Molecular Mechanisms of Preeclampsia

Tammy Hod[1], Ana Sofia Cerdeira[1,2], and S. Ananth Karumanchi[1,3]

[1]Department of Medicine, Obstetrics & Gynecology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02214

[2]Gulbenkian Program for Advanced Medical Education, 1067-001 Lisbon, Portugal

[3]Howard Hughes Medical Institute, Chevy Chase, Maryland 20815

Correspondence: sananth@bidmc.harvard.edu

Preeclampsia is a pregnancy-specific disease characterized by new onset hypertension and proteinuria after 20 wk of gestation. It is a leading cause of maternal and fetal morbidity and mortality worldwide. Exciting discoveries in the last decade have contributed to a better understanding of the molecular basis of this disease. Epidemiological, experimental, and therapeutic studies from several laboratories have provided compelling evidence that an antiangiogenic state owing to alterations in circulating angiogenic factors leads to preeclampsia. In this review, we highlight the role of key circulating antiangiogenic factors as pathogenic biomarkers and in the development of novel therapies for preeclampsia.

Preeclampsia is a leading cause of maternal morbidity and mortality worldwide, complicating 2%–8% of pregnancies (Duley 2009). It is a multisystemic disorder with both maternal and fetal manifestations. In the mother, alongside with hypertension and proteinuria, the disease can progress to widespread microangiopathy that mainly affects the kidney, liver, and brain. Thrombocytopenia, liver dysfunction, microangiopathic hemolytic anemia, acute renal failure, placental abruption, visual disturbances, stroke, seizures, and maternal death are serious consequences of preeclampsia. In the fetus, preeclampsia is associated with intrauterine growth restriction and prematurity. Besides adverse pregnancy outcomes, women with preeclampsia have an increased risk of future health complications (Irgens et al. 2001; Bellamy et al. 2007; Vikse et al. 2008; Wang et al. 2013b; Chen et al. 2014).

Recent data suggest that alterations in circulating angiogenic factors play a pathogenic role in preeclampsia. Angiogenesis, the process of new blood vessel formation from preexisting ones, is tightly regulated by angiogenic factors. Importantly, angiogenic factors are also essential for maintenance of normal vessel health; they provide important cues for organ development. Increased levels of the antiangiogenic factors, soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble Endoglin (sEng) trap circulating vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and transforming growth factor β (TGFβ) respectively, decreasing their free levels, leading to endothelial dysfunction and the clinical manifestations of the

disease (Maynard et al. 2003; Levine et al. 2004, 2006b; Venkatesha et al. 2006; Romero and Chaiworapongsa 2013). Interestingly, the preeclampsia-like signs and symptoms are also seen in cancer patients receiving antiangiogenic chemotherapy (Hurwitz et al. 2004; Patel et al. 2008; Vigneau et al. 2014).

The search for the ability to better diagnose, predict, and prevent preeclampsia as well as the mechanisms of its pathogenesis to develop a therapy that safely prolongs gestation has been extensive. Exciting data on angiogenic factors as central contributors to the pathogenesis of preeclampsia, candidate biomarkers, and therapeutic targets open clinically meaningful perspectives for the near future.

PATHOGENESIS

### Angiogenic Factors as Mediators of the Maternal Syndrome

Taylor and Roberts posited that a dysfunctional placenta releases "toxic" factors into the maternal circulation that trigger the clinical syndrome of preeclampsia (Roberts et al. 1989). The placenta is both necessary and sufficient to cause the disease, and delivery of the placenta is the only treatment (Powe et al. 2011). Over the last decade, preeclampsia has been recognized as an antiangiogenic state (Romero and Chaiworapongsa 2013). Excessive placental production of antiangiogenic factors sFlt-1 and sEng are liberated into the maternal circulation inducing the clinical syndrome. Preeclamptic placentas show overexpression of sFlt-1 and sEng. Their levels are increased in the serum of preeclamptic women weeks before the appearance of overt clinical manifestations of the disease and they correlate with disease severity (Levine et al. 2006b)

Importantly, sFlt-1 overexpression in pregnant rodents is sufficient to induce the hallmarks of the disease: hypertension, proteinuria, and the characteristic kidney lesion, glomerular endotheliosis (Maynard et al. 2003). In addition, we showed that alterations in circulating sFlt-1 may explain a number of risk factors for preeclampsia such as multiple gestation, trisomy 13,

nulliparity, and molar pregnancies (Powe et al. 2011). Addition of sEng to this model induces a more severe phenotype including thrombocytopenia, cerebral edema, and fetal growth restriction (Venkatesha et al. 2006; Maharaj et al. 2008).

Fms-like tyrosine kinase 1 (Flt-1), and soluble Flt-1 (sFlt-1) are the products of *FLT-1* generated by differential mRNA processing. Flt-1, also known as vascular endothelial growth factor receptor 1 (VEGFR-1), is a membrane spanning receptor for VEGF and PlGF. It comprises an extracellular domain with seven immunoglobulin-like domains (ligand-binding), a membrane domain, and a cytoplasmic tyrosine kinase domain. Soluble Flt-1 is a shorter isoform that retains the ligand-binding domain but lacks the cytoplasmic and transmembrane domains and is therefore secreted into the circulation (Kendall and Thomas 1993; Huckle and Roche 2004; Thomas et al. 2007). sFlt-1 can also be generated by cleavage of the membrane receptor by proteases, although the physiologic significance of this process is not known (Rahimi et al. 2009; Zhao et al. 2010). Soluble Flt-1 binds VEGF and PlGF in circulation and locally in tissues acting as a scavenger that prevents them from interacting with their membrane receptors on the endothelium (Fig. 1). Therefore, an increase in the concentration of sFlt-1 decreases free, and thus bioactive, PlGF and VEGF. Another splicing variant of VEGFR-1, sFlt1-14, generated primarily in nonendothelial cells, functions similarly to sFlt-1 as a potent VEGF inhibitor. Interestingly, conversion of VEGFR-1 mRNA to sFlt1-14 prevents VEGFR-1-mediated signal transmission, in contrast to sFlt-1, whose production in endothelial cells is accompanied by a large excess of the transmembrane receptor. Expression of sFlt1-14 has been shown to be significantly increased in preeclampsia. Specifically, syncytial knots were identified as the major source of local and circulating sFlt1-14 (Sela et al. 2008). The exact role of the different splicing variants of VEGFR-1 in antiangiogenic state remains to be elucidated.

The physiologic function of sFlt-1 is not clearly understood. sFlt-1 is crucial for maintenance of cornea avascularity (Ambati et al.
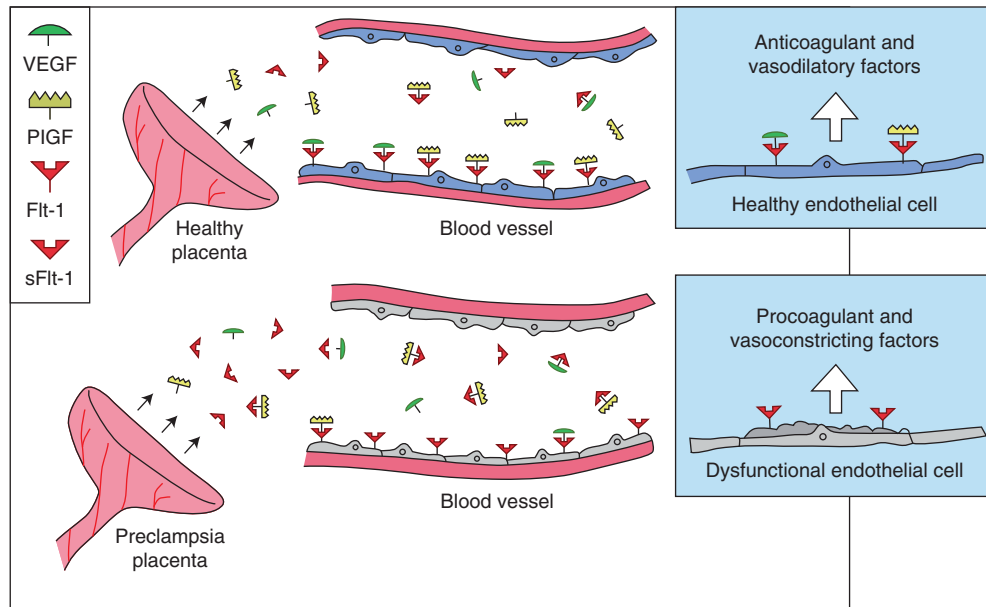
Figure 1. VEGF signaling and role of sFlt-1 in maternal endothelial dysfunction. There is mounting evidence that VEGF, and possibly PlGF, are required to maintain endothelial health in several tissues including the kidney and perhaps the placenta. In normal pregnancy, the placenta produces modest concentrations of VEGF, PlGF, and sFlt-1. In preeclampsia, excess placental sFlt-1 binds circulating VEGF and PlGF and prevents their interaction with endothelial cell-surface receptors leading to endothelial dysfunction. (From Karumanchi et al. 2005; reproduced, with permission, from the author.)

2006) and provides important cues for vessel sprouting (Chappell et al. 2009). Flt-1 signaling appears to be nonessential for normal placental development (Hiratsuka et al. 1998), and therefore has been debated as a viable therapeutic target in preeclampsia. Evolutionary biologists have argued that one important function of sFlt-1 may be to improve placental vascular perfusion for the benefit of the fetus (Yuan et al. 2005).

Plasma sFlt-1 levels are increased before the onset of clinical signs and symptoms and they are correlated with disease severity (Chaiworapongsa et al. 2004, 2005). This is mirrored by a decrease of free VEGF and PlGF (Maynard et al. 2003; Polliotti et al. 2003; Levine et al. 2004). The main source of sFlt-1 in pregnancy is the placenta (Maynard et al. 2003; Gu et al. 2008). sFlt-1 is highly overexpressed in preeclamptic placentas, in particular in syncytial knots. Additional sources for sFlt-1 production, such as peripheral blood mononuclear cells (Rajaku-

mar et al. 2005), have been described, but the clinical significance is unknown.

sFlt-1 contains heparin-binding domains in the third and fourth immunoglobulin loops that account for its strong avidity to the heparan sulfate on cell surfaces or on the extracellular matrix (Kendall and Thomas 1993; Park and Lee 1999; Sela et al. 2011). This chemical property raised questions about the mechanism by which sFlt-1 gains access to the maternal circulation. Heparanase releases sFlt-1 retained in these storages (Sela et al. 2011). However, expression of heparanases in the placenta is only modestly altered in preeclampsia (Ginath et al. 2014).

Release of trophoblast-derived material/ fragments into the maternal circulation has long been recognized (Redman and Sargent 2000). It is an intriguing phenomenon in which the physiological function remains to be elucidated. Trophoblast fragments are a significant source of sFlt-1 in the maternal circulation in

preeclampsia. The first report of trophoblast deportation comes from Schmorl who showed trophoblast material in lungs of patients that died from eclampsia (Lapaire et al. 2007). It is now recognized that the detachment of syncytial knots from the placenta results in free, multinucleated trophoblast microparticles that are metabolically active and capable of de novo gene translation, including the ability to synthesize sFlt-1 protein from endogenous stores of mRNA (Rajakumar et al. 2012). This phenomenon is enhanced in preeclampsia, suggesting that an accelerated shedding of syncytial aggregates is a proximal event in the pathogenesis of preeclampsia (Buurma et al. 2013).

VEGF signaling is not only important for angiogenesis but also for maintaining endothelial health in certain specialized vascular beds such as liver, kidney, brain as well as endocrine organs such as thyroid tissue, where it is constitutively expressed to maintain endothelial fenestrations (Kamba et al. 2006). There is compelling evidence that the growth of some tumors is dependent on VEGF-induced angiogenesis (Sitohy et al. 2011). VEGF inhibitor therapy caused early striking vascular changes in islet cell tumors. Within 24 hours, endothelial fenestrations and sprouts disappeared, patency was lost, and flow ceased in some vessels. Tumors vessels lacking fenestrations were less responsive to the inhibitors, suggesting that vessel phenotype may be predictive of responsiveness to VEGF inhibitors (Inai et al. 2004). Cessation of anti-VEGF therapy led to complete revascularization within the first week after the treatment stopped which fully regressed during a second treatment (Mancuso et al. 2006). VEGF, which is strongly expressed by podocytes, although its binding sites are localized on glomerular endothelial cells, plays a paramount role in maintaining glomerular integrity under normal physiologic conditions, such that when intraglomerular VEGF levels decrease capillary endothelial cells swell, capillary loops collapse, glomerular filtration is impaired, and proteinuria develops (Mattot et al. 2002; Eremina and Quaggin 2004). Moreover, after renal injury, there is a transient increase in VEGF expression followed by a decrease in its expression resulting

in glomerular and peritubular capillary loss, which correlates with the severity of glomerulosclerosis, interstitial fibrosis, and tubular atrophy. This shows that lack of persistence of VEGF expression may be a primary factor in the progression of renal insufficiency (Kang et al. 2001a,b).

VEGF treatment promoted glomerulogenesis and vascularization of implanted organoids and enabled the generation of vascularized nephrons from single cell suspensions (Xinaris et al. 2012). Genetic studies in mice have provided compelling evidence that VEGF is involved in the induction and maintenance of the fenestrae in glomerular capillary endothelial cells (Eremina et al. 2003). Moreover, slit-diaphragm proteins such as nephrin are significantly reduced in podocytes in animals receiving VEGF inhibitors (Sugimoto et al. 2003) as well as in renal biopsy studies in preeclamptic subjects (Garovic et al. 2007). Podocyte VEGF knockdown disrupts integrin activity via decreased VEGFR2 signaling, thereby damaging the glomerular filtration barrier, causing proteinuria and acute renal failure (Veron et al. 2012). VEGF also mediates endothelium-dependent vasodilatation and is thus important for blood pressure regulation (He et al. 1999; Facemire et al. 2009). Hypertension and proteinuria with glomerular damage resembling preeclampsia are adverse effects of VEGF inhibitors therapy given to nonpregnant cancer patients (Eremina et al. 2008; Patel et al. 2008; Vigneau et al. 2014). Furthermore, the treatment of pregnant rats or mice with sFlt-1 results in hypertension, proteinuria, and glomerular endotheliosis, the classic lesions of preeclampsia (Maynard et al. 2003; Lu et al. 2007). Taken together, these studies and others suggest that VEGF inhibition prompts endothelial dysfunction, hypertension, and proteinuria.

Other factors released by the placenta act synergistically with sFlt-1 to induce an antiangiogenic environment. Placental endoglin is upregulated in preeclampsia, releasing sEng to the maternal circulation. sEng interferes with TGF-β signaling and eNOS activation and thereby causes endothelial dysfunction (Venkatesha et al. 2006). Overexpression of sEng and sFlt-1

in mice also induces cerebral edema that resembles eclampsia and severe preeclampsia (Maharaj et al. 2008). sEng is increased in the sera of preeclamptic women 2–3 mo before clinical signs of preeclampsia develop, and its blood levels correlate with disease severity (Levine et al. 2006a; Noori et al. 2010). Semaphorin 3B, a novel trophoblastic secreted antiangiogenic protein may also synergize with sFlt1 by interfering with VEGF signaling and contributing to the pathogenesis of preeclampsia (Zhou et al. 2013).

Recently, a pathophysiological explanation was provided for the epidemiological association of peripartum cardiomyopathy (PPCM) with preeclampsia (Bello et al. 2013). Exogenous sFlt-1 alone caused diastolic dysfunction in wild type mice, and profound systolic dysfunction in mice lacking cardiac PGC-1α, a powerful regulator of angiogenesis. Furthermore, plasma samples from women with PPCM contained abnormally high levels of sFlt-1. These data indicate that PPCM is a two-hit combination of, first, systemic antiangiogenesis during late pregnancy which is substantially worsened in preeclampsia and/or in multiple gestation and second, a host susceptibility attributed to insufficient local proangiogenic defenses in the heart (Patten et al. 2012).

Mechanisms of Placentation Defects in Preeclampsia

Although high levels of sFlt-1 and sEng are widely recognized as contributors to the pathogenesis of the disease, the upstream mechanisms of placental damage remain to be elucidated. Several mechanisms have been proposed such as placental hypoxia, oxidative stress, endoplasmic stress, inflammation, altered NK cell signaling, autoantibodies against the angiotensin receptor, and others (Chaiworapongsa et al. 2014).

During normal placental development, the maternal uterine spiral arteries undergo an extensive remodeling, transforming them into low resistance, high bore blood vessels adequate to provide nutrients to the placenta and fetus (Brosens et al. 1967). In preeclampsia, cytotro-

phoblasts (CTBs) fail to invade the myometrium and the physiological changes of the spiral arteries are restricted to the decidua (Fig. 2). Invading CTBs up-regulate expression of molecules that are central to uterine invasion and pseudovasculogenesis (the process by which CTB switch their adhesion molecules to mimic that of vascular cells) (Damsky and Fisher 1998). Expression of molecules from the VEGF family changes as invasion of these cells through the uterine wall takes place. Invasive CTBs in early gestation express VEGF-A, VEGF-C, PlGF, VEGFR-1 and VEGFR-3 and at term, VEGF-A, PlGF, and VEGFR-1. The interactions between these molecules are critical for invasion and pseudovasculogenesis because interference with ligand binding altered integrin α1, an adhesion molecule highly expressed by endovascular CTB, resulting in increased apoptosis of these cells. Interestingly, in severe preeclampsia, expression of VEGF-A and VEGFR-1 in the cytotrophoblasts is down-regulated but sFlt-1 release is increased (Zhou et al. 2002). These findings suggest that dysregulation of angiogenic factors in the maternal–fetal interface is associated with failed pseudovasculogenesis and impaired placentation (Zhou et al. 2002; Fisher 2004).

Epidemiological data suggest that the immune system may play a role in the pathogenesis of preeclampsia (Robillard et al. 2011). Uterine natural killer (uNK) cells, which are abundant in decidualized endometrium, surround spiral arteries and secrete a range of angiogenic growth factors play that a major role in immunology-related placentation, vascular remodeling of uterine arteries, and angiogenesis (Croy et al. 2000). When chorionic plate arteries and spiral arteries are cultured with conditioned medium from uNK cells, disruption of vascular smooth muscle cells and breakdown of extracellular matrix components is induced. These results show that uNK cells contribute to the early stages of spiral artery remodeling (Robson et al. 2012). Genetic studies have associated natural killer (NK) cells with preeclampsia. Specific combinations of killer immunoglobulin-like receptors (NK cell receptors for receptors for MHC class I ligands) haplotypes in the mother and trophoblast HLA-C types in the fetus are
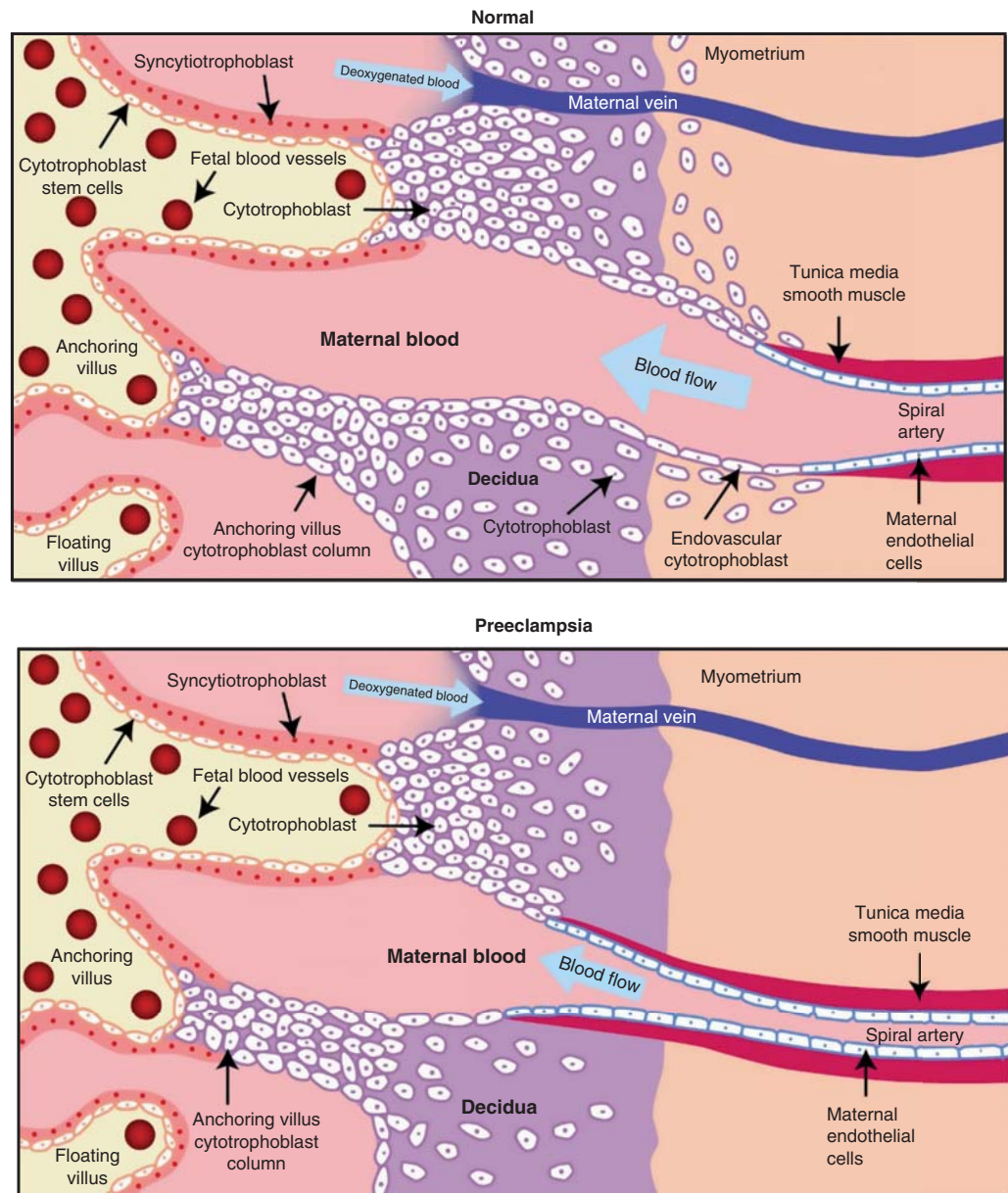
Figure 2. Spiral artery defects in preeclampsia. Exchange of oxygen, nutrients, and waste products between the fetus and the mother depends on adequate placental perfusion by maternal vessels. In normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries, transforming them from small-caliber resistance vessels to high-caliber capacitance vessels capable of providing placental perfusion adequate to sustain the growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from an epithelial phenotype to an endothelial phenotype, a process referred to as "pseudovasculogenesis" (*upper* panel). In preeclampsia, cytotrophoblasts fail to adopt an invasive endothelial phenotype. Instead, invasion of the spiral arteries is shallow and they remain small caliber, resistance vessels leading to placental ischemia (*lower* panel). (From Lam et al. 2005; reproduced, with permission, from the author.)

strongly associated with preeclampsia (Hiby et al. 2004). Moreover, uNK cells have a distinct phenotype, as well as a differential gene expression, compared with circulating NK cells (Koopman et al. 2003). Recently it has been shown that exposure to a combination of hypoxia, TGF-β1, and a demethylating agent results in transformation of peripheral NK cells to decidual NK-like cells, including expression of decidual NK cell markers, the ability to secrete VEGF, reduced cytotoxicity, and promotion of invasion of human trophoblast cell lines. These striking findings have potential therapeutic applications for placental disorders associated with altered NK cell biology (Cerdeira et al. 2013).

Impaired Corin expression or function in the pregnant uterus has been suggested as another mechanism for failed spiral artery remodeling. Local atrial natriuretic peptide (ANP) production by Corin, a cardiac protease that activates ANP, promoted trophoblast invasion and spiral artery remodeling to prevent hypertension in pregnancy (Cui et al. 2012). Pregnant Corin- or ANP-deficient mice develop hypertension and proteinuria. Moreover, in preeclamptic women, uterine, Corin mRNA and protein levels are significantly lower than in normal pregnancies. Of note, Corin levels in plasma, probably derived from the heart, are higher in preeclamptic women and did not reflect the levels in tissues (Cui et al. 2012).

Inadequate placentation, owing to deficient trophoblast invasion of uterine spiral arteries, a characteristic of preeclampsia, can lead to placental hypoxia that can lead to abnormal expression of angiogenic factors. Persistent placental hypoxia promotes hypoxia-inducible factor-1 alpha (HIF-1α) release, which fosters a proliferative noninvasive trophoblast phenotype (Rajakumar et al. 2005), further aggravating hypoxia. HIF-1α promotes transforming growth factor-β3 (TGF-β3) expression, an inhibitor of trophoblast differentiation (Caniggia et al. 2000). Endoglin (Eng), related to a decrease in invasive functions, is a coreceptor for TGF-β, which is expressed on cytotrophoblasts during the first trimester and is promoted by TGF-β1 and TGF-β3. TGF-β3 also promotes

the production of sEng (Mano et al. 2011). In addition, VEGF and PlGF are induced under hypoxic conditions (Tissot van Patot et al. 2004; Nishimoto et al. 2009) and are inhibited by sFlt-1. Interestingly, sEng and sFlt-1 expression are both stimulated under ischemic conditions, thus contributing to the systemic endothelial dysfunction (Gilbert et al. 2007, 2009).

The renin–angiotensin system (RAS) may be involved in the pathogenesis of preeclampsia. Decidual expression of AT1 receptor, the gene encoding the angiotensin (Ang II) type 1 receptor is higher in preeclampsia than in normal pregnancies (Herse et al. 2007). Ang concentration, which is also increased in the chorionic villi in preeclampsia, may promote uteroplacental dysfunction via vasoconstriction (Anton et al. 2008). Levels of stimulatory Ang type 1 receptor autoantibodies (AT1-AAs) are elevated in ∼70%–95% of preeclamptic women (Siddiqui et al. 2010). Injection of AT1-AAs from pregnant women to pregnant mice induced hypertension, proteinuria, placental abnormalities, and glomerular endotheliosis, which are the key features of preeclampsia (Zhou et al. 2008). The binding of AT1-AAs to AT1-R induces sFlt-1 and sEng production by human villous explants through tumor necrosis factor (TNF)-α pathways (Irani et al. 2010). TNF-α is also increased in preeclampsia owing to placental ischemia and it stimulates placental production of sEng and sFlt-1 (Parrish et al. 2010). Interestingly, high levels of AT1-AAs correlate with the disease severity because of their association with the presence of hypertension, proteinuria, and sFlt-1 (Siddiqui et al. 2010).

A pathogenic role of heme-oxygenase-1 (HO-1) in preeclampsia has been identified (Ahmed and Cudmore 2009). Heme breakdown metabolites generated by the enzymatic activity of HO-1 account for its angiogenic and vasodilatory properties. Placental HO-1 expression and exhaled CO levels were both found to be reduced in severe preeclamptic women. Interestingly, in vitro experiments showed that HO-1 induction increased CO production and down-regulated secretion of sFlt-1 (Levytska et al. 2013). More recently, endometrial VEGF up-regulation has also been shown in a mouse

model to induce placental sFlt1 production and contribute to preeclampsia (Fan et al. 2014).

Placental underperfusion, secondary to deficient conversion of the spiral arteries was recently linked to endoplasmic reticulum (ER) stress, which can lead to activation of proinflammatory pathways, contributing to maternal endothelial cell activation (Burton and Yung 2011). The ER is a hub for proper folding and export of peptides, guided by ER-specific chaperones. ER stress can dysregulate the function of chaperones, resulting in export of misfolded proteins into the circulation (Redman 2008). Administration of aberrant transthyretin, immunoprecipitated from PE serum to pregnant mice induces a full spectrum of preeclampsia-like features, whereas immunodepletion of the transthyretin from preeclamptic serum ameliorates most of the disease features and reduces sFlt-1 and sEng. Moreover, aggregation of transthyretin protein is found in serum and in placental tissue from preeclamptic pregnancies. It is possible that hypoxia, which has been shown to control transthyretin expression and uptake, and ER stress destabilize transthyretin into a misfolded conformation and aggregation acquiring the potential to induce antiangiogenic factors (Kalkunte et al. 2013). The presence of β-amyloid aggregates in placentas of women with PE and fetal growth restriction (Buhimschi et al. 2014) may further support the pathogenic role of ER stress and misfolded proteins in preeclampsia.

Placental pathological lesions similar to preeclampsia also occur in cases of intrauterine growth restriction (IUGR), but without maternal disease (Khong et al. 1986). Increased syncytiotrophoblast microparticles are found in women with early onset preeclampsia, but not in cases of normotensive IUGR suggesting that syncytial pathology may be unique to preeclampsia (Goswami et al. 2006). These biologically active syncytial l microparticles can transport toxic proteins, such as sFlt1 and sEng into the maternal circulation, where they mediate the major manifestations of preeclampsia (Fig. 3) (Rajakumar et al. 2012). We and others have hypothesized that the absence of a second insult of syncytial debris shedding, crucial for the ap-

pearance of the systemic disease in PE, protects women with fetal IUGR from the full blown maternal syndrome observed in preeclamptic women. Changes not only in quantity, but also in the size of the circulating microparticles, might be important in the pathogenesis of preeclampsia (Redman et al. 2012). Moreover, differences in physical and antigenic characteristics of normal and preeclamptic placental micro- and nanovesicles produced by placental perfusion or mechanical disruption have been observed (Tannetta et al. 2013). It is therefore possible that specific differences in microparticles of preeclampsia versus normal or IUGR pregnancies are the basis for their distinct pathology.

The mechanisms by which angiogenic factor expression in the placenta contribute to the pathophysiology of preeclampsia are yet to be defined. However, most models described above and others show increased sFlt-1 and sEng secretion, which further strengthens its role in the stage II or the effector pathway that leads to the clinical syndrome of preeclampsia (Fig. 4).

ANGIOGENIC FACTORS AS BIOMARKERS

Measurement of angiogenic factors using automated assays (sFlt-1 and PlGF) has proven to be clinically useful for routine diagnosis of preeclampsia (Ohkuchi et al. 2010; Schiettecatte et al. 2010; Sunderji et al. 2010; Verlohren et al. 2010; Benton et al. 2011; Knudsen et al. 2012). Several groups have shown that sFlt-1 and free PlGF levels can be used to differentiate preeclampsia from diseases that mimic preeclampsia, such as chronic hypertension, gestational hypertension, kidney disease, and gestational thrombocytopenia (Salahuddin et al. 2007; Sunderji et al. 2010; Perni et al. 2012; Rolfo et al. 2012; Verdonk et al. 2012).

Similar to the field of cardiovascular medicine with the introduction of troponins, risk stratification of women presenting at obstetrical triage with suspected preeclampsia may be an important advance in obstetrical clinical practice. Rana et al. tested the performance of angiogenic factors as predictors of adverse outcomes in patients presenting at obstetrical triage with suspected preeclampsia (Rana et al. 2012).
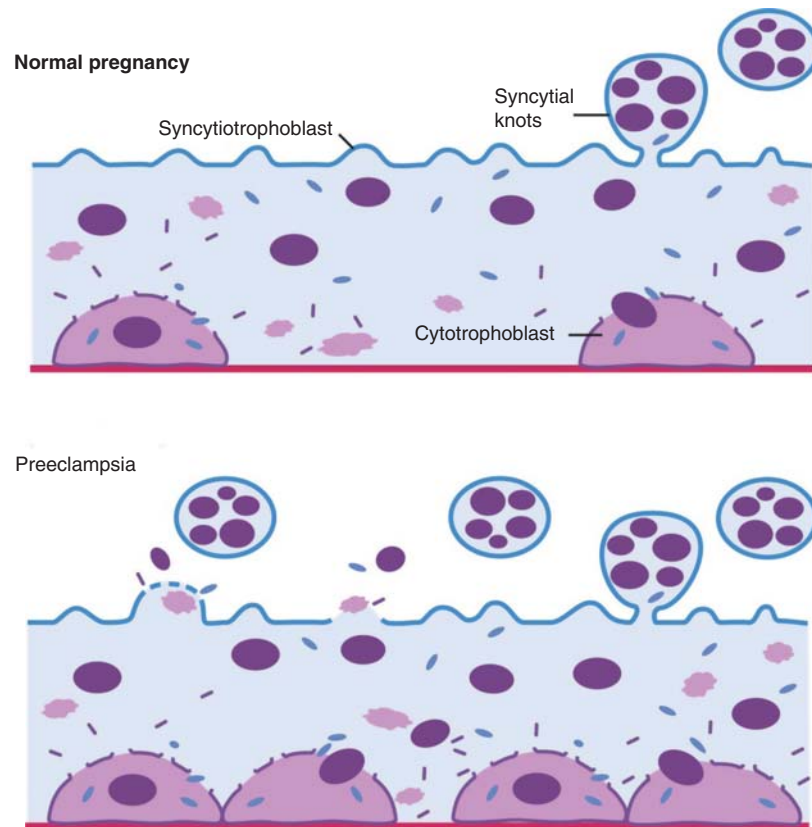
Figure 3. Schematic of syncytiotrophoblast pathology in preeclampsia. Syncytialization, knot formation, and subsequent microparticle shedding appear to be normal events of pregnancy. Preeclampsia rises from an augmentation of this sequence. Syncytial fragments shed into the maternal circulation are a significant source of circulating sFlt-1 in preeclampsia. (Created from data in Huppertz and Herrler 2005.)

In women presenting at <34 wk, plasma sFlt-1/PlGF ratio predicted adverse maternal and perinatal outcomes better than current clinical assessment (AUC 0.93) and importantly correlated strongly with duration of pregnancy (Rana et al. 2012). An extension of this study showed that plasma sEng, for which levels correlated with sFlt-1, also performed better than clinical current standards and had a performance similar to sFlt-1/PlGF ratio (Rana et al. 2012). Similarly, Chaiworapongsa et al. showed a good performance of plasma angiogenic factors assessed in obstetric triage as prognostic predictors of risk for developing preeclampsia requiring preterm delivery (Chaiworapongsa et al. 2011). In patients presenting at <34 wk gestation, plasma PlGF/sFlt-1 ratio of <0.033 MoM identified patients who delivered within 2 wk because of preeclampsia with a sensitivity of 93% and a specificity of 78%, likelihood ratio for a positive test of 4.23, AUC 88. Likewise, Moore et al. also showed a robust association of serum angiogenic biomarkers with maternal and neonatal complications in women presenting to triage with suspected preeclampsia (Moore et al. 2012). Verlohren et al. (2012) also assessed the value of serum sFlt-1/PlGF as prognostic markers, showing an increased risk of imminent delivery in preeclamptic women with high sFlt-1/PLGF ratios. In addition, Rana et al. (2013) showed that women diagnosed with preeclampsia, but with a normal plasma angiogenic profile, are not at risk for adverse maternal or fetal outcomes. Chappell et al. (2013) studied the per-
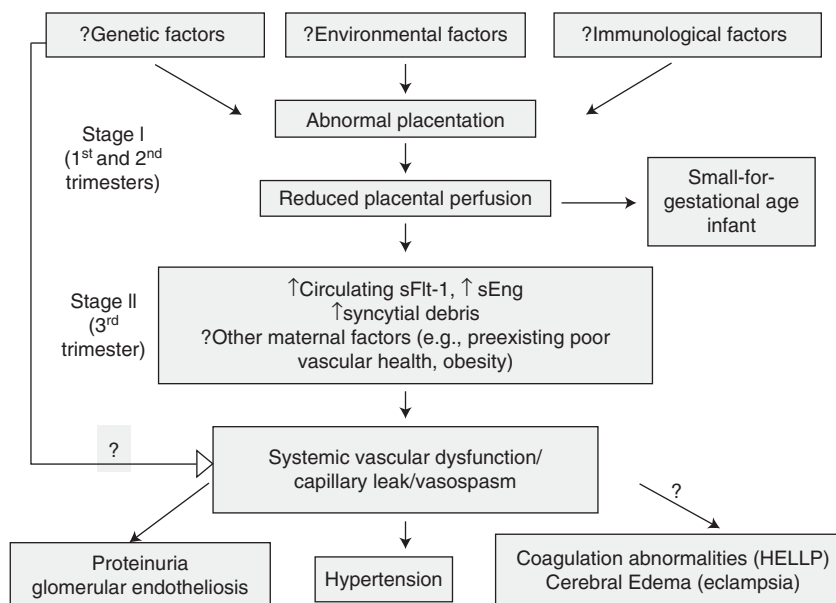
Figure 4. Summary of the pathogenesis of preeclampsia.

formance of plasma PlGF alone for prediction of delivery for preeclampsia within 14 d in women at <35 wk with suspected preeclampsia. They showed a high sensitivity and negative predictive value for PlGF levels <5th centile (Chappell et al. 2013). Taken together, these studies suggest that angiogenic factors have a significantly better prognostic accuracy than the current clinical standard and may be useful for risk stratification and management. Automated assays are already available for sFlt-1 and PlGF measurements in various biological fluids (serum plasma or urine), with results available in <20 min (Schiettecatte et al. 2010), which makes these factors easily translatable to clinical decision making in labor and delivery.

Numerous studies have evaluated the performance of angiogenic factors in prediction of preeclampsia alone or in combination with other markers (for a detailed review, see Hagmann et al. 2012). In particular, first trimester prediction has been developed using models that incorporate angiogenic factors, maternal characteristics, biophysical, and other biochemical markers. A large prospective study of 7797 women with singleton pregnancies at 11–13 wk of gestational age evaluated the performance of

an algorithm that combined PlGF, uterine pulsatility index, pregnancy-associated plasma protein A, mean arterial pressure, body mass index, and presence of nulliparity or previous preeclampsia, for prediction of preeclampsia. At a 5% false positive rate, the detection rate for early preeclampsia was 93.1% (Poon et al. 2009). The calculated positive likelihood ratio was 16.5, and negative likelihood ratio was 0.06 (Levine and Lindheimer 2009). The same group subsequently developed variations of this algorithm (including PlGF and sEng) that also showed a good performance (Akolekar et al. 2011, 2013). Other groups have studied the performance of angiogenic factors in the second trimester. Kusanovic et al. (2009) conducted a longitudinal study of 1622 consecutive pregnancies, analyzing the performance of angiogenic factors sEng, PlGF and sFlt-1 alone or combined in an angiogenic index. PlGF/sEng ratios in the mid trimester (20–25 wk) showed a sensitivity of 100% and a specificity of 98.3%, a positive likelihood ratio of 57.6 (95%CI:37.6–57.6) and a negative likelihood ratio of 0.0 (95%CI:0.0–0.3) for prediction of early onset preeclampsia (Kusanovic et al. 2009), suggesting that angiogenic factors could be reliable tools for second trimester prediction

of preeclampsia. However, there is no evidence that interventions or close follow up may improve maternal and/or fetal outcome even if one were to robustly predict early onset preeclampsia. Recent guidelines of the American College of Obstetrics and Gynecology Task Force also do not support the use of biomarkers to predict preeclampsia (ACOG Task Force on Hypertension and Pregnancy 2013). Studies evaluating the utility of low-dose aspirin to reduce the incidence of preeclampsia in those at high risk based on abnormal first trimester biochemical profile are currently in progress.

ANGIOGENIC FACTORS AS THERAPEUTIC TARGETS

To date, delivery of the placenta is the only treatment, which in the case of preterm delivery may adversely affect neonatal outcomes. The development of a therapy that safely prolongs gestation would constitute a major advance in this field. Several strategies have been developed to target angiogenic factors using both in vitro and in vivo models to reestablish the angiogenic balance. Depletion of sFlt-1 in preeclamptic villous condition media by immunoprecipitation reverses the antiangiogenic properties in vitro (Ahmad and Ahmed 2004). Administration of VEGFA-121 into a rodent model of preeclampsia improved hypertension, proteinuria, glomerular endotheliosis, and reverted sFlt-1 induced changes in gene expression (Li et al. 2007). Coadministration of adenovirus expressing VEGF in a similar model, resulted in a significant reduction of free sFlt-1, rescued the renal damage and normalized blood pressure (Bergmann et al. 2010). VEGF-121 infusion has also been reported to lower blood pressure and improve renal function in rats with placental ischemia-induced hypertension (Gilbert et al. 2010). Furthermore, increased expression of placental growth factor induced by pravastatin decreased the levels of circulating sFlt-1 and ameliorated preeclamptic symptoms in a preeclampsia mouse model of placental-specific sFlt-1 overexpression (Kumasawa et al. 2011).

In humans, resolution of signs of preeclampsia were observed in cases of parvovirus-induced hydrops (Stepan and Faber 2006), mirror syndrome (Llurba et al. 2012), fetal demise in a twin pregnancies (Hladunewich et al. 2009), all of which correlated with fall in sFlt1 levels. These experiments of nature have strengthened the notion that reduction of sFlt-1 during pregnancy may be safe without generating significant adverse effects to the fetus.

Thadhani et al. (2011) recently translated some fundamental discoveries to the bedside. Taking advantage of the positive charge of sFlt-1, they used a negatively charged dextran sulfate cellulose column for extracorporeal removal of sFlt-1. In a pilot study of eight women with preterm preeclampsia, dextran sulfate apheresis lead to a reduction in sFlt-1 levels and improvement in proteinuria and blood pressure, without evident adverse effects to the mother and fetus. Five women were treated once, two women twice, and a third patient treated four times. Of the two women undergoing two apheresis treatments, one remained pregnant for 15 d and the other for 19 d. A third woman, treated four times, remained pregnant for 23 d. In all of these cases, there was evidence of continued fetal growth. In untreated women, average prolongation of pregnancy was 3.6 d (Thadhani et al. 2011). This is an exciting study that opens the way for a safe prolongation of gestation in severe preterm preeclamptic patients. Other modalities for targeting the angiogenic imbalance are administration of agents that scavenge sFlt-1, such as sFlt-1 antibodies, PlGF, VEGF, or decrease sFlt-1 production by siRNA strategies or small molecules.

Relaxin, a peptide hormone that is increased in pregnancy, is evolving as a potential target for preeclampsia, given its vasodilatory and vascular remodeling properties (Conrad and Shroff 2011). A phase I safety study in women with severe preeclampsia is currently ongoing (Unemori et al. 2009). Interestingly, its effects could be partially mediated by VEGF and PlGF (McGuane et al. 2011). Thus, relaxin could offset the increased sFlt-1 levels in preeclampsia by potentiating local VEGF and PlGF signaling in the vasculature.

During pregnancy, heme oxygenase-1 (HO-1) negatively regulates sFlt-1 and the loss of

HO-1 activity early in pregnancy could be partly responsible for the cascade of events observed subsequently in preeclamptic pregnancies, such as the elevation in sFlt-1 as well as the increase in oxidative stress or inflammation (Ahmed, 2011). Carbon monoxide (CO), one of the metabolites of the HO system, has also been implicated in PE, as exposure to CO reduced endothelial and placental sFlt-1 and sEng release (Cudmore et al. 2007). Statins were found to increase HO-1 expression, which in turn leads to the subsequent production of CO and bilirubin, which inhibit sFlt-1 and sEng (Ramma and Ahmed 2014). Pravastatin treatment in injected adenovirus carrying sFlt-1 mice reduced sFlt-1 and sEng concentrations to levels similar to control group whereas placental PlGF and VEGF expression were up-regulated (Saad et al. 2014). Cardiac glycosides such as ouabain have also been reported to be potent inhibitors of sFlt-1 protein and mRNA synthesis through the HIF-1α pathway. Furthermore, in an animal model of pregnancy-induced hypertension, ouabain therapy improved hypertension without any adverse consequences to the pups (Rana et al. 2014). Additional studies are needed to evaluate the role of HO-1 and HIF-1 as potential therapeutic targets in preeclampsia.

Finally, complement activation downstream from the endothelial dysfunction has also been recently reported to be critical to the maternal phenotype in preeclampsia (Burwick et al. 2013). Eculizumab, a monoclonal antibody that targets C5, has been used in one patient with severe HELLP syndrome (Burwick and Feinberg 2013). More studies are needed to evaluate whether specific subgroups of preeclampsia may benefit from complement inhibition.

LONG TERM COMPLICATIONS OF PREECLAMPSIA

Endothelial dysfunction, which has been linked to atherosclerosis, persists in formerly preeclamptic women many years after an affected pregnancy (Chambers et al. 2001; Sattar et al. 2003; Saxena et al. 2010). Large retrospective epidemiologic studies have shown an elevated risk for many types of cardiovascular disease in women with a history of preeclampsia (Smith et al. 2001; Wilson et al. 2003; Ray et al. 2005; Lykke et al. 2009). The prevalence of hypertension in women with previous preeclampsia is >50% an average of 14 yr after pregnancy. This is three to four times the risk found in women without preeclampsia (Bellamy et al. 2007). Similarly, the risk of death from cardiovascular disease and cerebrovascular disease is about twofold greater in women with a history of preeclampsia. Women who have had preeclampsia before 34 wk or preeclampsia complicated by fetal growth restriction, have even higher risks of death from cardiovascular disease (Ray et al. 2005; Bellamy et al. 2007; Lykke et al. 2009). Studies have shown that levels of sFlt-1 remain modestly higher in women with a history of preeclampsia compared with those without preeclampsia in the postpartum period, which may explain the elevated cardiovascular risk in women with a history of preeclampsia (Wolf et al. 2004; Saxena et al. 2010). Although persistent alterations in antiangiogenic proteins levels leading to endothelial dysfunction can be a potential explanatory mechanism, it is possible that shared risk factors may jointly predispose to preeclampsia, endothelial dysfunction, and cardiovascular disease, and that pregnancy as a stress test reveals subclinical cardiovascular disease phenotypes long before manifestation of overt disease. Further investigation is necessary to determine whether preeclampsia itself may cause permanent vascular damage from inflammatory stress, coagulation dysregulation and endothelial damage, contributing directly to cardiovascular disease pathogenesis.

Preeclampsia is also linked to endocrine and metabolic disorders, specifically subsequent development of hypothyroidism, hyperlipidemia, and diabetes mellitus (Hubel et al. 2000; Levine et al. 2009; Lykke et al. 2009). A significantly increased risk of hypothyroidism was observed among nulliparous women with late-onset preeclampsia in a cohort of 15,935 women with 20–40 yr of follow-up (HR: 1.82, 95%CI:1.04–3.19) (Mannisto et al. 2013). Women with preeclampsia have higher thyrotropin (TSH) levels in late pregnancy and women with history of recurrent preeclampsia have been shown to have a higher

risk of developing subsequently reduced thyroid function many years after preeclampsia. The association was stronger if the high concentration of TSH was combined with absence of thyroid peroxidase antibodies, and particularly strong if preeclampsia had occurred in two pregnancies, suggesting that the hypothyroid function in preeclampsia may occur independent of the autoimmune mechanisms accepted as the most likely cause of hypothyroidism in iodine replete women (Levine et al. 2009). The increased risk for thyroid dysfunction among women with preeclampsia is thought to be mediated through antiangiogenic proteins, which act as antagonists to VEGF and PlGF, causing endothelial dysfunction and capillary regression in several tissues, including the thyroid (Maynard et al. 2005; Levine et al. 2006b). This theory is further strengthened by studies in mice using VEGF inhibitors such as sFlt-1, which have shown substantial thyroid capillary regression and increased TSH concentrations (Kamba et al. 2006; Kamba and McDonald 2007).

Hypertensive diseases are also associated with worsened renal outcomes, mainly increased albuminuria and an increased risk of future chronic kidney disease (CKD) and end-stage renal disease (ESRD). A meta-analysis confirms the association between preeclampsia and albuminuria in the long term. Thirty-one percent of women who had preeclampsia developed microalbuminuria at an average of 7.1 yr postpartum, in contrast to only 7% of women who had uncomplicated pregnancies (McDonald et al. 2010). An increased risk of renal disease was shown in a large retrospective cohort study of 570,433 women and an average follow-up of 17 yr after initial pregnancy. The relative risk of ESRD in women who had preeclampsia in the first pregnancy was close to five. Women with two or three episodes of preeclampsia had a 15-fold increase in the risk of developing ESRD (Vikse et al. 2008). Moreover, the severity of hypertensive disease during pregnancy correlated with progression to ESRD (Wang et al. 2013a).

In addition to the maternal complications of preeclampsia detailed above, preeclampsia significantly increases perinatal and neonatal morbidities. Maternal preeclampsia is a fre-

quent cause of preterm birth (Basso et al. 2006) and serum levels of sFlt-1 in the mother are inversely related to gestational age and birth weight of the newborn (Veas et al. 2011). sFlt-1, which is markedly increased in amniotic fluid during the second and third trimesters of pregnancies complicated by preeclampsia (Vuorela et al. 2000; Wang et al. 2010), may directly reach the fetal lung via fetal breathing or through an intramembranous pathway and fetal swallowing to enter the fetal lung. Studies have shown that VEGF inhibition during early neonatal period results in persistent abnormalities of alveolar and pulmonary vascular structures into and beyond infancy, which are characteristic of pathological changes in human bronchopulmonary dysplasia (BPD) (Le Cras et al. 2002; Tang et al. 2004). Furthermore, excess intra-amniotic sFlt-1, causing transient impairment of VEGF signaling in the fetus, was sufficient to cause sustained abnormalities of lung structure during infancy as well as biventricular hypertrophy most probably secondary to pulmonary and systemic hypertension in 14-d-old rats (Tang et al. 2012). These findings are interesting because offspring of mothers with preeclampsia are at risk for pulmonary vascular dysfunction later in life (Jayet et al. 2010). Preeclampsia-induced vascular dysfunction may have other clinical consequences in offspring, such as an increased risk of hypertension (Seidman et al. 1991; Vatten et al. 2003) and stroke (Kajantie et al. 2009). Interestingly, the antiangiogenic state of gestational hypertension/preeclampsia protects the infant from retinopathy of prematurity (Yu et al. 2012), a disorder characterized by the overproduction of VEGF (Pierce et al. 1995; Robbins et al. 1997).

Taken together, all of the maternal and fetal complications mentioned above suggest a central role of antiangiogenic factors, not only in the pathogenesis of preeclampsia but also as leading contributing factors to unfavorable maternal as well as fetal long term consequences.

CONCLUSION

Preeclampsia is a pregnancy-specific disease characterized by an antiangiogenic state (see

Fig. 4, for summary). Many questions remain to be answered, namely the upstream mechanisms of the deregulation of angiogenic factors. The angiogenic imbalance can be quantified in plasma or serum by automated assays and used for clinical decision making and therapeutic monitoring in clinical trials. Administration of proangiogenic factors or removal of antiangiogenic factors is a promising approach for preeclampsia treatment. Preeclampsia is also associated with long-term health risks to both mother and child. More studies are needed to evaluate the underlying mechanisms leading to long-term cardiovascular disease in women exposed to preeclampsia.

COMPETING INTEREST STATEMENT

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