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REVIEW ARTICLE

Novel vascular roles of human endoglin in pathophysiology

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Abstract

Endoglin, alias CD105, is a human membrane glycoprotein highly expressed in vascular endothelial cells. It is involved in angiogenesis and angiogenesis-related diseases, including the rare vascular pathology known as hereditary hemorrhagic telangiectasia type 1. Although endoglin acts as an accessory receptor for members of the transforming growth factor- β family, in recent years, emerging evidence has shown a novel functional role for this protein beyond the transforming growth factor- β system. In fact, endoglin has been found to be an integrin counterreceptor involved in endothelial cell adhesion processes during pathological inflammatory conditions and primary hemostasis. Furthermore, a circulating form of endoglin, also named as soluble endoglin, whose levels are abnormally increased in different pathological conditions, such as preeclampsia, seems to act as an antagonist of membrane-bound endoglin and as a competitor of the fibrinogen-integrin interaction in platelet-dependent thrombus formation. These studies suggest that membrane-bound endoglin and circulating endoglin are important components involved in vascular homeostasis and hemostasis.

KEYWORDS

cell adhesion, endoglin, hemostasis, HHT, integrins, platelets, vascular homeostasis

1 | INTRODUCTION

Endoglin (Eng), also known as CD105, is a membrane glycoprotein expressed on the endothelial cell (EC) surface [1–3]. In humans, it is encoded by the *ENDOGLIN* gene (*ENG*) located on chromosome 9q34.11 [4]. Mutations in *ENG* are responsible for hereditary hemorrhagic telangiectasia type 1 (HHT1; Mendelian Inheritance in Man 131195), a vascular disease characterized by arteriovenous malformations (AVMs), telangiectases, and epistaxis [5–7]. HHT1 is one of the 2 most common forms of hereditary hemorrhagic telangiectasia (HHT) [8,9], and in spite of the great advances in the clinical and molecular diagnosis of the disease as well as in the understanding of the pathogenic mechanisms involved using molecular, cellular, and animal models, the current treatments for patients with HHT remain at the palliative level [5–7].

Endoglin has been found not only on mature ECs but also on endothelial progenitor cells [10] and syncytiotrophoblasts [11] and at lower levels in macrophages [12], T lymphocytes [13,14], epithelial cells, fibroblasts, smooth muscle cells, and minor subsets of the hematopoietic lineage [1,15,16]. Interestingly, Eng is also one of the essential markers for mesenchymal stromal cell (MSC) identification [17]. An upregulated expression of endothelial endoglin has been observed in neoangiogenic vessels during inflammation and in solid tumors, a finding compatible with its involvement in cardiovascular diseases and cancer [1]. Moreover, endoglin is not expressed by platelets [18,19], but endothelial endoglin has been reported to be involved in platelet-dependent hemostasis [20]. Functionally and structurally, the extracellular part of endoglin contains 2 separate regions: (i) the juxtamembrane zona pellucida domain (ZPD) involved

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in integrin binding [10,21,22] and (ii) the orphan domain responsible for the interaction with the physiological ligands BMP9 and BMP10 of the transforming growth factor- β (TGF- β) family [23,24].

In addition to membrane-bound endoglin, there is a circulating form of endoglin, also named as soluble endoglin (sEng), that can be generated upon catalytic activation of at least the metalloproteases (MMPs) MMP-12 and MMP-14, which are known to be upregulated during inflammation [25-27]. Of note, a correlation between increased circulating levels of sEng with severity of preeclampsia, and the development of a rare life-threatening pregnancy complication of preeclampsia named hemolysis, elevated liver enzymes, and low platelets (HELLP) has been reported [28–31]. Also, the pathogenic role of sEng in preeclampsia-associated hypertension and renal involvement has been demonstrated in several in vivo studies [32-35]. Abnormal high levels of sEng are also found in other cardiovascularrelated conditions like atherosclerosis, hypercholesterolemia [3,36], diabetes mellitus [37–39], diabetic retinopathy [40], hypertension [37], circulatory failure in septic shock syndrome [41], coronary artery disease [42-44], acute myocardial infarction [45], and reperfusion after cerebral large-vessel occlusion [46]. Recently, sEng was also found to be increased in Carmat total artificial heart (C-TAH), where it appears to contribute to microvascular dysfunction [47].

Despite the recognized involvement of membrane-bound and circulating endoglin in cardiovascular-related conditions, their molecular mechanisms of action are still unclear. While most of the functional studies have analyzed the role of endoglin only as an auxiliary receptor of the TGF- β family of cytokines, mainly BMP9 and BMP10, the aim of this review is to highlight the new role of both endoglin proteins as integrin counterreceptors and the impact of this function in vascular homeostasis and hemostasis.

1.1 | Endoglin in the inflammatory setting and its function in cell adhesion and transmigration

Basal endoglin expression levels vary in the vascular endothelium of different tissues, whereas they are clearly upregulated in ECs from inflamed tissues with an associated inflammatory cell infiltrate [48]. Furthermore, an inflammation model of dextran sodium sulfate-induced chronic colitis revealed more leukocyte infiltration in the gut and a more severe colitis phenotype in endoglin heterozygous ($Eng^{+/-}$) mice than in control animals [49–51]. These findings prompted us to investigate the role of endoglin under the inflammatory setting.

We first studied an $Eng^{+/-}$ mouse model [52] in which inflammation was induced using carrageenan or lipopolysaccharide. In wildtype (WT) control animals (Eng+/+), we observed a reduction in leukocyte numbers in blood and a concomitant increase in peritoneal fluid and lung parenchyma, while these inflammation-dependent parameters were only mildly changed in $Eng^{+/-}$ mice [53]. These data suggested the involvement of endoglin in leukocyte adhesion, a process in which integrins are heavily engaged [54]. Supporting this hypothesis, the protein primary sequence of human endoglin contains an arginine-glycine-aspartic acid (RGD) motif, known to be recognized by

Essentials

- Membrane-bound endothelial endoglin (Eng) binds to integrin α5β1 of leukocytes, regulating their trafficking.
- Eng and its circulating form (sEng) are involved in hemostasis and thromboinflammatory processes.
- sEng binds to integrin αllbβ3 of platelets, interfering thrombus formation and stabilization.
- Eng and sEng are implicated in angiogenesis, revascularization, and cell permeability.

integrins [55], and already highlighted by Michelle Letarte's group in the 1990s [56]. Using truncated endoglin constructs, we showed that the ZPD, encompassing the RGD motif, is involved in integrin binding [22]. In addition, the active role of the RGD motif in endoglinmediated cell adhesion was supported by the following: (i) in vitro experiments using human endoglin mutated on the RGD sequence (ie, arginine-glycine-alanine) [22]; (ii) inhibition of endoglin-dependent cellular adhesion and transmigration in the presence of peptides containing the RGD sequence [22,53]; and (iii) identification of endoglin binding integrins $\alpha 5\beta 1$ and $\alpha IIb\beta 3$, both belonging to the RGD subtype of integrins [20,53,57]. While the RGD motif of endoglin appears to be involved in the binding to integrins, it should be noted that additional sequences of the ZPD are implicated and needed to fulfill the optimal endoglin-integrin binding. Moreover, the endoglin binding to non-RGD subtype integrin family members cannot be excluded [22].

We were able to demonstrate for the first time that under inflammatory conditions endothelial endoglin is involved in leucocyte adhesion and transmigration through its interaction at least with the activated integrin $\alpha 5\beta 1$ [53], a fibronectin receptor abundantly expressed on the majority of leukocyte populations, including lymphocytes, monocytes, and granulocytes [54]. Furthermore, α 5 β 1 integrin is one of the major adhesion molecules of human hematopoietic progenitor cells. Among these, granulocyte colony-stimulating factormobilized peripheral blood progenitor cells are the most important source for clinical hematopoietic cell transplantation today [58]. It should be noted that the inflammatory setting contains certain cytokines that may promote an optimal endothelial-endoglin/leukocyteintegrin interaction. Thus, the chemokine CXCL12 can activate integrins to properly bind endoglin [53]. In addition, tumor necrosis factor- α (TNF- α) has been found to significantly increase the expression of α 5 β 1 integrin on monocytes [59], likely facilitating their binding to endoglin in ECs. TNF- α also seems to induce a redistribution on the EC surface, concentrating endoglin in cell-cell contacts [53]. These data provide evidence that endothelial endoglin participates in leukocyte adhesion and transmigration during inflammation (Figure 1A, B), a highly regulated and complex process sensitive to the action of cytokines and in which many other cell surface receptors, including integrins, are also involved [54]. Among the 24 different α/β heterodimeric integrins,

FIGURE 1 Involvement of membrane endoglin in cell adhesion. (A) Scheme of a blood vessel stimulated by inflammation. (B) Detail of leukocyte transmigration after activation with cytokines, involving homodimeric endoglin-integrin $\beta 1$ ($\alpha 5\beta 1$) interaction. For simplicity, all the other molecules involved in leukocyte rolling and adhesion, whose existence and role are known, have been omitted for simplification. (C) Inflammation can be one of the second hits inducing arteriovenous malformations. Created with BioRender. com.



assembled between 8 β subunits and 18 α subunits [55], representative players in the leukocyte adhesion and extravasation cascade include the β 2-integrin family, such as the α L β 2 (CD11a/CD18) and α M β 2 (CD11b/ CD18) integrins, or the β 1-integrin family, such as the α 4 β 1 (CD49d/ CD29; VLA-4) and α 5 β 1 integrins [53,54,60]. While the interaction between endoglin and α 5 β 1 integrin has been reported, it will be of interest to investigate whether endoglin can also interact with the other β 2- and β 1-integrin family members.

Inflammation has been postulated as a second hit to explain the existence of localized vascular lesions over a systemic background of germinal *ENG* mutations in HHT1 [61]. As inflammation-induced leukocyte infiltration is a process required for proper vascular remodeling, when endoglin protein levels do not reach a minimum threshold to achieve its function as an integrin counterreceptor, endoglin haploinsufficiency may impair this process [62]. Therefore, as a consequence of a defective vascular repair/remodeling, the capillary network gradually disappears and only a preferential vessel remains that eventually becomes the arteriovenous shunt (Figure 1C).

1.2 | Membrane-bound endoglin in hemostasis

In 2018, we demonstrated that membrane-bound endoglin may act as an adhesion molecule involved in the interaction between ECs and platelets through integrin recognition [20]. Thus, the extracellular domain of human endoglin promotes specific platelet adhesion under static conditions and also confers resistance of adherent platelets to detachment upon exposure to flow. Moreover, platelets adhere to confluent ECs in an endoglin-mediated process owing to the interaction between endoglin and platelet integrin α IIb β 3 by a mechanism involving the RGD motif of endoglin. Remarkably, platelets from patients with Glanzmann's thrombasthenia lacking the α IIb β 3 integrin [63] showed a significant reduction of adherence to human ECs (~70%) or endoglin-coated plates (~80%) compared with control healthy platelets, suggesting a defective endoglin-dependent adhesion to ECs [20]. The above experimental evidence led us to postulate the importance of the binding between endoglin and the α IIb β 3 integrin in hemostasis [20], mainly defined by α IIb β 3, which is a platelet-specific integrin that plays a key role in platelet functions [64]. This hypothesis could explain the bleeding severity seen in patients with HHT. Thus, while bleeding in HHT is mainly due to the rupture of the fragile nasal and gastrointestinal telangiectases, additional involvement of abnormal hemostasis has been proposed in HHT animal models of the disease [20,65]. The 2 major target genes in HHT are ENG and ALK1, giving rise to HHT1 and HHT type 2 (HHT2) variants, respectively; both are characterized by fragile mucocutaneous telangiectases that frequently break causing recurrent epistaxis and gastrointestinal bleeding [7-9]. As measured by an Epistaxis Severity Score, it was found that patients with HHT1 and HHT2 who experience severe bleeding do not have prolonged clotting times or alterations in clotting factors [65]. In addition, we showed that bleeding time, but not prothrombin time, is significantly prolonged in endoglin-haplodeficient $(Eng^{+/-})$ mice compared with $Eng^{+/+}$ animals [20]. As coagulation is only one of the processes involved in hemostasis, other hemostasis mechanisms in HHT1 ($Eng^{+/-}$) and HHT2 ($Alk1^{+/-}$) mouse models, which do not show HHT vascular phenotypes in the context of spontaneous bleeding, were investigated [65]. The comparative study in terms of bleeding between HHT1 and HHT2 animal models revealed distinct behaviors. In Eng^{+/-} mice, the results of in vivo and in vitro assays suggested deficient platelet-endothelium interactions that impair robust and stable thrombus formation. Consequently, the thrombus could be torn off and dragged by the mechanical force exerted by the bloodstream, leading to reappearance of hemorrhages [20,65]. In contrast, in $Alk1^{+/-}$ mice, overactivation of the fibrinolysis system was observed. These results support the idea that endoglin and Alk1 haploinsufficiency leads to a common phenotype of impaired hemostasis, but through different mechanisms [65].

Furthermore, thanks to the generation and characteristics of the transgenic mice ubiquitously overexpressing human endoglin (*ENG*⁺) [66], we showed that *ENG*⁺ mice complete a stable carotid occlusion after FeCl₃ administration at a faster pace than their control $Eng^{+/+}$ animals or $Eng^{+/-}$ mice [65]. In addition, a significantly increased



FIGURE 2 Membrane endoglin implication in platelet adhesion. (A) Scheme of a blood vessel in which a lesion is present in an endothelium where endoglin is normally expressed (wild-type mice, $Eng^{+/+}$). Endothelial endoglin is involved in platelet rolling and thrombus stabilization. (B) Reduced endoglin expression in endothelial cells (Eng^{+/-} mice) could impair thrombus formation. Due to deficient platelet aggregation and anchorage, the thrombus can be torn off and dragged by the mechanical force exerted by the bloodstream. (C) Overexpression of endoglin in ENG⁺ mice. In this condition, the thrombus seems to be much more stable. For simplicity, all the other known molecules involved in platelet rolling and adhesion have been omitted for simplification. Created with BioRender.com.

adhesion of platelets to ECs isolated from *ENG*⁺ mice was observed [65], further supporting the hypothesis that endoglin in the endothelium is involved in platelet interactions (Figure 2A–C).

Our data also support the notion that the interaction of endothelial endoglin with integrins expressed by circulating leukocytes or platelets is regulated by blood flow. In fact, human leukocytes bound to plates coated with endoglin and CXCL12 and subjected to physiologic shear stress (1-10 dynes/cm²) showed a strong adhesive capacity that was resistant to up to 8 dynes/cm² of stress, whereas adherence of leukocytes to control plates showed a much weaker resistance to flow [53]. In addition, perfusion at 2 dynes/cm² of CXCL12-activated platelets bound to EC monolayers showed that platelet resistance to flow was strongly reduced in Eng^{+/-} compared with Eng^{+/-} mouse ECs [65]. Related to the link between flow and endoglin function, it has been reported that endoglin modulates blood vessel diameter through EC shape changes in response to hemodynamic cues [67]. In addition, an endoglin-dependent directional change of EC migration under flow has been described. Thus, in vivo experiments mimicking blood flow in vessels have shown that when tracking cell migration no difference in overall migratory distance between endoglinsilenced and control cells was observed. However, the majority of control cells moved against flow while the majority of endoglin knockdown cells instead migrated with the flow [68]. Whether the flow-dependent interaction of endothelial endoglin with integrins of circulating leukocytes or platelets affects blood vessel diameter or the migratory behavior of ECs remains to be elucidated. Because shear stress, induced by the tangential force of the blood flow, has been described as a second hit in HHT [61], the above findings suggest that the loss of endoglin in the endothelium of patients with HHT1 may have an impact on the development of telangiectases and AVMs as previously postulated [68].

1.3 | Circulating endoglin in hemostasis

Up to now, we have considered membrane endoglin and their interactions with blood components, notably platelets, in the context of primary hemostasis (or "platelets phase"). Primary hemostasis begins immediately after injury and creates a platelet plug on the wound surface. This platelet plug is strengthened by the conversion of fibrinogen to fibrin via the coagulation cascade during secondary hemostasis (or "coagulative phase"). Previous studies suggest that the presence of membrane-bound endoglin stabilizes platelet aggregation and consequently the thrombus [20,65]. Because the circulating form of endoglin (sEng) seems to act as a competitor of membrane-bound endoglin, the role of sEng was then investigated in this biological context.

To better decipher the role of sEng in hemostasis, we recently analyzed the bleeding parameters and thrombus formation using a transgenic mouse line that overexpresses human sEng (hsEng⁺). No differences between hsEng⁺ and WT mice were observed in terms of hematologic parameters, such as platelet count, coagulation factors, or prothrombin time. However, we observed that hsEng⁺ mice had significantly longer bleeding time than those of controls. Furthermore, the role of sEng in hemostasis was also assessed in vivo using a model of carotid artery thrombosis. Thus, *hsEng*⁺ mice showed a significantly longer time to occlusion than that shown by controls, and the thrombus appeared less stable, as suggested by more frequent emboli in hsEng⁺ mice compared with WT animals [57]. These new data support the novel conclusion that sEng is a decoy molecule that exerts an opposite effect to membrane-bound endoglin in thrombus formation and development (Figure 3A, B). To further analyze the mechanism of action of sEng, several techniques, such as surface plasmon resonance, artificial intelligence, and cytometric binding, were used. These studies have demonstrated that sEng competes with the binding of fibrinogen to allbß3 integrin, reducing thrombus formation or making it less stable [57]. Of note, this inhibitory activity is more likely to occur at high concentrations of sEng, which are found in certain pathological conditions. For example, although plasma levels of sEng in healthy individuals are approximately 2 to 6 ng/mL, in preeclampsia, sEng concentrations can reach approximately 40 ng/mL, and in some cases of a more severe pregnancy syndrome named HELLP, patients can present with up to 400 ng/mL [29]. HELLP syndrome can affect

FIGURE 3 Involvement of membrane endoglin and sEng in platelet adhesion. (A) Scheme of a blood vessel in which a lesion is present in an endothelium where endogenous endoglin is normally expressed, but also an excess of sEng is present ($hsEng^+$ mice). The binding of sEng to α Ilb β 3 integrin is postulated. This interaction would interfere with thrombus stabilization by competing with fibrinogen binding. (B) Magnification of the sEng-integrin interaction in platelets. Created with BioRender.com.



pregnant and postpartum women and is characterized by hemolysis with a microangiopathic blood smear, elevated liver enzymes, and a low platelet count [69]. Although HELLP represents a severe form of preeclampsia, the relationship between the 2 disorders remains controversial. In this regard, HELLP may be considered a separate disorder from preeclampsia because as many as 15% to 20% of patients with HELLP do not have antecedent hypertension or proteinuria, although they display very high levels of sEng [70] and low platelet numbers that can develop disseminated intravascular coagulation; this is a blood clotting disorder that can lead to heavy bleeding. In addition to the low number of platelets, it can be postulated that the high levels of sEng could also contribute to destabilizing thrombus formation by competing with fibrinogen binding to platelet α IIb β 3 [57], thus underlying the origin of the frequent bleedings. Further studies are needed to unveil the potential pathogenic role of sEng in plateletrelated conditions such as preeclampsia and HELLP syndrome.

1.4 | Circulating endoglin as a marker and decoy molecule in pathology

Circulating endoglin was initially described just as a marker of preeclampsia, a pregnancy disease that combines high blood pressure and proteinuria [30,32]. Furthermore, a prognostic value of sEng in the prediction of adverse outcomes such as HELLP syndrome was proposed [71]. Elevated levels of sEng have also been described in several cardiovascular-related conditions, as described in the Introduction section. Moreover, increased circulating levels of sEng have also been proposed as a prognosis marker in cancer, including breast cancer, prostate cancer, colorectal cancer, or head and neck paragangliomas, and a marker of cancer metastasis [57]. Recently, high levels of sEng have also been reported to be associated with cerebral edema and hemorrhage, which are, in turn, also seen in cerebral ischemia/ reperfusion injury [46]. Considering the significant shedding of sEng after hypoxia/reoxygenation, possible autocrine stimulation of

recombinant human sEng on the human brain endothelium was proposed. Indeed, sEng stimulation seems to cause an inflammatory phenotype in the human brain endothelium and compromise barrier function [46]. These findings are in line with several in vivo and in vitro studies supporting an active role of sEng in endothelial dysfunction and vascular remodeling [47,72,73]. As demonstrated by in vitro studies using endothelial colony-forming cells (ECFCs), treatment with sEng has revealed antiangiogenic abilities leading to decreased pseudotube formation and a decreased cellular migration capacity [47]. These antiangiogenic effects on vasculogenic cells, associated with previously described effects of endoglin in vascular permeability [22], are in agreement with an increase in endothelial dysfunction in C-TAH-implanted patients, with the device being operated in manual mode. The increased levels of sEng after C-TAH could contribute to the microvascular dysfunction observed in one of the patients after C-TAH implantation in manual mode, with the appearance of cutaneous bleeding. It is worth noting here that vascular permeability is a passive process that allows plasma to pass through the endothelium [22]. Thus, the increased levels of sEng observed in the manual mode of C-TAH could be at the origin of edema by this mechanism and justified by a venous stasis process that upregulates the production of MMPs, which could cleave membrane-bound endoglin, in turn releasing sEng. As sEng is a competitor of membrane-bound endoglin and encompasses the integrin-interacting region, including the RGD motif [53], when present at the circulatory level, it can interact with leukocytes (Figure 4).

1.5 | Endoglin in cell permeability and cytoskeletal structures

Endoglin also seems to be implicated in vascular permeability both at the level of cell-cell interaction and at the level of the cell itself in terms of cytoskeletal reorganization. Thus, the retina of endoglin heterozygous mice shows more permeability foci compared with



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FIGURE 4 sEng as a decoy molecule. Scheme of leukocytes under inflammatory stimuli. Cytokines, such as TNF- α and CXCL12, stimulate integrin expression and/or activation on leukocytes, as illustrated with monocytes and granulocytes. When present, sEng can bind integrin α 5 β 1 via the RGD motif. Created with BioRender. com.

controls, where endoglin was normally expressed [22]. In this study it was demonstrated that not only the absence of membrane-bound endoglin but also the increase of sEng, acting as a competitor of membrane endoglin, determined the loss of integrin-dependent endothelial interaction with mural cells [22], leading to increased vascular permeability. Of note, blood-brain barrier permeability increases in preeclamptic women due to plasma circulating factors, such as sEng, whose levels are markedly increased in this condition [74,75], with the brain being one of the deleteriously affected organs in preeclampsia. Additional mechanisms appear to be involved in the increase in cell permeability upon endoglin reduction. In fact, endoglin deficiency leads to EC hyperpermeability through constitutive activation of RhoA and destabilization of endothelial barrier function [76]. Interestingly, RhoA is expressed in ECs and is involved in the pathological angiogenesis of retinal diseases [77], and dysregulated levels of membrane-bound endoglin in the retina seem to modulate vascular remodeling, the formation of AVMs, and vascular permeability [78]. The hyperpermeability associated with endoglin loss appears to also be related to an impaired response to vascular endothelial growth factor (VEGF) and TGF- β cytokines [79], which in turn may negatively affect the adherents' junctions regulated by vascular endothelialcadherin [80]. However, the exact link between vascular endothelialcadherin and endoglin and its impact on endoglin-dependent barrier disruption of the endothelium remains barely speculative.

Recent findings proposed a correlation between Eng presence and calcium mobilization [81]. In fact, endoglin-silenced ECFC displayed a significantly higher permeability than that displayed by controls, and this increase seems to be associated with a higher Ca²⁺ mobilization and reorganization of the actin network in a cofilin-dependent fashion [81]. These data suggest that endoglin modulates cofilin activity and in turn the actin cytoskeleton. Accordingly, a reduced level of endoglin may result in a disorganized cytoskeleton and EC breaking associated with changes in shear stress leading to vessel hemorrhages. Furthermore, endoglin silencing reduces ECs sprouting while increasing the diameter of the ECs pseudotubes using microcarrier beads. The role of

endoglin in the TNF- α -induced permeability of EC monolayers was also analyzed. Thus, the loss of endoglin markedly potentiated the TNF- α -induced increased permeability [81]. A putative interaction between TNF- α ligand and membrane endoglin supports the hypothesis that TNF- α would be less effective in the presence of endoglin. Conversely, when endoglin is completely or partially silenced, TNF- α is fully active to increase cell permeability. Therefore, endoglin could be considered a membrane protective agent on ECs by limiting the TNF- α effect on endothelial permeability [81].

1.6 | Endoglin in revascularization, angiogenesis, and therapeutic strategies

Endoglin is predominantly expressed in the vascular endothelium and is essential for angiogenesis, but dispensable for vasculogenesis [82-85]. This is in agreement with the abnormal angiogenesis observed in patients with HHT and endoglin haploinsufficiency, and with the promising results obtained with bevacizumab (Avastin, Genentech), a humanized monoclonal antibody anti-VEGF used as a treatment for HHT [86,87]. Interestingly, the endoglin ligands BMP9 and BMP10 are also involved in angiogenesis-mediated vascular homeostasis and vascular pathology. Thus, (i) BMP9 and BMP10 are 2 vascular guiescence and endothelial-protective factors that are essential for postnatal retinal vascular remodeling [88]; (ii) in ECs, endoglin is required for BMP9 signaling [89], and BMP9 regulates endoglin-dependent responses to the chemokine CXCL12 [90], an activator of integrins involved in endoglin-mediated cell adhesion [20,53]; and (iii) patients with pathogenic mutations in GDF2, the gene encoding BMP9, present with a vascular anomaly syndrome overlapping the HHT phenotype and with pulmonary arterial hypertension [91-93]. It remains to investigate whether BMP9 and/or BMP10 are also involved in endoglin-dependent hemostasis.

Mice lacking endoglin (knock-out mice) are not viable as the fetuses die after a few weeks of gestation due to defects in vascular development [52,81,85]. In this pioneering article, it was also proposed that loss of endoglin causes poor vascular smooth muscle development and arrested endothelial remodeling. The critical involvement of endoglin in angiogenesis is in agreement with its pathogenic role in HHT1. Indeed heterozygous ($Eng^{+/-}$) mice are the closest genetic animal model of HHT1 patients in terms of genotype. However, $Eng^{+/-}$ mice have a very mild phenotype, and HHT-like features appear at a low frequency [52]. More recently, genetic tools, such as Cre-lox technology, have been used to deplete (or "knock out") the mouse endoglin gene, resulting in robust AVMs that resemble those seen in patients with HHT [85].

The above evidence underlines how important endoglin is in the structure of blood vessels. This conclusion is also supported by the finding that loss of endothelial endoglin impairs vascular mural cell interactions with the endothelium. Thus, endothelial endoglin, via integrin binding, is involved in the recruitment of mural cells, including pericytes expressing β 1 integrins, and in vessel stabilization [22]. This is in line with previous reports showing the following: (i) in yolk sacs from

FIGURE 5 Role of endoglin in revascularization. Scheme of nude mice that have undergone hindlimb ischemia by femoral artery ligation. (A) Vessel regeneration is optimally achieved by coinjection of endothelial colony-forming cells (ECFCs) and mesenchymal stromal cell (MSCs). (B) Vessel regeneration is markedly impaired upon coinjection of ECFCs and MSCs, when endoglin is silenced in ECFCs. Created with BioRender.com. siRNA, small interfering RNA.



endoglin knock-out mice, the levels of α -smooth muscle actin were strikingly decreased; (ii) vascular smooth muscle cells fail to differentiate and associate with ECs when endoglin is lost; and (iii) in the absence of endoglin, blood vessels become fragile and dilated [81,94].

The role of endoglin in revascularization and cell therapy strategies has been investigated. Therapeutic interventions using progenitors of ECs and vascular smooth muscle cells have been used to make up for a lack of circulation, as in cases of ischemia of the lower limbs. One of the strategies was the therapeutic angiogenesis promoted by autologous transplantation of bone-marrow cells for patients with limb ischemia [95]. While this therapeutic approach induces active angiogenesis in the ischemic and distal parts of the treated limb, the results proved to be patient-dependent and with various rates of success [96]. Alternatively, improved vascularization can also be achieved by injecting a specific subpopulation of endothelial progenitors (ECFCs) and MSCs, which are able to differentiate into vascular mural cells when in contact with ECFCs [97,98]. By coinjecting human ECFCs and MSCs in nude mice, the role of endoglin in vessel regeneration upon hind limb ischemia was assessed [99]. Remarkably, when silencing endoglin in ECFCs, it was found that the improved revascularization was abolished; the presence of human cells in murine vascular tissues was significantly reduced, compromising perfusion efficiency and vascular density; and tissue necrosis was significantly increased [99]. These data highlight that inhibition of endoglin in ECFCs reduces the beneficial effect of ECFCs-MSCs coinjection, suggesting that an underlying mechanism of cell-cell interaction could be at the origin of this defect, in agreement with previous reports [10,22]. Taken together, the above results support the hypothesis that the interaction between ECs and perivascular cells is mediated by endoglin and that the loss of endoglin causes poor vascular smooth muscle development and arrested endothelial remodeling (Figure 5A, B).

The selective high expression of endothelial endoglin in neoangiogenic tumor vessels and its correlation with poor survival in

FIGURE 6 Schematic representation of membrane and soluble endoglin involvement in different pathophysiological conditions, and associated biological processes. (A) Membrane endoglin in its expression is increased (ie, inflammation) or decreased (ie, hereditary hemorrhagic telangiectasia [HHT] type 1). (B) Soluble endoglin as a marker of pathology found in plasma/serum. Circles dimension indicates the relevance (in term of increased/ decreased expression) of endoglin in the specific condition. HELLP, hemolysis, elevated liver enzyme, low platelets.



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patients with cancer have also led to the investigation of endoglin as an imaging and therapeutic target for cancer [100]. Indeed, the chimeric monoclonal antibody TRC105 (Carotuximab, Tracon Pharmaceuticals) that targets CD105 has been used, conjugated with positron emission tomography, single-photon emission computed tomography, and nearinfrared fluorescence imaging modalities, for efficient tumor labeling. Targeting endoglin of the tumor vasculature with TRC105 to improve cancer therapy has also been tested in phase I to III clinical studies in patients with cancer, as both a monotherapy and in combination with other chemotherapeutic and antiangiogenic therapies [100-102]. Thus, treatment with TRC105 and anti-VEGF (bevacizumab) antibodies improved clinical outcomes [101], while combined TRC105 and PD-1 antibody treatment promotes tumor regression through intratumoral suppression of regulatory T cells and enhanced cytotoxic T-cell response in human colorectal cancer [14]. Moreover, TRC105 has been studied in clinical trials to counteract abnormal angiogenesis in agerelated macular degeneration [103]. Interestingly, some vascularrelated adverse events in cancer patients treated with TRC105 or an ALK1 inhibitor (dalantercept; ligand trap for BMP9 and BMP10) phenocopied characteristic HHT symptoms (mucocutaneous telangiectasia. epistaxis, and gingival bleeding) [101,102,104,105], suggesting that inhibiting endoglin or ALK1 leads to their haploinsufficiency, as postulated in HHT1 and HHT2, respectively [9].

2 | DISCUSSION

Membrane-bound and circulating endoglin are involved in different pathologies such as HHT, preeclampsia, hypertension, cancer, or several cardiovascular-related conditions (Figure 6A). For many years the molecular function of these 2 endoglin forms has been ascribed only to their activity as components of the TGF- β system, where membranebound endoglin acts as an auxiliary receptor for TGF- β family members, while this ligand binding can be competed out by circulating endoglin. However, during the last decade, evidence is emerging for new endoglin functions beyond TGF-β. Among these, a novel function of endoglin, which encompasses an RGD motif in its extracellular region, is related to its role as a cell adhesion molecule through its binding to integrins of the RGD subtype, including $\alpha 5\beta 1$ and $\alpha IIb\beta 3$. It will be of interest to investigate the binding capacity of endoglin to other integrin family members. The role of endoglin in cell adhesion has relevant implications in inflammation, leukocyte adhesion and extravasation, vascular permeability, angiogenesis, or the therapeutic activity of endothelial progenitors in revascularization upon ischemia. In addition, by binding to integrin α IIb β 3 of platelets, both endoglin forms appear to have a key role in primary hemostasis by regulating the formation of the thrombus (Figure 6B). Based on the above findings, endoglin appears to be a novel therapeutic target in conditions associated with ischemia, abnormal angiogenesis, thrombosis, or bleeding.

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AUTHOR CONTRIBUTIONS

E.R. designed the review and wrote the first version of the paper. E.R. and C.B. revised the paper and provided funding support.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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