Soluble endoglin: a biomarker or a protagonist in the pathogenesis of preeclampsia?

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■ INTRODUCTION

Pre-eclampsia is a pregnancy-specific disorder characterized by endothelial dysfunction, hypertension (> 140/90 mmHg) and proteinuria (> 300mg/24hours) in the third trimester of pregnancy. It affects about 3-5% of all pregnancies worldwide[1,2]. It remains a major cause of maternal mortality (15–46%) and is responsible for a 5-fold increase in perinatal mortality[3]. The World Health Organization has recognized the importance of pre-eclampsia by launching a global programme to fight pre-eclampsia-eclampsia (World Health Organization. Policy and Coordination Committee 16th meeting. Department of Reproductive Health and Research. Summary Medium-term Programme of Work 2004-2009 HRP/ PCC (16)/2003/8.1). In addition, women who have suffered pre eclampsia and their offspring are at greater risk of developing cardiovascular disease later in life[4].

Several risk factors are associated for pre-eclampsia; these include nulliparity, multifetal gestations, previous history of pre-eclampsia, obesity, diabetes *mellitus*, vascular and connective tissue disorders like systemic lupus erythematosus and antiphospholipid antibodies. Severe pre-eclampsia can lead to the appearance of the HELLP (haemolysis, elevate liver function tests, low platelets) syndrome, the most critical variant of pre-eclampsia, and is associated with a higher risk of maternal and neonatal adverse outcomes[5].

At the present, there are no effective treatments for this disease, and delivery remains the only solution for maternal symptoms. In many cases, the newborn is often premature and there are significant perinatal morbidity and mortality among such babies.

Until recently, the molecular pathogenesis of pre-eclampsia was largely unknown, but recent observations support the hypothesis that altered expression of placental anti-angiogenesis factors are responsible for the clinical manifestation of this disease. Two anti-angiogenic factors soluble fms-like kinase 1 (sFlt1) and soluble endoglin (sEng) have been reported to have increased plasma concentration in the maternal circulation weeks before the onset of pre-eclampsia[6,7] and decreased quickly after delivery[8]. The concentration of these factors in plasma is correlated with the severity of pre-eclampsia and bad prognossis. Thus, they have been proposed as useful biomarkers for the diagnostic and prognosis of pre-eclampsia[9,10].

Also, Gilbert *et al* showed that sEng and sFlt1 were increased in the serum of uteroplacental ischaemia animal model (RUPP)[11,12]. sFtl1 is a soluble form of the VEGF receptor-1, a cell membrane receptor for VEGF (vascular endothelial growth factors) and PIGF

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(placental growth factor-1), whereas sEng is the soluble form for a membrane co-receptor of several members of the TGFβ superfamily. Both, sFlt1 and sEng, are secreted by the placenta[13,14].

PLACENTA IS THE ORIGIN OF PRE-ECLAMPSIA

Placenta plays a central role in pre-eclampsia as it is evidenced by rapid disappearance of disease symptoms after delivery and maintenance of these symptoms when placenta is retained. Abnormalities in the placental development have been studied in order to understand the early stage of this disease. The molecular basis for placental dysregulation of pathogenic factors it is not clear. The first event in the early gestation of pre-eclampsia is the abnormal placentation by alterations in the placenta vascular remodeling[15]. It has been reported unappropriated vascular remodeling of uterine spiral arteries by impaired invasion of these arteries by trophoblasts. On this way, the blood supply to the placenta is reduced to a level unable of providing adequate placental perfusion to sustain the growing fetus[16] and generates a hypoxic environment. In humans, trophoblasts stem cells are differentiated into two cell types, villous trophoblasts and extravillous trophoblasts (EVTs). Earlier in pregnancy, invading trophoblasts, called interstitial EVTs, migrate into the decidualized endometrium and endovascular EVTs migrate along the lumen of spiral arterioles[17]. This EVT invasion creates the large-diameter and low-resistant vessels that carry blood to the placenta. After 12 weeks, endovascular EVTs invade the uterine spiral arteries, replace the endothelial cells, and participate in the degradation of tunica media smooth muscle cells. This remodeling of the spiral arteries is essential for proper placental perfusion to sustain fetal growth. Pre-eclampsia is considered to be caused by poor placentation that can be explained by the shallow invasion by trophoblast until 20 weeks of gestation. This impairment is closely related with a failure of vascular remodeling. The consequence is shallow spiral arteries that contribute to the hypoxic environment in the placenta[18,19]. Therefore, the hypoxic environment has been postulated as a critical signal that initiates the pathogenic process of pre-eclampsia[20]. There are a lot of evidence supporting the central role of hypoxia in pre-eclampsia. It was reported that the placentas of pre-eclamptic women and models of placental hypoxia show similar gene expression[21]. In addition, reductions in utero placental blood flow in animal models induced

hypertension, proteinuria and endothelial dysfunction, which are the typical sign of this disease[22]. This relationship between uteroplacental ischaemia and pre-eclampsia was demonstrated in several animals, such as dogs[23], rabbits[24,25], primates[26,27] and, more recently, it has been established a reduced uterine perfusion pressure model in the pregnant rat (RUPP) in which the utero placental perfusion is reduced by approximately 40% and shows many features of pre-eclampsia[28-32].

The possibility that sFlt1 and sEng are a key pathogenic link between placental pathology and maternal endothelial damage provides hope that these substances, in addition to serving as biomarkers of pre--eclampsia, could also play some role in the pathogenesis of the disease and, thus, could possibly also be effective therapeutic targets for the disease. In this editorial we will focus on sEng, in order to analyze if it is only a biomarker or if it could also be a protagonist in the pathogenesis of pre-eclampsia.

ENDOGLIN

Endoglin (Eng), also called CD105, is a glycosylated transmembrane protein that acts as an auxiliary receptor of several members of the TGF-β superfamily of proteins[13]. Endoglin is highly expressed in endothelial cells and syncytiotrophoblast[33,34]. Although the most studied physiological effect of Eng is regulating angiogenesis [1], we have reported that Eng plays a major role in the regulation of vascular tone[13]. It has been described that the vasodilator effect of Eng is mediated by regulating endothelial nitric oxide synthase (eNOS) expression and NO synthesis[35]. It has been proposed that Eng plays a major role in pre-eclampsia[36]. In this review, we will describe the mechanisms of Eng overexpression and sEng release in pre-eclampsia, as well as the possible role of sEng in hypertension, proteinuria and abnormal placentation, all of them characteristic of pre-eclampsia.

Mechanisms of placental endoglin release in pre-eclampsia

Hypoxia seems to be involved in increased endoglin expression in the placenta

Endoglin is overexpressed in placenta during pre--eclampsia. Eng mRNA expression in chorionic villi

at 11-week's gestation were significantly higher in women that will develop pre-eclampsia. These findings suggest that this increase could be implicated in the early pre-eclampsia[37]. The development of the placenta during the early normal pregnancy is carried out in an environment of low oxygen tension until 10 or 12 weeks of gestation. In that moment, the intravillous space is filled to maternal blood and the oxygen levels begin to increase. High level of endoglin is found in the early stage of gestation when the oxygen is low, but in the later stage the levels of endoglin is decreased[38,39]. However, in pre-eclamptic placentas the amount of endoglin is always elevated. In trophoblasts obtained from placentas of pre-eclampsia patients, the release of sEng in hypoxia conditions (2% O2 tension) was also higher than in trophoblast from normal pregnant women[40]. These facts confirm that the hypoxic environment induces endoglin expression and plays an important role in the pathogenesis of pre-eclampsia[41-43]. The mechanism explaining high endoglin expression in the presence of placentary hypoxia is based in the fact that endoglin gene promoter has a hypoxia responsive element (HRE) region. HIF-1, in cooperation with Sp1 and the coactivator CBP/P300 is bound to this region stimulating endoglin expression. This stimulatory effect is further enhanced when the HIF-1α/Sp1 complex collaborates with the TGFβ/Smad3 signaling pathway[41].

On the other hand, there are data suggesting that the increase in Eng expression observed in pre-eclampsia can also be mediated, at least in part, by HIF-1α, probably through activation of the TGF-β3 pathway[43,44]. Tal R. et al have demonstrated the effects of HIF-1 overexpression in pregnant mice using adenovirus which express a constitutively active and stabilized HIF-1α. They observed an elevation of sFlt1 and sEng in the plasma of these mice[45].

Increased oxidative stress can be involved in increased Eng expression by the placenta

A crosstalk between hypoxia and oxidative stress has been described by several authors. Under hypoxic conditions, reactive oxygen species (ROS) are produced by a flavoprotein-containing NAD(P)H or by mitochondria as a mechanism that contributes to stabilize HIF-1α and consequently mediates the transcriptional response to hypoxia[46]. Reactive oxygen

species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia, nuclear translocation and transcriptional activation by a mechanism of O2 sensing[46]. Thus, hypoxic conditions may favor the generation of ROS in pre-eclampsia. Indeed, oxidative stress has been shown to play a pivotal role in the etiopathogenesis of pre-eclampsia[20]. Furthermore, it has been demonstrated that a decrease in endogenous antioxidant factors, such as the antioxidant hemeoxygenase-1 (HO-1), superoxide dismutase and glutathione peroxidase, may contribute to the increase release of sEng[47-49].

Several studies have demonstrated high levels of LDL in the serum from women with pre-eclampsia and these LDL show a higher susceptibility to oxidation giving oxidized LDL (oxLDL) rich in oxysterols[50].

Endogenously oxygenated cholesterol derivatives, such as 24(S)-hydroxycholesterol or 22(R)-hydroxycholesterol (oxysterols), are detected at relatively high levels in the placenta[51]. Oxysterols, a product of cholesterol oxidation, can play a role mediating sEng release in pre-eclampsia. Oxysterols have been identified as endogenous agonists of liver X receptors (LXRs). LXRs are ligand-activated transcription factors, which have two isoform: LXRα and LXRβ. LXRα is present in tissues with high lipid metabolism, such as liver, intestine, adipose tissue and kidney, whereas LXRβ is ubiquitously expressed[52]. It has been suggested a role of LXRs in the reproductive system and a potential application of LXR agonists in the treatment of reproductive pathologies[53]. It has been reported the association of the LXRβ activation and pre-eclampsia. Mouzart et al. showed that the single nucleotide polimorphisms rs2695121 in LXRβ gene was strongly associated with pre-eclampsia. In contrast, they did not find a significant association between the polimorphisms in LXRα gen and this disease[54]. On the other hand, Henry-Berger et al. have shown that endoglin is one of the target gene of LXR. Endoglin has six putative response elements (LXRE) in its proximal promoter region. The heterodimer LXR/RXR is able to bind them and stimulate its transcription. It has been reported that endoglin was up-regulated by administration of LXR agonists in human choriocarcinoma JAR cells[55]. Therefore, the LXR activation is able to up-regulate the inappropriate expression and function of endoglin and contribute with the complication of pregnancy[56].

Mechanisms involved in the release of sEng in the placenta

In pre-eclamptic women, the high levels of sEng seems be derived from membrane isoform of the placental cells[14].The release of sEng occurs by proteolytic cleavage of the membrane isoform. It is known that this proteolytic cleavage is mediated by membrane metalloproteinases, which are increased in pregnancy. Hawinkels *et al*, reported that metalloproteinase-14 (MMP14 or MT1-MMP) is involved in the release of sEng[57]. Kaitu'u-Lino *et al.* treated the syncitiotrophoblast cell line (BeWo) with metalloproteinase inhibitor, GM6001, and observed a significant decreased in sEng release. The GM6001 inhibits other MMPs besides MMP14, so they administered MMP-13 or MMP-2/9 specific inhibitors, and did not observe differences in sEng release compared with its controls[58]. Furthermore Kaitu'u-Lino, *et al.,* also demonstrated that there are other metalloproteinase present in placenta, such as MMP-16,-24,-25, but they were not associated with pre-eclampsia. Furthermore, MMP-17 and -15 were also up-regulated in pre-eclampsia, but neither of them were involved in the increased release of sEng[59,60].

Recently, a study from our group demonstrated that hypoxia induces sEng shedding in cultured human trophoblasts (JAR cells) and this release was increased by ROS. This study also demonstrated that oxysterols are involved in the sEng shedding through LXR pathway and that oxysterols and activators of LXR pathway increase cell surface expression and activity of MMP14 and inhibit the TIM-3 expression which is a MMP14 inhibitor[55]. Thus, we can suggest that oxysterols induce sEng release by increasing cell surface endoglin[56], and by increasing MMP14 and inhibit its inhibitor TIMP-3[55]. Experiments *in vivo* have also demonstrated that administration of LXR agonist or oxysterols induced the increase in plasma levels of sEng in mice supporting the hypothesis that the LXR activation increases sEng release[55].

On the other hand, Lamarca *et al*, in the uteroplacental ischaemia animal model (RUPP), have reported high levels of antibodies to angiotensin receptor type I, AT1-AA. They also demonstrated that the increase of AT1-AA induces the rise of TNF-α, which stimulates the release of sEng and sFlt1[61].

Thus, from our own results and those of the literature we can hypothesize that soluble endoglin release is based on placental hypoxia, which causes oxidative stress and the formation of oxysterols. As a consequence, there is an increase in placental levels of membrane endoglin and the metalloprotease MMp-14, which cleaves membrane endoglin releasing the soluble form, thus increase plasma levels of this form (Fig. 1).

### ■ Possible effects of endoglin in pre-eclampsia

Effects of sEng in arterial pressure

The possible role of sEng in pre-eclampsia is not elucidated yet. It has been hypothesized that sEng plays its antiagiogenic and pro-hypertensive effects in pre-eclampsia due to its ability to bind circulating TGFβ. TGFβ, in the presence of membrane Eng, produce vasodilatation mediated by eNOS overexpression[62]. Thus, the binding of TGF-β by sEng could prevent the interaction of these TGFβ molecules with the cell-surface Eng and, consequently, its normal effects in the endothelium[63]. By this mechanism, sEng leads abolishes TGFβ-dependent eNOS activation, thus leading to vasoconstriction[14]. Several studies reported that decreased NO availability is one of the principal causes of endothelial dysfunction in pre-eclampsia and is involved in haemodynamics changes characteristics of pre-eclampsia[64,65]. However, this hypothesis needs to be adequately confirmed. Other authors have suggested that sEng alone cannot scavenge TGFβ for which it has very low affinity, and suggest that sEng binds with high affinity to BMP9, which stimulates secretion from endothelial cells of the vascoconstrictor endothelin-1, also implicated in endothelial cell stabilization[66]. It remains to be determined if scavenging of circulating BMP9 by sEng is important in pre-eclampsia and regulation of hypertension. It should be noted that administration of LXR agonist or oxysterols, in addition to inducing the increase in plasma levels of sEng, also induces hypertension in mice, whereas when sEng release was prevented by a blocking peptide, hypertension was not observed[55]. Furthermore, male mice transgenic for sEng and with high plasma sEng levels also show hypertension[55]. In the RUPP model in rats, the hypertension is associated to high plasma levels of sEng[11]. Thus, all these data suggest that soluble endoglin plays a major role in the pathogenesis of pre-eclampsia-associated hypertension.

Figure 1

Mechanisms proposed for soluble endoglin (sEng) release and effects of sEng in the pathogenesis of pre-eclampsia.

sEng release is based on placental hypoxia (with increase of cytosolic levels of HIF-1), which causes oxidative stress and the formation of oxysterols. This situation activates the LX receptors and NFκB pathway. As a consequence, there is an increase in placental levels of membrane endoglin and the metalloprotease MMP-14, which cleaves membrane endoglin releasing the soluble form, thus increasing plasma levels of this endoglin form. High plasma levels of endoglin decrease vessel relaxation ability, thus contributing to placental hypoperfusion (and, consequently, to placental hypoxia) and increased peripheral resistance (and, consequently, increased arterial pressure). sEng also impairs normal trophoblast invasion and normal placentation. In addition, sEng could also alter renal glomerular filtration membrane, thus inducing proteinuria.
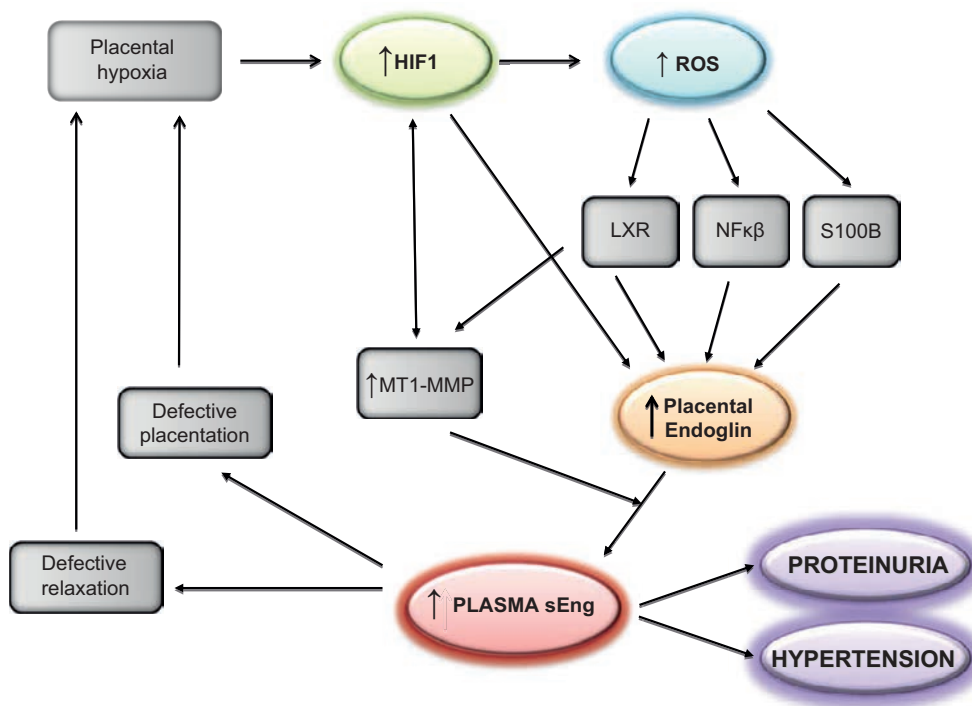
Effects of sEng in proteinuria

Classically, glomerular endothelial injury was considered responsible for the renal impairment present in pre-eclampsia. Proteinuria, another major characteristic of pre-eclampsia, has been explained by "endotheliosis" of glomerular capillaries, an alteration characterized by endothelial cell swelling and loss of endothelial fenestrae in these capillaries. It has been demonstrated that neutralization of TFG-β by neutralizing TGF-beta1 antibodies results in loss of glomerular fenestrae and proteinuria, thus suggesting that the glomerular endotheliosis lesions of pre-eclampsia are at least partially due to the placenta-derived sEng, which block the TGF-beta/endoglin signaling, respectively, causing the loss of glomerular endothelial cell fenestrae, cell swelling and proteinuria[67].

Podocytes are modified glomerular epithelial cells that cover the outside surface of glomerular capillaries, and play a major role in regulating glomerular permeability to proteins. Recent evidence suggests that appearance of podocytes in urine is associated with the appearance and prognosis of pre-eclampsia[68]. There are some data suggesting that podocytes are crucial in explaining the loss of filtration capacity of the pre-eclamptic kidney[69]. Also, recent studies from our laboratory have demonstrated that sEng is able to prevent leukocyte binding to endothelium by interfering in the binding of leukocyte integrins to the RGD domain of endothelial endoglin[70]. Thus, it can be hypothesized that by a similar mechanism, sEng is able to reduce the binding of the podocytes to glomerular basal membrane (GBM) by impairing the binding of integrin α3β1 in the podocyte to

the GBM component laminin-521, one of the major mechanism for podocyte attachment[71]. This could induce the detachment of podocytes from the GBM, as these cells are subjected to a great force by the fluid that is filtered through the wall of the glomerular capillaries.

Effects of sEng in placentation

As above described, endoglin is greatly expressed in syncyotrophoblast and extravillous trophoblast (EVT). It was shown that the inappropriate levels of endoglin could induce a wrong trophoblast differentiation though a negative regulation by TGFβ1 and TGFβ3[72]. Studies have demonstrated that overexpression of endoglin decreased cell migration and invasion in mouse fibroblast or endothelial cells. Endoglin also has an important role in trophoblast biology[43], through its ligands, TGFβ1 and TGFβ3[72]. TGFβ signaling is involved in several inhibitory signals, such as antiproliferative, antimigratory and anti-invasive signals. Furthermore, it is demonstrated that LXRβ activation reduced EVT invasion and migration through TGFβ pathway[73].

In addition, sEng can be also involved in inadequate placentation. It has been reported that sEng reduces the invasive ability of cytotrophoblasts through regulation of the expression of MMP-2 and MMP-9, metalloproteases necessary for a correct trophoblast invasion[74]. It has been reported that the invasion and vascular remodeling under hypoxia were significantly reduced in autophagy-deficient EVT cells compared with wild-type EVT cells. Interestingly, sEng suppressed EVT invasion by inhibiting autophagy. This effect of sEng was recovered by TGF-β1 treatment in a dose-dependent manner. A high dose of sEng inhibited the vascular construction by EVT cells and endothelial cells, whereas a low dose of sEng inhibited the replacement of endothelial cells by EVT cells[75]. Although the evidences are very limited, we can hypothesize that sEng is involved in the inadequate placentation, which is the primary cause of pre-eclampsia. Thus, we can hypothesize, in the basis of the above described, that increased plasma levels of sEng are not only an early biomarker of pre-eclampsia but are also involved in the pathogenesis of the disease (defective placentation, hypertension and proteinuria) (Fig. 1)

■ CONCLUSION AND PERSPECTIVES

In summary, placenta plays the central role in pre-eclampsia. The first event in early gestation is an abnormal placentation by alterations in vascular remodeling. The triggering of the hypoxia pathway induces an oxidative stress in the placenta. The oxidative stress and high levels of cholesterol in pre-eclamptic women induce an increased production of oxysterols in maternal plasma. This leads to activation of the oxysterols receptors LXR, which induces sEng release, and increased plasma levels of sEng contributes to the clinical manifestation of pre-eclampsia: hypertension and proteinuria, and contributes to defective placentation, thus creating a vicious circle that amplifies the disease. A schematic representation of these mechanisms is shown in Fig. 1. According with these results, it can be speculated that one of the therapies for pre-eclampsia should be the reduction of sEng shedding. We have hypothesized that inhibition of the enzymatic activity of MMP14 could prevent sEng release. We have described a peptide (P583), which corresponds to the aminoacids 583-590 of endoglin sequence. This sequence includes the MMP14 cleavage site GL and interferes with its proteolytic activity. It was proven *in vitro* that P583 inhibits the sEng shedding. Administration of p583 to mice treated with LXR agonist or oxysterols reduced plasma levels of sEng and reverted the appearance of hypertension in these mice[55]. These studies suggest that a therapeutic strategy that inhibits sEng release or eliminates sEng from the circulation could be effective in preventing pre-eclampsia, a disease without treatment at present.

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