Vasculogenesis and Angiogenesis in the IUGR Placenta

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Placenta vascular formation is important for fetal growth and development. Proper development of the placenta ensures the exchange of oxygen/nutrients and blood flow necessary for fetal growth. In this chapter, we will discuss the processes of vasculogenesis, angiogenesis, and pseudovasculogenesis during placental development and in pregnancies complicated by intrauterine growth restriction. Some of the factors controlling these processes include oxygen, the VEGF family of growth factors, and their receptors. Disruption in the balance of these controlling factors may explain the vascular malformations seen in pregnancies complicated by intrauterine growth restriction.

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The human placenta is a highly invasive and proliferative structure during the first half of pregnancy. While the growth rate of the human placenta decreases, its maturation continues throughout gestation. In contrast, fetal growth is largely exponential throughout gestation. In fact, the fetal/placental ratio increases markedly as gestation advances, suggesting that placental development precedes fetal development. The placenta is responsible for the exchange of oxygen and nutrients from the mother to the fetus. Normal placentation and placental development are critical for a successful pregnancy and mediate important steps, such as implantation, immune protection of the fetus, maternal blood flow to the placenta, and delivery of nutrients to the fetus. Placental dysfunction is known to be a major cause of pregnancy complications, such as intrauterine growth restriction (IUGR).1,2 Placentae of growth-restricted pregnancies are characterized by a number of pathologic findings, such as reduced syncytiotrophoblast surface area, increased thickness of the exchange barrier formed by the trophoblast and fetal capillary endothelium, and an increase in placental apoptosis.3-10 This suggests that fetal growth and development are largely dependent on adequate placental development and function.

One important part of placental development is the formation of an adequate vascular system during gestation to meet the demands of the growing fetus. In this chapter, we will discuss the processes of vasculogenesis, angiogenesis, and pseudovasculogenesis during normal pregnancies and pregnancies affected by IUGR.

Vasculogenesis and Angiogenesis

Blood vessel formation develops through two different processes, namely vasculogenesis and angiogenesis.11 The process of vasculogenesis involves the de novo formation of blood vessels from precursor cells, whereas angiogenesis involves the creation of new vessels from already existing ones.12 During vasculogenesis, hemangiogenic stem cells (derived from mesenchymal cells) differentiate to hemangioblastic stem cells, giving rise to angioblastic cells. These cells in turn differentiate into endothelial cells forming new vascular networks.11-14 The formation of these networks is important for organogenesis during fetal development. Signals that regulate the organization of endothelial cells into tubal networks involve complex processes with a number of factors identified thus far.12 Shortly after the endothelial tubes are formed, they become associated with pericytes (vascular smooth muscle cells) derived from the mesenchyme surrounding the endothelium.15,16 These pericytes then proliferate and migrate, coating the endothelial cell tubes and forming the vessels.15,17,18
In contrast to vasculogenesis, angiogenesis can be accomplished by either the migration of endothelial cells from preexisting vessels through the sprouting of endothelial cells (sprouting angiogenesis) or by the elongation or increase in length of the existing vessels (non-sprouting angiogenesis). Angiogenesis is normally observed during wound healing and in the ovary and endometrium during the menstrual cycle. Aberrant angiogenesis has been identified in several clinical conditions, such as rheumatoid arthritis, chronic inflammation, cancer, and diabetes. Both processes (vasculogenesis and angiogenesis) are critical to form a vascular system needed for effective transport of nutrients, oxygen, and waste products. Several factors have been identified as important regulators of these processes, including basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), angiopoietins-1 and -2 (Ang-1 and Ang-2), and the vascular endothelial growth factor (VEGF) family of proteins.

The VEGF proteins are the most studied family of growth factors known to regulate the processes of vasculogenesis and angiogenesis. The VEGF family consists of seven proteins designated VEGF-A (also known as VEGF) to VEGF-F and Placenta Growth Factor (PIGF). These growth factors are known to induce signal transduction by binding to their specific transmembrane receptors. Receptors for the VEGF family include VEGFR-1 (flt-1), VEGFR-2 (KDR), and VEGFR-3 (flt-4). VEGF-C and VEGF-D bind VEGFR-3 regulating lymphangiogenesis and will not be discussed further. VEGF-A binds to both VEGFR-1 and VEGFR-2, whereas PIGF only binds to the VEGFR-1 receptor. The VEGF family of proteins also bind to the neuropilin receptors (NP-1 and NP-2), resulting in various downstream activations, which is further described in articles by Otrock and coworkers and TAvoid and coworkers. It is important to note that VEGFR-1 also has a soluble form (sVEGFR-1), which contributes to both physiologic and pathologic conditions by blocking the normal function of the VEGF and PIGF growth factors.

When these growth factors bind to their transmembrane receptors, a series of downstream proteins are activated, which induce endothelial cell proliferation, migration, and tube formation. VEGF-A, aside from being a potent endothelial survival factor, is also known to induce vasodilation by increasing nitric oxide (NO) production, another function which facilitates blood flow. Whereas PIGF shares many of the same functions as VEGF, expression of PIGF is primarily restricted to the trophoblast cells in the placenta, suggesting a role for this growth factor in placental development.

**Placental Vasculogenesis and Angiogenesis**

Both vasculogenesis and angiogenesis are critical for normal placental development in the human. It is important to appreciate that the maternal and fetal vascular systems do not connect and that their development, although coordinated, occurs separately. In this chapter, we will discuss the processes of fetal vasculogenesis and angiogenesis as well as maternal vascular remodeling. In the early placenta, the mesenchymal tissue is located centrally within the villous core and surrounded by the villous trophoblasts. Vasculogenesis is evident by about 21 days post conception. At this point that mesenchymal cells inside the villi transform into hemangiogenic precursor cells. These precursor cells then migrate toward the periphery and develop into hemangioblastic cell cords, which will ultimately form the first vessels within the villus. By about 28 dpc, a defined lumen is observed, and by 32 dpc, erythrocytes can be observed within the vascular lumen. Vasculogenesis is regulated by growth factors present in the placenta. The human placenta is known to express VEGF-A (VEGF), PIGF, VEGF-B, and VEGF-C isoforms from the VEGF family of proteins.

Over gestation, PIGF increases, whereas there is a decrease in VEGF-A. Expression of VEGF-A is high in the cytotrophoblast cells in the early placenta and is known to be responsible for the formation of the hemangioblastic cords from the precursor cells. In fact, studies in VEGF knockout mice demonstrate a lack of precursor cell differentiation into endothelial cells during vasculogenesis, impaired differentiation of blood islands, as well as impaired sprouting angiogenesis, suggesting an important role for this growth factor during placental development. The villi start to mature as vasculogenesis continues and angiogenesis begins.

Vascular growth is necessary to increase placental fetal blood flow over gestation. Placental vascular growth through angiogenesis begins around 32 dpc within the villi. Angiogenesis during pregnancy can be separated into different periods. The first period starts around 32 dpc until about 24 weeks of pregnancy. This period includes the formation of capillary networks by branching angiogenesis. During the branching angiogenesis, prevascular networks form in response to growth factors derived from the cytotrophoblasts, such as VEGF-A. In this process, the endothelial tube segments formed by vasculogenesis are extended into primitive capillary networks by two mechanisms: elongation of preexisting tubes and lateral ramification of these tubes (sprouting angiogenesis). Sprouting angiogenesis is a multistep process composed of: (1) increased vascular permeability, (2) degradation of basement membrane, (3) increase in endothelial cell proliferation and migration, (4) formation of endothelial cell tubes, and (5) recruitment of pericytes to the outside of the capillary to form a stable vessel. At this point, there is an increase in the expression of VEGF-A produced by the trophoblast, whereas PIGF expression is low. As pregnancy progresses, the branching angiogenesis is replaced by nonbranching angiogenesis. Nonbranching angiogenesis is observed from 24 weeks of gestation to term. During the nonbranching angiogenesis, there is decreased trophoblast proliferation, increased endothelial cell proliferation, a dramatic growth of the terminal villi, and an increase in PIGF and sVEGFR-1 levels with a decrease in VEGF-A expression.

Both VEGF and PIGF are regulated by metabolic changes such as hypoxia. Hypoxia experiments demonstrate an increase in VEGF expression, whereas PIGF expression de-
creases. Before 8 weeks of gestation, the pO₂ in the intervillous space is less than 12 mm Hg, but this pO₂ increases to greater than 50 mm Hg after 12 weeks of gestation. The maximum pO₂ is reached at about 16 weeks of gestation (60 mm Hg) but starts to gradually decline until term (45 mm Hg).

During pregnancy, the trophoblast cells remodel the already existing maternal uterine vessels through a process called pseudovasculogenesis. Early in gestation (less than 6 weeks of gestation), the uterine spiral arteries are characterized as high-resistance and low-capacity vessels (Fig. 2A). Endovascular cytotrophoblasts invade, breakdown the smooth muscle cells, and acquire characteristics resembling the maternal endothelial cells they ultimately replace. This process modifies these spiral arteries to high-capacitance and low-resistance vessels (Fig. 2B). Although this process also occurs to a limited extent on the venous side of the maternal circulation, it is remarkably directed to the arterial side. On the arterial side, this remodeling extends the entire distance of the decidua and into the myometrium, where the vessel transitions from being composed of fetal to maternal cells. This remodeling is completed around 20 weeks of gestation and results in an increase in the uteroplacental perfusion to meet the requirements of the developing fetus.

### Placental Vasculogenesis and Angiogenesis During IUGR

Poor vascular development is known to cause intrauterine embryonic death characterized by low vascular density in the placental villi along with fibrosis and other deficiencies. Aberrant angiogenesis is also associated with the development of compromised pregnancies, such as IUGR. IUGR is a disease responsible for neonatal morbidity and mortality affecting 8% of all pregnancies. This disease is characterized by hypoxia, asphyxia, and intrauterine demise. Studies by
Chen and coworkers showed a significant decrease in villi vascular density within the IUGR placentae, suggesting a decrease in branching angiogenesis during this disease. Other reports have shown a decrease in surface area, volume, and number of terminal villi as well as a reduced number of capillaries in the stroma of IUGR placentae as compared with placentae from normal pregnancies. These findings suggest that aberrant vascular formation could be a factor associated with the increased umbilical blood flow resistance observed during IUGR. Although the exact causes of the abnormal vascularization during IUGR are not known, deficiencies in angiogenic growth factors have been demonstrated during this disease. For example, VEGF-A immunostaining is decreased in the villi of IUGR placentae, whereas PI GF staining is increased when compared with controls. Interestingly, mouse experiments have shown that inhibition of VEGF-A can induce IUGR in pregnant mice, whereas loss of PI GF did not. However, more recently, a decrease in PI GF but not VEGF in maternal and umbilical serum levels was noted in human IUGR at time of delivery. These apparently conflicting results will require further investigation to clarify. However, an alteration in levels of these two important angiogenic growth factors could be important in controlling vascular development in the IUGR placentae.

Summary

Vasculogenesis and angiogenesis are essential for proper placental development. Adequate vasculogenesis and angiogenesis support the required blood flow on the fetal side necessary for fetal growth and development. Additionally, adequate pseudovasculogenesis of the maternal vasculature is important for providing adequate maternal blood flow to the placenta. Abnormal vasculogenesis, angiogenesis, and pseudovasculogenesis are correlated with impaired placental and fetal development seen in complicated pregnancies, such as IUGR. The findings discussed in this chapter suggest that the balance between various angiogenic (such as VEGF-A and PI GF) and antiangiogenic factors (such as sVEGFR-1) can influence placental vascular development. Although the processes of angiogenesis, vasculogenesis, and pseudovasculogenesis have been extensively studied, a greater understanding of these processes in placental development is needed to develop rational therapeutic strategies that target these processes in an attempt to improve nutrient delivery in complicated pregnancies like IUGR.

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