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Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularization of tumor and ischemic tissue

Hans-Georg Kopp^a, Carlos A. Ramos^b, and Shahin Rafii^a

^aDepartment of Genetic Medicine, Howard Hughes Medical Institute, Weill Medical College of Cornell University, New York, USA

^bDepartment of Medicine, Memorial Sloan-Kettering Cancer Center, New York, USA

Abstract

Purpose of review—During the last several years, a substantial amount of evidence from animal as well as human studies has advanced our knowledge of how bone marrow derived cells contribute to neoangiogenesis. In the light of recent findings, we may have to redefine our thinking of endothelial cells as well as of perivascular mural cells.

Recent findings—Inflammatory hematopoietic cells, such as macrophages, have been shown to promote neoangiogenesis during tumor growth and wound healing. Dendritic cells, B lymphocytes, monocytes, and other immune cells have also been found to be recruited to neoangiogenic niches and to support neovessel formation. These findings have led to the concept that subsets of hematopoietic cells comprise proangiogenic cells that drive adult revascularization processes. While evidence of the importance of endothelial progenitor cells in adult vasculogenesis increased further, the role of these comobilized hematopoietic cells has been intensely studied in the last few years.

Summary—Angiogenic factors promote mobilization of vascular endothelial growth factor receptor 1-positive hematopoietic cells through matrix metalloproteinase-9 mediated release of soluble kit-ligand and recruit these proangiogenic cells to areas of hypoxia, where perivascular mural cells present stromal-derived factor 1 (CXCL-12) as an important retention signal. The same factors are possibly involved in mobilization of vascular endothelial growth factor receptor 2-positive endothelial precursors that may participate in neovessel formation. The complete characterization of mechanisms, mediators and signaling pathways involved in these processes will provide novel targets for both anti and proangiogenic therapeutic strategies.

Keywords

endothelial progenitor cells; hematopoietic progenitor cells; neoangiogenesis

Introduction

The occurrence of adult vasculogenesis remains an area of intense debate and of tremendous interest. There is now compelling evidence that recruitment of circulating vascular as well as hematopoietic cells contributes to the initiation and maintenance of postnatal neoangiogenic processes. Comobilization of subsets of hematopoietic and vascular cells, collectively referred

to as hemangiogenic cells, support revascularization of ischemic limbs and tumor growth. Several studies have indicated that bone marrow derived hemangiogenic cells and their progeny functionally contribute to neoangiogenesis during wound healing [1–6], postmyocardial ischemia [7–12], cerebral ischemia [13], limb ischemia [14–18], endothelialization of vascular grafts [19–22], atherosclerosis [23], retinal neovascularization [24,25], as well as tumor growth [3,26–32]. Previously, vascular endothelial growth factor receptor 1-positive (VEGFR-1+) myelomonocytic cells have been implicated in neoangiogenic responses [26], but recently there has been published evidence of proangiogenic subpopulations in almost all hematopoietic cell lineages [33•,34,35•,36–38].

The elucidation of the mechanisms by which different hematopoietic cells contribute to vasculogenesis will provide novel targets for the treatment of cardiovascular disease as well as malignancies. In addition, the identification and quantification of circulating hemangiogenic cells may provide novel biological surrogate markers for metastatic risk and response to treatment. This report focuses on recent discoveries in the field and summarizes the current view of molecular and cellular pathways that support mobilization and recruitment of marrow derived cells to neoangiogenic niches, thereby accelerating the vascularization of ischemic organs and tumor tissue.

Endothelial progenitors support postnatal angiogenic processes

Circulating endothelial progenitor cells (EPCs) [39] have been shown to incorporate into ischemic limbs [2,40], and to functionally populate the surface of Dacron grafts [19] as well as left ventricular assist devices [41], facilitating the rapid formation of a nonthrombogenic vascular neointima. In addition to their role in wound healing, these cells play a role in promoting tumor angiogenesis [3,29,42]. Ingram *et al.* [43•,44] have recently shown that the vascular wall may also contain highly proliferative EPCs capable of forming colonies with replating potential (HPP-ECs) [43•,44]. The same group had previously described a hierarchy of endothelial cell precursors in cord and adult blood, by in-vitro analysis [45]. These data suggest that endothelial progenitors include a scarce population of clonogenic cells with serial replating potential that can contribute to ischemic revascularization and tumor neoangiogenesis.

The identity of circulating endothelial progenitor cells

The phenotype of EPCs is not consensual and they have been variably defined as cells mobilized from the bone marrow in response to chemotherapy, cytokines, ischemia, or tumor growth; that express various combinations of antigens traditionally associated with hematopoietic stem or progenitor cells as well as endothelial cells (such as c-kit, CD133, Sca-1, CD34, VEGFR2, vascular endothelial cadherin); that display phenotypical aspects of endothelial cells in in-vitro clonogenic assays (such as low-density lipoprotein-captation and endothelial nitric oxide synthase expression); or that integrate in blood vessel endothelium, where they can be detected by fluorescence in-situ hybridization (FISH) analysis, green fluorescent protein (GFP) positivity or β -galactosidase expression [46,47]. It is unclear whether EPCs are bone marrow derived from primitive cells (a putative hemangioblast) or from differentiated cells (like monocytes) that acquire an endothelial cell-like phenotype [48,49•]. Similarly, it remains to be clearly established to what degree vessel wall derived cells can contribute to the circulating pool of endothelial progenitors.

Evidence for the contribution of endothelial progenitors to tumor angiogenesis in humans

Several reports have demonstrated that human marrow-derived endothelial precursors incorporate, although in low numbers, into regenerating organs [50] and tumor neovessels [51]. In the latter study, in which secondary solid tumors in patients after hematopoietic stem cell transplantation were examined, 0.5–12% of tumor endothelial cells were found to be donor derived. Interestingly, the highest incorporation was reported in lymphomas. Another study has shown that CD133+ endothelial progenitors also incorporate into human lung carcinomas [52]. The absolute number of circulating endothelial progenitors has also been demonstrated to correlate with the degree of neoangiogenic activity [53,54], suggesting the use of quantification methods for circulating endothelial cells to provide surrogate markers for the response to antiangiogenic and chemotherapeutic agents. Remarkably, although the contribution of endothelial progenitors has been found to be inconsistent, all of these reports demonstrated that proangiogenic hematopoietic cells can contribute to neovessel assembly.

The variability in the incorporation of marrow-derived cells may be due to variations in tumor growth rate and in the extent of tumor ischemia and vascular injury. Another reason for variations in the reported contribution of endothelial progenitors to neovasculature might be difficulty in identifying the true repopulating clonogenic EPCs or in effectively infecting them with lentiviral or retroviral vectors [55]. When transplantation studies are used to quantify the contribution of bone marrow derived cells to neoangiogenesis, another caveat may be the low number of transplanted true repopulating endothelial progenitors. In studies in which 27 million LacZ+ marrow cells were transplanted (i.e. a larger number of cells than usual), there was significantly more engraftment of endothelial progenitors into the marrow and incorporation into tumor neovessels [26,56]. Therefore, a minimal dose-limiting effect may play a role when it comes to the contribution of transplanted, marrow-derived, true repopulating endothelial progenitors with the capacity to form HPP-ECs (CFU-ECs) [45]. Most of the bone marrow vasculature itself after lethal irradiation and whole bone marrow transplantation is host derived in humans [57] and in mice (H.G. Kopp, unpublished data), regardless of the degree of hematopoietic engraftment. If the bone marrow vasculature is the origin of bone marrow derived EPCs, endothelial chimerism in the bone marrow will be the decisive determinant of the percentage of donor-derived endothelial cells in neovascular beds. The tracking method used may also influence the results of such studies: whereas GFP by itself could alter the differentiation of endothelial progenitors [58], β -galactosidase may be less toxic to EPCs. Emerging evidence also shows that the contribution of circulating endothelial cells is highest during early phases of transformation of dormant to rapidly growing tumors [26].

Collectively, these data suggest that under permissive conditions EPCs have the capacity to contribute luminally to the generation of neovessels of specific tumors that undergo rapid growth and these findings are corroborated in human sex-mismatched transplantation studies. The corecruitment of specific subsets of hematopoietic cells, however, may be essential for the proper incorporation of circulating and locally derived endothelial cells.

The role of comobilized hematopoietic cells in the regulation of tumor angiogenesis

Proangiogenic hematopoietic cells support angiogenesis both during embryonic development and postnatally by delivering angiogenic factors, including VEGFs, matrix metalloproteinases (MMPs), and angiopoietins to neovessels [59–65].

The physiological significance of recruitment of myeloid cells in the regulation of tumor growth is underscored in studies performed with MMP-9-deficient mice, who display a defect

in tumor growth [60]. Transplantation of wild-type MMP-9^{+/+} bone marrow resulted in enhanced tumor growth in MMP-9^{-/-} mice due to the delivery of MMP-9-producing hematopoietic cells to the tumor vasculature. Our group has shown that VEGFR1⁺ progenitors contribute to tumor angiogenesis and metastasis [26]. In addition, Gr1⁺CD11b⁺ cells seem to contribute to tumor neoangiogenesis by releasing MMP-9 [66]. Immune dendritic-like cells may also incorporate into the vessel wall and contribute to tumor neoangiogenesis [34]. Okamoto *et al.* [67•] suggest that c-kit-positive hematopoietic cells regulate the angiogenic switch in a tumor graft model, when they studied the localization of hematopoietic cells during tumor development. The authors found a tumor-specific distribution of c-kit positive cells either to the center or to the rim of the forming tumor. Anti-c-kit antibodies had antiangiogenic effects in all used tumor cell models, which included mouse colon26 colon cancer cells and human PC3 prostate adenocarcinoma [67•]. These data suggest that the corecruitment of hematopoietic cells conveys signals that support the incorporation and differentiation of endothelial cells into functional neovessels.

Activated VEGFR1⁺ myeloid cells can release angiogenic factors such as VEGF, platelet derived growth factor (PDGF) and brain-derived neurotrophic factor (BDNF), enhancing vessel formation and stability [60,68,69]. Therefore, VEGFR1⁺ myeloid cells that are comobilized with VEGFR2⁺ endothelial progenitors could support neovessel formation through a paracrine mechanism by releasing angiogenic factors. Interestingly, inhibition of either VEGFR1 or VEGFR2 signaling alone is insufficient to induce tumor regression and necrosis in animal models [26,59]. Inhibition of VEGFR2 signaling results in decreased vessel density and diffuse hemorrhage, while inhibition of VEGFR1 diminishes the number of perivascular cells consistent with mutually supporting roles for these two cell types. A combination of neutralizing mAbs to both VEGFR2 and VEGFR1, however, was sufficient to completely block tumor growth and induce tumor necrosis. These data support the notion that the incorporation of neoangiogenic endothelial cells into rapidly expanding tumors requires the corecruitment of VEGFR1⁺ hematopoietic cells to grant stability to the newly formed vessels.

Therefore, angiogenic factors not only support the mobilization of endothelial progenitors, but also induce the comobilization of hematopoietic cells to the tumor vasculature. This corecruitment of various lineages, including myelomonocytic cells and possibly hematopoietic stem and progenitor cells, may provide necessary signals for sprouting and stabilization of endothelial cells [33••,70••].

Angiogenic factors promote mobilization of VEGFR1⁺ hematopoietic cells through MMP-9-mediated release of soluble kit-ligand

The rapid mobilization of endothelial and hematopoietic progenitors has been observed as a sequel of vascular trauma [71] or rapid tumor growth. Vascular trauma results in VEGF-A elevation within hours of insult. Similarly, tumor implantation results in rapid mobilization of hemangiogenic cells to the peripheral circulation.

Stem and progenitor cells in the bone marrow are localized in a microenvironment known as the stem cell 'niche' [72–74]. These niches are critical for regulating the self-renewal and cell fate decisions, yet molecular mechanisms governing recruitment to exit these niches are not well studied. Under steady state conditions, most stem cells are maintained in the G₀ phase of the cell cycle [75]. The equilibrium between different compartments is dictated by the bioavailability of stem cell-active cytokines, which are bound to the extracellular matrix or tethered to the membrane of stromal cells, thereby creating haptotactic gradients [76]. Stress, such as marrow ablation by cytotoxic agents, switches on sequences of events by which stem and progenitors are recruited from their niches to reconstitute hematopoiesis.

MMPs promote the release of extracellular matrix-bound or cell-surface bound cytokines [77], such as VEGF-A, which then can regulate angiogenesis [78] or osteoclast recruitment [79]. Marrow suppression results in activation of MMP-9. Activated MMP-9 promotes the release of the stem cell-active cytokine, soluble Kit-ligand, (sKitL), thereby directing stem and progenitor cell recruitment and facilitating hematopoietic reconstitution [80].

Our group has shown that marrow suppression or release of VEGF-A or placental growth factor (PlGF) by tumors results in a timely upregulation of MMP-9 within the marrow microenvironment and release of sKitL. Increased bioactive sKitL promotes stem cell cycling and enhances their motility. These data suggest that the activation of a metalloproteinase serves as the decisive checkpoint for the rapid recruitment of hemangiogenic progenitor cells.

It remains to be determined whether alterations in the cytokine repertoire influence the differentiation status of progenitors prior to being launched to the peripheral circulation. Large numbers of progenitors are detected in the peripheral circulation after myelosuppression or plasma elevation of VEGF-A. This strongly suggests that mobilized hematopoietic and endothelial progenitors differentiate only during their sojourn in the peripheral circulation or once they have arrived at the tumor vasculature.

Angiogenic factors recruit hematopoietic stem and progenitor cells through VEGFR1 activation

Engagement of VEGFR2 leads to MMP-9 activation and mobilization of VEGFR2+ckit+ endothelial progenitors [80]. The molecular transducer that promotes mobilization of hematopoietic stem cells, however, is not known. Within the VEGF family of cytokines, VEGF-A is the most potent mediator of the angiogenic switch [81,82]. VEGF-A exerts its effect through interaction with two tyrosine kinase receptors, VEGF receptor 1 (VEGFR1, Flt-1) and VEGF receptor-2 (VEGFR2, Flk-1, KDR). PlGF, a member of the VEGF family, functions as an angiogenic amplifier by signaling through VEGFR1 [6]. Although VEGFR1 and VEGFR2 convey signals that regulate angiogenesis, their role in the regulation of hematopoiesis is not known. Mice deficient in VEGFR2 display profound defects in vasculogenesis and hematopoiesis [83–85]. Because of early lethality of embryos, it is difficult to assess whether VEGFR2 expression is absolutely essential for hematopoietic stem cell proliferation or migration during adulthood. Similarly, VEGFR1^{-/-} mice die from vascular disorganization in early embryogenesis, and therefore, the role of VEGFR1 in the regulation of hematopoiesis during late embryonic development has been difficult to evaluate [86,87]. In contrast, mice deficient in VEGFR1 kinase domain have no apparent hematopoietic disorder [88], but they display angiogenic defects [89]. It remains to be determined whether VEGFR1-kinase deficient mice possess a normal ability of hematopoietic regeneration after myelosuppression.

Several lines of evidence suggest that VEGFR1 may play an essential role in regulating specific aspects of adult hematopoiesis. Functional VEGFR1 has been shown to be expressed on mature myelomonocytic cells, conveying signals that support their migration *in vitro* [90–92]. Our group has previously shown that plasma elevation of VEGF-A in conjunction with angiopoietin-1 stimulates hematopoiesis, by mobilizing bone marrow-repopulating cells [93]. In *Drosophila*, VEGF-A supports the motility of hemocytes [94], which are ancestral homologues of murine and human hematopoietic cells. In conclusion, VEGFR1 expression on hematopoietic stem cells may regulate hematopoiesis by influencing motility and thereby recruitment. PlGF as a prototypical angiogenic factor can augment the motogenic and mitogenic potential of VEGFR1+ stem cells promoting hemangiogenic reconstitution. This conclusion has a dual clinical implication: while the blockade of VEGF-R1 may inhibit angiogenesis and tumor growth, it may also lead to unwanted myelosuppression. By contrast,

PlGF and VEGF-A have potential clinical applications for accelerating angiogenic or hematopoietic recovery.

The emerging role of stromal-derived factor 1 (CXCL-12) and CXCR-4

Stromal-derived factor 1 (SDF-1) was found to be involved in the attraction and differentiation of hematopoietic progenitors to sites of ischemia. In a model of hindlimb ischemia induced by femoral vessel ligation, SDF-1 plasma levels are upregulated with concomitantly decreased bone marrow levels immediately after surgery. In addition, ischemic tissues were shown to express increased amounts of SDF-1. The authors concluded that SDF-1 is not only chemoattractive to hematopoietic progenitor cells, but that it may also contribute to their differentiation into EPCs [95]. Ceradini *et al.* [96,97•] found a new mechanism by which hypoxia through HIF1 α stimulates the endothelial expression of SDF-1 and consecutively results in homing of CXCR4-positive progenitor cells to areas of hypoxia. In bone marrow vascular endothelial and stromal cells, Dar *et al.* [98••] described how CXCR4-positive endothelial cells can uptake, transcytose and secrete SDF-1 to the abluminal surface, thereby enhancing homing of CD34-positive hematopoietic progenitor cells. This function of CXCR4 as an ‘interceptor’ for SDF-1 was not found in hematopoietic cells themselves. The importance of SDF-1 and CXCR4 for human coronary heart disease (CAD) is underscored by the finding that EPCs from patients with CAD displayed impaired CXCR4 signaling compared with EPCs from healthy donors. In the same study, the authors used CXCR4^{+/-} mice for an ischemic hindlimb procedure, demonstrating severely impaired regeneration capacity [99•].

In the face of mounting evidence for SDF-1/CXCR-4 playing a role in revascularization, Grunewald *et al.* [100••] found SDF-1 expressed by perivascular myofibroblasts to provide a retention signal for VEGF-A mobilized hematopoietic progenitors [100••,101]. These results stress both the importance of SDF-1/CXCR-4 as a master chemokine signaling pathway in angiogenesis and the fact that neoangiogenic processes do not rely on single cytochemokines, but rather reflect the concerted action of different, comobilized cells, which are guided by different, coactive signaling pathways.

Conclusion

Recent findings underscore the functional importance of marrow-derived, VEGF-responsive VEGFR1+ hematopoietic cells in promoting neoangiogenesis and hematopoiesis. If the comobilization of hematopoietic and endothelial progenitors plays an essential clinical role in human tumor angiogenesis, these cells should provide effective targets to inhibit tumor growth. By contrast, since endothelial progenitors and hematopoietic progenitors are endowed with the capacity to home to tumor vasculature, this unique property may be exploited to deliver lethal doses of toxins to the tumor microenvironment. Two studies have shown that CD34+ hematopoietic cells [102] or GFP+Tie2+ cells [55] can be stably transfected with the thymidine kinase gene and selectively decrease the growth of xenotransplanted tumors.

Rigorous characterization of the phenotype of truly repopulating endothelial progenitors is absolutely necessary to draw any meaningful conclusions as to whether circulating EPCs contribute to neoangiogenesis. Most transplantation studies published to date have used either very few endothelial progenitors or their lentiviral/retroviral and promoter driven tracking has failed to detect repopulating EPCs. Most of so-called endothelial specific promoters – Tie2, Scl/Tal-1 – seem to track mature endothelial progenitors rather than repopulating endothelial progenitors. Therefore, determining the number of engrafted viable and functionally intact clonogenic GFP+ or LacZ+ endothelial progenitors capable of generating CFU-ECs and HPP-ECs is essential to formally determine the contribution of these cells to tumor neoangiogenesis or ischemic revascularization. Nonetheless, the extent of mobilization of hemangiogenic

progenitors may provide for a reliable and validated surrogate biomarker to evaluate the extent of neoangiogenesis. In addition, activation of VEGFR1 and VEGFR2 may promote ischemic revascularization, while inhibition of these two receptors may provide an effective means to block growth of hemangiogenesis dependent tumors.

Plasma elevation of VEGF-A not only promotes mobilization of mature hematopoietic cells but also progenitors and multi-potent hematopoietic cells. Proangiogenic VEGFR1+ cells not only comprise mature hematopoietic cells but also repopulating hematopoietic stem and progenitor cells. Although many studies have shown that mature monocytes/macrophages can contribute to neovessel formation, the physiological significance of recruitment of hematopoietic 'progenitors' and pluripotent cells with repopulating potential, such as VEGFR1 + cells, has only recently been studied. It remains to be determined, however, whether VEGFR1 + progenitors can differentiate locally within the neoangiogenic niche and thereby amplify the delivery of larger amounts and possibly a wider repertoire of proangiogenic factors. In addition, it is unclear and currently under intensive study whether the recruitment of VEGFR1+ progenitors can grant perivascular stabilization to larger neovessels.

Abbreviations

EPC	endothelial progenitor cell
GFP	green fluorescent protein
HPP-EC	high proliferative potential endothelial colony
MMP	matrix metalloproteinase
SDF-1	stromal-derived factor 1
VEGF	vascular endothelial growth factor

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 192–194).

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