Endoglin (CD105) is a transmembrane glycoprotein expressed on activated vascular endothelial cells (1). It is an accessory protein of the transforming growth factor-β (TGF-β) receptor system and composed of two disulfide-linked subunits of 95 kDa each forming a 180-kDa homodimeric mature protein (2). In addition to its expression on endothelial cells, endoglin is found on the surface of several other cell types (Table 1; reviewed in refs. 2–5).

The endoglin gene is 40 kb long and located on human chromosome 9q34 (6, 7). Gene transcription results in a mRNA product with a length of 3.4 kb and 14 exons; exons 1 to 12 encode the extracellular domain, exon 13 encodes the transmembrane domain, and exon 14 encodes the cytoplasmic domain. Endoglin is expressed as two isoforms, designated long (L-CD105) and short (S-CD105), based on the length of the cytoplasmic domain (5, 8). Endoglin was assigned the cluster of differentiation number 105 at the Fifth International Workshop on Human Leukocyte Differentiation Antigens and thus is also known as CD105 (9).

The importance of endoglin in vascular homeostasis is reflected by the fact that mutations in endoglin or its downstream signaling mediator lead to hereditary hemorrhagic telangiectasias (Osler-Weber-Rendu syndrome; ref. 10). This syndrome, which affects 1 in 8,000 individuals worldwide, is an inherited autosomal-dominant and highly penetrant disorder characterized by vascular dysplasias, frequent episodes of epistaxis, mucocutaneous telangiectases, and arteriovenous malformations of the lung, brain, liver, and gastrointestinal tract (1, 2, 10–12). There are two genetic forms of the disease: hereditary hemorrhagic telangiectasia 1, characterized by a mutation of endoglin itself, and hereditary hemorrhagic telangiectasia 2, characterized by a mutation of the endoglin-associated protein activin-like kinase (ALK) 1 (10, 13). With both forms, patients have dilation of postcapillary venules and direct arteriovenous connections that bypass capillary networks (7). In a transgenic murine model of hereditary hemorrhagic telangiectasia with a heterozygous genotype for endoglin (eng+/−), mice exhibit large numbers of irregular, dilated, and thinner-walled vessels with fewer associated vascular smooth muscle cells than their wild-type counterparts. Whereas endoglin haploinsufficient mice survive to adulthood, homozgyous mutation of endoglin (eng−/−) results in embryologic lethality by day E11.5 due to defective yolk sac vascularization, heart valve abnormalities, and irregular ventricular development (11, 14, 15).

Overview of the TGF-β System and Endoglin

As mentioned above, endoglin is an accessory protein of the TGF-β receptor family. TGF-β ligands and receptors make up a complex signaling system involved in many developmental, physiologic, and pathologic cellular processes (16). The TGF-β family of ligands includes TGF-β1, TGF-β2, and TGF-β3 isoforms, activins, and bone morphogenetic proteins (BMP; ref. 2). There are seven type I (TβR-I), five type II (TβR-II), and two type III (TβR-III) receptors (11, 13, 17). TβR-I and TβR-II are serine/threonine kinase receptors and their kinase activity is necessary for all responses to TGF-β (1). In contrast, the type III receptors, endoglin and betaglycan, lack kinase activity and are regulators of binding and signaling. As shown by coimmuno-precipitation experiments, TGF-β does not bind endoglin alone
The inhibitory Smads Smad-6 and Smad-7 interfere with Smad-mediated signaling by competing with receptor-mediated Smads for receptor binding. Inhibitory Smads can also dephosphorylate the activated type I receptor and recruit ubiquitin ligases to the cell membrane to promote the degradation of the receptor (10, 13, 21).

### Endoglin and Endothelial Cell Signaling

TGF-β signaling in endothelial cells can mediate a variety of processes, including proliferation, migration, and adhesion (7). TGF-β is expressed in endothelial cells of healing wounds, embryos, infected tissues, psoriatic skin, inflamed synovial arthritis, and solid tumors (2, 20). Whether TGF-β stimulates or inhibits these processes depends on experimental conditions. For example, low doses of TGF-β in vitro (0.25-0.70 ng/mL) stimulate endothelial cell proliferation and migration, whereas higher doses inhibit them, causing endothelial cell quiescence (22–24).

The stimulatory phenotype in endothelial cells is mediated by ALK1-dominated signaling, and endoglin expression is necessary for activation of this pathway. ALK1 activation causes an increase in endothelial cell proliferation and migration as well as transcription of the proangiogenic genes Id1, interleukin-1 receptor-like 1, and endoglin itself (11, 25). In contrast, ALK5 signaling induces endothelial cell quiescence by inhibiting proliferation and migration and increases expression of maturation-specific genes, such as fibronectin, connexin-37, α3β1 integrin, and plasminogen activator inhibitor-1 (10, 11, 13).

ALK5 signaling on endothelial cells also promotes the recruitment and differentiation of vascular smooth muscle cells during vessel formation (11). ALK5 is the predominant mediator of TGF-β signaling in quiescent endothelial cells, but during angiogenesis, ALK1 is preferentially activated (11, 13). In addition, the complete absence of ALK5 in cells causes a decrease in ALK1 signaling, indicating that the presence of ALK5 is also necessary for signaling via ALK1 (26).

Alteration of the patterns of expression of molecules in these pathways has led to several interesting insights into their biological roles.
signaling. Overexpression of ALK1 in endothelial cells in vitro causes a decrease in cell attachment and a loss of proliferation and overall viability; additional forced overexpression of endoglin opposes this phenotype, thus underscoring the importance of endoglin in ALK1 signaling (17). In addition, reduction of ALK5 signaling in endothelial cells rescues the effect of small interfering RNA–mediated silencing of endoglin expression (26). Furthermore, focused gene chip array studies showed that constitutively active ALK1 led to an up-regulation of the angiogenic mediators interleukin-8 (18-fold), jagged-1 (3-fold), and endothelin-1 (3-fold; ref. 27). In contrast to TGF-β, BMP-9, another ALK1 and endoglin ligand, activates the ALK1/Smad-1/Smad-5/Smad-8 pathway but phenotypically leads to a decrease in endothelial cell migration and proliferation (18). BMP-9 also signals via BMP receptor II in these cells, and the interaction of signaling via these two receptor complexes may explain the seemingly contradictory results.

In endothelial cells, endoglin expression is up-regulated by hypoxia, TGF-β stimulation, and irradiation in vitro, whereas endoglin is down-regulated by tumor necrosis factor-α (13, 19). Endoglin is constitutively phosphorylated in vascular endothelial cells on two serine residues, Ser634 and Ser635 (8, 17, 20). TGF-β signaling in the presence of endoglin causes an activation of ALK1-associated Smad proteins as well as negative regulation of the ALK5-associated Smad-3 protein. Ectopic endoglin expression, in fact, inhibits Smad-3 transcriptional activity via c-Jun activation and thus negatively regulates ALK5 pathway activation (11, 13, 28). Interestingly, endoglin expression increases Smad-2 activity, leading to an increase in endothelial nitric oxide synthase (eNOS) expression (discussed below; refs. 8, 12). This overlapping regulation highlights the complexity of TβR-I/endoglin interaction and signaling. Interestingly, only 1% of endoglin molecules on an endothelial cell bind TGF-β in a receptor complex, but this finding has unknown functional significance (9, 29, 30).

Experimental alteration of endoglin transcription and translation has also led to other interesting findings that highlight the importance of endoglin. When endoglin expression was reduced by an antisense approach, the inhibitory effects of TGF-β on endothelial cell proliferation, migration, and tube formation were heightened (20). These effects were mediated by ALK5 signaling, emphasizing that endoglin is a negative regulator of ALK5 activity (13). Stable transfection of endothelial cells with small interfering RNA to endoglin also enhanced the ability of TGF-β to suppress growth and migration (26, 31). Furthermore, when endoglin small interfering RNA–transfected cells were irradiated in vitro, they showed a decrease in cell viability, with elevation of p53 (proapoptotic) and reduction of bcl-2 (antiapoptotic) levels compared with mock-transfected cells (31). Endoglin is thus important in endothelial cell survival during radiation exposure in addition to growth and migration.

Although it is clear that endoglin expression in tissue sections is a marker of proliferating endothelial cells, there is no consensus as to the exact role of endoglin in developmental angiogenesis. Endothelial cells from eng+/+ and eng−/− mouse embryos were isolated, immortalized, and cultured (32). eng−/− mouse embryonic endothelial cells showed a higher proliferation rate by [3H]thymidine incorporation and achieved higher cell densities relative to eng+/+ mouse embryonic endothelial cells. This finding is in contrast to previous work that showed an increase in proliferation of endoglin-overexpressing cells (26). Furthermore, eng+/+ cells showed increased levels of phosphorylated Smad-1/Smad-5/Smad-8 when compared with eng−/− mouse embryonic endothelial cells in the former study, whereas the latter study showed the opposite. It is hypothesized that the discordant results of these studies were due to the
differences between RNA interference technology and gene ablation. Specifically, small interfering RNA silencing of endoglin led to decreased ALK5 levels, whereas ALK5 levels in eng−/− mouse embryonic endothelial cells were elevated (32). The above reinforces the fact that endoglin signaling is complex and requires further study.

Endoglin downstream signaling also interacts with the nitric oxide pathway in endothelial cells. Transcription of eNOS is regulated by endothelial shear stress, hypoxia, hormones, and various growth factors and mediators including TGF-β1 (12). In endoglin haploinsufficient mice (eng+/−), levels of eNOS are constitutively reduced (33, 34). Modulation of endoglin expression has been shown to regulate nitric oxide–dependent vasodilation in addition to eNOS expression and activity in both in vitro and in vivo models (12). Using a doxycycline-inducible endoglin system in endothelial cells, it has been shown that increases in endoglin correspond with increases in eNOS mRNA and protein levels; similarly, reduction in endoglin causes a decrease in both basal and TGF-β1–induced eNOS expression (12). This regulation occurs through interaction of endoglin with ALK5/Smad-2, causing an increase in phosphorylation of the latter in a TβR-I–independent manner. These findings suggest that endoglin expression and nitric oxide regulation are intimately related and that eNOS plays a major role in endoglin-dependent angiogenesis.

Endoglin and Angiogenesis

Endothelial cells in normal quiescent endothelium are stable and have a very low turnover rate, with a doubling time of more than 1,000 days. Angiogenic endothelium, in contrast, has a rapid turnover and has been termed “activated” (35). One potential marker of this activated endothelium is endoglin. In several malignancies, endoglin is found on peritumoral and intratumoral vessels. Our laboratory has evaluated patterns of endoglin expression in primary colon adenocarcinomas and normal colonic mucosa, along with CD31 expression. Whereas anti-CD31 antibodies stained blood vessels in both normal and malignant colon equally, endoglin expression was observed primarily in malignant lesions, with little to no expression in the vessels of the nonmalignant mucosa (Fig. 2). Furthermore, many investigators have shown that endoglin expression serves as a better prognostic marker of patient outcomes than more traditional vascular markers, such as CD31 and von Willebrand factor (3, 4, 36, 37).

Table 2 summarizes studies that have examined endoglin (CD105) expression on tumor endothelium and clinicopathologic factors associated with its expression (4, 8, 38–54). In all malignancies studied, endoglin expression as determined by immunohistochemical staining was consistently associated with lower patient survival rates. This is not surprising because increased tumor vasculature is an established marker of poor prognosis. Furthermore, in gastrointestinal, breast, prostate, and head and neck malignancies, endoglin expression was associated with the presence of distant metastatic disease (38, 40, 41, 44, 46–48). Endoglin expression thus seems to have prognostic value in a variety of solid cancers.

Table 2. Endoglin expression and associated clinicopathologic variables in human solid malignancies

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Lymph node metastasis</th>
<th>Distant metastasis</th>
<th>Higher tumor grade</th>
<th>Decreased survival</th>
<th>Comments</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Gastric cancer</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Ascites</td>
<td>Ding et al. (38)</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nikiteas et al. (39)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>Saad et al. (40)</td>
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<td>Duff et al. (8)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>Response to neoadjuvant chemotherapy</td>
<td>Fonsatti and Maio (4)</td>
</tr>
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<td>Saad et al. (41)</td>
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<td>Akagi et al. (42)</td>
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<td>Duff et al. (8)</td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Fonsatti and Maio (4)</td>
</tr>
<tr>
<td>Non–small cell lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gomez-Esquer et al. (43)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Preoperative PSA</td>
<td>Li et al. (44)</td>
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<td></td>
<td></td>
<td>Beresford et al. (45)</td>
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<tr>
<td>Head and neck cancers</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>VEGF levels</td>
<td>Chien et al. (48)</td>
</tr>
<tr>
<td>Primary brain malignancies</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>VEGF levels</td>
<td>Chuang et al. (49)</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
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<td></td>
<td>+</td>
<td>+</td>
<td>VEGF levels</td>
<td>Marioni et al. (50)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>Suboptimal cytoreduction</td>
<td>Erdem et al. (53)</td>
</tr>
</tbody>
</table>

Abbreviations: PSA, prostate-specific antigen; VEGF, vascular endothelial growth factor.
cyclophosphamide) was associated with an increased clinical response rate (45). The differences between pathologic responders and nonresponders did not reach statistical significance, but this may be attributed to the elimination of complete responders from the analysis because they had no postoperative tumor to evaluate.

**Endoglin and Hypoxia**

Hypoxia is a potent stimulus for tumor-associated induction of angiogenic factors. Given the role of endoglin in activated endothelium, it is no surprise that there is a correlation between hypoxia and endoglin expression. In fact, hypoxia is one of the few known stimuli of endoglin induction. In *vitro*, hypoxia (0.2% O₂) causes an increase in endoglin mRNA levels as early as 1 h after the onset of hypoxia exposure, with maximum RNA transcription at 3 h; endoglin protein levels are elevated to maximum levels at 16 h after exposure to hypoxia (30). Regulation of endoglin expression by hypoxia occurs at the transcriptional level as evidenced by standard reporter assays (30). In *vivo*, hypoxia induced by occlusion of the middle cerebral artery in a mouse model caused up-regulation of endoglin on the endothelium of focally ischemic areas in the brain (55). Functionally, endoglin expression not only promotes angiogenesis by activating endothelial proliferation pathways but also activates antiapoptotic signaling in hypoxic endothelial cells (13). In cells treated with antisense to endoglin, TGF-β augments the apoptotic effect of hypoxia (29, 30). Induction of endoglin by hypoxia seems to be regulated by intermediates such as p38 and c-Jun NH₂-terminal kinase (55). These findings suggest that endoglin may be involved in the stimulation of tumor angiogenesis via hypoxic regulation.

**Endoglin in Diagnosis and Therapy**

As discussed above, endoglin is preferentially expressed in the angiogenic endothelium of tumors. The expression of endoglin on tumor endothelium may have diagnostic value if its expression can be imaged in *vivo*. Investigators attempted to show this principle using technetium-99m-labeled anti-endoglin monoclonal antibodies (mAb) to perfuse fresh nephrectomy specimens from patients with renal cell carcinoma (56). Radiographic hotspots corresponded to periprostatic magnetic resonance imaging–identified tumors in all seven cases studied. In one case, technetium-99m imaging was able to identify a malignant mass that was missed on preoperative magnetic resonance imaging–identified tumors in all seven cases studied. In another case, investigators injected iodine-125–labeled anti-endoglin mAbs into two dogs with spontaneous mammary carcinoma and imaged them 8 h later (57). Tumors showed rapid and efficient uptake of radioactivity with high signal-to-noise ratios. A diagnosis of ductal adenocarcinoma was verified by surgical excision 10 days later, and the dogs showed no systemic side effects during the 3-month follow-up period. Others used indium-111–labeled anti-endoglin mAbs to identify B16 melanoma-bearing tumors in mice by *in vivo* scintigraphy (58). Another group of investigators conjugated deglycosylated ricin A and treated mice bearing breast cancer xenografts (65). More than half of the mice showed tumor regression, and no progression was seen even after 100 days with no further therapy. Other investigators conjugated anti-endoglin mAbs to toxic molecules in an attempt to increase the efficacy of treatment.

A unique therapeutic approach involves sensitizing host immune cells to the endoglin protein prophylactically so that future angiogenic vessels are seen as antigenic and targeted by the host’s own immune system. This approach was used by investigators using a double-attenuated *Salmonella*-based oral vaccine containing a murine endoglin-expressing plasmid (68). After vaccination, mice were injected with D2F2 mouse mammary carcinoma, and tumor progression was evaluated. Unvaccinated mice had significantly more lung metastases, and their tumors had a more disseminated distribution. Tumors in vaccinated mice were less angiogenic, and the vaccinated mice had a longer overall survival. Given the above findings, endoglin seems to be a unique and optimal target for antitumor therapies and needs to be explored further.

**Conclusion**

Recent advances in antiangiogenic therapies for solid malignancies have improved patient survival and thus validated the tumor vasculature as a target in anticancer therapy.
Targeting the angiogenic mediator vascular endothelial growth factor has proven efficacious in several solid malignancies; however, targeting the tumor-associated endothelial cell directly may also be a successful strategy (69). Endoglin has been identified as a unique marker of proliferating (activated) endothelial cells in vitro and in vivo. Although the exact role of endoglin in endothelial cell signaling is complex and remains to be completely elucidated, its presence in solid tumor vasculature has prognostic value, suggesting its potential as a therapeutic target. Targeting endoglin on the activated endothelial cells that express this target has produced promising preclinical results and thus warrants further investigation.

References


Endoglin (CD105): A Marker of Tumor Vasculature and Potential Target for Therapy

Nikolaos A. Dallas, Shaija Samuel, Ling Xia, et al.


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