The Role of Hypoxia and Hypoxia-Inducible Factor-1Alpha in Preeclampsia Pathogenesis

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ABSTRACT

Preeclampsia, a pregnancy-specific disorder that affects 5%–8% of pregnancies and is the leading worldwide cause of maternal and fetal morbidity and mortality, is characterized by shallow trophoblast invasion and inadequate spiral artery remodeling, which are widely believed to lead to placental hypoxia, the putative culprit initiating the cascade of events that ultimately results in the maternal manifestations of the disease. Despite extensive research, however, the pathophysiology of this disease remains poorly understood, no effective prevention exists, and treatment is limited to symptomatic therapy. Recent research has introduced exciting new theories regarding the pathogenesis of preeclampsia. Clinical and experimental evidence implicating the circulating antiangiogenic molecules soluble Fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin (sENG), as well as endothelin-1 and the angiotensin II receptor type I autoimmune antibody (AT-1AA), have been especially promising. This review collates evidence for a role of hypoxia and hypoxia-inducible factor-1alpha (HIF1A; referred to as HIF-1α throughout) in the pathogenesis of preeclampsia and discusses potential links between hypoxia and the newly reported potential mediators of the disease’s manifestations.

INTRODUCTION

Preeclampsia is a pregnancy-specific disorder that affects 5%–8% of pregnant women and is responsible for significant maternal and fetal morbidity and mortality [1]. The syndrome is thought to begin in shallow trophoblast invasion and abnormal placentation, leading to persistent placental hypoxia and the release of various mediators into the maternal circulation. These are thought to cause endothelial dysfunction that ultimately results in the various fetal and maternal manifestations of the syndrome. It is characterized clinically by the onset of hypertension and proteinuria after 20 wk of gestation. Advanced-stage clinical symptoms include seizures, renal failure, intrauterine growth restriction (IUGR), and/or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. Currently, treatment for this devastating disorder is limited to symptomatic therapy, and the only known cure is rapid delivery of the placenta. Despite numerous studies and some promising recent research, the pathogenesis of this disease remains poorly understood.

ANGIOGENIC IMBALANCE IN THE PATHOPHYSIOLOGY OF PREECLAMPSIA

Evidence is growing that an imbalance between proangiogenic factors, such as vascular endothelial growth factor (VEGF), and antiangiogenic factors, such as soluble Fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin (sENG), is central to the pathogenesis of preeclampsia. The first studies reporting the presence of angiogenic imbalance in preeclampsia focused upon VEGF and described increased serum levels of this molecule in patients with preeclampsia [2–5]. Meanwhile, other authors have reported decreased systemic VEGF levels [6–8]. These seemingly conflicting results are explained by the methodology of the studies. All studies reporting decreased VEGF used a commercially available ELISA kit that measured free (unbound) VEGF, whereas all studies reporting increased VEGF in preeclampsia used different ELISA kits that measured total (bound and unbound) VEGF. Because in pregnancy circulating sFLT-1 is present at very high levels compared with the nonpregnant state, free VEGF levels, which more accurately reflect effective circulating bioactive VEGF, are substantially lower than total VEGF levels. In fact, due to the high levels of sFLT-1 in pregnancy, free VEGF levels have been shown to be uniformly below 30 pg/ml in patients with normal pregnancy and patients with preeclampsia [9] and below the lower limit of detection in most samples [2]. In 1998, Clark et al. [10] reported that a VEGF antagonist is produced by the human placenta and released into the maternal circulation. This factor was subsequently recognized as sFLT-1, a splice variant of VEGF receptor FLT-1, and was shown by Vuorela et al. [11] to be significantly elevated in the amniotic fluid of women with preeclampsia. Levine et al. [12] and Maynard et al. [13] later showed that sFLT-1 is elevated in placentas and sera of women with preeclampsia compared to women with normal pregnancy. This is accompanied by decreased serum and/or urinary levels
of free VEGF and PLGF [12, 14, 15], suggesting that sFLT-1 binds VEGF and PLGF in the maternal circulation and thereby blocks their angiogenic effects. Moreover, the increase in serum sFLT-1 presages the occurrence of clinical manifestations of preeclampsia and correlates with disease severity [12]. Two isoforms of soluble VEGF receptor 1 have been described: sFLT-1, which is generic, and sFLT1-14 [16], also known as sFLT1-e15a [17], which is human specific and the most common VEGF inhibitor produced by the human placenta in preeclampsia. Animal studies corroborated the clinical findings of a role for antiangiogenic factors in pathogenesis of preeclampsia. Injection of an adenovirus expressing sFLT-1 into pregnant or nonpregnant rats resulted in characteristic preeclamptic manifestations, including hypertension, proteinuria, and glomerular endotheliosis, the hallmark kidney lesion in preeclampsia [13]. The same group later demonstrated elevation in sENG, a circulating receptor of TGF-β, in sera of women with preeclampsia [18]. Interestingly, the coadministration of adeno virus expressing sFLT-1 and sENG into rodents caused a particularly severe form of the syndrome, women between 9 and 13 wk of gestation were studied. Whereas the authors found PLGF to be lower in women who went on to develop preeclampsia compared to women who did not develop the syndrome, they did not find differences in sFLT-1 and sENG levels between groups [26]. Moreover, even PLGF, the factor with the best predictive value in their study, identified less than 40% of patients destined to develop preeclampsia [26]. A recent meta-analysis found that concentrations of PLGF, sFLT-1, and sENG are significantly different before the onset of preeclampsia and that these differences are greatest from 19 wk of gestation onwards. However, test accuracies of all three markers were too poor for accurate prediction of preeclampsia in clinical practice [27], indicating either that the involvement of these agents in the pathophysiology of preeclampsia is a late event or that several pathologic phenotypes lead to the syndrome of preeclampsia. No single biomarker appears to have clinical utility for preeclampsia prediction, but several cohort studies have shown that a combination of patient factors, (multiple) biomarkers, and/or uterine artery Doppler result in better prediction [21, 25, 28–32]. These models still have to be externally validated.

It is thought that sFLT-1 and sENG neutralize their ligands, reducing the circulating concentration of VEGF, PLGF, and TGF-β, which results in a shift in the angiogenic balance towards antiangiogenesis, which in turn leads to endothelial damage and the clinical onset of the syndrome. Many of the manifestations of preeclampsia have been reported to be induced by iatrogenic VEGF inhibition in humans. Bevacizumab, a humanized monoclonal antibody against VEGF used as an anticancer drug, causes hypertension and proteinuria in up to 67% and 63% of patients, respectively [33]. Elevated liver enzymes and thrombocytopenia are also well-described side effects of treatment with VEGF-targeted inhibitors [34]. Bevacizumab treatment was recently reported to cause a fulminant preeclampsia-like syndrome in nonpregnant patients characterized by hypertension, proteinuria, HELLP-like syndrome, seizures, and characteristic brain neuroimaging, suggesting that interference with VEGF signaling is sufficient to mimic preeclampsia in these patients [35].

The imbalance between pro- and antiangiogenic factors in preeclampsia may be a target for therapeutic interventions. In other fields, peripheral arterial disease has been treated by proangiogenic gene therapy using intramuscular administration of VEGF either via a naked plasmid or using an adeno viral-mediated expression [36, 37]. Even as data supporting a significant role for antiangiogenic factors in producing the maternal syndrome in preeclampsia gradually accumulate, the mechanisms leading to the impaired placentation and the upregulation of antiangiogenic molecules in this disease are yet to be fully established.

ANGIOTENSIN RECEPTOR-1 AUTOANTIBODY

For a long time, the renin-angiotensin-aldosterone (RAS) system has been linked to the pathogenesis of preeclampsia. Plasma renin activity (PRA) in patients with preeclampsia is lower compared with that of women with normal pregnancy [38]. Renin, a key enzyme in the conversion of angiotensinogen precursor to angiotensin I, is a volume sensor that is triggered in response to a decrease in blood pressure and/or perfusion to the kidney. Low PRA has been associated with circulatory volume expansion [39], and this may suggest a compensatory response to hypertension in preeclampsia. It is also known that patients with preeclampsia have increased vascular responsiveness to angiotensin II [40]. In 1999, Wallukat et al. [41] identified an agonistic autoimmune antibody to angiotensin II receptor type I (AT1-AA) in the circulation of patients with preeclampsia but not in healthy pregnant women. Since then, an increasing number of studies suggest that AT1-AA is implicated in the pathogenesis of preeclampsia. Stimulation of AT1 receptor by AT1-AA was demonstrated to inhibit trophoblast invasiveness in vitro [42], a well-known characteristic of preeclampsia. Moreover, this autoantibody induces calcium release in vascular smooth muscle cells, which may therefore mediate the vascular alterations in preeclampsia [43]. Recently, it was shown that AT-1AA injection into pregnant mice induces the key features of preeclampsia, including hypertension, proteinuria, glomerular endotheliosis, placental abnormalities, and IUGR [44]. In addition, these preeclamptic manifestations are attenuated when losartan, an AT1 receptor antagonist, or a neutralizing peptide consisting of a specific epitope of AT1 receptor are administered, suggesting that the effect of AT1-AA is mediated via the AT1 receptor [44]. It was recently shown that AT1-AA autoantibody is present in the serum of more than 95% of women with preeclampsia and that its serum concentrations correlate with disease severity, further supporting a role for this antibody in the pathogenesis of preeclampsia [45]. Importantly, Zhou et al. [46, 47] recently provided a link between AT1-AA and antiangiogenic factor upregulation. They demonstrated that AT1-AA induces sFLT-1 and sENG production in both placental villous explants in vitro and mice in vivo. In addition, the same group demonstrated a correlation between circulating AT1-AA levels and sFLT-1 serum concentration in patients with preeclampsia [45], suggesting that this AT1-AA-mediated sFLT-1 induction may be clinically relevant. However, the underlying mechanism that leads to the production of AT1-AA in preeclampsia is still unknown.
Furthermore, several unanswered questions remain before one can ascribe a pathogenetic role for AT1-AA in preeclampsia. First, the fact that the incidence of preeclampsia is greatest in the first pregnancy rather than becoming worse with subsequent gestations is in contrast to an autoimmune basis for this disease. Second, AT1-AA is not specific to preeclampsia or to pregnancy; it also occurs in patients with graft rejection and accelerated hypertension [48, 49]. Moreover, levels of aldosterone, which is downstream to angiotensin II signaling, are decreased in preeclampsia rather than being upregulated [50].

**ENDOTHELIN-1**

Recent evidence from multiple studies implicates endothelin-1, a potent vasoconstrictor, as a downstream effector in the pathophysiology of preeclampsia. Endothelin-1 is increased in women with preeclampsia [51, 52] and is correlated with sFLT-1 and sENG levels in these patients [53]. Alexander et al. [54] demonstrated increased renal endothelin-1 expression in a pregnant rat model of uteroplacental ischemia that recapitulates many of the manifestations of preeclampsia. Using an endothelin-1 antagonist, those authors showed marked attenuation of the hypertension in their model, suggesting that endothelin-1 plays a major role in mediating hypertension in response to placental hypoxia. Significantly, both sFLT-1 and AT1-AA lead to increased renal and placental endothelin-1 expression in animal models of preeclampsia [55, 56], thus providing a link between endothelin-1 and the implicated mediators of preeclampsia. Moreover, in both of these models, hypertension is completely ameliorated by chronic endothelin-1 receptor blockade, suggesting that endothelin-1 plays an important role in mediating hypertension in response to excess sFLT-1 and AT1-AA during pregnancy [55, 56].

**EVIDENCE FOR HYPOXIA IN THE PATHOGENESIS OF PREECLAMPSIA**

Multiple pieces of evidence support the existence of hypoxia in preeclampsia. Lunell et al. [57] described a 50% reduction in uteroplacental circulation in patients with preeclampsia. In addition, preeclamptic placentas were shown to have increased incidence of infarcts relative to normal placentas [58]. The incidence of preeclampsia also is increased in women residing at high altitudes [59]. Recently, molecular evidence for hypoxia was demonstrated in preeclamptic placentas, showing a striking similarity in global gene expression to in vitro and in vivo models of placental hypoxia [60]. Finally, models of uteroplacental ischemia in rodents and primates can recapitulate many of the symptoms of preeclampsia, including hypertension, proteinuria, and glomerular endotheliosis [61, 62].

It is widely believed that shallow trophoblast invasion into the maternal decidua and lack of spiral artery remodeling [63], resulting in maintenance of high-resistance blood vessels, leads to uteroplacental hypoxia in preeclampsia. However, how this placental hypoxia leads to the maternal syndrome of preeclampsia remains open for debate. Several recent studies have made important progress in trying to answer this question. The group of Grainger and colleagues [54, 64, 65] demonstrated that uteroplacental ischemia in pregnant rats leads to preeclampsia-like manifestations accompanied by increases in sFLT-1, sENG, and endothelin-1. Elevation of sFLT-1 following reduced uteroplacental perfusion was also reported in a primate model of preeclampsia [62]. Importantly, these studies establish a causal relationship between placental ischemia and antiangiogenic factor upregulation. Furthermore, using the same rat model, Lamarcia et al. [66] recently provided a key link between placental hypoxia and another mediator postulated to play a role in the pathogenesis of preeclampsia.
FIG. 2. Early placental development is characterized by hypoxic environment and increased HIF-1α expression, leading to TGF-β3 upregulation and inhibition of trophoblast invasion. In normal placental development, increase in placental oxygen levels at 10–12 wk of gestation downregulates HIF-1α, restoring trophoblast-invasive capabilities. Failure to downregulate HIF-1α and its persistent expression promotes increased placental TGF-β3 expression and consequently shallow trophoblast invasion and persistent placental hypoxia, which in turn continues to stimulate HIF-1α expression. Failure to downregulate HIF-1α may be due to different mechanisms: decreased COMT expression leading to decreased placental production of 2-ME; decreased is oxygen-sensitive, being rapidly inactivated and degraded in normoxia. In normal oxygen tension, the interaction between the tumor suppressor von Hippel-Lindau (VHL) and HIF-1α is enabled by two prolyl hydroxylations within the HIF-1α degradation domain, at residues P402 and P564, by prolyl hydroxylases (PHDs) [69–72]. VHL functions as the recognition component of an E3 ubiquitin ligase that targets HIF-1α for ubiquitination and proteosomal degradation. In addition, asparaginyl hydroxylation at residue N803 within the C-transactivation domain of HIF-1α by the action of the asparaginyl hydroxylase factor-inhibiting HIF-1 (FIH) inhibits the interaction of HIF-1α with various coactivators, such as p300/CBP, resulting in its inactivation [73, 74]. During hypoxia, activity of the prolyl and asparaginyl hydroxylases, which is oxygen-dependent, is markedly reduced. The accumulating HIF-1α combines with HIF-1β to form the HIF-1 heterodimer, which binds to the hypoxia-responsive element of various genes, activating their transcription (Fig. 1).

The first evidence establishing a role for HIF-1α in the pathogenesis of preeclampsia originated from the pioneering work of Caniggia et al. Those authors demonstrated HIF-1α to be highly expressed in the low-oxygen environment of the placenta in early gestation, falling at around 9 wk of gestation, when placental oxygen levels increase [75]. Rajakumar et al. [76] reported similar findings, suggesting that HIF-1α plays an important role in placental development and function. In a related work, Caniggia et al. [77] showed that TGF-β3 placental expression parallels that of HIF-1α, with both being elevated in the low-oxygen environment of early gestation. In addition, they reported that TGF-β3 inhibits placental explant trophoblast differentiation and invasion, abnormalities characteristic of preeclampsia [77]. In an elegant experiment [75], these same authors used antisense inhibition of HIF-1α in placental explant trophoblasts. This resulted in downregulation of TGF-β3, restoring trophoblast differentiation and invasive capabilities and suggesting a role for HIF-1α in inhibition of trophoblast invasion, a key early step in the pathogenesis of preeclampsia. Furthermore, this shallow trophoblast invasion and its consequent placental hypoxia could in turn promote continued HIF-1α production, potentially creating a vicious cycle (Fig. 2). Subsequently, the same group as well as others...
showed that HIF-1α expression is upregulated in placentas of women with preeclampsia [68, 78]. More recently, cell-free HIF-1α RNA expression in maternal plasma was reported to be increased in pregnant women with preeclampsia and IUGR [79]. Further evidence relating hypoxia and HIF-1α to the pathogenesis of preeclampsia comes from studies of women residing at hypoxic high altitudes. These women have a 3- to 4-fold increased risk of developing preeclampsia [80], and their placentas overexpress HIF-1α [59].

A growing body of evidence supports HIF-1α being the molecular link between placental hypoxia and the downstream mediators of preeclampsia. Endothelin-1 is a well-known transcriptional target of HIF-1α in response to hypoxia [81, 82]. Similarly, HIF-1α was shown to induce the antiangiogenic factor sFLT-1 in placentas explants [67]. In addition, endoglin, the membrane-bound protein that gives rise to its proteolytic product sENG, is upregulated by HIF-1α [83], and sENG was recently shown to be induced by hypoxia in trophoblasts [84]. We recently utilized our previously described adenovirus expressing a constitutively active and stabilized HIF-1α to assess the effects of HIF-1α overexpression in pregnant mice. This resulted in elevation of sFLT-1 and sENG in the circulation of these mice [86]. Remarkably, the pregnant mice developed characteristic preeclamptic manifestations, including hypertension, proteinuria, glomerular endotheliosis, IUGR, and a HELLP-like syndrome [86]. Taken together, these data strongly suggest HIF-1α as a molecule involved in the pathogenesis of preeclampsia, leading to antiangiogenic factor upregulation following placental hypoxia. These data are corroborated by the recent study by Kanasaki et al. [87]. Those authors demonstrated that deficiency in catechol-O-methyl transferase (COMT), an enzyme that converts 17-hydroxyestradiol into 2-methoxyestradiol (2-ME), results in reduced 2-ME levels and a preeclampsia-like syndrome in pregnant mice. They also showed that 2-ME is reduced in placentas of women with preeclampsia, indicating the clinical importance of their animal findings. Notably, COMT deficiency in their model was associated with placental HIF-1α upregulation, and 2-ME administration to the mice resulted in HIF-1α downregulation and resolution of the preeclamptic syndrome [87]. Interestingly, the same group later showed in vitro that 2-ME treatment of hypoxic trophoblasts restores their invasive capabilities and is associated with suppression of HIF-1α-mediated TGF-β3 upregulation [88], in agreement with previous findings reported by Canigia et al. [75, 77]. Taken together, these data suggest that 2-ME physiologically acts to suppress HIF-1α in the hypoxic placenta, enabling proper trophoblast differentiation and invasion. On the other hand, reduced 2-ME levels may lead to HIF-1α overexpression, which in turn leads to upregulation of TGF-β3 and consequent shallow trophoblast invasion as well as antiangiogenic factor (sFLT-1 and sENG) and endothelin-1 upregulation, ultimately leading to the various maternal manifestations of the syndrome (Fig. 2). Recently, in a larger cohort of severe preeclamptic human placentas, Palmer et al. [89] did not find any differences in COMT expression between preeclamptic and normal placentas, thus challenging the clinical significance of the COMT-deficiency animal model. It remains to be determined what mechanisms may be responsible for decreased 2-ME in preeclampsia. Suppression of placental COMT activity was previously described in preeclampsia [90]. In addition, polymorphisms of the COMT gene have been described and demonstrated to be associated with fetal growth restriction [91, 92], and it would be interesting to explore this avenue further in relation to preeclampsia. Another important research area would be to investigate the possible involvement of HIF-1α in the AT1-AA pathway, thus further tying the knot between the antiangiogenic factors and the RAS system. In this respect, several studies performed on kidney and lung tissues revealed that HIF-1α leads to increased expression of angiotensinogen and angiotensin-converting enzyme [93, 94], whereas angiotensin II results in upregulation of HIF-1α [95]. In a recent study by Wenzel et al. [96], administration of AT1-AA together with angiotensin-II, but not separately, led to preeclampsia manifestations in pregnant rats along with increased placental expression of HIF-1α [96], suggesting that AT1-AA may affect HIF-1α indirectly by increasing tissue sensitivity to angiotensin-II.

Besides endothelin-1 and the antiangiogenic factors sFLT-1 and sENG, several other factors have been reported to be increased in preeclampsia via HIF-1α. Urotensin-II, a potent vasoconstrictor and angiogenic agent, has been reported to be overexpressed in placentas of women with preeclampsia and upregulated via HIF-1α in response to hypoxia in a syncytiotrophoblast model [97]. Moreover, urocortin-2 and urocortin-3, peptides showing homology to corticotropin-releasing factor and local regulators of myometrial contractility, have been shown to be upregulated in preeclamptic human placentas. Their placental expression is also increased by hypoxia in an HIF-1α-mediated fashion [98]. Recently, Rolfo et al. [99] found the myeloid cell leukemia factor-1 (MCL-1) E3 ubiquitin ligase, which targets MCL-1 for proteasomal degradation, to be overexpressed in preeclampsia and hypooxic placentals villous explants via an HIF-1α-mediated pathway, contributing to trophoblast cell death. Thus, the scope of HIF-1α-induced molecular dysregulation in preeclampsia appears to be wider than merely antiangiogenic factor upregulation. Future research should focus on identification of further HIF-1α-induced molecules that are dysregulated in preeclampsia and characterization of their potential roles in this disease.

In addition to placental hypoxya and decreased 2-ME levels, other possible causes of HIF-1α upregulation in preeclampsia have been suggested (Fig. 2). Previously, Rajakumar et al. [100, 101] demonstrated that proteosomal degradation of HIF-1α is reduced in early onset preeclamptic placentas and that this reduction is not due to VHL protein deficiency. Moreover, PHD-2 and FIH, the factors regulating HIF-1α stability and activation, respectively, have recently been shown to be downregulated in placentas from women who developed early onset preeclampsia [102], which could explain the reduction in HIF-1α proteosomal degradation. Interestingly, placentals explants of these women demonstrated lack of regulation of HIF-1α levels at various oxygen tensions, suggesting inability to sense a hypoxic environment [102]. It has been suggested that HIF-1α gene polymorphism may explain its upregulation in women who develop preeclampsia. However, no association of HIF-1α gene polymorphisms with preeclampsia have been found in studies of Korean [103] and Mexican [104] populations.

**SUMMARY**

Preeclampsia is a syndrome that is characterized by shallow trophoblast invasion and abnormal placentation leading to persistent placental hypoxia and the release of various mediators into the maternal circulation. These are thought to cause endothelial dysfunction that ultimately results in the various fetal and maternal manifestations of the syndrome. Accumulating evidence has put the antiangiogenic factors sFLT-1 and sENG, along with endothelin-1 and AT1-AA, at the forefront of efforts to understand the pathophysiology of preeclampsia. Whereas hypoxia has long been known to play...
an important role in placentaion, normal pregnancy, and the pathophysicsology of preeclampsia, the links between it and these newly proposed mechanisms were poorly understood until recently. This review attempted to show possible mechanistic links between hypoxia and the upregulation of the antiangiogenic factors and AT1-AA in preeclampsia. As discussed, placental hypoxia leads to upregulation of sFLT-1, sENG, endothelin-1, and AT1-AA. These effects are likely mediated, at least in part, via HIF-1α, a master regulator of the hypoxia response, because HIF-1α is overexpressed in women with preeclampsia and its overexpression results in upregulation of sFLT-1, sENG-1, and endothelin-1, ultimately leading to preeclamptic manifestations in animals. Taken together, these exciting new data suggest that HIF-1α suppression or antagonism of its downstream effectors may serve as targets for pharmacological intervention in preeclampsia.

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