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Hypoxia and sFlt-1 in Preeclampsia: The "Chicken-and-Egg" Question

Preeclampsia is a disorder of pregnancy that is usually characterized by the appearance of proteinuria and hypertension during the third trimester of pregnancy (1). It is a common disorder, occurring in 5% of all pregnancies, and often requires premature delivery of the baby. Occasionally, preeclampsia may lead to seizures and multiorgan dysfunction in the mother with severe morbidity and even mortality. Preeclampsia occurs only in the presence of placenta and remits dramatically postpartum, after the placenta has been delivered (2). The placentae of preeclamptic women appear abnormal, with evidence of underperfusion and ischemia. The initiating event in preeclampsia has been postulated to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles (3, 4). Overwhelming evidence points to endothelial dysfunction as the central mechanism in the pathogenesis of the maternal syndrome in preeclampsia (5). This has led to a hypothesis put forth by Roberts *et al.* (6, 7) that the ischemic placenta secretes soluble factors into the maternal vasculature. This, in turn, induces endothelial dysfunction and the clinical features of preeclampsia. Several groups including our laboratory have recently presented evidence that excess secretion of a naturally occurring antiangiogenic molecule of placental origin referred to as soluble fms-like tyrosine kinase-1 (sFlt-1, also referred to as sVEGFR-1) may cause the maternal syndrome (8–14). sFlt-1 acts by antagonizing proangiogenic molecules such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) (15). The excess sFlt-1 production in preeclampsia has been hypothesized to be secondary to placental ischemia/hypoxia (12). However, it was unclear how hypoxia would affect the net angiogenic balance in the placenta because VEGF, a proangiogenic molecule, is also up-regulated by hypoxia (16). A report in this issue of *Endocrinology* [Nagamatsu *et al.* (17)] provides new evidence that effects of hypoxia are cell type specific and that in placental cytotrophoblasts (in contrast to endothelial cells), hypoxia induces an excess production of sFlt-1. This leads to VEGF deficiency and, consequently, an antiangiogenic state.

Role of sFlt-1 in the Pathogenesis of the Maternal Syndrome in Preeclampsia

The search for one or more placentally derived factors leading to the maternal syndrome has been a source of great research interest for decades. Several groups have hypothesized that alterations in factors such as TNF-α, IL-6, IL-1α, IL-1β, oxidized lipid products, neurokinin-B, and syncytiotrophoblast debris may induce the maternal syndrome (1, 18–21). It is important to note that, although these factors have been reported to induce endothelial dysfunction in *in vitro* studies, none of these has been reported to induce the full-blown maternal syndrome including the appearance of glomerular endotheliosis, the signature lesion of preeclampsia.

sFlt-1, a splice variant of the VEGF receptor Flt-1 that lacks the transmembrane and cytoplasmic domains, is made in large amounts by the placental trophoblasts and is released into the maternal circulation (22, 23). sFlt-1 acts as a potent antiangiogenic molecule by binding circulating VEGF and PlGF. Several groups including our own have recently reported that sFlt-1 gene product is up-regulated in the preeclamptic placentae (11, 12, 14). The excess sFlt-1 noted in the bloodstream is associated with decreased free VEGF and free PlGF. After delivery, sFlt-1 levels fell, corresponding to the resolution of the clinical syndrome. Preeclamptic serum, but not normal pregnant serum, has also been reported to have an antiangiogenic activity that can be reversed by excess VEGF and PlGF (12). Adenoviral gene transfer of sFlt-1 into rats resulted in a preeclampsia-like illness including hypertension, proteinuria, and glomerular endotheliosis (12). Furthermore, loss of a single VEGF allele in the glomerulus of mice resulted in proteinuria and glomerular endotheliosis, providing genetic proof that VEGF deficiency leads to a preeclampsia-like state (24). Finally, we recently demonstrated that alterations in these angiogenic molecules (elevated sFlt-1, decreased free PlGF and decreased free VEGF) antedate the onset of clinical symptoms (13). Collectively, these data suggest that excess placental production of sFlt-1 has a causal role in the pathogenesis of the maternal syndrome. Whether sFlt-1 also induces the abnormal placentation/placental ischemia remains unknown at the present time.

Abnormal Placentation of Preeclampsia

During normal placental development, cytotrophoblasts differentiate and invade the maternal spiral arterioles and completely remodel them into large capacitance vessels with low resistance. This endovascular cytotrophoblast invasion involves replacement of not only the endothelium, but also the highly muscular tunica media. During normal differentiation, invasive trophoblasts alter their adhesion molecule expression from those that are characteristic of epithelial cells (integrin α_6/β_4, α_v/β_5, and E-cadherin) to those of endothelial cells (integrin α_v/β_3, platelet adhesion cell adhesion molecule-1 and E-cadherin), a process referred to as pseudovasculogenesis (25). In preeclampsia, endovascular invasion of cytotrophoblasts remains superficial and uterine blood vessels do not undergo adequate vascular transformation compared with normal pregnancy (3, 26). Furthermore, Fisher *et al.* (27) have elegantly demonstrated that in preeclampsia, these invasive trophoblasts fail to undergo

Abbreviations: PlGF, Placental growth factor; VEGF, vascular endothelial growth factor.

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pseudo-vasculogenesis. The aberrant vascular transformation is thought to lead to placental insufficiency and consequently placental hypoxia (28). This hypothesis has been further strengthened by clinical observations that in women destined to develop preeclampsia, uteroplacental blood flow is reduced by 50–70% (29). In more recent studies, hypoxia-inducible transcription factors have been shown to be selectively increased in the preeclamptic placentae (30, 31). In several species of animals including baboons, rhesus monkeys, rabbits, and rats, restriction of placental blood flow during pregnancy has resulted in a preeclampsia-like illness (32). These data lend credence to the hypothesis that placental insufficiency and placental hypoxia may lead to the maternal syndrome. However, it is worth noting that placental hypoxia can itself directly block the invasion and differentiation of cytotrophoblasts in primary culture studies, suggesting that there may be a positive feedback loop between hypoxia and abnormal placentation (33).

There is circumstantial evidence that excess sFlt-1 may play a direct role in the pathogenesis of the abnormal placentation noted in preeclampsia (28). Because VEGF is a known to convert hematopoietic stem cells to endothelial cells (34), it is likely that VEGF inhibition would block the endothelial differentiation process noted in invasive cytotrophoblasts. Indeed, sFlt-1 has been shown *in vitro* to inhibit placental cytotrophoblast invasion and differentiation in primary cytotrophoblast cultures (14). Furthermore, modest alterations in circulating angiogenic molecules in preeclamptic patients have been detected even as early as first trimester of pregnancy, long before the process of trophoblast invasion and differentiation (35). It was also recently reported that knocking out Flt-1 gene in mice does not affect placentation and the authors concluded that inhibition of sFlt-1 during pregnancy might not affect placentation (36). However, it is impossible to conclude that sFlt-1 has no role in placentation because sFlt-1 blocks signaling through Flt-1, Flk-1, and neuropilin. *In vivo* studies documenting the definitive role of sFlt-1 during placentation, such as a transgenic overexpression of sFlt-1 in the placenta are lacking.

The Connection between Placental Hypoxia and sFlt-1 Production

It has been hypothesized that the excess sFlt-1 production is a consequence of the placental hypoxia that occurs during abnormal placentation (12). However, because both Flt-1 and VEGF are hypoxia-inducible genes (16, 37), it was unclear up until recently what the net effect of placental hypoxia would be on the balance of these angiogenic factors. In this issue of *Endocrinology*, Nagamatsu *et al.* (17) have demonstrated that cytotrophoblasts and endothelial cells respond in opposite ways in response to hypoxia in terms of angiogenic balance. Lowering the oxygen percentage from $20\%O_2 \rightarrow 8\%O_2 \rightarrow 2\%O_2$ in a primary cytotrophoblast culture caused an increase in the number of cells, as well as a rise in sFlt-1 concentration. sFlt-1 mRNA was strikingly increased with reduced O_2 tension. Although total VEGF levels in these cells increased modestly with hypoxia, free VEGF levels were undetectable along with very low free PlGF concentrations in the media. These changes were unique to cytotrophoblasts and were not seen in human umbilical vein endothelial cells or villous fibroblasts. Furthermore, the authors did not find any changes in the PlGF mRNA in these primary cytotrophoblasts, suggesting that the alterations in PlGF noted during conditions of placental hypoxia are likely mediated by excess binding of sFlt-1 to PlGF. Although these studies do not provide any clues to the mechanisms of placental hypoxia in preeclampsia, they provide important evidence that excess sFlt-1 production that occurs in preeclampsia may be a consequence of placental hypoxia. Furthermore, because other cells such as monocytes and endothelial cells also produce sFlt-1, it has been argued that the trophoblasts may not be the only source of sFlt-1 production during preeclampsia. Recent data from Chaiworaponga, T., and R. Romero, personal communications (National Institute of Child Health and Human Development, Detroit, MI) have shown that the uterine vein sFlt-1 concentrations were significantly higher than the uterine arterial concentrations. This finding, along with study reported in this journal, would suggest that placental tissue is the major source of excess sFlt-1 production during preeclampsia.

The studies by Nagamatsu *et al.*, however, do not rule out the possibility that alterations in placental sFlt-1 during preeclampsia are primary and directly lead to the abnormal placentation/placental hypoxia. The placental hypoxia may then result in more sFlt-1 production, thus leading to a vicious cycle of sFlt-1 production that finally spills over to the systemic circulation, leading to the clinical syndrome of preeclampsia.

In summary, the maternal syndrome of preeclampsia is thought to be secondary to abnormal placentation and excess placental production of sFlt-1 (see Fig. 1). Although it is still unclear whether placental hypoxia or excess sFlt-1 production is the trigger event in the pathogenesis of preeclampsia, this study, along with other recent studies, provides evidence that the massive sFlt-1 production noted during clinical preeclampsia may be secondary to placental hypoxia. Further studies in transgenic animals looking at local sFlt-1 overexpression in the placenta may shed light on the role of sFlt-1 during early placental development. Why placental rather than endothelial hypoxia leads to predominantly sFlt-1 production remains unknown. Studies that identify regulatory factors that are present uniquely in the cytotrophoblasts of the placenta, but not in other cell types, may help understand this mystery. Future clinical studies directed at pharmacological

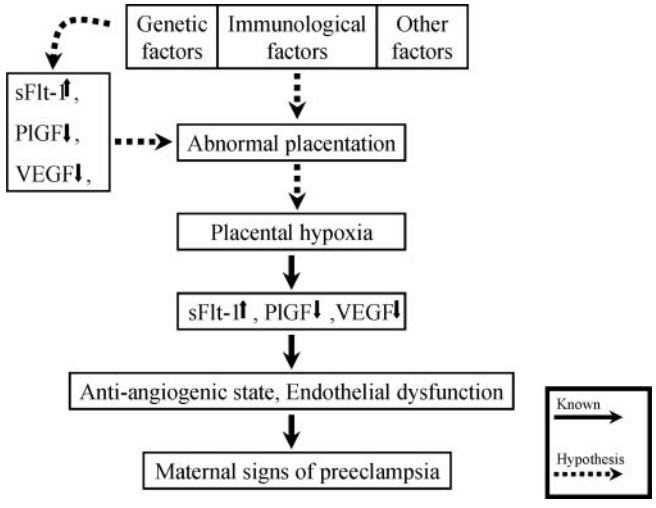

FIG. 1. Hypothesis of the role of sFlt-1 and placental hypoxia in the pathogenesis of preeclampsia

neutralization of sFlt-1 with proangiogenic agents should help further clarify the pathogenesis of this complex disease.

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