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Review

Endoglin (CD105): A Review of its Role in Angiogenesis and Tumor Diagnosis, Progression and Therapy

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Abstract. *Endoglin (CD105) is an accessory receptor for transforming growth factor beta (TGF-β) and its expression is up-regulated in actively proliferating endothelial cells. Endoglin has been suggested as an appropriate marker for tumor-related angiogenesis and neovascularization. Several studies demonstrate the potential of endoglin in tumor diagnosis, prognosis, and therapy. This review details the structure and function of endoglin, and investigates the role of endoglin in angiogenesis and tumor diagnosis, prognosis, and therapy.*

Recent movements towards antibody-based therapeutic strategies in cancers have resulted in the characterization of several potential antigens. Endoglin, also known as CD105, is one such antigen gaining widespread popularity.

Endoglin was originally characterized over two decades ago (1). It is classified as an accessory receptor for transforming growth factor beta (TGF-β), a pleiotropic cytokine, and has been shown to be expressed on endothelial cells (2). Remarkably, its expression is up-regulated in actively proliferating endothelial cells (3-5). Endoglin has therefore been suggested as an appropriate marker for tumor-related angiogenesis and neovascularization. Its roles in the prognosis, diagnosis, and treatment of neoplasms have recently been discussed (5-8). The endothelial cells of neoplasms are more prolific than endothelial cells of normal tissue and thus they express elevated endoglin levels (9, 10). Therefore, the goal of therapeutic cancer treatment with endoglin is to selectively target highly proliferating endothelial cells in order to inhibit metastasis and induce tumor shrinkage by preventing the vital delivery of nutrients to, and the exchange of waste from, tumor cells.

This review highlights the important structural and functional features of endoglin, and the role of endoglin in angiogenesis and its potential use as a diagnostic, prognostic, and therapeutic agent in patients with tumors.

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Structure and Expression

Human endoglin is a 633 amino acid, 180 kDa homodimeric disulfide-linked hypoxia-inducible transmembrane glycoprotein. It contains a large extracellular domain, a hydrophobic transmembrane domain, and a short intracellular domain (11, 12). The extracellular domain contains an Arg-Gly-Asp (RGD) tripeptide, and four *N*-linked glycosylation sites and a region of *O*-linked glycosylation (12). The RGD tripeptide is absent from murine endoglin (13). The intracellular domain contains many serine and threonine residues, some of which are phosphorylation sites (14). Two isoforms of endoglin exist, L and S, and they differ in the length of the intracellular domain, tissue distribution and degree of phosphorylation. L-Endoglin contains 47 amino acids in the cytoplasmic tail, has a high degree of phosphorylation and is predominantly expressed in endothelial cells,

whereas S-endoglin contains only 14 amino acids (15, 16). Both isoforms are constitutively phosphorylated and this is likely due to the constitutively active TGF-β receptor type II (TGF-βR2)(17, 18). A soluble form of endoglin has also been identified in the sera of healthy and cancer patients (8). Elevated levels of soluble endoglin have been noted in sera of patients with diseases such as metastatic melanoma, and breast cancer patients at risk of metastasis (19, 20).

The 14 exon endoglin gene is located on chromosome 9q34 (21, 22). Mutations in the gene, mostly those truncating the extracellular domain of the protein, can lead to hereditary hemorrhagic telangiectasia type 1 (HHT1) syndrome, an autosomal dominant vascular dysplasia (22, 23). Additionally, endoglin null mice die of vascular developmental defects, particularly of the primitive vascular plexus of the yolk sac, by gestinational day 11.5 (24).

TGF-β1 and hypoxic conditions can induce the up-regulation of the endoglin gene promoter (25). Cloning of the 2.6 kb endoglin promoter, which contains TGF-β response elements but does not contain either TATA or CAAT transcription start sites, demonstrated strong activity in endothelial cells and much weaker activity in epithelial cells and fibroblasts (25, 26). Two domains, one in the region of -1294 and -932 and the other near the 5' region of the promoter, have been identified as being involved in determining the endothelial cell-specific expression of endoglin. The region surrounding the transcription start site is essential for endoglin promoter function in both endothelial and non-endothelial cells (26).

In addition to the up-regulation of endoglin promoter activity, hypoxic conditions up-regulate endoglin mRNA and protein levels (27). Endoglin protein levels, and levels of other proteins such as those that are homologs of both the drosophilia protein, mothers against decapentaplegic (Mad) and the *C. elegans* proteins (Sma), (Smad6 and Smad7), inhibitor of differentiation proteins (id1 and id2), signal transducers and activators of transcription (STAT1), and interleukin 1 receptor-like 1 (IL1R1), have also been found to be up-regulated in human umbilical vein endothelial cells (HUVECs) transfected with an active form of activin receptor-like kinase (ALK)-1, a TGF-βR1 (28). TNF-α application resulted in the down-regulation of endoglin protein levels (29).

Endoglin is co-expressed with β-glycan, another component of the TGF-βR complex, in the microvascular endothelium of normal tissue (2). Endoglin is highly expressed in synctiotrophoblasts and more weakly expressed in stromal cells, fibroblasts and hematopoietic progenitor cells (30-33). In solid malignancies, endoglin is highly expressed on peri- and intratumoral endothelial cells and sometimes on the stroma of the tumor (3, 19, 34, 35).

Function

Endoglin is an accessory co-receptor for TGF-β, a pleiotropic cytokine regulating cellular proliferation, differentiation, migration and adhesion (2, 36-38). TGF-β signals *via* heterodimeric serine/threonine kinases TGF-β receptor type 1 (TGF-βR1) and TGF-βR2. TGF-β1 has been shown to function as a tumor suppressor and it also induces inflammation and release of angiogenic factors from inflammatory cells *in vivo* (39). This has not been replicated *in vitro* (40). Endoglin binds TGF-β1 and β3 with high affinity (K_D ~60 pmol/l) by associating with the constitutively active TGF-βR2 (16, 41). This activates the cytoplasmic kinase activity of TGF-βR2 and results in the phosphorylation of TGF-βR1 which interacts with downstream signaling molecules such as the SMAD family of proteins (42). There are two TGF-βR1 cascades that compete with each other. The first is ALK-5 inducing SMAD2/3 phosphorylation, which inhibits cellular responses to TGF-β. The second is ALK-1 inducing SMAD1/5 phosphorylation, which enhances cellular responses to TGF-β (17, 43). Some studies suggest that endoglin is only required for the ALK-1 pathway and not the ALK-5 pathway (43, 44), whereas others show that the cytoplasmic domain of endoglin is regulated by ALK-5 phosphorylation on serines 646 and 649 (45). However, the mechanism and location of phosphorylation seems to differ between disease states (46).

TGF-β1 binding reduces endoglin phosphorylation (18). Cells transfected with and overexpressing endoglin inhibited their normal responses to TGF-β1, including the inhibition of cell proliferation, *c-myc* mRNA down-regulation, cellular adhesion stimulation, homotypic aggregation, and phosphorylation of platelet/endothelial cell adhesion molecule-1 (CD31) (47, 48). It has been shown that endoglin inhibition enhances TGF-β1-induced growth and migration suppression (49). Taken together these data suggest that endoglin modulates the effects of TGF-β1 as part of the TGF-βR complex. However, endoglin has been identified as a component in the endothelial nitric oxide synthase pathway and modulates cyclooxygenase-2 (COX-2) activity (50, 51). In development, endoglin appears to modulate the transition from endothelial progenitors to functional endothelial cells (52). In addition, only a small percentage of TGF-β1 molecules bind endoglin and endoglin also binds other TGF-β superfamily molecules, including activin-A, bone morphogenetic proteins (BMP-2 and BMP-7), suggesting possible unknown functions for this molecule (16, 41).

Endoglin and HHT1

As previously mentioned, HHT1, also known as Osler-Weber-Rendu disease, is an autosomal dominant vascular dysplasia. HHT1 is characterized by recurrent hemorrhages

(telangiectasias) in mucocutaneous tissues and visceral arteriovenous malformations (AVMs) (53). The pathogenesis of AVM formation in the absence of endoglin has been recently described (54). Numerous mutations in the external domain of the endoglin gene leading to truncated endoglin mutants have been identified as mutations present in HHT1 (22, 23, 55, 56). Mice expressing only a single wild-type endoglin allele show similar phenotypes to human HHT1, suggesting that HHT1 is a haplo-insufficient disease (57, 58).

Endoglin and Angiogenesis

Angiogenesis, the neoformation of blood vessels from pre-existing microvessels, is essential to numerous physiological and pathological processes, such as cell nourishment, and cancer and ischemic disease progression. This complex process involves remodeling of the extracellular matrix and proliferation and migration of endothelial cells (59). Vascularization is necessary for tumor growth and metastasis (60). With insufficient supply of blood, tumor cells will undergo apoptosis/necrosis. Given its distinct tissue distribution and its known functional integration with the TGF-β system, it is not surprising that endoglin is involved in angiogenesis.

Support for the involvement of endoglin in angiogenesis is demonstrated by the death of endoglin knockout mice due to vascular development defects, particularly of the primitive vascular plexus of the yolk sac, by gestational day 11.5 (24). As previously mentioned, mutations in the external domain of the endoglin gene may lead to the development of HHT1 (22, 23, 55, 56, 61). A significant correlation was found between high endoglin levels on HUVECs and HUVEC proliferation (3, 19). A clear correlation has also been noted between endoglin levels and markers of cell proliferation such as cyclin-A and Ki-67 (4). Endoglin suppression in HUVECs resulted in inhibition of angiogenesis *in vitro* (49). Consistent with the fact that hypoxic conditions induce angiogenesis (62), it was found that endoglin promoter activity, mRNA, and protein levels were up-regulated by hypoxia-inducible factor (HIF)-1 (27). Hypoxic conditions also up-regulated ALK-1 activity along with endoglin activity both, *in vitro* and *in vivo* (63). Stronger staining levels of anti-endoglin mAb were also noted on endothelial cells actively undergoing angiogenesis, such as tumoral endothelial cells, compared to normal endothelium (3, 4, 8). It has also been shown that anti-CD31 mAb stains endothelial cells of both normal and cancerous colon tissue, whereas anti-endoglin mAb stains cancerous colon endothelial cells well but shows little staining of endothelial cells of non-malignant colons (64). Mice haplosufficient for endoglin showed lower Lewis lung carcinoma vascularization and growth when compared to control littermates, further suggesting the role of endoglin in tumor angiogenesis. This finding has sparked interest for studies investigating the role of endoglin in tumor diagnosis, prognosis and therapy.

Endoglin and Tumor Diagnosis and Prognosis

High levels of endoglin expression on actively proliferating tumoral endothelial cells on the luminal surface allows for immunoscintigraphy of tumors for diagnostic purposes. *Ex vivo* analyses of excised kidneys from patients with renal carcinoma showed localization of anti-endoglin mAb to the region of tumor lesions by scintigraphy (65). In fact, scintigraphy with anti-endoglin mAb revealed two tumor masses previously unidentified *in vivo* with MRI (65). This suggests the use of labeled anti-endoglin mAbs may be superior for detecting renal tumors compared to standard MRI. Whether this is true for other histotypes has yet to be investigated.

Radiolabelled anti-endoglin mAbs have been safely and effectively used to image human melanoma xenografts in mice and adenocarcinomas in canine models (6, 66). Anti-endoglin mAb uptake is rapid and without systemic side-effects for up to three months after imaging (6). Levels of anti-endoglin mAb are concentrated at the tumor periphery, where vessel density and active angiogenesis are prominent, and the half-life of the antibody in serum is less than 1 minute (66). To be used effectively *in vivo*, background activity of anti-endoglin mAb must be minimized by using small doses of the ligand in order to avoid quick saturation of the high affinity receptors (67).

Intratumoral microvessel density (IMVD) quantitated by immunohistochemical staining for endothelial cell markers has been suggested to have prognostic value, with increased IMVD correlating with shorter survival (68-71). Not all studies agree with this conclusion (72). Likely these differences are due to inconsistent staining methods, counting methods, and the use of antibodies against different pan-endothelial cell markers. Efforts to standardize these counting and staining approaches may help to ensure comparability of data, however, staining for appropriate markers is an absolute requirement (73). Increments of IMVD measured by anti-endoglin mAb from low- to high-grade colorectal dysplasias and high-grade colorectal dysplasias to carcinomas have been reported (74). IMVD quantified by anti-endoglin mAb has been inversely correlated with tumor prognosis in patients with astrocytomas and glioblastomas, whereas IMVD measured by the pan-endothelial marker CD31 did not show any prognostic value (75, 76). Similarly, IMVD quantified by anti-endoglin mAb inversely correlated with survival in patients with non-small cell lung cancer, hepatocellular carcinoma, and breast carcinoma, whereas IMVD evaluated by anti-CD34 mAb did not (7, 77-79). IMVD assessed by

anti-endoglin mAb also better correlated with vascular endothelial growth factor levels than IMVD measured by anti-CD34 mAb or anti-CD31 (76, 78). IMVD estimated by anti-endoglin mAb positively correlated with Gleason score in prostate cancer patients and tumor stage in squamous cell carcinomas of the oral cavity (5, 80). Staining with endoglin was more sensitive for capillaries in cervical tumors and better predicted lymph node metastasis than did staining with factor VIII (81). Endoglin staining, but not CD-31 staining, correlated with Ki-67 values in patients with glioblastoma (75). However, staining for endoglin in patients with pituitary adenoma did not correlate with Ki-67 values (82). It is noteworthy that not all reports show that IMVD measured by endoglin has prognostic value (83). Nevertheless, collectively these results suggest that IMVD as measured by endoglin is a superior marker for prognosis in disease and cancer when compared to IMVD measured by traditional markers such as CD34, CD31 and factor VIII.

Interestingly, patients with metastatic breast and colorectal tumor exhibited significantly higher serum endoglin levels than did controls (49, 84, 85). Serum endoglin levels have also been shown to be reduced by chemotherapy (84). Taken together these results suggest that serum endoglin levels may be used to classify patients with advanced disease and those at risk of developing metastases. Endoglin can also be used to monitor recurrence in cancer patients after chemotherapy(86).

Endoglin and Tumor Therapy

The identification of endoglin as an ideal marker of endothelial cell proliferation has prompted many questions regarding its therapeutic relevance in cancer. To date, the only humanized antibody with anti-angiogenetic activity receiving approval for clinical indications has been bevacizumab, an anti-VEGF mAb (87-90). There is a need for more antibodies that are highly expressed on tumor endothelium to be developed. The expression of endoglin on tumor endothelium has therapeutic potential if it can be targeted *in vivo*.

Initial support for the targeting of endoglin as a therapeutic agent stemmed from *in vitro* studies which showed that anti-endoglin mAbs were able to induce apoptosis in HUVECs (91). Since then, anti-endoglin mAbs working *via* cytotoxic T-cells have been developed and tested *in vitro* (92). Immunoliposomes generated by single chain Fv fragments and nanobodies are two possible tools for anti-endoglin mAb application (93, 94). *In vivo,* anti-endoglin mAb has been shown to inhibit tumor growth and metastasis in SCID mice (77, 95-100). This inhibition is either by the destruction of tumor vasculature and/or inhibition of tumor angiogenesis. The effectiveness of anti-endoglin mAb seems to be dependent on tumor localization and is enhanced by T-cell immunity, meaning immunocompromised patients may not benefit from endoglin therapy (98, 99). In addition to the SCID models, anti-endoglin mAb has been shown to inhibit tumor growth in mice inoculated with hepatoma cells (101). Anti-endoglin mAbs have been conjugated to several toxic substances, such as Auger electron emitters and deglycosylated ricin A, in an attempt to increase their therapeutic potential (77, 95).

There is one phase I study of TRC105, an anti-endoglin mAb, that is still ongoing in patients with advanced refractory cancer (102). To date, only one grade 4 bleeding that resolved spontaneously and few other minor adverse events have been reported. Endoglin binding sites were saturated at a dose of 0.3 mg/kg, although doses up to 1.0 mg/kg were tested. The full results of this study remain to be published, although preliminary analyses suggest that TRC105 can be tolerated up to doses of 1.0 mg/kg and may provide clinical benefits (102).

There are both pros and cons to the use of anti-angiogenetic agents (103, 104). Anti-angiogenic strategies are superior to traditional chemotherapeutic agents because: i) they can be easily administered to the tumor cells *via* the blood stream, ii) they are applicable to multiple tumor types since all solid tumors require a blood supply for growth, and iii) the destruction of a single vessel will result in the death of numerous tumor cells. However, it is important to note that anti-angiogenic strategies can potentially interfere with physiological angiogenic processes, such as regeneration after injury or disease. Lastly, not all tumor cells may be destroyed after antiangiogenic treatment, meaning that patients may require long-term application of these drugs.

Future Directions

The role of endoglin in the TGF-β signaling system and its elevated expression on actively proliferating endothelial cells make it an intriguing and promising molecule in tumor imaging and therapy. However, there is much to be learned about endoglin before it can be used in clinical settings. Firstly, only a small portion of TGF-β binds endoglin, suggesting that endoglin has other possible unknown endogenous effects. Next, different anti-endoglin mAbs demonstrate differences in reactivity to endothelial cells and this is likely to result in differences in diagnostic, prognostic and therapeutic efficacy. Optimal antibodies for distinct clinical purposes should be identified. Pre-clinical studies on the therapeutic effectiveness of anti-endoglin mAbs are promising and this subject warrants further investigation.

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