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● ● ● VASCULAR BIOLOGY

Comment on Prokopi et al, page 723

Platelet MPs obscure some EPC definitions

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The cell surface phenotype used to define an EPC, in one commonly used *in vitro* assay, may arise from an uptake of contaminating platelet MPs by cultured mononuclear cells, resulting in a gross misinterpretation of the assay results.

Since the first description of a circulating endothelial progenitor cell (EPC) in human blood possessing the ability to form blood vessels *de novo* (postnatal vasculogenesis), investigations of the role of EPCs in cardiovascular disease and repair have exploded.¹ The rapid translation of approaches showing favorable EPC-mediated amelioration of ischemic disease in animal models to clinical trials of EPC or EPC-related cell infusions into human patients suffering from peripheral arterial disease or myocardial infarction has also been remarkable, though the benefits reported thus far have been far less than anticipated from the preclinical data. Some of the potential reasons for the failure of EPCs to deliver more robust results in human clinical trials have already been reviewed.^{2,3}

One of the greatest challenges in studying human EPC biology is the lack of a specific marker to unequivocally identify this circulating cell subset.⁴ At present, human EPCs are identified and quantitated using 3 general approaches. One may use flow cytometry and monoclonal antibodies to delineate certain cell

surface antigens such as CD34, CD133, and vascular endothelial growth factor 2 receptor (KDR) that may enrich for cells that display some ability to participate in new blood vessel formation. However, the peripheral blood cells displaying these antigens do not possess actual *in vivo* vasculogenic activity, though ample data from clinical studies suggest some role for these cells in maintaining cardiovascular health.⁴

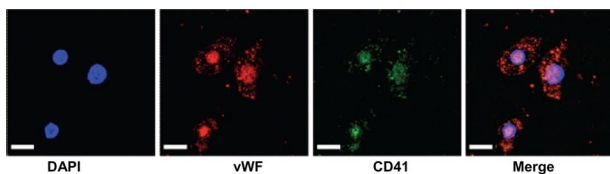
In another set of assays, one may quantify colony-forming cells using a colony forming unit-Hill (CFU-Hill) or an endothelial colony-forming cell (ECFC) assay. Recent studies indicate that the CFU-Hill assay measures a heterogeneous mixture of hematopoietic cells that ultimately permit outgrowth of alternatively activated macrophages having significant stimulatory effects on angiogenesis but fail to contribute to postnatal vasculogenic activity directly.⁵ In contrast, the ECFC assay permits identification of clonal colonies of endothelium with a hierarchy of levels of proliferative potential that form human blood vessels when seeded within 3-dimensional

collagen/fibronectin gels and implanted into immunodeficient mice.⁶

Finally, some investigators simply plate peripheral blood mononuclear cells on fibronectin-coated tissue culture plates and, after 3 to 4 days, remove and discard the non-adherent cells to permit recovery of the adherent cell fraction.⁷ The adherent cells are then generally assessed for the ability to ingest acetylated low-density lipoprotein (acLDL) and to bind *Ulex europaeus* agglutinin I (UEA1) plant lectin and, if positive, are defined as EPCs. The function of these cultured cells in promoting new vessel formation in preclinical animal models has led to use of a similar culture protocol to generate human progenitor cells for transplantation in some human clinical trials.⁸

In this issue, Prokopi et al⁹ report that proteomic analysis reveals the presence of platelet microparticles (MPs) in a standard assay for putative EPCs. Peripheral blood mononuclear cells (PBMNCs) were isolated and plated on fibronectin for EPC culture. MPs present in the EPC-conditioned medium were isolated and analyzed by proteomic approaches and numerous platelet enriched proteins were detected. Using a variety of tests, the authors demonstrate that platelets are a normal contaminant in the PBMNC population and these cells rapidly disintegrate to form MPs under the EPC culture conditions. Platelet MP-derived proteins were found to be taken up and displayed by the adherent PBMNC (despite lack of mRNA for the same proteins in the PBMNC; see figure). While the EPC-conditioned medium stimulated endothelial tube formation in the Matrigel assay, removal of the platelet MPs from the EPC-conditioned medium attenuated this angiogenic effect. Additional data from a large clinical trial indicated that, apart from circulating monocytes, only the platelet count emerged as a significant predictor for patient circulating EPC concentrations in the general population.

While the results of Prokopi et al implicate platelet MP transfer of proteins to PBMNC as a



Cultured monocytic cell line stained with DAPI for nuclear detection, von Willebrand factor (VWF), CD41 (glycoprotein IIb), and merged image, demonstrating the uptake of platelet-derived MPs by the adherent cells. Scale bar 25 μ m. See the complete figure in the article beginning on page 723.

major variable that could result in overestimation of EPC numbers in this particular assay, the study failed to examine which PBMNCs take up the MPs and whether all of the angiogenic effects of the adherent PBMNCs are platelet MP-derived. These results suggest that this particular assay for EPCs is not reliable due to the inability to discriminate between MP transfer of platelet cell surface proteins and host cell display of similar transcribed/translated proteins. These data also point out the ongoing need for development of specific functional assays to define EPCs and to continue to strive for discovery of novel unique EPC-restricted cell surface markers.

Conflict-of-interest disclosure: M.C.Y. is a cofounder and consultant for EndGenitor Technologies Inc in Indianapolis, IN. ■

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been largely measured in terms of overall response rate. Data on progression-free survival (PFS) and overall survival (OS) in the induction setting for high-risk patients (IFM⁵ and GEIMIMA⁶ data) is currently pending.

Now for the good news: risk may matter less. Reece et al evaluate the impact of risk in a relapsed myeloma trial and define high risk as the presence of deletion 13, t(4:14), or del 17p using FISH data. But are all these equally poor? Deletion of 13q was found to be significant as a prognostic marker when isolated by metaphase cytogenetics,⁷ but when present as the sole FISH abnormality, it was not noted to have an impact on survival.⁸ Similarly, t(4:14) patients with this abnormality and a low β 2M were not noted to have a significant survival decrement in an IFM analysis.⁹ In the current report, the median β 2M was 3.3, suggesting that half of the 28 patients in the analysis did not have poor-risk disease. This leaves the del 17p group, who generally do have poor outcomes regardless of the choice of therapy, and unfortunately, this is no different for LD-treated patients.

So what is the answer? Does risk matter, and can we all agree on how to define it? The simple answer is yes on both accounts. Risk does still matter, and it is unlikely in the high-risk setting that single agents are going to be the solution. Combination therapy with maintenance will likely be the minimum if we are to make significant improvements in PFS and OS for this group of patients. Second, the definitions of high risk are agreed upon for now. From the International Myeloma Working Group, there is a consensus document that codifies the currently agreed upon risk assessment, and the published version will provide support and data that will summarize how to best classify newly diagnosed patients.¹⁰

In the relapsed setting, this is less clear. It is clear that we likely eliminate the highest risk patients from analysis in relapsed trials simply because they do not survive long enough to receive salvage therapy. Thus, highest risk patients are likely best evaluated in the induction setting as an inherent selection bias has already set in by the time we get to relapsed trials.

It is clear from both trials that response rate alone is not sufficient to gauge the efficacy of a given approach in the context of high-risk disease. Both papers show no real difference between the overall response rate (ORR) for standard or high-risk patients except in the

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Comment on Reece et al, page 522, and Kapoor et al, page 518

Risky business in myeloma

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The present and future for patients with myeloma has never looked better. Yet for high-risk patients, much work remains to be done.

The term “multiple myeloma” was coined more than 100 years ago, based on a patient who was noted to have multiple sites of bone-based plasmacytomas or myelomas on postmortem examination. However, in the modern era of gene expression profiling and advanced diagnostics, the word “multiple” could just as easily refer to the heterogeneous nature of the disease that we collectively call multiple myeloma. In this issue of *Blood*, Kapoor and colleagues from the Mayo Clinic,¹ and Reece and colleagues from the Canadian lenalidomide compassionate use trial,² both report on the impact of high-risk FISH abnormalities on response and response duration in the context of myeloma. Given the move toward individualized therapy based on risk, these studies are critically important in helping to identify the relative utility of lenalidomide among a group of patients whose outcomes are historically quite poor.

However, interpretation of these 2 trials has its challenges and leaves the clinician with

both good and bad news. First, the bad news: risk does matter. Kapoor et al leave the reader with no doubt that, though the fraction of high-risk patients is lower than one would expect from a general population study (25% according to the IFM experience), patients with poor-risk myeloma have a significantly shorter duration of remission than standard risk patients. While these data exemplify the phrase “tyranny of small numbers”, similar data has been presented by the SWOG group in their phase 3 trial of lenalidomide/dexamethasone (LD) versus dexamethasone (D).³ While LD was superior to D, patients who received LD with high-risk criteria had a shorter remission than patients who received LD and had standard risk, though LD was superior to D regardless of risk (B. Barlogie, personal written communication, February 2009). While we accept that bortezomib is able to overcome high-risk features when combined with melphalan in the VISTA trial,⁴ in the transplant eligible population, this has