The Breast 18 (2009) S3, S48–S50

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original Article

Biomarkers for angiogenesis and antiangiogenic drugs in clinical oncology

Francesco Bertolini*

Laboratory of Hematology-Oncology, European Institute of Oncology, Milan, Italy

ARTICLE INFO

Keywords:
Angiogenesis
Anti-angiogenic therapy
Endothelial cells

SUMMARY

Aims: The clinical use of anti-angiogenic drugs, alone or in combination with other drugs, is increasing in medical oncology. However, identifying the best suited drug and the optimal dosage and schedule for treatment of patients remain challenging.

Methods and Results: We reviewed data about surrogate biomarkers of angiogenesis and anti-angiogenic drug activity currently available in the literature. Circulating endothelial cells (CECs) and circulating endothelial progenitors (CEPs) have been found to have some predictive potential in some clinical trials involving advanced breast cancer patients. Molecular surrogate markers, on the other hand, are more scanty at the present time, because the identification of truly endothelial-cell-restricted genes and/or antigens has been so far more elusive.

Conclusion: The search and validation of new biomarkers for angiogenesis and anti-angiogenic drug activity have many biological, technical and clinical facets which render this task particularly complex. An accurate planning of biomarker search and validation throughout future clinical studies is highly warranted.

Introduction

Anti-angiogenic drugs such as bevacizumab, sunitinib, and sorafenib are now available for clinical oncologists. Although clinical trials have demonstrated a benefit of these drugs in terms of prolonged survival of cancer patients, there is a compelling need for determining the optimal biologic dose (OBD) of these drugs, monitoring their biologic activity, selecting and stratifying the patients who are most likely to benefit from these treatments.[1] In medical oncology, problems related to the definition of the OBD for such drugs include the low frequency of tumor responses (tumor shrinkage); the lack, in some cases, of dose limiting toxicities (DLT) normally used to define a maximum tolerated dose (MTD), observed frequently when using cytotoxics but not as frequently when using certain anti-angiogenic drugs; and significant (if not optimal) therapeutic activity at doses below the MTD. Considering also that these drugs are extremely expensive, there is an urgent need for development and clinical validation of biomarkers of angiogenesis for patient selection and stratification and for OBD tailoring.[1,2]

Strategies to measure angiogenesis and anti-angiogenic drug activity

Functional and Imaging approaches

Preclinical angiogenesis assays currently rely on growth factor (VEGF or FGF) -induced generation and quantification of new vessels in the cornea or in the skin of animal models.[3] These procedures have limitations: some are poorly standardized, some are difficult to reproduce and in many cases reference values are scanty. Most of all, these invasive measurements are not adaptable to patients. Thus, the clinical evaluation of the efficacy of a given anti-angiogenic therapy so far has been mainly based on the measurement of microvessel density (MVD) in biopsy samples.[1,2] This approach has also major drawbacks. It is invasive, difficult to standardize, and the MVD of a biopsy does not sometimes correlate with the MVD of an entire lesion because of tumor tissue heterogeneity. In addition, changes in MVD are not necessarily induced with some antiangiogenic drugs even though the absolute numbers of blood vessels is diminished (reviewed by Kerbel[1] and by Bertolini et al.[2]).

Another approach is to measure circulating or urinary levels of angiogenic growth factors, such as VEGF, b-FGF, HGF and IL-8. Similarly, soluble VEGF receptors such as VEGFR1, VEGFR2 and VEGFR3 are currently being investigated in a variety of cancer indications, involving patients treated with anti-angiogenic therapies in order to understand their potential as surrogate biomarkers. More work is needed to ascertain whether these biomarkers can predict patients' survival or response to anti-angiogenic therapies.[1,2,4]

Functional imaging is another promising approach for the measurement of angiogenesis and anti-angiogenic drug activity. Dynamic contrast magnetic resonance imaging (DCE-MRI), for instance, measures changes in tumor blood flow and vascular permeability. However, standardization issues are still pending, and clinical validation has not yet been shown, despite some early promising indications. MRI-related techniques are also used for the imaging of the tumor vasculature, albeit with standardization

* Correspondence: Francesco Bertolini, MD, PhD. Laboratory of Hematology-Oncology, European Institute of Oncology, via Ripamonti 435, 20141 Milan, Italy.
Tel.: +39 02 57489535; fax: +39 02 57489537.
E-mail address: francesco.bertolini@ieo.it (F. Bertolini)

issues still remaining to be cleared. Another innovative approach is 3D-power Doppler ultrasound, which has recently been applied to functional studies on vasculogenesis in preclinical models.[5]

Molecular markers

A crucial obstacle has hampered so far the search for molecular markers of angiogenesis and of anti-angiogenic drug activity. Endothelial cells share the large majority (if not all) of their antigens with other hematopoietic or mesenchymal cells. CD31, CD34, vWf, CD105, and CD146, for example, which are antigens used for MVD quantification, are not only expressed by endothelial cells but also by hematopoietic cells, platelets and some fibroblasts subpopulations.[4] Thus, the quantification of these antigens as proteins released in circulation or their mRNA transcripts might result in information of limited clinical predictive value. Many attempts have been made to purify cancer-specific endothelial cells and to screen for genes or proteins expressed only by these cells. The transcriptome of endothelial cells purified from cancer patients has been investigated by different laboratories. These putative markers (or genetic signatures) should still be fully validated in the clinical setting, but they are potentially important also for the development of therapeutics specifically targeting tumor vessels.[6]

Only a small number of genes is considered to be truly endothelial-restricted or endothelial-specific. One of these is VE-cadherin. Interestingly, the number of copies of VE-cadherin transcripts in the blood of cancer patients is significantly increased when compared to healthy controls. However, caution should be applied when using this candidate biomarker to investigate the anti-angiogenic activity of a given drug or therapeutic strategy. In fact, VE-cadherin RNA expression is markedly reduced (or absent) in apoptotic endothelial cells. Thus, the number of circulating VE-cadherin transcripts most likely reflects either the number of circulating endothelial cells and their viability status.[2]

An increase of circulating transcripts for the endothelial progenitor cell-related CD133 antigen has been described in the blood of cancer patients. It should be noted, however, that CD133 is expressed also by hematopoietic progenitors. Thus, further work is needed to understand the cellular source of the CD133 transcript increase in the blood of these patients.[4]

Cellular markers

In healthy subjects, circulating endothelial cells (CECs) are a numerically very rare cell population representing 1/1,000–100,000 of circulating blood cells.[4] In many pathological conditions, such as cancer, the number of CECs is increased. The majority of CECs shows characteristics of mature, terminally differentiated and frequently apoptotic cells, only a subpopulation of which expresses antigens that suggest a stem- or progenitor-like phenotype. These putative circulating endothelial progenitors (CEPs) might home to sites of active vasculogenesis.

CEPs maintain a proliferative potential that mature CECs have lost, so that clonogenic cell culture assays should be possible. However, recent studies indicate that the large majority of colonies generated in commercially available assays for endothelial CEPs are of myeloid origin and have no vasculogenic potential.[7,8] Therefore, a more endothelial-specific strategy is needed.

Differentiating CEPs from CECs based on different expression of surface molecules is very difficult due to the antigenic promiscuity amongst hematopoietic cells and progenitors, platelets, CECs and CEPs. In fact, there is no single antigen to discriminate between CECs, platelets and hematopoietic cells.[4] Multiparametric flow-cytometry is thus used for CEC and CEP enumeration. Endothelial cells are identified by the expression of markers such as CD31, CD146 or VEGFR2; CD45 expression is used to exclude hematopoietic cells from the analysis. The use of a nuclear staining for DNA is crucial to exclude aggregated platelets and/or endothelial micro and macro particles (that share surface markers with CECs and CEPs) from the CEC count.[2,9] How to discriminate CEPs in the CEC population is still a matter of controversies. Some authors have shown endothelial potential in CD133+ cells, some others have failed to reproduce this approach and have found in vitro endothelial cell potential only in CD45−CD34+ cells.[7,8,10]

Methodological inconsistency between flow cytometry procedures, involving differences in the combinations of markers, gating strategies, and the occasional use of a pre-enrichment step, has led to different CEC values reported in the literature.[4] Thus, there is a need for standardization of flow cytometry procedures to minimize intra- and inter-laboratory variability.[9]

In preclinical models, a highly significant positive correlation was found between classic angiogenesis assays in the cornea or in the skin and the absolute number of CECs and CEPs. Also, quantification of CECs and CEPs has been used to determine the OBD of targeted anti-angiogenic drugs in mice.[3]

CECs and CEPs as surrogate markers of angiogenesis and anti-angiogenic drug activity in medical oncology

CEC and CEP levels are increased in the peripheral blood of patients affected by some types of cancer, and return to normal values in patients undergoing complete remission.[4] Thus, CEC/CEP numbers and viability have been measured in different clinical trials involving cancer patients treated with various anti-angiogenic therapies.[11–15] In metastatic breast cancer patients treated with low dose metronomic chemotherapy using CTX and methotrexate, the CEC count after two months of continuous (daily) therapy was a particularly good predictor of disease-free and overall survival after a follow-up of more than two years. Patients showing a CEC count above physiological levels after two months of therapy had a significantly improved progression-free and overall survival.[11] When the humanized anti-VEGF antibody bevacizumab was added to the metronomic chemotherapy for the treatment of metastatic breast cancer, patients who showed a clinical response in a phase II clinical trial (as well as a larger population of patients who had a clinical benefit from the treatment) had significantly greater baseline levels of viable CECs than did patients who failed to respond; furthermore, the number of apoptotic CECs before therapy initiated was associated with prolonged progression-free survival.[14] In patients treated with the small molecule anti-angiogenic agent sunitinib, changes in CECs differed between the patients with clinical benefit and those with progressive disease.[13] In a study where 36 locally advanced patients received regular-dose chemotherapy, plus endocrine therapy plus bevacizumab before surgery, baseline CEP count was positively associated with a clinical response.[15] Taken together, our studies indicate that assessment of CECs might be an estimation tool for prediction of response in patients with advanced breast cancer receiving metronomic chemotherapy alone or in association with bevacizumab. On the other hand, CEPs might be more promising for predicting response in patients receiving regular-dose chemotherapy plus anti-angiogenic drugs. These possibilities await confirmation in prospective randomized clinical trials.

Conclusions

Surrogate biomarkers of angiogenesis are urgently needed to better design preclinical studies and clinical trials involving anti-angiogenic drugs, alone or in association with other therapies.[1,2] As more anti-angiogenic agents enter the clinical arena for more indications, it is becoming clear that these drugs may induce unforeseen side effects.[1] The prediction and management of these

side effects might thus become another goal for this new generation of biomarkers.

Acknowledgements: We apologize to the numerous investigators whose papers could not be cited because of space limitations.

Competing interests: None

Funding: Supported in part by AIRC (Associazione Italiana per la Ricerca sul Cancro), ISS (Istituto Superiore di Sanità), Ministero della Salute grant RF-IMI-2006-411189 and the the sixth EU Framework Programme (Integrated Project 'Angiotargeting'; contract no 504743) in the area of "Life sciences, genomics and biotechnology for health".

References

1. Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008;**358**:2039–49.
2. Bertolini F, Mancuso P, Shaked Y, Kerbel RS. Molecular and cellular biomarkers for angiogenesis in clinical oncology. *Drug Discov Today* 2007;**12**:806–12.
3. Shaked Y, Bertolini F, Man S, Rogers MS, Cervi D, Foutz T, et al. Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell* 2005;**7**:101–11.
4. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. *Nat Rev Cancer* 2006;**6**:835–45.
5. Eckelman WC, Reba RC, Kelloff GJ. Targeted imaging: an important biomarker for understanding disease progression in the era of personalized medicine. *Drug Discov Today* 2008;**13**:748–59.
6. Seaman S, Stevens J, Yang MY, Logsdon D, Graff-Cherry C, St Croix B. Genes that distinguish physiological and pathological angiogenesis. *Cancer Cell* 2007; **11**:539–54.
7. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007;**109**:1801–9.
8. Case J, Mead LE, Bessler WK, Prater D, White HA, Saadatzadeh MR, et al. Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. *Exp Hematol* 2007;**35**:1109–18.
9. Mancuso P, Antoniotti P, Quarna J, Calleri A, Rabascio C, Tacchetti C, et al. Validation of a standardized method for enumerating circulating endothelial cells and progenitors: flow cytometry and molecular and ultrastructural analyses. *Clin Cancer Res* 2009;**15**:267–73.
10. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* 2002;**2**: 826–35.
11. Mancuso P, Colleoni M, Calleri A, Orlando L, Maisonneuve P, Pruneri G, et al. Circulating endothelial cell kinetics and viability predict survival in breast cancer patients receiving metronomic chemotherapy. *Blood* 2006;**108**:452–9.
12. Fürstenberger G, von Moos R, Lucas R, Thürlimann B, Senn HJ, Hamacher J, et al. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. *Br J Cancer* 2006;**94**:524–31.
13. Norden-Zfoni A, Desai J, Manola J, Beaudry P, Force J, Maki R, et al. Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. *Clin Cancer Res* 2007;**13**:2643–50.
14. Dellapasqua S, Bertolini F, Bagnardi V, Campagnoli E, Scarano E, Torrisi R, et al. Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. *J Clin Oncol* 2008;**26**:4899–905.
15. Torrisi R, Bagnardi V, Cardillo A, Bertolini F, Scarano E, Orlando L, et al. Preoperative bevacizumab combined with letrozole and chemotherapy in locally advanced ER- and/or PgR-positive breast cancer: clinical and biological activity. *Br J Cancer* 2008;**99**:1564–71.