Angiogenic factors and preeclampsia

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1. ABSTRACT

Preeclampsia is a major cause of maternal and neonatal morbidity and mortality worldwide. Although the etiology of preeclampsia is still unclear, recent studies suggest that its major phenotypes, hypertension and proteinuria, may be due to an excess of circulating anti-angiogenic growth factors, most notably soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng). sFlt1 is an endogenous protein that is produced by the placenta. sFlt1 is able to bind to the angiogenic growth factors vascular endothelial growth factor and placental growth factor, thereby neutralizing their functions. High serum concentrations of sFlt1 and low concentrations of free vascular endothelial growth factor and free placental growth factor have been observed during and prior to clinical manifestation of preeclampsia. More recently, serum levels of sEng were also shown to be significantly elevated in preeclamptic women and levels of sEng correlated strongly with disease severity. Therefore, measurement of sFlt1 and sEng in the maternal circulation may be a useful diagnostic and screening tool for preeclampsia. The availability of such a test to predict preeclampsia would have significant impact on current obstetrical care and may help reduce preeclampsia-induced morbidity and mortality. This review will focus on the role of angiogenic factors in normal and abnormal placental development and indicate how measurement of circulating angiogenic factors may help identify women at risk of preeclampsia.

2. INTRODUCTION

Preeclampsia is a hypertensive disorder specific to pregnancy and contributes significantly to maternal and neonatal morbidity and mortality (1-3). Characterized by new-onset hypertension, proteinuria and edema after 20 weeks of gestation, preeclampsia is often complicated by intrauterine growth restriction (IUGR) and preterm delivery. The only definitive treatment of preeclampsia is delivery of the placenta (1-3). Other complications of preeclampsia include cerebral hemorrhage, pulmonary edema, liver rupture, renal failure, disseminated intravascular coagulation, HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, and eclampsia (defined as the occurrence of seizures in a pregnant woman that cannot be attributed to other causes).

Preeclampsia is a complex disease in which numerous genetic, immunological, and environmental factors interact. Based on the observation that the only definitive cure for preeclampsia is delivery of the placenta and that women suffering from molar pregnancy, in which a placenta develops without a fetus, frequently develop severe preeclamptic signs (4), it is reasonable to assume that the placenta plays a vital role in the pathogenesis of the disease. It has been suggested that preeclampsia is a two-stage disease (2,3,5). The first stage is asymptomatic, characterized by abnormal placental development during the first trimester resulting in placental insufficiency and the release of excessive amounts of placental materials into the maternal circulation. This is followed by the
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symptomatic stage, characterized by generalized endothelial dysfunction, during which the pregnant woman develops hypertension and proteinuria.

A leading hypothesis is that an imbalance of angiogenic growth factors in the maternal circulation caused by the release of excessive quantities of anti-angiogenic factors from the placenta contributes to the pathogenesis of preeclampsia (6,7).

3. PLACENTAL DEVELOPMENT AND ANGIGENESIS

3.1. Early Placental Development

Humans belong to the placentalia, mammals who develop a placenta during pregnancy, including rodents, domestic animals, and other primates. The placenta differs structurally between the species, but its functions remain essentially uniform: regulation of maternal adaptation to pregnancy and providing for the optimal exchange of oxygen, nutrients, and waste between mother and fetus. The vascularization of the placenta is vital for placental development and includes the processes of vasculogenesis (de novo formation of new types of blood vessels) and angiogenesis (increase in the number of existing blood vessels).

Two to three weeks after conception, the placenta is composed of primary villi, structures which consist of a core of cytotrophoblast cells surrounded by a layer of syncytiotrophoblast. As mesenchymal cells begin to fill the cores, secondary villi are formed. The mesenchymal cells transform into hemangiogenic precursor cells, which differentiate to form the first placental blood vessels. Mesenchymal-derived macrophages (Hofbauer cells) appear in the mesenchyme of the secondary villi just before formation of the first blood vessels (8). The early appearance of these macrophages and the fact that they express angiogenic growth factors such as vascular endothelial growth factor (VEGF) suggest that they may have a role in initiating vasculogenesis (8,9,10).

Expression patterns of various angiogenic growth factors have been studied mostly during late pregnancy (9,11,12,13), when it has been demonstrated that receptors for angiogenic growth factors are essential for the formation of blood vessels (14,15). Knockout experiments in mice have shown that VEGFR1 (VEGF receptor 1, also referred to as fms-like tyrosine kinase 1 or Flt1) plays a role in the organization and arrangement of early endothelial cells into tube-like structures covered by endothelial cells (16). Similarly, knockout experiments with VEGFR2 (VEGF receptor 2, also referred to as KDR/Flk1) have pointed to a crucial role for VEGFR2 in the differentiation of hemangiogenic precursor cells into endothelial cells (17). Both receptors are expressed by vasculogenic and angiogenic precursor cells. VEGF itself is highly expressed by cytotrophoblast cells and later by Hofbauer cells (placental macrophages) (9,12). Studies have documented the presence of angiogenic growth factors from very early pregnancy.

During the first trimester of pregnancy the number of capillaries within the placenta increases dramatically. Branching angiogenesis from existing capillaries forms the basis for development of a primitive capillary network (18). By the sixth week of gestation, a basal lamina surrounds the capillaries, which are arranged into a weblike structure within the smaller, mesenchymal villi. In the larger intermediate villi, the capillaries are found closer to the villous surface, just beneath the trophoblast layer. New placental arteries and veins are formed within these villi, when the precursors of fetal endothelium form long, dense cellular aggregates. These tube-like structures become surrounded by cells that express alpha and gamma smooth muscle actins, vimentin, and desmin (10). Fusion of the adventitia of these vessels occurs around 15 weeks of gestation. The primitive capillary network slowly regresses as these new stem villi expand outwards towards the syncytiotrophoblast. The formation of new villous trees and a vascular bed is a continuous process, as the peripheral ends of the newly differentiated stem villi expand and give rise to new capillary networks, which in turn form new stem villi (10). The data indicate that vascularization of the placenta is a highly organized process and involves both trophoblast and mesenchyme.

3.2. Physiological Remodeling of Maternal Spiral Arteries

Apart from de novo formation of blood vessels within the villous structure, normal placental development is also dependent on the invasion of extravillous trophoblast cells into the maternal decidua. Extravillous trophoblast cells (EVTs) arise from cytotrophoblast cells. Their invasive potential is determined by the expression of cell adhesion molecules such as integrin alpha-1, integrin alpha-V beta-3, VE-cadherin, VCAM-1, and PECAM-1, which promote differentiation and invasion (19). Cytotrophoblast cells within the villi usually do not express these antigens, but rather express integrins alpha-6 beta-4 and alpha-V beta-6, and E-cadherin, adhesion molecules that inhibit invasion (19). The ability of EVTs to adopt an invasive phenotype plays an important role in the process by which EVT connections form connections with the uterine vasculature. During invasion, a subset of EVTs migrates towards and invades the maternal spiral arteries present in the maternal decidua (20). The normal muscular and elastic structures of the distal portion of the spiral arteries are replaced by a layer of fibrin in which trophoblast cells are embedded. This converts the normally narrow and rigid vessels into dilated, flaccid structures at least threefold wider in diameter than the original vessels (21,22). Plugs of endovascular trophoblast cells are formed within the distal segments of the maternal spiral arteries, which act like filters and keep the maternal blood from reaching the intervillous space.

Trophoblast invasion is initiated in a hypoxic environment. Measurement of oxygen tension levels within the intervillous space during early pregnancy have revealed that oxygen concentrations are less than 20mm Hg until week 10 of gestation (23). Towards the end of the first trimester, the trophoblastic plugs begin to dislocate,
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allowing maternal blood to flow towards the intervillous space. Oxygen tension levels rise threefold, starting at the periphery of the placenta and gradually making its way towards the center (23,24). New vessels originate in the center of the placenta, into which maternal arterial blood is delivered through the spiral arteries. The villi in this central region of the placenta are generally more immature than villi in the peripheral region, and oxygen tension levels are highest in this region. Blood from the intervillous space is drained from the more peripheral regions of the placenta into the maternal venous compartments.

Failure of cytotrophoblast cells to adopt an invasive phenotype is probably one of the first steps leading to preeclampsia. Brosens and coworkers were the first to show that physiological remodeling of the maternal spiral arteries was absent in women with preeclampsia (25). As shown in various histological studies, trophoblast invasion in preeclamptic pregnancies is shallow and confined to the superficial portions of the decidua (26, 27). Biopsy specimens from preeclamptic placentas which show narrow constricted vessels indicate a defect in trophoblast differentiation (26,28). This is supported by in vivo and in vitro models in which cytotrophoblast cells from preeclampsic pregnancies are unable to differentiate into an invasive phenotype and remodeling is suboptimal (29, 30). Defective vascular remodeling has also been observed in pregnancies resulting in IUGR (26); and in cases of spontaneous miscarriage, there is often a complete absence of trophoblast invasion (24, 31). The net result of this vascular pathology is a marked reduction of blood flow to the intervillous space.

It has been suggested that variation in oxygen tension is a prerequisite for trophoblast differentiation and invasion. Reduced oxygen tension can be due either to preplacental hypoxia or to defective transformation of spiral arteries. In preplacental hypoxia the supply of oxygen in maternal blood is reduced before reaching the placenta on account of high altitude (hypobaric hypoxia) or medical conditions such as maternal asthma or chronic anemia. Gene expression profiles of preeclamptic placentas, placentas delivered after pregnancy at high altitude (hypobaric hypoxia) or suffering from severe chronic anemia, have been shown to be similar to those of preeclampsic placentas (32). It may be that hypoxia-induced genes, including the hypoxia-inducible transcription factors. Angiogenic growth factors have also been shown to be abnormally expressed in placentas from women with preeclampsia or IUGR (6, 33, 34). Importantly, primary cytotrophoblasts and villous explants in culture have been shown to up-regulate anti-angiogenic factor such as sFlt1 in culture. Therefore, the most probable cause of abnormal expression levels of angiogenic factors is the prolongation of placental hypoxia beyond the first trimester of pregnancy. Whether placental hypoxia alone is responsible for the development of preeclampsia is not known. Risk factors for preeclampsia include pre-existing hypertension, diabetes, and obesity, all of which predispose to vascular insufficiency (35). However, it is generally believed that abnormal placentation with accompanying hypoxia does stimulate abnormal secretion of placental proteins into the maternal circulation, which in turn may induce endothelial dysfunction and support the development of preeclampsia.

4. ANGIOGENIC GROWTH FACTORS AND PREECLAMPSIA

4.1. Circulating Angiogenic Factors in the Maternal Syndrome

All of the clinical manifestations of preeclampsia can be attributed to endothelial dysfunction leading to end-organ damage and hypoperfusion. Based on this observation, numerous laboratories over nearly two decades have focused their attention on the identification of circulating factors which induce endothelial cell dysfunction during pregnancy. Since the placenta plays an important role in the pathogenesis of preeclampsia, researchers have focused their search for identification of such factors primarily on the placenta. With the advent of more sensitive molecular technology, it has been possible to use small amounts of placental material to learn which genes or proteins are expressed by the placenta during healthy or complicated pregnancy. Using microarray technology, new genes were recently discovered by our laboratory that play an important role in placental biology (6). These include soluble fms-like tyrosine kinase 1 (sFlt1), a receptor for vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF).

Placental production of sFlt1 is increased during preeclampsia (6, 36). sFlt1 acts by adhering to the receptor-binding domains of placental growth factor (PIGF) and vascular endothelial growth factor (VEGF), preventing their interaction with cell surface endothelial receptors, thus inducing endothelial dysfunction (6, 7). Circulating concentrations of sFlt1 are increased and free PIGF and free VEGF decreased during active disease and several weeks before onset of symptoms (37). More recently, another anti-angiogenic factor, soluble endoglin (sEng) was discovered to be up-regulated in preeclampsia (38). In rodent studies it was reported that sEng may act in concert with sFlt1 to induce a severe preeclampsia-like illness (38). These findings have opened a new chapter in understanding the pathogenesis of preeclampsia and in developing therapeutic strategies.

4.2. sFlt1, VEGF and PIGF in Preeclampsia

Flt1, the receptor for both VEGF and PIGF, exists in two forms: a membrane-bound form and a soluble form (sFlt1). sFlt1 is made in large amounts by the placenta and is released into the maternal circulation (6). By binding and neutralizing the pro-angiogenic proteins VEGF and PIGF (placental growth factor) in maternal blood, sFlt1 acts as a potent anti-angiogenic factor. Circulating sFlt1 concentrations are increased in women with established preeclampsia (6, 39). Consistent with the antagonistic effect of sFlt1, free (or unbound) VEGF and free (or unbound) PIGF concentrations are decreased in preeclamptic women at disease presentation and even before the onset of clinical symptoms (6, 40, 41). In vitro studies have indicated that the anti-angiogenic state in preeclampsia induced by excess placental production of sFlt1 can be rescued by administering VEGF and PIGF.
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(42). Adenovirus expression of sFlt1 in rats produces a syndrome of hypertension, proteinuria, and glomerular endotheliosis that mimics the human syndrome of preeclampsia (6). The fact that this was also observed in nonpregnant animals suggested that the effects of sFlt-1 on maternal vasculature were direct and independent of the presence of a placenta. When pregnant rats were given a soluble form of VEGF receptor-2 antagonist (sFlk-1), which does not antagonize PI GF, they did not develop a preeclamptic phenotype, indicating that antagonism of both VEGF and PI GF was necessary to induce the maternal syndrome (6). In a knockdown model in mice, a 50% reduction in renal VEGF resulted in substantial glomerular endotheliosis and proteinuria, similar to the kidney pathologies found in preeclamptic women (42). These experimental data suggested that excess sFlt1 made by the placenta of women who subsequently develop preeclampsia causes a paucity of VEGF and PI GF, thereby creating an anti-angiogenic state and resulting in the characteristic hypertension, glomerular endotheliosis, and proteinuria of preeclampsia.

Recently, we and others have observed that there is a marked rise in circulating sFlt1 concentration beginning about five to six weeks before the onset of clinical preeclampsia, accompanied by decreases in circulating free PI GF and VEGF (6). Moreover, alterations in these circulating angiogenic proteins correlated with disease severity, earlier onset of preeclampsia, and the birth of an infant small-for-gestational age. Many studies have examined the potential use of circulating sFlt1 measurements as a predictor of preeclampsia (37, 43, 44). While all have concluded that elevated sFlt1 levels during the second and third trimesters indeed indicate impending preeclampsia, some pregnant women who go on to develop the disorder have sFlt1 levels well within the normal range and vice versa. Despite the promising results of investigational studies, sFlt1 measurements will probably have to be combined with other markers and with preeclampsia risk factors to develop a good screening test for preeclampsia.

PI GF was first cloned from a term placental cDNA library (45). Subsequent experiments showed it is expressed by both syncytiotrophoblast and extravillous trophoblast cells, as well as by other placenta-derived tissues such as choriocarcinoma cell lines and human umbilical vein endothelial cells (46, 47). The PI GF gene contains a PDGF domain which is 53% homologous to a similar domain encoded by the VEGF gene (45). Two isoforms of PI GF are known, PI GF-1 and PI GF-2, the latter possessing an additional exon containing a heparin-binding domain, which allows PI GF-2 to bind to heparin (46,47). Due to its structural homology to VEGF, PI GF has also been shown to exert potent angiogenic effects, and PI GF-1, in particular, strongly induces neo-vascularization and influences the migration and proliferation of endothelial cells (48). PI GF expression is regulated by oxygen concentration (10, 49). Hypoxia has been shown to downregulate the expression of PI GF (49). An important role for PI GF in trophoblast survival is its anti-apoptotic function. High levels of PI GF protect cells from apoptosis by activating the stress-activated protein kinase (SAPK)-pathways, the c-Jun-N-terminal kinase, and the p38 kinase (50).

During normal pregnancy PI GF levels rise steadily until the beginning of the third trimester when they peak around 29-32 weeks and decline thereafter (37, 51, 55). This is consistent with the timing of the role attributed to PI GF in protecting trophoblast cells from apoptosis while inducing migration and vascularization. In women destined to develop preeclampsia lower levels of circulating PI GF have been documented as early as 10-12 weeks of gestation (52). In one study, women who developed early onset preeclampsia or preeclampsia with a small-for-gestational-age infant had significantly lower serum PI GF levels at 21-32 weeks of gestation than women who developed late onset preeclampsia or preeclampsia with an appropriate-for-gestational-age infant (37). In general, PI GF levels begin to decrease approximately 9 to 11 weeks before the first sign of preeclampsia, with considerable reduction during the five weeks immediately before the onset of the disease (37,55,56). The decreased PI GF in preeclampsia has been attributed to excess circulating sFlt1 that binds PI GF and to decreased placental production of PI GF, perhaps the result of downregulation by hypoxia, although formal evidence for the latter is lacking. Recently it was also reported that decreased urinary concentrations of free PI GF during mid-gestation predict the subsequent development of clinical preeclampsia (53). In parallel, there have been reports of markedly lower serum PI GF during the third trimester in normotensive women who gave birth to small-for-gestational-age (SGA) infants, as compared with gestational age-matched women who delivered infants whose size was appropriate-for-gestational age (AGA) (39, 54, 55).

As expected, due to higher levels of sFlt1, free VEGF was lower in preeclampsia than in normal pregnancies (6, 37). In specimens obtained before onset of preeclampsia, free VEGF levels were lower in the women with subsequent preeclampsia than in normotensive controls only within 5 weeks before onset of preeclampsia signs (37). Although VEGF may be the most biologically active of the pro-angiogenic molecules, since VEGF levels in preeclampsia are close to the detection limit, it appears that circulating free VEGF assays will not be useful for preeclampsia screening unless technologies can be developed to detect free VEGF below 1 pg/ml (37).

4.3. Soluble Endoglin In Preeclampsia

Another soluble factor secreted by the placenta that appears to be elevated in women with preeclampsia is soluble endoglin (sEng) (38). Endoglin (Eng) is an angiogenic receptor expressed mainly on the surface of endothelial cells, but also placental syncytiotrophoblasts (57, 58, 59). Eng acts as a co-receptor for transforming growth factor-beta, a potent pro-angiogenic molecule, signaling in endothelial cells (57, 58, 59). We recently found that Eng mRNA is up-regulated in the preeclamptic placenta (38). Moreover, we have discovered that the extracellular region of endoglin is proteolytically cleaved and that sEng is released in excess quantities into the
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Figure 1. Schematic depicting the hypothesis that angiogenic imbalance may underlie pregnancies complicated by preeclampsia. Reproduced with permission from Bdolah et al (63).

circulation of preeclamptic patients. In pregnant rats sEng appeared to exacerbate the vascular damage mediated by sFlt1 resulting in severe preeclampsia-like illness including the development of HELLP syndrome and fetal growth restriction (38). The precise role of these molecules during pregnancy and placentation is unclear, but there is evidence that Eng via TGF-beta may play a role in the hypothesized contribution of placental hypoxia/ischemia to the pathogenesis of PE. Human syncytiotrophoblasts express high levels of Eng throughout placental development, as well as briefly during the transition from polarized trophoblasts to the non-polarized invasive type. In cell lines derived from human choriocarcinoma, a disease characterized by uncontrolled trophoblast invasion, endoglin expression has been demonstrated to increase with methotrexate treatment, which induces differentiation into syncytiotrophoblast-like cells (60). In explant cultures of trophoblasts from 5-8 weeks of gestation, monoclonal antibodies to Eng and antisense endoglin oligonucleotides stimulated trophoblast outgrowth and migration (61). TGF-beta 1 and/or TGF-beta 3 inhibit trophoblast migration and invasion, and it seems that Eng mediates this effect. Therefore, we speculate that sEng is produced by the placenta as a compensatory mechanism to limit the effects of surface endoglin. In preeclampsia, excessive production of surface endoglin leads to increased sEng in the maternal circulation which together with sFlt1 may be responsible for the clinical manifestations of preeclampsia.

More recently in clinical studies, sEng appears to be elevated not only during the disease but also before onset of symptoms (Levine, Karumanchi, unpublished observations). Elevations in sEng were particularly pronounced - therefore, potentially most useful for prediction - in women who developed preterm preeclampsia or preeclampsia with a small-for-gestational-age infant. Although the gestational pattern of sEng concentration tended to parallel the trajectory of the sFlt1/PIGF ratio, multivariate analysis indicated that each was associated with preeclampsia. Indeed, a composite measure incorporating all three molecules, (sFlt1 + sEng) / PIGF, was more strongly predictive of preeclampsia than the individual biomarkers.

5. SUMMARY AND PERSPECTIVE

Successful pregnancy requires a balance between pro- and anti-angiogenic proteins made by the placenta. Early in normal pregnancy pro-angiogenic factors such as PIGF and VEGF dominate, while later in pregnancy anti-angiogenic factors such as sFlt1 and sEng are preeminent, possibly in preparation for delivery. We hypothesize that if the physiological rise in anti-angiogenic factors towards the end of pregnancy occurs too soon and/or if levels are excessive, preeclampsia may result (see Figure 1). Understanding the regulation of the normal angiogenic balance in pregnancy should help clarify the pathogenesis of preeclampsia, and possibly also IUGR.

An imbalance of angiogenic proteins can also lead to the opposite condition, an excess of pro-angiogenic factors, resulting in excessive vascularization of the uteroplacental unit. Placenta accreta is a life-threatening condition in which placental villi attach to and invade the maternal myometrium. Whereas preeclampsia is characterized by shallow placental implantation, in placenta accreta there is excessive trophoblast invasion of maternal
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tissues. It has been demonstrated recently that, in contrast to preeclampsia, VEGF protein levels are significantly higher and KDR levels significantly lower in tissue from placenta accreta than in normal placenta, while sFlt1 and PI GF levels did not differ (62). If the placent al balance between VEGF, PlGF, and their receptors tips in one direction, it may lead to suboptimal angiogenesis; if it tips in the other direction, excessive angiogenesis may occur, resulting in placentas that are highly invasive. Further studies are needed to elucidate the functions of angiogenic proteins during normal placental development and their role in maternal vascular health and disease.

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