

IN THE SPOTLIGHT

Circulating Endothelial Progenitors and Tumor Resistance to Vascular-Targeting Therapies

Michele De Palma^{1,2} and Silvia Nucera^{2,3}

Summary: Acute mobilization of circulating endothelial progenitors has been implicated in tumor resistance to vascular-disrupting agents. In the current issue of *Cancer Discovery*, Taylor and colleagues provide novel insight into the kinetics of endothelial progenitor mobilization by vascular-disrupting agents in both mouse tumor models and cancer patients. *Cancer Discov*; 2(5); 395-7. ©2012 AACR.

Commentary on Taylor et al., p. 434 (12).

INTRODUCTION

Endothelial cells (EC) are highly specialized cells that form a monolayered tissue, called endothelium, which lines the inner surface of the blood vessels. ECs also circulate (albeit at low numbers) in the mouse and human blood (1). Although most of these circulating ECs represent mature ECs that are possibly shed from the endothelium as a consequence of microvascular damage or remodeling, a small population of immature ECs also has been detected in the blood that originates from the bone marrow (1). The latter have been termed endothelial progenitor cells or circulating endothelial progenitors (CEP) and are distinguished from mature circulating ECs on the basis of their high proliferative potential and expression of defined cell-surface markers (2-4).

CEP counts are low in the blood of both naïve and tumor-bearing mice, with figures generally below or around 1 cell per microliter of blood in most mouse strains (5). The exceedingly low frequency of CEPs, together with the difficulty of distinguishing them from circulating hematopoietic progenitor cells, has generated significant uncertainty and controversy regarding their phenotype, enumeration, and functional properties (3). Nevertheless, EC colonies growing as monolayers can be established by culturing blood-derived mononuclear cells under conditions that favor EC proliferation, indicating that the blood is in fact a reservoir of rare CEPs with high clonogenic potential (2-4).

In early studies, researchers suggested that bone marrow-derived CEPs represent an essential source of vascular ECs in rapidly growing mouse tumors (2). Subsequent studies in which authors scrutinized the contribution of CEPs to tumor

blood vessels have had conflicting results, and a consensus on the extent and functional significance of this phenomenon is still lacking (6-8). In more recent studies, investigators also have suggested that certain cytotoxic drugs, such as vascular-disrupting agents (VDA) and taxanes, increase the number of CEPs in the peripheral blood of mice and enhance their incorporation in the tumor vasculature (9, 10). VDAs are microtubule-destabilizing drugs that cause the rapid collapse of intratumoral blood vessels, leading to tumor necrosis and shrinkage. However, tumors rapidly regrow after an initial response phase (11). Interestingly, the acute recruitment of bone marrow-derived CEPs to VDA-treated tumors has been causally linked to tumor revascularization and relapse in mouse tumor models (9, 10).

Shaked and colleagues (9) have previously shown that a single VDA dose induces a transient CEP spike in the mouse circulation as soon as 4 hours after drug administration. Neutralization of this CEP spike with the use of a blocking antibody against vascular endothelial growth factor receptor-2 (VEGFR-2) inhibited the incorporation of CEPs in the tumor blood vessels and delayed tumor revascularization and regrowth (9). These findings suggest that the therapeutic efficacy of VDAs may be improved by blocking drug-induced CEP rebounds into the tumor vasculature. In this issue of *Cancer Discovery*, Taylor and colleagues (12) report carefully performed studies that provide novel insight into the involvement of CEPs in tumor resistance to the VDA combretastatin-A4-phosphate (CA-4-P).

STUDY RESULTS

Taylor and colleagues (12) have extended previous findings by Shaked and colleagues (9, 10) by demonstrating that a single dose of CA-4-P induces 2—rather than 1—distinct CEP spikes in treated mice. Although the first CEP spike occurs a few hours after the administration of the VDA in both naïve and tumor-bearing mice, as previously reported (9), the second CEP spike occurs 3 to 4 days after the administration of VDA and is specifically observed in tumor-bearing mice (12). Of importance, the authors also detected bimodal CEP kinetics in cancer patients treated with a clinically validated VDA (ombrabulin). At variance with earlier findings (9, 10), anti-VEGFR-2 antibodies or sunitinib (an inhibitor of VEGFR

Authors' Affiliations: ¹The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland; ²Angiogenesis and Tumor Targeting Unit, and San Raffaele-Telethon Institute for Gene Therapy, Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Institute; and ³Vita-Salute San Raffaele University, Milan, Italy

Corresponding Author: Michele De Palma, The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology Lausanne (EPFL), CH-1015, Lausanne, Switzerland. Phone: 41-21-69-37271; Fax: 41-21-69-37201; E-mail: michele.depalma@epfl.ch

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and platelet-derived growth factor-receptor kinase activity) did not block the early CEP spike but effectively blunted the late CEP spike. Repeated administrations of sunitinib inhibited tumor regrowth for 2 to 3 weeks after a single dose of CA-4-P, whereas CA-4-P or sunitinib alone had no detectable antitumor activity in the same experimental tumor models. Together, these data suggest that the late CEP spike supports tumor regrowth after VDA therapy.

The late CEP spike may be induced by soluble factors that are upregulated in the tumor after VDA-induced ischemic damage. The authors observed significant serum elevation of granulocyte colony-stimulating factor (G-CSF) and matrix metalloproteinase-9 (MMP-9), which paralleled the late CEP spike in the VDA-treated mice. These data suggest that tumor-derived G-CSF and/or MMP-9 had promoted the second wave of CEP mobilization to the peripheral circulation. Although not experimentally validated by the authors, this hypothesis would be consistent with previous studies in which investigators showed that MMP-9 activity is required for CEPs to egress from the bone marrow (2).

To investigate whether VDA-induced CEP mobilization also leads to their enhanced incorporation in the tumor blood vessels, the authors used a bone marrow transplantation strategy that included green fluorescent protein (GFP)-tagged bone marrow cells. They found that vascular incorporation of GFP-tagged ECs is extremely infrequent in untreated tumors, in agreement with previous reports (9, 13–15). However, the occurrence of vessel-incorporated bone marrow-derived ECs increased in CA-4-P-treated mice a few days after the late CEP spike, possibly suggesting a direct relationship between increased CEP numbers and their enhanced rate of incorporation. As shown in previous studies (9, 10), anti-VEGFR-2 or sunitinib decreased the incorporation of bone marrow-derived ECs in the blood vessels of VDA-treated tumors. Because combined VDA/antiangiogenic treatment delays tumor regrowth, these findings suggest that CA-4-P-induced CEP mobilization and subsequent incorporation in the tumor blood vessels are important determinants of tumor revascularization and regrowth after VDA-based therapy.

OUTSTANDING QUESTIONS

The study of Taylor and colleagues (12) attests to the significance of “CEP rebounds” for tumor resistance to VDA therapy in mouse models of cancer. However, it also raises important questions. One key issue is represented by the ill-defined nature of the CEPs described in this study (12). Indeed, it is unclear from the data presented whether the second wave of cells mobilized in response to the VDA (late CEP spike) truly represent *bona fide* cell progenitors, that is, immature cells endowed with clonogenic activity. Does treatment with VDA increase the frequency of colony-forming unit ECs (3) in the blood of tumor-bearing mice? If yes, do colony-forming unit ECs specifically increase concomitant to the late CEP spike? Furthermore, the anatomic site at which CEPs proliferate in response to VDA treatment has not been defined. Do CEPs proliferate in the bone marrow or in the tumors? If CEPs proliferate after tumor colonization, does this occur before or after they become incorporated in the tumor

endothelium? Finally, do CEPs undergo a differentiation program and lose expression of “progenitor” markers (e.g., c-kit or Sca-1) once they integrate in the tumor endothelium? At present, these questions remain largely unanswered. Perhaps most importantly, future studies should also formally demonstrate that CEPs give rise to the bone marrow-derived ECs that incorporate in the tumor blood vessels. Because CEP-tracing experiments (16) were not performed, the current study (12) provides only indirect evidence that the late CEP spike gives rise to the pool of bone marrow-derived ECs.

Another unresolved issue, which may merit further investigation, is the modality by which CEPs promote tumor revascularization after VDA therapy. The authors report occasional luminal incorporation of bone marrow-derived (GFP-tagged) ECs in the blood vessels of the VDA-treated tumors, with less than 10% of the tumor blood vessels displaying at least one cell apparently incorporated in the endothelial layer. These data argue against the possibility that the “building-block” function of CEPs (i.e., their ability to structurally contribute to the tumor endothelium) could account for their blood vessel-forming activity in the VDA-treated tumors. As properly pointed out by the authors, antiangiogenic treatment by VEGFR-2 blockade or sunitinib not only suppressed the late CEP spike and bone marrow-derived EC incorporation in the tumor blood vessels, but it also dramatically reduced tumor infiltration by bone marrow-derived hematopoietic cells, which represent the vast majority of the cells recruited from the bone marrow after treatment with the VDA. These bone marrow-derived cells include macrophages and other myeloid-lineage cells, which are known to provide proangiogenic and/or reparative signals/factors to the tumor vasculature after radiation- or VDA-induced tumor damage, as shown in recent studies (14, 17, 18).

The finding that antiangiogenic drugs can reverse VDA-induced tumor infiltration by macrophages and other bone marrow-derived hematopoietic cells is of special interest. Of note, sunitinib or VEGFR-2 blockade did not affect hematopoietic cell infiltration in tumors that had not received the VDA. These observations may suggest that VDAs can sensitize tumors to the activity of the antiangiogenic drugs, whose crucial targets—besides CEPs—are the angiogenic tumor blood vessels and their associated perivascular cells (e.g., pericytes). VDA-induced tumor sensitization to antiangiogenic drugs may thus directly improve angiogenesis inhibition and also limit the extravasation of (proangiogenic/reparative) bone marrow-derived hematopoietic cells in the tumor. In such a scenario, CEP blockade might not critically account for the antiangiogenic and antitumoral effects of the combined treatments.

In conclusion, the study by Taylor and colleagues (12) reports exciting and thought-provoking findings that may have important implications for the design of more effective vascular-targeting treatments based on optimized drug scheduling. Moreover, it corroborates the notion that CEPs represent useful biomarkers of drug activity and/or resistance in mouse tumor models (4, 5, 9, 10). Nevertheless, further studies are needed to more rigorously establish the functional importance of CEPs and to better explore the biologic mechanisms whereby CEPs contribute to tumor resistance to VDA-based therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. De Palma

Writing, review, and/or revision of the manuscript: M. De Palma, S. Nucera

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