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review

New marker of angiogenesis CD105 (endoglin): diagnostic, prognostic and therapeutic role

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Background. *The well established notion that malignant tumours depend on angiogenesis to grow and metastasize focused the investigators' 'interest on tumour vasculature' into visualization and validation. Pan-endothelial markers (CD31, CD34, F8) and CD105 are differentially expressed in angiogenic and normal vessel endothelial cells. Since the former are excellent markers for the normal vasculature, CD105 (endoglin) is more suitable for identifying tumour angiogenesis. Endoglin is a transforming growth factor (TGF) - beta binding receptor, preferentially expressed on endothelial cells of angiogenic tissues, essential for angiogenesis and vascular development.*

Conclusions. *Tumour microvessel density expressed by CD105 immunohistochemical staining in paraffin-embedded tissue sections correlates significantly with tumour aggressiveness and prognosis in many solid tumours. Also, targeting of tumour neovasculature specific antigens offers the possibility of future therapeutic approaches.*

Key words: neoplasms – blood supply; neovascularisation, pathologic; angiogenesis factor; prognosis

Importance of tumour angiogenesis

In 1971, Folkman proposed that tumour growth is dependent on angiogenesis.[1] Angiogenesis is an essential process in the progression of malignant tumours because solid tumours cannot grow beyond 1-2 mm in diameter without angiogenesis.[2] Tumour neovascularization promotes growth because the new vessels allow the exchange of nutrients, oxygen and waste products by a crowded cell population for which the simple diffusion is no longer adequate.[3] Next to perfusion effect, endothelial cells of vessels release important paracrine growth factors for tumour cells (like insulin growth factor-2, basic fibroblast growth factor, platelet-derived growth factors). By releasing collagenases, urokinases and plasminogen activators they facilitate spread of tumour into the adjacent fibrin-gel matrix and connective tissue stroma.[4,5]

Tumour neovasculature has structural and functional abnormalities, increasing the opportunity for tumour cells to enter the circu-

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lation.[6] They have the abnormal vessel wall: incomplete or missing endothelial lining, interrupted or absent basement membrane, lack of pericytes, pharmacological and physiological receptors. Abnormal vascular architecture with contour irregularities, tortuosity, elongation of vessels, as well as loss of hierarchy is found in tumour vasculature, plenty of arteriovenous shunts and abnormal vascular density (chaotic network). Altered morphology results in functional abnormalities: shunt perfusion, absence of vasomotion, unstable blood circulation, obstruction of microvessels by leucocytes and tumour cells. Changed tumour perfusion results in platelet aggregation, micro- and macrothrombosis and in the increase of viscous resistance. Consequences of increased vascular permeability are hemoconcentration, interstitial bulk flow, extravasation of blood cells and hemorrhages.[6]

Neoangiogenesis is often a significant independent prognostic indicator for both the overall and the disease-free survival. Intratumoural microvessel density (MVD) - commonly measured with a histomorphometric method on tissue sections - is a widely regarded predictor of tumour growth, metastasis and patient's survival. Many studies have shown that MVD correlates with tumour aggressiveness of many different tumour types.[4,7,8] Meta-analysis by Uzzan and coworkers[9] of 88 published studies on MVD as a prognostic factor in women with breast cancer showed that high MVD significantly predicted the poor survival.

Validating tumour angiogenesis

The most important question in validating tumour angiogenesis is what proportion of tumour vascular network is due to pre-existing parent tissue vessels or newly formed vessels. We know plenty of pan-endothelial markers, such as CD34 - a cell surface sialomucin-like glycoprotein expressed by endothelial cells, CD31 - platelet-endothelial cell adhesion molecule and von Willebrand factor - also known as F8.[10] These markers detect both, tumour and parenteral vessels, but the former not to the same degree. Assessing tumour microvessel density with immunohistochemistry by antibodies against CD31, CD34 and von Willebrand factor may not be accurate, since these markers are expressed also in normal vessels, and on the other hand, they are not always expressed in all tumour vessels.[11,12] Besides, they are generally better expressed in larger vessels than in microvessels.[13]

In summary, because endothelial cells are heterogeneous, the markers of normal endothelial cells are apparently unfit for the studies of angiogenesis in tumour tissues. The growth of tumours includes not only the increase of blood vessels in number, but also the change of protein molecules in structure of endothelial cells. An ideal marker for angiogenesis should detect the newborn vessel quality as well as its quantity.[14]

In last years, imaging of tumour neovasculature by targeting a proliferation-associated endothelial marker CD105, called also endoglin, gave fruitful results.[15] CD105 is a new kind of cell adhesion molecules, first found in a human pre-B cell line.[16] It is a receptor that is strongly up-regulated in proliferating endothelial cells, and - as such - an optimal indicator of proliferation of endothelial cells also in tumour neovasculature. In contrast to pan-endothelial markers, CD105 is preferentially expressed on endothelial cells of all angiogenic tissues, including tumours, but weakly or not at all with those of normal tissues,[17-20] giving the superiority of CD105 as a marker of tumour angiogenesis.

CD105 (endoglin) is a disulfide-linked homodimeric cell membrane glycoprotein of 180 kDa. It is a transmembrane phosphorylated glycoprotein, a component of the receptor complex of transforming growth factor (TGF)-β, which is a pleiotropic cytokine that modulates

angiogenesis by the regulation of different cellular functions, including proliferation, differentiation and migration.[19] CD105 binds several components of the TGF-β superfamily, in particular TGF-β1 and TGF-β2. The overexpression of CD105 antagonizes several cellular responses to TGF-β1, while down-regulation of CD105 potentiates cellular responses to TGF-β1.18 Endoglin is essential for angiogenesis and vascular development.[21-23] The inhibition of CD105 expression on human umbilical vein endothelial cells (HUVEC) by an antisense approach, enhanced the ability of TGF-β1 to suppress their growth, migration and capacity to form capillary tubes. Much evidence supports an important role of endoglin in cardiovascular development and vascular remodelling in humans and chicken.[24,25] Endoglin is highly expressed at the endocardial cushion during heart septation by mesenchimal cells[24] and it is up-regulated in response to tissue injury and atherosclerosis.[26-28] The gene of CD105 is located on 9q34.[20] The loss of function in the human endoglin gene causes hereditary hemorrhagic teleangiectasia Type 1.[23,29]

The detection of CD105 with immunohistochemical staining using anti-endoglin monoclonal antibody shows that CD 105 is almost exclusively expressed on endothelial cells of both peri- and intratumoural blood vessels.[18] Staining is very selective for the blood vessel endothelium and reacts specifically with endothelial cells without the significant cross-reactivity of inflammatory or stromal cells within the neoplasm.[30,31] Endoglin staining reduces false-positive staining of blood vessels compared with other commonly used panendothelial markers. It can be readily performed on formalin-fixed, paraffin-embedded tissues. It is a good illuminator of tumour vasculature in solid malignancies (Figure 1).[17-19] CD105 is also expressed on non-endothelial cells including haemopoetic progenitor cells, fibroblasts, follicular dendritic cells, melanocytes, vascular smooth muscle cells, macrophages and mesangial cells, however, this expression is very weak.[18,19] Since CD105 is expressed on the most immature cellular subtypes in acute leukaemias, it can also be used in diagnozing haemopoietic tumours.[16,19]

The experience shows that, in many types of cancer, MVD counted by CD105 is a better estimator of tumour prognosis and survival than MDV counted by pan-endothelial markers. In colorectal cancer CD105 demonstrated significantly more proliferating neoplastic microvessels than CD31 and was a more specific and sensitive marker for tumour angiogenesis than commonly used panendothelial markers.[31] Also CD105, but not other markers, correlated significantly with liver metastases and lymph node invasion. Akagi *et al*[32] quantified MVD detected using monoclonal antibodies CD34 and CD105 in 54 cases of colorectal adenoma and in 20 cases of carcinomas. A significant increment of MVD detected by anti CD105 was found from low-grade to high-grade dysplasia and from high-grade dysplasia to carcinoma. In contrast, no significant difference of MVD assessed by anti CD34 was observed in the colorectal adenoma-carcinoma sequence. Microvessels positive for CD105 were preferentially observed on the surface area of adenomas (whereas CD34 staining was distributed uniformly in the sections), suggesting that angiogenesis mainly took place in this area.

The similar findings were shown in patients with head and neck squamous cell carcinomas,[33] where patients with high CD105-MVD had a significantly shorter disease-free interval and overall survival; but CD34-MVD was not associated with the survival. The evaluation of angiogenesis in non-small cell lung cancer,[34] determined with CD105 as well as CD34 immunostaining, also proved CD105 expression superior in the evaluation of angiogenesis. Five- year survival rate was significantly lower in patients with high CD105 expression regarding patients with a low CD105 expression. The difference in the longevity of

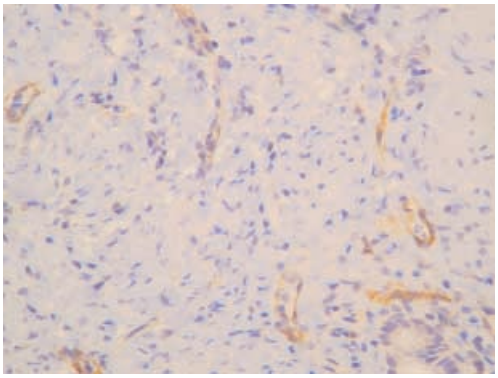

Figure 1. Expression of CD105 on endothelial cells of tumour vessels in gallbladder carcinoma (immunohistochemical staining using anti-endoglin monoclonal antibody on paraffin-embedded tissue section), magnification: x400.

survival between patients with high CD34 expression and low CD34 expression was, however, the same, but statistically insignificant. The hypothesis that the use of CD105 antibody should reduce the incidence of false-positive staining of normal blood vessels entrapped within a tumour and those located within the close vicinity of a cancerous mass was confirmed in the study of Kumar *et al*[35], who reported that vascular density determined using CD105 antibody correlates with the tumour prognosis in breast carcinoma.

However, not in all types of tumours MVD correlated with the prognosis. There was also a lot of discrepancies between different studies due to the diversity of technical approaches, variation in tissue pre-treatment protocols and non-standardized counting methods.[35,36] There is a trend to standardise the procedures so that results from different studies would be comparable. In clear cell renal carcinoma,[37] there was the inverse relationship between MVD and patient's survival: tumours with higher vascular density were associated with a greater post-operative 5-year survival rate than tumours with lower vascular density. Decreased MVD was associated with tumour fibrosis (which has morphological effect of decreasing MVD in a given tumour) and the development of large diameter vascular channels. It was concluded that the association between tumour microvessel density and the prognosis is not identical for all forms of malignancy but may be modified by architectural remodelling during tumour evolution. Besides, lower scores of MVD-CD105 were found in larger sized and more aggressive hepatocellular carcinomas,[38] however, the study did not provide significant any prognostic information. But active angiogenesis as highlighted by diffuse CD105 staining microvessels in the adjacent non-tumorous liver tissues was predictive for the early recurrence in this study.

Clinical potential of CD105 in human malignancies

As angiogenesis is crucial for tumour development and progression, the antiangiogenic therapy represents a promising approach for the cancer treatment. CD105 therapeutic targeting was investigated *in vitro*[39] and in animal models.[14,40,41]

She *et al*[42] investigated the mechanisms by which anti-endoglin monoclonal antibodies (mAbs) - termed SN6 series mAbs, suppress the growth of proliferating endothelial cells. They found that four SN6 series mAbs suppressed the growth of human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. Matsuno and co-workers[39] induced a long-lasting complete regression of distinct solid tumours in immunodeficient mice with the intravenous administration of antiendoglin conjugates, but not with the control conjugate. The same *in vivo* evidence was shown in a canine mammary carcinoma model.[40] In fact this study[40] was the first *in vivo* evidence that targeting of CD105 could represent an effective strategy to image solid malignancies. The antiangiogenic therapy of the mouse chimeras bearing established human skin tumours using various anti-endoglin monoclonal antibodies SN6, was effecti-

ve in the suppression of tumours. The efficacy was enhanced by combining a chemotherapeutic drug (cyclophosphamide).[43] Nowadays, next to systemic intravenous drug approach, a transcriptional targeting of conditionally replicating adenovirus with drug-substance to dividing endothelial cells is possible. Savontaus *et al*[41] utilized the regulatory elements of endoglin genes to construct two conditionally replicating adenoviruses (CRAD). *In vitro* studies it was demonstrated that both CRADs controlling the endoglin promoter, inhibited by 83-91% the capillary network formation in an *in vitro* angiogenesis assay in HUVECs, compared with the non-replicating control virus. This principle may be incorporated into novel therapeutic agents to develop anti-angiogenic treatment for cancer.

Endoglin has been detected also in the circulation of cancer patients, next to some other angiogenic growth factors. Increased CD105 in the circulation of patients with cancer results from angiogenesis both within and the immediate vicinity of the tumour mass. The main question was whether soluble CD105 levels associate with the disease progression. Li *et al*[44] demonstrated in 92 breast cancer patients that serum CD105 might be a valuable novel angiogenetic marker for identifying high risk breast cancer patients, since plasma levels of soluble CD105 (measured with indirect ELISA assay) correlated with metastasis. In 2001, Takahashi *et al*[45] reported about the association of serum endoglin with metastasis in patients with colorectal, breast and other solid tumours. In addition, they showed that chemotherapy exerts a suppressive effect on the serum endoglin. They suggested that serum endoglin may be a useful marker for monitoring early signs of metastasis and cancer relapse in a long-term follow-up of solid tumour patients. In 2003, Li and his group[36] compared the expression of CD105 in vasculature of resected colorectal cancer by MVD assessment and CD105 levels in the blood of the patients. CD105-MVD was an independent prognostic parameter for the survival of patients with colorectal cancer, and the plasma levels of CD105 were useful parameter for assessing the disease progression (serum-CD105 positively correlated with Dukes' stage).

Conclusions

It is likely that evaluating tumour angiogenesis will become an integral part of more consistent tumour staging system and routine prognostic evaluation. CD105 (endoglin) is proliferation-associated endothelial cell adhesion molecule, showed as an optimal indicator of tumour neovasculature. Moreover, targeting of tumour neovasculature specific antigens (like CD105) offers the possibility of future therapeutic approaches.

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