Angiogenic Imbalance in the Pathophysiology of Preeclampsia: Newer Insights

Yuval Bdolah, Vikas P. Sukhatme, and S. Ananth Karumanchi

Angiogenesis is the process of neovascularization from preexisting blood vessels, whereas vasculogenesis is the process of blood vessel generation from angioblast precursor cells. The human placenta undergoes high levels of angiogenesis and vasculogenesis during fetal development. Additionally, the placenta undergoes a process of vascular mimicry (also referred to as pseudovasculogenesis) in which the placental cytrophoblasts convert from an epithelial to an endothelial phenotype during normal fetal development. Failure of placental angiogenesis and pseudovasculogenesis during placental development has been linked to the pathogenesis of preeclampsia. It currently is believed that soluble factors released by the diseased placenta lead to clinical findings of preeclampsia. This article discusses placental vascular development in health and in disease, with a focus on accumulating recent evidence that the maternal clinical syndrome of preeclampsia is an antiangiogenic state resulting from an excess of anti-endothelial factors liberated by the diseased placenta.

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Angiogenesis is the formation of new capillaries from pre-existing ones, whereas vasculogenesis refers to in situ differentiation of endothelial precursors (angioblasts) into endothelial cells. Both angiogenesis and vasculogenesis are widely prevalent during normal placental development. Moreover, invasive trophoblasts of the placenta undergo a unique program of conversion from an epithelial to an endothelial phenotype, a process referred to as pseudovasculogenesis. Ligand-receptor systems influencing angiogenesis and vasculogenesis include vascular endothelial growth factor (VEGF)/VEGF receptors, angiopoietins/tie receptors, basic fibroblast growth factor/fibroblast growth factor receptor, and ephrin family members. Several members of these angiogenic proteins are expressed abundantly in the placenta and are thought to play an important role in normal placental vascular development. When placental vascular development is deranged, serious complications such as intrauterine growth retardation and preeclampsia can occur.

Preeclampsia is a multi-organ disorder that complicates approximately 5% to 7% of all pregnancies. It is associated with significant morbidity and mortality for the mother and the baby, but resolves completely postpartum. Women with preeclampsia have a 3- to 25-fold increased risk for severe obstetric complications. The clinical manifestations of preeclampsia consist mainly of hypertension, proteinuria, and in some cases progresses to seizures and/or the development of the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets). Although the underlying cause of this syndrome is much debated, the placenta is thought to play a central role in the pathogenesis of preeclampsia. It has been hypothesized that in preeclampsia, placental ischemia is an early event, leading to placental production of soluble factors that cause maternal endothelial dysfunction, resulting in the clinical findings of hypertension, proteinuria, and edema.

In this article, we describe placental vascular development in health and in disease (preeclampsia), and discuss in detail the role of soluble antiangiogenic proteins released by pre-
The vascularization of placental villi starts at day 21 of pregnancy, vascularization of the trophoblast to establish a fetoplacental vasculature, and, finally, complete maternal vascular remodeling at the level of the spiral arteries to gain an uteroplacental circulation.

The chorionic villi that are made up of trophoblasts are the basic building blocks of the placenta. During the first few weeks of placentation, a subpopulation of mesenchymal precursor cells transform into hemangioembryonic cells from which a primitive vascular network arises through vasculogenesis. This is then followed by fetal villous angiogenesis, the formation of capillaries. The functional importance of this transformation program is underscored by the fact that failure of this program is associated with maternal-fetal dysfunction. A normal pregnancy depends on the formation of the placenta as the organ for gas and nutrient exchange. The placentation program is tightly regulated, and the macrophages that are thought to mediate capillary formation of the villi. The villous trees and their capillary beds expand continuously until about week 26 of gestation. From the 26th week until term, the villous vascular growth undergoes non-branching angiogenesis owing to the formation of mature intermediate villi that contain long, poorly branched capillary loops. As gestation progresses, the terminal capillaries locally dilate to form large sinusoids that allow optimal exchange of gas and nutrients. Within the anchoring villi, cytotrophoblasts leave the trophoblast basement membrane, where they are anchored in the placenta, and form columns that attach the conceptus to the uterine wall. These invading cytotrophoblasts invade the maternal spiral arteries and execute a novel epithelial-to-endothelial phenotypic transformation termed by Damsky and Fisher as pseudovasculogenesis (see article, “Abnormal Placentation and the Syndrome of Preeclampsia”, by McMaster et al in this issue for details). This phenomenon is very analogous to endothelial conversion of aggressive cancer cells that is referred to as vascular mimicry. Once these unique cells invade the uterus, they display a new array of adhesion molecules and endothelial markers. Cytotrophoblast stem cells, which express adhesion molecules typical of any epithelial cells, such as E-cadherin and αβ4 integrin, alter their profile and start expressing adhesion molecules typical of endothelial cells, such as vascular endothelial-cadherin and αβ3 integrin. The functional importance of this transformation program is suggested by the fact that failure of this program is associated with preeclampsia.

Numerous pro- and antiangiogenic proteins made in the placenta are thought to be involved during the stages of placental vascularization and development. The VEGF family and its receptors deserve a special note owing to its central role in angiogenesis. This family includes placental growth factor, VEGF-A, VEGF-B, VEGF-C, and VEGF-D. VEGF-A is an endothelial-specific mitogen and a survival factor that exists in 4 isoforms: VEGF121, VEGF165, VEGF189, and VEGF206, with 20% homology to platelet-derived growth factor. The high-affinity receptor tyrosine kinases for VEGF-A include Flt-1 (also referred to as VEGFR-1) and Kinase-insert Domain containing Receptor [KDR] (human)/Flk1 (murine), also known as VEGFR-2. VEGF, as well as its receptors, are up-regulated dramatically by hypoxia in vivo as well as in cell culture models, and VEGF is capable of inducing proliferation and migration of endothelial cells and increases in vascular permeability. Knock-out studies in mice show the crucial role of VEGF in embryonic development. Inactivation of a single VEGF gene resulted in embryonic lethality in heterozygous embryos at days 11 to 12, and significant defects in placental vasculature were observed, implicating VEGF in placental vascular development. Three main isoforms of VEGF-A (121, 165 and 189) are expressed in the placenta, isofom 121 being predominant. VEGF-A and placental growth factor (PIGF) are produced by villous cytotrophoblasts, syncytiotrophoblasts, and extravillous trophoblasts. Both VEGF receptors Flt-1 and KDR are expressed on human trophoblast cells in addition to endothelial cells. Besides VEGF, the other major pro-angiogenic protein is PIGF. Similar to VEGF, PIGF also exhibits 4 different isoforms as a result of alternative splicing—PIGF-1, -2, -3, and -4. PIGF-1 and PIGF-3 (similar to VEGF121) have no heparin-binding domains, thus allowing these molecules to diffuse freely, whereas PIGF-2 and PIGF-4 have the heparin-binding domain and hence are largely matrix-associated. PIGF binds to Flt-1 but not KDR. Although VEGF is up-regulated by hypoxia, PIGF is paradoxically down-regulated by hypoxia in trophoblast cell cultures grown in vitro. The expression of PIGF in the human placenta appears to predominate in the syncytiotrophoblast layer, which is in direct contact with the maternal circulation. Although PIGF can potentiate the angiogenic activity of VEGF, reproduction was not affected in mice with an isolated PIGF--/-- genotype, and no placental or embryonic angiogenesis defects were reported. It has been shown that both VEGF-A as well as PIGF can induce trophoblast invasion and differentiation by using in vitro trophoblast cultures. More recently, VEGF-C also was shown to be expressed abundantly by the invading cytotrophoblasts and similar to VEGF-A induced trophoblast invasion in vitro.

In addition to VEGF family members, angiopoietins that act in the later stages of angiogenesis also are expressed abundantly in the normal placenta. The Tie-2 receptor, the functional receptor for both angiopoietins (Ang-1 and Ang-2), is expressed on both cytotrophoblasts and endothelial cells. Ang-1 activation of Tie-2 stabilizes newly formed vessels by recruiting perivascular support cells such as pericytes. Ang-2 is a competitive antagonist to Ang-1 at the Tie-2 receptor and may cause destabilization of the vasculature. Ang-1 stimulates trophoblast growth and migration in vitro. Ang-2 is expressed by the endovascular invasive trophoblasts during pseudovasculogenesis. Although sev-
eral studies have described the expression of the Ang family proteins during normal placentation, functional studies addressing the role of these proteins during normal and abnormal pregnancies still are lacking. It has been hypothesized that VEGF-A/Flt-1 and Ang1/Tie-2 may be involved in trophoblast differentiation and invasion, VEGF-A/KDR and Ang-1/Tie-2 may trigger fetoplacental vascular development, and Ang-2/Tie-2 may support the remodeling processes of the maternal vasculature. The human placenta is also an abundant source of other pro-angiogenic proteins such as basic fibroblast growth factor, proliferin, transforming growth factor-β 1, ephrins, and interleukin-8; however, the exact role of these factors during normal placentation still is unclear.

In contrast to pro-angiogenic proteins, little attention has been paid to the characterization of antiangiogenic proteins in the placenta. The first major endogenous inhibitor of angiogenesis that has been known to be made in abundant quantities is a soluble version of the VEGF receptor known as soluble fms-like tyrosine kinase 1 (sFlt-1), a potent circulating antiangiogenic molecule. This potent antiangiogenic molecule is generated by alternative splicing of the Flt-1 gene, leading to a truncated extracellular domain that still retains the ability to bind to VEGF and PlGF. Thus, if present in circulation, sFlt-1 can bind to VEGF and PlGF and prevent them from binding to cell-surface receptors, leading to a state of endothelial dysfunction (Fig 1). Clark et al have shown by in situ hybridization that trophoblasts express the sFlt-1 messenger RNA and that expression increases with advancing gestational age. Binding assays in endothelial cells and Western blotting of villus-conditioned media have confirmed the production of sFlt-1. Serum from pregnant women has been found to contain a VEGF-binding protein that later was confirmed to be sFlt-1. Taken together, these results suggest that the placenta secretes sFlt-1 that would be expected to be a VEGF antagonist and regulate normal placentation. Recent data from several laboratories have implicated excess sFlt-1 production as a possible mechanism for the maternal syndrome of preeclampsia (see later). Other antiangiogenic proteins that have been found to be expressed in the placenta include thrombospondin, endostatin, and prolactin. However, the precise role of these proteins during normal placentation is unclear. In general, early on in pregnancy, the proangiogenic proteins are overexpressed and probably account for placental angiogenesis and the increase in placental mass that accompanies fetal development. Toward the end of pregnancy, antiangiogenic factors increase in expression, possibly in preparation for delivery. In addition to the gestational

![Figure 1](image1.png)

**Figure 1** Mechanism of action of sFlt-1. sFlt-1 protein, derived from alternative splicing of Flt-1, lacks the transmembrane and cytoplasmic domains but still has the intact VEGF and PlGF binding extracellular domain. During normal pregnancy, VEGF and PlGF signal through the VEGF receptors (Flt-1) and maintain endothelial health. In preeclampsia, excess sFlt-1 binds to circulating VEGF and PlGF, thus impairing normal signaling of both VEGF and PlGF through their cell-surface receptors. Thus, excess sFlt-1 leads to maternal endothelial dysfunction.
age-dependent distribution of these proteins, there also are spatial differences in the expression of the angiogenic proteins in the human placenta. For example, at term, placental extracts derived from the decidual side have been found to have stronger antiangiogenic activity as compared with chorionic villus extracts.\(^3^9\)

### Angiogenic Imbalance in Preeclampsia

In preeclampsia, endovascular invasion of cytotrophoblasts remains superficial and the uterine blood vessels do not undergo adequate vascular transformation compared with what is seen in normal pregnancy.\(^4^0\) Furthermore, Zhou et al.\(^1^1\) have shown that the invasive trophoblasts fail to undergo pseudovasculogenesis. The functional consequences of these abnormalities are unknown, but it is likely that there is compromise of blood flow to the placenta, leading to placental ischemia. Several studies have shown that VEGF, Flt-1, and sFlt-1 are increased in the placenta of preeclamptic patients.\(^2^2-^4^1\) On the other hand, both VEGF and Flt-1 have been found to be decreased in the placental bed biopsy specimens obtained from preeclamptic patients.\(^2^2-^2^8\) Cytotrophoblasts isolated from preeclamptic patients when grown in vitro have been found to produce excess sFlt-1.\(^2^8\) Placenta obtained from preeclamptic patients also has been found to have reduced levels of Ang-2, whereas there were no differences in Ang-1 levels,\(^4^2\) a finding that remains to be characterized fully. Hypoxia-inducible factor-1 also is up-regulated in preeclampsia and others have suggested that hypoxia-inducible factor-1 target genes such as transforming growth factor \(\beta\) 3 and sFlt-1 may block the cytotrophoblast invasion.\(^3^3-^4^6\)

It has been hypothesized that the diseased placenta leads to the elaboration of soluble factors, which in turn induces the maternal syndrome. The concept of endothelial cell dysfunction as a major mechanism for the pathogenesis of the maternal syndrome was suggested in the late 1980s.\(^6,^7\) Roberts et al. proposed that endothelial cell injury caused increased sensitivity to pressor agents, vasoconstriction, and activation of the coagulation cascade as the basis of preeclampsia. Evidence of endothelial cell injury was provided by the classic renal lesion of preeclampsia, glomerular endotheliosis, in which glomerular capillary endothelial cells are engorged with intracellular inclusions.\(^4^7\) Data from many studies support the notion that the maternal serum in preeclampsia has soluble factors that mediate endothelial dysfunction. Preeclamptic patients' blood reveals increased endothelial permeability, vasoconstriction, and activation of the coagulation cascade. Work is in progress in our laboratory to determine if the excess sFlt-1 also can induce the placentation phenotype noted in human preeclampsia, so as to clarify whether the excess sFlt-1 concentrations made in
preeclampsia is a primary phenomenon or secondary to placental ischemia.

We also recently have performed a nested case-control study using archived blood specimens from the Calcium for Preeclampsia Prevention trial, which evaluated circulating angiogenic markers in patients with preeclampsia and matched controls to determine if alterations in these angiogenic proteins antedate the clinical symptoms of preeclampsia. During the last 2 months of pregnancy in healthy controls, the level of sFlt-1 increased and the level of PlGF decreased.64 These changes occurred prematurely and were more pronounced in the women in whom preeclampsia later developed, confirming the hypothesis that the antiangiogenic state noted toward the end of normal pregnancy is exacerbated in preeclamptic patients (see Fig 4). The sFlt-1 level increased beginning approximately 5 weeks before the onset of preeclampsia, which was accompanied by decreases in both free PlGF and VEGF. Alterations in the levels of sFlt-1 and free PlGF were greater in women with an earlier onset of preeclampsia and in women in whom preeclampsia was associated with a small-for-gestational-age infant. Alterations in sFlt-1 were useful to predict proximity to the clinical disease, whereas alterations in PlGF were found to be very useful to predict preterm preeclampsia.64 Other groups have shown similar alterations in PlGF during the second trimester in women who were destined to develop preeclampsia.66-68 The decreases in PlGF levels occurred as early as the first trimester (although not as dramatically as in the second trimester), which also has been used in other clinical studies as a possible prediction tool for the early diagnosis of preeclampsia.66-68

There is circumstantial evidence that antagonism of VEGF and PlGF may have a role in hypertension and proteinuria. VEGF induces nitric oxide and vasodilatory prostacyclins in endothelial cells, suggesting a role in decreasing vascular tone and blood pressure.69,70 Even a 50% decrease in VEGF production in the glomerulus of the kidney in mice resulted in proteinuria and glomerular endotheliosis.71 Exogenous VEGF has been found to accelerate renal recovery in rat models of glomerulonephritis and experimental thrombotic microangiopathy.72,73 More recently, exogenous VEGF was shown to ameliorate postcyclosporine-mediated hyperten-

Figure 2 Preeclampsia is an antiangiogenic state caused by excess sFlt-1. Endothelial tube assay was performed using serum from 4 normal pregnant controls (N) and 4 patients with preeclampsia (P) before and after delivery. A representative experiment from 1 normal control and 1 patient with preeclampsia is shown. (A) N t = 0 (5% serum from a normal pregnant woman at term), (B) N t = 48 (5% serum from normal pregnant woman 48 hours after delivery), (C) N t = 0 + exogenous sFlt-1 (10 ng/mL), (D) P t = 0 (5% serum from preeclamptic woman before delivery), (E) P t = 48 (5% serum from preeclamptic woman 48 hours after delivery), and (F) P t = 0 + exogenous VEGF (10 ng/mL) + PlGF (10 ng/mL). The tube assay was quantified and the mean tube length ± SEM in pixels is given at the bottom of each panel for all the patients analyzed. Recombinant human VEGF, human PlGF, and human sFlt-1 Fc were used for the assays. Reproduced with permission from Maynard et al.60
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High circulating levels of VEGF may be the common factor that might protect certain subgroups of patients (such as smokers) from developing preeclampsia. Collectively, these data suggest that VEGF is important not only in blood pressure regulation, but also in maintaining the integrity of the glomerular filtration barrier and that antagonism of VEGF signaling, as with excess sFlt-1, might lead to endothelial dysfunction, proteinuria, and hypertension.

However, there are limitations and several unanswered questions to the sFlt-1 story. The precise mechanisms of excess sFlt-1 production by the placenta are not known and, importantly, the role of sFlt-1 in normal placental development and in placental pseudo-vasculogenesis is not clear. No coagulation or liver function abnormalities or brain abnormalities (eclampsia) were reported in sFlt-1–treated animals. Serum concentrations of sFlt-1 have been found to be increased modestly in patients with intrauterine growth retardation without preeclampsia, a finding that has not been confirmed by others (Shibita et al, Serum level of sFlt-1 is increased in preeclampsia but not in small for gestational age pregnancies, J Soc Gynecol Investig, 11: A573, 2004). Although sFlt-1 was increased in most patients with preeclampsia, it was not increased in some patients with mild preeclampsia. Moreover, the relationship of sFlt-1 with known risk factors for preeclampsia is unclear; one hypothesis is that a threshold for sFlt-1 to cause disease exists, below which a normal pregnancy proceeds and above which preeclampsia results, and that women with risk factors may represent a group whose threshold has been effectively lowered, rendering them more susceptible to sFlt-1 and resulting in the maternal syndrome at levels that match those of normal

Figure 3  sFlt-1 induces glomerular endotheliosis. Histopathologic analysis of renal tissue from one representative Fc pregnant (control) (upper panel) and one sFlt-1–treated pregnant rat (lower panel) is shown here. Periodic acid-Schiff (PAS) stain of the sFlt-1–treated rat shows PAS-negative swollen cytoplasm of endocapillary cells with capillary occlusion and enlarged glomeruli (endotheliosis) compared with Fc control animal. Numerous protein resorption droplets also are seen in the PAS section. Electron micrographs of glomeruli from sFlt-1–treated rat confirmed cytoplasmic swelling of the endocapillary cells. There is relative preservation of the podocyte foot processes and the basement membranes. These pathologic changes are absent in the Fc-treated rat. All light photomicrographs were taken at 60× original magnification, the EM pictures were taken at 2,400× original magnification. Reproduced with permission from Maynard et al.60
pregnancy. Additional synergistic factors that are elaborated by the placenta may yet be identified that play a role in the pathogenesis of the generalized endothelial dysfunction noted in preeclampsia. Finally, the pathogenesis of proteinuria induced by sFlt-1 and/or other VEGF antagonists still is unclear.

More recently, endostatin (a circulating fragment of collagen XVIII and an endogenous inhibitor of angiogenesis) also was reported to be increased modestly in patients with preeclampsia. However, it is unclear at the present time if these alterations are functionally relevant in the pathogenesis of preeclampsia or whether this reflects an increase of endothelial basement membrane turnover.

Conclusions

In summary, we believe that for a successful pregnancy, there needs to be a balance of pro- and antiangiogenic proteins that are made by the placenta. Early on in pregnancy, the proangiogenic factors dominate whereas later on in pregnancy the antiangiogenic factors take over (possibly in preparation for delivery) (Fig 4). We hypothesize that if the normal physiologic increase in antiangiogenic factors toward the end of pregnancy occurs too soon and/or if there is an excess production of the antiangiogenic proteins, preeclampsia may result. Further studies are needed to better define the role of these angiogenic proteins during normal placental development and maternal health. Understanding the regulation of the normal angiogenic balance in pregnancy should help clarify not only the pathogenesis of preeclampsia, but also intrauterine growth retardation. The identification of circulating antiangiogenic factors such as sFlt-1 that may mediate some of the maternal syndrome of preeclampsia in experimental animals raises the possibility that pharmacologic strategies aimed at neutralizing sFlt-1 might ameliorate the clinical disease. Future prospective clinical studies are needed to determine if alterations in angiogenesis-related molecules might serve as diagnostic markers for the early diagnosis of preeclampsia.

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References

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