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Angiogenic Factors and Natural Killer (NK) Cells in the Pathogenesis of Preeclampsia

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Abstract

Preeclampsia is a pregnancy-specific complex disease in which numerous genetic, immunological and environmental factors interact. Characterized by new onset hypertension, proteinuria and edema after 20 weeks of gestation, preeclampsia is often complicated by small-for-gestational-age (SGA) babies and preterm delivery, and is therefore a significant cause of maternal and fetal morbidity and mortality. The only definitive treatment of preeclampsia is delivery of the placenta. Recent data suggest that the anti-angiogenic state induced by excess circulating anti-angiogenic factors of placental origin may be responsible for the clinical signs and symptoms of preeclampsia. Natural killer (NK) cells at the maternal/fetal interface, which are thought to play an important role in normal placental development, have been noted recently to induce angiogenic factors and vascular remodeling. Moreover, genetic studies suggest that susceptibility to preeclampsia may be influenced by polymorphic HLA-C ligands and killer cell receptors (KIR) present on NK cells. This review summarizes our current understanding of the role of angiogenic factors and NK cells in the pathogenesis of preeclampsia.

Keywords

Angiogenesis; proteinuria; glomerular endotheliosis; sVEGFR1; hypertension; endoglin; VEGF; PlGF; NK cells; pregnancy

Introduction

Preeclampsia is the most frequently encountered medical complication of pregnancy. It is characterized by the new onset of hypertension and proteinuria, usually during the last trimester of pregnancy, and is commonly associated with edema and hyperuricemia (Karumanchi et al., 2005; Sibai et al., 2005). The placenta plays a central role in the pathogenesis of preeclampsia, as evidenced by the rapid disappearance of clinical signs and symptoms of preeclampsia following delivery of the placenta. Preeclampsia is characterized by widespread systemic vascular endothelial dysfunction and microangiopathy in the mother, but not in the fetus (Karumanchi et al., 2005). Severe preeclampsia may be associated with small-for-gestational-

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age (SGA) fetuses that is probably related to severe placental vascular insufficiency. The predominant target organ may be the brain (seizures or eclampsia), the liver (HELLP syndrome) or the kidney (glomerular endotheliosis and proteinuria). It is currently believed that preeclampsia has its origin in abnormal vascular development of the placenta which, in turn, leads to widespread maternal endothelial effects (Fisher, 2004). In this review, we summarize the role of angiogenic factors and natural killer cells - two distinct biological mechanisms that may be related in the pathogenesis of preeclampsia.

Angiogenic factors in the pathogenesis of preeclampsia

Angiogenesis is the process of neovascularization from pre-existing blood vessels, while vasculogenesis is the process of blood vessel generation from angioblast precursor cells. The human placenta undergoes high levels of both angiogenesis and vasculogenesis during fetal development (Demir et al., 1989). Additionally, the human placenta undergoes a process of vascular mimicry (also referred to as pseudovasculogenesis) where placental cytotrophoblasts convert from an epithelial to endothelial phenotype during normal placental development (Zhou et al., 1997b). The major pathological abnormality in the preeclamptic placenta is insufficient maternal spiral artery remodeling. The decrease in remodeling of uterine spiral arterioles is thought to be due to defective endovascular invasion by cytotrophoblasts and impaired pseudovasculogenesis (Red-Horse et al., 2004; Zhou et al., 1997a). The hypothesis that defective trophoblastic invasion with accompanying utero-placental hypoperfusion may lead to preeclampsia is supported by both animal and human studies. Pathologic examination of placentas from pregnancies with advanced preeclampsia often reveals numerous placental infarctions and sclerotic narrowing of arterioles (Moldenhauer et al., 2003). In one-third to one-half of preeclamptic women, however, these changes are not pronounced. Ultrasound estimation of utero-placental blood flow is usually diminished and uterine vascular resistance is usually increased, in preeclamptic women prior to clinical symptoms (Harrington et al., 1996). Placental ischemia induced by mechanical constriction of the uterine arteries or aorta produces hypertension, proteinuria and, variably, glomerular endotheliosis in several animal species (Podjarny et al., 1999). However, placental ischemia alone, as in intrauterine growth restriction, does not appear sufficient to produce preeclampsia. Thus, although uteroplacental ischemia is an important trigger of preeclampsia, the response to this ischemia – either at the placental or the maternal systemic level - must be variable.

The list of pro- and anti-angiogenic molecules thought to play a role in normal placental vascular development is expanding exponentially, and has been reviewed in detail elsewhere (Zygmunt et al., 2003), but the family of vascular endothelial growth factor (VEGF) gene products has been the most extensively studied. VEGF-A is an endothelial-specific mitogen and a survival factor that exists in 4 isoforms: VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆. The high-affinity receptor tyrosine kinases for VEGF-A include Flt1 (also referred to as VEGFR1) and Kinase-insert Domain-containing Receptor (KDR, human/Flk1, murine), also known as VEGFR2 (Shibuya, 2001). KDR mediates the major growth and permeability actions of VEGF, whereas Flt1 may have a negative role, either by acting as a decoy receptor or by suppressing signaling through KDR. Placental growth factor (PlGF) was the first VEGF relative identified to be abundantly expressed in the placenta and it acts by binding to Flt1 but not KDR (Persico et al., 1999). VEGF-C and VEGF-D, based on their ability to bind the lymphatic-specific Flt3 receptor (also known as VEGFR3), seem to be important for lymphatic development (Jussila and Alitalo, 2002; Olofsson et al., 1999). Alternative splicing of Flt1 results in production of an endogenously secreted anti-angiogenic protein referred to as sFlt1, which lacks the cytoplasmic and transmembrane domain but retains the ligand-binding domain (Kendall and Thomas, 1993). Thus, sFlt1 can antagonize VEGF and PlGF by binding to them and preventing interaction with their endogenous membrane receptors (He et al., 1999; Kendall and Thomas, 1993).

Recently, gene expression profiling has been used to search for candidate factors produced by the placenta in preeclampsia. Using this approach, it has been found that mRNA message for sFlt1 (soluble FMS-like tyrosine kinase1) is upregulated in preeclamptic patients (Maynard et al., 2003). Circulating in blood, it acts as a potent antagonist to VEGF and (PlGF). Both VEGF and PlGF are made by the placenta and circulate at high concentration during pregnancy. Circulating sFlt1 levels are greatly increased in women with established preeclampsia and prior to onset of clinical symptoms (Chaiworapongsa et al., 2004, 2005; Hertig et al., 2004; Koga et al., 2003; Levine et al., 2004; Maynard et al., 2003). Consistent with the action of the circulating protein to bind PlGF, free (or unbound) PlGF levels are decreased in preeclamptic women well before the onset of clinical symptoms (Levine et al., 2004). When administered to pregnant and non-pregnant rats, sFlt1 produces a syndrome of hypertension, proteinuria and glomerular endotheliosis resembling the human syndrome of preeclampsia. The glomerular lesion in these experimental animals, consisting of severe glomerular endothelial swelling along with preserved foot processes in association with heavy proteinuria, is striking in its resemblance to the human counterpart of preeclampsia (Maynard et al., 2003). The organs targeted in preeclampsia (such as the glomerulus or hepatic sinusoids) have fenestrated endothelia. It has been shown that VEGF induces endothelial fenestrae *in vitro* and the loss of even 50% decrease in VEGF production in the glomerulus in mice leads not only to glomerular endotheliosis but also to loss of glomerular endothelial fenestrae (Eremina et al., 2003). The use of VEGF inhibitors in some cancer patients may not only induce hypertension and proteinuria but, occasionally, produce reversible posterior leukoencephalopathy – a syndrome characteristic of patients with eclampsia (Glusker et al., 2006; Hinchey et al., 1996; Yang et al., 2003). Furthermore, exogenous VEGF/PlGF or an antibody against sFlt1 can reverse the anti-angiogenic effects of preeclamptic blood, as assessed by studies of angiogenesis *in vitro* (Ahmad and Ahmed, 2004; Maynard et al., 2003). Thus, the anti-angiogenic effects of sFlt1 may account for many of the manifestations of preeclampsia, including the unique glomerular changes. Several risk factors for development of preeclampsia can also be explained by increases in sFlt1 levels. These include multi-gestational pregnancies, high altitude pregnancies, trisomy 13, and nulliparity (Bdolah et al., 2006a,b; Nevo et al., 2006; Wolf et al., 2005). Furthermore a decrease in circulating sFlt1 levels amongst smokers may explain the decreased incidence of preeclampsia in this subgroup (Levine et al., 2006; Powers et al., 2005).

Another soluble anti-angiogenic factor secreted by the placenta that appears elevated in women with preeclampsia is soluble endoglin (sEng) (Venkatesha et al., 2006). Endoglin (Eng) is an angiogenic receptor expressed mainly on the surface of endothelial cells, but also placental syncytiotrophoblasts (Cheifetz et al., 1992; Gougos et al., 1992; St-Jacques et al., 1994). Eng acts as a co-receptor for transforming growth factor-beta (TGF-beta), a potent pro-angiogenic molecule, signaling in endothelial cells. Eng mRNA is up-regulated in the preeclamptic placenta (Venkatesha et al., 2006). Moreover, the extra-cellular region of endoglin is proteolytically cleaved and sEng released in excess quantities into the circulation of preeclamptic patients. In pregnant rats, sEng appeared to exacerbate the vascular damage mediated by sFlt1, resulting in severe preeclampsia-like illness including the development of HELLP syndrome and fetal growth restriction (Venkatesha et al., 2006). The precise role of these molecules during pregnancy and placentation is unclear, but there is evidence that Eng via TGF-beta may play a role in the hypothesized contribution of placental hypoxia/ischemia to the pathogenesis of preeclampsia. It has been speculated that sEng is produced by the placenta a compensatory mechanism to limit the effects of surface endoglin. In preeclampsia, excessive production of surface endoglin leads to increased sEng in maternal circulation, which together with sFlt1, may be responsible for the clinical manifestations of preeclampsia. More recently, in clinical studies, sEng appears to be elevated not only during the disease but also before onset of symptoms (Levine et al., 2006). Elevations in sEng were particularly pronounced – therefore, potentially most useful for prediction - in women who developed pre-

term preeclampsia or preeclampsia with a SGA infant. Although the gestational pattern of sEng concentration tended to parallel the trajectory of the sFlt1/PlGF ratio, multivariate analysis indicated that each was associated with preeclampsia. Indeed, a composite measure incorporating all three molecules, (sFlt1 and sEng) / PlGF, was more strongly predictive of preeclampsia than the individual biomarkers (Levine et al., 2006).

NK cells during normal pregnancy and in preeclampsia

Natural killer (NK) cells are lymphocytes characterized by high cytolytic potential against virus-infected and tumor-transformed cells. They are regulated by the balance of signals emanating from multiple activating and inhibitory receptors whose expression and specificity does not require genetic rearrangements. NK cells are thus part of the innate immune system (Orange et al., 2002). NK cell activation can lead to cytokine secretion or cytotoxicity of targets presenting ligands for NK cell activating receptors. Damage of healthy host cells by NK cells is prevented by engagement of inhibitory receptors with host classical MHC class I molecules. Expression of NK cell receptors is not uniform among the NK cells of an individual. Different NK cell subsets express different combinations of activating and inhibitory receptors, generating a broad range of NK cell specificities (Parham, 2005).

During the first trimester of human pregnancy NK cells are a major cell population at the maternal-fetal interface, accounting for 70% of the local lymphocytes (Moffett-King, 2002). In contrast, peripheral blood NK cells (pNK) account for only up to 15% of circulating lymphocytes. The abundance of NK cells in decidua has prompted the idea that these cells might play a role in pregnancy support and maintenance (Moffett-King, 2002). Initial evidence in this direction came from studies involving NK-cell deficient mice. In mice uterine NK cells concentrate in a pregnancy-induced region of the uterine musculature called the metrial gland (Peel, 1989). A murine transgenic strain, *tge26*, carrying high copy numbers of the human CD3 ϵ gene, is deficient in NK and T cells (Wang et al., 1994). *tge26* females fail to develop metrial glands at implantation sites, present abnormal decidual cellularity and thickened blood vessel walls in the decidua (Guimond et al., 1996, 1997). About 50% litter lethality by mid-gestation correlates with the manifestation of these pathologies. Interestingly, engraftment of *tge26* females with scid/scid bone marrow, which contributes to the development of NK cells but not to the development of T-cells, results in restoration of uterine NK cell populations, development of metrial glands, fetal viability and reduction of decidual abnormalities including decidual blood vessels anomalies (Guimond et al., 1998).

Murine uterine NK cells seem to promote decidual artery modifications through secretion of interferon-gamma (IFN- γ) (Ashkar et al., 2000). Double knock-out mice for a lymphoid recombining activating gene (RAG-2 $^{-/-}$) and common cytokine receptor chain γ ($\gamma_c^{-/-}$) lack all lymphocyte populations including uterine NK cells. RAG-2 $^{-/-}$ $\gamma_c^{-/-}$ mice breed but present similar decidual and decidual vessel anomalies as *tge26* mice (Ashkar et al., 2000). Bone marrow engraftment from wild type C57BL6 or scid/scid mice into RAG-2 $^{-/-}$ $\gamma_c^{-/-}$ female recipients establishes uterine NK cell populations and reverses host vascular and decidual pathologies during pregnancy. However bone marrow engraftment from IFN- $\gamma^{-/-}$ mice, while also establishing uterine NK cell populations, fails to correct vascular anomalies. Moreover, injection of recombinant IFN- γ to RAG-2 $^{-/-}$ $\gamma_c^{-/-}$ pregnant females results in decidual vessel modification in the absence of uterine NK cells (Guimond et al., 1998). Interleukin 15 (IL-15) knock-out mice, a third strain deficient in NK cells, present similar decidual anomalies, lacking decidual arteries modifications and decidual integrity (Ashkar et al., 2003; Barber and Pollard, 2003). Thus, in mice, it appears that uterine NK cells are involved in promoting placental development and decidual vessel modifications.

In humans, NK cells are disseminated throughout the decidua and represent 30% of all the decidual cells towards the end of the first trimester of pregnancy. Human decidual NK (dNK) cells are phenotypically and functionally different from pNK cells. Human NK cells are CD3 – CD56+. Two main subsets of pNK cells have been identified. The prominent CD56^{dim} (~95%) and the less abundant CD56^{bright} (~5%). CD56^{dim} pNK cells express CD16 and Killer cell Immunoglobulin-like Receptors (KIRs), are granular and cytotoxic. CD56^{bright} pNK play a regulatory role secreting cytokines, they do not express CD16 or KIRs, are devoid of lytic granules and are not cytotoxic (Cooper et al., 2001a,b). They express L-selectin (CD62L), which is not expressed by CD56^{dim} pNK cells (Frey et al., 1998; Koopman et al., 2003). Decidual NK cells are similar to CD56^{bright} pNK cells in terms of CD56 and CD16 expression; however, like CD56^{dim} pNK cells dNK cells express KIRs and have granules with cytolytic content, i.e. perforin and granzymes (Koopman et al., 2003; Kopcow et al., 2005). They express CD9 which is not expressed by any of the peripheral subsets. Gene expression profiling studies evaluating a large number of genes revealed that dNK cells constitute a distinct subset from the two pNK cell types, expressing a number of immunosuppressive and angiogenic genes (Hanna et al., 2006; Koopman et al., 2003). Functionally, dNK cells have severely reduced cytotoxicity compared to pNK cells, despite presenting cytolytic granules and expressing activating receptor known to trigger cytotoxicity by CD56^{dim} pNK cells (Kopcow et al., 2005).

In preeclampsia, the degree and depth of spiral artery modifications are reduced compared to normal pregnancies, resulting in diminished blood perfusion and increased maternal blood pressure. It is believed that decidual NK cells play a role in preeclampsia pathogenesis as certain combinations of maternal KIR (receptors expressed on NK cells) and paternal HLA haplotypes have been associated with the disease (Hiby et al., 2004). A study analyzing KIR and HLA–C genotypes of 200 pregnant women with normal pregnancies and 201 pregnant women with preeclampsia revealed preeclampsia to be prevalent in AA women, thus devoid of all or most activating KIRs (Hiby et al., 2004). In relation to KIR specificity, HLA-C alleles can be classified into two groups, C1 or C2, depending on the residue that occupies position 80 of the α 1 domain (Colonna et al., 1993). C1 allotypes have asparagine at position 80 and are ligands of inhibitory KIR2DL2 and KIR2DL3. C2 allotypes have lysine in position 80 and are ligands of inhibitory KIR2DL1 and activating KIR2DS1. C2 allotypes are stronger ligands than C1 allotypes (Winter et al., 1998). Interestingly, preeclampsia prevalence in KIR AA females was due exclusively to pregnancies in which the fetus had at least one C2 allele (Hiby et al., 2004). Engagement of HLA-C2 would trigger stronger inhibitory signals on NK cells than engagement of HLA-C1 that cannot be counterbalanced by activating KIR2DS1 in AA females as the activating receptor is not expressed. Analysis of various human populations revealed an inverted correlation between the frequency of KIR AA and HLA-C2 haplotypes, in agreement with preeclampsia selecting against the coexistence of the two genotypes (Hiby et al., 2004).

Based on these observations, a hypothesis was formulated indicating that, in normal pregnancies, dNK cell activation through interaction with HLA-C on extravillous trophoblasts would promote placental development and maternal decidual spiral artery modifications by extravillous cytotrophoblasts. Insufficient dNK cell activation would halt this process prematurely, resulting in poor decidual artery remodeling and increasing the risk of preeclampsia (Hiby et al., 2004; Parham, 2004).

Recent evidence showing secretion of trophoblast migration-promoting factors and angiogenic factors by human dNK cells upon activation support this hypothesis (Hanna et al., 2006). *Ex vivo* human dNK cells were shown to express interleukin-8 (IL-8) and interferon-inducible protein-10 (IP-10), two trophoblast invasion-promoting cytokines. Indeed, a block in trophoblast trophoblast migration was demonstrated by the addition of anti-IL-8 and anti-IP-10 monoclonal antibodies in culture (Hanna et al., 2006). *Ex vivo* dNK cells were shown also to

express angiogenic and endothelial cell mitogenic factors, including VEGF and PlGF. IL-15-stimulated dNK cells secreted these molecules at higher levels than IL-15-stimulated pNK cells. Media conditioned by IL-15-stimulated dNK cells, but not media conditioned by IL-15-stimulated pNK cells, were able to promote human umbilical vascular endothelial cell (HUVEC) migration and formation of accelerated network-like structures *in vitro*. Both effects were partially inhibited by Flt1-Fc fusion protein which blocks VEGF and PlGF signaling. Subcutaneous co-injection of human dNK cells, but not of pNK cells, with JEG-3 choriocarcinoma cells into nude mice promoted JEG-3 tumor growth and vascularization (Hanna et al., 2006). These data are consistent with the hypothesis that decidual NK cells secrete cytokines and angiogenic factors that play an important role during normal placental vascular remodeling and differentiation.

Conclusions

Current evidence suggests that the clinical signs and symptoms of preeclampsia may be mediated by excess circulating anti-angiogenic factors of placental origin. The increase in circulating anti-angiogenic factors is likely due to abnormal placental vascular remodeling and accompanying placental ischemia. Numerous hypotheses, such as genetic factors, hypoxia and immunological factors, have been suggested to be responsible for the primary placental vascular defect noted in preeclampsia. Recent data suggest that abnormal NK cell signaling at the maternal-fetal interface may be the culprit. More work is needed to define better the role of NK cells and angiogenic factors during normal and complicated pregnancies.

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References

- Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. *Circ Res* 2004;95:884–891. [PubMed: 15472115]
- Ashkar AA, Black GP, Wei Q, He H, Liang L, Head JR, Croy BA. Assessment of requirements for IL-15 and IFN regulatory factors in uterine NK cell differentiation and function during pregnancy. *J Immunol* 2003;171:2937–2944. [PubMed: 12960317]
- Ashkar AA, Di Santo JP, Croy BA. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000;192:259–270. [PubMed: 10899912]
- Barber EM, Pollard JW. The uterine NK cell population requires IL-15 but these cells are not required for pregnancy nor the resolution of a *Listeria monocytogenes* infection. *J Immunol* 2003;171:37–46. [PubMed: 12816981]
- Bdolah Y, Lam C, Rajakumar A, Venkatesha S, Mutter W, Sachs BP, Lim KH, Epstein FH, Karumanchi SA. Preeclampsia in twin pregnancies: Hypoxia or bigger placental mass? (Abstract). *J Soc Gynecol Investig* 2006a;13:A670.
- Bdolah Y, Palomaki GE, Yaron Y, Bdolah-Abram T, Goldman M, Levine RJ, Sachs BP, Haddow JE, Karumanchi SA. Circulating angiogenic proteins in trisomy 13. *Amer J Obstet Gynecol* 2006b; 194:239–245. [PubMed: 16389038]
- Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee Kim Y, Goncalves LF, Gomez R, Edwin S. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. *Amer J Obstet Gynecol* 2004;190:1541–1547. [PubMed: 15284729]discussion 1547-1550
- Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, Bujold E, Goncalves L, Gomez R, Edwin S, Mazor M. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. *J Matern Fetal Neonatal Med* 2005;17:3–18. [PubMed: 15804781]

- Cheifetz S, Bellon T, Cales C, Vera S, Bernabeu C, Massague J, Letarte M. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *J Biol Chem* 1992;267:19027–19030. [PubMed: 1326540]
- Colonna M, Borsellino G, Falco M, Ferrara GB, Strominger JL. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. *Proc Natl Acad Sci USA* 1993;90:12000–12004. [PubMed: 8265660]
- Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001a;22:633–640. [PubMed: 11698225]
- Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaaheri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001b;97:3146–3151. [PubMed: 11342442]
- Demir R, Kaufmann P, Castellucci M, Erbenig T, Kotowski A. Fetal vasculogenesis and angiogenesis in human placental villi. *Acta Anat (Basel)* 1989;136:190–203. [PubMed: 2481376]
- Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 2003;111:707–716. [PubMed: 12618525]
- Fisher SJ. The placental problem: Linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. *Reprod Biol Endocrinol* 2004;2:53. [PubMed: 15236649]
- Frey M, Packianathan NB, Fehniger TA, Ross ME, Wang WC, Stewart CC, Caligiuri MA, Evans SS. Differential expression and function of L-selectin on CD56bright and CD56dim natural killer cell subsets. *J Immunol* 1998;161:400–408. [PubMed: 9647249]
- Glusker P, Recht L, Lane B. Reversible posterior leukoencephalopathy syndrome and bevacizumab. *N Engl J Med* 2006;354:980–982. [PubMed: 16510760]discussion 980-982
- Gougos A, St Jacques S, Greaves A, O'Connell PJ, d'Apice AJ, Buhning HJ, Bernabeu C, van Mourik JA, Letarte M. Identification of distinct epitopes of endoglin, an RGD-containing glycoprotein of endothelial cells, leukemic cells, and syncytiotrophoblasts. *Int Immunol* 1992;4:83–92. [PubMed: 1371694]
- Guimond MJ, Luross JA, Wang B, Terhorst C, Danial S, Croy BA. Absence of natural killer cells during murine pregnancy is associated with reproductive compromise in TgE26 mice. *Biol Reprod* 1997;56:169–179. [PubMed: 9002646]
- Guimond MJ, Wang B, Croy BA. Engraftment of bone marrow from severe combined immunodeficient (SCID) mice reverses the reproductive deficits in natural killer cell-deficient tg epsilon 26 mice. *J Exp Med* 1998;187:217–223. [PubMed: 9432979]
- Guimond MJ, Wang B, Fujita J, Terhorst C, Croy BA. Pregnancy-associated uterine granulated metrial gland cells in mutant and transgenic mice. *Am J Reprod Immunol* 1996;35:501–509. [PubMed: 8792932]
- Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006;12:1065–1074. [PubMed: 16892062]
- Harrington K, Cooper D, Lees C, Hecher K, Campbell S. Doppler ultrasound of the uterine arteries: the importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby. *Ultrasound Obstet Gynecol* 1996;7:182–188. [PubMed: 8705410]
- He Y, Smith SK, Day KA, Clark DE, Licence DR, Charnock-Jones DS. Alternative splicing of vascular endothelial growth factor (VEGF)-R1 (FLT-1) pre-mRNA is important for the regulation of VEGF activity. *Mol Endocrinol* 1999;13:537–545. [PubMed: 10194760]
- Hertig A, Berkane N, Lefevre G, Toumi K, Marti HP, Capeau J, Uzan S, Rondeau E. Maternal serum sFlt1 concentration is an early and reliable predictive marker of preeclampsia. *Clin Chem* 2004;50:1702–1703. [PubMed: 15331514]
- Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, Moffett A. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* 2004;200:957–965. [PubMed: 15477349]

- Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, Pessin MS, Lamy C, Mas JL, Caplan LR. A reversible posterior leukoencephalopathy syndrome. *N Engl J Med* 1996;334:494–500. [PubMed: 8559202]
- Jussila L, Alitalo K. Vascular growth factors and lymphangiogenesis. *Physiol Rev* 2002;82:673–700. [PubMed: 12087132]
- Karumanchi SA, Maynard SE, Stillman IE, Epstein FH, Sukhatme VP. Preeclampsia: a renal perspective. *Kidney Int* 2005;67:2101–2113. [PubMed: 15882253]
- Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci USA* 1993;90:10705–10709. [PubMed: 8248162]
- Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, Takeda S, Yano T, Tsutsumi O, Taketani Y. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. *J Clin Endocrinol Metab* 2003;88:2348–2351. [PubMed: 12727995]
- Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ, Strominger JL. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003;198:1201–1212. [PubMed: 14568979]
- Kopcow HD, Allan DS, Chen X, Rybalov B, Andzelm MM, Ge B, Strominger JL. Human decidual NK cells form immature activating synapses and are not cytotoxic. *Proc Natl Acad Sci USA* 2005;102:15563–15568. [PubMed: 16230631]
- Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;355:992–1005. [PubMed: 16957146]
- Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004;350:672–683. [PubMed: 14764923]
- Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111:649–658. [PubMed: 12618519]
- Moffett-King A. Natural killer cells and pregnancy. *Nat Rev Immunol* 2002;2:656–663. [PubMed: 12209134]
- Moldenhauer JS, Stanek J, Warshak C, Khoury J, Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. *Amer J Obstet Gynecol* 2003;189:1173–1177. [PubMed: 14586374]
- Nevo O, Soleymanlou N, Wu Y, Xu J, Kingdom J, Many A, Zamudio S, Caniggia I. Increased expression of sFlt-1 in *in vivo* and *in vitro* models of human placental hypoxia is mediated by HIF-1. *Amer J Physiol Regul Integr Comp Physiol* 2006;291:R1085–1093. [PubMed: 16627691]
- Olofsson B, Jeltsch M, Eriksson U, Alitalo K. Current biology of VEGF-B and VEGF-C. *Curr Opin Biotechnol* 1999;10:528–535. [PubMed: 10600689]
- Orange JS, Fasset MS, Koopman LA, Boyson JE, Strominger JL. Viral evasion of natural killer cells. *Nat Immunol* 2002;3:1006–1012. [PubMed: 12407408]
- Parham P. NK cells and trophoblasts: partners in pregnancy. *J Exp Med* 2004;200:951–955. [PubMed: 15492121]
- Parham P. Immunogenetics of killer cell immunoglobulin-like receptors. *Mol Immunol* 2005;42:459–462. [PubMed: 15607799]
- Peel S. Granulated metrial gland cells. *Adv Anat Embryol Cell Biol* 1989;115:1–112. [PubMed: 2658485]
- Persico MG, Vincenti V, DiPalma T. Structure, expression and receptor-binding properties of placenta growth factor (PlGF). *Curr Top Microbiol Immunol* 1999;237:31–40. [PubMed: 9893344]
- Podjarny E, Baylis C, Losonczy G. Animal models of preeclampsia. *Semin Perinatol* 1999;23:2–13. [PubMed: 10102166]
- Powers RW, Roberts JM, Cooper KM, Gallaher MJ, Frank MP, Harger GF, Ness RB. Maternal serum soluble fms-like tyrosine kinase 1 concentrations are not increased in early pregnancy and decrease more slowly postpartum in women who develop preeclampsia. *Amer J Obstet Gynecol* 2005;193:185–191. [PubMed: 16021077]

- Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, Fisher SJ. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. *J Clin Invest* 2004;114:744–754. [PubMed: 15372095]
- Shibuya M. Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. *Cell Struct Funct* 2001;26:25–35. [PubMed: 11345501]
- Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005;365:785–799. [PubMed: 15733721]
- St-Jacques S, Forte M, Lye SJ, Letarte M. Localization of endoglin, a transforming growth factor-beta binding protein, and of CD44 and integrins in placenta during the first trimester of pregnancy. *Biol Reprod* 1994;51:405–413. [PubMed: 7528549]
- Venkatesha S, Toporsian M, Lam C, Hanai JJ, Mammoto T, Kim YM, Bdelah Y, Lim KH, Yuan HT, Libermann TA, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;12:642–649. [PubMed: 16751767]
- Wang B, Biron C, She J, Higgins K, Sunshine MJ, Lacy E, Lonberg N, Terhorst C. A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy numbers of the human CD3E gene. *Proc Natl Acad Sci USA* 1994;91:9402–9406. [PubMed: 7937778]
- Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J Immunol* 1998;161:571–577. [PubMed: 9670929]
- Wolf M, Shah A, Lam C, Martinez A, Smirnakis KV, Epstein FH, Taylor RN, Ecker JL, Karumanchi SA, Thadhani R. Circulating levels of the antiangiogenic marker sFLT-1 are increased in first versus second pregnancies. *Amer J Obstet Gynecol* 2005;193:16–22. [PubMed: 16021053]
- Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX, Rosenberg SA. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003;349:427–434. [PubMed: 12890841]
- Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest* 1997a;99:2152–2164. [PubMed: 9151787]
- Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, Damsky CH. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *J Clin Invest* 1997b;99:2139–2151. [PubMed: 9151786]
- Zygmunt M, Herr F, Munstedt K, Lang U, Liang OD. Angiogenesis and vasculogenesis in pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2003;110(Suppl 1):S10–18. [PubMed: 12965086]