Regulation of Vasculogenesis by Platelet-Mediated Recruitment of Bone Marrow–Derived Cells

Daniel C. Rafii, Bethan Psaila, Jason Butler, David K. Jin, David Lyden

Abstract—Bone marrow–derived cells contribute to physiological and pathological vascular remodeling throughout ontogenesis and adult life. During tissue regeneration and tumor growth, the release of cytokines and chemokines mediates the recruitment of hematopoietic and endothelial progenitor cells that contribute to the assembly of neovessels. Current evidence implies that platelets contribute structurally and instructively to vascular remodeling. Platelets adhere almost immediately to exposed or activated endothelium, and they are major storage and delivery vehicles for pro- and antiangiogenic growth factors including VEGF-A and thrombospondin (TSP), and cytokines and chemokines, such as stromal-derived factor 1 (SDF-1). By site-specific deployment of these factors, platelets orchestrate the local angiogenic stimulus within a tissue and direct the recruitment and differentiation of circulating bone marrow–derived cells. These insights have profound clinical implications; inhibition of platelet-deployed growth factors, or their receptors may be an effective strategy to block tumor growth, whereas activation of these pathways may be used to accelerate revascularization and tissue regeneration. (Arterioscler Thromb Vasc Biol. 2008;28:000-000.)

Key Words: angiogenesis ■ hematopoietic progenitor cells ■ endothelial progenitor cells ■ ischemia ■ platelets

Accumulating evidence indicates that bone marrow–derived cells contribute to postnatal vasculogenesis in tumor growth,1–4 wound healing,1,5 postmyocardial ischemia,6–11 cerebral ischemia,12 limb ischemia,1,13–19 endocardialization of vascular grafts,20–23 atherosclerosis,24 and retinal neovascularization.25–27 The majority of studies have focused on the role of endothelial progenitor cells (EPCs) in vascular remodeling. In addition to VEGFR2+EPCs, a subset of hematopoietic progenitor cells (HPCs) are mobilized from the bone marrow and are thought to contribute to the early initiation and stabilization of newly-forming vessels,2,5 however their precise role is not yet clear. Platelets are integral to vasculogenesis, delivering angiogenic growth factors and cytokines that direct the site-specific recruitment and differentiation of bone marrow–derived progenitor cells at vasculogenic sites.28–31 This review summarizes recent insights into the synergistic role of platelets and bone marrow–derived progenitor cells in vasculogenesis.

Bone Marrow–Derived EPCs and Postnatal Vasculogenesis

During embryonic development, the majority of blood vessel formation occurs by vasculogenesis from the assembly of mesoderm-derived angioblasts. Traditionally, it was thought that once an intact vascular system was established, further vessel growth occurred only by sprouting of endothelial cells from existing vessels, in angiogenesis and arteriogenesis. In 1997, Ashahara et al isolated a population of CD34+ endothelial cells with a progenitor-like phenotype from human peripheral blood, indicating that vasculogenesis, or progenitor cell-driven de novo generation of new vessels, occurred postnatally.32 EPCs are distinguished from mature, circulating endothelial cells by their expression of stem cell markers such as AC133 and c-kit, and by their clonogenic properties in vitro.33 The precise ontogeny and functional nature of circulating EPCs remains incompletely understood. Although immature endothelial cells with clonogenic potential are found embedded in vessel walls,33–35 many studies have indicated that circulating EPCs are bone marrow–derived.1,2,7,20,36–39 Both human and animal studies indicate that bone marrow–derived EPCs incorporate into regenerating organs, ischemic tissue, and tumor neovessels.2,4,36,40–42

Hematopoietic Progenitor Cells in Revascularization and Tumor Vasculogenesis

In addition to EPCs, there is evidence that HPCs play a role in postnatal vasculogenesis,2,27,41,43 In murine models of hindlimb ischemia, mobilization of nonendothelial, VEGFR1+CXCR4+ HPCs was found to be a major determinant of tissue revascularization.19 One mechanism by which HPCs may promote vasculogenesis is via the paracrine release of angiogenic factors,44 thereby enhancing the recruitment and incorporation of EPCs into neovessels. Activated VEGFR1+CXCR4+ hematopoietic cells can release angiogenic factors...
such as VEGF-A, platelet-derived growth factor (PDGF), angiopoietins, and brain-derived neurotrophic factor (BDNF), which serve to enhance vessel formation and stability.\textsuperscript{45–47} Proteolytic enzymes, predominantly matrix metalloproteinases (MMPs) including MMP-9 are also secreted by HPCs and mature myelomonocytic cells. HPCs may also contribute structurally to neovessel formation as part of the pericellular network that stabilizes endothelial cell alignment and prevents vascular leakage, although this has not yet been definitively shown.

**Clarifying the Contribution of Bone Marrow–Derived Cells in Vasculogenesis: Future Directions**

The extent to which new endothelium in postnatal neovascularization is derived from bone marrow–derived progenitor cells versus mature or locally-derived cells is still a matter of controversy.\textsuperscript{48–50} The duration of dependence of human tumor vessels on bone marrow–derived cells appears to be dictated by tumor type, and it is likely that some of the variability between reports on the contribution of bone marrow–derived cells is a result of the different tumor models used. A study of patients who developed solid tumors subsequent to receiving sex-mismatched bone marrow transplants showed that the degree of incorporation of bone marrow cells varied from 1% to 12%, with the highest degree in patients with lymphoma.\textsuperscript{40} There is also evidence that bone marrow–derived cells are more critical earlier in tumor expansion, in the initiation of vasculogenic growth (“release” from tumor dormancy), and are only detectable at small levels in the mature, stable vasculature of developed tumors.\textsuperscript{4} Secondly, in vitro studies have indicated that with angiogenic stimulation, isolated CD34\textsuperscript{+} cells yield HPCs in vitro with no vessel-forming activity.\textsuperscript{52,53} Secondly, in vitro studies have indicated that with angiogenic stimulation, isolated CD34\textsuperscript{+}CD14\textsuperscript{+}VEGFR2\textsuperscript{+} cells isolated from umbilical cord blood or adult peripheral blood yield HPCs in vitro with no vessel-forming activity.\textsuperscript{52,53} Furthermore, the host genetic background also appears to be a determining factor of the extent of endothelial progenitor mobilization and incorporation into neovessels in murine studies.\textsuperscript{51}

As yet, there is no clear definition of either an EPC or an HPC, and the precise roles of these subpopulations remain incompletely understood. The identifying cell surface markers used for EPCs are also present on primitive HPCs, and it has been reported that human CD34\textsuperscript{+}AC133\textsuperscript{+}VEGFR2\textsuperscript{+} cells isolated from umbilical cord blood or adult peripheral blood yield HPCs in vitro with no vessel-forming activity.\textsuperscript{52,53} Secondly, in vitro studies have indicated that with angiogenic stimulation, isolated CD34\textsuperscript{+}CD14\textsuperscript{+} myeloid cells may develop an endothelial phenotype, expressing endothelial surface markers including von Willebrand factor (vWF) and VE-cadherin and forming tubular-like structures.\textsuperscript{54} Further clarification of the expression profiles, differentiation potentials, and in vivo phenotypes of bone marrow–derived cell subpopulations in human pathophysiology is likely to be forthcoming.

Despite the uncertainties that remain, an inflammatory infiltrate of bone marrow–derived cells is clearly an important factor in tissue recovery and tumor progression independent of their vessel-forming capacity. The existence of a renewable reservoir of bone marrow–derived vasculogenic cells has attracted much interest given the therapeutic implications for revascularization therapy and as neovascularation development is the rate-limiting step in tumor progression. The clinical utility of quantifying EPCs/HPCs as biomarkers for cardiovascular disease risk and to evaluate response to antiangiogenic cancer therapy is under investigation.\textsuperscript{55} Preclinical studies have examined the effect of genetically-modifying bone marrow progenitor cells to inhibit tumor vascularization, and given their tumor-homing capacities these cells may also be manipulated to deliver antigrowth therapies.\textsuperscript{3,41}

**Angiogenic Factors VEGF-A and SDF-1α Induce the Recruitment of Bone Marrow–Derived Cells to Sites of Vasculogenesis**

Once mobilized from the bone marrow, a complex array of signaling pathways mediated by direct cell-cell interactions and secreted factors guides the site-specific homing of bone marrow–derived cells to sites of vasculogenesis. VEGF family proteins, predominated by VEGF-A, are master regulators of physiological bone marrow homeostasis and potent mediators of the angiogenic switch that occurs after ischemic injury and tumor growth. These scenarios induce upregulation of VEGF-A through induction of hypoxia-inducible factor-1.\textsuperscript{56} Plasma elevation of VEGF-A in conjunction with other growth factors such as angiopoietin-1 stimulates the mobilization of EPCs/HPCs.\textsuperscript{43} The VEGF receptors, VEGFR-1, -2, -3 and neuropilin receptors, NP-1 and -2 have both independent and synergistic functions. VEGFR1 signaling mediates mobilization of hematopoietic cells and influences their cellular migration and chemotaxis.\textsuperscript{43} VEGFR1 also induces paracrine release of tissue-specific growth factors.\textsuperscript{57} VEGFR2 activation is important for endothelial cell growth and promotes endothelial cell survival and vascular permeability.\textsuperscript{58} Whereas VEGF-A binds to both VEGFR1 and VEGFR2, placental growth factor (PIGF, a VEGF family member) and VEGF-B signal exclusively through VEGFR1. In the early stages of hematopoietic recovery after myelosuppression, PIGF promotes the cellular replication and recruitment of VEGFR1 \textsuperscript{1}c-Kit\textsuperscript{+} HPCs from their quiescent state by its direct interaction with VEGFR1 and from its indirect effects, via MMP-9 secretion increasing available stem cell factor (SCF, also known as soluble Kit-ligand, sKitL).\textsuperscript{58}

In conjunction with the VEGF family proteins, the chemokine SDF-1 also contributes to the site-specific homing pattern of bone marrow–derived cells in both normal physiology and during pathological stress.\textsuperscript{59–62} Local gradients of SDF-1 influence the localization of cells within the bone marrow, as hematopoietic cells migrate toward high concentrations of SDF-1, binding via the chemokine receptor CXC\textsuperscript{4}, also known as soluble Kit-ligand, sKitL). In vivo CXCR4 also mediates recruitment of CXCR4-expressing metastasizing tumor cells.\textsuperscript{64–66} Impaired CXCR4-mediated progenitor cell recruitment may contribute to the pathogenesis of coronary artery disease and to the poor vascular maintenance associated with chronic diseases such as renal failure and diabetes.\textsuperscript{67}
Platelets direct site-specific recruitment of bone marrow–derived progenitor cells to sites of vasculogenesis. The VEGF and SDF-1/CXCR4 signaling pathways and the molecular interactions between platelets and progenitor cells are shown. VEGF indicates vascular endothelial growth factor; SDF-1, stromal-derived factor 1; CXCR4, chemokine receptor 4; PSGL-1, P-selectin glycoprotein ligand.

Platelets Influence the Homing and Differentiation of Bone Marrow–Derived Progenitor Cells

There is accumulating evidence that platelets mediate the effect of hematopoietic cytokines to recruit bone marrow–derived cells to the vasculogenic niche (Figure). Thrombopoietic cytokines thrombopoietin (TPO) and stem cell factor (SCF) are significantly elevated shortly after ischemic tissue injury, and transgenic mice deficient in TPO or the TPO receptor (c-MPL) demonstrate significantly impaired hindlimb revascularization and inhibited angiogenic tumor growth. The integral role of platelets was vividly portrayed in studies using real-time in vivo fluorescence microscopy, illustrating that aggregation of activated platelets was necessary for the recruitment of bone marrow–derived c-Kit+ Sca1+ Lin– progenitor cells and CD34+ cells to sites of endothelial disruption. Hematopoietic cells were unable to adhere directly to the exposed extracellular matrix, but were tethered to platelet surface P-selectin via P-selectin glycoprotein ligand (PSGL-1) binding (Figure). Platelet release of SDF-1 provided an ongoing retention signal for bone marrow–derived cells at these sites. The mechanism by which platelets release SDF-1, VEGF and other growth factors in a site-specific fashion is likely to involve glycoprotein (GP) Ib–dependent platelet aggregation, inward receptor signaling, and MMP-9–mediated SDF-1 release. In addition to platelet-derived SDF-1, exposed smooth muscle cells also produce SDF-1 after arterial injury, and platelet activation via CXCR4 signaling leads to P-selection upregulation, further promoting arrest of bone marrow–derived cells to activated endothelium. In vitro studies of human CD133+ VEGFR2+ EPC-platelet interactions have confirmed that these phenomena are not limited to animal models, and other chemokine axes in addition to SDF-1/CXCR4 may further contribute to platelet-mediated progenitor cell recruitment in vascular remodeling. Human EPCs expressing the chemokine receptor CXCR2 show greater adhesion to activated endothelium than CXCR2-null EPCs, colocalizing with surface-adherent platelets.

In addition to directing the migration and adherence of bone marrow–derived cells to sites of new vessel growth, platelets may also induce the differentiation of EPCs into mature endothelial cells, and the differentiation of CD34+ cells into foam cells. Macrophage/foam cell generation was abrogated by 3-hydroxy-3-methylglutaryl (HMG) coenzyme A reductase inhibitors and peroxisome proliferators-activated receptor (PPAR) agonists via inhibition of MMP-9. Taken together, these data suggest that aggregation and activation of platelets at sites of exposed subendothelium and vasculogenesis play a major role in the recruitment, differentiation, and incorporation of bone marrow–derived progenitor cells.

Proangiogenic Effects of Platelets Are Mitigated Through Its Release of Antiangiogenic Factors Such as Thrombospondins

Platelets contain not only proangiogenic factors but also antiangiogenic components, including platelet factor 4 and thrombospordin 1 (TSP). These proteins are stored together with coagulation factors in platelet α-granules which on activation may degranulate and thereby determine the local angiogenic stimulus. Platelets prevent hemorrhage from nascent vessels. Pathways activated by the release of antiangiogenic cytokines temporize the proangiogenic effect of SDF-1 and VEGF-A, thereby stabilizing the process of new vessel growth.

The factors that determine the balance between release of VEGF-A, SDF-1, or TSP and the promotion or inhibition of angiogenesis are not yet clear. There is evidence that pro- and antiangiogenic proteins are organized into separate platelet α-granules and that the selective binding of specific proteinase-activated receptors (PARs) counter-regulate the release of endostatin and VEGF-A from human platelets. In these studies, a specific PAR4 agonist elicited endostatin release while suppressing VEGF-A release, and PAR1 stimulation induced VEGF release over endostatin. Therefore, tumors may modulate the release of angiogenic factors from platelets by secretion of PAR ligands or proteolytic degradation of certain PAR receptors. It is conceivable that the net proangiogenic/antiangiogenic effect may be further modulated by the neoangiogenic microenvironment after platelet degranulation. For example, TSPs can undergo proteolytic

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inactivation, thereby allowing either SDF-1 or VEGF-A to exert their proangiogenic effects. The precise constitution of proteases that could dictate the proangiogenic effects of platelets is not fully understood and remains under extensive scrutiny. Alternatively, the “angiogenic phenotype” of circulating platelets may be altered during tumor growth or ischemic injury by systemic effects of angiogenic cytokines on megakaryocytes and the bone marrow microenvironment, via alterations in the packaging of pro- and antiangiogenic factors into platelets.

Conclusions

After ischemic tissue injury and during vascularization of tumor growth, the systemic release of cytokines such as VEGF-A, TPO, and SCF promotes the mobilization of stem/progenitor cells from the bone marrow. Studies have shown that an inflammatory infiltrate of mature monocytes and macrophages and less differentiated bone marrow–derived progenitor cells is critical in tumor vasculogenesis and nonmalignant vascular processes. Emerging evidence implicates a key role for platelets in the site-specific recruitment of hematopoietic cells to ischemic tissues and the tumor microenvironment, modulating the local angiogenic stimulus via cytokine-mediated activation and deployment of granular contents, such as VEGF-A, SDF-1, and TSPs. Many questions remain unanswered. First, the precise functional contribution of nonendothelial hematopoietic progenitor cells to new vessel formation is not yet known. The fate of these cells, and whether they differentiate locally within the neovascular niche or maintain their progenitor state is unclear. Second, the pathways that govern the net balance of pro- and antiangiogenic effects of platelet release remain to be elucidated. Despite these uncertainties, the clinical implications of these data are significant. The utility of manipulating the platelet-mediated recruitment of bone marrow–derived cells for the treatment of vasculopathies and as treatment for malignancy will be a subject of ongoing study.

Disclosures

None.

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