsia, and studies in Finland and India have supported that older women are at higher risk of developing preeclampsia than young women.^{72,73} The incidence of preeclampsia 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 50.⁷⁴ Preexisting medical condition such as heart disease, chronic respiratory conditions, diabetes, renal disorders, systemic lupus erythematosus, mental stress, reproductive tract surgery and history of antepartum hemorrhage may also increase the risk for preeclampsia.⁶ Importantly, cardiovascular and pulmonary disorders are associated with changes in tissue expression/activity of MMPs, which could contribute to the inadequate uteroplacental and vascular remodeling in preeclampsia.

5. ABNORMAL PLACENTATION AND PLACENTAL ISCHEMIA IN PREECLAMPSIA

During early pregnancy, the placenta is developed as a maternal-fetal interface through several processes including vasculogenesis, angiogenesis, trophoblast invasion and vascular remodeling. Vasculogenesis is the development of *de novo* vessels from pluripotent mesenchymal stem 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 growth factors and the invasive capability of trophoblast cells.⁷⁵ Healthy pregnancy requires sufficient 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.⁷⁶ 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 (Fig. 2).

The symptoms of preeclampsia remit after delivery of the baby and the placenta, implicating the placenta as a central culprit in the disorder. Abnormal placentation, RUPP and placental ischemia/hypoxia are important initiating events in preeclampsia.^{18,20,56} Inadequate placentation could be caused by abnormal inflammatory and immune responses and accumulation of natural killer (NK) cells and macrophages, apoptosis of trophoblast cells and decreased invasion of spiral arteries, and abnormal expression of integrins and MMPs leading to decreased ECM remodeling, shallow trophoblast invasion and poor spiral arteries remodeling (Fig. 2).

5.1. Immune Responses and Inadequate Placentation in Preeclampsia

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 excessive maternal immune response.⁷⁷ Preeclampsia is associated with augmented immune and inflammatory responses and increased production of the pro-inflammatory cytokines TNF- α and IL-6. In support of altered immune response in preeclampsia, HIV-positive women, who often have suppressed immune response, show lower incidence rates of hypertensive disorders and preeclampsia.⁷⁸

During normal pregnancy, cytotrophoblasts overexpress 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.⁷⁹ A decrease in the HLA-C/KIR interaction would lead to increased activity of NK cells, which would in turn attack placental and fetal tissues and lead to preeclampsia.⁸⁰

Healthy pregnancy is associated with moderate activation of the complement system. Increased complement activation products Bb, C3a and C5a have been associated with preeclampsia.⁸¹ Also, small subcutaneous vessels from preeclamptic women show more neutrophils adherent to the endothelium than vessels from normal pregnant women, which may contribute to the endothelial dysfunction in preeclampsia.⁸² Interestingly, inhibition of complement activation or depletion of neutrophils decreases blood pressure in the RUPP rat model of placental ischemia, supporting a role of complement activation and innate immune response in hypertension in pregnancy.^{81,83}

MMPs are released by inflammatory cells and MMP expression/activity is increased in various inflammatory and autoimmune disorders. An increase in the inflammatory and immune responses is expected to alter uteroplacental and vascular MMP expression/activity and consequently uteroplacental and vascular remodeling in preeclampsia.

5.2. Integrins and Reduced Trophoblast Invasion and Spiral Artery Remodeling

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 (Fig. 2).⁸⁴ These phenotypic changes in integrins may be impaired during placental hypoxia and preeclampsia. Hypoxia alters the placental expression of integrins and fibronectin, causing increased expression of integrin α_5 and fibronectin and decreased expression of integrin α_1 .⁸⁵ Also, during preeclampsia, abnormal expression of epithelial cell-type adhesion molecules and apoptosis of cytotrophoblasts cause limited invasion of spiral arteries, placental ischemia and RUPP.^{84,86,87} Ezrin is one of the integrins involved in cell adhesion, organization and migration. Ezrin is downregulated in syncytiotrophoblast microvesicles from preeclamptic women, resulting in reduced invasiveness of cytotrophoblasts, shallow placentation and defective vascularization of the placenta.⁸⁸ The decreased trophoblast invasion and replacement of vascular cells also leads to retention of VSM cells in the spiral arteries, which promote vasoconstriction,⁸⁹ and further decrease uteroplacental blood flow and aggravate placental ischemia (Fig. 2).

Endothelial cell adhesion molecules such as soluble intracellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) are downregulated during normal pregnancy, thus minimizing leukocyte adhesion to endothelial cells, and maintaining patency and blood flow in the spiral arteries. The plasma levels of ICAM-1 and VCAM-1 are increased in preeclampsia, leading to increased leukocyte adhesion to endothelial cells and restricted blood flow in the spiral arteries.⁹⁰

Preeclampsia is also associated with increased placental expression of microRNA miRNA-125b-1-3p which could reduce the expression of S1PR1, a G-protein coupled receptor that facilitates invasion of human trophoblasts.⁹¹ Preeclampsia is also associated with increased expression of placental miRNA-517a/b and miRNA-517c, which have been shown to be expressed and to decrease trophoblast invasion in extravillous trophoblasts under hypoxic conditions⁹².

5.3. MMPs, Abnormal Placentation, and Placental Ischemia

MMPs such as MMP-2 and MMP-9 may be involved in ECM remodeling and trophoblast invasion of the spiral arteries during pregnancy.³¹⁻³⁴ The amount and activity of MMP-2 and MMP-9 are increased in the aorta of normal pregnant rats, suggesting a role of MMPs in the pregnancy-associated vascular remodeling.^{37,93} In support of a role of MMPs in uteroplacental remodeling, in first trimester trophoblasts suppression of MMP-9 expression inhibits the invasive capability of trophoblasts.⁹⁴ Also, MMP-9 ablation in MMP-9 knockout mice shows a phenotype that mimics preeclampsia possibly due to impaired trophoblast differentiation and invasion.⁹⁵ Genetic polymorphisms in MMP-2 and MMP-9 transcription have been described in preeclampsia.⁹⁶ Decreased levels of MMP-9 have also been observed in preeclamptic compared with normal placenta.^{97,98} In preeclampsia, increased expression of miRNA-519d-3p and miRNA-204,⁹⁹ could target MMP-2 and MMP-9 and decrease trophoblast invasion of spiral arteries.⁹⁴ Collectively, these observations suggest a relationship between decreased MMP-2 and MMP-9 and impaired trophoblast invasion in

preeclampsia. On the other hand, measurements of the levels of MMPs have not been consistent in preeclampsia, with some studies showing an increase in serum levels of MMP-2 and MMP-9,¹⁰⁰ while other studies showing a decrease in circulating MMP-9 (Table 1).⁷ Further measurements of the plasma levels of MMPs and their correlation with MMP levels in the placenta and other maternal tissues are needed in both human preeclampsia and animal models of hypertension in pregnancy.

Because collagen is a major substrate of MMPs, a decrease in MMP-2 and MMP-9 is expected to cause excessive collagen deposition, and in turn decrease uteroplacental vascularization and spiral arteries remodeling.⁸ However, gelatinases mainly degrade collagen IV, and partially degrade collagen I, suggesting that other MMPs are involved. 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 preeclamptic versus normal pregnant women, and the low MMP-1 levels are correlated with the severity preeclampsia.¹⁰¹ Other studies suggest a role of MMP-1 in the pathogenesis of preeclampsia.¹⁰² Also, the matrilysin MMP-7 could play a role in endometrial tissue remodeling during the menstrual cycle and pregnancy.¹⁰³ Studies have also shown that cytotrophoblasts and VSM release MMP-12, which could mediate elastolysis and remodeling of the uterine spiral arteries during pregnancy.¹⁰⁴ Whether the uteroplacental and vascular MMP balance is altered in hypertension in pregnancy is a subject of topical interest. Also, the upstream mechanisms influencing MMPs activity and the downstream substrates and pathways via which MMP imbalance could affect uteroplacental remodeling and vascular function are emerging new areas for research.

We have examined whether alteration of MMP expression/activity is a potential pathogenic mechanism in the uteroplacental and vascular remodeling and placental ischemia in hypertension in pregnancy. We measured the hemodynamic and uteroplacental changes in normal pregnant rats and the RUPP rat model of placental ischemia. Blood pressure was increased, and the litter size and individual pup weight were decreased in RUPP versus normal pregnant rats.^{105,106} We further 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 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 the uterus, placenta and vasculature of RUPP rats. Also, the fetal litter size and individual pup weight are decreased in RUPP compared with normal pregnant rats.⁸

In search for the mechanisms involved in the changes in uteroplacental and vascular remodeling, Western blots, gelatin zymography and immunohistochemical analysis revealed that MMP-2 and MMP-9 were abundantly expressed in tissues of normal pregnant rats, supporting a role of MMPs in the uteroplacental and vascular remodeling during normal pregnancy.³⁷ MMPs immunostaining was particularly apparent in the aortic media, consistent with reports that VSMCs are a major source of MMPs.^{107,108} The levels of

MMP-2 and MMP-9 appear to be decreased in the uterus, placenta and aorta of RUPP compared with normal pregnant rats (Table 2). Western blots and gelatin zymography revealed decreases in protein amount and gelatinase activity of MMP-2 and MMP-9 in tissues of RUPP rats. MMP-2 and MMP-9 immunostaining was also reduced in uterine and placenta tissue sections and less intense in the aortic media of RUPP versus normal pregnant rats. The decreases in MMPs expression/activity, in parallel with the decreases in uterine, placental, and aortic tissue weight and cross-sectional area suggest a role for reduced MMPs expression/activity in growth-restrictive remodeling in tissues of RUPP rats.⁸

While the changes in MMPs in the aorta of RUPP rats suggest a role in hypertension in pregnancy, the mechanisms linking localized RUPP to the systemic changes in MMPs and the potential bioactive factors involved need to be examined.

6. CIRCULATING BIOACTIVE FACTORS and MMPs IN PREECLAMPSIA

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 TNF- α and IL-6, HIF, ROS and AT₁-AA (see Fig. 1).^{6,57–59,62,109–111} These factors could target uteroplacental and vascular MMPs and cause further vasoconstriction of spiral arteries and placental ischemia, as well as generalized vasoconstriction and increase in blood pressure in preeclamptic women and animal models of hypertension in pregnancy.^{21,112}

6.1. Pro-angiogenic and Anti-angiogenic Factors in Preeclampsia

6.1.1 Vascular Endothelial Growth Factor (VEGF)—The *VEGF* gene has a gene locus on chromosome 6p21.3, which 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.¹¹² 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 placental blood vessels.¹¹² VEGF regulates endothelial cell proliferation, angiogenesis and vascular permeability.^{21,112} In endothelial cells, VEGF increases $[Ca^{2+}]_i$, Ca^{2+} /calmodulin, endothelial nitric oxide synthase (eNOS) activity, and prostacyclin (PGI₂).^{113–115} VEGF could also promote Ca^{2+} -independent generation of NO by promoting Akt activation and eNOS Ser¹¹⁷⁷ phosphorylation in human umbilical vein endothelial cells (HUVECs).¹¹⁵

Some studies show an increase in circulating VEGF in preeclampsia.^{116–118} Also, villous explants from preeclampsia produce greater amounts of VEGF than those from normal pregnant women.¹¹⁹ It is likely that the severe vasoconstriction in preeclampsia would increase vascular shear-stress, and in turn increase circulating VEGF.⁶ Other studies have shown a decrease or unchanged serum levels of VEGF in preeclampsia.^{120,121} Women with the T allele of VEGF 936C/T have lower levels of VEGF and a higher risk of preeclampsia than women with VEGF 936C/C.¹²² Plasma VEGF levels are decreased in RUPP rat model of hypertension in pregnancy,²⁰ although as with the findings in human villous explants, placenta from RUPP rats show greater production of VEGF.¹²³ The differences in the results may be related to differences in the methods of VEGF measurement.¹²⁰ Also, in preeclampsia, an increase in circulating anti-angiogenic factors may bind to VEGF. Thus, total (bound and unbound) VEGF measured using radioimmunoassay or competitive enzyme

immunoassay could be higher while free VEGF measured using enzyme-linked immunosorbent assay (ELISA) may be lower in preeclamptic than normal pregnant women.¹²⁴

A decrease in VEGF may also play a role in the glomerular endotheliosis and proteinuria in preeclampsia. 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 has been detected in genetic glomerular VEGF deficiency.¹²⁵ Also, in clinical cancer trials the use of VEGF-neutralizing antibodies is associated with proteinuria.¹²⁶ In mice, infusion of VEGF antibodies leads to glomerular endotheliosis and proteinuria.¹²⁷ Also, mice lacking one VEGF allele in renal podocytes develop a renal pathology similar to that in preeclampsia. Thus, a decrease in VEGF could cause proteinuria and other renal pathology in preeclampsia. 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 hypertension in pregnancy.^{128,129}

MMPs may induce the release of growth factors by cleaving the growth factor-binding proteins or matrix molecules, and these effects may contribute to the altered tissue remodeling in preeclampsia. MMPs may also be regulated by growth factors.¹³⁰ Platelet derived growth factor-BB increases MMP-2 expression in rat VSMCs, possibly via Rho-associated protein kinase, extracellular signal-regulated kinases, and p38 mitogen-activated protein kinase (MAPK).¹³¹ Also, in carotid plaques, EGF enhances MMP-9 activity and increases MMP-1 and MMP-9 mRNA transcripts in VSMCs.¹³² Angiogenic growth factors such as VEGF and TGF- β are secreted by endothelial cells and other cells and act in an autocrine or paracrine fashion to accelerate angiogenesis. 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.³⁹ 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 PI₃K and MAPK pathways.¹³³ The interaction between MMPs and VEGF in the setting of uteroplacental and vascular remodeling in normal pregnancy and preeclampsia should be further examined.

6.1.2 Placental Growth Factor (PlGF)—PlGF is a pro-angiogenic factor that binds to VEGFR-1 and enhances the angiogenic effects of VEGF.¹³⁴ 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. PlGF promotes endothelial cell growth, placental vasculogenesis and development, and vasodilation of uterine vessels.²¹

Plasma PlGF levels are low in non-pregnant women (~44 pg/mL), and markedly increase during normal pregnancy.¹³⁴ PlGF levels are ~353 pg/mL during gestational weeks 21 and 22, rising steadily to ~574 pg/mL after gestational weeks 29 and 30.¹³⁵ Circulating PlGF levels decrease in preeclampsia^{116,136–138} and the decrease is more apparent in early than

late preeclampsia.¹³⁹ PIGF has four alternatively spliced mRNA forms (PIGF 1–4), and its predominant isoform PIGF-1 is downregulated in preeclampsia.¹²⁴ Circulating levels of PIGF are also decreased in RUPP and deoxycorticosterone acetate (DOCA)-salt hypertensive rats.^{20,140}

In addition to its growth promoting effects, PIGF promotes vasodilation via VEGFR-1 and endothelium-derived hyperpolarizing factor (EDHF)-mediated activation of small conductance Ca^{2+} -activated K^+ channels (SK_{Ca}).^{141,142} In small mesenteric arteries of pregnant rats treated with L-NAME and indomethacin, a second exposure to PIGF produces greater vasodilation and greater reduction in VSM $[\text{Ca}^{2+}]_i$ than the first PIGF application. VEGF and PIGF may promote VEGFR-1 dimerization, and the initial exposure to PIGF may facilitate the formation of receptor homodimers and their submembrane signaling, leading to augmented vasodilator responses to repeated PIGF stimulation.¹⁴² A decrease in the levels of PIGF may be partly responsible for the decreased vasodilator responses in preeclampsia.

6.1.3 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 PIGF 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.¹⁴³ Trophoblast cells express sFlt-1 mRNA, and the sFlt-1 level increases to ~1.5 ng/mL in normal pregnant women compared to ~0.15 ng/mL in non-pregnant women.²¹ 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 PIGF levels. Preeclamptic women show imbalance between sFlt-1, VEGF and PIGF.^{116,120,137,139,144–149} 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 levels of sFlt-1, reduced PIGF and increased risk of preeclampsia.¹⁵⁰ Several reports have shown higher circulating levels of sFlt-1 in early and late preeclampsia.^{116,136–139,149} Serum sFlt-1 is also higher in women with previous preeclampsia (~0.5 ng/mL) than in women with previous normal pregnancy (~0.3 ng/mL), and the increases can be detected even 6 months or more after delivery.¹⁴⁹ sFlt-1 levels are also greater in villous explants from preeclamptic compared with normal pregnant women.¹¹⁹

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 up-regulation of sFlt-1.^{119,120} 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-1.⁹² 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 preeclamptic versus normal pregnant women.¹⁵¹

Because of the increased levels of sFlt-1, a 53% decrease in VEGF/sFlt-1 ratio and a 70% decrease in PIGF/sFlt-1 ratio have been observed in preeclamptic placenta.¹¹⁹ The

circulating sFlt-1/PlGF ratio is higher in preeclamptic than normal pregnant women from second trimester onwards and could be a potential predictor of the onset of preeclampsia,^{138,139} However, some studies suggest that the circulating sFlt-1/PlGF ratio could be lower in late versus early preeclampsia.^{139,152} 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.^{153,154} 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 has also been implicated in the changes in endothelin-1 (ET-1) levels. Preeclamptic women with sFlt-1/PlGF ratio >85 have higher levels of ET-1 than women with sFlt-1/PlGF ratio <85.¹⁵⁵ Importantly, extracorporeal removal of circulating sFlt-1 from preeclamptic patients decreases sFlt-1/PlGF ratio, improves symptoms and prolongs pregnancy,¹⁵⁶ further supporting a role of sFlt-1 in preeclampsia.

RUPP rats show increases in plasma and placental levels of sFlt-1 and plasma sFlt-1/PlGF ratio.^{20,123,157} Other animal models of hypertension in pregnancy show either increased or little change in circulating levels of sFlt-1.^{140,158–162} Importantly, infusion of exogenous sFlt-1 or adenoviral overexpression of sFlt-1 in pregnant rats causes increases in blood pressure, decreased plasma VEGF, proteinuria, and glomerular endotheliosis with occlusion of renal capillaries and focal fibrin deposition in glomerular cells.^{120,163–166} Also, mice treated with sFlt-1 show increased vascular response to ET-1.¹⁶⁷ Treatment of endothelial cells with plasma of preeclamptic patients decreases angiogenesis, and removal of sFlt-1 or treatment with VEGF or a sFlt-1 antibody reverses the anti-angiogenic effects of sFlt-1 and restores endothelial cell angiogenesis.¹¹⁹

Of note, VEGF through an action on VEGFR-2 stimulates the production of sFlt-1 in human placental explants.¹⁶⁸ This feedback modulation of VEGF by sFlt-1 may represent a local protective mechanism at the maternal-fetal interface to control the levels of VEGF and prevent any damage to the placenta or fetus caused by excess VEGF during normal pregnancy.¹⁶⁸ Dysregulation of this VEGF-sFlt-1 feedback mechanism may be involved in the pathogenesis of preeclampsia.

It has been suggested that sFlt-1 could cause generalized endotheliosis in blood vessels leading to hypertension, in the glomeruli leading to proteinuria, and in the brain vessels leading to seizures and eclampsia.¹⁶⁶ Whether sFlt-1 could target tissue MMPs particularly during pregnancy is not clear. Some studies suggest that sFlt-1-induced inhibition of VEGFR-2 could decrease endothelial VEGF production and MMP-2 and MMP-9 expression/activity.¹⁶⁹ Also, 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.¹⁷⁰ Our recent studies have supported a role of sFlt-1 as a potential upstream mechanism linking placental ischemia to the decrease in MMPs in hypertension in pregnancy.⁸ We found that sFlt-1 reduced MMPs expression/activity in uterine, placental and vascular tissues of normal pregnant rats. VEGF reversed the sFlt-1 induced decreases in MMPs in tissues of normal pregnant rats. Also, VEGF increased MMPs levels in tissues of RUPP rats to levels similar to those in normal pregnant rats. These observations are

consistent with the reports that infusion of VEGF reduces blood pressure in RUPP rats.^{171,172}

6.1.4 Soluble Endoglin (sEng)—Transforming growth factor- β 1 (TGF- β 1) is known to bind to TGF receptors and to induce proliferation and migration of endothelial cells.¹¹² Endoglin (Eng) is a co-receptor for TGF- β 1 and TGF- β 3 that is highly expressed on cell membrane of endothelial cells and syncytiotrophoblasts, where it mediates proliferation of angiogenic endothelial cells and possibly trophoblasts.¹⁷³ Mutations in *Eng* gene are associated with loss of capillaries, arteriovenous malformations, and hereditary hemorrhagic telangiectasia.¹⁷⁴ In comparison with Eng, sEng is an anti-angiogenic protein that binds TGF- β 1 and prevents it from binding to its natural angiogenic receptor, and thereby inhibits TGF- β 1-induced eNOS activation and vasodilation.¹¹² Hypoxia induces the release of sEng. In placental extracts, exposure to hypoxia increases the expression of sEng.¹⁷⁵

Serum levels of sEng are barely detectable in non-pregnant women and are much lower in normal pregnant women.¹⁷⁶ The levels of sEng are 3-, 5- and 10-fold higher in women with mild preeclampsia, severe preeclampsia and HELLP syndrome, respectively, compared with gestational age-matched control pregnant women.¹⁷⁶ Serum levels of sEng may be increased in both early and late preeclampsia.^{136,177} However, one study showed an increase in sEng levels at gestational weeks 10–17 in women who developed early preeclampsia, but not in those who developed late preeclampsia.¹⁵²

In RUPP rat model of hypertension in pregnancy, the levels of sEng are increased in the serum and placenta, and the serum levels of TGF- β are decreased.^{62,178} However, sEng levels did not show detectable change in DOCA-salt or L-NAME treated rat models of hypertension in pregnancy.^{140,158} It is likely that sEng acts in concert with the antiangiogenic factor sFlt-1 to promote vascular permeability, proteinuria, IUGR and severe hypertension.¹⁷⁶ In support, pregnant rats infused with both sEng and sFlt-1 show HELLP syndrome-like characteristics.¹⁷⁹ In cultured HUVECs, sEng impairs endothelial formation.¹⁷⁶ Whether sEng targets MMPs and affects uteroplacental and vascular remodeling in hypertension in pregnancy is unclear. Of note, MMP-14 cleaves Eng, the TGF- β co-receptor, and thereby inhibits its angiogenic effects,¹⁸⁰ and whether these effects play a role in preeclampsia need to be examined.

6.2. Cytokines, TNF- α , and Interleukins

Placental ischemia/hypoxia causes the release of pro-inflammatory cytokines.^{11,21,181} During placental reperfusion injury, reestablished blood flow causes the releases of TNF- α and interleukins (ILs).⁷⁷ The circulating levels of TNF- α are greater in preeclamptic than normal pregnant women,^{182–184} although the placental levels of TNF- α may not be different in preeclampsia versus normal pregnancy.¹⁸⁵ LIGHT, or TNF superfamily member 14, is also increased in preeclampsia and may contribute to placental ischemia.¹⁸⁶ The plasma levels and CD4⁺T cell production of TNF- α are increased in RUPP versus normal pregnant rats.^{109,110,178,181,187} Infusion of TNF α causes hypertension and proteinuria in late pregnant mice, rats, and baboons.^{161,162,188} Similarly, infusion of the TNF superfamily member LIGHT in pregnant mice causes increases in blood pressure, proteinuria, and the expression

of ET-1 and sFlt-1.¹⁸⁶ TNF- α may work in concert with other inflammatory cytokines such as IL-6 to increase ET-1 levels and cause hypertension in RUPP rats.¹⁸¹ TNF- α may also function in synergy with sFlt-1 to promote a pro-inflammatory and antiangiogenic state. Treatment of HUVECs with both TNF- α 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.¹¹⁵ In support of a role of TNF- α in hypertension in pregnancy, blockade of TNF- α with the TNF- α decoy receptor etanercept reduces blood pressure in RUPP rats. Also, treatment of HUVECs with serum from RUPP rats treated with the TNF- α blocker etanercept produces less ET-1 than serum from nontreated RUPP rats.¹⁸¹

TNF- α is an important modulator of the immune response. TNF- α increases vascular permeability, fibroblast proliferation and lymphocyte activation, and promotes the production of IL-6 and IL-8. TNF- α downregulates eNOS and mitochondrial biogenesis, leading to mitochondrial dysfunction, oxidative stress and increased production of ROS.¹⁸⁹ TNF- α can also alter the expression of adhesion molecules in placental vessels¹⁸¹ and the production of MMPs in preeclampsia.⁹⁶

Another pro-inflammatory cytokine that could be elevated in preeclampsia is IL-6.^{136,182} RUPP rats show increased plasma levels and higher CD4⁺T cell production of IL-6.^{178,187} Chronic infusion of pregnant rats with IL-6 causes hypertension, proteinuria,¹⁹⁰ enhanced vascular contraction and reduced endothelium-dependent NO-cGMP relaxation pathway.⁵⁸ IL-6 may promote dimerization of the surface receptor GP-130 on endothelial cells leading to abnormal cell signaling and vascular dysfunction. IL-6 may also increase vascular permeability by disrupting the tight junctions in endothelial cells.¹⁹¹

IL-1 β is another pro-inflammatory cytokine that could promote the inflammatory response and disrupt endothelial function in preeclampsia. The production of IL-1 β is greater by monocytes from preeclamptic women compared with those from normal pregnant women.¹⁹²

IL-10 is an anti-inflammatory cytokine whose levels are reduced in the plasma and placenta of preeclamptic women^{183,185} and in the plasma of RUPP rats.¹⁷⁸ Also, in placental trophoblasts, exposure to hypoxia is associated with increased pro-inflammatory cytokines and decreased IL-10.¹⁹³

The source of pro-inflammatory cytokine in preeclampsia is mostly in the maternal circulation. Monocytes and macrophages are the main reservoirs of pro-inflammatory cytokines and are the first cells to be activated in nonspecific immune response.¹⁹⁴ Monocytes produce more TNF- α and IL-6 when treated with plasma from preeclamptic than normal pregnant women.¹⁹⁴ IL-10 may have regulatory effects on monocytes and the inflammatory response during normal pregnancy by controlling TNF- α and IL-1 β gene expression,¹⁹² and these IL-10-mediated regulatory effects appear to be lost in preeclampsia. Interestingly, uric acid stimulates monocytes to release cytokines, and hyperuricemia is often observed in preeclamptic patients. Also, monocytes from preeclamptic patients with high levels of uric acid produce more TNF- α and IL-1 β than monocytes from normal pregnant

women.¹⁹² In addition to their proteolytic activities, MMPs may increase the release of cytokines in preeclampsia.⁶⁰

6.3. 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-1 α and HIF-2 α subunits and a constitutively expressed HIF-1 β subunit. While hypoxia is an important inducer of HIF, *de novo* synthesis of HIF-1 α may occur in response to non-hypoxic stimuli such as pro-inflammatory factors. For example, TNF- α upregulates HIF-1 α mRNA expression.¹⁶² Also, a large number of genes are regulated by HIF-1 including VEGF, leptin, TGF- β 3, and NOS. Of note, DNA microarray analysis in arterial endothelial cells have shown that more than 2% of human genes are regulated directly or indirectly by HIF-1.¹¹

HIF expression may increase during pregnancy, and the changes may be related to the pregnancy-related increase in production of estrogen and progesterone. Estrogen stimulates uterine HIF-2 α , and progesterone primarily up-regulates uterine HIF-1 α expression.¹⁹⁵ HIF shows further increase in preeclampsia.¹⁹⁶ The circulating levels of HIF-1 α are increased in preeclamptic compared with normal pregnant women.¹⁹⁷ HIF-1 α may participate in the pathogenesis of preeclampsia by upregulating the antiangiogenic factors sFlt-1 and sEng, binding to ET-1 gene and regulating ET-1 mRNA expression, reducing the trophoblast invasion capability, and inducing AngII-converting enzyme (ACE) expression in the lungs and kidney and AngII production.^{11,198} In support, HIF-1 α increases the production of sFlt-1 in human villous trophoblasts.¹⁹⁹

The placental levels of HIF-1 α are elevated in the RUPP rat model of hypertension in pregnancy.⁶² Also, downregulation of HIF-1 α mRNA using siRNA reverses the increases in blood pressure, proteinuria, renal damage and serum levels of sFlt-1 in mice models of hypertension in pregnancy.¹⁹⁹

In addition to the role of HIF in oxygen homeostasis and its regulation by oxygen,²⁰⁰ cytokines, hormones, metallic ions and mechanical stretch may induce HIF expression.^{200,201} Prolonged mechanical stretch increases HIF-1 α and HIF-2 α mRNA expression and protein levels in skeletal muscle fibers.^{202,203} Also, upregulation of HIF-1 α has been observed in rat cardiac myocytes, aortic VSM cells and fibroblasts exposed to mechanical stretch.^{204–206} Studies have shown upregulation of HIF-1 α mRNA in VSM cells subjected to cyclic stretch for 4 hours.²⁰⁴ Similar increases in HIF-1 α protein have been shown in fibroblasts subjected to cyclic stretch for 24 hours.²⁰⁶ The mechanisms via which mechanical stretch upregulate HIF are unclear, but may involve PI₃K and MAPK.^{201,202,204} Studies have suggested that the expression/activity of MMP-2 and MMP-9 are regulated by HIF.^{207,208} We have also shown that mechanical stretch is associated with increased HIF-1 α expression, and that HIF could increase MMP expression in rat inferior vena cava.²⁰⁹ Whether HIF functions as a transducing signaling mechanism between vascular mechanical stretch and the expression of MMPs during pregnancy needs to be examined.

6.4. Reactive Oxygen Species (ROS)

ROS such as superoxide anion ($O_2^{\bullet-}$), H_2O_2 and hydroxyl ion (OH^-) contain highly reactive O_2 . Normal pregnancy may represent a state of oxidative stress caused by increased maternal metabolism and metabolic activity of the placenta. The generation of ROS is increased during pregnancy;²¹⁰ however, the placental production of ROS is normally counterbalanced by antioxidants.¹¹ In preeclampsia, defective trophoblast invasion and decreased uteroplacental blood flow result in periods of ischemia/reperfusion and a hypoxic environment that favors oxidative stress, inflammation, and vascular dysfunction.¹⁸⁹ In preeclampsia, the levels of antioxidants may be too low to counterbalance the increased ROS production.²¹¹ The expression of antioxidants such as hemeoxygenase-1, hemeoxygenase-2, copper/zinc superoxide dismutase, glutathione peroxidase and catalase is decreased in preeclampsia. Also, the total antioxidant capacity is lower in serum from preeclamptic compared with normal pregnant women.²¹² The ROS/antioxidants imbalance would then lead to lipid peroxidation, increased TXA2 and loss of glutathione peroxidase activity in the placenta.⁵⁹ Antioxidant levels were reduced in women who were later diagnosed with early preeclampsia,²¹³ suggesting a role of oxidative stress in the pathogenesis of preeclampsia. Interestingly, in preeclampsia women, brachial artery flow-mediated dilation is reduced and is associated with decreased plasma levels of the antioxidant ascorbate, and administration of ascorbic acid improved flow-mediated dilation, supporting a relation between oxidative stress and endothelial dysfunction in preeclampsia.²¹⁴ Also, the placental levels of hemeoxygenase-1 are reduced in RUPP compared with normal pregnant rats,⁶² supporting a link between ROS and hypertension in pregnancy.

Neutrophils and monocytes are major sources of ROS in preeclampsia. Monocytes from preeclamptic women produce more H_2O_2 and $O_2^{\bullet-}$ and cause more endothelial cell damage compared to monocytes from normal pregnant women.^{215,216} We should note that neutrophils also produce NO, which can protect cells from the damaging effects of $O_2^{\bullet-}$ during normal pregnancy. However, in preeclampsia, excess $O_2^{\bullet-}$ scavenge the NO produced by neutrophils to form peroxynitrite ($ONOO^-$), thus reducing NO bioavailability and causing endothelial cell damage.²¹⁶ 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 preeclamptic women.²¹⁷ In HUVECs, treatment with serum from preeclamptic women increases the mRNA expression of the NADPH oxidase subunit gp91^{phox}, and augments $O_2^{\bullet-}$ production.²¹⁸ Treatment of HUVECs with preeclamptic serum also causes overexpression of iNOS,²¹⁸ which could produce excessive amounts of NO and in turn increase the production of ROS and promote endothelial cell injury. Interestingly, in the RUPP rat model of hypertension in pregnancy treatment with specific inhibitors of iNOS is associated with a decrease in blood pressure, aortic levels of ROS and NADPH-dependent production of ROS.²¹⁹ Biopterin (BH_4) promotes eNOS dimerization and activity. Hypoxia reduces BH_4 and in turn causes eNOS uncoupling, increased ROS production, and decreased bioavailability of NO.²²⁰ In DOCA-salt hypertensive rats, supplementation with a BH_4 such as sepiapterin decreases the production of $ONOO^-$ and $O_2^{\bullet-}$ and increases the production of NO.²²⁰

Other markers of lipid peroxidation and oxidative stress such as malondialdehyde and prostaglandin $F_{2\alpha}$ are increased in serum of preeclamptic women at gestational weeks 10–14. This may cause gradual oxidative damage in the placenta, even before overt symptoms of preeclampsia.²²¹ The plasma levels of the oxidative stress marker 8-isoprostane, and the total aortic and placental levels of ROS are higher in RUPP compared with normal pregnant rats.^{178,219} In first-trimester villous trophoblasts, excessive oxidative stress affects the expression of several miRNAs including those involved in angiogenesis, apoptosis, immune response and inflammation, and this could be a potential mechanism in the pathogenesis of preeclampsia.²²² MMPs may also contribute to the increases in ROS in preeclampsia.⁶⁰

6.5. AngII and AT₁ Receptor Agonistic Autoantibodies (AT₁-AA)

AngII is an important circulating hormone in the control of water and electrolyte homeostasis and blood pressure. AngII activation of vascular AT₁R promotes vascular growth, inflammation, and vasoconstriction by increasing $[Ca^{2+}]_i$ and Rho-kinase activity in VSM. AngII activation of endothelial AT₂R is coupled to increases in eNOS activity and NO production, release of PGI₂, and vasodilation, and thereby counteracts AngII-induced vasoconstriction. Therefore, while normal pregnancy is associated with increased plasma levels of renin and AngII, the pressor response to AngII is decreased due to decreased expression of AT₁R or increased expression of AT₂R. On the other hand, the dose of AngII required to elicit a 20 mmHg pressor response in the diastolic blood pressure in women at gestational weeks 23–26 is lower in women who subsequently developed preeclampsia compared with normal pregnant women who remained normotensive,²²³ suggesting that the increased pressor response to AngII occurs long before overt clinical manifestations of preeclampsia.

AngII levels and AT₁R mRNA expression are increased in chorionic villi and placenta of preeclamptic versus normal pregnant women.^{224,225} Studies have shown that plasma hemopexin activity increases during normal gestation from week 10 onward, and that active hemopexin downregulates AT₁R in human monocytes and endothelial cells, and decreases functional AT₁R and AngII-induced contraction in rat aortic rings. These observations have suggested that during preeclampsia inhibition of hemopexin activity may result in enhanced AT₁R expression and increased vasoconstriction.²²⁶

AT₁-AA is a bioactive factor with vasoconstrictor and growth promoting properties via AT₁R. The serum levels of AT₁-AA are elevated in preeclamptic compared with normal pregnant women,^{160,227} and are further elevated in severe preeclampsia and in early versus late preeclampsia.²²⁸ The circulating levels of AT₁-AA are also increased in RUPP compared with normal pregnant rats.^{178,229–231} Also, infusion of AT₁-AA in pregnant mice causes some of the manifestations of preeclampsia including increased blood pressure, proteinuria and increased plasma sFlt-1 levels.¹⁶⁰ Also, infusion of AT₁-AA in pregnant rats is associated with increases in ET-1 levels approximately 4-fold in the placenta and 11-fold in the renal cortex.²³² Interestingly, 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 the pathogenesis of hypertension in pregnancy. Of note, the impaired ACh-induced vasodilation in AT₁-AA

infused pregnant rats was abolished by concomitant treatment with an ET_AR antagonist, suggesting an interplay between the AngII and endothelin system in the setting of endothelial dysfunction and hypertension in pregnancy.²³³ AT₁-AA has been linked to increased blood pressure, reduced trophoblast invasion, increased sFlt-1, ROS and cellular Ca²⁺, activation of coagulation tissue factor and thrombosis, vascular damage in the adrenal glands, and reduced aldosterone secretion in preeclampsia.^{160,234} AT₁-AA also promotes collagen-induced platelet aggregation, which may contribute to the hypercoagulability observed in preeclampsia.²²⁷ In cultured trophoblasts, stimulation of AT₁R with IgG isolated from preeclamptic women causes increases in sFlt-1 levels.²³⁵ In HUVECs, treatment with AT₁-AA isolated from preeclamptic women induces the release of the cell death and necrosis marker lactate dehydrogenase,²³⁶ suggesting that AT₁-AA may cause endothelial cell damage and necrosis. Also in HUVECs, AT₁-AA induces the activity of caspase-3 and caspase-8, suggesting that it may promote endothelial cell apoptosis.²³⁶ While the mechanisms causing the release of AT₁-AA in preeclampsia are not clearly understood, the plasma levels of AT₁-AA are increased in pregnant rats infused with TNF- α , suggesting the involvement of cytokine-induced pathways.²³¹

7. MMPs AND VASCULAR DYSFUNCTION IN HYPERTENSIVE PREGNANCY and PREECLAMPSIA

7.1 MMPs and Extracellular Matrix Abnormalities in Preeclampsia

MMPs degrade different substrates including collagen, gelatin, and other proteins.^{9,10,237} We have investigated the changes in MMPs substrates in the RUPP rat model of hypertension in pregnancy. Picro-Sirius Red staining revealed an increase in collagen content in uterus, placenta and aorta of RUPP compared with normal pregnant rats.⁸ Because MMPs facilitate cell growth and migration by promoting proteolysis of ECM, the decreased MMPs and increased collagen deposition in RUPP tissues could impede smooth muscle cell growth, proliferation and migration, and thus interfere with uteroplacental tissue invasion and decrease 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. In support, MMP-2 knockout (KO) is associated with decreased VSMC migration and neointima formation in the mouse carotid ligation model.^{238,239} Also, MMP-9 KO is associated with reduced VSMC migration and neointima formation in mouse carotid occlusion model,²⁴⁰ and reduced VSMC migration and proliferation and neointima formation in mouse model of carotid artery injury.²⁴¹ The decreased MMP activity and increased vascular collagen content could also increase vessel rigidity and decrease its plasticity and thus contribute to increased vascular resistance and hypertension. This is consistent with reports that MMP-1, MMP-2, and MMP-9 gelatinolytic activity is decreased and collagen deposition is increased in internal mammary artery from hypertensive compared with normotensive patients undergoing coronary artery bypass surgery.²⁴² AngII infusion and high salt diet in mice are also associated with hypertension and increased MMP-9 activity in carotid artery, and MMP-9 KO is associated with vessel stiffness and increased pulse pressure, suggesting a beneficial role of MMP-9 in preserving vessel compliance and alleviating the increase in blood pressure in early hypertension.²⁴³ Also, in

adult spontaneously hypertensive rat, a decrease in MMP-1, MMP-2, and MMP-3 may contribute to remodeling of resistance arteries and the setting of hypertension.²⁴⁴ It is important to note that collagen has 18 types and different subtypes.²⁴⁵ 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.^{9,10,237} Studies should measure the changes in various collagen types and subtypes in hypertension in pregnancy. RT-PCR experiments should also determine whether any increases in a specific collagen subtype are solely due to decreased degradation or could also involve decreased *de novo* collagen mRNA expression and protein biosynthesis.

In contrast with collagen, elastin staining of uterus and placenta was sparse and diffuse and not different in RUPP versus normal pregnant rats, suggesting that elastin is less likely to be involved in the observed changes in tissue growth and remodeling. Although prominent and well-defined elastin bands were observed in the aorta, no significant changes were noted between RUPP and normal pregnant rats, suggesting little role of elastin in the observed changes in aortic growth and remodeling.⁸

We should note that MMPs is a large family of at least 28 proteolytic enzymes.^{9,10,237} While changes in MMP-2 and MMP-9 have been observed in RUPP rats, other MMPs have been detected in the uterus, placenta and aorta, and the changes in these MMPs in hypertension in pregnancy need to be investigated. Studies have shown that upregulation of MMP-1 enhances flow-induced VSMC motility in cell culture, and MMP-1 inhibition attenuates flow-induced migration.²⁴⁶ Importantly, MMPs activity could be influenced by other MMP activators and inhibitors. For example, some MMPs may cleave other pro-MMPs, and MT1-MMP is a key activator of proMMP-2.^{9,10,237,247} On the other hand, MMP expression/activity could be influenced by TIMPs and other modulators of MMPs activity.^{9,10,237,247} For instance, TIMP-2 or specific MMP-2 blocking antibody inhibits cytotrophoblast invasion *in vitro*,³⁴ and the role of modulators of MMP expression/activity in hypertension in pregnancy and preeclampsia needs to be further examined. Also, the time course and the progressive changes in MMPs during the course of pregnancy and their reversal in the postpartum period need to be examined. Plasma levels of MMPs may also vary at different stages and with the severity of preeclampsia. Furthermore, plasma MMPs represent the global changes in different maternal tissues, and the changes in MMPs in specific uteroplacental and vascular tissues need to be measured.

While MMPs are largely known for their proteolytic effects ECM, we and others have identified novel MMP-induced downstream pathways that could affect uteroplacental remodeling and vascular function.^{40,41,93,102} Identifying the changes in the MMP-induced downstream pathways in hypertensive pregnancy would help define the molecular mechanisms of the increased vasoconstriction and blood pressure. In addition to their proteolytic effects on ECM proteins, MMPs may affect vascular function and the mechanisms of VSM contraction. We have shown that MMP-2 and MMP-9 cause relaxation of precontracted rat aorta⁴⁰ and inferior vena cava.^{41,42} Also, changes in endothelial cell and VSM function have been observed in RUPP rats.¹⁹ Changes in vascular MMPs expression/activity could play a role in the endothelial dysfunction and increased vascular contraction in hypertension in pregnancy.

7.2 MMPs and Endothelial Dysfunction In Preeclampsia

The presence of functional endothelium is important for healthy gestation, and ensures a favorable prognosis for the mother and fetus.²⁴⁸ During normal pregnancy, there is an increase in brachial artery diameter and flow-mediated dilation as gestation progresses.²⁴⁹ Also, endothelium-dependent bradykinin-induced relaxation is increased in human small subcutaneous arteries from pregnant compared with non-pregnant women.²⁵⁰ Experimental studies corroborated endothelial cell adaptations during pregnancy. ATP causes periodic bursts in intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) that are more frequent in uterine artery endothelial cells from pregnant compared with non-pregnant ewes,²⁵¹ which could influence the vasodilator response and in turn attenuate the uterine artery myogenic tone and ensure adequate uterine blood flow during pregnancy.²⁵²

In addition to their effects on the uterus, gonadal hormones may contribute to the hemodynamic and vascular changes during pregnancy. Estrogen promotes endothelium-dependent vascular relaxation by increasing the release of NO, prostacyclin and EDHF.²⁵³ Estrogen also causes relaxation of endothelium-denuded vessels by inhibiting the mechanisms of VSM contraction including $[\text{Ca}^{2+}]_i$ and protein kinase C.^{26,254–256} Estrogen may have additional effects on the vascular cytoskeleton, ECM, lipid profile and inflammatory response.²⁵³ Progesterone also causes vasodilation by mechanisms similar to estrogen.^{253,256,257} 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.³⁸

In contrast with normal pregnancy, women with preeclampsia show systemic endothelial cell dysfunction and hypertension, glomerular endotheliosis causing renal injury and proteinuria, and cerebral endotheliosis which could lead to cerebral edema and seizures.¹¹ Brachial artery flow-mediated dilation is less in preeclamptic than normal pregnant women.^{248,258} Preeclamptic women also show less vasodilation in the radial artery (~7.9%) when compared to normal pregnant women (~17.4%).²⁵⁹ Bradykinin-induced relaxation is decreased in small subcutaneous arteries of preeclamptic compared with normal pregnant women.²⁵⁰ Circulating endothelial cells and other markers of endothelial activation/injury such as soluble VCAM-1, E-selectin and endocan are increased in preeclamptic compared with normal pregnant women.^{149,184,260,261} Other studies have shown a decrease in circulating endothelial progenitor cells as a marker of endothelial damage in preeclamptic women.²⁶²

The RUPP rat has shown some of the characteristics of preeclampsia including high blood pressure, proteinuria, decreased glomerular filtration rate and renal plasma flow, and IUGR, and therefore has been used to study the vascular mechanisms of preeclampsia.^{56,172,263} 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.^{19,172} 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 (TXA2). Endothelial dysfunction is associated with an imbalance in the release of endothelium-derived vasodilator versus vasoconstrictor factors.

7.2.1 Changes in Nitric Oxide (NO) in Preeclampsia—Nitric oxide (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 $[\text{Ca}^{2+}]_i$ and causes VSM relaxation. Nitrites are important metabolites of NO that are increased in serum of normal pregnant compared with non-pregnant women.²⁶⁴ The plasma concentration and urinary excretion of cGMP, a second messenger of NO, are also increased in normal pregnancy. NOS expression and activity are also increased in human uterine artery and in the placenta with gestational age,^{265,266} supporting increased NO production during normal pregnancy. Also, the urinary levels of nitrites, the mRNA expression of eNOS, iNOS and nNOS, and the protein level of activated phospho-eNOS are increased in normal pregnant compared with virgin rats.²⁶⁷

Polymorphisms of *eNOS* gene could be a risk factor for preeclampsia. The VNTRa and 894T alleles of *eNOS* gene are associated with early and late severe preeclampsia, 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 subject to selective proteolytic cleavage in endothelial cells and vascular tissues, and this could account for the reduced vascular NO production in homozygous subjects for this variant.²⁶⁸ The T786C allele is also increased in preeclamptic compared with normal pregnant women.^{269,270} Genetic polymorphisms of eNOS could affect NO production. For instance, normal pregnant women with the TT phenotype for the T-786C allele have lower plasma nitrite levels than those with the CC phenotype²⁷¹, and the TT phenotype has been proposed as a risk factor for preeclampsia in Tunisian women.²⁶⁹

Endothelial dysfunction is often associated with decreased NO bioavailability due to decreased synthesis or increased degradation.²⁷² Clinical studies have shown increased²⁷³ or decreased^{274–277} plasma nitrite levels in preeclamptic compared with normal pregnant women. Also, urinary nitrite levels may not differ in preeclamptic versus normal pregnant women.²⁷⁴ The discrepancies in nitrite measurement could be related to the difficulty in controlling nitrate intake in diet. However, a study that carefully controlled dietary nitrate/nitrite intake did not show decreased NO production in preeclamptic women.²⁷⁸

The lack of change in whole-body NO despite the increase in blood pressure and the renal damage in preeclampsia suggest tissue-specific changes in NOS expression and NO bioavailability such that whole-body NO may not accurately reflect NO activity in the vasculature or the kidneys.¹¹ Studies have shown a decrease in nitrites in placentae from preeclamptic women.²⁷⁷ Also, eNOS expression is decreased in umbilical cord of preeclamptic compared with normal pregnant women,²⁷⁹ and the decrease is greater in women with severe preeclampsia.^{280,281} However, some studies showed an increase in eNOS mRNA expression in placenta of preeclamptic women.²⁸² Also, while the levels of cGMP are increased during normal pregnancy, the plasma and urinary cGMP levels are not different in preeclamptic versus normal pregnant women.²⁷⁴

The role of NO has also been examined in animal models of hypertension in pregnancy. In mid- to late pregnant rats, NOS blockade with N_ω -nitro-L-arginine methyl ester (L-NAME) causes some of the changes observed in preeclampsia including increases in blood pressure

and renal vasoconstriction, proteinuria, thrombocytopenia and IUGR,^{23,158,283} supporting that NO production is increased during gestation. Similar to the observation in humans, measurements of NO production in hypertensive pregnant animals have not been consistent. Studies showed no difference in nitrite levels in L-NAME treated versus nontreated normal pregnant rats.¹⁵⁸ Also, while plasma nitrite levels were lower in RUPP than normal pregnant rats,²⁶³ no differences were observed in urinary nitrite levels.^{56,284} Also, consistent with the studies in human, no changes were observed in the NOS substrate L-arginine in the circulation of RUPP versus normal pregnant rats.²⁸⁵ Vascular function studies have shown increased aortic vascular reactivity to phenylephrine in L-NAME treated pregnant rats.²³ Also, ACh-induced relaxation, eNOS expression, and NO production are reduced in mesenteric artery and aorta of RUPP versus normal pregnant rats, supporting specific reduction in NO synthesis in the vasculature.^{19,172,263} In rat model of hypertension in pregnancy produced by infusion of deoxycorticosterone acetate and replacement of drinking water with a 0.9% saline (DOCA-salt), NO-dependent relaxation was reduced in mesenteric vessels despite elevation of eNOS mRNA expression.²²⁰ MMPs may affect NO production by endothelial cells, and whether changes in MMPs during preeclampsia affect NO production should be examined.

7.2.2 MMPs and Endothelium-Derived Hyperpolarizing Factor (EDHF)—EDHF is a relaxing factor with specialized function in the control of small resistance vessels, local organ blood flow, peripheral vascular resistance and blood pressure. 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-gated Ca^{2+} channels and suppression of the activity of phospholipase C, an enzyme involved in transduction pathways 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.²⁸⁶ 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, hydrogen peroxide (H_2O_2) may mimic EDHF-mediated responses by mechanisms involving K_{Ca} activation²⁸⁷. Thus multiple EDHFs may exist and the identity of EDHF could vary depending on the vascular bed and animal species studied.²⁸⁸

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.^{289,290} The gap junction proteins connexins 37, 40 and 43 are partly involved in EDHF-mediated vascular response during normal pregnancy.²⁹¹ An increase in endothelial cell $[Ca^{2+}]_i$ may activate IK_{Ca} and SK_{Ca} and promote EDHF-mediated dilation in uterine radial arteries of pregnant rats.²⁹² 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.²⁹³

Studies in subcutaneous arteries from normal pregnant women have shown that MEGJs alone may be the main pathway of EDHF-mediated relaxation, while in women with preeclampsia MEGJs alone or in combination with H₂O₂ 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 preeclampsia.²⁹⁴ 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 × 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.²⁹⁵ 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 hypertension in pregnancy. Although studies in mesenteric microvessels have suggested that the EDHF relaxation may not be compromised in RUPP versus normal pregnant rats,¹⁷² decreased EDHF-mediated relaxation is thought to contribute to the vasoconstriction observed in hypertension and diabetes, and its role in preeclampsia needs to be further examined.

Our data suggest that prolonged increases in intravascular pressure and wall tension cause increases in MMP-2 and MMP-9 expression⁴⁰⁻⁴². Also, MMP-2 and MMP-9 cause relaxation of phenylephrine precontracted rat aorta. Thus during normal pregnancy, plasma volume expansion could lead to increased vascular MMP-2 and MMP-9, vasodilation and decreased blood pressure. The decrease in vascular MMP-2 and MMP-9 is expected to decrease relaxation in RUPP rats. Our data show that ACh-induced relaxation is reduced in blood vessels of RUPP versus pregnant rats. To test if the decreased vascular relaxation in RUPP rats reflects reduction in MMP-mediated NO-cGMP or PGI₂-cAMP pathway, the effects of MMPs on NO and PGI₂ production needs to be measured. To test if MMP-mediated EDHF is reduced in RUPP rats, MMP induced changes in membrane potential needs to be determined. We have shown that MMP-2 induces vascular relaxation partly via hyperpolarization and activation of K⁺ channels.^{41,42} Thus, the decrease in MMP-2 and MMP-9 mediated relaxation may contribute to the enhanced vascular contraction and increased blood pressure in RUPP versus normal pregnant rats.^{19,172,296}

7.2.3 MMPs and Endothelin-1 (ET-1) in Preeclampsia—ET-1 is a major endothelium-derived vasoconstrictor that could play a role in preeclampsia.²⁹⁷ ET-1 synthesis is initiated by the production of 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. Some of the circulating factors in preeclampsia including cytokines, hypoxia and AT₁-AA may stimulate endothelial cells to produce ET-1,²⁹⁷ This is supported by reports that serum from preeclamptic women causes HUVECs to produce greater amounts of ET-1 than normal pregnant serum.²⁹⁸ Some studies suggest that plasma ET-1 levels are elevated in preeclampsia.²⁹⁹ ET-1 levels are higher during later stages of preeclampsia and return to normal levels within 48 hours after delivery,³⁰⁰

suggesting that ET-1 may be involved in the progression rather than the initiation of preeclampsia. However, in most studies serum ET-1 levels do not differ in preeclamptic versus normal pregnant women, and higher levels of ET-1 are observed mainly in patients with HELLP syndrome.^{117,301,302} Of note, ET-1 is released in a paracrine fashion from endothelial cells directly toward VSMCs, and the increases in ET-1 levels in preeclampsia may be localized in tissues. Studies have shown a 4- to 8-fold increase in ET-1 levels in umbilical cord cells³⁰³ and in renal tissues during later stages of preeclampsia.³⁰⁰ In perfused placentas under hypoxia, both the maternal and fetal side produced increased levels of ET-1.³⁰⁴ In RUPP rats, preproET levels show a 45% increase in renal cortex and 22% increase in renal medulla.³⁰⁵ Thus, measurements of circulating ET-1 may not always reflect ET-1 levels locally in tissues. It is possible that in severe PE and in HELLP syndrome the production of ET-1 is so augmented that ET-1 release “loses” its paracrine directionality and consequently leads to an increase in circulating ET-1 levels. This is supported by reports that rat models that mimic severe PE and HELLP syndrome have increased plasma levels of ET-1.^{179,306}

ET-1 may play a role in the pathogenesis of preeclampsia by inducing apoptosis of trophoblast cells and increasing oxidant and anti-angiogenic substances.^{297,307} ET-1 activates endothelin receptor type A (ET_AR) and endothelin receptor type B (ET_BR).^{308–315} ET-1 activation of VSM ET_AR stimulates Ca²⁺ release from the intracellular stores and Ca²⁺ entry through Ca²⁺ channels, and causes protein kinase C-dependent inhibition of K⁺ channels leading to increased [Ca²⁺]_i and VSM contraction.³⁰⁸ ET-1-induced vasoconstriction is reduced in mesenteric vessels of normal pregnant compared with non-pregnant rats,³¹⁶ and VSM ET_AR are reduced in aortic media and VSMCs of late-pregnant rat.³¹⁷ Also, treatment with ET_AR antagonist reduces blood pressure in RUPP rat and other animal models of hypertension in pregnancy.^{305,318,319} Interestingly, among Brazilian women with preeclampsia, 52% of the patients diagnosed with severe preeclampsia exhibited increases in ET_AR agonistic autoantibodies (ET_A-AA) which could target ET_AR and increase vasoconstriction.³²⁰

ET-1 also activates ET_BR in endothelial cells and stimulates the release of NO, PGI₂, and EDHF which in turn reduce myogenic vascular tone, promote vasodilation of renal arteries and hyperfiltration in pregnant rats.^{308,321} Downregulation of ET_BR may impair trophoblast invasion in preeclampsia. Downregulation of ET_BR decreases microvascular vasodilator activity in pregnant rats. ET_BR expression is also reduced in endothelial cells 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 hypertension in pregnancy.¹⁷²

MMPs break big-ET-1 and into different endothelin forms that have different affinities for ET_AR and ET_BR and in turn differentially affect vasoconstriction in normal pregnancy and preeclampsia. MMPs could degrade big-ET into ET-1, which largely stimulates ET_AR and promotes vasoconstriction.³²² Studies have suggested a role of ET-1 and ET_AR in some forms of hypertension including hypertension in pregnancy.^{309,318,323–328} In omental vessels of normal pregnant women, MMP-1 causes vasoconstriction and enhances reactivity to AngII via an endothelium-dependent protease-activated receptor (PAR) and ET-1

pathway.¹⁰² ET-1 in turn can stimulate VSM contraction mechanisms^{309,315,329} including $[Ca^{2+}]_i$,^{330–332} protein kinase C,^{333–339} and Rho-kinase.^{340–342}

MMP-2 and MMP-9 could degrade big-ET to ET_{1–32} which preferentially stimulates endothelial ET_BR and promotes relaxation. We have shown increases in MMP-2 and MMP-9^{37,93} and ET_BR in normal pregnant rats.³¹⁶ Also, in line with our observed decrease in MMP-2 and MMP-9, ET_BR is downregulated in RUPP rats, and infusion of the ET_BR antagonist BQ788 increased blood pressure in pregnant rats.¹⁷²

7.3 MMPs and Smooth Muscle Dysfunction in Preeclampsia

7.3.1 VSM Ca²⁺ in Preeclampsia—Ca²⁺ is a major determinant of VSM contraction and growth. Ca²⁺ release from the intracellular stores and Ca²⁺ entry from the extracellular space increase $[Ca^{2+}]_i$ in VSM. Ca²⁺ binds calmodulin to form Ca²⁺-calmodulin complex which activates myosin light chain kinase, causes myosin phosphorylation, initiates actin-myosin interaction and produces VSM contraction. Decreased Ca²⁺ in VSM activates myosin phosphatase to dephosphorylate myosin light chain and dissociate the Ca²⁺-calmodulin complex. Endothelium-derived relaxing factors act on VSM to decrease $[Ca^{2+}]_i$. In hypoxia, decreased relaxing factors reduce VSM Ca²⁺ extrusion, while increased contracting factors increase $[Ca^{2+}]_i$ and VSM contraction. In normal pregnancy, increased K_{Ca} channel activity decreases uterine artery tonicity and increases uteroplacental blood supply. In preeclampsia, K_{Ca} channel activity is suppressed leading to increased uterine artery $[Ca^{2+}]_i$, vasoconstriction and reduced fetal blood supply.³⁴³ Myometrial vessels may show similar vasoconstriction response to high KCl, phenylephrine and AngII in normal pregnancy and preeclampsia.³⁴⁴ However, basal and agonist-stimulated $[Ca^{2+}]_i$ are reduced in renal arterial VSM of normal pregnant rats, and increased in pregnant rats treated with L-NAME.³⁴⁵ AT₁R activation increases $[Ca^{2+}]_i$ in the platelets, erythrocytes, and lymphocytes of preeclamptic women, and these effects subside 6 weeks after delivery.²³⁴ AngII- and caffeine-induced contraction and $[Ca^{2+}]_i$ in Ca²⁺-free solution are similar in VSM of normal pregnant and RUPP rats, while KCl-induced maintained $[Ca^{2+}]_i$ in a Ca²⁺-containing medium is greater in VSM of RUPP than normal pregnant rats, suggesting that it is not the release of intracellular Ca²⁺ but the increase in Ca²⁺ entry from extracellular space that increases vasoconstriction in hypertension in pregnancy.³⁴⁶

MMP-2 and MMP-9 may cause vascular relaxation by decreasing Ca²⁺ influx into VSM,⁴⁰ and a decrease in these MMPs could lead to increased Ca²⁺ influx, vasoconstriction and hypertension in pregnancy.

7.3.2 Protein Kinase C (PKC), MAPK, and Rho-kinase in Preeclampsia—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 $[Ca^{2+}]_i$, suggesting that PKC increases Ca²⁺ sensitivity of the contractile proteins. PKC activity and contraction are reduced in the uterine artery of late pregnant ewes and gilts and the aorta of late pregnant

rats.³⁴⁷⁻³⁴⁹ 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.^{348,350} PKC may increase the production of AT_1 -AA which stimulates AT_1 R. In cultured rat cardiomyocytes treatment with IgG obtained from preeclamptic women enhances AT_1 R-mediated response which is ameliorated with the PKC inhibitor calphostin C.³⁵¹ 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} .³⁴³ PKC inhibitors decrease TXA_2 mediated contraction in uterine and mesenteric arteries of non-pregnant rats and in mesenteric artery of pregnant rats, supporting a role of PKC in mediating VSM contraction during pregnancy.³⁵² Blocking PKC can prevent PKC-mediated vasoconstriction in preeclampsia. Cicletanine is an anti-hypertensive compound that prevents the increase in PKC and lower blood pressure in hypertensive pregnant rats. MMP-2 may reduce vascular contraction by degrading the actin-binding protein calponin.³⁵³ A decrease in MMP-2 would spare calponin and affect VSM contraction in hypertension in pregnancy.

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 hypertension in pregnancy could affect MAPK/caldesmon phosphorylation and VSM contraction.³⁵⁴ This is supported by reports that inhibition of p38 MAPK reduces TXA_2 -induced contraction in uterine and mesenteric arteries of virgin and pregnant rats.³⁵²

Rho is a family of small GTP-binding proteins that are involved in cell migration, cytoskeletal reorganization and VSM contraction. Rho-kinase is activated by GTP binding and inactivated by hydrolyzing GTP to GDP. There are two isoforms of Rho-kinase, rock I ($\text{ROK}\beta$) and rock II ($\text{ROK}\alpha$), which may be important in the formation of microvilli structures during pregnancy. Human placental villi show abnormal expression of rock II and apoptosis of the syncytium in preeclampsia.³⁵⁵ Rho-kinase increases Ca^{2+} sensitivity of the contractile proteins in subcutaneous resistance arteries of preeclamptic women.³⁵⁶ Also, AngII via AT_1 R induces RhoA/Rho-kinase activity in L-NAME treated hypertensive rats.³⁵⁷ Rho-kinase may stimulate IL-17 to phosphorylate the inhibitory eNOS Thr495 residue thus decreasing NO production in preeclampsia.³⁵⁸ Inhibition of Rho-kinase reduces TXA_2 -induced contraction in uterine vessels of non-pregnant rats.³⁵² However, some studies show decreased expression of Rho-kinase in umbilical arteries of preeclamptic women.³⁵⁹

Specific MMP subtypes may increase the production of ET-1, which could activate ET_A R in VSM, leading to activation of PKC, MAPK or Rho-kinase dependent pathway, and in turn increase the myofilament force sensitivity to Ca^{2+} , and enhance vasoconstriction in the setting of hypertension in pregnancy.

8. MMPs AND UTERINE CONTRACTION DURING PREGNANCY AND LABOR

The uterus responds to different mechanical and chemical stimuli, and its response varies in the non-pregnant compared to the pregnant state, and during parturition. The non-pregnant uterus contracts in response to mechanical stretch. On the other hand, during pregnancy several mechanisms are activated in order to reduce the excitability of the myometrium, maintain uterine quiescence, and allow sufficient time for the development of the fetus before it is ready to be delivered.³⁶⁰ The uterine smooth muscle cells undergo sequential steps of hyperplasia, hypertrophy, and ECM elaboration in order to adapt to the physiological demands of pregnancy. These processes are regulated by both mechanical and endocrine factors and are accompanied by extensive remodeling of the uterine ECM.^{361–363} During pregnancy, the uterus dramatically expands and its volume markedly increases. Both hypertrophy and distension of the pregnant uterus allow sufficient space for the growing fetus. Also, the balance between uterine contraction and relaxation is tightly-controlled in order to maintain healthy and full-term pregnancy. Any disturbance in the mechanisms that maintain the uterus in a quiescent relaxed state could cause preterm uterine contraction and premature birth. At full-term, other uterine mechanisms are activated in order to terminate uterine quiescence, increase electrical excitability of the myometrium, coordinate uterine smooth muscle contraction, and ensure uncomplicated parturition. Uterine stretch, and steroid and neurohypophysial hormones could affect uterine quiescence and excitability, partly through MMPs.

8.1. Uterine stretch, sex hormones and MMPs during pregnancy

MMPs could play a role in the endometrial tissue remodeling during estrous and menstrual cycles and during pregnancy.^{28–30,103,364} MMP-2 and MMP-9 are expressed in the bovine uterus,^{365,366} and MMP-2, MMP-7 and MMP-9 are expressed in the rat uterus.^{37,93} Alterations in MMP-2, MMP-7 and MMP-9 expression/activity could be involved in the endometrial changes associated with menstrual disorders and endometriosis^{367,368} The plasma levels of MMP-2 and MMP-9 increase during normal pregnancy.³⁶⁹ MMP-2 and MMP-9 have been detected in uterine natural killer cells during early pregnancy in humans.³⁷⁰ Increases in MMP-2 expression/activity have also been observed in the final ripening stages and collagen denaturation of the cervix during late pregnancy.³⁷¹ MMP-2 and MMP-9 have also been detected in the bovine endometrium and myometrium during pregnancy.^{28,103,366} We have shown an upregulation of MMP-2 and MMP-9 in the myometrium of late pregnant rats, and suggested a role of MMPs in pregnancy-related uterine remodeling.⁹³ MMP-2 and MMP-9 degrade ECM proteins and could promote remodeling of the uterus and cervix during pregnancy.³⁷² However, the mechanisms causing the changes in uterine MMPs during pregnancy are not clearly understood.

MMP expression/activity is regulated by multiple biophysical and biological factors including mechanical stretch and sex hormones. Mechanical stretch of skeletal muscle fibers is associated with increased MMP-2 expression.²⁰³ We have shown that protracted vein wall stretch and increases in vein wall tension are associated with increased MMP-2 and MMP-9 expression and decreased contraction of rat inferior vena cava.^{41,42,373} We have also tested if increased uterine stretch during pregnancy is associated with upregulation of MMPs, which

could in turn cause inhibition of uterine contraction and promote uterine relaxation. We measured contraction in response to oxytocin, a nonapeptide produced by the hypothalamus-pituitary and other tissues such as adrenal medulla,³⁷⁴ corpus luteum,^{375,376} and placenta,³⁷⁷ in isolated uterine strips from virgin, mid-pregnant (day 12), and late-pregnant rats (day 19), and assessed uterine MMP expression and activity using RT-PCR, Western blots, and gelatin zymography. Consistent with the concept of uterine quiescence during the course of pregnancy, we found that oxytocin contraction of myometrium strips was reduced in mid- and late-pregnant rats compared with virgin rats. Some studies have shown that oxytocin contraction of mid-term and late-pregnant uterus is greater than that in non-pregnant uterus,³⁷⁸ which is opposite from our findings. This is likely because during late pregnancy the uterus is bigger and often thicker, and studies often measure uterine contraction in absolute grams. In effect, when we measured the uterine contraction in grams, the contraction in late-pregnant uterus was greater than that in mid-pregnant uterus, but less than that in virgin uterus. On the other hand, when the uterine contraction was normalized to the uterine tissue weight, the contraction was less in late-pregnant than mid-pregnant uterus and far less than that in the virgin uterus, supporting that uterine contraction is reduced during pregnancy.

We tested whether the pregnancy-associated reduction in uterine contraction reflect changes in MMP expression/activity. We observed that the reduced contraction in the mid-pregnant and late-pregnant rat uterus was reversed by the MMP inhibitors SB-3CT, BB-94 and Ro-28-2653, supporting a potential role of MMPs in the pregnancy-related reduction in uterine contraction. Also, the mRNA expression and protein levels of MMP-2 and MMP-9 were enhanced in mid- and late-pregnant compared with virgin rat uterus. Furthermore, MMP-2 and MMP-9 activity, as measured by gelatin zymography, was enhanced in mid- and late-pregnant compared with virgin rat uterus. Interestingly, SB-3CT, but not BB-94 or Ro-28-2653, enhanced oxytocin contraction in mid-pregnant uterus, while the three MMP inhibitors enhanced contraction in late-pregnant uterus. This is likely due to differences in uterine MMP activity during the course of pregnancy. In mid-pregnant uterus, moderate increases in MMPs occur, and therefore the most potent inhibitor SB-3CT potentiates the contraction. On the other hand, in late pregnant uterus, further increases in MMP expression/activity, and therefore MMP inhibitors with different potencies increase uterine contraction.⁹³

We also simulated the changes associated with uterine expansion during pregnancy and tested the effects of induction of mechanical stretch in virgin uterus on uterine contraction and MMP expression/activity. Prolonged stretch of myometrium strips of virgin rats under 8 g basal tension for 18 hour was associated with reduced contraction and enhanced expression/activity of MMP-2 and MMP-9, that were reversed by MMP inhibitors. Also, treatment with MMP-2 and MMP-9 reduced oxytocin-induced contraction in myometrium of virgin rat. These observations support that prolonged increases in uterine wall tension (stretch) during pregnancy are associated with increased MMP-2 and MMP-9 and decreased contraction, leading to uterine expansion and better accommodation of the growing fetus.^{8,93}

We examined whether the pregnancy-associated changes in MMPs are specific to MMP-2 and MMP-9, or involve other MMPs. MMP-7 (matrilysin-1) is expressed in the uterus.⁹

Studies have shown changes in MMP-7 during gestation, but not labor.³⁷⁹ We found that uterine MMP-7 expression did not increase during pregnancy in rats. In effect, MMP-7 showed a pregnancy-related decrease in expression, suggesting that MMP-7 may function via a mechanism different from that regulating MMP-2 and MMP-9.

Steroid and neurohypophysial hormones undergo marked changes during the menstrual cycle, pregnancy and parturition.³⁸⁰ Steroid and neurohypophysial hormones could influence the uterine structure and architecture and markedly affect its mechanical response. The plasma levels of estradiol are increased during the proliferative phase of the menstrual cycle. A decrease in plasma estrogen levels initiates endometrial shedding, and the plasma levels of progesterone are increased during the luteal phase of the menstrual cycle.³⁸¹ Endometrial cellular concentrations of both estrogen and progesterone are positively correlated with the plasma levels of estrogen during the proliferative phase of the menstrual cycle.³⁸¹ Uterine tissue remodeling and endometrium shedding during menstruation could involve estrogen-induced changes in MMPs activity.^{364,382,383} Progesterone may also control the initiation, maintenance and progression of endometrial lesions partly by affecting MMP expression/activity.³⁸⁴ During pregnancy the uterus is exposed to not only mechanical stretch caused by progressive fetal growth, but also hormonal changes that could influence the myometrium structure and mechanical properties. The plasma levels of estrogen and progesterone markedly increase during pregnancy,^{25,385} and may play a role in the pregnancy-related uterine relaxation and expansion.^{93,386-388} A large concentration of progesterone receptors is detected in the endometrium during early pregnancy endometrium.³⁸¹ Studies have shown that estrogen causes relaxation of VSM of rat aorta and uterine artery,^{26,27} as well as the uterus.³⁸⁶ Also, progesterone inhibits contraction of rat blood vessels and human uterus.^{26,387} We have also shown that estrogen and progesterone decrease contraction of uterine strips of virgin rats, and further reduce contraction in uterine strips exposed to prolonged stretch.⁹³

Uterine contraction could also be affected by changes in the levels of the neurohypophysial hormone oxytocin. The plasma levels of oxytocin are relatively low in the non-pregnant state, and do not detectably change during the menstrual cycle. The plasma oxytocin levels increase at gestational week 12 and progressively increase during the course of pregnancy.³⁸⁹ Oxytocin promotes cervical dilation before birth and stimulates uterine contraction during the second and third stages of labor. Steroid and neurohypophysial hormones target uterine receptors and other local enzymes and proteins that modulate uterine structure and function, including MMPs.

The uterine changes during pregnancy may involve effects of gonadal hormones on MMP expression/activity. The activity of MMP-2 and MMP-9 is higher in pregnant than non-pregnant bitches, and serum MMP activity correlates with the serum levels of estrogen,⁵¹ suggesting a relationship between sex hormones and MMP expression/activity. MMP-9 modulates uterine biology during various reproductive processes. In mouse uterus, gelatin zymography revealed that estrogen alone or in combination with progesterone increased MMP-9 activity, whereas Northern blot analysis showed that estrogen decreased MMP-9 mRNA expression. In contrast, uterine MMP-2 expression/activity was not affected by steroidal treatment, suggesting that estrogen and progesterone regulate uterine MMPs

expression/activity and in turn uterine tissue remodeling via a complex mechanism.³⁶⁴ We should note that estrogen and progesterone are important regulators of myometrial growth and contractility, via both genomic and non-genomic mechanisms. It is generally thought that estrogen augments myometrial contractility and excitability, while progesterone sustains the pregnant state and promotes myometrial relaxation.³⁹⁰ While estrogen may have a stimulatory effects on uterine contractility at the time of parturition, these effects appear to be neutralized during the course of pregnancy, partly due to decreased uterine expression of estrogen receptors,³⁹⁰ or estrogen-induced expression of other factors that decrease myometrial contractility such as MMPs.⁹³ For instance, the last phase of pregnancy in rats shows a correlation between the plasma level of estrogen, the increase in uterine contractility, and the density of $\alpha 1$ -adrenergic receptors.³⁸⁵ On the other hand, uterine contraction is reduced in day-12 and day-19 of gestation in rats.⁹³ Also, estrogen causes rapid non-genomic relaxation of spontaneous and depolarization-induced contraction of uterine strips of non-pregnant rat.³⁸⁶ We have also shown that prolonged treatment of virgin rat uterus with estrogen is associated with decreased uterine contraction.⁹³ With regard to progesterone, in addition to its relaxing effects during pregnancy, it also decreases uterine contractility and promotes relaxation during the luteal phase, a phenomenon crucial for maximizing uterine receptivity to embryo implantation during *in vitro* fertilization cycles.³⁹¹ For example, in women undergoing *in vitro* fertilization, administration of vaginal progesterone gel starting 2 days before embryo transfer reduces the frequency of uterine contraction at the time of embryo transfer, increases uterine relaxation, propitiates embryo permanence in the endometrial cavity, and thereby promotes implantation.³⁸⁷ Other studies have examined the long-term effects of estrogen and progesterone replacement on uterine contractility in women aged 25 to 41. Following 2 weeks of transdermal estrogen to duplicate the pattern of estrogen production normally seen in the late follicular phase, vaginal administration of sustained release progesterone gel every two days from cycle day 15 caused time-dependent decrease in uterine contractility over the course of 5 days (Cycle days 15–20).³⁹²

MMPs play a major role in uterine tissue remodeling, and some of the effects of sex hormones on the uterus may involve MMPs. Uterine tissue remodeling and endometrium shedding during menstruation may involve estrogen-induced changes in MMPs activity.^{364,382,383} Progesterone may also regulate important factors in the formation and maintenance of endometrial lesions partly by affecting MMPs expression.³⁸⁴ In the pregnant bitches, significant correlations are observed between the elevated serum MMP-2 and MMP-9 activity and the elevated serum levels of estrogen and progesterone.⁵¹ In mouse uterus, estrogen alone or in combination with progesterone increases MMP-9 activity.³⁶⁴ We have found that treatment of virgin uterus with estrogen+progesterone increases MMP-2 and MMP-9 expression/activity. These findings are consistent with reports that estrogen increases MMP-2 expression in the immature rat uterus,³⁸² and suggest that estrogen and progesterone regulate uterine MMPs expression/activity during uterine tissue remodeling.

We have found that concomitant treatment of stretched myometrium of virgin rats with 17β -estradiol, progesterone, or estrogen+progesterone was associated with further reduction in contraction and increase in MMP expression/activity. The reduced contraction in sex hormone-treated uterus was reversed by MMP inhibitors. Also, estrogen and progesterone

treatment of stretched virgin uterus was associated with greater increases in MMP-2 and MMP-9 mRNA expression, protein level and enzymatic activity. These observations suggest a synergistic relationship between mechanical stretch and sex hormones in the reduced uterine contraction and enhanced MMP expression/activity. The mechanisms via which estrogen enhances uterine MMP expression/activity are unclear, but may involve estrogen receptor-mediated MAPK pathway.³⁹³ Also, while some studies suggest anti-inflammatory effects of estrogen,^{394–396} estrogen may increase inflammatory cytokines such as TNF- α and IL-6, which could in turn increase MMP expression/activity.^{364,393,397–399}

8.2. Uterine MMP Inducers and EMMPRIN during Pregnancy

Another factor that could affect uterine MMP expression during pregnancy is EMMPRIN, a known inducer of MMPs.^{29,52,366,400} We have examined whether the changes in uteroplacental MMPs expression/activity during pregnancy and in response to sex hormone involve changes in EMMPRIN.³⁷ Using the uterus, placenta and aorta from virgin and pregnant rats we found that MMP-2 and MMP-9 expression/activity were upregulated in the uterus and aorta during mid and late pregnancy. Treatment of the uterus and aorta of virgin rats with estrogen and progesterone caused increases in MMP-2 and MMP-9 expression/activity, that were blocked by EMMPRIN antibody. EMMPRIN was upregulated in the uterus and aorta of pregnant rats and in uterus and aorta of virgin rats treated with sex hormones.³⁷ These findings are consistent with reports that endometrial expression of EMMPRIN and MMPs is regulated by ovarian sex hormones in cycling baboons and that their expression patterns are dysregulated in endometriotic baboons.⁴⁰¹ These observations are also in agreement with reports that EMMPRIN expression increases in the bovine endometrium during estrous cycle and early gestation,²⁹ and support a role of EMMPRIN in inducing the changes in uterine MMPs expression during pregnancy. On the other hand, MMP-2 and MMP-9 expression/activity and EMMPRIN expression were downregulated in the placenta of late-pregnant compared with mid-pregnant rats,³⁷ suggesting different regulatory mechanisms of MMPs and EMMPRIN in the placenta compared with the uterus and blood vessels.

8.3 MMPs, Uterine Extracellular Matrix, and Uterine Relaxation Mechanisms

The effects of MMPs are generally thought to involve degradation of ECM components and extensive tissue remodeling. However, MMP inhibitors cause relatively rapid enhancement of contraction in uterine strips of pregnant rats, suggesting other effects in addition to uterine tissue remodeling. We have shown that MMP-2 and MMP-9 inhibit VSM contraction in the absence of detectable tissue degradation.^{40,42} Also, MMP-2 may cause membrane hyperpolarization and reduction in Ca²⁺ influx in rat inferior vena cava.^{41,42} Treatment of uterine strips with MMP-2 or MMP-9 caused relaxation of oxytocin-induced contraction.⁹³ These observations are consistent with our reports in vascular tissues.^{40–42} and point to inhibitory effects of MMPs on the mechanisms of uterine smooth muscle contraction (Fig. 3). Whether MMPs affect the plasma membrane channels or other mechanisms of uterine smooth muscle contraction remains to be examined.

Consistent with other reports in the rat uterus,^{378,402} we have shown that oxytocin causes a steady increase in contraction and an additional spontaneous phasic contractile response.⁹³

$[Ca^{2+}]_i$ is a key regulator of uterine contraction. Membrane depolarization mainly stimulates Ca^{2+} influx into uterine smooth muscle cells. Uterine smooth muscle $[Ca^{2+}]_i$ can also be modulated by different physiological and pharmacological agonists that affect the frequency, amplitude, and duration of uterine contraction.^{403,404} Studies in human myometrial cells have shown that oxytocin causes an initial $[Ca^{2+}]_i$ transient followed by uniform relatively low frequency $[Ca^{2+}]_i$ oscillations.^{405–407} The oxytocin-induced $[Ca^{2+}]_i$ oscillations in myometrial cells are attenuated by caffeine and the voltage-dependent Ca^{2+} channel antagonist verapamil, and blocked by the inorganic Ca^{2+} antagonist La^{3+} and the Ca^{2+} -ATPase inhibitor 2,5-di-tert-butylhydroquinone.⁴⁰⁸ Similarly, in rat uterine segments, oxytocin induces simultaneous $[Ca^{2+}]_i$ oscillations and phasic contractions that are inhibited by the Ca^{2+} channel blocker nifedipine.⁴⁰² Collectively, these studies support oxytocin-induced $[Ca^{2+}]_i$ oscillations that are mediated by intracellular Ca^{2+} release from inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} stores combined with voltage-dependent and capacitative Ca^{2+} influx. Interestingly, we have shown that MMP-2 causes hyperpolarization and reduction in Ca^{2+} influx in rat inferior vena cava,^{41,42} a smooth muscle preparation that also shows phasic contraction. These observations make it important to investigate the pregnancy-associated changes and the effects of MMPs on the magnitude and frequency of phasic uterine contractions, $[Ca^{2+}]_i$ and Ca^{2+} regulatory mechanisms.

We should note that we measured uterine contraction and MMP expression at gestational day 12 and day 19.³⁷ Other studies have shown that the contractility of rat uterus and the mRNA expression of the contractile agonists oxytocin, prostaglandin 2α , and ET-1 and their receptors are induced after gestational day 20,³⁷⁸ and that the last phase of pregnancy in rats shows an increase in uterine contractility and the density of $\alpha 1$ -adrenergic receptors,³⁸⁵ further highlighting the importance of measuring MMPs and EMMPRIN during the different stages of pregnancy. Likewise, our uterine stretch experiments were limited to 8 g basal tension for 18 hours, and the progressive effects of different levels of basal tension for extended time periods would be more analogous to the progressive uterine stretch imposed by the growing fetus over the course of pregnancy. Also, while our studies suggest a role of MMP-2 and MMP-9 in the reduced uterine contraction during pregnancy and in response to uterine tissue stretch, that should not minimize the possibility of involvement of other MMPs.

9. DYSREGULATION OF UTERINE MMPs DURING PRETERM LABOR

Myometrium activity is tightly regulated during pregnancy. At the first and mid-trimester, myometrium relaxation is needed to accommodate fetal growth. As fetal growth nears its completion during late pregnancy the uterine activity is first stabilized then starts to increase in preparation for delivery. We have demonstrated a relationship between uterine stretch, MMPs expression and uterine relaxation during gestation. We have also shown a role of sex hormones in promoting the effects of uterine stretch on MMPs expression and uterine relaxation. MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 are found in the amniotic fluid and fetal membranes during normal pregnancy. MMP-2 and MMP-3 are expressed constitutively while MMP-9 is barely detectable until labor. At labor, MMP-9 is the major MMP responsible for gelatinolytic activity in the membranes, while MMP-2 is dominant in the decidua. These findings may have clinical relevance as a disturbance in the balance of

MMPs or TIMPs could disturb uterine activity and lead to premature labor. The MMP/TIMP imbalance may be further aggravated by changes in the sex hormone levels or their uterine receptors.

Preterm labor complicates 10% to 15% of all pregnancies, and is a leading cause of perinatal morbidity and death;¹⁴ however, the mechanisms involved are not fully understood. MMP-2 and MMP-9 exhibit cell-specific expression in the human placenta. Studies have suggested that an increase in MMP-9 expression may contribute to degradation of ECM in the fetal membranes and placenta, thereby facilitating fetal membrane rupture and placental detachment from the maternal uterus at labor, both term and preterm.⁴⁰⁹ Also, studies on samples of amniochorion and amniotic fluid collected from women undergoing cesarean delivery before term, with either premature rupture of membranes or with preterm labor with no rupture of membranes, demonstrated an increased mRNA expression of MMP-2, MMP-9, and MT1-MMP and a decreased expression of TIMP-2 in prematurely ruptured membranes compared with preterm labor membranes. Enzyme-linked immunosorbent assay (ELISA) showed increases in the amniotic fluid concentrations of immunoreactive and bioactive MMP-2 and MMP-9 and immunoreactive MMP-3 and a decreased TIMP-2 concentration in fluids obtained from the premature rupture of membranes group compared with the preterm labor group.¹⁴ In contrast, in a study of 25 patients at preterm or term, MMP-2 protein and MMP-2 and MMP-9 pro-enzyme activities in the amnion markedly increased with labor at term, and were much higher than at preterm labor. There were no changes in chorion MMPs under any condition. These observations support a role of MMP-2 and MMP-9 in regulation of membrane rupture and other labor-associated mechanisms at term versus preterm parturition.⁴¹⁰ Cervicovaginal and/or intrauterine infection may be associated with preterm premature rupture of membranes (PPROM) and spontaneous preterm birth, likely due to the triggered inflammatory response. Microbial invasion of the amniotic cavity is associated with marked reduction in the levels of active MMP-2.^{409,411} A decrease in uterine MMP-2 and MMP-9 in preeclampsia, intrauterine infection and other pregnancy-related risk conditions is expected to hinder uterine expansion and cause IUGR and premature birth (Fig. 3).^{8,93} Other MMPs may also be involved in the regulation of membrane rupture and uterine contraction in term and preterm labor. Studies have suggested that inflammation could induce myometrial activator protein-1 (AP-1) which could in turn drive the production of stromelysins MMP-3 and MMP-10 and result in preterm labor in mice.⁴¹² Also, a cross-sectional study in 275 women examined whether parturition (either term or preterm), premature rupture of the membranes, and microbial invasion of the amniotic cavity are associated with changes in the levels of matrilysin MMP-7 in the amniotic fluid. MMP-7 was detected in 97.4% (268/275) of the samples, and showed an increase with advancing gestational age. Parturition at term and premature rupture of membranes without microbial invasion of the amniotic cavity (either term or preterm) was not associated with a change in MMP-7. On the other hand, preterm parturition in the absence of microbial invasion of the amniotic cavity and intra-amniotic infection in both patients with preterm labor and patients with preterm premature rupture of membranes were associated with marked increase in MMP-7. It was concluded that MMP-7 is a physiologic constituent of amniotic fluid, and its levels increase with advancing gestational age, and markedly increase during microbial invasion of the amniotic cavity in preterm gestations,

and the changes in MMP-7 may represent a maternal regulatory mechanism during infection and preterm labor.⁴¹³ Studies have also shown that plasma progesterone levels are lower in some preterm delivery patients compared to normal term pregnancies. For example, progesterone concentration was ~30% lower at 28 to 34 weeks gestation in women who delivered prematurely than in women who delivered at term.⁴¹⁴ Studies have suggested that progestin supplementation may prevent initiation of preterm labor or treat it once it is already established.⁴¹⁵ Whether changes in MMPs and sex hormones could interfere with uterine relaxation and trigger uterus contraction and pre-term delivery needs to be further examined. Also, whether the potential beneficial effects of progesterone in preterm labor are mediated via modulation of MMP expression/activity warrant further investigation.

10. MMPs in PREDICTION AND MANAGEMENT OF COMPLICATIONS OF PREGNANCY

Because preeclampsia has a relatively long preclinical phase before clinically manifesting in late gestation, the identification of women at risk, early diagnosis using various biomarkers, and prompt management could improve the maternal and perinatal outcome.¹¹ A decrease in brachial artery flow-mediated vasodilation could be an early indicator of compromised endothelial cell function between the 24th and 28th gestational weeks and before the clinical diagnosis of preeclampsia. The sensitivity of flow-mediated vasodilation is 87.5% and 95.5% for the prediction of early and late preeclampsia, respectively.²⁴⁸ Angiogenic/anti-angiogenic imbalance is an important feature in preeclampsia. Measurements of plasma VEGF, PIGF, sFlt-1 and sEng may help early detection in asymptomatic pregnant women, especially those at high risk for preeclampsia.¹¹ In preeclampsia, circulating levels of sFlt-1 are increased more than one month before the onset of clinical symptoms, and PIGF is decreased in women who subsequently develop preeclampsia from the end of the first trimester.⁴¹⁶ The sFlt-1/PIGF ratio is increased in preeclamptic versus normal pregnant women and in both early and late preeclampsia.¹³⁹ A meta-analysis of 20 different studies revealed that the overall diagnostic accuracy of sFlt-1/PIGF ratio for preeclampsia is relatively high, and its diagnostic efficiency is higher in early than late preeclampsia.⁴¹⁷

Assessment of the immune system could be useful in predicting preeclampsia in early pregnancy. An abnormal maternal immunological response could be presented as a change in monocytes and NK cells, increased inflammatory factors, altered release of cytokines, increased AT₁-AA, and activation of pro-inflammatory AT₁R.¹¹ TNF- α levels could be an early predictor of preeclampsia. Plasma obtained at gestational weeks 11–13 showed higher TNF- α levels in women who later developed preeclampsia.⁴¹⁸ Studies have suggested that elevated plasma TNF- α levels in association with changes in uterine artery Doppler at 11–13th gestational weeks have a 100% sensitivity in predicting preeclampsia.⁴¹⁹ Other reports suggest that plasma TNF- α levels may be useful in predicting preeclampsia in the early third trimester, but not the first or second trimesters.⁴²⁰ Some studies suggest that at gestational week 28, miRNA-206, which interacts with several genes directly involved in the pathology of preeclampsia, is elevated in plasma and placenta from women who subsequently develop preeclampsia,⁴²¹ although other studies show little predictive value of miRNAs.⁴²² Measurements of the levels of MMPs have not been consistent in preeclampsia, with some

studies showing an increase in MMP-2 and MMP-9,¹⁰⁰ while other studies showing a decrease in MMP-9.⁷ The discrepancy in the results may be due to the fact that plasma MMPs represents global changes in MMPs in different tissues, and localized changes in MMPs in uteroplacental tissues and fluids may carry more predictive value. Because of the existence of several biomarkers, their predictive value should be further assessed in order to identify the best marker combinations for predicting preeclampsia.¹¹

Currently, inducing labor is the most effective treatment for preeclampsia. International guidelines recommend the use of one of the antihypertensive agents methyldopa, labetalol, other beta-blockers (acebutolol, metoprolol, pindolol, and propranolol), or Ca²⁺ channel blockers (nifedipine).⁴²³ ACE inhibitors should not be used due to their teratogenic effects, and atenolol and prazosin are not recommended prior to delivery. If preeclampsia evolves to eclampsia, magnesium sulfate is often used to prevent eclamptic seizures.⁴²⁴

Sildenafil has been proposed for women with severe early-onset IUGR, as it has been shown to increase fetal growth with no maternal side effects.⁴²⁵ Sildenafil citrate vasodilates myometrial arteries isolated from women with IUGR-complicated pregnancies. Also, treatment with sildenafil citrate restored endothelial cell integrity in the placental vessels of L-NAME treated mouse model of hypertension in pregnancy.⁴²⁶ Eculizumab, an anti-C5 antibody, normalized laboratory values and prolonged pregnancy by 17 days in a woman with preeclampsia/HELLP syndrome, suggesting the benefits of manipulating the complement system during preeclampsia. However, complement inhibitors could increase susceptibility to infection and their long-term use requires close monitoring.⁴²⁷

Studies suggest potential benefits of correcting the angiogenic/anti-angiogenic imbalance in preeclampsia. In trophoblast cells and HUVECs treated with cobalt chloride to simulate hypoxic conditions, the free radical scavenger edaravone inhibit sFlt-1 expression in trophoblast cells and protects against the decrease in vascular development and tube formation in HUVECs.⁴²⁸ VEGF could improve the angiogenic/anti-angiogenic imbalance, but may impair bradykinin-induced vascular relaxation and enhance basal tone and vascular permeability in preeclampsia.⁷⁶ Modulators of PIGF could be more promising in preeclampsia, and low molecular weight heparin therapy increases circulating PIGF levels during third trimester pregnancy.⁴²⁹

TNF- α antagonists such as etanercept decrease blood pressure, increase eNOS expression, decrease ET-1 levels and prevent cardiac changes in RUPP rats.^{181,430} Also, IL-17 soluble receptor C inhibits IL-17, prevents the recruitment of host defense cells, suppresses the inflammatory response, decreases AT₁-AA and ROS, and ameliorates hypertension and improves pup and placental weight in RUPP rats.²²⁹

It is possible that RUPP causes the release of cytoactive factors which in turn disrupt MMP balance and lead to inadequate uteroplacental remodeling, vascular dysfunction and hypertension in pregnancy. Thus one way to manage hypertension in pregnancy is to target the cytoactive factors acting upstream of MMPs. If downregulation of MMP-2 and MMP-9 is a target of anti-angiogenic sFlt-1, then counteracting sFlt-1 by PIGF should reverse the reduction in MMP expression, improve MMP-mediated vascular relaxation and reduce

vasoconstriction and blood pressure. In support, infusion of pro-angiogenic PIGF or VEGF reduces blood pressure in RUPP rats,^{171,431} and infusion of anti-inflammatory IL-10 decreases blood pressure in DOCA/salt hypertensive pregnant rats.⁴³² An alternative approach is to aim at the common target affected by cytoactive factors. If MMPs are a central target in hypertension in pregnancy, then correcting MMP imbalance should promote vasodilation and reduce blood pressure. Doxycycline is a non-specific MMP inhibitor that was proposed to alleviate hypertension and vascular dysfunction in preeclampsia, but was found to decrease placenta weight and cause IUGR in both normal pregnant and hypertensive pregnant rats, and to reduce trophoblast invasion and placental perfusion in hypertensive pregnant rats.⁹⁶ Thus, novel approaches to indirectly or directly correct MMP imbalance should provide new strategies in the management of hypertension in pregnancy and preeclampsia.

11. CONCLUDING REMARKS

Normal pregnancy is associated with uteroplacental and vascular remodeling in order to adapt for the growing fetus and the hemodynamic changes in the maternal circulation. Normal pregnancy is also associated with increased MMPs expression/activity. It is likely that myometrium stretch and the increase in the sex hormone progesterone during pregnancy alter the expression/activity of specific MMPs, and in turn inhibit uterine contraction and promote uterine relaxation. The pregnancy-associated upregulation of uterine MMPs is paralleled by increased expression/activity of vascular MMPs, and both appear to be mediated by EMMPRIN and induced by estrogen and progesterone, suggesting similar role of MMPs in uterine and vascular tissue remodeling and function during pregnancy. The decrease in MMP expression/activity and EMMPRIN expression in the placenta of late-pregnant rats suggest reduced role of MMPs in the fetoplacental circulation during late pregnancy.

Preeclampsia is a complication of pregnancy manifested as maternal hypertension and often IUGR. Genetic and environmental factors may cause localized abnormal placentation, altered maternal immune response, and abnormal expression of integrins, inflammatory cytokines and MMPs, leading to increased apoptosis of trophoblast cells, shallow trophoblastic invasion and inadequate spiral artery remodeling, and causing RUPP and placental ischemia/hypoxia. Ischemic/hypoxic placenta causes the release of bioactive factors such as sFlt-1, sEng, TNF- α , IL-6, HIF, ROS and AT₁-AA which could target MMPs in ECM and alter uteroplacental and vascular remodeling. Bioactive factors could also affect endothelial cells, cause endothelial dysfunction, decrease vasodilators or increase ET-1 and other vasoconstrictors and lead to hypertension in pregnancy.

Animal models of hypertension in pregnancy such as the RUPP rat have helped in understanding the mechanisms of preeclampsia. Maternal blood pressure is higher, and the litter size and pup weight are reduced in RUPP versus normal pregnant rats. MMP-2 and MMP-9 expression/activity is reduced and collagen deposition is increased in uterus, placenta and aorta of RUPP versus normal pregnant rats, suggesting a role for MMPs in growth-restrictive remodeling in hypertension in pregnancy. Anti-angiogenic factors such as sFlt-1 decrease MMPs expression/activity in uterus, placenta and aorta of normal pregnant

rats, and the angiogenic factor VEGF reverses the effects of sFlt-1 in tissues of normal pregnant rats and the decreases in MMPs expression/activity in tissues of RUPP rats. Thus placental ischemia and anti-angiogenic sFlt-1 decrease uterine, placental and vascular MMP-2 and MMP-9, leading to increased uteroplacental and vascular collagen, and growth-restrictive remodeling in hypertension in pregnancy. Angiogenic factors and MMP inducers/activators may reverse the decrease in MMPs and enhance growth-permissive remodeling in preeclampsia.

While marked changes have been detected in uterine, placental and aortic MMPs in hypertensive pregnant rats, that should not minimize the importance of measuring the structural changes and MMPs expression in other tissues particularly the small resistance vessels which affect blood pressure. Also, RUPP during pregnancy may cause the release of not only anti-angiogenic factors, but also cytokines, ROS and HIF, which could in turn lead to uteroplacental and vascular dysfunction during hypertension in pregnancy.^{20,57–59,62,109,110} Interestingly, cytokines, ROS and HIF have been shown to affect MMPs expression/activity,^{209,398} and studying their differential effects as well as the effects of their inhibitors on MMPs in normal pregnancy and hypertension in pregnancy should be examined.

Further understanding of the interaction between bioactive factors, MMPs, and vascular mediators and cellular mechanisms should help design more specific and efficient measures for prevention, early detection, and management of preeclampsia. Targeting MMPs could provide a new approach for the detection and management of hypertension in pregnancy, preeclampsia, and premature labor.

Acknowledgments

This work was supported by grants from National Heart, Lung, and Blood Institute (HL-65998, HL-111775). Dr. J. Chen was a visiting scholar from the Department of Obstetrics & Gynecology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, P.R. China.

ABBREVIATIONS

ACh	acetylcholine
AngII	angiotensin II
AT₁R	AngII type 1 receptor
AT₁-AA	AngII AT ₁ R agonistic autoantibodies
[Ca²⁺]_i	intracellular free Ca ²⁺ concentration
cGMP	cyclic guanosine monophosphate
DOCA	deoxycorticosterone acetate
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor

eNOS	endothelial nitric oxide synthase
EMMPRIN	extracellular MMP inducer
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
ET-1	endothelin-1
H₂O₂	hydrogen peroxide
HIF	hypoxia-inducible factor
HELLP	hemolysis elevated liver enzymes low platelets
HUVECs	human umbilical vein endothelial cells
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IUGR	intrauterine growth restriction
L-NAME	N _ω -nitro-L-arginine methyl ester
MAPK	mitogen-activated protein kinase
MEGJ	myoendothelial gap junction
MMP	matrix metalloproteinase
NO	nitric oxide
O₂^{•-}	superoxide anion
PGI₂	prostacyclin
PIGF	placental growth factor
PKC	protein kinase C
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
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor

VCAM-1 vascular cell adhesion molecule-1

VSM vascular smooth muscle

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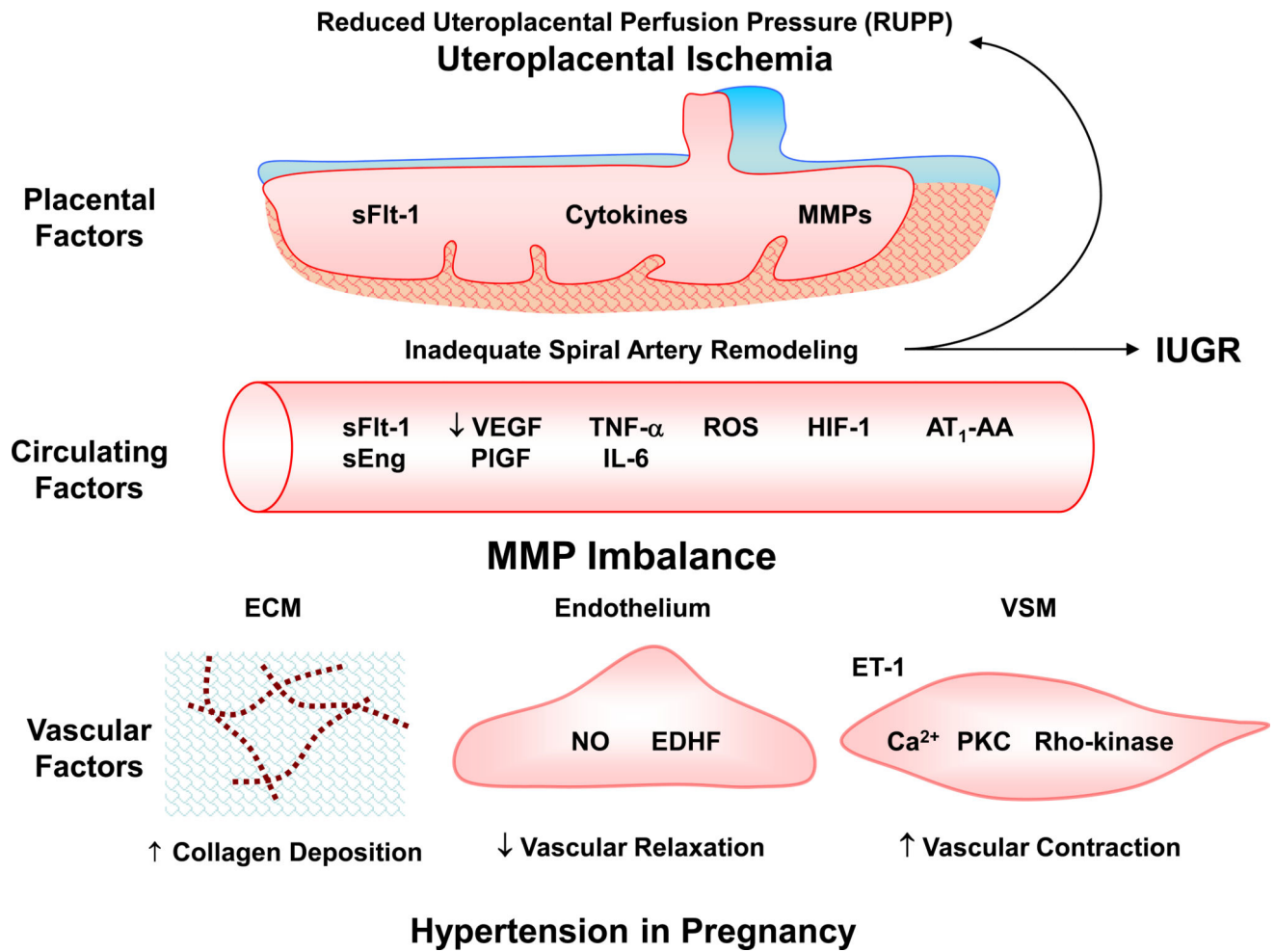
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**Fig. 1.**

Role of MMPs in hypertension in pregnancy and IUGR. Initial reduction of uteroplacental perfusion pressure (RUPP) and uteroplacental ischemia causes the release of cytoactive and circulating factors, which target MMPs in uteroplacental tissues leading to intrauterine growth restriction (IUGR) and in blood vessels leading to increased collagen deposition in extracellular matrix (ECM), decreased endothelium-dependent vascular relaxation pathways, and increased endothelin-1 (ET-1) and mechanisms of VSM contraction, resulting in increased vascular resistance and hypertension in Pregnancy.

AT₁-AA, AngII AT₁R agonistic autoantibodies; EDHF, endothelium-derived hyperpolarizing factor; HIF, hypoxia-inducible factor; IL-6, interleukin-6; NO, nitric oxide; PKC, protein kinase C; PIGF, placental growth factor; ROS, reactive oxygen species; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial growth factor; VSM, vascular smooth muscle

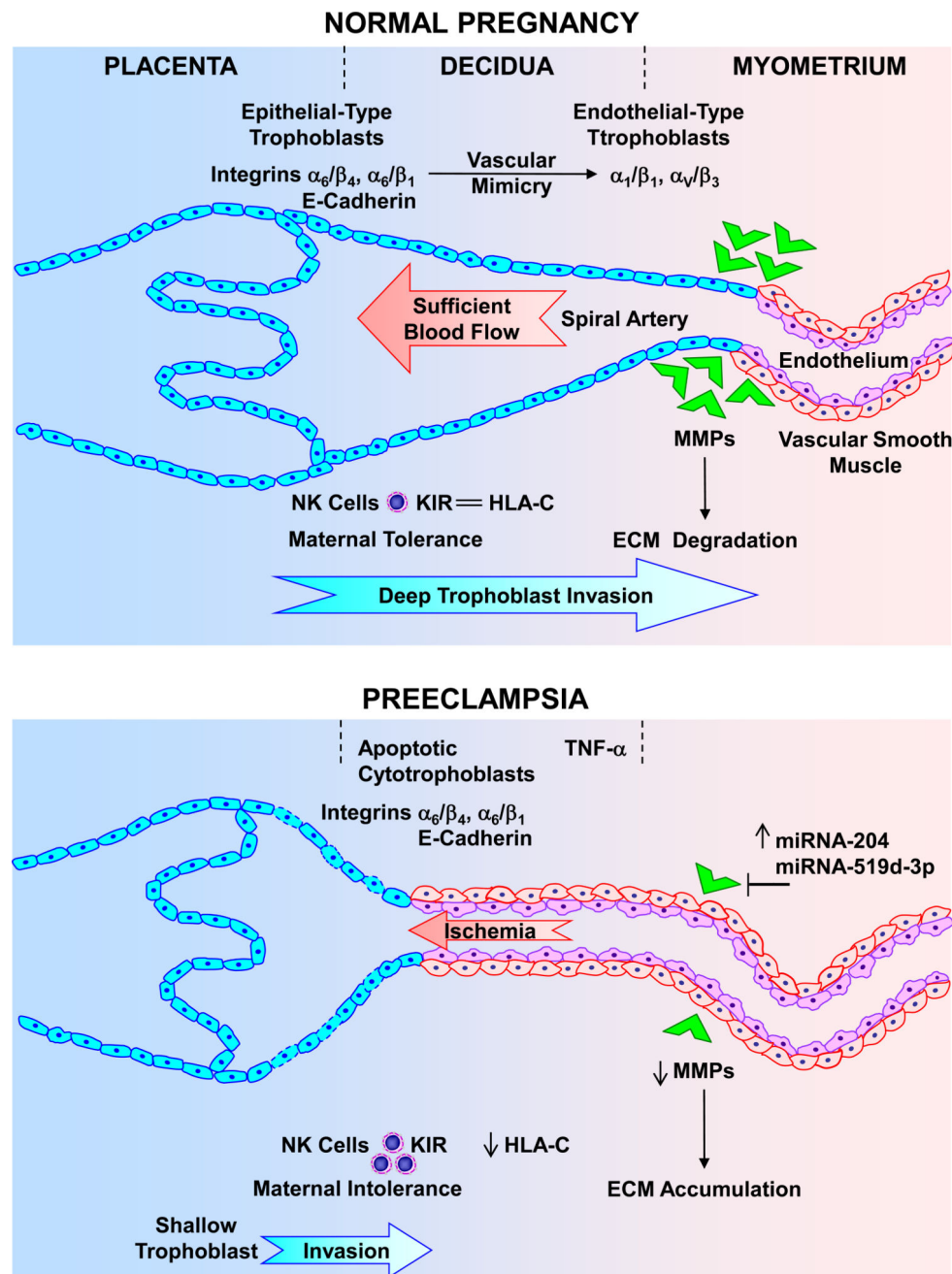


Fig. 2. Deficient placentation in preeclampsia. During normal pregnancy, cytotrophoblasts initially express epithelial-type adhesion molecules such as integrins α_6/β_4 and α_6/β_1 , and E-cadherin. As cytotrophoblasts become invasive, they express endothelial-type integrins α_1/β_1 and α_V/β_3 (“vascular mimicry”). Matrix metalloproteinases (MMPs) also cause degradation of extracellular matrix (ECM) and allow vascular remodeling. Cytotrophoblasts overexpress HLA-C that interact with the inhibitory KIR receptor and decrease natural killer (NK) cells, thus contributing to maternal tolerance. As a result, cytotrophoblasts invade the decidua to one-third of the myometrium, causing extensive remodeling of the spiral arteries

from small-caliber resistance vessels to high-caliber capacitance vessels, and providing sufficient placental blood flow. In preeclampsia, increased immune response causes the release of cytokines such as TNF- α , apoptosis of cytotrophoblasts and maintained expression of epithelial-type integrins α_6/β_4 and α_6/β_1 , and E-cadherin. Increased miRNA-519d-3p and -204 also decrease MMPs leading to decreased ECM degradation and vascular remodeling. Decreased HLA-C interaction with the inhibitory KIR receptor increases NK cells and decreases maternal tolerance. The decrease in trophoblast invasion of spiral arteries leads to shallow placentation to only superficial layers of the decidua, resulting in decreased blood flow and placental ischemia.

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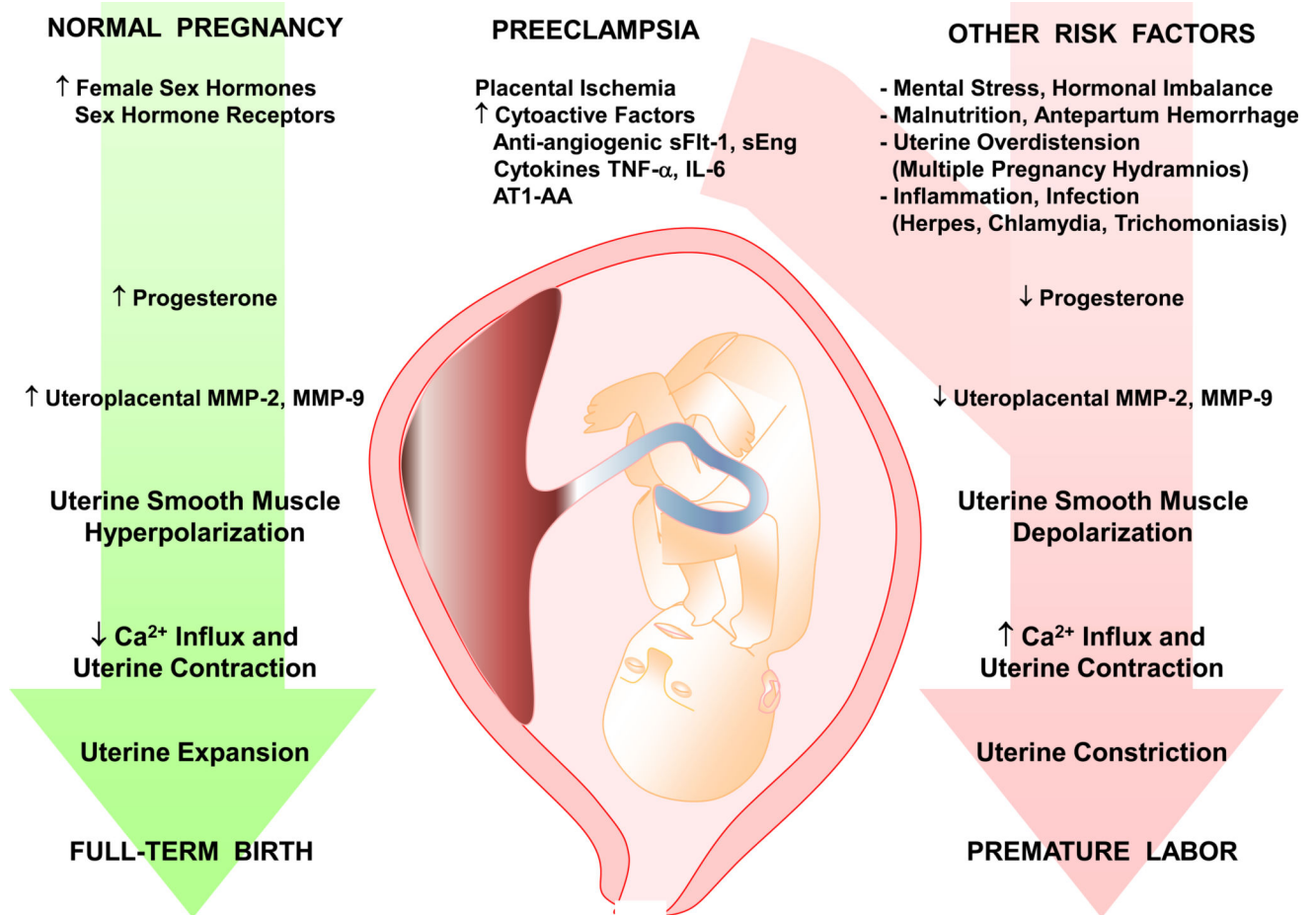


Fig. 3. Role of MMPs in normal and premature labor. During normal pregnancy increases in progesterone levels cause increases in uteroplacental MMP-2 and MMP-9 expression/activity, leading to uterine smooth muscle hyperpolarization and relaxation, uterine expansion to accommodate the growing fetus, and therefore full-term birth. Preeclampsia and other risk factors during pregnancy are associated with decreases in progesterone, leading to decreased MMP-2 and MMP-9 expression/activity, increased uterine smooth muscle depolarization, uterine contraction and premature birth. AT₁-AA, AngII AT₁R agonistic autoantibodies; IL-6, interleukin-6; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1; TNF-α, tumor necrosis factor-α

Table 1

MMP and TIMP levels in human normal pregnancy and preeclampsia

MMP/TIMP	Specimen (Units)	Normal pregnancy	Preeclampsia	Reference
MMP-1	Umbilical cord serum (pg/mL)	294.33±11.53	177.67±12.63	101
MMP-2	Serum (ng/mL)	669 (560–760)	834 (656–1002)	7100
	Plasma (ng/mL)	241.1±35.3 <i>SD</i>	290.5±48.4 <i>SD</i>	
MMP-9	Serum (ng/mL)	390 (277–569)	290 (280–470)	7100
	Plasma (ng/mL)	240.0±197.7 <i>SD</i>	262.4±153.8 <i>SD</i>	
TIMP-1	Serum (ng/mL)	148 (121–188)	213 (212–220)	7100101
	Plasma (ng/mL)	142.8±39.2 <i>SD</i>	187.1±35.4 <i>SD</i>	
	Umbilical cord serum (pg/mL)	1304.20±69.66	1363.00±71.50	
TIMP-2	Serum (ng/mL)	228 (207–267)	232 (225–245)	7
	Plasma (ng/mL)	158.3±32.3 <i>SD</i>	194.3±49.3 <i>SD</i>	100

Values represent means±standard error of the mean.

SD indicates standard deviation. Numbers in parenthesis indicates range.

Table 2

MMP levels in normal pregnant and RUPP rats

MMP	Specimen (Units)	Normal Pregnant	RUPP	Reference
MMP-2	Uterus (OD)	~1.0	~0.7	8
	Placenta (OD)	~0.9	~0.5	
	Aorta (OD)	~0.9	~0.6	
MMP-9	Uterus (OD)	~0.4	~0.2	8
	Placenta (OD)	~0.3	~0.2	
	Aorta (OD)	~0.4	~0.2	

OD: optical densitometry of Western blot bands normalized to β -actin

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