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Review

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Highlights on endoglin (CD105): from basic findings towards clinical applications in human cancer

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Abstract

Antibody targeting of tumor-associated vasculature is a promising therapeutic approach in human cancer; however, a specific cell membrane marker for endothelial cells of tumor vasculature has not been discovered yet. Endoglin (CD105) is a cell-surface glycoprotein most recently identified as an optimal indicator of proliferation of human endothelial cells. The finding that CD105 is over-expressed on vascular endothelium in angiogenetic tissues has prompted several pre-clinical studies designed to get a deeper understanding on the role of CD105 in angiogenesis, and to evaluate the most appropriate clinical setting(s) to utilize CD105 as a therapeutic target. In this review, the foreseeable clinical applications of CD105 in human cancer are discussed.

Background

The availability of new and more sophisticated technologies, together with the improved knowledge on tumor-host interactions, have allowed the identification and characterization of different tumor-associated antigens (TAA) to be used as molecular targets for immunotherapeutic approaches in patients with solid or hematologic malignancies. Prompted by encouraging pre-clinical evidences, significant clinical results in cancer treatment have been obtained through antibody-based therapeutic regimens, such as those that target CD20 on malignant B cells [1] or HER2 in breast cancer [2]. However, due to the heterogeneous expression of TAA in neoplastic tissues, these approaches raise some critical issues such as "patients' eligibility" to specific TAA-based treatment modalities. Moreover, the efficacy of TAA targeting is frequently limited by the inadequate accessibility of therapeutic antibodies or their derived molecules within the tumor mass [3].

Currently, great interest is focused on angiogenesis and on its potential clinical implications in cancer, and vascular targeting represents a highly promising alternative to the direct engagement of therapeutic TAA on neoplastic cells [4,5]. Among potential therapeutic strategies to induce tumor regression by blocking tumor blood supply, an intriguing approach relies on the selective targeting of cell surface molecules over-expressed on endothelial cells of tumor-associated blood vessels [4,5]. In this setting, emerging *in vitro* and *in vivo* pre-clinical evidence identifies CD105 as a cell membrane glycoprotein representing a prime vascular target to implement innovative antibody-based diagnostic and therapeutic strategies shared by human neoplasia of different histotype.

Biological features of CD105

Tissue distribution

CD105 is a 180 kDa transmembrane glycoprotein constitutively phosphorylated [6-10], with a marked tissue-specificity [11]. Supporting this notion, CD105 is

Table 1: *In vivo* distribution of CD105 on non-endothelial cells.

Histotype
Activated monocytes
Differentiated macrophages
Early B cells
Erythroid precursors
Fibroblasts
Follicular dendritic cells
Melanocytes
Heart mesenchimal cells
Vascular smooth muscle cells
Mesangial cells
Syncytiotrophoblasts

predominantly expressed on endothelial cells [11-13] and its promoter is strongly and selectively active in endothelial cells [14,15]. Consistently, elevated levels of CD105 expression were detected on human microvascular endothelium [16] and on vascular endothelial cells in tissues undergoing active angiogenesis, such as regenerating and inflamed tissues or tumors [11,12,17-21]. However, CD105 was also weakly expressed on selected non-endothelial cells of different histotype (Table 1 and ref [22,23] for review).

In solid neoplasia, CD105 is present on endothelial cells of both peri- and intra-tumoral blood vessels and on tumor stromal components [11,17,22-24]. In particular, CD105 is largely expressed in small and likely immature tumor vessels as demonstrated in breast, prostate and gastric cancer [24-26]; rarely, CD105 is expressed in the cytoplasm of neoplastic cells [23]. In lung carcinoma, staining for CD105 was reported to be strong at the areas of active angiogenesis including tumor edge, while it was less intense in the central area of the tumor and not detectable in the adjacent normal tissue [12].

Functional activity

CD105 is a component of the receptor complex of Transforming Growth Factor (TGF)-β [27-29], a pleiotropic cytokine involved in cellular proliferation, differentiation and migration [30]. It binds several components of the TGF-β superfamily [27,29]. Interestingly, binding of TGF-β1 to CD105 reduces the levels of CD105 phosphorylation [10] and the levels of CD105 expression modulate the effects of TGF-β1 [28,31-35]. In this respect, it is of interest that the inhibition of CD105 expression enhanced the ability of TGF-β1 to suppress growth, migration and capacity to form capillary tubes of cultured endothelial cells [32].

In the absence of TGF-β1, CD105 shows an anti-apoptotic effect in endothelial cells under hypoxic stress, suggesting for a protective role of CD105 against pro-apoptotic factors [36].

In addition, the discovery that levels of CD105 regulate the expression of different components of the extracellular matrix including fibronectin, collagen, PAI-1 and lumican [34,37,38], is also suggestive for a crucial role of CD105 in cellular transmigration [38].

Modulation

Different environmental factors and cytokines involved in angiogenesis modulate CD105 expression. The levels of CD105 protein, mRNA and promoter activity are up-regulated by hypoxia [39] and by TGF-β1 [28,39-41], which cooperate to induce the expression of CD105 at transcriptional level [39]. Instead, TNF-α down-regulates CD105 protein levels but it has no effect at the transcriptional level [42].

Furthermore, CD105 expression was up-regulated on human umbilical vein endothelial cells (HUVEC) infected with a recombinant adenovirus carrying a constitutively active form of activin receptor-like kinase (ALK)-1, a type I TGF-β receptor [43].

Supporting the *in vivo* modulation of CD105 by pro-angiogenetic stimuli, elevated levels of CD105 were associated with high levels of vascular endothelial growth factor in non-small cell lung cancer lesions positive for angiopoietin-2, a regulatory factor of survival of endothelial cells, considerably expressed at sites of vascular remodelling and in highly vascularized tumors [44].

CD105 and vascularization

Even if its functional role is not fully understood, several findings suggest for the involvement of CD105 in angiogenesis and vascular development, and in maintaining vessel wall integrity.

Table 2: Intra-tumor microvessel density determined by immunohistochemical staining for CD105: an indicator of poor prognosis in patients with solid neoplasia.

Tumor histotype	References
Breast carcinoma	[54-55, 75-76]
Cervical cancer	[77]
Colorectal cancer	[78-79]
Endometrial carcinoma	[80-81]
Gastric carcinoma	[26]
Melanoma	[82]
Nonseminomatous testicular germ cell tumors	[83]
Non-small cell lung cancer	[56, 84]
Prostate cancer	[25]
Renal cell carcinoma	[85]
Squamous cell carcinoma of the oral cavity	[19]

Table 3: CD105 as a marker of survival in patients with solid tumors of different histotype.

Tumor histotype	CD105-MVD[a] correlation with survival	References
Breast carcinoma	The number of CD105-positive microvessels correlated significantly ($p = 0.001$) with poor overall survival.	[55]
Colorectal cancer	Patients with CD105-MVD above the median showed the worst prognosis; similar results were obtained when CD105-MVD was divided in quartiles.	[78]
Endometrial carcinoma	Patients with the lower quartiles for CD105-MVD showed reduced survival compared to those with the higher quartiles.	[80]
Non-small cell lung cancer	5-year survival rate of patients with the lower CD105-MVD was higher compared to that of patients with the higher CD105-MVD.	[84]
Prostate cancer	Median survival time were shorter for patients with CD105-MVD above the median.	[25]

[a]CD105-MVD, intratumor microvascular density as determined by anti-CD105 monoclonal antibodies.

First, CD105 expression is up-regulated on proliferating endothelial cells in culture [11-13] and on endothelial cells of angiogenetic blood vessels [11,12,24,33,45]. Furthermore, *CD105* knockout mice died of defective vascular development during early gestation [46], as observed in *TGF-β1*- and *ALK-1*-null mice [47,48]. In particular, *CD105* null mice showed important structural defects in the primitive vascular plexus of the yolk sac that prevented the formation of normal mature vessels [46]. Additionally, both in humans and mice, *CD105* gene mutations are associated with hereditary hemorrhagic telangiectasia type 1, an inherited disease characterized by arteriovenous malformations and bleedings [49-51]. Finally, CD105 has been most recently suggested as a regulator factor of nitric oxide-dependent vasodilatation. In fact, the levels of CD105 expression modulated the amounts of endothelial nitric oxide synthase (eNOS) in kidney and femoral arteries of mice. Furthermore, over-expression or suppression of CD105 in cultured endothelial cells induced a marked increase or decrease in the protein levels of eNOS, respectively [52].

Interestingly, an increment in microvessel density, as determined by immunohistochemical staining for CD105, was found during the progressive stages of colorectal carcinogenesis [53]. In line with this finding, the assessment of neovascularization by CD105 staining was found to represent a potential predictor of prognosis in different solid malignancies (Table 2 and Table 3). For instance, the CD105-positive blood vessels count was prognostic for survival in patients with prostate cancer of Gleason score 5–7 [25], and correlated with overall survival of node-negative patients affected by breast carcinoma [54,55].

mAb directed to CD105 but not to the pan-endothelial marker CD34 revealed an inverse correlation between intra-tumoral microvessel density and apoptotic index of neoplastic cells in non-small cell lung cancer patients [56]. Since angiogenesis is crucial for tumor development and progression [57], this finding provided supportive evidence to the usefulness of CD105 targeting in antiangiogenetic therapy of cancer [56].

CD105 targeting

Ex vivo *background*

Selected anti-CD105 monoclonal antibodies (mAb) significantly inhibit the proliferation of cultured human microvascular and macrovascular endothelial cells [11,58,59], thus supporting the notion that CD105 is a promising vascular target to implement innovative antibody-based therapeutic strategies in human cancer. Noteworthy, differences in the growth suppression of endothelial cells have been found among 4 anti-CD105 mAb defining different epitopes [59]; nevertheless, TGF-β1 and each of the 4 anti-CD105 mAb showed synergistic suppression of HUVEC proliferation [59].

A bispecific single-chain diabody directed to the adenovirus fiber knob domain and to CD105 was shown to be effective in enhancing adenovirus transduction in HUVEC, thus sustaining the use of CD105 protein as target for therapeutic gene transfer in endothelial cells [60]. Along this line, a vector constructed with the CD105 promoter was efficiently utilized to deliver gene expression specifically to endothelial cells of mouse blood vessels [61]. Additionally, human CD105 promoter fragments were successfully utilized in pigs to drive CD59 expression in the small vessels of heart, kidney and lung, but not in the large vessels of these organs [62].

Most recently, another bispecific single-chain diabody was proposed for therapeutic approaches aiming to destroy tumor-associated vasculature. This engineered antibody is directed to human CD105 as well as CD3 and it is effective to mediate killing of CD105-positive endothelial cells by cytotoxic T lymphocytes [63]. An alternative strategy of CD105 targeting for anti-angiogenetic treatment of cancer might derive by the use of conditionally replicating adenoviruses (CRAD). In fact, CRAD obtained by utilizing Flk-1 and CD105 regulatory elements have been transcriptionally targeted towards proliferating endothelial cells, with specificity and efficacy in killing HUVEC [64].

In vivo *diagnostic targeting*

The over-expression of CD105 on proliferating endothelial cells of the tumour vasculature suggested that CD105 might also represent a good target for the immunoscintigraphy of tumors. In keeping with this idea, targeting of CD105 by radiolabeled mAb was described as a safe and effective procedure to image tumors in animal models [13,65]. The intravenous administration of a ^{125}I-labeled anti-CD105 mAb efficiently imaged spontaneous mammary adenocarcinomas in dogs. The immunoscintigraphy performed 8 hours after mAb injection demonstrated that the uptake of the radiolabeled mAb was rapid and intense, and no systemic side effects were observed in the injected dogs during a 3 months follow-up after imaging procedures [13]. Consistently, the scintigraphy performed 15 minutes after administration of low doses of ^{111}In-labeled anti-CD105 mAb in C57BL/6 mice demonstrated an accumulation of radioactivity in xenografts of human melanoma. The autoradiography and immunohistology showed a marked concentration of the mAb in the periphery of the tumor mass with an heterogeneous distribution in its centre. Noteworthy, the 97% of the injected dose of the radiolabeled anti-CD105 mAb was removed from the circulation within 15 min, and the blood half-life of the anti-CD105 mAb was estimated to be <1 minute [65].

The immunoscintigraphy performed after renal artery perfusion of ^{99}Tcm-labeled anti-CD105 mAb in the freshly excised kidney from a patient with renal carcinoma identified 2 distinct hot spots of radioactivity, which matched the positions of the tumors, as demonstrated by the subsequent histopathologic examination; noteworthy, only one of the two tumor masses was identified by a pre-surgery magnetic resonance imaging scan [66].

In vivo *therapeutic targeting*

Targeting of CD105, as therapeutic antiangiogenetic approach in cancer, has been extensively investigated in severe combined immunodeficiency [SCID] mice bearing human breast tumors. The results of these studies demonstrated a long lasting suppression of tumor growth and metastasis by systemic administration of radiolabeled or immunotoxin-conjugated anti-CD105 mAb [67-69]. Furthermore, naked anti-CD105 mAb, which reacted strongly with proliferating human endothelial cells but weakly with murine endothelial cells, showed synergism with conventional chemotherapeutic regimens in a human skin/SCID mouse chimera model [70]. Interestingly, in all these animal models the anti-tumor efficacy and the anti-metastatic activities were identified in the ability of the anti-CD105 mAb to inhibit tumor-associated angiogenesis and/or to obliterate tumor-associated vasculature [67-70].

Conclusions and future directions

A number of convincing experimental findings suggest that selected anti-CD105 mAb can strongly localize to the endothelium of tumor-associated vasculature and that they are efficient to inhibit tumor angiogenesis, tumor growth and metastasis in mice, pointing to CD105 as a suitable vascular target to implement antibody-based therapeutic approaches in cancer.

However, concern about the therapeutic applications of anti-CD105 mAb and their derived molecules in cancer patients emerged by discrepancies observed in the expression of CD105 within normal and tumor tissues [71-74]. In this respect, it has been suggested that not all anti-CD105 mAb are useful for anti-angiogenetic targeting

since different mAb have different reactivity with the vasculature of normal tissues [45,59,66,73]. Based on these findings, the comparative evaluation of the reactivity of a large panel of anti-CD105 mAb in the same tumor specimen has been proposed to identify the most reactive with tumor endothelium [45].

Nevertheless, the information on CD105 so far obtained by *ex vivo* studies and in animal models warrants additional efforts to further define the most appropriate therapeutic setting [s] for CD105 in human cancer, and to translate pre-clinical evidences into phase I/II clinical trials.

Abbreviations

ALK, activin receptor-like kinase

CRAD, conditionally replicating adenoviruses

eNOS, endothelial nitric oxide synthase

HUVEC, human umbilical vein endothelial cells

TAA, tumor-associated antigens

TGF, transforming growth factor

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