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It takes 2 to thrombopoies in the vascular niche

Eric Gars and Shahin Rafii
WEILL CORNELL MEDICAL COLLEGE; HOWARD HUGHES MEDICAL INSTITUTE

In this issue of Blood, Pitchford et al demonstrate that intravenous administration of VEGF-A promotes megakaryocyte (Mk) maturation and localization to bone marrow sinusoidal endothelial cells (BMECs), stimulating thrombopoiesis. Using specific agonists of VEGFR1 and VEGFR2, the authors show VEGF-A acts upstream of VEGFR1 to up-regulate CXCR4 on Mks and stimulate their migration from the endosteal region to the vascular niche. These intriguing results implicate VEGFR1 activation as a trigger for reactive thrombocytosis in the context of hypoxia and angiogenesis.

While platelet production was once thought to be a product of systemically acting cytokines, our group and others have firmly established a critical role for the vascular niche in Mk maturation, platelet production, and recovery of thrombopoiesis after bone marrow injury. Genetic studies using thrombopoietin (TPO)– and TPO–receptor (c–Mpl)–deficient mice show that TPO is not solely responsible for Mk development. Chemokine-mediated interactions between Mk progenitors and bone marrow niche cells, including BMECs, have been shown to augment Mk maturation, endomitosis, and platelet production. For instance, intravascular delivery of FGF-4 and SDF-1 is sufficient to increase circulating platelet counts in Tpo−/− and Mpl−/− mice by enhancing survival and maturation of Mks, promoting redistribution of Mks adjacent to BMECs, and stimulating the expression of adhesion molecules that induce transendothelial migration of pro-platelets. We have previously shown that the bone marrow of mice treated with 5-fluorouracil (5-FU) had a decrease in megakaryocytic lineage cells after administration of VEGFR1 blocking antibodies. Further, selective inhibition of platelet recovery was observed after coadministration of myelosuppressive doses of 5-FU with a vascular disrupting agent (combretastatin–A4–phosphate), demonstrating that BMEC integrity is essential for thrombopoiesis. Indeed, the supportive role of the bone marrow niche on thrombopoiesis is reciprocal—platelets deploy angiogenic factors (VEGF, FGF, SDF-1, etc) that promote vascular activation and angiogenesis in the context of tissue injury and malignancy, further reinforcing vascular niche support of thrombopoiesis (see figure).

The hematopoietic effects of VEGF-A are not only mediated indirectly through its angiogenic activity, but also directly via receptors (eg, VEGFR1) expressed on the hematopoietic progenitors. Thus, this 1 signal can potentially orchestrate complex hematopoietic processes such as bone marrow regeneration after cytotoxic damage. Pitchford et al make a significant contribution by elucidating how VEGF-A promotes Mk migration toward the vascular niche—a critical step in the reconstitution of the bone marrow and initiation of thrombopoiesis after myeloablation. The authors demonstrate that VEGF-A, acting via VEGFR1, up-regulates CXCR4, resulting in redistribution of Mks to the vascular niche. Although the number of bone marrow Mks is not affected by VEGF-A, the redistribution is associated with Mk maturation and, apparently, platelet shedding. Notably, CXCR4-dependent migration of Mks results in localization of mature Mks to the vascular niche and platelet release into bone marrow sinusoids. VEG1 agonists PIGF-2 and VEGF-A stimulate VEGFR1 on megakaryocytes (Mks) leading to CXCR4 upregulation and endomitosis. CXCR4-dependent migration of Mks results in localization of mature Mks to the vascular niche and platelet release into bone marrow sinusoids. PIGF indicates placental growth factor.
this report poses several questions. For instance, it is unclear that these effects are due to direct stimulation of VEGFR1 on MKs or indirectly via other niche components. Because vascular cells and myeloid cells also express VEGFR1, it is possible that VEGFR1 signaling may be induced in multiple cell types that each could, in turn, secrete paracrine/cytokine signals leading to heretofore undefined combinatorial effects that contribute to up-regulation of CXCR4 and increased thrombopoiesis. In this regard, delineation of the cell type–specific contribution of VEGFR1 would advance our understanding of this process. In addition, the molecular mechanism mediating CXCR4 up-regulation by VEGFR1 also needs to be elucidated. Whether VEGFR1 stimulation increases CXCR4 expression levels or prevents receptor internalization could also provide insight into possible cross-talk between 2 signaling pathways, especially during platelet production.

Although SDF-1 and CXCR4 signaling is essential for vascular niche–supported hematopoiesis, the cellular source of SDF-1 is not known. Pitchford et al noted that despite the prominent role of the spleen during regenerative hematopoiesis in the mouse, VEGF-A does not induce Mk changes in this organ. This may point to the fact that organ–specific vascular niche cells, including the specialized VEGFR3+ sinusoidal ECs and perivascular cells, might play a decisive role in this response.

The clinical implications of this study are potentially significant. Treatment–related thrombocytopenia contributes to the morbidity and mortality associated with cytotoxic chemotherapy and hematopoietic stem cell transplantation. While treatment of noniatrogenic thrombocytopenia with c-Mpl agonists has been effective, Mk-active cytokines have not been approved to decrease the latency of platelet recovery after cytotoxic and myeloablative chemotherapies. We have previously shown that megakaryopoiesis promotes endothelial regeneration after myelosuppressive therapy. This study highlights the mutually supportive role of MKs and the marrow’s vasculature and suggests that cytotoxic damage to the vascular niche renders megakaryopoiesis refractory to TPO analogs because Mk precursors cannot relocalize to the sinusoids. Novel strategies to promote the association of MKs with BMECs could potentially enhance platelet recovery and help restore the integrity of the bone marrow vascular niche and its supportive role in thrombopoiesis.

Conflict-of-interest disclosure: The authors declare to competing financial interests.

REFERENCES
1. Pitchford SC, Lodie T, Rankin SM. VEGFR1 stimulates a CXCR4-dependent translocation of megakaryocytes to the vascular niche, enhancing platelet production in mice. Blood. 2012;120(14):2787-2795.
2. Avecilla ST, Hattori K, Heissig B, et al. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nat Med. 2004;10(1):64-71.
3. Hattori K, Heissig B, Wu Y, et al. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1+ stem cells from bone marrow microenvironment. Nat Med. 2002;8(8):841-849.
4. Kopp H, Hooper AT, Broekman MJ, et al. Thrombospondins deployed by thrombopoietic cells determine angiogenic switch and extent of revascularization. J Clin Invest. 2006;116(12):3277-3291.
5. Wang JF, Liu ZY, Groopman JE. The alpha,

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Carfilzomib in multiple myeloma: gold, silver, or bronze?

Donna E. Reece Princess Margaret Hospital

In this issue of Blood, Siegel and colleagues report that the next-generation proteasome inhibitor carfilzomib, when administered as a single agent, can produce meaningful disease control in heavily pretreated patients with relapsed/refractory multiple myeloma.1 The treatment of multiple myeloma typically involves the judicious use of sequential regimens, each designed to induce a prolonged remission with acceptable toxicity. The introduction of novel agents, particularly the immunomodulatory derivatives (IMiDs) thalidomide and lenalidomide as well as the proteasome inhibitor bortezomib, has increased the achievement and durability of remissions, and led to improved survival in this malignancy.2-4 However, virtually all patients develop resistance to these agents at some point, and eventually succumb to the disease.5 The term “double refractory” has been coined to refer to patients who are resistant and/or intolerant to both lenalidomide and bortezomib and who have a poor prognosis. The identification of newer agents effective in double refractory myeloma represents a high priority and certainly merits a medal in the field of malignant hematolgy.

Siegel et al report the results of a large phase 2 trial of the new proteasome inhibitor carfilzomib in patients with progressive disease who had received all of the effective classes of agents, including bortezomib. Carfilzomib differs from bortezomib, the prototype proteasome inhibitor, in that it irreversibly binds to the proteasome more selectively, primarily inhibiting the chymotrypsin-like activity of this enzyme. In the current trial, patients had been exposed to a median of 5 prior lines of therapy, 28% were known to have adverse cytogenetic abnormalities, and 80% were considered to be double refractory. With single-agent carfilzomib, the overall response rate was 23.7%, and patients who were double refractory had a response rate of 20.1% (15.4% excluding those in this subset based on intolerance to the agent(s)). Although the progression–free survival was relatively short at 3.7 months, the duration of response for
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