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Editorial Comment

Biomarkers of response to angiogenesis inhibitors: An open and unsolved question

*Domenico Ribatti**

Department of Human Anatomy and Histology, University of Bari Medical School, Piazza G. Cesare, 11, Policlinico, 70124 Bari, Italy

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Surrogate markers of angiogenesis or antiangiogenesis are needed to demonstrate the activity and efficacy of antiangiogenic agents in clinical trials and for the future monitoring of antiangiogenic treatments in clinics. The measurement of changes in tumour angiogenesis as a means of predicting and/or assessing the efficacy of antiangiogenesis therapies has mainly been based on the evaluation of the microvascular density (MVD) in tumour biopsy samples. Several studies on MVD and prognosis gave positive results in patients with solid and haematological tumours.[1] Nevertheless, despite the initial confirmatory publications, numerous reports have subsequently appeared in the literature that fail to show a positive association between increasing tumour vascularity and reduced patient outcome, and caution as to the clinical utility of tumour angiogenesis is being urged.

Another approach to predict and/or assess the efficacy of antiangiogenic therapy is the measurement of plasma or urinary levels of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF) and interleukin-8 (IL-8). Circulating levels of many angiogenic factors depend on a variety of factors, including perfusion, protease activity and the hypoxic status of the tumour. In some types of cancer the circulating levels of one or more of these factors have been reported as indicators for predicting patient survival.[2] Circulating blood platelets contain several factors with the potential to be angiogenic markers, including VEGF and other cytokines. Folkman and Klement proposed measuring the 'platelet proteome' of cancer patients to determine whether specific patterns of angiogenic proteins might yield important information. They have demonstrated that changes in platelet-associated PF-4 detect malignant growth across a spectrum of human cancers in mice and that PF-4 appeared to be up-regulated in the early growth of human liposarcoma, mammary adenocarcinoma and osteosarcoma.[3]

Soluble VEGFRs (sVEGFRs) have been investigated as surrogate markers in experimental models and in patients treated with antiangiogenic therapies. sVEGFR-1 has been detected in sera from patients with colorectal and breast cancer, but not in healthy individuals.[4] Norden-Zfoni et al.[5] demonstrated that in patients with metastatic imatinib-refractory gastrointestinal stromal tumours treated with SU11248, VEGF increased by 2.2 fold and sVEGFR-2 decreased by 25% during the first 2 weeks of treatment.

Increasing levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) were found in serum of women with progressing advanced breast cancer, while women with stable disease or disease responding to hormonal therapy had stable or decreasing sVCAM-1 levels.[6] In a phase I clinical trial with endostatin in patients with advanced solid tumours, VEGF,

* Tel.: + 39 080 5478240; fax: +39 080 5478310.
E-mail address: ribatti@anatomia.uniba.it

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FGF-2, sVCAM-1, and SE-selectin were measured before and during therapy. Pre-treatment levels were highly heterogeneous and no consistent changes in circulating proteins levels were observed during therapy.[7] In patients with advanced colorectal cancer, the levels of SE-selectin and sTie-2 were not altered by PTK 787/ZK222584 which inhibits VEGFRs.[8] On the other hand, SE-selectin was increased by the angiogenesis inhibitors SU5416[9,10] and CM-101[11] or highly variable during celecoxib plus metonomic vinblastine or cyclophosphamide treatment.[12]

Modern medical imaging techniques can play an important role in the evaluation of antiangiogenic treatment efficacy. Common imaging techniques such as dynamic contrast-enhanced perfusion magnetic resonance imaging (MRI), perfusion computed tomography (CT) and others, give only an indirect estimation of angiogenesis. New molecular imaging techniques can give an overall estimation of angiogenesis and antiangiogenic therapy effects. These new approaches include nuclear imaging techniques such as positron emission tomography (PET), which has been fused with CT to obviate shortcoming in anatomical information,[13] particularly MRI, that uses paramagnetic nanoparticles to track angiogenesis by targeting $\alpha v\beta 3$ integrins[14] and other specific angiogenesis markers, sonography with novel contrast agents such as gas-filled microbubbles directed against specific target endothelial cell receptors[15] and optical techniques.[13]

Circulating endothelial cells (CECs) and circulating endothelial precursor cells (CEPs) are present in most cancers at diagnosis compared with normal controls and their quantities change in response to treatment. CEC levels are increased in a number of cancer patients and their levels return to normal values as a result of complete remission.[16–18] VEGF and other angiogenic cytokines mobilise CEPs from the bone marrow to form new tumour blood vessels. High baseline levels of both cell types predicted responses to antiangiogenic therapies in animal models treated with a range of drugs. Mancuso et al.[19] demonstrated that CEC kinetics and viability predict survival in patients with metastatic breast cancer who were treated with metronomic cyclophosphamide and metotrexate therapy. The CEC count after 2 months of continuous therapy was a good predictor of disease-free and overall survival after a prolonged follow up of more than 2 years.[19] The maximum tolerated dose of cyclophosphamide caused a short-term suppression of viable CECs and CEPs immediately after the drug was given, which was followed by an increase in the number of viable CECs and CEPs.[20] Fürstenberg et al.[18] demonstrated that in patients with breast cancer who have received neoadjuvant chemotherapy, the number of CECs, found to be increased in patients compared with healthy control individuals, was decreased by chemotherapy, whereas CEP mobilisation was significantly increased during the drug-free break periods.

Inhibition of EPC mobilisation by systemic administration of anti-VEGFR-1 monoclonal antibodies reduced tumour growth.[21] Using a murine model of human lymphoma, a significant increase in the frequency of CECs was observed starting 2 weeks after tumour inoculation, at a time of rapid tumour growth.[22] This rise in CEC correlated with tumour volume and tumour-derived circulating VEGF levels. Treatment of tumour-bearing mice with endostatin caused an increase in the frequency of apoptotic cells in the endothelial cell compartment and most CECs were apototic or dead, while cyclophosphamide had no such effect, because most of the circulating apoptotic cells were haematopoietic and not endothelial in nature, and a relevant proportion of CECs was still viable.[22] Continuous infusion of endostatin inhibited the mobilisation and differentiation of EPCs in mice bearing an angiogenic human lymphoma, and this effect was paralleled by the inhibition of tumour growth.[23]

More recently, Taylor et al.[24] demonstrated that high levels of circulating VEGFR-2 positive CEPs correlated with metastatic disease in patients with paediatric solid malignancies and they found no correlation between angiogenic factor levels and CECs, circulating VEGFR-2 positive CEPs, or clinical status. Vroling et al.[25] demonstrated that CECs increase during treatment of renal cell cancer with sunitinib in parallel to plasma VEGF and erythropoietin (EPO) levels, whereas haematopoietic progenitor cells and monocytes decrease. Greenfield et al.[26] documented an increase in EPCs in patients with gliomas and reported an inverse correlation between the percentage of these cells defined by their co-expression of CD133 and VEGFR-2, and length of survival after resection of tumour.

Microarray-based techniques can be applied to identify transcripts expressed in endothelial or stromal cells and plasma can be analysed by proteomics to detect differentially expressed proteins. By comparing the results of these analyses, proteins produced in the tumour vasculature and present in the plasma should be identified, but they should be absent from non-tumour tissue and from the plasma of tumour-free individuals. For example, the number of copies of VE-cadherin transcripts in the blood of cancer patients was significantly increased compared to healthy controls[27] and an increase in circulating transcripts of CD133 in the blood of cancer patients has been reported.[28,29]

Angiogenesis inhibitors are now being approved and introduced into medical practice throughout the world and inhibition of angiogenesis is a major area of therapeutic development for the treatment of cancer. A clinical challenge in antiangiogenesis is the finding of biological markers that will help to identify subsets of patients more likely to respond to a given antiangiogenic therapy, to detect early clinical benefit or emerging resistances and to decide whether to change therapy in second-line treatments. Assessments of the validity of neovascularisation markers in clinical trials are needed. In this context, CECs and CEPs could assess treatment efficacy during and after therapy and determine the optimum biological dose of antiangiogenic drugs.

Conflict of interest statement

None declared.

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