Biomarkers for Monitoring Antiangiogenic Therapy
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Abstract
A variety of antiangiogenic agents are currently available for the treatment of renal cell carcinoma. With these exciting new therapeutic agents comes the challenge of elucidating useful biomarkers to monitor patients receiving these therapies. Although many patients benefit from antiangiogenic therapies, it is often by achieving stability of their disease. Thus, development of noninvasive biomarkers of disease response and relapse is a crucial objective to aid in the management of patients. The current technologies being explored in this field include circulating plasma proteins, cells, and nucleic acids and novel imaging techniques. Surrogate markers of angiogenesis could help with selecting patients for therapy, determining optimal dosing of therapy, deciding whether to change therapy, and assessing appropriate salvage therapy.

Antiangiogenic therapy represents an exciting advance in the management of cancer. Targeting of the vasculature has been shown to benefit patients with several types of malignancies. This method of treatment has been shown to have activity when combined with chemotherapy and, in the case of renal cell carcinoma, when used as single agents. The major target of current antiangiogenic therapy is the vascular endothelial growth factor (VEGF) pathway. VEGF promotes endothelial proliferation, survival, and permeability and thereby influences tumor angiogenesis and vasculogenesis. VEGF is especially linked to clear cell renal cell carcinoma biology in that mutation of the von Hippel-Lindau (VHL) tumor suppressor gene leads to subsequent up-regulation of hypoxia-inducible factor–regulated genes, including VEGF (1). Renal cell carcinoma is also especially amenable to antiangiogenic therapy as evidenced by the recent approval of two new antiangiogenic agents, sorafenib and sunitinib, for its treatment. Sorafenib is a multikinase inhibitor of which the targets include Raf, VEGF receptor 2, and platelet-derived growth factor receptor. It has shown tumor shrinkage in up to 80% of patients with renal cell carcinoma and delayed progression in a large placebo-controlled randomized phase 3 trial (2). Sunitinib is another multitypered tyrosine kinase inhibitor of VEGF receptor, platelet-derived growth factor receptor, and c-kit with impressive antitumor activity in phase 2 trials (3). In addition to their observed clinical activity in cytokine refractory patients, these drugs have the benefit of being oral medications with adverse effects that are generally manageable on an outpatient basis.

Potential Use of Surrogate Biomarkers
There are many clinical uses for biomarkers of angiogenesis. Because there are several agents that can be used to treat patients with renal cell carcinoma, stratification of patients (using baseline indicators) to likelihood of response would be useful. In addition to developing biomarkers for prediction of response, it will also be important to develop surrogate markers of clinical efficacy because the therapeutic effects can be seen even in the absence of classically defined tumor response. Tumor burden biomarkers that are more sensitive and specific and that can yield information earlier than current imaging techniques would thus provide better means of following up patients receiving therapy. Another clinical need in this field is to identify markers that predict the proper target dosage of a particular agent to adjust doses to limit toxicity without compromising efficacy. If reliable biomarkers for the angiogenic state of a tumor could be identified early, salvage therapies could be initiated in a more timely fashion for patients with tumors that show evidence of angiogenic escape.

Despite showing a high proportion of tumor shrinkage, antiangiogenic therapy produces few, if any, complete tumor regressions and patient tumors typically develop resistance after a median of 6 to 12 months of receiving therapy. Thus, biomarkers of resistance or relapse that offer mechanistic insight are just as important to identify as markers of response. Although little is known about the mechanisms of resistance to antiangiogenic therapy, it has been postulated that resistance develops as a result of activation of compensatory pathways driving angiogenesis in the setting of VEGF receptor-2 blockade. Resistance could develop by three possible mechanisms: (a) incomplete inactivation of the VEGF pathway; (b) activation of other hypoxia-inducible factor–driven genes, such as CXCR4 and TGF-α; and (c) selection of tumor cell populations able to survive in the presence of VEGF receptor-2 blockade, perhaps by activation of VEGF-independent pathways such as fibroblast growth factor and interleukin-8 (4, 5). It should also be noted that neo-vascularization encompasses more that just angiogenesis. Thus,
tumors may potentially develop resistance by other less well-studied mechanisms such as vessel co-option, vasculogenesis (e.g., stromal cell–derived factor 1), and vascular mimicry.

To develop useful biomarkers, one must consider the characteristics of a good biomarker. These include cost-effectiveness, low baseline levels in normal individuals, accessibility by noninvasive means such as blood and urine, robustness in the clinical setting, and reproducibility in multiple clinical centers. Although noninvasive means of following up patients is the ultimate goal, we will likely need to be guided initially by information obtained by invasive methods. For example, there is much interest in obtaining tissue biopsy specimens from patients who are receiving targeted therapies to assess the molecular features of the tumor exposed to a specific agent. The current hope is that pathologic features of a tumor will have clinical and laboratory correlates. This paradigm is addressed in the neoadjuvant administration of targeted agents with biopsy specimens obtained before therapy and then with tissue acquired at the time of nephrectomy. Similarly, surgery or radiofrequency ablation of limited metastatic disease could be a valuable source of tumor tissue that has been exposed to antiangiogenic therapy. It is hoped that the findings in tissue will guide the selection of biomarkers in patient blood. Correlation of pathologic data with markers in patient blood will likely lead to useful surrogate markers of the local tumor angiogenic state.

One recognized caveat of pathologic analyses is sampling error. Indeed, tumors may have differential areas of response and/or relapse to therapy within them. Pathologic correlation with imaging to select appropriate areas of a tumor for study may prove a useful way of minimizing sampling bias.

Noninvasive Markers

Currently, there are several candidates for biomarkers of antiangiogenic therapy that can be assessed in patient plasma.

Circulating cytokines. Classes of proteins that may prove useful to measure are those that promote or suppress angiogenesis, including cytokines/growth factors such as VEGF and its family members, basic fibroblast growth factor, and several chemokines (both proangiogenic and antiangiogenic members), although no single predictive cytokine has been found to date. Soluble shed receptors and the proteases that cleave them, such as soluble Flt-1, soluble VEGF receptor 2, soluble vascular cell adhesion molecule, and various matrix metalloproteinases, could also prove to be useful biomarkers. The use of cytokines as measures of angiogenic activity can be complicated by the fact that platelets contain and could release many angiogenic and antiangiogenic factors that could confound accurate measurements in patient samples.

In vitro functional assays. Another potential means of measuring the angiogenic state of patient plasma is by in vitro biological assays in which cultured endothelial cells are incubated with patient plasma and then subjected to various analyses, including proliferation, migration, and endothelial tube formation (6). Although these assays have the benefit of providing an integrated assessment of the cumulative angiogenic capability of a patient’s plasma, they are labor-intensive, expensive, and difficult to standardize for large multicenter trials.

Circulating cells. A promising area of antiangiogenic monitoring is the measurement of circulating endothelial cells (CEC) in the peripheral blood of patients. This may be achieved by using cell-surface marker–based purification strategies, in vitro colony formation, or reverse transcription-PCR. Reverse transcription-PCR for markers such as CD146 and VE-cadherin has been shown to correlate with CEC number (7, 8). However, very few data about measurement of RNA in patient peripheral blood samples are available; thus, this is a method that still needs considerable further development. Moreover, the optimal collection tube and processing method for RNA purification is still being optimized.

Currently, cell-surface marker–based strategies are the most commonly used means of quantitating CECs. It is thought that, under physiologic conditions, blood vessels shed endothelial cells into the circulation and endothelial precursor cells are mobilized from the bone marrow, which are likely to participate in the formation of new vessels. This process can be perturbed by proangiogenic neoplasms and by antiangiogenic therapies. The total CECs are composed of mature CECs and circulating endothelial progenitors (also referred to as endothelial precursor cells). Multiple surface markers have been used to define CEC populations by multiparameter flow cytometry. Strategies make use of endothelial markers such as CD31 and CD146, the absence of leukocyte markers such as CD45, and the absence of the stem cell marker CD133 to define mature CECs, with circulating endothelial progenitors having the same characteristics as mature CECs with the exception that they are CD133+. In turn, each of these populations could be viable or apoptotic.

Several studies have shown that with antiangiogenic therapy, the number of these cells changes. For example, Beaudry et al. (9) showed in a murine Lewis lung carcinoma model that in tumor-bearing mice subjected to VEGF inhibition, the number of mature CECs increased. Beerepoot et al. (10) found that patients with progressing cancer had higher levels of viable mature CECs than healthy patients or patients with stable disease. Mansuco et al. (11) found that, in breast cancer patients treated with metronomic dosing of chemotherapy, patients who showed clinical benefit had an increase in apoptotic CECs from baseline to the second month of therapy. Ebbinghouse et al. (12) showed that baseline CEC numbers may be a possible prognostic marker for patients treated with the thrombospondin mimetic peptide ABT-510. A hypothetical model for CEC kinetics that is consistent with the above data is shown in Fig. 1. However, a recent study found that treatment with an antivascular agent led to an increase in circulating endothelial progenitors early in the course of therapy (13). These studies have highlighted the fact that sampling at multiple time points may be critical for the interpretation of data from clinical trials.

CECs can also be measured by the Veridex method developed by Immunicon (Huntingdon Valley, PA). This method, although automated and less operator dependent than multiparameter flow cytometry, requires that samples be processed within 72 h of collection. Thus, batching and selection of samples, a necessity for multicenter trials, is not possible. The CD133 antibody, which is required to enumerate circulating endothelial progenitors with this approach, is still being developed, however. As the process of identifying and quantifying cell populations is not yet uniform and conflicting data exist, a consensus has yet to be established on the effect of antiangiogenic therapy on these various cell populations or the optimal approaches and times to measure them. Moreover, the analysis must be done in an
of tumor vessel-specific markers or of endothelial apoptosis could be developed in the near future, and these techniques could provide extremely useful surrogates of angiogenic activity and have the benefit of providing a view of the tumor itself and not of a surrogate for tumor activity (i.e., the patient blood). These imaging modalities have the added benefit of viewing intratumoral heterogeneity in response to targeted agents. A more comprehensive view of imaging modalities as a means of following up patients is described by Rosen and Schnall (19).

**Future Perspectives**

Antiangiogenic drugs represent a new field of cancer therapy. With the rapid increase in the number of patients with renal cell carcinoma receiving antiangiogenic therapy, there is a critical need to develop biomarker algorithms to follow up these patients. The future calls for development and improvement of technologies for such monitoring. These include multiplex proteome analytic platforms. The rapid concomitant analysis of multiple cytokines in small amounts of blood could prove useful even in therapy selection. For example, if a specific patient was found to have elevated levels of plasma basic fibroblast growth factor and VEGF, perhaps agents that target both pathways should be used for initial therapy. Currently, the Luminex platform shows promise, but the current technology does not include many angiogenic and antiangiogenic targets. The standardization of methods such as multiparameter flow cytometry or further development of the Veridex technology is also necessary for the enumeration of CECs to be a standard, robust, easy, and reproducible means of following up patients.

The future of this field also involves the collection of a large number of patient samples on antiangiogenic therapy. This involves collection of blood for analysis of plasma and peripheral blood mononuclear cells and for nucleic acid preparation. Ideally, the collection of samples would include serial samples of patients before therapy, while receiving therapy, and at the time of disease progression. However, the analysis of serial patient samples needs to be done with care. For example, there may be multiple mechanisms for relapse. CECs may serve as a common early indicator of relapse, with different patients showing different compensatory pathways to overcoming VEGF blockade. Thus, care should be taken to consider individual differences as this field progresses. This should lead to optimization and individualization of therapy for patients, especially because multiple choices for antiangiogenic therapy exist and more are being developed.

In addition to development of a large database of patient blood samples, it is imperative that tissue samples from patients before therapy and while receiving therapy be collected for pathologic analyses to best guide the study of noninvasive biomarkers. Correlation of noninvasive and invasive analytic platforms. The rapid concomitant analysis of multiple cytokines in small amounts of blood could prove useful even in therapy selection. For example, if a specific patient was found to have elevated levels of plasma basic fibroblast growth factor and VEGF, perhaps agents that target both pathways should be used for initial therapy. Currently, the Luminex platform shows promise, but the current technology does not include many angiogenic and antiangiogenic targets. The standardization of methods such as multiparameter flow cytometry or further development of the Veridex technology is also necessary for the enumeration of CECs to be a standard, robust, easy, and reproducible means of following up patients.

**Open Discussion**

**Dr. Atkins:** Do you have a sense of what the focus of big multi-institutional trials should be? Also, how would you prioritize items to be tested in small single-institution translational research trials?
Dr. Sukhatme: There are no data in any animal models of renal cell carcinoma, but we could get some guidance from animal models of other tumor types treated with the inhibitors in question. We can also learn from correlative studies in trials outside renal cell carcinoma with similar drugs, such as the breast cancer and colon cancer trials. I am also in favor of trying to do intensive studies on small groups, as pilots first, to generate hypotheses.

Dr. Figlin: There are some data that you should be aware of, although they have not yet appeared in the abstract form. We published at American Society of Clinical Oncology last year a report of more than 100 kidney cancer patients who were evaluated on an ABT-510 thrombospondin mimetic trial looking at CECs. One noted that the frequency of CECs is different by Memorial Sloan-Kettering Cancer Center risk group. Therefore, one can look at these cells prognostically as a surrogate for clinical parameters of risk. Then, if you are going to look at the same patient over time, you may be able to see bigger changes that might correlate with treatment.

Dr. George: Maybe another way of phrasing this would be to form it around a hypothesis. There is enough preclinical modeling in basic science behind this to form those hypotheses and then test. We will not form anything definitive in small single-arm studies, but we can’t get to the large randomized populations unless we have at least tested some of these hypotheses preliminarily in some of these small settings.

Dr. McDermott: As we create a state of more complete VEGF deprivation with combination therapy and more potent VEGF receptor inhibitors, do you think we are going to see more cardiovascular toxicity in our patients?

Dr. Sukhatme: I do. Some of the effects occur because of the effects of VEGF deprivation on endothelial cells, which we know a fair amount about. These effects include hypertension, proteinuria, posterior leukoencephalopathy, and possibly thrombosis. In addition, direct effects on cardiac function may occur since VEGF receptors are expressed on cardiac myocytes and such effects may only manifest themselves clinically over longer periods of time.

Dr. McDermott: In the three kidney cancer patients we treated on a very potent inhibitor of the VEGF receptor, one had two transient ischemic attacks and stopped taking the drug because of high blood pressure. The second patient came in after taking the drug for a year and a half with fatigue and small pleural effusions. We decided to perform an echocardiogram. His ejection fraction was 15%, so we took him off the drug. His ejection fraction 6 months later increased to 50%. The third patient, who had been tolerating the drug for a year and a half, just recently had a non-Q-wave myocardial infarction. While these events may not be drug related, I tend to think that they reflect effective, long-term inhibition of the VEGF receptor pathway.

Acknowledgments

We thank John Heymach, Michael Atkins, and members of the DF/HCC Renal Cancer Program for valuable discussions.

References

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