A surrogate marker to monitor angiogenesis at last

The first angiogenesis inhibitor has recently been approved for cancer treatment. Nonetheless, optimizing the dose of novel angiogenesis inhibitors remains a formidable challenge—primarily because we lack reliable surrogate markers of tumor angiogenesis. In this issue, Shaked et al. provide evidence that the levels of circulating endothelial precursor cells (CEPs), which contribute to the formation of tumor vessels, are genetically predetermined and regulated by angiogenic factors, and reflect the antitumor efficacy of angiogenesis inhibitors. These findings highlight the potential usefulness of CEPs as a surrogate marker to monitor and adjust antiangiogenic therapy.

It is now just over 15 years ago that the VEGF gene was cloned, and already anti-VEGF antibodies have been approved to inhibit angiogenesis as a treatment for colorectal cancer (Hurwitz et al., 2004). In spite of this rapid leap forward from bench to bedside, our ability to monitor—or even predict—the efficacy of novel antiangiogenic compounds has not progressed at the same pace. This poses a formidable medical problem, as more than 300 angiogenesis inhibitors have now been identified, of which 80 are currently being tested in clinical trials (Park et al., 2004). Several surrogate markers of angiogenesis have been considered, but few have proven to be clinically useful. In this issue of *Cancer Cell*, Shaked and colleagues (Shaked et al., 2005) report that the levels of circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs) vary greatly among animals with different genetic constitution, but correlate well with the degree of tumor angiogenesis or the response to angiostatic therapy (Figure 1). Moreover, antiangiogenic therapy can be optimized by monitoring CECs and CEPs. These exciting results suggest that the kinetics of these cells in peripheral blood are useful surrogate markers of pathological angiogenesis and are likely to positively impact the further clinical development and application of antiangiogenic therapies.

Historically, blood vessels in tumors were believed to grow only via sprouting of preexisting vessels—a process termed angiogenesis. However, less than 10 years ago, circulating endothelial progenitor cells derived from the bone marrow were proposed to contribute to the formation of new vessels in tumors—a process termed adult vasculogenesis (Asahara et al., 1997). Preclinical trials in animal models, in which genetically marked donor bone marrow cells were transplanted into lethally irradiated hosts, indicated that, depending on the tumor type, bone marrow fraction, and method of analysis, incorporation of CEPs into tumor vessels varied from less than 1% to up to 50% of new endothelial cells. While the mere presence of CEPs in vessels may suggest that they contribute to vessel growth, this finding does not establish their functional importance.

An interesting question is whether, and to what extent, CEPs functionally contribute to vessel growth in health and disease. Although bone marrow transplantation rescues impaired tumor angiogenesis in mice lacking inhibitors of differentation (Id), PlGF, or eNOS (Carmeliet et al., 2001; Rafii et al., 2002), extrapolation of such rescue experiments should be done cautiously, as the predominant angiogenic mechanism of endothelial cell sprouting was crippled in these mice. Another complication is that often similar signals (VEGF, PlGF, angiopoietins) regulate endothelial cell sprouting and CEP recruitment and, thus, selective inhibitors cannot be easily used to identify their relative contribution. However important biologically, the question to what extent CEPs functionally contribute to tumor angiogenesis is not affecting their use as surrogate markers.

Why do we need surrogate markers to monitor antiangiogenic therapy, and could we not simply administer the maximal tolerable dose to cancer patients? In part, because "the dose makes the poison," as Paracelsus stated over 400 years ago. Intuitively, one might reason that "the more vessels that can be induced to regress, the more efficacious the angiostatic therapy would be." However, elimination of the entire tumor vascular network will impair vascular delivery of cytotoxic drugs and, thus, an optimal angiostatic regimen is required to "normalize" the density and shape of tumor vessels for maximal cytotoxic drug delivery (Jain, 2001). In addition, a supramaximal dose of an angiostatic compound may induce undesired side effects by attacking the quiescent vasculature in the rest of the body—this will become increasingly relevant when more and more cancer patients are treated at earlier stages during their disease

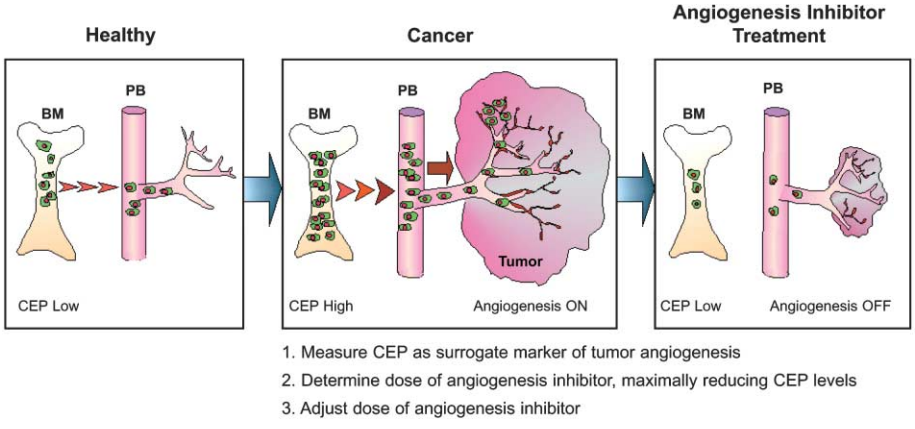

Figure 1. CEP levels monitor tumor angiogenesis and growth

Left panel: In healthy mice, a low number of circulating endothelial progenitors (CEPs) are mobilized from the bone marrow (BM) into the peripheral blood (PB). Middle panel: In tumor-bearing mice, increased numbers of CEPs correlate with enhanced tumor angiogenesis and growth. CEP levels can be used as a surrogate marker to monitor tumor angiogenesis and to adjust the dose of angiogenesis inhibitors. Right panel: Treatment of tumor-bearing mice with angiogenesis inhibitors reduces CEP levels in the peripheral blood and shrinks the tumor.

course. Optimizing angiostatic therapy is thus of great medical importance, and will likely determine the future success of angiogenesis inhibition.

Currently, the degree of angiogenesis or the response to angiostatic compounds is evaluated indirectly by measuring the microvascular density, size, or interstitial fluid pressure in tumors, or by determining the levels of angiogenic molecules (such as VEGF) in the plasma or in tumor tissue. These parameters are not always predictive and/or technically challenging in daily hospital practice. In addition, current technology to image the disorganized and highly permeable tumor microvasculature is still in a preclinical phase of development (McDonald and Choyke, 2003). There is thus an urgent need to discover novel and more reliable surrogate markers of tumor angiogenesis. In a recent study, Willett et al. reported that treatment of colorectal cancer patients with an anti-VEGF antibody not only blocked tumor growth but also lowered the numbers of CEPs—thus suggesting that CEPs might be surrogate markers of angiostatic therapy (Willett et al., 2004).

In a series of elegant genetic and pharmacological studies, Shaked et al. provide further evidence that the levels of CECs and CEPs could indeed be useful surrogate markers of tumor angiogensis (Shaked et al., 2005). First, the authors showed that CEP levels correlated with the angiogenic response to VEGF and bFGF in the avascular cornea and matrigel plug, taking advantage of the variable degree of angiogenic responsiveness in different mouse strains: mouse lines which are hyporesponsive to angiogenic molecules formed few blood vessels and also had low numbers of CEPs, while the opposite was true for the hyperresponsive mouse strains. Second, loss of the angiogenic inhibitor TSP-1 or genetic upregulation of VEGF or Tie2 elevated CEP levels, while pharmacological inhibition of TSP-1 or VEGFR-2 lowered CEP levels in the peripheral blood. Third, an anti-VEGFR-2 antibody inhibited CEP levels and tumor growth at a comparable dose range—the implication of this finding being that it is possible to select an optimal angiogenesis inhibitor dose for tumor inhibition by determining the inhibitor dose that maximally reduces CEP levels. Fourth, CEP levels seemed to be a generally applicable surrogate marker, as they were elevated in tumor-bearing mice and reduced to background levels using antiangiogenic drugs—regardless of the type of tumors (transplanted versus spontaneous, solid versus leukemic), angiogenesis inhibitor, or mouse strain. Together, these data show that the levels of cells of endothelial lineage, circulating in the peripheral blood, correlate with the degree of angiogenesis in the adult. The insight that CECs and CEPs are useful surrogate markers to monitor angiogenesis will likely aid future development and application of antiangiogenic compounds in the clinic.

Apart from the excitement that these findings raise, a number of outstanding issues will need to be resolved in the future. For instance, will CEP and CEC levels also vary 20-fold in humans, and can we identify the genes responsible for this predisposition? Will these levels be predictive surrogate markers of angiogenesis in cancer or ocular or inflammatory disease in humans as well? Do the levels of circulating cells in the peripheral blood correlate with the percentage of CEP-derived cells in growing vessels in tumors? CEP counts in tumor-bearing mice and patients are low—will this assay be sufficiently robust and sensitive in daily hospital practice, and will most tumors mobilize sufficient CEPs to be detected as a surrogate marker? Apart from cancer, CEP levels may also be affected by cardiovascular disorders (Hill et al., 2003)—how specific will these surrogate markers turn out to be? The findings that CEP levels can be used as surrogate markers also raise the intriguing question whether hematopoietic stem cells, which also affect tumor angiogenesis, will have any value as surrogate markers as well.

Notwithstanding these outstanding questions, the authors' findings provide a strong rationale to further evaluate the usefulness of measuring CEC and CEP levels as surrogate markers for tumor angiogenesis and its treatment.

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