Commentary

Angiogenic Monocytes

Another Colorful Blow to Endothelial Progenitors

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Ever since the first description of endothelial progenitor cells (EPCs),¹ the concept of a circulating pool of distinct bone marrow-derived cells destined to become endothelial cells at sites of angiogenesis has attracted enormous support. This enthusiasm is easily understood, given the far reaching implications of the existence of EPCs for both therapeutic angiogenesis to ischemic tissues, as well as for tumor angiogenesis suppression.^{2,3} In support of this latter concept, a landmark study⁴ described blockade of angiogenesis and hence tumor growth by ablating the vascular endothelial growth factor (VEGF)-recruitable bone marrow cells, which were believed to be EPCs.

Efforts to challenge the significance of EPCs are often met with disdain,⁵ however, even if such studies are based on solid conclusive experimental evidence.⁶ Still, throughout the past decade many other studies have shown only marginal importance of EPCs in models of ischemic or tumor-induced angiogenesis.² The model systems studied form the core of this controversy: whereas some models using chemical-, radiation-, or genetically-induced myeloablation and bone marrow transfer show high numbers of EPCs incorporated in the newly formed vessels, an equal number of other models show almost complete lack of incorporation of bone marrow-derived cells. In the current issue of *The American Journal of Pathology*, Sun-Jin Kim and co-workers⁷ describe an elegant series of experiments to assess the contribution of bone marrow-derived cells to tumor angiogenesis in a physiological, nonmyeloablative setting. They conclude that the actual angiogenic cell type incorporated in the newly formed vessels is monocytes/macrophages.

The model used by Kim and colleagues⁷ is based on parabiosis: surgically joining the circulation of two mice, one wild-type and one GFP-transgenic, to establish a common circulation. The contribution of the GFP-positive cells to angiogenesis in the wild-type mouse is studied

via three established techniques: wound healing, implanted gel foam fragments drenched in angiogenic growth factors, and, finally, in subcutaneous tumors. Using a panel of markers and multicolor confocal microscopy, Kim and colleagues⁷ show that the majority of GFP-positive cells in newly formed vessels in the wild-type mouse actually stain positive for the widely-used and well-established macrophage marker F4/80. These F4/80- and CD31-positive cells do not express EPC markers, and no GFP-positive cells that would qualify as EPCs were found. In contrast, GFP-positive circulating monocytes stained positive for both CD14 (monocyte marker) and VEGFR2 (endothelial cell marker). Interestingly, CD31-negative GFP-positive cells were also found, surrounding the newly formed vessels. Given the currently unquestioned reputation of F4/80 as genuine macrophage marker, Kim and colleagues⁷ conclude that it is circulating, bone marrow-derived monocytes that actually home to tumors to engage in angiogenesis.

In itself, the concept of monocytes being able to contribute to angiogenesis is not novel. As early as 2003, Urbich and colleagues⁸ showed that EPCs have distinct monocytic features, and EPCs can be cultured from CD14-positive cells. In addition, monocytes were shown *in vivo* to be able to contribute to angiogenesis as EPCs.⁹ Later, in the stem cell era, these monocytes were even classified as multipotent monocytic cells acting as endothelial stem cells.¹⁰

Still, although it has become quite established in recent years that myeloid cells play a pivotal role in tumor angiogenesis, their exact nature remains elusive. Myeloid cells expressing Tie-2¹¹ and VEGFR2, formerly established endothelial markers, have been described.¹² This means that there might be specific subclasses of monocytes destined to home to sites of ischemia or tumor development, using Tie-2 and/or VEGFR2 to home to gradients of angiopoietin or VEGF, respectively.¹³ Thus,

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these cells might function as novel targets for anti-tumor therapy. In fact, a landmark study by De Palma and colleagues¹⁴ used Tie2-expressing monocytes to inhibit tumor growth and metastasis by targeted interferon- α delivery.

Is this the final knockout for the EPCs role in tumor angiogenesis? Much of the evidence for the existence of EPCs is based on *in vitro* culture experiments. The concept of an EPC as a circulating angioblast or endothelial stem cell was fuelled by a number of studies that showed the presence of a circulating cell in both adult¹⁵ and umbilical cord blood¹⁶ that was capable of almost unlimited growth in culture. Characterization during culturing identified these cells as being endothelial, based not only on surface markers, but also on function, ie, formation of capillary-like tubes. Unfortunately, culturing prohibits fate-mapping, preventing the characterization of the original cell from which the culture was derived. Was it a genuine angioblast/EPC, or was it a CD14-positive myeloid cell capable of transdifferentiating into endothelium?

Progenitor cells positive for both endothelial markers (KDR/VEGFR2) and the stem cell marker CD133 have been identified. But, as mentioned previously and again established in the current study by Kim and colleagues,⁷ CD14-positive myeloid cells can be positive for VEGFR2, and they can be cultured to resemble endothelium given the appropriate conditions of matrix support, endothelium medium, and growth factors.⁸ The paucity of angioblast-like cells in blood makes their potential contribution to postnatal angiogenesis questionable, and indeed no such cell type was shown to be relevant in the parabiosis model described by Kim,⁷ consistent with recent reports.⁶ Instead, cells positive for F4/80 and VEGFR2 were the predominant type contributing in these *in vivo* tumor angiogenesis models.

It will be quite important to establish, however, whether these cells constitute a specific subclass of monocytes destined to become angiogenic, based on specific surface markers. Specific subclasses of monocytes have been recently established to home to atherosclerotic lesions.^{17,18} Such angiogenic monocytes might be functionally interchangeable with the term EPC (of myeloid origin), although they cannot be named angioblasts. The concept of cross talk between hematopoietic and endothelial lineage cells is not entirely hypothetical; during embryogenesis both endothelial cells and hematopoietic cells derive from a common ancestor: the hemangioblast.

The difference between EPCs and angiogenic monocytes is not semantics, but exclusion of cellular mimicry.¹⁹ Single surface markers do not type a cell (except in fluorescence-activated cell sorting); genetic programming determines the function of a cell *in vivo*. This genetic programming is, however, subject to change, as exemplified by recent progress in stem cell technology.²⁰ Therefore, appropriate culturing might expand cells of whatever origin to serve as therapeutic progenitor cells.^{21,22} Whether these cells will actually incorporate in newly formed vessels as genuine endothelium, or supply the necessary growth factors and conditions for adult endothelium to expand, is not an issue of practical importance.²³ After all, by now it is well established that

many different bone marrow-derived cells are essential for VEGF-induced angiogenesis, many of which are recruited to sites of ischemia and retained by SDF1 until perfusion is restored.²⁴ In this light, the presence of bone marrow-derived GFP-positive, but CD31/F4/80-negative cells in the tumors as described by Kim and colleagues,⁷ is highly interesting and their future typing would give essential clues as to what types of bone marrow-derived cells are actually involved in maintaining tumor angiogenesis.⁴

In conclusion, the study by Kim and colleagues⁷ in the current issue of the AJP again fuels the controversy regarding the *in vivo* role of genuine EPCs (angioblast), but also establishes a pivotal role of F4/80-positive myeloid cells (angiogenic monocytes) in tumor angiogenesis in a nonmyeloablative, physiological setting. In its purest essence, it adds the already established marker molecule F4/80 to the list of things to type in vascular progenitor cell typing. As such it will not only fuel controversy, but also adds another piece to the puzzle of defining the true circulating pro-angiogenic progenitor cell.

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