

The physiological role of endoglin in the cardiovascular system

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López-Novoa JM, Bernabeu C. The physiological role of endoglin in the cardiovascular system. *Am J Physiol Heart Circ Physiol* 299: H959–H974, 2010. First published July 23, 2010; doi:10.1152/ajpheart.01251.2009.—Endoglin (CD105) is an integral membrane glycoprotein that serves as a coreceptor for members of the transforming growth factor- β superfamily of proteins. A major role for endoglin in regulating transforming growth factor- β -dependent vascular remodeling and angiogenesis has been postulated based on the following: 1) endoglin is the gene mutated in hereditary hemorrhagic telangiectasia type 1, a disease characterized by vascular malformations; 2) endoglin knockout mice die at midgestation because of defective angiogenesis; 3) endoglin is overexpressed in neoangiogenic vessels, during inflammation, and in solid tumors; and 4) endoglin regulates the expression and activity of endothelial nitric oxide synthase, which is involved in angiogenesis and vascular tone. Besides the predominant form of the endoglin receptor (long endoglin isoform), two additional forms of endoglin have been recently reported to play a role in the vascular pathology and homeostasis: the alternatively spliced short endoglin isoform and a soluble endoglin form that is proteolytically cleaved from membrane-bound endoglin. The purpose of this review is to underline the role that the different forms of endoglin play in regulating angiogenesis, vascular remodeling, and vascular tone, as well as to analyze the molecular and cellular mechanisms supporting these effects.

nitric oxide; vascular malformations; angiogenesis; development

ENDOGLIN (also known as CD105) is a type I integral membrane glycoprotein that belongs to the zona pellucida (ZP) family of proteins (64, 105). It is highly expressed on proliferating vascular endothelial cells (ECs) (25, 119) and has been identified as an accessory receptor for transforming growth factor- β (TGF- β) (30). The human *ENDOGLIN* (*ENG*) gene has been localized to chromosome 9q34ter (53), and it is mutated in hereditary hemorrhagic telangiectasia (HHT) type 1 (118). Mice lacking endoglin die during embryonic development because of defective angiogenesis (6, 23, 103), and endoglin plays a major role in tumoral and nontumoral adult angiogenesis (13, 79). A soluble form of endoglin also plays a central role in preeclampsia, a disease characterized by hypertension and severe alterations in placental circulation (172). Overall, these results support the view that endoglin has a pivotal function in vascular development and disease (13, 50, 93, 158). The purpose of this review is to critically assess the role of endoglin in vascular function as well as the mechanisms involved.

Structure of Endoglin

Human endoglin is a type I integral membrane protein with a large extracellular domain (561 amino acids), a single hydrophobic transmembrane domain, and a short cytosolic domain (64) (Fig. 1A). The expression of two different alternatively spliced isoforms, long (L) endoglin and short (S) endoglin, has been demonstrated in human and mouse tissues (12, 64, 130). Human S-endoglin and L-endoglin proteins vary from each other in their cytoplasmic tails that contain 14 and 47 amino acids, respectively, with a sequence of only 7 residues being specific for S-endoglin (Fig. 1B). Because L-endoglin is the predominantly expressed isoform, unless stated otherwise, the functional studies in this review will be referred to this isoform. Endoglin is a highly glycosylated protein expressed as a 180-kDa disulfide-linked homodimer (63). The primary structure of endoglin suggests that there are five *N*-linked glycosylation sites in the NH₂-terminal domain and a probable *O*-glycan domain, which are rich in serine and threonine residues proximal to the membrane-spanning domain (64). Human endoglin also contains an Arg-Gly-Asp (RGD) peptide sequence (64) that is known as a cell recognition site for numerous adhesive proteins present in the extracellular matrix (ECM), but this motif is absent from mouse (60), porcine (177), rat, and canine (105) endoglin proteins. Structurally, endoglin belongs to the ZP family of proteins that share a ZP domain of ~260 amino acid residues in their extracellular

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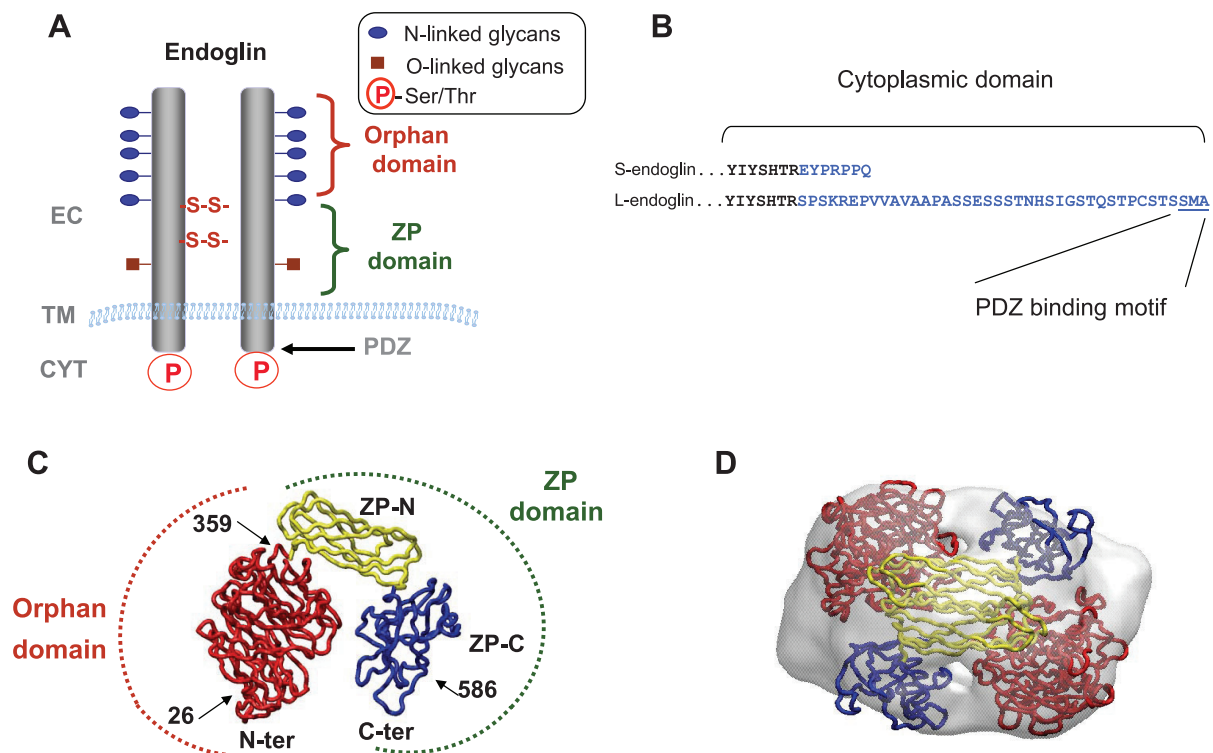


Fig. 1. Structural representation of endoglin. *A*: endoglin is a type I membrane protein with a large extracellular domain that contains a zona pellucida (ZP) domain of 260 amino acids in the juxtamembrane region and an NH₂-terminal (N-ter) orphan domain. Endoglin forms dimers and the corresponding monomers are disulphide linked. Consensus motifs to attach *N*-linked glycans and *O*-linked glycans to the extracellular domain have been identified. The cytoplasmic domain of endoglin is phosphorylated (P) at Ser/Thr residues and contains a consensus postsynaptic density 95/*Drosophila* disk large/zonula occludens-1 (PDZ)-binding motif present at the carboxyl terminus (C-ter). The cytoplasmic (CYT), transmembrane (TM), and extracellular (EC) domains of the protein are indicated. The scheme is not to scale. *B*: amino acid sequences from short (S)-endoglin and long (L)-endoglin cytoplasmic tails. Sequences that differ between L and S isoforms are in blue. The PDZ-binding motif in L-endoglin is underlined. *C* and *D*: 3-dimensional model of endoglin. *C*: the atomic model predicted in silico shows the presence of 3 different subdomains in red, yellow, and blue. The orphan domain contains amino acid residues Glu26-Ile359 (red), whereas the ZP domain encompasses the fragment Gln360-Gly586. The ZP-N and ZP-C subdomains are colored in yellow and blue, respectively. The amino acid numbers corresponding to the border regions of the globular domains are indicated. *D*: the electron microscopy density map of soluble endoglin (gray volume) allows the fitting of the atomic model of dimeric endoglin. S-S-, disulfide bridge. Adapted from Llorca et al. (105) and used with permission.

region (81, 105). The three-dimensional structure of the extracellular domain of endoglin at 25Å resolution, using single-particle electron microscopy, has been elucidated (105). Endoglin arranges as a dome made of antiparallel-oriented monomers enclosing a cavity at one end. Each subunit comprises one ZP domain in the juxtamembrane region. The NH₂-terminal domain does not show any significant homology to any other protein family/domain and thereby has been named an “orphan” domain (Fig. 1, *A*, *C*, and *D*).

The cytosolic domain of endoglin is constitutively phosphorylated, and it can be targeted by serine and threonine kinases (89, 177), including the TGF-β type I and II receptors (73, 83). It has been shown that the endoglin phosphorylation status can influence its subcellular localization (83) and cellular migration (144). Endoglin cytoplasmic domain contains a consensus postsynaptic density 95/*Drosophila* disk large/zonula occludens-1 (PDZ)-binding motif (Ser-Ser-Met-Ala) present at the carboxyl terminus that mediates endoglin interaction with several PDZ domain-containing proteins and endoglin phosphorylation of distal threonine residues (13).

Regulation of Endoglin Expression

The cellular and tissue distribution of endoglin suggests its role in vascular development, angiogenesis, and vascular ho-

meostasis. Endoglin is expressed at low levels in resting ECs, but it is highly expressed in vascular ECs at sites of active angiogenesis, during embryogenesis (80, 131), and in inflamed tissues and healing wounds (163), psoriatic skin (145), inflamed synovial arthritis (155), upon vascular injury (21), and in tumor vessels (13, 25, 55, 119). Endoglin is also overexpressed after ischemia and reperfusion in the kidney (47), hindlimbs (79), and heart (168). Endoglin is expressed not only in ECs but also in several other cell types involved in the cardiovascular system. For example, whereas endoglin expression is low in normal smooth muscle cells (2), its expression is upregulated in vascular smooth muscle cells (VSMCs) of human atherosclerotic plaques (36). Endoglin is expressed in cardiac fibroblasts and modulates the profibrogenic actions of angiotensin II (31). Endoglin is also expressed in other tissues undergoing fibrosis such as the kidney (143) and liver (34). Endoglin is present on monocytes, and it is upregulated during the monocyte-macrophage transition (87). During the development of the cardiovascular system, endoglin is found on the vascular endothelium of human embryos during all developmental stages from 4 wk onward, and it is transiently upregulated on cushion tissue mesenchyme during heart septation (135). Furthermore, an altered expression of endoglin was observed in human fetuses with cardiac defects (9).

The mechanisms responsible for the increased endoglin expression in activated vessels are probably multifactorial, hypoxia, vascular injury, and related cytokines being the most likely stimuli. In fact, endoglin expression is upregulated after ischemia in the heart (168), kidney (47), and hindlimbs (79), as well as upon arterial injury (21, 110). Also, in murine cerebral microvascular ECs, hypoxia induces the expression of endoglin at both the mRNA and protein levels, and this induction is regulated by p38 and probably JNK pathways (179). Furthermore, a hypoxia-responsive element downstream of the main transcription start site of the endoglin gene has been characterized. Thus, under hypoxic conditions, the hypoxia-inducible factor-1 (HIF-1) complex binds a functional consensus hypoxia-responsive element in the endoglin gene promoter (146). TGF- β signaling, via Smad transcription factors, also potently stimulates endoglin expression (20, 88, 142). By contrast, tumor necrosis factor- α decreases endoglin protein levels in ECs (98). Whereas hypoxia alone moderately stimulates endoglin transcription, the addition of TGF- β 1 under hypoxic conditions results in a transcriptional cooperation between both signaling pathways, leading to a marked stimulation of endoglin expression. This synergic stimulation involves the formation of a transcriptional multicomplex containing Smad3/Smad4, stimulating protein 1 (Sp1), and HIF-1, leading to a cooperative effect of these factors on endoglin transcription (146). Also, upon vascular injury, a transcriptional activation of endoglin mediated by the cooperative interaction between Sp1 and Krüppel-like factor 6 transcription factors has been reported (21).

Modulation by Endoglin of TGF- β -Dependent Cell Responses

Endoglin is an auxiliary TGF- β receptor that modulates TGF- β 1- and TGF- β 3- but not TGF- β 2-dependent responses in several cell types. In human monocytic cells, TGF- β 1, but not TGF- β 2, responses are abrogated in the presence of endoglin (88). In a variety of cell types, including myoblasts and fibroblasts, endoglin opposes TGF- β 1-dependent responses such as the inhibition of cellular proliferation (88), the expression of the ECM proteoglycan lumican (22), as well as the increased expression of ECM components, including plasminogen activator inhibitor type 1 (PAI-1), collagen, or fibronectin (46, 72, 88, 96, 125). Moreover, neutralizing anti-endoglin antibodies or antisense oligonucleotides for endoglin enhance the inhibitory effect of TGF- β on proliferation and migration (100, 152), whereas endoglin overexpression counteracts the antiproliferative effect of TGF- β 1 in ECs (100). The inhibition of endoglin expression on ECs increases the antiproliferative effect of TGF- β 1 and enhances EC apoptosis induced by hypoxia and TGF- β 1 (100, 101). These findings are compatible with the fact that endoglin is markedly upregulated in the proliferating endothelium of tissues undergoing angiogenesis (13, 18, 25, 56, 85).

While it is widely accepted that endoglin is expressed at high levels in proliferating ECs, the direct role of endoglin in mediating EC proliferation and migration is controversial. Several experimental evidences support the hypothesis that endoglin promotes EC proliferation and migration (92). However, other authors have reported that an *Eng*^{-/-} EC line proliferates faster than *Eng*^{+/+} control cells (128) and that

Eng^{-/-} progenitors can be expanded and differentiated in culture (33). With the use of a mouse with a conditional mutation in the *Eng* gene, it was shown that subcutaneous Matrigel implants in adult mice were populated by reduced numbers of new blood vessels compared with controls, whereas their endoglin-deficient retinas exhibited increased proliferation of ECs (112). These variable results suggest that the endoglin role in cell proliferation might be context dependent and they should be interpreted with caution.

Endoglin cytoplasmic and extracellular domains specifically interact with those of the activin-like kinase 1 (ALK1), a TGF- β type I receptor (15). Also, the colocalization of endoglin and ALK1 has been demonstrated in vascular endothelia (113). Moreover, studies using *Eng*^{-/-} and *Eng*^{+/-} embryonic ECs indicate that endoglin promotes EC proliferation via the TGF- β /ALK1 pathway (92), suggesting the involvement of endoglin and ALK1 in a common signaling pathway (15, 93, 157). Analyses of the downstream target genes regulated by endoglin and ALK1 have been carried out by gene expression fingerprinting of endoglin-deficient human ECs from HHT patients with pathogenic mutations in either endoglin or ALK1 genes. These studies allowed the identification of hundreds of downregulated and upregulated genes, including those involved in angiogenesis, cytoskeleton organization, cell guidance, intercellular connections, cell migration and proliferation, or nitric oxide (NO) synthesis (51, 159).

Role of Endoglin in Vascular Pathology

The importance of endoglin in vascular biology is reflected by the fact that mutations in the *ENG* gene lead to a vascular disease called the Rendu-Osler-Weber syndrome or HHT1 (118). HHT is an inherited autosomal-dominant and highly penetrant disorder characterized by vascular dysplasias, frequent episodes of epistaxis, mucocutaneous telangiectases, and arteriovenous malformations (AVMs) of the lung, brain, liver, and gastrointestinal tract (69). A second form of HHT (HHT2) is caused by mutations in the gene coding for the TGF- β type I receptor known as activin receptor-like kinase-1 (*ACVRL1* or *ALK1*). Either of the two genes, *ENG* or *ACVRL1*, is mutated in more than 90% of patients with HHT (1, 19, 52, 57, 94, 95). Two additional loci for HHT have been mapped to chromosomes 5 and 7, but the corresponding mutant genes have not been identified yet (10, 35). Moreover, a combined syndrome of juvenile polyposis (JP) and HHT was described to be caused by mutations in *SMAD4* that encodes a transcription factor of the TGF- β signaling pathway (59). This combined syndrome (JP-HHT) occurs only in 1 to 2% of persons clinically diagnosed with HHT, as evidenced by detected mutations in *SMAD4*.

A common feature in HHT patients is the presence of vascular lesions (telangiectases and AVMs) that lead to a loss of the intervening capillary network that connects the arteriole with the venule (24, 74). Interestingly, the frequency of pulmonary AVMs in HHT2 (8%) is far less than in HHT1 (45%) patients. Expression studies in lung vessels showed that endoglin and ALK1 have distinct expression profiles in the pulmonary vasculature and are only coexpressed in the distal (precapillary) arteries, distal veins, and capillaries, consistent with the tendency for pulmonary AVMs to form in the distal pulmonary vessels in HHT (113). Despite the important patho-

logical implications of these lesions, the mechanisms by which they are generated have not been fully elucidated. Because of the predominant expression of endoglin in ECs, it is tempting to speculate that an endoglin loss of function of the mutant allele in this cell type is the cause of the lesion. One of the functions described for endoglin is the protective role against apoptosis in ECs subjected to hypoxia and TGF- β stimuli (101). Thus endoglin haploinsufficiency in HHT may lead to a massive apoptosis in those capillary ECs where endoglin function is required for survival (Fig. 2). As a consequence of the EC apoptosis, the capillary network gradually disappears and only a preferential vessel remains that eventually becomes the arteriovenous shunt (Fig. 3). An interesting question that arises in HHT patients is why the vascular lesions appear only at distinct sites within certain organs, rather than being present throughout the body and in all organs/tissues. To explain this finding, one can postulate the need for an external trigger, or second hit, such as inflammation, infection, vascular injury, ischemia, or trauma that synergizes with endoglin haploinsufficiency to generate the lesion. Of note, these potential hits can upregulate endoglin expression (21, 34, 47, 90, 110, 146, 163, 167, 168), suggesting that endoglin function is required under those stressing conditions. Experimental support for the second hit hypothesis has been recently reported using a mouse with a conditional mutation in *Eng*, demonstrating that AVMs develop when an angiogenic stimulus is combined with endoglin depletion (112). Thus, in the HHT setting, endoglin protein levels may not reach the minimum threshold to achieve the optimal function and may be critical to generate the vascular lesion (Fig. 3).

Endoglin and Regulation of Angiogenesis

Angiogenesis is a complex and highly regulated physiological homeostatic process by which the body maintains the supply of oxygen and metabolites depending on the requirements of a given organ or tissue (3, 141). It involves the formation of new vessels with two separate, but coordinated, phases: activation and maturation. The whole process consists of a series of EC responses to angiogenic stimulation; including degradation of ECM, budding, proliferation, migration, tube formation, maturation, and maintenance of quiescent endothelium. It should be noted that ECs in normal quiescent endothelium have a very low turnover rate, with a doubling time of more than 1,000 days. Angiogenic endothelium, in contrast, has a rapid turnover, and it has been termed “activated” endothelium (85). In the activation phase, new sprouts form at distinct locations in the preexisting vessel. Sprout formation is initiated by EC activation, degradation of the ECM by ECs, followed by the development of a new bud from the EC layer. This bud will elongate by EC proliferation and migration toward the source of the angiogenic stimuli. The process culminates with tube formation and maturation. The maturation phase consists of a progressive decrease in EC proliferation and the recruitment of mesenchymal cells to form mural cells, which can be pericytes or VSMCs. Pericytes are thought to stabilize capillaries, whereas VSMCs are critical for arterial structure and function (3, 28, 76, 141).

These processes are driven by a complex interaction of different growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor, and TGF- β and their specific receptors. This complexity is eventually combined

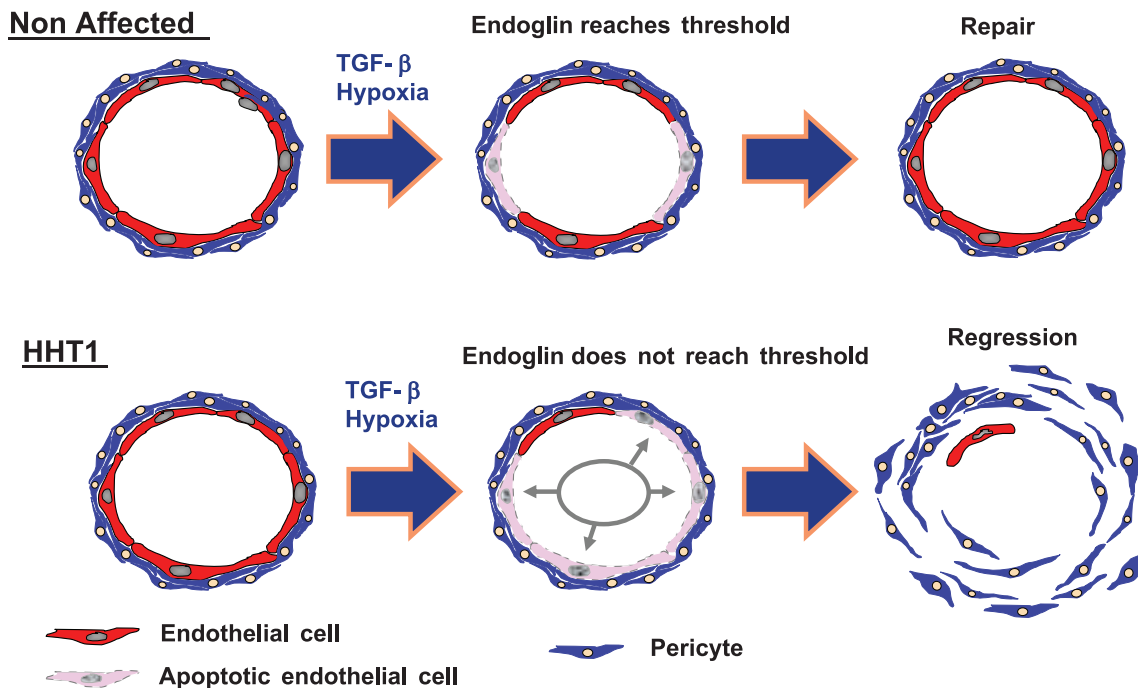


Fig. 2. Hypothetical model for the role of endoglin in endothelial cell apoptosis and its relevance in HHT vascular lesions. Under certain stimuli, such as TGF- β and hypoxia, endothelial cells undergo apoptosis that is prevented by the induced expression of endoglin in healthy subjects. In patients with hereditary hemorrhagic telangiectasia (HHT), endoglin haploinsufficiency may lead to a massive apoptosis in endothelial cells where endoglin function is required for survival leading to capillary regression. A cross section of an individual vessel is depicted with endothelial cells in red, pericytes in blue, and apoptotic endothelial cells in pink.

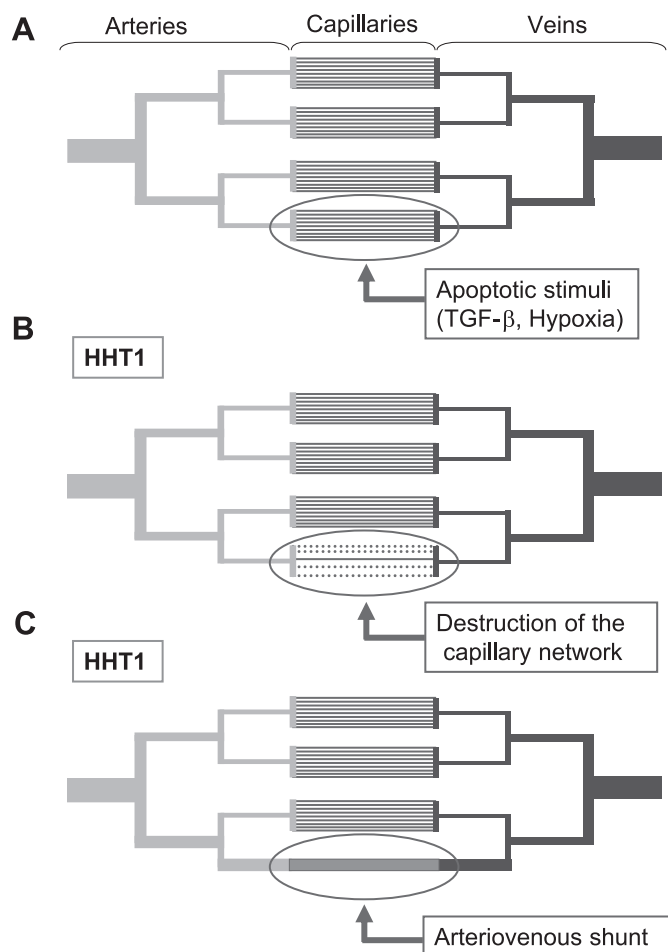


Fig. 3. Hypothetical model for the generation of arteriovenous malformations in HHT. The capillary network subjected to the apoptotic stimuli, as shown in Fig. 2, is not affected in normal subjects (A). However, in HHT patients (B and C), as a consequence of the endothelial cell apoptosis, the capillary network gradually disappears and only a preferential vessel remains that eventually becomes the arteriovenous shunt (C).

with other types of angiogenic stimuli such as hypoxia. The directionality of new vessel growth is driven by leading endothelial tip cells in response to guidance molecules. Recent studies have shown how endothelial tip, stalk, and phalanx cells form sprouts and how ECs arrange in tubular structures in a highly organized way (28). Platelet-derived growth factor signaling is important for the initial recruitment of mesenchymal cells that differentiate to VSMCs in response to TGF- β signaling. Both the stimulatory and inhibitory effects of TGF- β on angiogenesis have been reported (129, 157). Whether TGF- β stimulates or inhibits these processes depends on the experimental conditions. For example, low doses of TGF- β in vitro (0.25–0.50 ng/ml) stimulate EC proliferation and migration, whereas higher doses show an inhibitory effect, leading to EC quiescence (17, 68, 91).

A major evidence for the pivotal role of endoglin in angiogenesis is that mice lacking endoglin ($Eng^{-/-}$) die from cardiovascular defects at midgestation (E10.5–11.5) with major defects in yolk sac vasculature (6, 23, 103). In these embryos, the first stages of differentiation from hemangioblasts to ECs and the formation of the primitive vascular structures, a process called vasculogenesis, occurs normally, but this primitive

system does not develop to a mature vascular network (angiogenesis), indicating a critical role for endoglin in angiogenesis rather than in vasculogenesis (80, 131).

$Eng^{-/-}$ embryos also show a defective development of VSMCs (103), and several explanations have been proposed to account for this deficiency. First, the ectopic expression of endoglin in neural crest stem cells causes pericardial hemorrhage associated with altered smooth muscle cell investment in the walls of major vessels, suggesting a direct role for endoglin in myogenic differentiation (115). Alternatively, in the yolk sac, the absence of endoglin in ECs of $Eng^{-/-}$ mice results in a reduced availability of active TGF- β protein to promote the recruitment and the differentiation of mesenchymal cells into VSMCs, thus leading to weak vessel walls (29). More recently, it has been shown that endoglin, via the venous-specific marker COUPTFII, plays distinct and cell-autonomous roles in VSMC recruitment and arteriovenous specification in angiogenesis that may contribute to HHT (114).

Endoglin haploinsufficient ($Eng^{+/-}$) mice have been used to investigate the function of endoglin in adult neovascularization because these adult mice have endoglin protein levels reduced by 50%, as in HHT1 patients (23, 77, 162). $Eng^{+/-}$ mice have a delayed reperfusion following hindlimb ischemia induced by femoral ligation (79). Also, endoglin heterozygous mice implanted with Matrigel plugs, to measure EC outgrowth and invasion into the ECM in vivo, showed significantly less vascular structures compared with wild-type mice (79). Moreover, $Eng^{+/-}$ mice with myocardial infarction induced by coronary artery ligation showed a defective angiogenesis (168) in agreement with data from a femoral artery-ligated hindlimb model (79).

Endoglin and Tumor Angiogenesis

Most of the events occurring during “physiological” angiogenesis also occur during the process of tumor angiogenesis. Tumor vessels can exist either in an immature state (lack of mural cells) or in association with pericytes/VSMCs. Angiogenesis is a crucial process in tumor growth, as a continuous supply of oxygen and nutrients is necessary to support the anabolic cell metabolism involved in cell proliferation. Increased tumor size is accompanied by new vessel formation stimulated by hypoxia and by the increased metabolism necessary for tumoral cell growth. If angiogenesis is fully blocked, tumor size cannot reach more than a few cubic millimeters. Thus angiogenesis is considered as a major target for the treatment of tumors.

Endoglin is overexpressed in tumor vessels (13, 25, 49, 56, 119) but also in tumor cells (4, 13). Endoglin is a better marker of tumor capillary density than other classical markers of ECs, and the assessment of microvascular density with endoglin staining is a good prognostic indicator as it has been demonstrated that the density of endoglin-positive blood vessels negatively correlates with overall survival, disease-free survival, and metastasis in a great variety of solid tumors (reviewed in Ref. 13). Moreover, $Eng^{+/-}$ mice bearing subcutaneous lung carcinomas show decreased tumor growth rates and lower capillary densities compared with wild-type mice (50), suggesting endoglin involvement in tumor growth (Fig. 4). Given the high density of endoglin in tumor ECs, endoglin can be also used to target these vessels with anti-endoglin antibody-

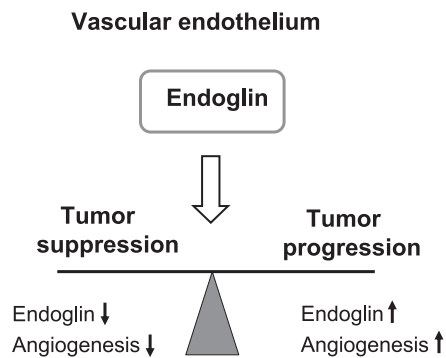


Fig. 4. Role of endoglin in tumor vascular endothelium. In vivo and in vitro studies support the involvement of endoglin in tumor suppression and progression, modulating angiogenesis and tumor proliferation. Increased endoglin expression correlates with increased tumor angiogenesis, probably because of the proangiogenic role of endoglin in endothelial cells.

ies, bound or not to toxic substances, to destroy tumoral vessels and limit tumor growth. In this regard, there are several reported approaches using experimental animal models (55, 117, 156, 164, 165). Among these, the approach of using naked anti-endoglin monoclonal antibodies as a tumor vascular targeting agent is clinically relevant. In fact, a multicenter phase I clinical trial using an anti-endoglin antibody (TRC105) has been recently approved by the United States Food and Drug Administration, and it is now in progress in patients with advanced and/or metastatic cancer (13).

Endoglin and Regulation of Vascular Repair

Mononuclear cells (MNCs) contribute to the formation of new blood vessels (7, 106, 140), and activated MNCs express upregulated levels of endoglin (87). These findings prompted van Laake et al. (168) to investigate whether endoglin expression in MNCs might regulate vascular repairing in an infarcted myocardium by injecting MNCs from healthy human donors or from HHT1 patients, which express half levels of endoglin. MNCs from healthy human donors significantly improved heart function in *Eng*^{+/-} mice, whereas MNCs from HHT1 patients were without significant effect (168). Because MNCs from HHT1 patients showed a reduced homing ability to infarcted mouse myocardium, the possible involvement of stromal cell-derived factor-1 α (SDF-1 α) and its corresponding chemokine receptor CXCR4, which are crucial for homing and are negatively influenced by CD26, were explored (132). Thus it was shown that a decreased homing of HHT1-MNCs is caused by an impaired ability of the cells to respond to SDF-1 α . Interestingly, modulating the dipeptidyl peptidase IV CD26 levels using inhibitors like diprotin A restored homing in cases where an increased expression of CD26 contributes to the underlying pathological mechanism (132).

The low angiogenic activity shown by *Eng*^{+/-} mice and the abnormal behavior of MNCs from HHT1 patients in the heart infarction experiments suggest a defective homing of endoglin-expressing circulating cells. VEGF, in addition to directly stimulating EC proliferation and migration, also induces the expression of SDF-1 α , a chemokine that regulates MNCs adherence to the vessel walls, where they can act in a paracrine fashion to enhance proliferation of resident ECs (70). It should be noted that VEGF production has been observed to be

decreased in ECs derived from *Eng*^{+/-} mice (79), and this finding could explain the defects in MNC recruitment observed in these animals. MNC adherence to the vessel wall is regulated by a family of membrane molecules called cell adhesion molecules (CAMs), including ICAM-1, VCAM-1, PECAM-1, and P-selectin. After renal ischemia-reperfusion, both CAM expression and MNC infiltration were reduced in *Eng*^{+/-} compared with wild-type mice (47). Thus a defective CAM expression in endoglin-deficient cells (either in endothelial or circulating cells) could also explain the low angiogenesis rate associated with endoglin deficiency. TGF- β also regulates the formation of podosomes that are highly dynamic structures involved in adhesion, migration, invasion, and circulating cell recruitment (104, 120). As reduced endoglin expression affects TGF- β signaling in monocytes (88), this may lead to a defective podosome formation and impaired recruitment to inflamed tissues.

Endoglin and Regulation of Vascular Tone

Mice deficient in endoglin (*Eng*^{+/-}) show a defective vasodilator response to endothelium-dependent vasodilator substances such as acetylcholine or bradykinin (77). In agreement with these data, *Eng*^{+/-} mice show a decreased NO synthesis and/or a decreased endothelial NO synthase (eNOS) expression (77, 78, 160). However, opposite results have been reported, in terms of vasodilation, using *Eng*^{+/-} mice. Thus Jerkic et al. (77) observed a defective vasodilator response to acetylcholine or bradykinin in the perfused hindlimb. By contrast, a decreased myogenic vasoconstrictory response and an increased endothelium-dependent vasodilatation in phenylephrine-precontracted mesenteric arteries have been reported (160). In addition, the same group has reported that in pulmonary arteries the endothelium-dependent relaxation in response to acetylcholine of adult *Eng*^{+/-} mice was higher than that of *Eng*^{+/+} control vessels (11). Accordingly, the interpretations provided to explain these opposite results are also different. On one hand, Jerkic et al. (77) postulate that the decreased vasodilatation in *Eng*^{+/-} mice is due to a decreased NO production as a consequence of decreased levels of eNOS. On the other hand, while agreeing with a reduced NO production in these animals, Toporsian et al. (160) hypothesize that endoglin haploinsufficiency in ECs leads to the uncoupling of eNOS that is associated with a defective eNOS/heat shock protein 90 (Hsp90) association and a subsequent decrease in NO release and increase in O₂⁻ production (160). Because of the increased levels of O₂⁻, these authors explain the increased vasodilatory response in *Eng*^{+/-} mice based on the fact that O₂⁻ has been reported to directly inhibit smooth muscle contraction in vitro (82) and that the H₂O₂, generated from O₂⁻ by dismutation, is a vasorelaxant in mouse mesenteric arteries. However, a wide number of studies have shown that O₂⁻ decreases endothelium-dependent vasodilatation as it removes NO upon chemical reaction, producing peroxynitrites (137). In addition, endogenous H₂O₂ may act as a vasoconstrictor in murine resistance vessels (154), although other studies have shown that H₂O₂ is a major endothelium-dependent relaxing factor in mice aorta (27) or cerebral arteries (48).

Because some of these studies were performed in perfused hindlimbs (77) and others in isolated mesenteric arteries (160), a possible explanation for these contradictory results is that the

responses and mechanisms involved in endothelium-dependent vascular relaxation may vary with the different vascular beds and the specific experimental approach. For instance, in mouse mesenteric arteries, a chronic rise in blood flow induces a diameter enlargement involving NO and O_2^- (37), a finding compatible with the myogenic response data of Toporsian et al. (160). Moreover, the infusion of mice with angiopoietin II, characterized by a hyperproduction of O_2^- and ONOO⁻, causes an impairment in the NO-mediated component of endothelium-dependent relaxation in response to acetylcholine. This inhibitory effect is mediated by increased O_2^- and ONOO⁻ in the VSMCs of mesenteric arteries (174), at variance with the increased endothelium-dependent vasodilatation of mesenteric arteries reported in *Eng*^{+/-} mice (160).

Regarding muscular arteries that are involved in the hindlimb perfusion experiments of *Eng*^{+/-} animals (77), it has been reported that mice fed a high-salt diet show an increased generation of O_2^- in the skeletal muscle microcirculation and an impaired endothelium-dependent dilation through reduced NO bioavailability. Specifically, arteriolar dilation in response to acetylcholine was ~50% smaller in high-salt mice than in normal-salt mice, whereas the inhibition of NO synthase (NOS) with *N*^G-monomethyl-L-arginine significantly reduced the resting diameters and responses to acetylcholine in normal-salt mice but not in high-salt mice (124). These data are in agreement with the defective vasodilator response to acetylcholine in the perfused hindlimb of *Eng*^{+/-} mice (77).

With respect to pulmonary arteries and the pathogenesis of pulmonary hypertension, an increased production of NO in eNOS transgenic mice prevented the increase in right ventricular systolic pressure, lung vascular remodeling, and right ventricular hypertrophy induced by chronic hypoxia, thus suggesting that a decreased eNOS production contributes to the pathogenesis of pulmonary hypertension (127). Furthermore, a decreased eNOS/Hsp90 interaction has been suggested to play a role in the pathogenesis of hypoxia-induced pulmonary hypertension on the basis of decreased eNOS activity and NO bioavailability (84, 122), a result that is in agreement with the pulmonary hypertension observed in *Eng*^{+/-} mice (161). However, a recent study in lungs of caveolin knockout mice showed that an increased eNOS/Hsp90 interaction was involved in the mechanism of pulmonary hypertension because of the persistent activation of eNOS and the resultant increased formation of ONOO⁻ (178). Thus high levels of O_2^- and ONOO⁻, similarly to those reported in *Eng*^{+/-} mice (160), decreased NO availability and led to the nitration of PKG, a critical mediator of the NO-dependent vasodilatation. Consequently, an impaired endothelium-dependent relaxation was observed (178), as opposed to the increased endothelium-dependent vasodilatation found in *Eng*^{+/-} mice (160), although both animal models share the pulmonary hypertension phenotype (161, 178).

Taken together, these data provide evidence that there is a dysregulated vascular tone in endoglin-deficient animals, while underlining the complexity in the regulation of the vascular tone by NO, O_2^- and their derivatives and in the pathways present in different vascular beds, which may explain the apparently discrepant results observed in the *Eng*^{+/-} mice by different groups.

TGF- β 1 leads to an increased vasodilatation in control mice that is severely impaired in *Eng*^{+/-} mice, suggesting the

involvement of endoglin in the TGF- β -regulated vascular homeostasis (147). The decreased vasodilatation shown by *Eng*^{+/-} mice is not associated with increased arterial pressure because these animals also show increased cyclooxygenase-2 (COX-2) expression and activity with the corresponding increase in the production of COX-2-derived vasodilator eicosanoids (78). Accordingly, the simultaneous inhibition of COX-2 and NOS markedly increases arterial pressure in *Eng*^{+/-} mice (78).

The altered vasodilator response in endoglin-deficient animals suggests a potential mechanism in the genesis of AVMs present in HHT1 patients. Under normal conditions, NO regulates the dilatation of precapillary sphincters. However, if this mechanism is impaired, the precapillary sphincters remain closed, whereas the blood circulates only through the preferential ways, lacking precapillary sphincters, existing in the capillary beds. This could lead to capillary EC apoptosis induced by hypoxia and may be reinforced by endoglin haploinsufficiency as in HHT1. At the same time, the preferential ways may react to the increased flow by widening their diameter and recruiting smooth muscle cells (arterialization), thus leading to the typical vascular malformations. This hypothesis is supported by the observation that adult *Eng*^{+/-} mice display pulmonary arterial hypertension accompanied by a rarefaction of peripheral vessels and dilatation of central large vessels (161). It should be noted that the lung is a frequent place for arteriovenous shunts in HHT1 patients (69, 74). Further support for a dysregulated vascular tone in HHT has been recently reported (11). Adult, but not newborn, *Eng*^{+/-} mice show pulmonary vascular eNOS uncoupling. In agreement with this finding, pulmonary arteries from adult *Eng*^{+/-} mice are more dilated and have an enhanced endothelium-dependent smooth muscle relaxation potential. This increased vasorelaxation may play a role in the formation of pulmonary AVMs later in life and could explain the generally late onset of pulmonary clinical manifestations in HHT (11). In summary, these results further support a role for endoglin in the regulation of vascular tone.

NO Mediates Endoglin Involvement in Angiogenesis and Vascular Homeostasis

NOS-derived NO is a major regulator of vascular tone and angiogenesis following arterial occlusion. The ischemic tissue shows an increase in eNOS mRNA, protein expression, and NO synthesis (77, 109). The deficiency of the NO pathway either by pharmacological inhibition or by gene disruption of eNOS diminishes ischemia-induced angiogenesis. Conversely, a supplementation of NO by the use of exogenous sources restores ischemia-induced angiogenesis. (109).

The transcription of eNOS is regulated by endothelial shear stress, hypoxia, several hormones, and various mediators and growth factors, including TGF- β 1. Endoglin plays a major role in regulating eNOS abundance and NO synthesis. The endoglin-dependent regulation of eNOS abundance seems to be based on two different mechanisms. First, endoglin regulates eNOS mRNA expression (77, 78, 147). Thus *Eng*^{+/-} mice and ECs derived from these mice show reduced levels of both basal and TGF- β 1-induced eNOS mRNA and protein levels, without changes in inducible or neuronal NOS (77, 78, 160). Furthermore, the ectopic expression of endoglin in ECs in vitro results

in increased levels of Smad2 protein, leading to an enhanced TGF- β receptor-dependent induction of eNOS mRNA expression (147). The second mechanism involved in the regulation by endoglin of eNOS abundance is the regulation of the half-life of eNOS protein and eNOS activity (160). In this regard, functional ECs lacking endoglin lose the capacity to generate NO in response to calcium-dependent eNOS activation. Thus endoglin associates with eNOS and Hsp90 and stabilizes the activation complex, resulting in NO production (Fig. 5A). In addition, eNOS activity was reported to be uncoupled in *Eng*-deficient murine ECs, as evidenced by a severely reduced eNOS/Hsp90 association and an increased eNOS-derived O_2^- , H_2O_2 , and, presumably, $ONOO^-$ production (Fig. 5B). Accordingly, it has been suggested that endoglin modulates the coupling of eNOS activity by acting as a scaffolding protein and bringing cytoplasmic Hsp90 into close proximity with caveolar eNOS (160). Furthermore, it has been reported that in *Eng*^{+/-} mouse tissues and in *Eng*^{-/-} cells, eNOS is uncoupled, leading to a decreased NO availability and increased O_2^- and H_2O_2 production (11, 161).

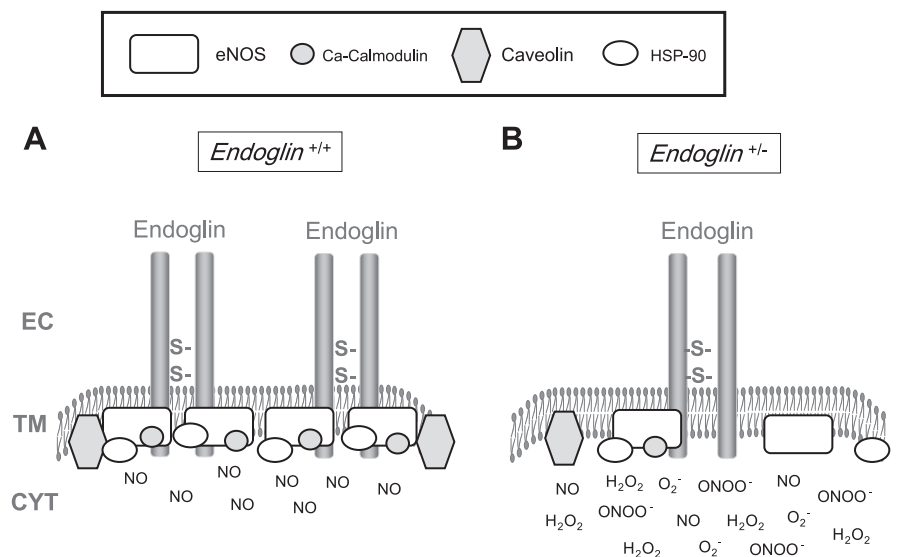
These findings suggest that endoglin expression and NO regulation are intimately related. Consequently, a major role for eNOS in endoglin-dependent angiogenesis and vascular tone has been postulated. A demonstration of the importance of NO in mediating the stimulation of angiogenesis by endoglin comes from the fact that a blockade of NO synthesis with *N*^ω-nitro-L-arginine methyl ester (L-NAME) decreases angiogenesis after hindlimb ischemia in normal mice but not in endoglin-deficient mice (79). Similar results were observed in a model of tumor-induced angiogenesis in *Eng*^{+/-} mice. In these mice, tumor growth and vessel density was lower than in control mice (50). Also, NO synthesis blockade with L-NAME induced a marked inhibition of angiogenesis in control mice that was much lower in *Eng*^{+/-} mice (A. Duwell, N. Eleno, C. Bernabeu, and J. M. López-Novoa; unpublished data). Taken together, these data support the hypothesis that eNOS-derived NO plays a major role in the endoglin-dependent regulation of angiogenesis and vascular tone.

Endoglin, the TGF- β Signaling Pathway, and Vascular Homeostasis

TGF- β superfamily members, including bone morphogenetic protein (BMP), activin, and TGF- β subfamilies, critically regulate many different processes within the cardiovascular system, including cardiac development and angiogenesis. The importance of TGF- β signaling in the cardiovascular system is underlined by the observation that the genetic deletion of several TGF- β family members, their receptors, or downstream signaling proteins in mice results in the death of most of the mutants because of severe defects in yolk sac vasculature formation (67). Also, alterations in this pathway, including either germ-line mutations or alterations in the expression of members of these signaling pathways, may lead to cardiovascular pathology (40, 62, 66, 157).

Members of the TGF- β superfamily signal through specific cell surface receptor complexes containing a heterodimeric association between signaling receptors types I and II and nonsignaling type III receptors or coreceptors. The receptors types I and II are serine/threonine kinases and are involved in the downstream signaling, whereas the coreceptors, including endoglin and betaglycan, are proteins without known signaling motifs. The core TGF- β signaling pathway comprises at least seven type I (also known as ALK receptors) and five type II receptors, where type I acts downstream of type II and whose combinatorial heterodimeric association determines the specificity of the ligand signaling. The type I receptors include BMP (ALK1, ALK2, ALK3, and ALK6), activin (ALK1, ALK2, and ALK4), and TGF- β (ALK1, ALK2, and ALK5) receptors. Upon ligand binding, the type II receptor transphosphorylates the type I receptor, which subsequently propagates the signal by phosphorylating the receptor-regulated Smad (R-Smad; and Smad1, -2, -3, -5, and -8) family of proteins. Once phosphorylated, R-Smads form heteromeric complexes with a cooperating homolog named Co-Smad (Smad4) and translocate into the nucleus where they regulate the transcriptional activity of target genes. (62, 116, 157).

Fig. 5. Hypothetical model on the role of endoglin in endothelial nitric oxide (NO) synthase (eNOS) expression and activation. **A:** in *Endoglin*^{+/+} cells, a pool of endoglin resides in caveolae, a cholesterol-rich plasma membrane domain containing caveolin and eNOS. Endoglin acts as a molecular scaffold facilitating calcium-bound calmodulin and heat shock protein-90 (Hsp90) association to eNOS during endothelial activation (160), thus producing normal amounts of NO and the consequent vasodilatation. **B:** in endoglin haploinsufficiency (*Endoglin*^{+/-}), both the amount of eNOS (77, 78) and the eNOS/Hsp90/calmodulin association are reduced, leading to decreased NO as well as increased eNOS uncoupling and formation of eNOS-derived superoxide anion (O_2^-) (160). In turn, O_2^- produces hydrogen peroxide (H_2O_2) by dismutation (11, 161) and presumably reacts with NO to generate peroxynitrite ($ONOO^-$). Thus the removal of the vasodilator NO and the presence of oxygen free radicals results in an impaired vasomotor tone.



Endoglin forms a protein complex with the TGF- β type I (ALK1 and ALK5) and type II receptors and the ligand (14, 30, 73). Several members of the TGF- β superfamily, including TGF- β 1 and TGF- β 3 (but not TGF- β 2) activin-A, BMP-7, and BMP-2, are able to bind endoglin, and this binding requires the presence of the corresponding signaling receptors (8, 30, 96). By contrast, endoglin is able to bind BMP-9 in the absence of signaling receptors (149), in agreement with the endoglin-dependent increase of the cellular response to BMP-9 (40). Interestingly, BMP-9 has also been shown to be a specific ligand of ALK1. Among the BMP-9-dependent effects are the inhibition of EC proliferation and migration in vitro, as well as the inhibition of neoangiogenesis in vivo (40–42, 166). These BMP-9 effects appear to be mediated by a receptor complex formed by ALK1, the BMP receptor type II, and endoglin (40). Endoglin modulates ligand binding and signaling by an association with ALK1 and ALK5. These type I receptors activate signaling pathways via Smad1, -5, and -8 (ALK1) or Smad2 and -3 (ALK5) to regulate, among others, the proangiogenic inhibitor of DNA binding 1 (Id1) or PAI-1 target genes, respectively. The balance between ALK1 and ALK5 signaling pathways in ECs and VSMCs plays a crucial role during vascular remodeling and angiogenesis, although the exact molecular mechanisms remain to be elucidated (41, 93, 126, 151, 157). In the ALK1/ALK5 setting, endoglin inhibits the TGF- β /ALK5/Smad3-mediated cellular responses (15, 71, 92, 96, 150, 169) and enhances ALK5/Smad2-mediated responses (29, 73, 147). In addition, endoglin promotes TGF- β 1/ALK1 (15, 92) and BMP-9/ALK1 (40) signaling in ECs. Also, endoglin enhances the BMP-7 signal via Smad1/Smad5 pathway in myoblasts (150). Thus endoglin appears to be a critical modulator of the balance between ALK1 and ALK5 signaling (92). The mechanism by which endoglin potentiates TGF- β /ALK1 signaling involves the direct association of ALK1 with the cytoplasmic and extracellular domains of endoglin, whereas the extracellular domain mediates the enhancement of ALK1 signaling (15). These studies support the view that endoglin and ALK1 participate in a common signaling pathway that is critical for EC responses to TGF- β family members. This conclusion agrees with the fact that pathogenic mutations in endoglin or ALK1 genes result in HHT (1) and that ALK1 and endoglin null mice have similar vascular phenotypes (6, 23, 103, 126, 153). The extracellular and cytoplasmic domains of endoglin also interact with ALK5 and the type II receptor, but ALK5 interacts with the endoglin cytoplasmic domain only when the kinase domain is inactive. Upon association, ALK5 and the type II receptor phosphorylate the endoglin cytoplasmic domain; ALK5, but not the type II receptor, then dissociates from the complex (73). These data suggest the hypothesis that the extracellular and cytoplasmic domains of endoglin play distinct roles in receptor signaling that are downstream of ligand binding and receptor activation.

Role of Other Endoglin Forms in Vascular Physiopathology

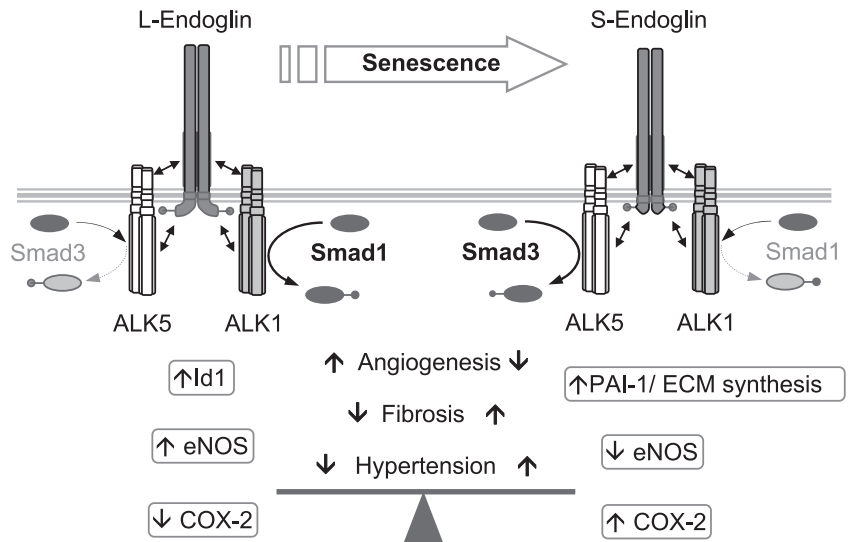
S-endoglin isoform. The endoglin data described in the previous sections of this review are referred to the most abundant form of endoglin, the membrane-bound full-length L-endoglin. However, another isoform of membrane endoglin has been described, which is generated by an alternative splicing of the same gene, giving rise to S-endoglin (12, 130).

In humans, S-endoglin protein contains a cytoplasmic domain that is 33 amino acids shorter than that of L-endoglin (Fig. 1B). Comparative studies between L-endoglin and S-endoglin have revealed distinct functions for each isoform. Thus S-endoglin seems to have an antiangiogenic effect, in contrast to the proangiogenic role attributed to L-endoglin. Mice transgenic for human S-endoglin (170) exhibit a deficient angiogenic phenotype that drives to a significant delay in tumor growth (130), similar to that shown by mice deficient in L-endoglin (50, 136). S-endoglin is also involved in the senescence of ECs. The ratio of S-endoglin to L-endoglin isoforms is increased during the senescence of human ECs in vitro, as well as during the aging of mice in vascularized tissues (14). Furthermore, transgenic mice overexpressing S-endoglin in ECs showed hypertension, decreased hypertensive response to NO inhibition, decreased vasodilatory response to TGF- β 1, and decreased eNOS expression in lungs and kidneys, supporting the involvement of S-endoglin in the NO-dependent vascular homeostasis (14). These results suggest that S-endoglin is induced during endothelial senescence and may contribute to age-dependent vascular pathology.

Signaling by S-endoglin seems to be also different from that by L-endoglin. In myoblasts and ECs, L-endoglin enhanced the ALK1/Id1 pathway, whereas S-endoglin promoted the ALK5/PAI-1 route (14, 169). These effects on signaling are supported by the biological effects on TGF- β 1-induced collagen-I expression and the inhibition of cell proliferation. Thus, whereas L-endoglin decreased TGF- β 1-induced collagen-I and connective tissue growth factor expression and increased TGF- β 1-induced proliferation, S-endoglin strongly increased TGF- β 1-induced collagen-I and connective tissue growth factor expression and reduced TGF- β 1-induced cell proliferation (169). The mechanism underlying the different behavior of S- and L-endoglin might reside in their different interaction with the signaling TGF- β receptors. In ECs, S-endoglin interacts with both ALK5 and ALK1, although the interaction with ALK5 is stronger than with ALK1 (14), at variance with L-endoglin that shows a higher affinity for ALK1 versus ALK5 (15). Moreover, S-endoglin behaves differently than L-endoglin in relation to several TGF- β -responsive specific reporter constructs with different specificities. Thus S-endoglin expression increased the ALK5 signaling pathway, whereas L-endoglin inhibited the same pathway. On the other hand, L-endoglin, but not S-endoglin, stimulated the ALK1 signaling pathway (14, 169). These results suggest that the S-endoglin-to-L-endoglin ratio in ECs may contribute to balancing the TGF- β signal through ALK5 or ALK1 and their important roles in vascular pathophysiology (Fig. 6).

A role for S-endoglin in the regulation of vascular tone has been postulated. Mice transgenic for human S-endoglin (*S-Eng*⁺) show a defective NO synthesis and a decreased eNOS expression in lungs and kidneys (14), in contrast with the positive relationship between levels of L-endoglin and eNOS previously reported in both mice and cultured ECs (77, 78, 160). However, at variance with *Eng*^{+/-} mice, in which the arterial pressure was normal, *S-Eng*⁺ mice were hypertensive (14). Furthermore, in both cultured ECs and endoglin-deficient mice, low levels of L-endoglin are associated with high COX-2 expression (77), and this increased COX-2 expression is also found in tissues from *S-Eng*⁺ mice (14). Moreover, *S-Eng*⁺ transgenic mice show a reduced hypotensive response to

Fig. 6. Hypothetical model of S-endoglin functions during endothelial senescence. In the normal state, the TGF- β response is modulated by L-endoglin, but upon senescence of endothelial cells, S-endoglin is upregulated, interacting with the TGF- β receptor complex containing activin-like kinase 1 (ALK1) and ALK5. As a consequence of this interaction, S-endoglin regulates the expression of different target genes including *plasminogen activator inhibitor type 1* (PAI-1), *inhibitor of DNA binding 1* (Id1), *eNOS*, and *cyclooxygenase-2* (COX-2). Thus S-endoglin allows a switch that triggers the cardiovascular pathology: 1) up-regulation of PAI-1/extracellular matrix (ECM) synthesis may lead to increased fibrosis, 2) downregulation of Id1 is associated with decreased angiogenesis, and 3) downregulation of eNOS and upregulation of COX-2 are involved in endothelial dysfunction and impaired vascular relaxation. The involvement of TGF- β receptor type II (TBRII), TGF- β , and S-endoglin/L-endoglin heterodimers has been omitted for simplification. Adapted from Blanco et al. (14) and used with permission.



TGF- β 1 administration (14). This observation is in agreement with the finding that TGF- β 1 regulates eNOS expression (148) and induces vasodilatation in wild-type mice, this vasodilatation being severely impaired in *Eng*^{+/-} mice (147). Thus an in vivo overexpression of S-endoglin appears to result in the same phenotype as L-endoglin deficiency, suggesting opposing functional effects of both isoforms on the NO and COX-2 systems.

Soluble endoglin. A soluble form of endoglin (sEng) has been detected by ELISA and Western blot analysis in plasma, serum, and urine from patients with different pathologies, including preeclampsia and cancer. Circulating sEng has been purified from preeclampsia patients, and its partial peptide sequence suggests that it is an NH₂-terminal cleavage product of full-length membrane-bound endoglin (172).

Preeclampsia is a systemic syndrome of pregnancy clinically characterized by a new onset of proteinuria and hypertension associated with significant morbidity and mortality to both mothers and fetuses. In these patients, sEng plasma levels are upregulated in a pattern similar to that of a soluble VEGF receptor known as soluble fms-like tyrosine kinase-1 (sFlt1) (97, 172). Interestingly, levels of cell surface endoglin are significantly increased in preeclamptic placentas (97). Because endoglin is expressed at high levels in the syncytiotrophoblast and invading cytotrophoblasts (65) and membrane-type metalloprotease-1 (MT1-MMP or MMP-14) is expressed in trophoblasts (175), it has been postulated that increased sEng levels in preeclampsia derive from the proteolytic action of MT1-MMP on the full-length membrane-bound endoglin expressed in trophoblasts or in nearby cells (107) (Fig. 7). Experimental support for this hypothesis has been recently reported, demonstrating that MT1-MMP is in fact a major endoglin-shedding protease (75). This is compatible with the fact that β -glycan, another TGF- β coreceptor with a partial sequence homology to endoglin, can be shed by MT1-MMP (171). Of note, increased levels of membrane-bound endoglin have also been reported in circulating cells of women with preeclampsia, thus suggesting that this is not only a local, but a systemic, endoglin upregulation (134). It has been shown that uterine ischemia and/or hypoxia plays a major role in increased sEng release. In an experimental model with pregnant rats, placental ischemia induced by a reduction of uterine perfusion pressure increases

the expression of sEng and provokes hypertension, thus mimicking the pathophysiological features of preeclampsia (61). Compatible with these findings, women with preeclampsia have alterations in placental HIF-1 and its targets (138), and hypoxia has been shown to upregulate the expression and secretion of sFlt1 protein in primary trophoblast cultures from first-trimester placentas (123). Hypoxia has been shown to upregulate endoglin expression in ECs (146, 179), although this upregulation was not observed in cultured villous trophoblasts (121). Preeclampsia has been attributed to increased oxidative stress in the placenta (139), whereas heme oxygenase-1 (HO-1) and its metabolite carbon monoxide exert protective effects against oxidative stimuli (39). In this regard, it has been reported that an overexpression of HO-1 in ECs inhibited VEGF-mediated sFlt-1 release and interferon- γ - and tumor necrosis factor- α -induced sEng release, whereas HO-1 inhibition potentiated sFlt-1 and sEng production from ECs and placental villous explants. Furthermore, mice lacking HO-1 produced higher levels of sFlt-1 and sEng compared with wild-type mice (39).

In addition to being a reliable biomarker of the disease, it has been suggested that sEng plays a major role as an antiangiogenic

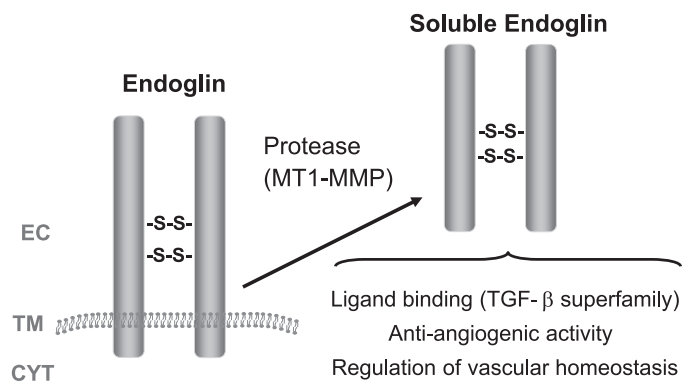


Fig. 7. Generation of soluble endoglin by proteolytic processing of membrane-bound endoglin. A recent report suggests that membrane-type metalloprotease-1 (MT1-MMP) is a major endoglin-shedding protease acting on the juxtamembrane region and leading to the secretion of the large ectodomain of endoglin (75). Several functions reported for soluble endoglin are indicated.

genic factor in preeclampsia. Thus sEng amplifies the vascular damage mediated by sFlt1 in pregnant rats, inducing a severe preeclampsia-like syndrome with features of the hemolysis-elevated liver enzymes and low platelet syndrome (172). Moreover, an overexpression of sFlt1 and sEng in rodents was found to induce focal vasospasm, hypertension, and increased vascular permeability that were associated with brain edema, producing images reminiscent of reversible posterior leukoencephalopathy associated with human eclampsia (111). This effect may be mediated by the interference of the NO-mediated vasodilation. In vitro studies demonstrate that sEng impairs EC proliferation and capillary formation (172). Interestingly, the angiogenic process is disturbed in preeclamptic placentas, thus suggesting that sEng has antiangiogenic properties. Compatible with this finding, it has been shown that an injection of sEng in rats induced hypertension (172). To explain the mechanism involved, it has been proposed that sEng plays its antiangiogenic and prohypertensive effects through an interaction with circulating endoglin-binding molecules, such as the TGF- β protein superfamily, thus preventing the binding of these molecules to the cell membrane TGF- β receptor complex (108). In fact, in vitro studies have shown that sEng inhibits TGF- β 1 signaling and competes for TGF- β 1 binding to its receptors, abolishing ALK5 signaling-dependent responses in ECs and consequently the proangiogenic effects of TGF- β 1 in the normal endothelium (172). Of note, the short form of membrane endoglin (S-endoglin), with only 14 amino acids in its cytoplasmic domain, shows opposite effects to the proangiogenic L-endoglin isoform (14, 130, 169), supporting a critical role for the extracellular domain in this process. Another possible mechanism involved in the antiangiogenic effects of sEng is based on its inhibitory effect on TGF- β 1-mediated eNOS activation in ECs (172). Thus, while there is an increased NO synthesis in pregnant rats, evidence for NO deficiency in preeclampsia has been obtained from experimental rat models of preeclampsia. Endothelial dysfunction has been reported in women with preeclampsia. Accordingly, it has been postulated that preeclampsia is a disease with a major endothelial dysfunction component that plays a role in preeclampsia-associated hypertension (43, 173). Endothelial dysfunction in preeclampsia has also been attributed to placental oxidative stress, the excess production of damaging reactive oxygen species, as markers of high oxidative stress are detected in preeclamptic placentas (134). A key animal model of preeclampsia is produced by infusion of the NO synthesis inhibitor of L-NAME in pregnant rats, leading to hypertension, proteinuria, and thrombocytopenia (176). As described in *NO Mediates Endoglin Involvement in Angiogenesis and Vascular Homeostasis*, decreased NO synthesis is associated with decreased angiogenesis, as occurs in endoglin-deficient mice (77, 78, 147), suggesting that sEng behaves as an antagonist of L-endoglin in endothelium-dependent responses such as angiogenesis. Supporting this view, sEng inhibits the proangiogenic activity of VEGF, another protein that plays a major role in angiogenesis (54, 75).

sEng also seems to be a regulator of vascular tone. It has been reported that the administration of sEng to mice induces an increase in arterial pressure by increasing vascular resistance (172). Most probably, this effect could be attributed to the inhibitory effect of sEng on TGF- β 1-mediated eNOS activation in ECs, and it has been suggested that high levels of

circulating sEng might contribute to the hypertension shown by women with preeclampsia (172). Thus these data suggest that sEng behaves as an antagonist of L-endoglin in endothelium-dependent responses such as vasodilatation and angiogenesis.

Recently, a role for sEng in brain AVMs has been postulated. Thus patients with brain AVMs had higher mean sEng levels compared with controls (32). To determine whether sEng affects the vasculature of the adult mouse brain, an injection of adenovirus-expressing human sEng into brain areas previously exposed to adeno-associated virus expressing VEGF was carried out. Indeed, an increased number of dysplastic blood vessels associated with increased membrane-type matrix metalloprotease-2 (MMP-2) and MMP-9 activity were revealed in the sEng-treated animals (172). These results suggest that elevated sEng may play a role in the generation of sporadic brain AVMs and may provide new targets for a therapeutic intervention in patients with brain AVMs.

In addition to preeclampsia and brain AVMs, altered sEng levels have been reported in several pathologies such as cancer (13, 26, 99), atherosclerosis and coronary artery disease (16, 102), hepatitis (34), diabetes (5), systemic sclerosis (44, 58), malaria (45), biliary atresia (133), or sickle cell disease (86). Interestingly, a number of laboratories have reported increased levels of sEng in serum, plasma, or other fluids from cancer patients as a marker of poor prognosis [reviewed by Fonsatti et al. (56) and Bernabeu et al. (13)]. Also, it has been reported that changes of sEng plasma levels after an acute myocardial infarct are accurate predictors of acute mortality in these patients (38). Although there are numerous reports on the possible participation of sEng in different diseases, the specific molecular mechanism of action of this soluble form of endoglin in these pathologies remains to be elucidated.

Taken together, these results suggest that both the membrane-bound short isoform (S-endoglin) and the soluble endoglin are involved in several pathological conditions and play opposite roles with respect to the predominant membrane-bound L-endoglin isoform.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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