**Pre-eclampsia** is a devastating disease of late (after week 20) pregnancy. The condition features hypertension, proteinuria, premature labor, haemolysis, liver abnormalities, thrombocytopenia (HELLP syndrome), seizures (eclampsia) and death [1]. Both mothers and children, if they survive delivery, can expect subsequent increased cardiovascular risk in the future. Pre-eclampsia affects about 5% of the pregnancies in affluent societies; in the rest of the world, the incidence is higher. Suffice it to say, pre-eclampsia is a major world health problem.

The group led by Anand Karumanchi *et al.* [2] has made major contributions in terms of our understanding pre-eclampsia. They showed that pre-eclampsia is associated with a circulating aberrant splice variant of the vascular endothelial growth factor (VEGF) receptor fms-like tyrosine kinase (Flt). This receptor is also an important target for placental growth factor (PLGF). The soluble fms-like tyrosine kinase (sFlt) receptor is a splice variant of the VEGF receptor Flt. The Karumanchi group showed that patients with pre-eclampsia have increased circulating levels of sFlt that putatively catches VEGF and PLGF before these mediators can interact with their ligands (Figure 1, panel A). As a result, endothelial and, particularly, placental (trophoblast) growth could be stunted, causing pre-eclampsia. The hypothesis was underscored by *in vitro* and *in vivo* experiments. Serum from pre-eclamptic patients inhibited tube formation by endothelial cells in culture. Giving sFlt to rats caused hypertension, proteinuria and glomerular endotheliosis.

Subsequently, the group collaborated with clinical investigators who had performed a randomized trial of calcium supplementation in women at risk for pre-eclampsia. The calcium story was negative; however, the women developing pre-eclampsia in these studies had increased circulating sFlt levels and decreased PLGF and VEGF concentrations [3]. The data appeared convincing [4].

Now, the Karumanchi group implicates an additional molecule in pre-eclampsia, namely endoglin (Eng) or CD105 [5]. The ENG gene encodes for the Eng protein. Mutations in ENG cause hereditary haemorrhagic telangiectasia Osler Rendu Weber Syndrome type 1 (HHT1), an autosomal-dominant disorder characterized by arteriovenous malformations and focal loss of capillaries. Eng is a proliferation-associated and hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells. Eng is a receptor for transforming growth factor (TGF)-β1 and -β3. The protein modulates TGF-β signalling by interacting with TGF-β receptors I and II. Eng is strongly expressed in blood vessels and has become a target for cancer researchers [6]. As a result, Eng has been labelled as a tumour therapy target [7].

Eng localizes to caveolae, where the molecule associates with nitric oxide synthase [8]. Here, various mechanisms join together to assure vascular function and integrity. The Karumanchi group now reports a soluble form of Eng (sEng), analogous to sFlt, is present in the sera of pregnant women. sEng is said to be elevated in pre-eclamptic women and cooperates with sFlt to induce endothelial dysfunction *in vitro* and pre-eclampsia *in vivo*. The findings implicate TGF-β1 signalling and nitric oxide-related mechanisms in the pre-eclampsia syndrome.

The investigators used an Affymetrix microarray chip and studied pre-eclamptic and normal placentas. They found that pre-eclamptic placentas over-expressed sFlt and also ENG mRNA. They confirmed this result by northern blotting and immunostaining.
for Eng. They next developed an assay for sEng and found a tight correlation between sEng and sFlt, as well as with disease severity. Studies similar to those performed with sFlt also indicated that sEng interfered with capillary formation. The investigators then used sEng and sFlt to induce features of pre-eclampsia in pregnant rats. An adenoviral expression system was employed in these studies. Blood pressure, liver enzymes, platelet counts and proteinuria all reflected the clinical features of the human disease. Moreover, in subsequent experiments, the authors showed that sEng inhibits TGF-β signalling in endothelial cells and blocks TGF-β1-mediated nitric oxide synthase activation. The data are interesting, but important questions remain unanswered. For instance, what is sEng and how does Eng signalling or their absences contribute to the disease? Or, even more basic, how does Eng work in the first place?

TGF-β superfamily members, including the bone morphogenic proteins, are potent regulatory cytokines with diverse functions in vascular cells [9]. Signalling occurs through heteromeric type-I and type-II receptor complexes, activating ‘sons-of-mothers-against-decapentaplegic’ (Smad)-dependent and Smad-independent signals that regulate vascular development, remodeling and function of practically all vascular cells. One of the participating transmembrane signalling molecules is Eng. Eng (CD105) is a 180 kDa type I integral membrane protein with an extracellular region of 561 amino acids, a hydrophobic transmembrane domain and a 47-residue cytoplasmic tail that is strongly expressed on endothelial cells [10]. The sequence contains an RGD tri-peptide. Eng binds to transforming growth factor β (TGF-β1) and TGF-β3 with high affinity and forms heteromeric associations with the TGF-β signalling receptors types I and II. The identification of the gene encoding activin receptor-like kinase-1 (ALK1), a type I TGF-β receptor as the second locus for HHT, strongly suggests that TGF-β is involved in the pathogenesis of HHT.

The many mutant alleles found in Eng suggested to Raab et al. [11] that the mutants would produce a soluble circulating protein. Raab et al. [11] then used viral transfection to express normal and truncated forms of Eng. They showed that a secreted dimeric form of Eng, namely sEng, could be produced. On the basis of this information, we can speculate that Eng is important to TGF-β signaling and sEng indeed, probably, interferes with this signalling similar to the interference to VEGF and PLGF provided by sFLt.

TGF-β1 exerts bi-functional effects on endothelial cells in vitro; the cytokine can both stimulate and inhibit the proliferation of endothelial cells [12]. Experimentally, low doses of TGF-β1 tend to stimulate, while high doses inhibit proliferation. TGF-β1 regulates the expression and/or activation of matrix metalloproteinases 2 and 9. These enzymes are involved in extracellular matrix degradation and initiate angiogenesis. The data from the Karumanchi group [5] indicate that angiogenesis is disturbed in pre-eclamptic placentas. ALK1 and ALK5 are particularly important to angiogenesis signalling. In endothelial cells, both ALK1 and ALK5 are expressed and bind TGF-β1. The ratio between ALK1 and ALK5 stimulation seems to be important in determining whether the endothelium is activated or whether it remains quiescent. The ALK1-Smad1/5 pathway appears to be activating and induces the expression of proangiogenic genes, including Id1, Eng and IL1RL1. The ALK5-Smad2/3 pathway signals expression of maturation-specific genes such as connexin37, βIH-H3 and PAI-1. Thus, ALK1-Smad1/5 pathway seems to be specifically involved in activating Eng signalling (Figure 2).
HHT is thus far the only vascular disorder specifically attributed to Eng and ALK1. Mice harbouring heterozygous Eng and ALK1 mutations resemble HHT in humans. Mice with deleted Eng or ALK1 die in utero. Pre-eclampsia now appears to be another Eng, or rather sEng-related disorder. A great mystery remains regarding the generation of the splice variant sFlt and the mechanisms regulating sFlt production. Equally mysterious is the production of sEng and the mechanisms regulating that production. Therapeutic implications (targets) are exciting. We are waiting impatiently for the next chapter.

References

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