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Role of endothelial progenitors and other bone marrow-derived cells in the development of the tumor vasculature

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Abstract

Increasing evidence suggests the importance of bone marrow-derived cells for blood vessel formation (neovascularization) in tumors, which can occur in two mechanisms: angiogenesis and vasculogenesis. Angiogenesis results from proliferation and sprouting of existing blood vessels close to the tumor, while vasculogenesis is believed to arise from recruitment of circulating cells, largely derived from the bone marrow, and de novo clonal formation of blood vessels from these cells. Although bone marrow-derived cells are crucial for neovascularization, current evidence suggests a promotional role of these cells on the existing blood vessels rather than de novo neovascularization in tumors. This is believed to be due to the highly proangiogenic features of these cells. The bone marrow-derived cells are heterogeneous, consisting of many different cell types including endothelial progenitor cells, myeloid cells, lymphocytes, and mesenchymal cells. These cells are highly orchestrated under the influence of the specific tumor microenvironment, which varies depending on the tumor type, thereby tightly regulating neovascularization in the tumors. In this review, we high-light some of the recent findings on each of these cell types by outlining some of the essential proangiogenic cytokines that these cells secrete to promote tumor angiogenesis and vasculogenesis.

Keywords

Angiogenesis; Bone marrow-derived cells; Endothelial progenitor cells; Lymphocytes; Monocytes; Macrophages; Myeloid cells; SDF-1; Vasculogenesis; VEGF

Endothelial progenitor cells and vasculogenesis

Endothelial progenitor cells (EPCs) are defined as precursor cells that have the ability to differentiate into mature endothelial cells when recruited to angiogenic sites. They are believed to arise from hemangioblasts, hematopoietic stem cells that are common precursors of EPCs [1]. Asahara and colleagues initially identified EPCs by isolating and culturing mononuclear blood cells from human peripheral blood using magnetic beads against CD34, an endothelial cell surface antigen [2]. In this study, EPCs were characterized by their spindle shape, incorporation of DiI-labeled acetylated low-density lipoprotein, expression of endothelial cell surface molecules such as CD31, Flk-1 (vascular endothelial growth factor receptor-2; VEGFR2), and Tie-2, and release of nitric oxide. The recent availability of transgenic mice where β-galactosidase (Lac-Z) or green fluorescent protein (GFP) are expressed either in endothelial cells only [3] or in all tissues [4] has led to improved understanding of the role of

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bone marrow-derived cells on blood vessel formation. Using these mouse models, numerous studies have reported that EPCs incorporate into the vasculature of solid tumor xenografts [5–7] and ischemic tissues [8–10].

However, the extent to which EPCs are incorporated into tumor vasculature has been controversial. While some reports have suggested that EPCs can comprise 50–100% of the tumor vasculature [6,11], the majority of studies, including ours, showed only rare or undetectable levels of EPCs in the tumor vasculature [12–17]. Table 1 summarizes some of the findings from these studies. The reason for this discrepancy is not clear, although, the characterization of the EPCs phenotype, different tumor models, and stages of tumor growth have been suggested as possibilities [12,18,19]. A recent study by Nolan and colleagues, for example, reported that EPCs are incorporated into the tumor vasculature at 20–35% efficiency on days 4–6 following tumor implantation, but then becomes diluted away by local non-bone marrow-derived endothelial cells resulting in only 1% of EPCs to be detected in the tumor vasculature after 4 weeks of growth [5]. Further studies are needed to address many questions on the fundamental characteristics of EPCs some of which include: whether EPCs lose proliferating abilities once recruited to the tumor vasculature despite their high self-renewal capabilities [2], whether the proliferating capabilities of EPCs are controlled by the tumor microenvironmental factors, whether local (non-bone marrow-derived) endothelial cells proliferate relatively faster than EPCs in tumors, and whether EPCs lose their identifying markers in daughter cells?

Despite the controversy regarding EPC incorporation into the tumor vasculature in animal models, a clinical study demonstrated the presence of EPC incorporation into the tumor vasculature of cancer patients, who had received gender-mismatched bone marrow transplantation [20]. The levels of EPCs were generally low (1–12%) and again dependent on the type of cancers (spindle cell sarcoma, Hodgkin's lymphoma, mucoepidermoid carcinoma, thyroid carcinoma, and osteogenic carcinoma) [20]. Recent clinical studies have also reported that EPCs are detected more frequently in the peripheral blood and in the tumors of patients with more invasive disease [21] or recurrence [22], indicating a possible involvement in tumor progression. Consistent with this, recent preclinical studies have shown that EPCs promote metastasis [23] and determine sensitivity of tumors to chemotherapeutic [24] or vascular disrupting agents [25].

Genetic mouse models have demonstrated that impaired EPC mobilization inhibits growth of some tumor models [6,26]. EPCs are proangiogenic secreting many angiogenic cytokines including VEGF [27,28], stromal-derived factor-1 (SDF-1) [27], angiopoietin-1 [7], angiopoietin-2 [8], and erythropoietin [28]. The proangiogenic activity of EPCs has been shown to be further enhanced by tumor cell- or stromal cell-secreted VEGF and placental growth factor (PlGF) in a paracrine manner [29]. However, further studies are needed to determine whether these effects are due to bona fide EPCs or via other populations of bone marrow-derived cells such as myeloid cells.

Myeloid cells and vasculogenesis

Myeloid cells are VEGFR1 positive hematopoietic cells that eventually give rise to monocytes, macrophages, and granulocytes/neutrophils. The importance of myeloid cells in promoting tumor growth was first underscored by a study by De Palma and colleagues, who showed that Tie2-expressing monocytes are recruited to perivascular regions of the tumor vasculature and that genetically targeting Tie2-expressing monocytes significantly inhibited tumor growth in mice [12]. Other investigators have reported similar findings that myeloid cells promote tumor angiogenesis [30] and progression [31,32].

Myeloid cells have features that are similar to EPCs in that they may express (under some circumstances) endothelial specific markers such as CD34, CD31, and VEGFR2 [33,34]. They are also functionally similar to EPCs or endothelial cells by integrating lectin and acetylated low-density lipoprotein [35]. In addition, myeloid cells are often observed in closely adjacent to blood vessels [12] and have been shown to improve blood flow in ischemic models [36, 37]. Although some studies have suggested that myeloid cells may differentiate into endothelial cells [38,39], this remains controversial and further studies are needed to define the precise relationship between myeloid and endothelial cells.

Many of the proangiogenic factors in myeloid cells that promote angiogenesis and vasculogenesis have been identified. For example, myeloid cells have shown to express matrix metalloprotease-9 (MMP-9) which induces the angiogenic switch by releasing VEGF from its membrane-bound form [40,41]. MMP-9 expressing myeloid cells have also been shown to be crucial for angiogenesis in multistep carcinogenesis of cervical [42,43] and pancreatic [44] mouse models. Another class of proteases, cathepsin cysteine protease has been reported to be expressed in myeloid cells regulating tumor growth, vascularity, and invasion [45]. A recent study by Shojaei and colleagues demonstrated that Bv8, prokineticin 2, is expressed in myeloid cells and this induces angiogenesis in tumors [46]. Further, this study showed that granulocyte colony-stimulating factor mobilizes myeloid cells and positively regulates the expression level of Bv8 in the myeloid cells [46]. Other recent studies indicate that Stat3 and its downstream effectors (such as VEGF, basic fibroblast growth factor, interleukin-1β, and MMP-9) [47] or semaphorin 4D, a proangiogenic ligand that interacts with plexin B1 receptor on endothelial cells [48], is involved in myeloid cell-mediated angiogenesis in tumors.

Myeloid cells are recruited to tumors and other angiogenic sites via VEGF and SDF-1 gradients. By using a mouse model in which VEGF levels could be conditionally modulated, Grunewald and colleagues have shown that activation of VEGF results in retention of the myeloid cells and other bone marrow-derived cells and that this is via VEGF-induced SDF-1 release from perivascular regions [49]. Similar findings have been reported by other investigators [50] including the fact that VEGF and SDF-1 can be controlled by their upstream regulator, hypoxia-inducible factor-1 (HIF-1) [10]. Recently, Du and colleagues have demonstrated that HIF-1 levels in tumors modulate the recruitment of these bone marrow-derived cells into glioblastomas, thereby regulating angiogenesis in these tumors [41].

Mast cells and platelets are also subpopulations of myeloid cells that have been shown to play an important role in tumor angiogenesis. Coussens and colleagues have shown that mast cells promote tumor progression in a mouse model of skin carcinogenesis by first infiltrating into the tumor, degranulating and activating dermal fibroblasts, and then by activating pro-MMP-9 (inactive form) to MMP-9 (active form) thereby promoting angiogenesis [51]. In this study, the authors showed that mast cells release chymase and tryptase, the mast cell-specific serine proteases [51]. Mast cells have also been shown to be a rich source of VEGF, basic fibroblast growth factor, and MMP-9 [52,53], and their functions are crucial for maintaining endothelial cell proliferation [53]. Platelets have been shown to be recruited to the activated endothelium providing VEGF [54,55] and SDF-1 [56]. Platelets have also been shown to release platelet-derived microvesicles to tumors, thereby stimulating expression of proangiogenic factors such as MMP-9, VEGF, and interleukin-8 in the tumor cells to promote angiogenesis [57].

We and others have shown that myeloid cells in the tumors are therapeutically important. We have recently shown that myeloid cells restore tumor re-growth after irradiation by supporting immature blood vessel development [17]. Shojaei and colleagues have demonstrated that these cells are responsible for resistance to anti-angiogenic therapy [58]. Other investigators have shown that these cells facilitate tumor metastasis [31,59,60], and there is strong clinical

evidence that the abundance of macrophage infiltration correlates with poor prognosis in breast, prostate, ovarian, and cervical cancers [61].

Thus, the myeloid cell appears to be an excellent target to inhibit tumor growth, angiogenesis, and metastasis. However, given the efficiency with which myeloid cells are rapidly replenished from stem/progenitor cells in the blood and bone marrow, it is difficult to target them efficiently from the blood stream. Despite this, we have obtained encouraging early results in potentiating the efficacy of cancer therapy by inhibiting myeloid cells by using antibodies against CD11b, a cell surface molecule expressed on monocytes and macrophages.

Lymphocytes and vasculogenesis

CD4 or CD8 lymphocytes are capable of rejecting tumors by immune surveillance [62]. However, some studies suggest that these lymphocytes are in certain cases associated with tumor promoting abilities. For example, Daniel and colleagues showed that infiltration of CD4 helper T cells enhance tumor progression in a skin cancer mouse model possibly by promoting the infiltration of MMP-9 producing myeloid cells [63]. In a similar multistep carcinogenesis mouse model, CD4 cells have also shown to promote angiogenesis and the progression of pancreatic tumors but only in the absence of tumor necrosis factor receptor-1 or interferon-γ (IFN-γ) signaling [64]. Although the evidence for a direct involvement of CD8 cytotoxic T cells in angiogenesis/vasculogenesis is rather weak, a recent study indicated that anti-angiogenic therapies disrupting the tumor vasculature can potentiate the immune response by increasing CD4 and CD8 lymphocytes and releasing IFN-γ in the tumor [65]. It has been suggested that the tumor microenvironment plays a crucial role in suppressing T cell activities which could in turn promote stability of the tumor vasculature [66].

Bone marrow-derived mesenchymal cells and vasculogenesis

Mesenchymal cells derived from the bone marrow are adherent stromal cells characterized by their ability to differentiate into mesenchymal tissues such as bone, cartilage, and fat [67]. Increasing evidence indicates that bone marrow-derived mesenchymal cells support tumor angiogenesis by providing a supportive role as carcinoma-associated fibroblasts [67,68] or perivascular mural cells [69]. These cells express α-smooth muscle actin [68], Tie-2 [70], and other pericyte markers [71]. Mesenchymal cells secrete SDF-1 [68] and VEGF [72] and are recruited to the sites expressing VEGF, platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) [72]. By exploiting this feature of being recruited to tumors, Studeny and colleagues showed that intravenous injection of human mesenchymal stem cells expressing interferon-β to MDA231 metastases bearing mice significantly prolonged the overall survival of the animals [73].

Conclusions

In this review, we have highlighted some of the recent findings on the role of bone marrow-derived cells and their proangiogenic cytokines for new blood vessel formation in tumors. Convincing evidence indicates a critical role played by bone marrow-derived cells in promoting tumor angiogenesis and progression. This suggests that solid tumors have the capability of modulating their tumor environment such that infiltrating immune cells favor, rather than inhibit tumor growth. Molecular mechanisms by which the bone marrow-derived cells promote tumor angiogenesis are not well-understood. This is largely because of the heterogeneity of the bone marrow-derived cells and the complex nature of these cells including the diverse interactions occurring among them. But, this is an exciting time for this field because of the development of powerful genetic mouse models as wells as the availability of specifically targeted therapies against each population of the bone marrow-derived cells. This will enable

future studies to dissect out the critical players promoting tumor angiogenesis as well as develop new anticancer strategies.

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References

1. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 2003;9:702–712. doi:10.1038/nm0603-702. [PubMed: 12778169]
2. Asahara T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–967. doi:10.1126/science.275.5302.964. [PubMed: 9020076]
3. Schlaeger TM, Qin Y, Fujiwara Y, Magram J, Sato TN. Vascular endothelial cell lineage-specific promoter in transgenic mice. Development 1995;121:1089–1098. [PubMed: 7743922]
4. Motoike T, et al. Universal GFP reporter for the study of vascular development. Genesis 2000;28:75–81. doi:10.1002/1526-968X(200010)28:2<75::AID-GENE50>3.0.CO;2-S. [PubMed: 11064424]
5. Nolan DJ, et al. Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. Genes Dev 2007;21:1546–1558. doi:10.1101/gad.436307. [PubMed: 17575055]
6. Lyden D, et al. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 2001;7:1194–1201. doi:10.1038/nm1101-1194. [PubMed: 11689883]
7. Asahara T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85:221–228. [PubMed: 10436164]
8. Takahashi T, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434–438. doi:10.1038/8462. [PubMed: 10202935]
9. Qin G, et al. Functional disruption of a4 integrin mobilizes bone marrow-derived endothelial progenitors and augments ischemic neovascularization. J Exp Med 2006;203:153–163. doi:10.1084/jem.20050459. [PubMed: 16401693]
10. Ceradini DJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–864. doi:10.1038/nm1075. [PubMed: 15235597]
11. Garcia-Barros M, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300:1155–1159. doi:10.1126/science.1082504. [PubMed: 12750523]
12. De Palma M, Venneri MA, Roca C, Naldini L. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. Nat Med 2003;9:789–795. doi:10.1038/nm871. [PubMed: 12740570]
13. Gothert JR, et al. Genetically tagging endothelial cells in vivo: bone marrow-derived cells do not contribute to tumor endothelium. Blood 2004;104:1769–1777. doi:10.1182/blood-2003-11-3952. [PubMed: 15187022]
14. Purhonen S, et al. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. Proc Natl Acad Sci USA 2008;105:6620–6625. doi:10.1073/pnas.0710516105. [PubMed: 18443294]
15. Shinde Patil VR, et al. Bone marrow-derived lin(−)c-kit(+) Sca-1+ stem cells do not contribute to vasculogenesis in Lewis lung carcinoma. Neoplasia 2005;7:234–240. doi:10.1593/neo.04523. [PubMed: 15799823]
16. Machein MR, Renninger S, de Lima-Hahn E, Plate KH. Minor contribution of bone marrow-derived endothelial progenitors to the vascularization of murine gliomas. Brain Pathol 2003;13:582–597. [PubMed: 14655762]

17. Ahn GO, Brown JM. Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. Cancer Cell 2008;13:193–205. doi:10.1016/j.ccr.2007.11.032. [PubMed: 18328424]
18. Kerbel RS, et al. Endothelial progenitor cells are cellular hubs essential for neoangiogenesis of certain aggressive adenocarcinomas and metastatic transition but not adenomas. Proc Natl Acad Sci USA 2008;105:E54. doi:10.1073/pnas.0804876105. [PubMed: 18715995]
19. Salven P, et al. EPCs are again claimed to be essential in yet other models despite the irreproducibility of the original experiments introducing them. Proc Natl Acad Sci USA 2008;105:E55. doi:10.1073/pnas.0805971105.
20. Peters BA, et al. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nat Med 2005;11:261–262. doi:10.1038/nm1200. [PubMed: 15723071]
21. Naik RP, et al. Circulating endothelial progenitor cells correlate to stage in patients with invasive breast cancer. Breast Cancer Res Treat 2008;107:133–138. doi:10.1007/s10549-007-9519-6. [PubMed: 18043899]
22. Igreja C, et al. Characterization and clinical relevance of circulating and biopsy-derived endothelial progenitor cells in lymphoma patients. Haematologica 2007;92:469–477. doi:10.3324/haematol.10723. [PubMed: 17488657]
23. Gao D, et al. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. Science 2008;319:195–198. doi:10.1126/science.1150224. [PubMed: 18187653]
24. Shaked Y, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for anti-angiogenic drugs as chemosensitizing agents. Cancer Cell 2008;14:263–273. doi:10.1016/j.ccr.2008.08.001. [PubMed: 18772115]
25. Shaked Y, et al. Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. Science 2006;313:1785–1787. doi:10.1126/science.1127592. [PubMed: 16990548]
26. Vidal A, et al. $p130^{Rb2}$ and $p27^{kip1}$ cooperate to control mobilization of angiogenic progenitors from the bone marrow. Proc Natl Acad Sci USA 2005;102:6890–6895. doi:10.1073/pnas.0405 823102. [PubMed: 15867156]
27. Yamazaki M, et al. Sonic hedgehog derived from human pancreatic cancer cells augments angiogenic function of endothelial progenitor cells. Cancer Sci 2008;99:1131–1138. doi:10.1111/j.1349-7006.2008.00795.x. [PubMed: 18422746]
28. Furstenberger G, et al. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary beast cancer. Br J Cancer 2006;94:524–531. doi:10.1038/sj.bjc.6602952. [PubMed: 16450002]
29. Li B, et al. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. FASEB J 2006;20:1495–1497. doi:10.1096/fj.05-5137fje. [PubMed: 16754748]
30. Yang L, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell 2004;6:409–421. doi:10.1016/j.ccr.2004.08.031. [PubMed: 15488763]
31. Yang L, et al. Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. Cancer Cell 2008;13:23–35. doi:10.1016/j.ccr.2007.12.004. [PubMed: 18167337]
32. Lin EA, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res 2006;66:11238–11246. doi:10.1158/0008-5472.CAN-06-1278. [PubMed: 17114237]
33. Rohde E, et al. Blood monocytes mimic endothelial progenitor cells. Stem Cells 2006;24:357–367. doi:10.1634/stemcells.2005-0072. [PubMed: 16141361]
34. Dineen SP, et al. Vascular endothelial growth factor receptor 2 mediates macrophage infiltration into orthotopic pancreatic tumors in mice. Cancer Res 2008;68:4340–4346. doi:10.1158/0008-5472.CAN-07-6705. [PubMed: 18519694]
35. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? Nat Rev Cancer 2002;2:826–835. doi:10.1038/nrc925. [PubMed: 12415253]
36. Fujiyama S, et al. Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial

progenitor cells. Circ Res 2003;93:980–989. doi: 10.1161/01.RES.0000099245.08637.CE. [PubMed: 14525810]

37. Luttun A, et al. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis, and atherosclerosis by anti-Flt1. Nat Med 2002;8:831–840. [PubMed: 12091877]
38. Bailey AS, et al. Myeloid lineage progenitors give rise to vascular endothelium. Proc Natl Acad Sci USA 2006;103:13156–13161. doi:10.1073/pnas.0604203103. [PubMed: 16920790]
39. Li B, et al. Low levels of tumor necrosis factor a increase tumor growth by inducing an endothelial phenotype of monocytes recruited to the tumor site. Cancer Res 2009;69:338–348. doi: 10.1158/0008-5472.CAN-08-1565. [PubMed: 19118019]
40. Bergers G, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000;2:737–744. doi:10.1038/35036374. [PubMed: 11025665]
41. Du R, et al. HIF1a induces the recruitment of bone marrow-derived vascularmodulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 2008;13:206–220. doi:10.1016/j.ccr.2008.01.034. [PubMed: 18328425]
42. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contribute to skin carcinogenesis. Cell 2000;103:481–490. doi:10.1016/S0092-8674(00)00139-2. [PubMed: 11081634]
43. Giraudo E, Inoue M, Hanahan D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 2004;114:623–633. [PubMed: 15343380]
44. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci USA 2006;105:12493–12498. doi: 10.1073/pnas.0601807103. [PubMed: 16891410]
45. Joyce JA, et al. Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. Cancer Cell 2004;5:443–453. doi:10.1016/S1535-6108(04)00111-4. [PubMed: 15144952]
46. Shojaei F, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature 2007;450:825–831. doi:10.1038/nature06348. [PubMed: 18064003]
47. Kujawski M, et al. Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. J Clin Invest 2008;118:3367–3377. doi: 10.1172/JCI35213. [PubMed: 18776941]
48. Sierra JR, et al. Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages. J Exp Med 2008;205:1673–1685. doi:10.1084/jem.20072602. [PubMed: 18559453]
49. Grunewald M, et al. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell 2006;124:175–189. doi:10.1016/j.cell.2005.10.036. [PubMed: 16413490]
50. Aghi M, Cohen KS, Klein RJ, Scadden DT, Chiocca EA. Tumor stromal-derived factor-1 recruits vascular progenitors to mitotic neovasculature, where microenvironment influences their differentiated phenotypes. Cancer Res 2006;66:9054–9064. doi: 10.1158/0008-5472.CAN-05-3759. [PubMed: 16982747]
51. Coussens LM, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 1999;13:1382–1397. doi:10.1101/gad.13.11.1382. [PubMed: 10364156]
52. Heissig B, et al. Low-dose irradiation promotes tissue revascularization through VEGF release from mast cells and MMP-9 mediated progenitor cell mobilization. J Exp Med 2005;202:739–750. doi: 10.1084/jem.20050959. [PubMed: 16157686]
53. Soucek L, et al. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. Nat Med 2007;13:1211–1218. doi:10.1038/nm1649. [PubMed: 17906636]
54. Salgado R, et al. Platelets and vascular endothelial growth factor (VEGF): a morphological and functional study. Angiogenesis 2001;4:37–43. doi:10.1023/A:1016611230747. [PubMed: 11824377]
55. Wartiovaara U, et al. Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation. Thromb Haemost 1998;80:171–175. [PubMed: 9684805]

56. Rafii S, Psaila B, Butler J, Jin DK, Lyden D. Regulation of vasculogenesis by platelet-mediated recruitment of bone marrow-derived cells. Arterioscler Thromb Vasc Biol 2008;28:217–222. doi: 10.1161/ATVBAHA.107.151159. [PubMed: 18096826]
57. Janowska-Wieczorek A, et al. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. Int J Cancer 2005;113:752–760. doi:10.1002/ijc.20657. [PubMed: 15499615]
58. Shojaei F, et al. Tumor refractoriness to anti-VEGF treatment is mediated by $CD11b^{+}Gr1^{+}$ myeloid cells. Nat Biotechnol 2007;25:911–920. doi:10.1038/nbt1323. [PubMed: 17664940]
59. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. Nat Cell Biol 2006;8:1369–1375. doi:10.1038/ncb1507. [PubMed: 17128264]
60. Kaplan RN, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005;438:820–827. doi:10.1038/nature04186. [PubMed: 16341007]
61. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 2004;4:71–78. doi: 10.1038/nrc1256. [PubMed: 14708027]
62. Koebel CM, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007;450:903–908. doi:10.1038/nature06309. [PubMed: 18026089]
63. Daniel D, et al. Immune enhancement of skin carcinogenesis by CD4+ T cells. J Exp Med 2003;197:1017–1028. doi:10.1084/jem.20021047. [PubMed: 12695493]
64. Muller-Hermelink N, et al. TNFR1 signaling and IFN-g signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. Cancer Cell 2008;13:507–518. doi:10.1016/j.ccr.2008.04.001. [PubMed: 18538734]
65. Manning EA, et al. A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. Clin Cancer Res 2007;13:3951–3959. doi: 10.1158/1078-0432.CCR-07-0374. [PubMed: 17606729]
66. Ganss R, Hanahan D. Tumor microenvironment can restrict the effectiveness of activated antitumor lymphocytes. Cancer Res 1998;58:4673–4681. [PubMed: 9788621]
67. Haniffa MA, Collin MP, Buckley CD, Dazzi F. Mesenchymal stem cells: the fibroblasts' new clothes? Haematologica 2009;94(2):258–263. [PubMed: 19109217]
68. Mishira PJ, et al. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res 2008;68:4331–4339. doi:10.1158/0008-5472.CAN-08-0943. [PubMed: 18519693]
69. Au P, Tam J, Fukumura D, Jain RK. Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. Blood 2008;111:4551–4558. doi:10.1182/blood-2007-10-118273. [PubMed: 18256324]
70. de Palma M, et al. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 2005;8:211–226. doi:10.1016/j.ccr.2005.08.002. [PubMed: 16169466]
71. Bexell D, et al. Bone marrow multipotent mesenchymal stromal cells act as pericyte-like migratory vesicles in experimental gliomas. Mol Ther 2009;17:183–190. doi:10.1038/mt.2008.229. [PubMed: 18985030]
72. Beckermann BM, et al. VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br J Cancer 2008;99:622–631. doi:10.1038/sj.bjc.6604508. [PubMed: 18665180]
73. Studeny M, et al. Mesenchymal stem cells: potential precursors for tumor stromal and targeted-delivery vehicles for anticancer agents. J Natl Cancer Inst 2004;96:1593–1603. [PubMed: 15523088]
74. Capillo M, et al. Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. Clin Cancer Res 2003;9:377–382. [PubMed: 12538491]
75. Suriano R, et al. 17Beta-estradiol mobilizes bone marrow-derived endothelial progenitor cells to tumors. Cancer Res 2008;68:6038–6042. doi:10.1158/0008-5472.CAN-08-1009. [PubMed: 18676823]

Table 1

Summary of studies showing the extent of EPCs incorporation to the tumor vasculature

EPC incorporation (%)	Type of tumor	BM donor	Markers used	Reference
<0.01	B16 melanoma	VEGFR-2/Lac-Z	Lac-Z, CD31, CD105, von Willebrand factor	[14]
<0.01		GFP	GFP, CD31	[17]
80–100	B6RV2 lymphoma	Lac-Z	Von Willebrand factor, Lac-Z	[6]
<0.01		End-SCL-Cre-ER^t	Lac-Z, CD31	[13]
<0.01	Lewis lung carcinoma	End-SCL-Cre-ER^t	Lac-Z, CD31	[13]
<0.01		GFP	GFP, CD31	[17]
0.01–0.15		GFP	GFP, CD31	[24]
0.02		Tie-2/GFP	GFP, CD31	[12]
1–2		GFP	Lin$^-$c-kit$^+$Sca-1$^+$, GFP, CD31	[15]
50–100		Lac-Z	Von Willebrand factor, Lac-Z	[6]
50	MCA/129 fibrosarcoma	Lac-Z	Von Willebrand factor, Lac-Z	[11]
7–10	MMTV-PyMT spontaneous mammary carcinoma	GFP	CD31, VE-cadherin, GFP	[5]
2.5–3.5	MT1A2 mouse mammary carcinoma	Tie2/Lac-Z	Lac-Z, CD31	[17]
7–10	Namalwa lymphoma	Human BM	Human CD31, CD34, CD133	[74]
10–25	Spontaneous tumor (NOD/SCID/β-glucuronidase $^{-/-}$)	NOD/SCID/β-glucuronidase	β-Glucuronidase, VE-cadherin	[29]
0.5	TG1-1 mouse mammary carcinoma	GFP	GFP, CD133	[75]
<0.01		GFP	GFP, CD31	[17]

The selected studies all used bone marrow transplantation followed at least 4 weeks later by tumor implantation. Models of parabiosis or ex vivo injection of EPCs were not included