Published in final edited form as:

J Cell Biochem. 2007 December 15; 102(6): 1375–1388.

Novel Biochemical Pathways of Endoglin in Vascular Cell

Physiology

Carmelo Bernabeu^{1,2}, Barbara A. Conley³, and Calvin P.H. Vary^{3,*}

¹ Centro de Investigaciones Biologicas, Consejo Superior de Investigaciones Cientificas (CSIC), 28040 Madrid, Spain

² Center for Biomedical Research on Rare Diseases (CIBERER), 28040 Madrid, Spain

³ Center for Molecular Medicine, Maine Medical Center Research Institute, 81 Research Drive, Scarborough, Maine 04074

Abstract

The broad role of the transforming growth factor beta (TGF β) signaling pathway in vascular development, homeostasis, and repair is well appreciated. Endoglin is emerging as a novel, complex, and poorly understood regulatory component of the TGF β receptor complex, whose importance is underscored by its recognition as the site of mutations causing hereditary hemorrhagic telangiectasia (HHT) [McAllister et al., 1994]. Extensive analyses of endoglin function in normal developmental mouse models [Bordeau et al., 1999; Li et al., 1999; Arthur et al., 2000] and in HHT animal models [Bordeau et al., 2000; Torsney et al., 2003] exemplify the importance of understanding endoglin's biochemical functions. However, novel mechanisms underlying the regulation of these pathways continue to emerge. These mechanisms include modification of TGF β receptor signaling at the ligand and receptor activation level, direct effects of endoglin on cell adhesion and migration, and emerging roles for endoglin in the determination of stem cell fate and tissue patterning. The purpose of this review is to highlight the cellular and molecular studies that underscore the central role of endoglin in vascular development and disease.

Keywords

endoglin; transforming growth factor-beta receptors; hereditary hemorrhagic telangiectasia; vascular pathology; vascular endothelium; vascular smooth muscle

The canonical transforming growth factor beta (TGF β) signaling pathway comprises seven type I and five type II TGF β receptors [Manning et al., 2002]. The TGF β type I receptors are serine and threonine kinases, which include activin-like kinase 1 (ALK1) and T β RI, also known as ALK5. ALK1 and ALK5 associate with, and are activated via ligand-dependent phosphorylation [Vivien and Wrana, 1995] by the type II TGF β receptor, T β RII [Wrana et al., 1992]. The activated type I receptor propagates canonical or Smad-dependent signals by phosphorylating Smad proteins [Shi and Massague, 2003; Feng and Derynck, 2005]. Another component of the TGF β system is endoglin. Endoglin is a transmembrane protein [Gougos and Letarte, 1990] that acts as an auxiliary receptor for TGF β [Cheifetz et al., 1992; Barbara et al., 1999]. Of note, mutations in endoglin (*ENG*) [McAllister et al., 1994] and *ALK1* [Johnson et al., 1996] genes cause the vascular dysplasia hereditary hemorrhagic telangiectasia (HHT), termed HHT1 and HHT2, respectively.

*Correspondence to: Calvin P.H. Vary, Maine Medical Center Research Institute, 81 Research Dr., Scarborough, ME 04074. E-mail: varyc@mmc.org.

Endoglin is expressed in vascular endothelial and smooth muscle cells and plays an important role in the homeostasis of the vessel wall. Evidence to support this view includes: (1) human endoglin mutations result in the vascular disorder, HHT1; (2) murine endoglin is necessary for the process of angiogenesis and vascular smooth muscle development [Li et al., 1999]; (3) endoglin is up-regulated in the endothelia of neovascularized tissues such as tumors [Burrows et al., 1995; Kumar et al., 1996, 1999; Bodey et al., 1998; Fonsatti et al., 2003], in the thyroid disorders, Grave's disease and Hashimoto's thyroiditis [Marazuela et al., 1995], in psoriasis [van de Kerkhof et al., 1998], scleroderma [Rulo et al., 1995; Leask et al., 2002], and in ischemic stroke [Kumar et al., 1996]; and (4) endoglin is up-regulated in the smooth muscle cells of human atherosclerotic plaques [Conley et al., 2000], and in smooth muscle cells that respond to vascular injury [Ma et al., 2000]. Vascular injury also results in increased endoglin expression in endothelial cells [Botella et al., 2002]. Transcriptional activation of endoglin and TGF β signaling components by cooperative interaction between Sp1 and KLF6 suggests that these factors play a role in the response to vascular injury [Botella et al., 2002]. These data support the view that understanding endoglin's role in development and disease will provide considerable insight into the processes of angiogenesis, smooth muscle cell regulation, and vascular homeostasis.

HHT is a genetic vascular disorder that affects about one in 10,000 people [Lux and Marchuk, 2001], although recent studies suggest that this prevalence may be 1/5,000 or higher [Guttmacher et al., 1995; Kjeldsen et al., 1999; Dakeishi et al., 2002; Westermann et al., 2003]. HHT shows a significant age-dependent onset of symptoms. Adults positive for a mutant HHT endoglin allele have significantly greater risk of cerebral arteriovenous malformation and epistaxis (nose bleeding), which increases with age [Aassar et al., 1991; Shovlin et al., 1995]. Up to 1/3 of HHT patients have multiple organ involvement, which can be disabling and life threatening. The detection and treatment of HHT are now the focus of at least 24 HHT Centers worldwide, including 8 in the United States.

Clinically, HHT sufferers present with vascular dysplasia characterized by arteriovenous malformation resulting from muscularization of postcapillary venules without obvious endothelial cell defects. Microscopically, vascular lesions originate as focal dilatations of postcapillary venules followed by thickening of the vessel wall with mononuclear cell infiltration (primarily lymphocytes) and proliferation of smooth muscle cells [Braverman et al., 1990; Aassar et al., 1991]. Pulmonary arteriovenous malformations occur in ~30% of patients and are associated with serious complications that include stroke and brain abscess.

HHT1 is a dominantly inherited disorder. More than 155 distinct mutations in *ENG* are linked to HHT1 [Prigoda et al., 2006]. These mutations tend to cluster as premature termination codons in exons that encode the extracellular domain of the protein, and lead to truncated forms of endoglin that are not readily detectable by immunological methods [McAllister et al., 1995; Berg et al., 1996; Shovlin et al., 1997; Yamaguchi et al., 1997; Gallione et al., 1998]. These observations strongly suggest that HHT results from reduced dosage or haploinsufficiency of endoglin protein [Abdalla and Letarte, 2006].

STRUCTURE OF ENDOGLIN: RELATIONSHIP TO FUNCTION

Endoglin was originally described as a type I integral membrane protein with an extracellular domain of 561 amino acids, a hydrophobic transmembrane domain, and a 47-residue cytosolic domain [Gougos and Letarte, 1990]. Comparative analysis of the primary structure reveals that endoglin belongs to the zona pellucida (ZP) family of extracellular proteins that share a ZP domain consisting of 260 amino acids with 8 conserved cysteine residues close to the transmembrane region [Bork and Sander, 1992; Jovine et al., 2005]. This consensus ZP domain

is divided in two ZP subdomains that are potentially involved in endoglin receptor oligomerization [Jovine et al., 2005; Llorca et al., 2007].

In humans, endoglin contains an RGD tripeptide located in the ZP domain of the extracellular region [Gougos and Letarte, 1990]. Although this motif led to the hypothesis that endoglin binds to integrins or other RGD-binding receptors [Gougos et al., 1992; Lastres et al., 1992], the function of the RGD sequence in human endoglin may reflect a recent adaptation because this motif is absent from mouse [Ge and Butcher, 1994], porcine [Yamashita et al., 1994], rat, and canine [Llorca et al., 2007] endoglin proteins.

The primary structure of endoglin suggests that there are four N-linked glycosylation sites in the N-terminal domain and a probable *O*-glycan domain, which is rich in Ser and Thr residues proximal to the membrane-spanning domain [Gougos and Letarte, 1990]. Experimental studies using specific glycosidases confirmed that endoglin is glycosylated [Gougos and Letarte, 1988]. This post-translational modification occurs in multiple stages when endoglin is overexpressed in COS cells, giving rise to partially and fully glycosylated species that are present at the cell surface [Lux et al., 2000].

The 47-residue cytosolic domain of the predominant L-isoform of endoglin constitutes the region of the protein with the highest degree of conservation among endoglins from different mammalian species, as well as with the homologous protein betaglycan [Lopez-Casillas et al., 1991]. A splicing isoform of human endoglin results in the expression of a short S-endoglin species with a distinct cytosolic domain of 14 residues [Bellon et al., 1993]. Both cytosolic domains can be phosphorylated by serine and threonine kinases [Lastres et al., 1994], including the TGF β type I and II receptors [Guerrero-Esteo et al., 2002; Koleva et al., 2006]. Recently, a short endoglin isoform was characterized in mice [Perez-Gomez et al., 2005]. Although the L-endoglin isoform and betaglycan contain a consensus PDZ-binding motif (SerSerMetAla) present at the carboxyl terminus, the S-endoglin isoform lacks this motif. As will be discussed below, the L-form of endoglin is linked to the regulation of the adhesive properties of endoglin, and thus isoform switching of the cytosolic domain of endoglin may have potential regulatory significance to the function of endoglin.

The three-dimensional structure of the extracellular region of endoglin at a resolution of 25 Å was determined using single-particle electron microscopy [Llorca et al., 2007]. The molecular reconstruction suggests that endoglin exists as a dome comprised of antiparallel-oriented monomers enclosing a cavity at one end. Using these data, a high-resolution structure of endoglin indicates that each endoglin subunit comprises three well-defined domains, including the two ZP regions and one orphan domain, which are organized into an open U-shaped monomer [Llorca et al., 2007] (Fig. 1). These studies were performed by using a soluble form of the extracellular domain of endoglin. Of note, a soluble form of endoglin was recently detected in pregnant women with preeclampsia and it appears to play a pathogenic role in this disease [Levine et al., 2006; Venkatesha et al., 2006]. The metalloprotease MMP-MT1 was suggested to play a role in soluble endoglin production [Venkatesha et al., 2006]. Interestingly, a structural analysis of the extracellular region of endoglin identified a potential protease cleavage site that is highly conserved among different mammalian species and is located between the two subdomains of the ZP consensus region of endoglin [Llorca et al., 2007]. However, whether this soluble protein is generated by protease cleavage of the membrane bound endoglin or by an alternative splicing mechanism remains to be determined.

ENDOGLIN AND BETAGLYCAN: REGULATION OF TGF β LIGAND ACCESS TO RECEPTORS

Endoglin and betaglycan bear a significant degree of sequence similarity [Ge and Butcher, 1994] and therefore, the search for functional attributes of endoglin has drawn upon results from the study of betaglycan. Betaglycan interacts with the TGF β type II receptor [Lin and Lodish, 1993] and plays a role in the presentation of the TGF β ligand to T β R β II [Lopez-Casillas et al., 1993]. TGF β binds to the N-terminal endoglin-related region of betaglycan, and mutational analysis suggests that the remainder of the extracellular and the cytosolic domains are not required for betaglycan-dependent enhancement of TGF β binding to T β R β II [Lopez Casillas et al., 1994].

Examination of the primary structure of betaglycan, especially in its cytosolic domain, indicated that this component of the TGF β receptor system was a homolog of human endoglin [Lopez-Casillas et al., 1991]. Based on this finding, it was established that endoglin binds TGF β 1 and TGF β 3 but not TGF β 2 [Cheifetz et al., 1992]. This difference in affinity of endoglin for the TGF β isoforms distinguishes it from betaglycan because betaglycan recognizes all three isoforms. These studies provided the basis for the examination of endoglin's functions as a component of the TGF β receptor system.

Because endoglin differs from betaglycan in its TGF β ligand-binding profile [Cheifetz et al., 1992], it was not surprising to learn that functional differences, as well as similarities, exist between these two proteins. For example, both L- and S-endoglin isoforms bind TGF β 1 [Bellon et al., 1993], which is consistent with an exclusive role for the extracellular domain in TGF β ligand binding. This view is supported by studies indicating that switching of the endoglin and betaglycan cytosolic domains has no effect on endoglin ligand binding [Letamendia et al., 1998]. However, in contrast to betaglycan, the binding of ligand to endoglin requires the presence of T β R β II [Letamendia et al., 1998], suggesting that endoglin participates in ligand binding only within the TGF β receptor complex. This result explains the observation that only a small fraction of the total cell surface endoglin binds ligand [Cheifetz et al., 1992].

THE ROLE OF ENDOGLIN WITHIN THE TGF β RECEPTOR COMPLEX

Endoglin bound to ligand is isolated as a complex with the TGF β type I receptor and the type II receptor, T β R β II [Yamashita et al., 1994]. The TGF β type I receptors include: ALK1, the bone morphogenetic protein (BMP) receptors ALK2, 3, and 6, as well as ALK5 and the activin receptors, ALK2 and ALK4. In addition to T β R β II, the various type I receptors can interact with the activin (ActRII) or BMP type II receptors [Shi and Massague, 2003; Feng and Derynck, 2005]. In vitro co-immunoprecipitation studies of the interaction of endoglin with type I and type II receptors indicates that endoglin interacts with the ligands activin-A, BMP-7, and BMP-2 [Barbara et al., 1999]. These results are supported, at least for BMP-7, by functional experiments demonstrating that endoglin overexpression enhances the BMP-7/Smad1/Smad5 pathway, while inhibiting the TGF- β 1-induced ALK-5/Smad3 signaling in myoblasts [Schnerer et al., 2007]. As discussed above, these interactions require coexpression of the respective ligand-binding kinase receptor [Letamendia et al., 1998; Barbara et al., 1999]. Thus, endoglin binds TGF β 1 and β 3 by associating with T β R β II, and interacts with activin-A and BMP-7 in association with the ActRII receptors ActRIIA and ActRIIB. In addition, endoglin binds BMP-2 by interacting with the BMP ligand-binding receptors ALK3 and ALK6 [Barbara et al., 1999]. Interestingly, BMP-9 binds with high affinity to endoglin without the TGF- β signaling receptors [Scharpfenecker et al., 2007]. In agreement with this finding, overexpression of endoglin increases the BMP-9 response, whereas silencing of both BMPRII and ActRIIA expressions completely abolishes it [David et al., 2007]. These studies indicate that endoglin complexes with most ligand-type I/II receptor complexes, potentially reflecting

a role for endoglin in the dynamics of type I/II receptor interactions and their downstream signaling pathways, or a regulatory role for phosphorylated endoglin occurring because of receptor activation, or both.

Studies of the interaction of endoglin with ALK5 and T β RII indicate that both ALK5 and T β RII interact with the extracellular and cytosolic domains of endoglin. However, ALK5 interacts with the endoglin cytosolic domain only when the kinase domain is inactive. Upon association, ALK5 and T β RII phosphorylate the endoglin cytosolic domain; then ALK5, but not T β RII, dissociates from the complex [Guerrero-Esteo et al., 2002]. These data suggest the hypothesis that endoglin's extracellular and cytosolic domains play distinct roles in receptor signaling that are downstream of ligand binding and receptor activation.

ROLE OF ENDOGLIN IN THE MODULATION OF TGF β -DEPENDENT CELL RESPONSES

Endoglin modulates TGF β -dependent cellular responses. In human monocytic U-937 cells, TGF β 1, but not TGF β 2 responses are abrogated in both L- and S-endoglin transfectants [Lastres et al., 1996]. In a variety of cell types, including myoblasts, the TGF β 1-dependent responses opposed by endoglin include inhibition of cellular proliferation, cellular adhesion, platelet/endothelial cell adhesion molecule 1 phosphorylation, homotypic cell aggregation, and the increased expression of extracellular matrix components, including collagen and fibronectin [Lastres et al., 1996; Letamendia et al., 1998; Guerrero-Esteo et al., 1999; Diez-Marques et al., 2002; Obreo et al., 2004], and the secreted extracellular matrix-associated protein lumican [Botella et al., 2004]. Interestingly, no changes in total ligand binding were observed in L-endoglin transfectants [Lastres et al., 1996], suggesting that endoglin's effects occur downstream of ligand binding. As with TGF β receptor signaling in general, endoglin-dependent regulatory effects are likely to be cell type specific, subject to conditions that include the specific TGF β type I receptors that are present and the relative levels of endoglin isoform expression.

Although TGF β is a potent inhibitor of cell proliferation, endoglin expression counteracts this inhibitory effect in several cell types, including endothelial cells [Lastres et al., 1996; Li et al., 2000]. The positive correlation between endoglin expression and endothelial cell proliferation was confirmed in several experimental models. Thus, endoglin is markedly up-regulated in the proliferating endothelium of tissues undergoing angiogenesis [Burrows et al., 1995; Kumar et al., 1996, 1999; Bodey et al., 1998; Fonsatti et al., 2003], and in vitro inhibition of its expression on endothelial cells impairs this process [Li et al., 2000]. In addition, suppression of endoglin not only increases the TGF β 1-dependent inhibition of endothelial cell proliferation, but also endothelial cell apoptosis induced by hypoxia and TGF β 1 [Li et al., 2003]. Furthermore, using mice bearing targeted endoglin (*eng*) alleles, studies of derived *eng*^{-/-} and *eng*^{+/-} embryonic endothelial cells indicate that endoglin promotes endothelial cell proliferation via a TGF β /ALK1 pathway [Lebrin et al., 2004]. An exception to this widely reported correlation between endoglin and endothelial cell proliferation is the finding that an endothelial cell line established from null *eng*^{-/-} 8.5-day-old embryos are responsive to TGF β and can proliferate faster than control mouse *eng*^{+/-} endothelial cells [Pece-Barbara et al., 2005]. Future studies should clarify the detailed mechanism of endoglin-dependent effects on endothelial cell proliferation.

How endoglin regulates these TGF β -dependent responses is unknown. A potential mechanism of action is via endoglin-dependent effects on TGF β receptor phosphorylation. T β RII is thought to be a constitutively active (ca) receptor that activates the type I receptor via phosphorylation upon ligand-induced association. Betaglycan functions by selectively binding the phosphorylated T β RII via its cytosolic domain to promote TGF β 2 signaling [Blobe et al., 2001]. Interestingly, endoglin association with T β RII results in an altered phosphorylation state

of T β RII and loss of ALK5 from the complex [Guerrero-Esteo et al., 2002], either of which could explain the inhibitory effects of endoglin on ALK5 signaling, which requires phosphorylation by the T β RII kinase after its association with TGF β 1. Additionally, studies in primary human umbilical vein endothelial cells suggest that endoglin phosphorylation opposes the activated ALK1-dependent inhibition of cell adhesion [Koleva et al., 2006]. These results suggest that by interacting through its extracellular and cytosolic domains with the signaling receptors, endoglin might affect TGF β responses.

As endoglin directly interacts with a variety of TGF β type I receptors [Barbara et al., 1999; Guerrero-Esteo et al., 2002; Blanco et al., 2005], this raises the possibility for additive or opposing effects of endoglin on TGF β receptor signaling. Thus, although endoglin shows an inhibitory effect on TGF β /ALK5/Smad3 cellular responses [Letamendia et al., 1998; Guo et al., 2004; Lebrin et al., 2004; Blanco et al., 2005; Scherner et al., 2007], it enhances ALK5/Smad2 signaling [Guerrero-Esteo et al., 2002; Carvalho et al., 2004; Santibanez et al., 2007]. In addition, endoglin may be required for TGF β 1/ALK1 signaling in some cell types, especially endothelial cells. This balance between ALK5 and ALK1 may play a role in the regulation of cell growth and differentiation in cells that express endoglin, as well as ALK1 and ALK5 [Lebrin et al., 2004]. The mechanism by which endoglin potentiates TGF β /ALK1 signaling appears to involve direct association of ALK1 with the cytosolic and extracellular domains of endoglin, with the extracellular domain mediating the enhancement of ALK1 signaling [Blanco et al., 2005]. These studies suggest that the functional association of endoglin with ALK1 is critical for endothelial cell responses to TGF β .

Recent studies indicate that endoglin regulates the levels of expression and the activities of proteins that mediate vascular tone. The vasoregulatory protein endothelial nitric oxide synthase (eNOS) is decreased in endoglin-deficient cells, whereas it is increased in endoglin-overexpressing cells [Jerkic et al., 2004; Toporsian et al., 2005]. At least in part, the endoglin-dependent increase of eNOS levels is mediated by increased stabilization of eNOS protein in caveolae, via a post-transcriptional mechanism that involves direct association of endoglin with caveolar proteins and potentially heat shock protein 90 [Toporsian et al., 2005]. In addition, endoglin stabilizes the Smad2 protein, potentially via reduction in the levels of the Smad ubiquitination response factor 2, Smurf2 [Santibanez et al., 2007]. Thus, in the presence of endoglin Smad2 protein levels are increased, leading to TGF β receptor-dependent induction of eNOS mRNA, and enhancement of Smad-dependent signaling. Because of the endoglin-dependent regulation of eNOS, changes in nitric oxide levels lead to altered COX-2 expression, which is suggestive of a Smad-independent mechanism underlying endoglin function [Jerkic et al., 2006].

A schematic model of the modulatory role of endoglin in the TGF β signaling pathways is depicted in Figure 2. Endoglin physically interacts and functionally modulates ALK1 and ALK5 signaling leading to the potentiation of Smad1 and Smad2 and inhibition of Smad3, which, in turn, regulates expression of Id1, eNOS, and plasminogen activator inhibitor-1 (PAI-1) genes, respectively. In the future, a complete identification of all the downstream genes affected by endoglin expression will be of interest, especially in HHT, in which endoglin haploinsufficiency is supposed to trigger the vascular lesions. In a step toward this goal, the gene expression fingerprinting of HHT endothelial cells revealed 277 down-regulated and 63 up-regulated genes that are potentially involved in biological processes relevant to the HHT pathology, including genes involved in angiogenesis, the cytoskeleton, cell migration, proliferation, and nitric oxide synthesis [Fernandez-L et al., 2007].

ENDOGLIN IN CELL ADHESION AND MIGRATION: ROLE OF THE CYTOSOLIC DOMAIN

As noted, endoglin possesses properties of an adhesion molecule. This view was extended by studies indicating that endoglin expression results in the inhibition of cell migration in a variety of in vitro [Guerrero-Esteo et al., 1999; Liu et al., 2002; Conley et al., 2004] and in vivo [Ma et al., 2000] models. Efforts to address potential mechanisms underlying these properties of endoglin were based on the high degree of sequence conservation within the endoglin cytosolic domain and the lack of HHT-causing mutations in this domain. Yeast two-hybrid and cell biological approaches identified zyxin and zyxin-related protein 1 (ZRP-1) as the first examples of cytosolic proteins that interact with endoglin's cytosolic domain [Conley et al., 2004; Sanz-Rodriguez et al., 2004]. Because these interactions are localized within endoglin's cytosolic domain, which contains the sites of serine and threonine phosphorylation [Koleva et al., 2006], these data suggest that the endoglin cytosolic domain is a site of protein-protein interactions that are regulated by phosphorylation.

Several studies have illustrated how endoglin-zyxin interactions influence cell migration. Expression of endoglin is associated with the inhibition of cell migration and redistribution of zyxin from sites of focal adhesion (FA). Expression of endoglin caused reduction in zyxin associated with an integrin-rich FA-associated protein fraction obtained using RGD-tagged magnetic microspheres [Conley et al., 2004]. This reduction was correlated with: (1) inhibition of cell migration, (2) reduction of FA-associated p130(Cas)/Crk protein levels, and (3) that FA-associated endoglin levels were strongly mediated by endoglin's cytosolic domain. It is noteworthy that the p130(Cas)/Crk interaction is required for the induction of cell migration [Klemke et al., 1998] and was implicated in vessel wall assembly [Foo et al., 2006].

Independently, it was discovered that endoglin also interacts with ZRP-1 [Sanz-Rodriguez et al., 2004]. Although zyxin and ZRP-1 share significant sequence homology, especially in the LIM3 domain, which contributes to endoglin binding [Conley et al., 2004], the amino terminal regions of zyxin and ZRP-1 are distinct. This distinction may underlie the different responses observed because of the interaction of endoglin with ZRP-1, which include the redistribution of ZRP-1 from sites of FA to F-actin stress fibers in endothelial cells, and dynamic rearrangement of F-actin fibers [Sanz-Rodriguez et al., 2004].

The interaction between endoglin and the ZRPs is exclusive because the interaction was not observed with betaglycan [Conley et al., 2004; Sanz-Rodriguez et al., 2004], even though their cytosolic domains are 70% identical. However, in addition to endoglin-specific protein-protein interactions, endoglin associates with proteins that also interact with beta-glycan. For example, beta-arrestin2 interacts with the conserved distal end of the betaglycan cytosolic domain and regulates betaglycan internalization [Chen et al., 2003]. This interaction also occurs with the endoglin cytosolic domain and results in endoglin internalization with beta-arrestin2 in endocytic vesicles [Lee and Blobel, 2007]. Endoglin's cytosolic domain also interacts with a member of the Tctex1/2 family of cytosolic dynein light chains, Tctex2b, linking endoglin to the microtubule-based transport machinery [Meng et al., 2006]. Interestingly, Tctex1 is phosphorylated by the BMP type RII receptor, BMPRII [Machado et al., 2003] further supporting a functional linkage between Tctex proteins, endoglin, and TGF β receptor complexes. Together, these studies point to a critical role for diverse protein-protein interactions involving the endoglin cytosolic domain in endoglin function (Fig. 3).

The importance of endoglin's cytosolic domain in cell adhesion was corroborated by Muenzner and colleagues, who showed that endoglin expression mediated an increase in cell adhesion that was dependent on an intact cytosolic domain as well as the expression of integrin β 1

[Muenzner et al., 2005]. These results further implicate endoglin in the regulation of integrin-mediated cell adhesion and detachment.

An interesting observation suggesting conservation of the endoglin–LIM domain interaction comes from the study of the *Drosophila* protein, piopio (*Pio*). *Pio* is an apically secreted extracellular matrix protein that has an important role in the regulation of tracheal tube growth. As with mammalian endoglin, *Pio* possesses an extracellular ZP domain, and a C-terminal sequence whose closest mammalian homolog is endoglin [Jazwinska et al., 2003]. Interestingly, other genes that mimic *Pio*-mutant phenotypes in *Drosophila* include steamer duck (*stdk*) [Prout et al., 1997]. *Stdk*, whose mammalian homolog is Pinch, is an evolutionarily conserved LIM-domain protein that is postulated to act as part of an integrin-dependent signaling complex that colocalizes to sites of actin filament anchorage in both muscle and wing epithelial cells. Thus, interactions involving *Pio* and *Stdk* may be functionally analogous to endoglin and Lim-domain proteins. Future studies are needed to clarify the evolutionary origins of endoglin and betaglycan and their overlapping and distinct networks of interactions.

REGULATION OF ENDOGLIN FUNCTION: TGF β RECEPTOR-MEDIATED PHOSPHORYLATION

Endoglin phosphorylation is a potential Smad-independent mechanism of endoglin function that regulates Smad-independent effects on endothelial cell growth and adhesion [Koleva et al., 2006]. Endoglin phosphorylation influences its subcellular localization [Koleva et al., 2006], potentially by modulating endoglin's interactions with adhesive proteins such as zyxin and ZRP-1, and thus modifying the adhesive properties of endoglin-expressing cells.

The regulation and pattern of endoglin phosphorylation by the TGF β receptors is complex. Koleva et al. [2006] conducted a detailed study of endoglin phosphorylation by *ca* forms of the TGF β receptors *ca*ALK1, *ca*ALK5, and wild type T β RII. Site-directed mutagenesis of endoglin suggests that *ca*ALK5 and T β RII phosphorylate the 634SerSer635 motif within endoglin's cytosolic domain. In contrast to serine phosphorylation, ALK1 phosphorylates wild type endoglin preferentially on threonine residues. Interestingly, mutation of the 634SerSer635 residues to 634AlaAla635 strongly reduces threonine phosphorylation of endoglin, suggesting that phosphorylation of 634SerSer635 is a prerequisite for subsequent endoglin threonine phosphorylation. This hypothesis was verified by replacement of one mutated alanine with a phospho-mimicking aspartate residue (634Asp Ala635), which restores threonine phosphorylation by *ca*ALK1.

Studies of additional endoglin site-specific mutations are also informative. For example, removal of endoglin's putative C-terminal PDZ-binding motif results in endoglin hyperphosphorylation of distal threonine residues [Koleva et al., 2006]. These data reveal that receptor-mediated phosphorylation of endoglin is a complex process involving negative regulation by the PDZ-binding motif and an unexpected sequential mechanism of serine and threonine phosphorylation. Future studies will be needed to gain a comprehensive understanding of the full range of functions that are mediated by endoglin phosphorylation.

ENDOGLIN AND ALTERNATIVE SMAD-INDEPENDENT TGF β SIGNALING

Involvement of endoglin in alternative Smad-independent TGF β signaling pathways is further supported by the phenotypic similarities between the *eng*^{−/−} and TGF β -activated kinase-1 (TAK1)^{−/−} developing mouse embryos [Jadrich et al., 2006]. TAK1 is a noncanonical Smad-independent effector of TGF β and BMP signaling. Similar to the *eng*^{−/−} mouse, smooth muscle cell development is impaired with normal endothelial cell development in the TAK1^{−/−} mouse [Jadrich et al., 2006], thereby raising the possibility that TAK1 may mediate Smad-independent

signals downstream of endoglin. Consistent with this idea, genetic data obtained combining Smad4 conditional inactivation with endoglin overexpression in cells of the embryonic neural crest suggest that endoglin operates in pathways that are separate from the canonical TGF β receptor signaling pathways required for smooth muscle cell fate determination [Mancini et al., 2007]. The aforementioned studies suggest that endoglin modulates multiple interactions between TGF β Smad-dependent and -independent signaling pathways.

ENDOGLIN AND VASCULAR SMOOTH MUSCLE CELL DEVELOPMENT

Although endoglin's expression was originally described as endothelial cell-restricted, it was later detected in the endocardium at 4 weeks of gestation and in the endocardial cushion mesenchyme by 5–8 weeks of gestation, suggesting a role in cardiac septation and valve formation [Qu et al., 1998]. Endoglin-targeted embryos die by E11.5 [Bourdeau et al., 1999; Arthur et al., 2000] due to defects in angiogenesis and cardiac morphogenesis, resulting in septation defects, thereby suggesting a loss of endocardial to mesenchymal transitions and a possible absence of vascular smooth muscle cells [Li et al., 1999]. However, it is unclear whether loss of endoglin results in a delay or loss of smooth muscle cell specification, differentiation, or both.

Endoglin is expressed on injured and atheromatous, but not in normal vascular smooth muscle [Adam et al., 1998; Conley et al., 2000; Ma et al., 2000], suggesting that endoglin plays a functional role in myofibroblast or pericyte responses to injury, and implicating a role for endoglin in vascular precursor cell physiology. This view is further supported by studies indicating that endoglin is expressed by circulating mesenchymal stem cells [Barry et al., 1999] and is a functional marker of long-term repopulating hematopoietic stem cells [Chen et al., 2002].

Evidence suggests that endoglin plays a role in myogenic specification during development. Because many of the smooth muscle cells that invest the large vessels and form the cardiac cushions are derived from the neural crest [Jiang et al., 2000], Mancini et al. [2007] examined whether endoglin plays a role in specification from the neural crest. These studies show that endoglin is required for the maintenance of neural crest stem cell myogenic potential. Moreover, expression of endoglin in neural crest stem cells declines with age, coinciding with a reduction in both smooth muscle differentiation potential and TGF β 1 responsiveness.

Endoglin also plays a role in bone marrow mesenchymal stem cell regulation [Yamada et al., 2007], and in the regulation of the epithelial-mesenchymal transformation during cardiac valve formation [Mercado-Pimentel et al., 2007]. In addition, endoglin affects the efficiency of formation of the hemangioblast, a common embryonic progenitor of the hematopoietic and endothelial lineages [Perlingeiro, 2007]. Finally, supporting the relevance of endoglin-expressing circulating precursors, it was reported that endoglin has a crucial role in blood mononuclear cell-mediated vascular repair [van Laake et al., 2006]. Together, these studies support the hypothesis that endoglin expression is required for multiple cell precursors to begin tissue formation, respond to injury, and suggest that age-dependent loss of endoglin underlies an impaired response to vascular injury.

These reports point to novel and important emerging roles for endoglin in the differentiation and determination of the differentiation fate of vascular precursor cells. Thus, endoglin may participate in the integration of diverse TGF β signals, and may directly mediate important cell-adhesive, proliferative, and migration processes in the developing and adult vasculature. Although a biochemical basis exists for understanding endoglin's diverse effects at the cellular level, much work remains to better understand the role of endoglin in vascular development and disease.

Acknowledgements

The authors acknowledge the contributions of many investigators that, although relevant to this subject of this review, could not be included due to space limitations. C.P.H.V. was supported by NIH grants RR15555 from the COBRE program of the National Center for Research Resources, R01-HL083151 from the NIH National Heart, Lung and Blood Institute, and the Maine Cancer Foundation. The research work of C.B. was supported by grants from the *Ministerio de Educación y Ciencia* (SAF2004-01390) and the *Instituto de Salud Carlos III* (ISCIII-CIBER CB/06/07/0038) of Spain.

Grant sponsor: National Center for Research Resources, NIH; Grant number: RR15555; Grant sponsor: NIH National Heart, Lung and Blood Institute; Grant number: R01-HL083151; Grant sponsor: Maine Cancer Foundation; Grant sponsor: Ministerio de Educación y Ciencia; Grant number: SAF2004-01390; Grant sponsor: Instituto de Salud Carlos III; Grant number: ISCIII-CIBER CB/06/07/0038.

References

- AAssar OS, Friedman CM, White RI Jr. The natural history of epistaxis in hereditary hemorrhagic telangiectasia. *Laryngoscope* 1991;101:977–980. [PubMed: 1886446]
- Abdalla SA, Letarte M. Hereditary haemorrhagic telangiectasia: Current views on genetics and mechanisms of disease. *J Med Genet* 2006;43:97–110. [PubMed: 15879500]
- Adam PJ, Clesham GJ, Weissberg PL. Expression of endoglin mRNA and protein in human vascular smooth muscle cells. *Biochem Biophys Res Commun* 1998;247:33–37. [PubMed: 9636649]
- Arthur HM, Ure J, Smith AJ, Renforth G, Wilson DI, Torsney E, Charlton R, Parums DV, Jowett T, Marchuk DA, Burn J, Diamond AG. Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Dev Biol* 2000;217:42–53. [PubMed: 10625534]
- Barbara NP, Wrana JL, Letarte M. Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. *J Biol Chem* 1999;274:584–594. [PubMed: 9872992]
- Barry FP, Boynton RE, Haynesworth S, Murphy JM, Zaia J. The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem Biophys Res Commun* 1999;265:134–139. [PubMed: 10548503]
- Belloni T, Corbi A, Lastres P, Cales C, Cebrian M, Vera S, Cheifetz S, Massague J, Letarte M, Bernabeu C. Identification and expression of two forms of the human transforming growth factor-beta-binding protein endoglin with distinct cytoplasmic regions. *Eur J Immunol* 1993;23:2340–2345. [PubMed: 8370410]
- Berg JN, Gutmacher AE, Marchuk DA, Porteous ME. Clinical heterogeneity in hereditary haemorrhagic telangiectasia: Are pulmonary arteriovenous malformations more common in families linked to endoglin? *J Med Genet* 1996;33:256–257. [PubMed: 8728706]
- Blanco FJ, Santibanez JF, Guerrero-Esteo M, Langa C, Vary CP, Bernabeu C. Interaction and functional interplay between endoglin and ALK-1, two components of the endothelial transforming growth factor-beta receptor complex. *J Cell Physiol* 2005;204:574–584. [PubMed: 15702480]
- Blobe GC, Schiemann WP, Pepin MC, Beauchemin M, Moustakas A, Lodish HF, O'Connor-McCourt MD. Functional roles for the cytoplasmic domain of the type III transforming growth factor beta receptor in regulating transforming growth factor beta signaling. *J Biol Chem* 2001;276:24627–24637. [PubMed: 11323414]
- Bodey B, Bodey B Jr, Siegel SE, Kaiser HE. Immunocytochemical detection of endoglin is indicative of angiogenesis in malignant melanoma. *Anticancer Res* 1998;18:2701–2710. [PubMed: 9703932]
- Bork P, Sander C. A large domain common to sperm receptors (Zp2 and Zp3) and TGF-beta type III receptor. *FEBS Lett* 1992;300:237–240. [PubMed: 1313375]
- Botella LM, Sanchez-Elsner T, Sanz-Rodriguez F, Kojima S, Shimada J, Guerrero-Esteo M, Cooreman MP, Ratzu V, Langa C, Vary CP, Ramirez JR, Friedman S, Bernabeu C. Transcriptional activation of endoglin and transforming growth factor-beta signaling components by cooperative interaction between Sp1 and KL F6: Their potential role in the response to vascular injury. *Blood* 2002;100:4001–4010. [PubMed: 12433697]

- Botella LM, Sanz-Rodriguez F, Sanchez-Elsner T, Langa C, Ramirez JR, Vary C, Roughley PJ, Bernabeu C. Lumican is down-regulated in cells expressing endoglin. Evidence for an inverse correlation between endoglin and lumican expression. *Matrix Biol* 2004;22:561–572. [PubMed: 14996436]
- Bourdeau A, Dumont DJ, Letarte M. A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest* 1999;104:1343–1351. [PubMed: 10562296]
- Bourdeau A, Faughnan ME, Letarte M. Endoglin-deficient mice, a unique model to study hereditary hemorrhagic telangiectasia. *Trends Cardiovasc Med* 2000;10:279–285. [PubMed: 11343967]
- Braverman IM, Keh A, Jacobson BS. Ultrastructure and three-dimensional organization of the telangiectases of hereditary hemorrhagic telangiectasia. *J Invest Dermatol* 1990;95:422–427. [PubMed: 2212727]
- Burrows FJ, Derbyshire EJ, Tazzari PL, Amlot P, Gazdar AF, King SW, Letarte M, Vitetta ES, Thorpe PE. Up-regulation of endoglin on vascular endothelial cells in human solid tumors: Implications for diagnosis and therapy. *Clin Cancer Res* 1995;1:1623–1634. [PubMed: 9815965]
- Carvalho RL, Jonker L, Goumans MJ, Larsson J, Bouwman P, Karlsson S, Dijke PT, Arthur HM, Mummery CL. Defective paracrine signalling by TGFbeta in yolk sac vasculature of endoglin mutant mice: A paradigm for hereditary haemorrhagic telangiectasia. *Development* 2004;131:6237–6247. [PubMed: 15548578]
- Cheifetz S, Bellon T, Cales C, Vera S, Bernabeu C, Massague J, Letarte M. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *J Biol Chem* 1992;267:19027–19030. [PubMed: 1326540]
- Chen CZ, Li M, de Graaf D, Monti S, Gottgens B, Sanchez MJ, Lander ES, Golub TR, Green AR, Lodish HF. Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. *Proc Natl Acad Sci USA* 2002;99:15468–15473. [PubMed: 12438646]
- Chen W, Kirkbride KC, How T, Nelson CD, Mo J, Frederick JP, Wang XF, Lefkowitz RJ, Blobel GC. Beta-arrestin 2 mediates endocytosis of type III TGF-beta receptor and down-regulation of its signaling. *Science* 2003;301:1394–1397. [PubMed: 12958365]
- Conley BS, Smith JD, Guerrero-Esteo M, Bernabeu C, Vary CPH. Endoglin, a TGF-beta receptor-associated protein, is expressed by smooth muscle cells in human atherosclerotic plaques. *Atherosclerosis* 2000;153:323–335. [PubMed: 11164421]
- Conley BA, Koleva RI, Smith JD, Kacer D, Zhang D, Bernabeu C, Vary CPH. Endoglin controls cell migration and composition of focal adhesions. *J Biol Chem* 2004;279:27440–27449. [PubMed: 15084601]
- Dakeishi M, Shioya T, Wada Y, Shindo T, Otaka K, Manabe M, Nozaki J, Inoue S, Koizumi A. Genetic epidemiology of hereditary hemorrhagic telangiectasia in a local community in the northern part of Japan. *Hum Mutat* 2002;19:140–148. [PubMed: 11793473]
- David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood* 2007;109:1953–1961. [PubMed: 17068149]
- Diez-Marques L, Ortega-Velazquez R, Langa C, Rodriguez-Barbero A, Lopez-Novoa JM, Lamas S, Bernabeu C. Expression of endoglin in human mesangial cells: Modulation of extracellular matrix synthesis. *Biochim Biophys Acta* 2002;1587:36–44. [PubMed: 12009422]
- Feng XH, Derynck R. Specificity and versatility in tgfbeta signaling through Smads. *Annu Rev Cell Dev Biol* 2005;21:659–693. [PubMed: 16212511]
- Fernandez-L A, Garrido-Martin EM, Sanz-Rodriguez F, Pericacho M, Rodriguez-Barbero A, Eleno N, Lopez-Novoa JM, Duwell A, Vega MA, Bernabeu C, Botella LM. Gene expression fingerprinting for human hereditary hemorrhagic telangiectasia. *Hum Mol Genet* 2007;16:1515–1533. [PubMed: 17420163]
- Fonsatti E, Altomonte M, Nicotra MR, Natali PG, Maio M. Endoglin (CD105): A powerful therapeutic target on tumor-associated angiogenic blood vessels. *Oncogene* 2003;22:6557–6563. [PubMed: 14528280]
- Foo SS, Turner CJ, Adams S, Compagni A, Aubyn D, Kogata N, Lindblom P, Shani M, Zicha D, Adams RH. Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. *Cell* 2006;124:161–173. [PubMed: 16413489]

- Gallione CJ, Klaus DJ, Yeh EY, Stenzel TT, Xue Y, Anthony KB, McAllister KA, Baldwin MA, Berg JN, Lux A, Smith JD, Vary CP, Craigen WJ, Westermann CJ, Warner ML, Miller YE, Jackson CE, Guttmacher AE, Marchuk DA. Mutation and expression analysis of the endoglin gene in hereditary hemorrhagic telangiectasia reveals null alleles. *Hum Mutat* 1998;11:286–294. [PubMed: 9554745]
- Ge AZ, Butcher EC. Cloning and expression of a cDNA encoding mouse endoglin, an endothelial cell TGF-beta ligand. *Gene* 1994;138:201–206. [PubMed: 8125301]
- Gougos A, Letarte M. Biochemical characterization of the 44G4 antigen from the HOON pre-B leukemic cell line. *J Immunol* 1988;141:1934–1940. [PubMed: 3262645]
- Gougos A, Letarte M. Primary structure of endoglin, an RGD-containing glycoprotein of human endothelial cells. *J Biol Chem* 1990;265:8361–8364. [PubMed: 1692830]
- Gougos A, St Jacques S, Greaves A, PJ OC, d'Apice AJ, Buhning HJ, Bernabeu C, van Mourik JA, Letarte M. Identification of distinct epitopes of endoglin, an RGD-containing glycoprotein of endothelial cells, leukemic cells, and syncytiotrophoblasts. *Int Immunol* 1992;4:83–92. [PubMed: 1371694]
- Guerrero-Esteo M, Lastres P, Letamendia A, Perez-Alvarez MJ, Langa C, Lopez LA, Fabra A, Garcia-Pardo A, Vera S, Letarte M, Bernabeu C. Endoglin overexpression modulates cellular morphology, migration, and adhesion of mouse fibroblasts. *Eur J Cell Biol* 1999;78:614–623. [PubMed: 10535303]
- Guerrero-Esteo M, Sánchez-Elsner T, Bernabeu C. Extracellular and cytoplasmic domains of endoglin interact with the TGF-b receptors I and II. *J Biol Chem* 2002;277:29197–29209. [PubMed: 12015308]
- Guo B, Slevin M, Li C, Parameshwar S, Liu D, Kumar P, Bernabeu C, Kumar S. CD105 inhibits transforming growth factor-beta-Smad3 signalling. *Anticancer Res* 2004;24:1337–1345. [PubMed: 15274293]
- Guttmacher AE, Marchuk DA, White RI Jr. Hereditary hemorrhagic telangiectasia. *N Engl J Med* 1995;333:918–924. [PubMed: 7666879]
- Jadrich JL, O'Connor MB, Coucouvanis E. The TGF{beta} activated kinase TAK1 regulates vascular development in vivo. *Development* 2006;133:1529–1541. [PubMed: 16556914]
- Jazwinska A, Ribeiro C, Affolter M. Epithelial tube morphogenesis during *Drosophila* tracheal development requires Piopio, a luminal ZP protein. *Nat Cell Biol* 2003;5:895–901. [PubMed: 12973360]
- Jerkic M, Rivas-Elena JV, Prieto M, Carron R, Sanz-Rodriguez F, Perez-Barriocanal F, Rodriguez-Barbero A, Bernabeu C, Lopez-Novoa JM. Endoglin regulates nitric oxide-dependent vasodilatation. *FASEB J* 2004;18:609–611. [PubMed: 14734648]
- Jerkic M, Rivas-Elena JV, Santibanez JF, Prieto M, Rodriguez-Barbero A, Perez-Barriocanal F, Pericacho M, Arevalo M, Vary CP, Letarte M, Bernabeu C, Lopez-Novoa JM. Endoglin regulates cyclooxygenase-2 expression and activity. *Circ Res* 2006;99:248–256. [PubMed: 16840721]
- Jiang X, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cardiac neural crest. *Development* 2000;127:1607–1616. [PubMed: 10725237]
- Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, Speer M, Pericak Vance MA, Diamond A, Guttmacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous ME, Marchuk DA. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet* 1996;13:189–195. [PubMed: 8640225]
- Jovine L, Darie CC, Litscher ES, Wassarman PM. Zona pellucida domain proteins. *Annu Rev Biochem* 2005;74:83–114. [PubMed: 15952882]
- Kjeldsen AD, Vase P, Green A. Hereditary haemorrhagic telangiectasia: A population-based study of prevalence and mortality in Danish patients. *J Intern Med* 1999;245:31–39. [PubMed: 10095814]
- Klemke RL, Leng J, Molander R, Brooks PC, Vuori K, Cheres DA. CAS/Crk coupling serves as a “molecular switch” for induction of cell migration. *J Cell Biol* 1998;140:961–972. [PubMed: 9472046]
- Koleva RI, Conley BA, Romero D, Riley KS, Marto JA, Lux A, Vary CP. Endoglin structure and function: Determinants of endoglin phosphorylation by transforming growth factor-beta receptors. *J Biol Chem* 2006;281:25110–25123. [PubMed: 16785228]
- Kumar P, Wang JM, Bernabeu C. CD 105 and angiogenesis. *J Pathol* 1996;178:363–366. [PubMed: 8691311]

- Kumar S, Ghellal A, Li C, Byrne G, Haboubi N, Wang JM, Bundred N. Breast carcinoma: Vascular density determined using CD105 antibody correlates with tumor prognosis. *Cancer Res* 1999;59:856–861. [PubMed: 10029075]
- Lastres P, Bellon T, Cabanas C, Sanchez-Madrid F, Acevedo A, Gougos A, Letarte M, Bernabeu C. Regulated expression on human macrophages of endoglin, an Arg-Gly-Asp-containing surface antigen. *Eur J Immunol* 1992;22:393–397. [PubMed: 1537377]
- Lastres P, Martin Perez J, Langa C, Bernabeu C. Phosphorylation of the human-transforming-growth-factor-beta-binding protein endoglin. *Biochem J* 1994;301:765–768. [PubMed: 8053900]
- Lastres P, Letamendia A, Zhang H, Rius C, Almendro N, Raab U, Lopez LA, Langa C, Fabra A, Letarte M, Bernabeu C. Endoglin modulates cellular responses to TGF-beta 1. *J Cell Biol* 1996;133:1109–1121. [PubMed: 8655583]
- Leask A, Abraham DJ, Finlay DR, Holmes A, Pennington D, Shi-Wen X, Chen Y, Venstrom K, Dou X, Ponticos M, Black C, Bernabeu C, Jackman JK, Findell PR, Connolly MK. Dysregulation of transforming growth factor beta signaling in scleroderma: Overexpression of endoglin in cutaneous scleroderma fibroblasts. *Arthritis Rheum* 2002;46:1857–1865. [PubMed: 12124870]
- Lebrin F, Goumans MJ, Jonker L, Carvalho RL, Valdimarsdottir G, Thorikay M, Mummery C, Arthur HM, Dijke Pt P. Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J* 2004;23:4018–4028. [PubMed: 15385967]
- Lee NY, Blobe GC. The interaction of endoglin with beta-arrestin2 regulates transforming growth factor-beta-mediated ERK activation and migration in endothelial cells. *J Biol Chem* 2007;282:21507–21517. [PubMed: 17540773]
- Letamendia A, Lastres P, Botella LM, Raab U, Langa C, Velasco B, Attisano L, Bernabeu C. Role of endoglin in cellular responses to transforming growth factor-beta. A comparative study with betaglycan. *J Biol Chem* 1998;273:33011–33019. [PubMed: 9830054]
- Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;355:992–1005. [PubMed: 16957146]
- Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP. Defective angiogenesis in mice lacking endoglin. *Science* 1999;284:1534–1537. [PubMed: 10348742]
- Li C, Hampson IN, Hampson L, Kumar P, Bernabeu C, Kumar S. CD105 antagonizes the inhibitory signaling of transforming growth factor beta1 on human vascular endothelial cells. *FASEB J* 2000;14:55–64. [PubMed: 10627280]
- Li C, Issa R, Kumar P, Hampson IN, Lopez-Novoa JM, Bernabeu C, Kumar S. CD105 prevents apoptosis in hypoxic endothelial cells. *J Cell Sci* 2003;116:2677–2685. [PubMed: 12746487]
- Lin HY, Lodish HF. Receptors for the TGF-beta superfamily: Multiple polypeptides and serine/threonine kinases. *Trends Cell Biol* 1993;3:14–19. [PubMed: 14731534]
- Liu Y, Jovanovic B, Pins M, Lee C, Bergan RC. Over expression of endoglin in human prostate cancer suppresses cell detachment, migration and invasion. *Oncogene* 2002;21:8272–8281. [PubMed: 12447690]
- Llorca O, Trujillo A, Blanco FJ, Bernabeu C. Structural model of human endoglin, a transmembrane receptor responsible for hereditary hemorrhagic telangiectasia. *J Mol Biol* 2007;365:694–705. [PubMed: 17081563]
- Lopez Casillas F, Payne HM, Andres JL, Massague J. Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: Mapping of ligand binding and GAG attachment sites. *J Cell Biol* 1994;124:557–568. [PubMed: 8106553]
- Lopez-Casillas F, Cheifetz S, Doody J, Andres JL, Lane WS, Massague J. Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system. *Cell* 1991;67:785–795. [PubMed: 1657406]
- Lopez-Casillas F, Wrana JL, Massague J. Betaglycan presents ligand to the TGF beta signaling receptor. *Cell* 1993;73:1435–1444. [PubMed: 8391934]
- Lux A, Gallione CJ, Marchuk DA. Expression analysis of endoglin missense and truncation mutations: Insights into protein structure and disease mechanisms. *Hum Mol Genet* 2000;9:745–755. [PubMed: 10749981]

- Lux, A.; Marchuk, DA. Hereditary hemorrhagic telangiectasia. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. Molecular and metabolic basis of inherited disease. 8. IV. NY: McGraw Hill; 2001. p. 5419-5431. Chapter 212
- Ma X, Labinaz M, Goldstein J, Miller H, Keon WJ, Letarte M, O'Brien E. Endoglin is overexpressed after arterial injury and is required for transforming growth factor- β -induced inhibition of smooth muscle cell migration. *Arterioscler Thromb Vasc Biol* 2000;20:2546–2552. [PubMed: 11116051]
- Machado RD, Rudarakanchana N, Atkinson C, Flanagan JA, Harrison R, Morrell NW, Trembath RC. Functional interaction between BMPR-II and Tctex-1, a light chain of dynein, is isoform-specific and disrupted by mutations underlying primary pulmonary hypertension. *Hum Mol Genet* 2003;12:3277–3286. [PubMed: 14583445]
- Mancini ML, Verdi JM, Conley BA, Nicola T, Spicer DB, Oxburgh LH, Vary CP. Endoglin is required for myogenic differentiation potential of neural crest stem cells. *Dev Biol* 2007;308:520–533. [PubMed: 17628518]
- Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science* 2002;298:1912–1934. [PubMed: 12471243]
- Marazuela M, Sanchez-Madrid F, Acevedo A, Larranaga E, de Landazuri MO. Expression of vascular adhesion molecules on human endothelia in autoimmune thyroid disorders. *Clin Exp Immunol* 1995;102:328–334. [PubMed: 7586686]
- McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, Helmbold EA, Markel DS, McKinnon WC, Murrell J, McCormick MK, Pericak-Vance MA, Heutink P, Oostra BA, Haitjema T, Westerman CJ, Porteous ME, Guttmacher AE, Letarte M, Marchuk DA. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet* 1994;8:345–351. [PubMed: 7894484]
- McAllister KA, Baldwin MA, Thukkani AK, Gallione CJ, Berg JN, Porteous ME, Guttmacher AE, Marchuk DA. Six novel mutations in the endoglin gene in hereditary hemorrhagic telangiectasia type 1 suggest a dominant-negative effect of receptor function. *Hum Mol Genet* 1995;4:1983–1985. [PubMed: 8595426]
- Meng Q, Lux A, Holloschi A, Li J, Hughes JM, McCarthy JE, Heagerty AM, Kioschis P, Hafner M, Garland JM. Identification of Tctex2beta a novel dynein light chain family member interacting with different TGF-beta receptors. *J Biol Chem* 2006;281:37069–37080. [PubMed: 16982625]
- Mercado-Pimentel ME, Hubbard AD, Runyan RB. Endoglin and Alk5 regulate epithelial-mesenchymal transformation during cardiac valve formation. *Dev Biol* 2007;304:420–432. [PubMed: 17250821]
- Muenzner P, Rohde M, Kneitz S, Hauck CR. CEACAM engagement by human pathogens enhances cell adhesion and counteracts bacteria-induced detachment of epithelial cells. *J Cell Biol* 2005;170:825–836. [PubMed: 16115956]
- Obreo J, Diez-Marques L, Lamas S, Duwell A, Eleno N, Bernabeu C, Pandiella A, Lopez-Novoa J, Rodriguez-Barbero A. Endoglin expression regulates basal and TGF-beta1-induced extracellular matrix synthesis in cultured L(6)E(9) myoblasts. *Cell Physiol Biochem* 2004;14:301–310. [PubMed: 15319534]
- Pece-Barbara N, Vera S, Kathirkamathamby K, Liebner S, Di Guglielmo GM, Dejana E, Wrana JL, Letarte M. Endoglin null endothelial cells proliferate faster, and more responsive to TGFbeta 1 with higher affinity receptors and an activated ALK1 pathway. *J Biol Chem* 2005;280:27800–27808. [PubMed: 15923183]
- Perez-Gomez E, Eleno N, Lopez-Novoa JM, Ramirez JR, Velasco B, Letarte M, Bernabeu C, Quintanilla M. Characterization of murine S-endoglin isoform and its effects on tumor development. *Oncogene* 2005;24:4450–4461. [PubMed: 15806144]
- Perlingeiro RC. Endoglin is required for hemangioblast and early hematopoietic development. *Development* 2007;134:3041–3048. [PubMed: 17634194]
- Prigoda NL, Savas S, Abdalla SA, Piovesan B, Rushlow D, Vandezande K, Zhang E, Ozcelik H, Gallie BL, Letarte M. Hereditary haemorrhagic telangiectasia: Mutation detection, test sensitivity and novel mutations. *J Med Genet* 2006;43:722–728. [PubMed: 16690726]
- Prout M, Damania Z, Soong J, Fristrom D, Fristrom JW. Autosomal mutations affecting adhesion between wing surfaces in *Drosophila melanogaster*. *Genetics* 1997;146:275–285. [PubMed: 9136017]

- Qu R, Silver MM, Letarte M. Distribution of endoglin in early human development reveals high levels on endocardial cushion tissue mesenchyme during valve formation. *Cell Tissue Res* 1998;292:333–343. [PubMed: 9560476]
- Rulo HF, Westphal JR, van de Kerkhof PC, de Waal RM, van Vlijmen IM, Ruiter DJ. Expression of endoglin in psoriatic involved and uninvolved skin. *J Dermatol Sci* 1995;10:103–109. [PubMed: 8534608]
- Santibanez JF, Letamendia A, Perez-Barriocanal F, Silvestri C, Saura M, Vary CP, Lopez-Novoa JM, Attisano L, Bernabeu C. Endoglin increases eNOS expression by modulating Smad2 protein levels and Smad2-dependent TGF-beta signaling. *J Cell Physiol* 2007;210:456–468. [PubMed: 17058229]
- Sanz-Rodriguez F, Guerrero-Esteo M, Botella LM, Banville D, Vary CPH, Bernabeu C. Endoglin regulates cytoskeletal organization through binding to ZRP-1, a member of the Lim family of proteins. *J Biol Chem* 2004;279:32858–32868. [PubMed: 15148318]
- Schampfenecker M, van Dinther M, Liu Z, van Bezooijen RL, Zhao Q, Pukac L, Lowik CW, ten Dijke P. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. *J Cell Sci* 2007;120:964–972. [PubMed: 17311849]
- Schnerer O, Meurer SK, Tihaa L, Gressner AM, Weiskirchen R. Endoglin differentially modulates antagonistic transforming growth factor-beta1 and BMP-7 signaling. *J Biol Chem* 2007;282:13934–13943. [PubMed: 17376778]
- Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003;113:685–700. [PubMed: 12809600]
- Shovlin CL, Winstock AR, Peters AM, Jackson JE, Hughes JM. Medical complications of pregnancy in hereditary haemorrhagic telangiectasia. *Q J Med* 1995;88:879–887.
- Shovlin CL, Hughes JM, Scott J, Seidman CE, Seidman JG. Characterization of endoglin and identification of novel mutations in hereditary hemorrhagic telangiectasia. *Am J Hum Genet* 1997;61:68–79. [PubMed: 9245986]
- Toporsian M, Gros R, Kabir MG, Vera S, Govindaraju K, Eidelman DH, Husain M, Letarte M. A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. *Circ Res* 2005;96:684–692. [PubMed: 15718503]
- Torsney E, Charlton R, Diamond AG, Burn J, Soames JV, Arthur HM. Mouse model for hereditary hemorrhagic telangiectasia has a generalized vascular abnormality. *Circulation* 2003;107:1653–1657. [PubMed: 12668501]
- van de Kerkhof PC, Rulo HF, van Pelt JP, van Vlijmen-Willems IM, De Jong EM. Expression of endoglin in the transition between psoriatic uninvolved and involved skin. *Acta Derm Venereol* 1998;78:19–21. [PubMed: 9498020]
- van Laake LW, van den Driesche S, Post S, Feijen A, Jansen MA, Driessens MH, Mager JJ, Snijder RJ, Westermann CJ, Doevedans PA, van Echteld CJ, ten Dijke P, Arthur HM, Goumans MJ, Lebrin F, Mummery CL. Endoglin has a crucial role in blood cell-mediated vascular repair. *Circulation* 2006;114:2288–2297. [PubMed: 17088457]
- Venkatesha S, Toporsian M, Lam C, Hanai JI, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;12:642–649. [PubMed: 16751767]
- Vivien D, Wrana JL. Ligand-induced recruitment and phosphorylation of reduced TGF-beta type I receptor. *Exp Cell Res* 1995;221:60–65. [PubMed: 7589256]
- Westermann CJ, Rosina AF, De Vries V, de Coteau PA. The prevalence and manifestations of hereditary hemorrhagic telangiectasia in the Afro-Caribbean population of the Netherlands Antilles: A family screening. *Am J Med Genet A* 2003;116:324–328. [PubMed: 12522784]
- Wrana JL, Attisano L, Carcamo J, Zentella A, Doody J, Laiho M, Wang XF, Massague J. TGF beta signals through a heteromeric protein kinase receptor complex. *Cell* 1992;71:1003–1014. [PubMed: 1333888]
- Yamada Y, Yokoyama S, Wang XD, Fukuda N, Takakura N. Cardiac stem cells in brown adipose tissue express CD133 and induce bone marrow nonhematopoietic cells to differentiate into cardiomyocytes. *Stem Cells* 2007;25:1326–1333. [PubMed: 17289932]

- Yamaguchi H, Azuma H, Shigekiyo T, Inoue H, Saito S. A novel missense mutation in the endoglin gene in hereditary hemorrhagic telangiectasia. *Thromb Haemost* 1997;77:243–247. [PubMed: 9157574]
- Yamashita H, Ichijo H, Grimsby S, Moren A, ten Dijke P, Miyazono K. Endoglin forms a heteromeric complex with the signaling receptors for transforming growth factor-beta. *J Biol Chem* 1994;269:1995–2001. [PubMed: 8294451]

Fig. 1.

Atomic model and electron microscopy of endoglin. **A:** The predicted atomic model was generated as described [Llorca et al., 2007]. The amino acid numbers corresponding to the approximate location of disordered regions connecting globular domains are indicated. The molecule is colored according to the three types of domains defined. The orphan domain encompasses amino acid residues Glu26-Ile359 (red), whereas the ZP domain is contained within the fragment Gln360-Gly586. The ZP-N and ZP-C sub-domains are colored in yellow and blue, respectively. **B:** Fitting of the atomic model into the electron microscopy density map of soluble endoglin. Side (i) and top (ii) views of the electron microscopy density containing the fitted monomer are shown. The fitting of dimeric endoglin based on the atomic prediction of the monomer is also included (iii). **C:** Cartoon model for the domain organization of endoglin within the dimer. Adapted from Llorca et al. [2007].

Fig. 2.

Hypothetical model for endoglin in TGFβ/ALK-1 and TGFβ/ALK-5 pathways. Endoglin extracellular and cytoplasmic domains interact with ALK1 [Blanco et al., 2005] and ALK5 [Guerrero-Esteo et al., 2002], as indicated with brown arrows. Endoglin plays a crucial role on TGFβ signaling by potentiating ALK1/Smad1, ALK5/Smad2 (green arrows), and inhibiting ALK5/Smad3 (red arrow) pathways which lead to the regulation of Id1 [Lebrin et al., 2004; Blanco et al., 2005], eNOS [Santibanez et al., 2007], and PAI-1 genes [Letamendia et al., 1998; Guerrero-Esteo et al., 1999], respectively. The involvement of TβRII and TGFβ has been omitted for simplification. Adapted from [Blanco et al., 2005].

Fig. 3.

Hypothetical model for endoglin cytosolic domain-mediated functions. **A:** Endoglin cytosolic domain is constitutively phosphorylated [Lastres et al., 1994] by serine and threonine kinases, including the TβRII, ALK1, and ALK5 receptors [Guerrero-Esteo et al., 2002; Koleva et al., 2006]. This endoglin phosphorylation potentially regulates multiple protein–protein interactions involving the cytosolic domain. **B:** Endoglin interacts with the cytosolic proteins zyxin, ZRP-1, Tctex2b, and beta-arrestin [Conley et al., 2004; Sanz-Rodriguez et al., 2004; Koleva et al., 2006; Meng et al., 2006; Lee and Blobel, 2007]. These interactions likely mediate downstream functions, including F-actin dynamics, focal adhesion composition, and protein transport via endocytic vesicles. In turn, these processes regulate cell adhesion, migration, and proliferation.