Angiogenic Factors in Preeclampsia and Related Disorders

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During fetal development, the human placenta undergoes high levels of both angiogenesis and vasculogenesis. Additionally, the developing placenta undergoes a process of vascular mimicry (referred to as pseudovasculogenesis) as cytotrophoblasts convert from an epithelial to an endothelial phenotype. The initiation, maturation, and maintenance of the placental vasculature are of critical importance. Failure to do so can lead to adverse obstetric outcomes such as preeclampsia and/or intrauterine growth restriction (IUGR). Furthermore, the foundation of many aspects of adult health is laid in utero. In this context, normal placental function is not only critical for normal fetal development but can also permanently influence long-term health and disease. Understanding the mechanisms that regulate placental vasculogenesis and angiogenesis is therefore of critical importance. This chapter will focus on placental vascular development with a particular emphasis on the role of angiogenic factors in the pathogenesis of the maternal syndrome of preeclampsia and related disorders.

The placenta is a remarkable organ, as it enables fetal development within a protective maternal environment (Cross et al. 1994). It performs an impressive array of functions ranging from anchoring the conceptus and preventing its rejection by the maternal immune system to providing critical exchange of gases, nutrients, and waste products. The placenta is a highly vascularized organ, containing both maternal and fetal blood vessels. The formation, maturation, and maintenance of the placental vasculature are of critical importance. Failure to do so can lead to adverse obstetric outcomes such as preeclampsia (Brosens et al. 1972), IUGR (Macara et al. 1996), and miscarriage (Meegdes et al. 1988). Furthermore, the placenta plays an active role in programming the fetal development, which, when deranged, may lead to disease in adult life (Irving et al. 2000; Barker 2003; de Rooij et al. 2010; Schulz 2010). Thus, although transient in existence, derangement of placental vessel formation can have a lifelong impact. Understanding the mechanisms that regulate placental vasculogenesis and angiogenesis is therefore of critical importance.

During embryonic development, the blastocyst separates into an outer polarized cell layer...
termed trophectoderm which gives rise to extra-embryonic trophoblasts (the specialized epithelial cells of the placenta) and the nonpolarized inner cell mass that forms the embryo proper. Implantation of the blastocyst involves a complex interplay between the trophoblasts and maternal cells involving invasion of trophoblasts through the uterine wall to ultimately form the placenta.

Invading trophoblasts give rise to placental villi, the structural unit of the placenta. In addition, invasion of trophoblast creates open endings in the maternal vessels leading to maternal blood release into the intervillous space. Placental villi contain an inner layer of mononuclear cytotrophoblasts and an outer layer of continuous multinucleated cytoplasm referred to as syncytiotrophoblasts. It is in the spaces surrounding these structures that the maternal blood bathes fetal villi. Around day 13, first sprouting of trophoblast can be found protruding towards the intervillous space forming the first primary villi. Shortly after, extraembryonic mesoderm penetrates into the primary villi transforming them into secondary villi. The first fetal capillaries can be observed within this mesenchyme leading to the formation of tertiary villi (Huppertz 2008).

Continuing growth and differentiation of the trophoblast leads to branching of the villi and the shaping of the placental labyrinth where fetal–maternal exchange of oxygen and nutrients occurs. The ramifications of the villous trees can be subdivided in segments based on their caliber, stromal characteristics, vessel structure, and appearance during pregnancy as immature intermediate, stem, mature intermediate, and terminal villi (Castellucci et al. 2000). This chapter will focus on placental vascular development with a particular emphasis on the role of angiogenic factors in this process.

PLACENTAL VASCULAR DEVELOPMENT

Placental Vasculogenesis

Placental vascular development begins when cells from mesenchymal origin residing within the placental secondary villi differentiate into endothelial progenitor cells, which are believed to arise from the hemangioblast, a common precursor for endothelial and hematopoietic lineages. The subsequent assembly of these differentiated cells leads to the formation of primitive blood vessels.

In humans, placental vasculogenesis is evident by approximately 21–22 days post-conception (pc). At this stage, cords of hemangioblasts are present and some show primitive lumen formation. These cords further develop so that by day 32 pc most villi show the presence of capillary structures (Demir et al. 1989). Lumen formation occurs via establishment of intercellular spaces between hemangiogenic cell cords (Demir et al. 1989, 2004). Fusion of intracytoplasmic vacuoles of few cells lead to small microvascular connecting tubes that establish bridge-like connections to the larger vessels, resulting in a primitive connecting network (Demir et al. 2004).

Hematopoietic stem cells, also derived from hemangiogenic stem cells, can be seen at 23–26 pc in the primitive lumen, surrounded by endothelial progenitor cells (Demir et al. 2004). At this time, endothelial tubes are still isolated, as the placental vascular network is not yet connected to the embryonic vascular structures. This connection is established via the connective stalk (forerunner of the umbilical cord) around day 32 pc.

Placental Angiogenesis

During the angiogenesis phase, the endothelial tube segments formed through vasculogenesis will be transformed into an organized vascular network by means of branching angiogenesis through lateral sprouting and nonbranching angiogenesis through elongation of existing tubes.

From day 32 pc until the end of the 24th week, branching angiogenesis prevails. Multiple sprouting of microvessels produces a complex, multiply branched capillary web. From week 25 pc until term, patterns of villous vascular growth switch from the prevailing branching angiogenesis to a prevalence of nonbranching angiogenesis in which the capillaries
predominantly increase in length by elongation of existing capillary loops. Under normal conditions it takes place in combination with branching angiogenesis (Kaufmann et al. 2004). By this time, mesenchymal villi start to develop into mature intermediate villi and then later start producing terminal villi. Analysis of proliferation markers at this stage reveals a decrease in trophoblast proliferation and an increase in endothelial proliferation along the entire length of these villous structures. The final length of these peripheral capillary loops exceeds 4000 µm (Kaufmann et al. 1985, 1987) and they grow at a rate that exceeds that of the villi themselves, resulting in coiling of the capillaries. The looping capillaries bulge toward and obtrude into the intervillous space, and thereby contribute to formation of the terminal villi. Each of the former is covered by an external thin (<2 µm) layer of trophoblast that contributes to the so-called vasculosynci- tial membranes, separating the maternal and fetal circulations. These are the principal sites of exchange of gases via diffusion between mother and fetus.

Recently, Jirkovska and colleagues challenged the hypothesis that nonbranching angiogenesis prevails in the second half of pregnancy, reporting regular findings of branched terminal villous capillaries and blindly ending capillary sprouts in 3D reconstructions of images captured by confocal microscopy (Jirkovska et al. 2008). Further studies are necessary to clarify this.

Also important in these phases is the recruitment of supporting cells, pericytes, and smooth muscle cells which stabilize the developing vessels and are critical to the integrity of the developing vasculature. From the earlier stages, stromal cells are recruited as pericytes. First spots of basal lamina material are observed around week 6 pc, but a basement membrane with complete envelopment of the capillaries is only observed in the third trimester (Demir et al. 1989). In addition, stromal cells within the villi differentiate in a defined centripetal gradient with increasing cytoskeletal complexity from the superficial trophoblasts toward the blood vessels in the center of stem villi. They range from proliferating mesenchymal cells (precursor cells) to highly differentiated myofibroblasts and possibly even myocytes and, together with stromal fibrosis, help to form thin media and adventitia-like structures in stem vessels in stem villi (Demir et al. 1989; Kohnen et al. 1996). These vessels are the fore-runners of villous arteries and veins. A continuous transformation and ripening of the villous vessel system takes place until term (Fig. 1).

**Pseudovasculogenesis: Maternal Vascular Remodeling**

The formation of adequate maternal–placental circulation requires remodeling of maternal uterine spiral arteries. Invasion of the maternal spiral arteries by fetal cytotrophoblasts is a key event in this process. The created open endings of the maternal vessels release maternal blood into the placental labyrinth which subsequently flows around the placental villi and is drained by spiral veins.

During normal pregnancy, cytotrophoblast cells of the developing placenta migrate and invade the endothelium and the muscular layer of the maternal spiral arteries up to the inner one-third of the myometrium (Fig. 2). Along the process of invasion, the expression of a number of different classes of molecules such as integrins, cadherins, and metalloproteinases is modified in a process termed pseudovasculogenesis, since these cytotrophoblasts lose the epithelial phenotype and acquire an endothelial-like phenotype (Zhou et al. 1997b). Besides invading, cytotrophoblast cells replace the endothelial lining of the arteries, forming a pseudoendothelium compounding intriguing vessels, part fetal and part maternal. This process causes a remodeling of the artery wall, transforming the high-resistance muscular maternal arteries in high-flow, low-resistance vessels capable of providing adequate placental perfusion to sustain the growing fetus. This process peaks about 12 weeks and is complete near 20 weeks of gestation (De Wolf et al. 1980).

Placental oxygen tension has been suggested to be one of the major regulators of cytotrophoblast migration and differentiation (Genbacev
Figure 1. Representation of a normal human term placental (A) and villous structure (B). (A) Schematic representation of a normal human term placenta depicting the maternal and fetal circulation. (B) Representation of a group of terminal villi.
Figure 2. Placental vascular remodeling in health (upper panel) and in disease, preeclampsia (lower panel). In normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries, transforming them from small-caliber resistance vessels to high-caliber capacitance vessels capable of providing placental perfusion adequate to sustain the growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from an epithelial phenotype to an endothelial phenotype, a process referred to as “pseudovascularogenesis” or “vascular mimicry” (top). In preeclampsia, cytotrophoblasts fail to adopt an invasive endothelial phenotype. Instead, invasion of the spiral arteries is shallow, and they remain small-caliber resistance vessels (bottom). (Adapted from Lam et al. 2005; reprinted, with permission, from Wolters Kluwer Health © 2005.)
et al. 1997); however, data regarding this topic are conflicting (James et al. 2006). It has also been hypothesized that decidual natural killer cells and/or activated macrophages may play a role in this process (Hanna et al. 2006). Finally, the hemodynamic changes that take place during spiral artery remodeling are considerable and have also been hypothesized to play a role in the spiral artery remodeling process (Osol and Mandala 2009).

ANGIOGENIC FACTORS AS REGULATORS

We still know very little about the details of the genetic program required to pattern the developing vasculature in the placenta. A number of candidates have been proposed, such as vascular endothelial growth factor (VEGF), angiopoietins, placental growth factor (PIGF), basic fibroblast growth factor, and transforming growth factor-β, etc. Among these, VEGF and its two receptors, Flt-1 and Flk-1/KDR have been shown to be crucial regulators of physiological and pathological blood vessel growth (Folkman and Shing 1992; Dvorak 2002) and, together with the angiopoietin family, have been the most extensively studied during placental development.

Members of the VEGF family include VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF. There are three VEGF family receptors, known as Flt-1, KDR, and Flt-4, as well as a soluble form of Flt-1 (sFlt-1, also referred to as sVEGFR1), which is generated by alternative mRNA processing and lacks the transmembrane domain and the cytoplasmic domain (Kendall and Thomas 1993; Huckle and Roche 2004; Thomas et al. 2007). VEGF-A, B, and PIGF bind to Flt-1, VEGF-A binds to KDR, and VEGF-C and D bind to Flt-4. Flt-1 and VEGF also bind to sFlt-1, which sequesters them from acting on their cognate membrane receptors. VEGF is highly expressed in the early human placenta. According to immunohistochemical and in situ hybridization studies, villous trophoblast and Hofbauer cells are the main source of this cytokine (Geva et al. 2002; Demir et al. 2004). Its cognate receptor KDR is present on endothelial cells or mesenchymal cells from which the endothelial cells differentiate (Clark et al. 1996; Vuckovic et al. 1996; Helske et al. 2001; Demir et al. 2004). Moreover, first trimester trophoblast and Hofbauer cells have been shown to secrete VEGF in vitro (Lash et al. 2010). Flt-1 was detected in the primitive vascular lumens and angiogenic cell cords (Demir 2009). This suggests that the initial steps necessary for building the primary vascular network are mediated by the VEGF family in a paracrine way. Immunohistochemical studies on the expression patterns of VEGF, PIGF, and their receptors in the placenta as well as maternal serum levels throughout pregnancy suggest an important role in villous angiogenesis. Expression of VEGF and KDR decline as pregnancy advances (Vuckovic et al. 1996) or moderately increase but less than placental weight does (Kaufmann et al. 2004). In contrast, expression of Flt-1 and PIGF increase towards term (Clark et al. 1998; Kumazaki et al. 2002). Maternal serum levels of free VEGF decline as pregnancy advances and sFlt-1 levels rise at 33–36 weeks. PIGF concentrations rise during pregnancy increasing markedly at 28–32 weeks (Levine et al. 2004).

A decreasing expression of VEGF and KDR, but increasing expression of PIGF, have been correlated with a switch from branching to non-branching angiogenesis in the development of the placental villous angioarchitecture (Kaufmann et al. 2004). It has thus been hypothesized that the balance between VEGF, PIGF, and their receptors determines to some extent the geometry of the villous vascular bed (Kaufmann et al. 2004). VEGF is a known important inducer of branching angiogenesis (Carmeliet et al. 2009). PIGF is expressed in the normal placenta (Clark et al. 1998) and PIGF knockout mice are viable and show normal reproduction (Carmeliet et al. 2001). PIGF has been shown to be proangiogenic but also to inhibit branching angiogenesis in some cases (Bjorndahl et al. 2004; Fischer et al. 2008). In addition, it may also regulate other activities such as those of decidual natural killer cells (Tayade et al. 2007). sFlt-1 binds VEGF and PIGF and thus can inhibit their activity. Besides, sFlt-1 participates
in the local guidance of nascent vessel sprouts (Kearney et al. 2004; Chappell et al. 2009) and thus may be able to directly regulate placental vessel formation.

Maturation of primitive endothelial tubes into mature blood vessels requires the recruitment of surrounding mesenchymal cells and their differentiation into vascular smooth muscle cells and pericytes (Carmeliet 2003). This process is largely mediated by the angiopoietins and their receptors Tie-2, PDGF, and TGF-β. In human placental tissues, expression of Ang-1, Ang-2, Tie-1, and Tie-2 has been detected at both protein and mRNA levels (Dunk et al. 2000; Goldman-Wohl et al. 2000; Zhang et al. 2001; Geva et al. 2002; Kayisli et al. 2006; Seval et al. 2008). Ang-1 mRNA increases with gestation (Geva et al. 2002; Wulff et al. 2002), whereas Ang-2 decreases. Ang-1 and Ang-2 have been primarily localized to trophoblast whereas moderate to weak immunoreactivity was observed in endothelial cells (Seval et al. 2008). Tie-1 and Tie-2 have been shown to be expressed in trophoblast and endothelial cells (Seval et al. 2008). Hofbauer cells express Ang-2, Tie-1, and Tie-2 (Kayisli et al. 2006; Seval et al. 2008). In the early placenta, Ang-1 showed immunoreactivity in endothelial cells of vessels within immature intermediate villi and no immunoreactivity in angiogenic cell cords of mesenchymal villi (Seval et al. 2008). At term, Dunk and colleagues localized Ang-1 to the surrounding perivascular tissues of the mature stem villi (Dunk et al. 2000). In primates, Tie-2 has been shown to be expressed at high levels in the endothelium of chorionic vessels and at low levels in the capillaries of the villi (Wulff et al. 2002). In humans, Kayisli and colleagues reported an increasing expression of Tie-2 in the endothelium parallel to vascular maturation (Kayisli et al. 2006). This is consistent with the role of Ang-1/Tie-2 as a vascular maturation and stabilizing agent. Moreover, the high levels of Ang-2 in early stages might prevent stabilization of the newly formed vessels, thus permitting the placental vessels to remain in a plastic state so that they can respond to the sprouting signal of VEGF, thus promoting branching angiogenesis (Hashizume et al. 2010). Reports of localization of angiopoietins and its receptors at the time of vasculogenesis and initial stages of angiogenesis (namely in angiogenic cell cords) (Kayisli et al. 2006; Seval et al. 2008) raise the possibility that this signaling system may also regulate initial steps of vessel formation in the placenta.

The localization of receptors of VEGF and angiopoietin families in the trophoblast suggests that, in addition to regulation of vascular development, they may also regulate trophoblast function. Tie-1 and Tie-2 are expressed in the villous trophoblast, and Ang-2 has been shown to enhance trophoblast DNA synthesis and NO release whereas Ang-1 acts as a potent trophoblast chemotactic factor (Dunk et al. 2000). Ang-1 mRNA expression by decidual cells suggests that they may act as a chemoattractant to promote extravillous cytotrophoblast migration (Dunk et al. 2000). In addition, the high expression of Ang-2 in early gestation makes it a possible contributing factor for induction of maternal vascular transformation as it destabilizes the vasculature. Indeed, Ang-2 has been localized to trophoblast cells whereas Tie-2 is expressed in the maternal endothelium (Goldman-Wohl et al. 2000), indicating a paracrine mechanism for vascular remodeling. Decidual cells also express VEGF (Charnock-Jones et al. 1994; Cooper et al. 1995), whereas invading cytotrophoblasts express both VEGF and Flt-1 (Charnock-Jones et al. 1994; Cooper et al. 1995; Clark et al. 1996). The initial phases of extravillous cytotrophoblast differentiation take place within columns of trophoblast cells which are formed in the upper part of villi anchored to the maternal decidua. Flt-1, KDR, Tie-1, and Tie-2 were shown to be expressed within these columns and to have a differential expression pattern throughout the column (Zhou et al. 2002; Kayisli et al. 2006). Under in vitro conditions, VEGF-A induces cytotrophoblast invasion, an effect that is blocked by addition of sFlt-1 (Zhou et al. 2002). In vivo evidence for this observation is lacking because normal invasion of trophoblast is relatively shallow even at baseline in rodents, compared to human placentas which show robust invasion.
Other signaling systems involved in vessel maturation are PDGF and its receptor (PDGFR-β) and TGF-β superfamily (Carmeliet 2003; Jain 2003). PDGF has essential roles in the stabilization of nascent blood vessels by recruiting PDGFR-β-positive mesenchymal progenitors. PDGF and its receptor have been shown to be present in the human placental vasculature (Holmgren et al. 1991). Null mutant placentas for Pdgfb and Pdgfrb show reduced numbers of pericytes, reduced fetal vessel density, and increased vessel diameter (Ohlsson et al. 1999). An activating mutation of Pdgfrb caused a disorganization of the fetal blood vessel compartment of the labyrinth (Looman et al. 2007). Taken together, these results suggest that PDGF and its receptor may also be important in placental vessel maturation. TGF-β has shown to be expressed by trophoblasts along pregnancy (Xuan et al. 2007). Knockout models of TGF-β family members show abnormal vessel development and many are embryonic lethal (Pardali et al. 2010). Although abnormalities in placental vasculature have been reported (Larsson et al. 2001; Hong et al. 2007), the mechanisms of TGF-β family members’ regulation of human placental vascular development remain to be elucidated.

Interestingly, pregnancy-specific growth factors and hormones, such as human chorionic gonadotropin (Zygmunst et al. 2002) and alphafetoprotein (Liang et al. 2004), and insulin-like growth factor-II (IGF-II) (Herr et al. 2003) have been shown to be proangiogenic, and therefore may also play a role in placental vascular development.

In summary, angiogenic factors are important not only for placental vascular development but also for other aspects of placentation such as regulation of trophoblast growth and differentiation. It is interesting to note that vessel formation may regulate placental development and vice versa through a number of different signaling systems. In this regard, studies in mice indicate that there is a complex interplay between the trophoblast and the development of its associated fetal-derived vasculature (Rossant and Cross 2001).

The characterization of antiangiogenic proteins in the placenta, in contrast to proangiogenic proteins, appears to have received far less attention. An important anti-angiogenic protein produced by the placenta that has recently been extensively studied is sFlt-1, an endogenous inhibitor of VEGF which, together with soluble endoglin (sEng), an endogenous inhibitor of TGF-β, have been shown to play an important role in the pathogenesis of preeclampsia Other anti-angiogenic proteins expressed in the placenta include thrombospondin-1, endostatin, and prolactin fragments; however, their role in placentation is unclear (Bdolah et al. 2004). Human placenta is thus a rich source of angiogenic substances and these may play an important role not only in the regulation of placental vessel formation but also, when released to the circulation, play a role in maternal vascular adaptation to pregnancy.

**PREECLAMPSIA**

Preeclampsia is a multisystem disease, characterized by new-onset hypertension and proteinuria after 20 weeks of gestation, which complicates 3%–8% of pregnancies (Duley 2009). It is a leading cause of maternal and perinatal morbidity and mortality worldwide (Duley 2009). In the mother, the disease can progress to widespread endothelial dysfunction affecting mainly the liver, the brain, and the kidney. Preeclampsia is also frequently associated with IUGR and prematurity (Sibai et al. 2005). As of 2011 treatment is supportive and the only known cure is delivery of the placenta.

The observation that this syndrome occurs solely in the presence of placental tissue (i.e., in pregnancy or with hydatidiform mole) (Sibai et al. 1995; Soto-Wright et al. 1995) and remits dramatically in the postpartum period after the placenta is delivered suggest that the placenta is both necessary and sufficient for the development of preeclampsia. Naturally, research focused on the placenta as the source of the disease. It has been proposed that preeclampsia is caused by placental dysfunction followed by the release of factors by the diseased placenta into the maternal circulation inducing endothelial dysfunction, which
heralds the classical manifestations of the disease (Fig. 3).

The major pathological abnormality in the preeclamptic placenta is insufficient maternal spiral artery remodeling. Cytotrophoblast cells fail to acquire the endothelial-like phenotype and are unable to invade the myometrial spiral arteries. As a result, the myometrial segments remain narrow (Zhou et al. 1997a; Naicker et al. 2003; Kadyrov et al. 2006). The histological and physiological characteristics of these vessels do not change, they retain their original endothelial linings and muscular walls remaining high resistance vessels (Fig. 2) (Lin et al. 1995; Khaw et al. 2008). It has been theorized that this failure of conversion of spiral arteries...
results in persistent placental hypoxia and dysfunction. However, it should be noted that placental hypoxia/ischemia alone may not be sufficient to produce preeclampsia because it is detected in many instances of IUGR in women without preeclampsia, and it should also be noted that histological evidence of spiral artery remodeling may be absent in some cases of preeclampsia. Thus other factors may concur to cause placental dysfunction. Various pathways including deficient heme oxygenase expression, genetic factors, oxidative stress, inflammation, altered natural killer cell signaling, and, more recently, deficient catechol-O-methyl transferase (Redman and Sargent 2005; Cudmore et al. 2007; Kopcow and Karumanchi 2007; Kanasaki et al. 2008; Zhao et al. 2009) have also been proposed to have key roles in inducing placental disease (Fig. 3).

The role of maternal endothelial dysfunction in producing the clinical manifestations of preeclampsia has been studied extensively since the 1980s (Roberts et al. 1989; Ferris 1991) and extensive data support the notion that the maternal serum in preeclampsia has soluble factors which mediate endothelial dysfunction. The search for these factors led to the characterization of soluble Fms-like tyrosine kinase 1 (sFlt-1), an antiangiogenic protein that is overproduced by the placenta and induces systemic endothelial dysfunction.

In 2003 it was shown that placental expression of sFlt-1 is strikingly increased in preeclampsia and this is associated with increased levels of maternal circulating sFlt-1 and decreased levels of free bioactive VEGF and PlGF (Maynard et al. 2003), a finding that has been confirmed by several groups (Buhimschi et al. 2005; Levine et al. 2005; Aggarwal et al. 2006). It was also observed that serum from patients with preeclampsia inhibited endothelial tube formation in vitro and that 48 hours postpartum, this antiangiogenic effect had disappeared from the serum. Moreover, when injected into pregnant rats, sFlt-1 induced hypertension, proteinuria, and glomerular endotheliosis, the hallmarks of the disease (Maynard et al. 2003). Circulating levels of sFlt-1 begin to rise before the onset of clinical symptoms (Levine et al. 2004) and correlate with the severity of the disease (Chaivorapongs et al. 2004), suggesting important diagnostic and predictive potential (Cerdeira and Karumanchi 2010). Several risk factors for preeclampsia can also be correlated with increased sFlt-1 such as multigestational pregnancies (Bdolah et al. 2008; Maynard et al. 2008), hydatidiform mole (Koga et al. 2010), trisomy 13 (Bdolah et al. 2006), and nulliparity (Wolf et al. 2005). Potential additional contributors as a source of sFlt-1 besides trophoblasts include peripheral blood mononuclear cells (Rajakumar et al. 2005) and proteolytic shedding of extracellular fragment of Flt-1 in endothelial cells (Rahimi et al. 2009; Zhao et al. 2010), but the clinical significance of these sources is unknown.

Placental syncytiotrophoblasts and in particular syncytial knots were identified as a major source of sFlt-1 production (Sela et al. 2008). Syncytial knots are induced by placental hypoxia and are noted predominantly in preeclamptic placentas. Interestingly syncytial knots and placental debris have been shown to be released into the maternal circulation suggesting an additional source of increased sFlt-1 in the maternal blood besides secretion by the placenta (Lok et al. 2008). Much work has been done regarding placental hypoxia and its possible role in preeclampsia. It has been shown to increase sFlt-1 expression. Many other factors have also been shown to increase placental production of sFlt-1 such as angiotensin II and autoantibodies against angiotensin receptor-1 (Zhou et al. 2007). Furthermore, several animal models of preeclampsia show an increase in sFlt-1, suggesting that this molecule may be a central culprit in the pathogenesis of preeclampsia which originates with different insults to the placenta converging on a final common pathway mediated by excess release of sFlt-1 into the maternal circulation.

The other two VEGF receptors (KDR and Flt-4) are also expressed in human placentas; Flt-4 expression has been shown to remain unchanged (Helske et al. 2001) and KDR has been shown to be not changed (Helske et al. 2001) or reduced (Tripathi et al. 2009; Groten
et al. 2010). A soluble form of human KDR was described (Ebós et al. 2004), and appears to be decreased in preeclampsia (Chaiworapongsa et al. 2010); however, its role in preeclampsia is unknown.

There is circumstantial evidence that antagonism of VEGF and PlGF may have a pathogenic role in the appearance of hypertension and proteinuria. VEGF stabilizes endothelial cells in mature blood vessels and is particularly important in maintaining the health of the endothelium in the kidney, liver, and brain (Esser et al. 1998). VEGF induces nitric oxide and vasodilatory prostacyclins in endothelial cells suggesting a physiological role in decreasing vascular tone and blood pressure (He et al. 1999). A 50% reduction of glomerular VEGF leads to glomerular endotheliosis and proteinuria similar to that seen in preeclampsia (Eremina et al. 2003) and VEGF has been shown to ameliorate cyclosporine-related hypertension, endothelial dysfunction, and nephropathy (Kang et al. 2001). Moreover, cancer patients receiving VEGF inhibitors therapeutically present hypertension and proteinuria along with glomerular damage as adverse effects that resembling preeclampsia (Zhu et al. 2007; Patel et al. 2008). Collectively these data suggest that VEGF is important not only in blood pressure regulation but also in maintaining the glomerular filtration barrier. Thus antagonism of VEGF signaling, such as with an excess of sFlt-1, may lead to endothelial dysfunction, proteinuria, and hypertension.

It is likely that other factors elaborated by the placenta act synergistically with sFlt-1. This is supported by the observation that while overexpression of sFlt-1 in rats reproduced the hallmarks of the disease, the severe phenotype was not reproduced. The search for additional factors identified soluble endoglin (sEng) as another antiangiogenic molecule deregulated in preeclampsia.

sEng is a truncated form of endoglin generated by shedding of the extracellular domain. Endoglin is a co-receptor for transforming growth factor (TGF)-β1 and 3 (Cheifetz et al. 1992) and is highly expressed on cell membranes of vascular endothelium and syncytiotrophoblasts (Gougos et al. 1992). sEng interferes with TGF-β signaling and eNOS activation and thereby causes endothelial dysfunction (Toporsian et al. 2005; Venkatesha et al. 2006). When overexpressed by adenoviral vector in rats, sEng causes proteinuria and hypertension that are milder then seen with sFlt-1 overexpression alone. However, when sEng is co-expressed with sFlt-1, the rats develop severe proteinuria, hypertension, and IUGR of the pups, as well as hemolysis, elevated liver enzymes, and low platelets similar to the HELLP syndrome. Placental endoglin is up-regulated in preeclampsia, releasing sEng to the maternal circulation (Venkatesha et al. 2006). Clinically, it was shown to be increased in the sera of preeclamptic women 2–3 months before the onset of the clinical signs of preeclampsia and to correlate with disease severity (Levine et al. 2006). Several unanswered questions remain related to the role of sFlt-1 and sEng in the pathogenesis of preeclampsia. The exact role of sFlt-1 and sEng in normal placental development and in placental pseudovascularogenesis is not clear.

Angiogenic Factors in Preeclampsia

Considerable evidence suggests that preeclampsia predisposes women to late cardiac and vascular diseases (Funai et al. 2005; Irgens et al. 2001). Whether preeclampsia per se is a cause of future hypertension at heart disease, or if this represents the sequestration of women predestined to these disorders into the preeclamptic population remains unknown. Recent study of cardiovascular risk factors present before and after pregnancy suggests that nearly half of the elevated risk for future hypertension after preeclampsia can be explained by prepregnancy risk factors (Romundstad et al. 2010). Therefore, pregnancy may be viewed as a stress test that can reveal subclinical cardiovascular disease phenotypes long before overt disease. It is also tempting to speculate that the long-term cardiovascular complications noted in some patients who have had preeclampsia may be due to a chronic antiangiogenic state resulting...
from polymorphisms in genes such as sFlt-1. Additionally, patients with preeclampsia are said to have a decreased long-term incidence of malignancy (Vatten et al. 2002), a provocative observation disputed by some (Mogren et al. 2001), which may suggest that the antiangiogenic state of preeclampsia may reflect a more permanent maternal milieu.

INTRAUTERINE GROWTH RESTRICTION

Normal fetal growth is a complex interplay of fetal, maternal, and placental health. An abnormality in any of these three systems leads to small for gestational age (SGA) babies. SGA babies that arise as a result of placental vascular insufficiency are referred to as IUGR. Although IUGR is frequently associated with severe premature preeclampsia, IUGR can also occur in the absence of any evidence of preeclampsia. Low birth weight in IUGR babies has also been suggested as a predisposing factor for increased hypertension and cardiovascular diseases later in life (Baschat and Hecher 2004). Normal pregnancy results in a significant increase in diastolic blood flow velocity as a result of physiological maternal vascular remodeling. In pregnancies complicated by IUGR there is severe placental vascular insufficiency and the Doppler waveforms are similar to the nonpregnant state (Harrington et al. 1996). This can be shown by several methods including Doppler ultrasound and MRI imaging of the uterine vessels. Similar to preeclampsia, placental bed biopsies in human IUGR show impaired migration of the extravillous trophoblasts and remodeling of the maternal spiral arterioles (Brosens et al. 1972). It is still unclear why some patients with IUGR have the full-blown maternal syndrome of preeclampsia, while others do not. Human IUGR placentas, but not preeclamptic placentas, have also been found to have defective villous capillarization (Mayhew et al. 2004). The morphology of the trophoblast covering these diseased villi are also abnormal with reduced amounts of proliferating villous cytotrophoblasts, and an apoptotic syncytiotrophoblast. It is believed that less oxygen extraction and consumption by the IUGR fetus and the resulting higher levels of pO2 have been hypothesized to inhibit the fetoplacental angiogenesis (Chaddha et al. 2004). However, studies documenting lack of relative hypoxia in IUGR placentas (without preeclampsia) are still lacking. Impairment of insulin-like growth factor (IGF) I and II signaling pathways in experimental animals leads to IUGR (DeChiara et al. 1990; Baker et al. 1993; Crosse et al. 2002). Two babies with IUGR were reported to have mutations of the IGF-1R gene (Abuzzahab et al. 2003). Although abnormalities in IGF signaling are a relatively uncommon cause of IUGR, impaired IGF-II signaling through IGF1R is the first molecular abnormality that has been reported in this complex disease. IGFs are potent proangiogenic molecules both in vitro and in vivo (Herr et al. 2003; Lopez-Lopez et al. 2004; Rabinovsky and Draghia-Akli 2004). Abnormalities in other angiogenic proteins have been shown in small clinical studies, but definitive pathogenic roles for these factors have not been demonstrated so far. Serum concentrations of sFlt-1 have been found to be increased modestly in patients with intrauterine growth retardation without preeclampsia (Tsatsaris et al. 2003), a finding that has not been confirmed by others (Shibata et al. 2005). Endostatin concentrations in the fetus and neonate were reported to be lower in pregnancies complicated by IUGR (Malamitsi-Puchner et al. 2005). Leptin and asymmetric dimethylarginine have also been reported to be elevated during placental insufficiency (Leperc et al. 2003; Savvidou et al. 2003), but the cause-and-effect relationship of these molecules has not yet been established.

CONCLUSIONS

Normal placental development is characterized by vasculogenesis, angiogenesis, and pseudovasculogenesis, which are tightly regulated by a balance between pro- and antiangiogenic factors. Abnormalities in this regulation may lead to adverse obstetric outcomes such as preeclampsia and IUGR and also compromise adult health. Preeclampsia is characterized by...
an increased placental production of antiangiogenic molecules such as sFlt-1 and sEng which are implicated in the pathogenesis of the disease. Several recent clinical studies suggest that angiogenic biomarkers such as sFlt-1 and PLGF may serve to diagnose and predict preeclampsia and its related complications. In addition, depleting or antagonizing sFlt-1 and sEng in the maternal circulation may prove to be a valuable approach for preeclampsia treatment. Understanding the mechanisms that regulate placental vascular development might allow the development of new preventive strategies for pregnancy complications.

REFERENCES


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