Angiogenic Factors in Preeclampsia
From Diagnosis to Therapy
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Preeclampsia is one of the most frequently encountered hypertensive complication of pregnancy that affect ≈5% of pregnant women worldwide.1 It is often characterized by new onset of hypertension and proteinuria, usually during the last trimester of pregnancy, and is commonly associated with edema and hyperuricemia. Preeclampsia occurs only in the presence of the placenta, even when there is no fetus (as in hydatidiform mole) and usually remits when the placenta is delivered. The placenta in preeclampsia is often abnormal, with evidence of hypoperfusion and ischemia. The renal biopsy and autopsy studies from preeclamptic patients showed renal glomerular endotheliosis, a unique form of microvascular endothelial injury that is described as the classic lesion of this disorder.2,3 Besides affecting kidneys, preeclampsia can affect the liver, hemotologic system leading to hemolysis and disseminated intravascular coagulation, brain resulting in seizures (eclampsia), renal failure, abortion, fetal growth restriction and in certain cases fetal and maternal death. Preeclampsia remains a major cause of maternal and fetal morbidity and mortality worldwide.4 Because of a general lack of access to advanced pre- and postnatal care, most of these deaths occur in the developing world.

Preeclampsia is about twice as common in first pregnancies as in multigravidae. Other predisposing factors include pre-existing hypertension, chronic renal disease, obesity, diabetes mellitus, thrombophilies, trisomy 13, and multiple gestations.5,6 It occurs more frequently in women whose mothers had preeclampsia and in women whose fathers were products of a preeclamptic pregnancy.7 The incidence of preeclampsia is higher in women who live in high altitudes, suggesting that hypoxia may contribute to the development of the syndrome.8 In vitro fertilization has also emerged as an important risk factor for preeclampsia.9 Although none of these risk factors is fully understood, they have provided insights into pathogenesis. The exact cause of preeclampsia is heretofore unknown, but improper implantation and a subsequent imbalance of proangiogenic and antiangiogenic circulating factors are considered to be at the root of this disorder.5,10,11 In this review, we will discuss the pathogenic role of angiogenic factors in the maternal syndrome and will highlight the role of novel angiogenic factors in early diagnosis and in the development of therapies for preeclampsia.

Biology of Antiangiogenic State in Preeclampsia

Using gene expression profiling, our group identified upregulated soluble fms-like tyrosine kinase 1 (sFlt1) mRNA in preeclamptic placentas.12 sFlt1 protein, a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1 or vascular endothelial growth factor receptor 1, is a circulating antiangiogenic protein that inhibits proangiogenic factors—VEGF and placental growth factor (PIGF) signaling in the vasculature.13 Heterologous expression in rodents produces a syndrome of hypertension, proteinuria, and glomerular endotheliosis resembling the human syndrome of preeclampsia.12,23-26 Antagonism of sFlt1 has been shown to ameliorate preeclampsia phenotype in mouse models.23,27,28 Reduction of VEGF expression by 50% in the glomeruli using genetically modified mice leads to proteinuria and glomerular endothelial damage that resemble preeclampsia.29 Furthermore, VEGF antagonists, used in patients with cancer, sometimes produce a preeclampsia-like phenotype including hypertension, glomerular endothelial damage, and reversible posterior leukoencephalopathy that is often noted in eclampsia.30-33 These data support the hypothesis that inhibition of VEGF signaling in the maternal vasculature by high circulating sFlt1 may lead to preeclampsia-like signs/symptoms.

The studies on sFlt1 and VEGF in preeclampsia biology have also led to new insights into basic biology of vascular and placental homeostasis in health and in disease. The cause of hypertension by sFlt1 has been described to be because of decreased endothelial nitric oxide production and increased endothelin secretion.34,35 However, other factors such as prostacyclins and hydrogen sulfide that are downstream of the VEGF receptor may also be involved.36,37 The microvasculature of organs affected in preeclampsia such as the glomeruli and hepatic sinusoidal vasculature are more permeable because of the presence of intracellular perforations referred.
Soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) cause endothelial dysfunction by antagonizing vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 signaling. There is mounting evidence that VEGF and TGF-β1 are required to maintain endothelial health in several tissues, including the kidney and other vascular beds. During normal pregnancy, vascular homeostasis is maintained by physiological levels of VEGF and TGF-β1 signaling in the vasculature. In preeclampsia, excess placental secretion of sFlt1 and sEng (2 endogenous circulating antiangiogenic proteins) inhibits VEGF and TGF-β1 signaling, respectively, in the vasculature. This results in endothelial cell dysfunction, including decreased prostacyclin, nitric oxide production, and release of procoagulant proteins. Reprinted from Powe et al5 with permission of the publisher. Copyright © 2012, the American Heart Association, Inc.

Although placenta is the major source of sFlt1 production, immunohistochemistry studies have suggested that syncytiotrophoblast tissue (degenerating syncytiotrophoblast tissue) in the placenta is the major site of sFlt1 production.40,41 These syncytiotrophoblast tissue easily detach from the syncytiotrophoblast, resulting in free, multinucleated aggregates (50- to 150-μm diameter) that are loaded with sFlt1 protein and mRNA, and are capable of de novo gene transcription and translation.40,42 Other studies using autopsy material have suggested that shed syncytiotrophoblast tissue may contribute to circulating sFlt1 in preeclampsia.43 Because these syncytial microparticles are of fetal origin, this process of syncytial microparticle shedding may lead to chimerism, as fetal cells can be retained in the maternal blood and organs for decades after delivery. The long-term consequences to the mother exposed to excess fetal material are unknown.

Soluble endoglin (sEng), another antiangiogenic protein, that is expressed in the syncytiotrophoblast may also contribute to preeclampsia.44 The role of this protein in producing preeclampsia phenotypes was evaluated based on the hypothesis that sEng may impair transforming growth factor-β1 binding to its cell surface receptors and decreasing endothelial nitric oxide signaling45 (Figure). sEng is placental in origin, is present in the sera of pregnant women, and is elevated in preeclamptic individuals and correlates with disease severity.46,47 Administration of both sFlt1 and sEng using an adenoviral expression system in pregnant rats produces a severe preeclampsia-like animal model with hypertension, proteinuria, glomerular endotheliosis, thrombocytopenia, and fetal growth restriction.46 Patients who presented with high levels of both sEng and sFlt1 had more severe and premature form of the disease.46–48 Patients with eclampsia, a type of severe preeclampsia, have also been reported to have very high levels of sFlt1 and sEng and very low levels of free PIGF.49 However, more data on the precise mechanisms of how sEng contributes to preeclampsia are needed.

Numerous epidemiological studies also demonstrate that alterations in circulating sFlt1 may explain several risk factors for preeclampsia, such as multiple gestation, trisomy 13, nulliparity, and molar pregnancies.5,50–53 These epidemiological studies further lend support to the hypothesis that alterations in circulating angiogenic factors plays a causal role in mediating the maternal syndrome of preeclampsia.

The underlying events that induce placental disease activating the cascade of placental damage and antiangiogenic factor production remain unknown.5,54 An array of insults may contribute to placental damage that is proximally linked to the production of soluble pathogenic factors. Numerous pathways have been proposed to have key roles in inducing placental disease, including deficient heme oxygenase expression, placental hypoxia, genetic factors, corin deficiency, autoantibodies against the angiotensin receptor, oxidative stress, inflammation, altered natural killer cell signaling, deficient catechol-O-methyl transferase, complement activation, and more recently aberrant vasopressin production.11,55–62 Interestingly, several of these pathways were shown to increase placental production of the antiangiogenic factors in cell culture or animal models. However, human studies describing the temporal relationship between these pathways and preeclampsia remain to be established.

Figure. Soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) causes endothelial dysfunction by antagonizing vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 signaling. There is mounting evidence that VEGF and TGF-β1 are required to maintain endothelial health in several tissues, including the kidney and other vascular beds. During normal pregnancy, vascular homeostasis is maintained by physiological levels of VEGF and TGF-β1 signaling in the vasculature. In preeclampsia, excess placental secretion of sFlt1 and sEng (2 endogenous circulating antiangiogenic proteins) inhibits VEGF and TGF-β1 signaling, respectively, in the vasculature. This results in endothelial cell dysfunction, including decreased prostacyclin, nitric oxide production, and release of procoagulant proteins. Reprinted from Powe et al5 with permission of the publisher. Copyright © 2012, the American Heart Association, Inc.
between the angiogenic factors and the upstream pathways are still lacking before one can draw definitive conclusions.

**Biomarker Studies in Preeclampsia**

Free concentrations of plasma VEGF during pregnancy are low and often below the detection of most commercially available diagnostic kits. As a surrogate of VEGF impairment, PI GF levels during pregnancy has emerged as an attractive candidate. PI GF, a member of the VEGF family, is a proangiogenic protein that is made abundantly during pregnancy and it binds to Flt1 receptor but not the other VEGF receptors. Because sFlt1 levels rise, free PI GF levels drop and ratio of sFlt1/PIGF correlates with preeclampsia phenotypes. Working with industry partners, we and others have developed highly sensitive, specific, and robust assays with rapid throughput to quantitate levels of total sFlt1 and free PI GF in plasma and serum. Using these automated assays, we have attempted to answer the following questions: (1) How do plasma/serum sFlt and PI GF differentiate between disease and no disease? (2) How well do these biomarkers predict progression in patients with suspected preeclampsia? (3) Do alterations in angiogenic biomarkers serve as early predictive markers for adverse outcomes related to preeclampsia? and (4) Can we use the angiogenic biomarkers as an intermediate outcome to measure response to a specific treatment?

Several groups have demonstrated that measurement for sFlt1 and PI GF can be used to differentiate preeclampsia from other diseases that mimic preeclampsia, such as chronic hypertension, gestational hypertension, kidney disease, and gestational thrombocytopenia. Angiogenic markers have also been shown to be promising in preeclampsia-related disorders, such as idiopathic fetal growth restriction and stillbirth. To demonstrate clinical use of these biomarkers, we led a large clinical study to evaluate the role of angiogenic biomarkers in the prediction of preeclampsia-related adverse outcomes among women evaluated at our institution for suspected preeclampsia. We demonstrated that the plasma sFlt1/PI GF ratio on presentation predicts adverse maternal and perinatal outcomes (occurring within 2 weeks) in the preterm setting. This ratio alone performed better than the standard battery of clinical diagnostic measures, including blood pressure, proteinuria, uric acid, and other laboratory assays. Several recent studies have confirmed that levels of sFlt1 and PI GF in the triage setting can be used as a robust prognostic test and these levels correlate with the duration of pregnancy. In addition, we have also provided evidence that women with clinically diagnosed preeclampsia, but who have normal angiogenic profile have no adverse maternal or fetal outcomes. sFlt1 and PI GF testing may be, therefore, useful in women with symptoms of preeclampsia to identify those with normal angiogenic profile and a reduced risk of preeclampsia-related complications, thereby avoiding unnecessary intervention. Recently, Zeisler et al demonstrated in a prospective multicenter clinical trial that serum sFlt/PI GF can be used to rule out preeclampsia among patients with suspected disease with negative predictive value >99%.

Numerous studies evaluated the performance of angiogenic factors in prediction of preeclampsia alone or in combination with other markers. In particular, first trimester prediction has been developed using models that incorporated angiogenic factors, maternal characteristics, biophysical and other biochemical markers. Poon et al evaluated 7797 women with singleton pregnancies, during gestational weeks 11 to 13. This study yielded very good results using an algorithm developed by logistic regression that combined the logs of uterine pulsatility index, mean arterial pressure, PAPP-A (pregnancy associated plasma protein A), serum-free PI GF, body mass index, and presence of nulliparity or previous preeclampsia. At a 5% false-positive rate, the detection rate for early preeclampsia was 93.1%. The calculated positive likelihood ratio was 16.5 and negative likelihood ratio was 0.06. The same group more recently confirmed that maternal factors when combined with PI GF levels and uterine artery pulsatility index can detect 75% of cases of preterm preeclampsia in a study of >35,000 patients. Circulating angiogenic factors measured during early gestation have a high negative predictive value in ruling out the development of severe adverse maternal and perinatal outcomes among patients with systemic lupus erythematosus or antiphospholipid antibody syndrome. In a prospective multicenter study, we demonstrated that among high-risk subjects with systemic lupus erythematosus or antiphospholipid antibody syndrome, the combination of sFlt1 and PI GF was most predictive of severe adverse pregnancy outcomes when measured early in pregnancy (16–19 weeks), with risk greatest for subjects with both PI GF in lowest quartile (<70.3 pg/mL) and sFlt1 in highest quartile (>1872 pg/mL; odds ratio, 31.1; 95% confidence interval, 8.0–121.9; positive predictive value, 58%; negative predictive value, 95%). Severe adverse outcome rate in this high-risk subgroup was 94% (95% confidence interval, 70%–99%), if lupus anticoagulant or history of high blood pressure is additionally present. We have therefore proposed that among a high-risk population, measurement of angiogenic profile would allow clinicians to identify subjects with low risk for severe adverse outcomes and in this group the number of medical visits could be substantially reduced. Patients at low risk can be reassured and healthcare costs for their pregnancies decreased, whereas those at high risk can be managed by specialists with close monitoring and delivery for severe maternal or fetal disease. Future studies in other high-risk populations to evaluate the clinical use of early prediction using angiogenic biomarkers because it relates to preeclampsia-related adverse maternal and fetal outcomes are needed.

Angiogenic factor levels have also been used as a surrogate marker in clinical trials. In summary, data from multiple groups support the hypothesis that angiogenic factors are useful in risk stratification of women with preeclampsia to accurately diagnose preeclampsia and predict development of complications. More studies are needed to evaluate the impact of clinical decisions based on results of sFlt1 and PI GF testing on health outcomes, and assess the cost-effectiveness of management strategies based on sFlt1 and PI GF testing.

**Therapeutic Studies**

Human and animal studies as outlined above have strongly suggested that targeting angiogenic pathway may be a viable strategy to prevent or treat preeclampsia. We summarize 3 different strategies that have been evaluated in either preclinical or early clinical studies.
Therapeutic Apheresis

Removal of toxic circulating factors in preeclampsia has met with limited success. Plasmapheresis to remove circulating antibodies and toxic proteins has not prolonged pregnancy in preterm preeclampsia. Because sFlt1 has a large volume of distribution and that circulating plasma levels of sFlt1 represent <20% of the total body sFlt-1 burden,96 we hypothesized that a selective adsorption column would create a concentration gradient and augment its removal. We identified dextran sulfate (a polyanionic derivative of dextran) columns as one potential strategy for use in preeclampsia as dextran sulfate bound sFlt1 efficiently.85 Dextran sulfate columns bind apolipoprotein B–containing low-density lipoprotein cholesterol and is Food and Drug Administration–approved for use as an apheresis strategy in patients with familial homozygous hypercholesterolemia.84 Among pregnant patients with familial homozygous hypercholesterolemia, dextran sulfate apheresis using whole blood has been used throughout pregnancy without any major complications. In a small pilot proof-of-concept study, we demonstrated that a 30% to 40% reduction in circulating sFlt1 levels using dextran sulfate apheresis is sufficient to ameliorate preeclampsia signs and symptoms and prolong pregnancy duration by 2 to 4 weeks with no neonatal or maternal morbidity.65 During the course of the treatment, the extracorporeal adsorption device only lowered soluble sFlt1 levels by 35% on average, validating the idea that modest lowering of circulating sFlt1 levels is sufficient to successfully prolong preeclamptic pregnancies. In a follow-up study, Thadhani et al86 using plasma-specific apheresis strategy further validated this concept and demonstrated ameliorated proteinuria and stabilization of blood pressure by reducing sFlt1 levels on average by 18%. Interestingly, among women treated once, pregnancy continued for an average of 8 days and, among women treated multiple times, pregnancy continued on average for 15 days, which is in contrast to 3 days in untreated contemporaneous preeclampsia subjects. A more specific apheresis cartridge that selectively removes sFlt1 and other toxic proteins such as sEng with antibody affinity columns may be a more effective strategy with predictable clinical outcomes for patients and with less side effects.

Recombinant Ligands

In animal models, several groups have tested whether the naturally occurring ligands for sFlt1, such as VEGF or PlGF, would be of benefit in ameliorating preeclampsia.23,27,28,89,90 However, no data are available in humans, if this strategy would be safe. Experimental studies suggest that the hormone relaxin may also be used to ameliorate preeclampsia by improving vascular compliance and upregulating VEGF locally.71 Clinical studies to test the safety of human relaxin in women with preeclampsia is ongoing.92

Small Molecules

Compounds that upregulate proangiogenic factors such as statins have been used to ameliorate preeclampsia in animal models.24 A clinical trial to test safety of statins in women with established preeclampsia has been initiated in the United Kingdom and in the United States.93,94 More recently, hypoxia-inducible factor inhibitors have also been evaluated in preclinical models. In a rat model of placental ischemia–induced hypertension, ouabain acting as hypoxia-inducible factor-1α inhibitor reduced mean arterial pressure without any adverse effects on pups.95 Several groups have also demonstrated efficacy in animal models of preeclampsia for sildenafil and endothelin antagonists, both drugs acting on pathways downstream of the VEGF receptor.96

Long-Term Complications of Preeclampsia

Epidemiological studies have shown an increased risk of chronic hypertension, cardiovascular disease (CVD), and chronic kidney disease in women with a history of preeclampsia.97–100 Recently, the American Heart Association has recognized preeclampsia as a novel risk factor for CVD in women.101 It is currently thought that the background metabolic milieu of women (ie, shared risk factors) confer risk for both preeclampsia and for long-term CVD. Romundstad et al102 have suggested that at least 50% of the long-term hypertension can be explained by pre-existing risk factors. However, it is also possible that seemingly transient vascular injury induced by preeclampsia, subclinical in nature, predisposes to the development of chronic hypertension, and CVD. The absence of hypertension in the siblings of women with preeclampsia who might be expected to be at similar risk of CVD and increased risk of CVD in women with recurrent preeclampsia supports this hypothesis.103,104 Data from a recent epidemiological study suggested that increased risk of chronic kidney disease after preeclampsia seems to be explained by the pregnancy exposure per se and not by familial aggregation of risk factors.105 Recently, using the sFlt1 overexpression model in mice, we demonstrated that preeclampsia exposure potentiates the adverse vascular remodeling response to injury later in life.106 The vascular injury response in mice is enhanced after preeclampsia despite complete normalization of blood pressure and other cardiovascular parameters after delivery, as in women with preeclampsia. Moreover, examination of uninjured vessels reveals that in the absence of a vascular injury stimulus, there is no difference in vascular remodeling after preeclampsia. Experimental preeclampsia in mice has also been shown to induce long-term changes in the global plasma protein profile (proteome) that correlate with changes associated with CVD.107 This supports a new paradigm in which preeclampsia causes changes in vascular physiology that enhance the response to future vascular damage that may be mediated by pre-existing or new risk factors to which women are exposed after preeclampsia. A greater understanding of the mechanism of postpreeclampsia CVD will improve screening and suggest novel targets for prevention of future CVD in this high-risk population of women.108

Summary

During the past decade, epidemiological, experimental, and therapeutic studies have provided evidence that altered antiangiogenic state because of altered circulating sFlt1 and related proteins, such as PlGF and sEng leads to preeclampsia.109 Recent study suggests that sFlt1 and sEng are largely expressed in syncytial knots in the placenta and released into maternal circulation as syncytial microparticles.42 Understanding the dysregulated antiangiogenic pathway in the syncytiotrophoblast and its role in mediating maternal vascular disease marks a significant advance in our efforts to explain the origins of preeclampsia. Further study on the basic biology of placentation and syncytialization may shed clues on fundamental molecular defect.
S.A. Karumanchi has financial interest in Aggamin LLC and re-

I thank Dr Ravi Thadhani for help with this article and for providing

Acknowledgments

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Hypertension. published online April 11, 2016;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2016/04/18/HYPERTENSIONAHA.116.06421.citation

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