Tumor stroma fosters neovascularization by recruitment of progenitor cells into the tumor bed

Ruth Ganss *

Laboratory for Cancer Medicine, Western Australian Institute for Medical Research, Centre for Medical Research, University of Western Australia, WA, Australia

Received: April 27, 2006; Accepted: July 20, 2006

- **Introduction**
 - Tumor angiogenesis revisited
 - A critical role for pericytes in blood vessel maturation
 - Fibroblasts as regulators of angiogenic activity
 - Inflammation-induced angiogenesis
- **Distal origin of stromal cells**
 - Contribution of endothelial progenitors to tumor neovascularization
- **Multistep endothelial progenitor recruitment during tumor-induced angiogenesis**
- **Recruitment of perivascular cells from the bone marrow**
- **Proangiogenic bone marrow-derived mononuclear cells within the tumor bed**
- **The "endothelial capacity"**
- **Tumor therapy *via* progenitor cells**
- **Concluding remarks**

Abstract

The tumor stroma is an active player during carcinogenesis and contains a variety of cell types such as vascular cells, fibroblasts and inflammatory cells which directly or indirectly foster neovascularization. During tumor progression stromal cells, in particular the neovasculature, acquire new characteristics distinct from their normal counterparts and display a high degree of plasticity to meet the tumor's demands. The local environment may, to some extent, shape pre-existing, tumor-resident stromal cells. However, there is accumulating evidence that new endothelial and other stromal cells are actively recruited into tumors, and that this recruitment is essential for a unique and tumor-specific proangiogenic environment.

Keywords: tumor stroma • angiogenesis • vasculogenesis • endothelial cells • pericytes • myofibroblasts • bone marrow-derived progenitor cells

Introduction

Cancer is not just an accumulation of tumor cells. Tumors depend on a supporting environment to thrive. Malignant cells are in close association with vascular cells, fibroblasts, infiltrating immune cells,

and extracellular matrix components, collectively termed the tumor stroma. Stromal cells produce a variety of growth factors, chemokines, collagens and matrix-degrading enzymes that provide a three

* Correspondence to: Ruth GANSS, PhD
Western Australian Institute for Medical Research, Level 5,
MRF Building Rear, 50 Murray St., Perth WA 6000, Australia.

Tel.: +61-8-9224 0354
Fax: +61-8-9224 0322
E-mail: ganss@waimr.uwa.edu.au

dimensional tumor scaffold, enhance cancer cell proliferation, and promote the invasive capacity of tumors. The single most studied component of the tumor stroma is endothelial cells and the blood vessels they form. It was recognized more than 30 years ago that the growth of new blood vessels within tumors, a process termed angiogenesis, is a prerequisite for tumour expansion and might well be the “Achilles” heel of cancer [1]. Studies on human tumor biopsies have been mainly performed on advanced cancer, hence our knowledge is biased towards late-stage tumors. However, using mouse models of multistep tumorigenesis we are able to gain insights into the complex processes which are part of a tumor’s progression from a premalignant stage into a solid cancer with a distinct, aberrant microenvironment. By doing so, we are beginning to discover that the formation of a tumor-supporting stroma is not just an adaptation to the metabolic needs of the tumor, *i.e.* oxygen and nutrients. Instead, we encounter a fine-tuned program, in which tumor and stromal cells regulate each other and show an astonishing plasticity. Together tumor and stromal cells serve a common goal, to foster tumor growth.

Tumor angiogenesis revisited

Tumor-induced angiogenesis was originally defined as the proliferation of endothelial cells and sprouting of new blood vessels from pre-existing vessels. Pioneering work by Judah Folkman and Douglas Hanahan has demonstrated that the onset of blood vessel formation, the “angiogenic switch”, is a distinct and integral stage of tumour growth which can occur surprisingly early during tumor progression [2, 3]. The first signs of angiogenic activity in pre-neoplastic lesions are vessel dilatation, which precedes a stage of increasing heterogeneity in vessel diameter and distribution. The vasculature of late-stage tumors eventually loses all hierarchical features and becomes chaotic and tortuous [4, 5].

Angiogenesis is orchestrated by a variety of stimulatory and inhibitory growth factors, and the list of potential regulators continues to grow [3, 6]. While vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the best known inducers of vascular growth, it is evident that they are not the sole players. Angiogenic regulators have largely been studied using *in vitro* model systems that

mimic vessel formation under defined conditions and simply by assessing endothelial cell proliferation. However, such studies do not recapitulate the spatial and temporal formation of a tumor-induced vascular network. Furthermore, angiogenesis research has focused for decades on endothelial cells as the major constituents of the vasculature and tumour cells as the main source for growth-stimulating factors. It is now appreciated that angiogenesis represents a more complex morphogenetic process, during which the whole vascular structure of the tumor stroma is remodeled. Moreover, non-neoplastic tumor stromal cells secrete factors capable of stimulating vessel sprouting and perpetuating the angiogenic process.

A critical role for pericytes in blood vessel maturation

Neovascularization is tightly regulated during embryogenesis and during a variety of biological processes in adults. In response to angiogenic factors, pre-existing vessels become dilated and supporting cells such as smooth muscle cells and pericytes detach from the vessel wall. Endothelial cells then migrate into the perivascular space by degrading basement membrane and extracellular matrix, multiply and form new vascular sprouts. Under normal circumstances, this transient proliferation phase is followed by the establishment of a stable, hierarchical vascular network. Pericytes are again recruited to the vasculature in response to growth factors such as platelet derived growth factor B (PDGF-B); these pericytes reduce endothelial cell proliferation and stabilize the newly formed vasculature [7].

This self-limiting process of vascular growth is profoundly disturbed during tumor angiogenesis, leading to a chaotic, highly aberrant vasculature. Whereas abnormalities in the endothelial cell compartment are well documented [4, 5, 8], only limited studies have so far examined pericytes within tumors. However, there is increasing evidence that pericytes, characterized as α -smooth muscle actin-, desmin-, NG2 proteoglycan- or PDGFR β -positive cell populations, play an important role during tumor growth [9]. Tumor pericytes are described as being loosely associated with the endothelium, having cell processes that extend away from the vessel wall, and in contrast to their normal counterparts, being sensitive to PDGF signaling [10,

11]. The importance of pericytes for ongoing angiogenesis has recently been documented in an experimental system in which selective ablation of PDGF-B signaling reduced tumor angiogenesis and substantially improved the efficacy of anti-VEGFR treatment [12]. Thus, similar to endothelial cells, intratumoral pericytes are clearly distinct from the mural cells of normal tissue [13]. This observation is consistent with reports describing pericytes as a pluripotent cell population which can change appearance upon cytokine activation [14].

Fibroblasts as regulators of angiogenic activity

Vessel sprouting and maturation are finely tuned by growth factors and physical contact with adjacent cells. Fibroblasts represent a prominent stromal cell type which secretes large amounts of growth factors and extracellular matrix proteins and which actively participate in tumor formation [15]. In addition, during tissue stress and tumor growth, fibroblast differentiation towards an α -smooth muscle actin-positive contractile myofibroblast phenotype is frequently observed. *In vitro*, myofibroblasts have been shown to enable invasion of endothelial cells into tumor cell clusters and to regulate the formation of three-dimensional vessel-like structures [17, 18]. TGF β , an abundant growth factor within the tumor microenvironment, may trigger transformation of fibroblasts into myofibroblasts and subsequently into smooth muscle cells or pericytes which, in turn, support angiogenesis [14, 16]. However, *in vivo*, these events are not well documented, in part because of striking heterogeneity in the expression of marker proteins between different tumors and within individual tumors [19]. Therefore, it is not fully understood whether smooth muscle cells within solid tumors described as activated, abnormal or immature result from fibroblast phenotype switching or arise from a common mesenchymal precursor cell.

Inflammation-induced angiogenesis

Upon activation inflammatory cells like monocytes, macrophages, neutrophils, mast cells and leukocytes release pro-angiogenic cytokines like VEGF and CXC chemokines that stimulate endothelial cell prolifera-

tion and motility. There is ample evidence these immune cells are recruited into the tumor stroma. Within the tumor stroma, the local cytokine milieu blocks potential immunological anti-tumor functions and instead modifies their phenotype to promote tumor progression [20, 21]. This also involves secretion of factors which promote proliferation and migration of endothelial cells, matrix remodeling and the formation of stabilized vessels. Increased numbers of tumor-associated macrophages, for instance, promote tumor malignancy, and the number of these cells within tumors is significantly correlated with angiogenic activity and poor prognosis in cancer patients [22].

Distal origin of stromal cells

The tumor shapes its own stroma and, in turn, diverse stromal cell types contribute to tumor neovascularization in a tightly regulated fashion. Precursor cells may reside in the local tumor environment and display a high degree of plasticity with the potential to transdifferentiate into various cell types [23, 24]. However, establishment of the tumor stroma is a less localized phenomenon than previously believed. There is increasing evidence from the literature that bone marrow-derived precursor cells populate the newly established stroma and contribute directly or indirectly to neovascularization. This process of postnatal vasculogenesis differs from classical angiogenesis, in which sprouts arise from endothelial cells of pre-existing vessels. There is still debate as to the extent to which bone marrow-derived vascular precursor cells contribute to tumor progression and their function within the tumor environment. Although there is little evidence for bone marrow contribution to steady-state tissue regeneration [25], it is well established that circulating endothelial precursor cells contribute to neovessels after wounding and ischemic injury [26–28].

Endothelial progenitors contribute to tumor neovascularization

Recent evidence strongly suggests that growth factors like VEGF that are secreted by tumors not only act locally but also promote mobilization of circulating endothelial progenitor cells from the bone marrow.

Fig. 1 Tumor progression in AlbTag mice involves incorporation of bone marrow-derived cells into the vasculature of late-stage tumors. (A) In AlbTag mice SV40 Large T antigen is expressed in hepatocytes under the control of the albumin promoter and tumors develop through hyperplasia, dysplasia, and eventually hepatocellular carcinoma at the age of 14–16 weeks. Endothelial cells were isolated from cancerous tissue of EGFP bone marrow chimeric AlbTag mice at different stages during tumor progression and the frequency of EGFP⁺ "green" endothelial cells quantified by FACS analyses [34]. (B) Tumor vessels of a 16 week-old AlbTag mouse, reconstituted with bone marrow from an EGFP reporter mouse at the age of 4 weeks. Incorporation of EGFP⁺ "green" bone marrow-derived cells into lectin-perfused (red) vessels is shown. Scale bar = 20 μ m.

These progenitor cells are released into the peripheral and circulation home into the vascular bed, where they contribute to the vascular network [29]. Incorporation of marrow-derived precursor cells into neovessels was first documented in bone marrow chimeric mice with subcutaneously implanted tumors. In these studies, bone marrow-derived endothelial cells could be distinguished from tumor-resident vessels due to the expression of a marker gene, usually β -galactosidase or green fluorescent protein. The studies revealed an estimated precursor contribution ranging from approximately 50 to 90%

[30, 31] (Table 1). Integration of progenitor cells into the vasculature of spontaneously growing tumors has been documented, however, with a greater variability (0 to 40%) [32–34]. Variable involvement of bone marrow-derived endothelial cells in these models of de novo tumor growth clearly reflects different tumor types and grades. Interestingly, in a spontaneous tumor model in which progenitor integration into the vasculature could be monitored over time, only late-stage cancers relied on stem cell incorporation into tumor vasculature, possibly due to massive expansion of tumor mass and increasing metabolic demands

Table 1 Progenitor cell frequency in tumor stroma

Tumor type	Stromal compartment	Frequency [%]	Reference
T: fibrosarcoma/melanoma	neovessels	50	[31]
T: lymphoma lewis lung carcinoma	neovessels	95	[30]
	neovessels	50	
	and VEGFR1 ⁺ mononuclear cells in vicinity to neovessels	nd	
T: mammary carcinoma/ lewis lung carcinoma/ melanoma	neovessels	0	[42]
	Tie2 expressing mononuclear cells in vicinity to neovessels	nd	
S: prostate adenocarcinoma (TRAMP): poorly differentiated well differentiated	neovessels	14	[33]
	neovessels	0	
S: lymph hyperplasia (Pten +/-) uterine carcinoma (Pten +/-)	neovessels	0	[32]
	neovessels	16	
	and VEGFR1 ⁺ mononuclear cells in vicinity to neovessels	nd	
S: hepatocellular carcinoma (AlbTag) early tumor nodules advanced tumor nodules	neovessels	6	[34]
	neovessels	27	
S: insulinoma (RIPTag) early lesions advanced tumors	neovessels	0	[34]
	neovessels	40	
S: insulinoma (RIPTag)	pericytes	> 1	[40]
S: insulinoma (RIPTag)	myofibroblasts	25	[41]
H: lymphoma/carcinoma/sarcoma	neovessels	5	[35]

T: murine transplantation tumors; S: murine spontaneous tumor models; H: human tumors, nd: frequency not determined.

[34] (Fig. 1). To date, there are only few quantitative studies in humans addressing the integration of bone marrow-derived cells into the vascular network. Compared to murine tumor studies, numbers seem to be lower. Peters *et al.* for instance have documented an average contribution to tumor endothelium of 5% stem cells in bone marrow transplanted patients using sex chromosome-specific probes [35].

Multistep nature of endothelial progenitor recruitment during tumor-induced angiogenesis

The release of VEGFR2⁺ endothelial precursors along with VEGFR1⁺ hematopoietic stem cells from

the bone marrow is a complex, multifactorial process, requiring pro-angiogenic factors like VEGF and matrix metalloproteinase-9 (MMP-9). As elegantly shown by Heissig *et al.*, MMP-9 promotes release of soluble cKitL which in turn stimulates cell proliferation and motility bone of marrow-derived cells [36]. Mobilized circulating endothelial progenitors must eventually find their way to sites of angiogenesis. So far, mechanisms of homing and integration into the vessel wall are largely unknown. It was recently demonstrated, in a model of spontaneous liver carcinogenesis, that tumor-resident endothelial cells secrete a variety of CC chemokines which attract CCR⁺ endothelial progenitors into the tumor bed. In this novel mechanism endothelial cells, as components of the tumor-induced stroma, enhance

neovascularization in a self-amplifying loop, rather than tumor cells [34, 37].

Intriguingly, tumor-activated fibroblasts in human breast carcinomas secrete stromal cell-derived factor 1 (SDF-1), which promotes angiogenesis by recruiting SDF1 receptor-positive (CXCR4⁺) endothelial progenitors into the tumor [24]. Again, these data demonstrate that the tumor stroma acquires distinct characteristics which lead to the recruitment of bone marrow-derived precursors to amplify the angiogenic process. Once at the tumor site, endothelial progenitors form cell clusters, bridge pre-existing vessels, and integrate into the vascular network. Intravital studies on vascular integration of embryonic endothelial precursors and *in vivo* blocking experiments have provided evidence that initial progenitor cell arrest is mediated by E- and P-selectin [38]. Thus integration itself appears to be a multistep process, which remains largely uncharacterized.

Recruitment of peri-vascular cells from the bone marrow

Few studies on the role of bone marrow-derived precursor cells in tumor-induced angiogenesis have focused on the perivascular compartment. However, there is an increasing number of reports describing the existence of bone marrow-derived, pericyte-like cells within the tumor stroma (Table 1). For instance, in a melanoma model, angiogenesis recruited periendothelial cells that shared expression of hematopoietic markers like CD11b and CD45 but also expressed the pericyte-specific NG2 proteoglycan [39]. In a spontaneous tumor model, a subset of PDGFR β ⁺ perivascular progenitors was found in the tumor bed; specific ablation of these cells resulted in endothelial cell death and severe impairment of angiogenesis [40]. Again, this demonstrates the existence of immature bone marrow-derived pericytes that are crucial for tumor neovascularization. In a model of pancreatic islet cell carcinoma, a considerable number of myofibroblasts and fibroblasts originating from bone marrow were found embedded in tumor stroma [41]. This finding suggests that tumor-induced myofibroblasts described in other models may also be recruited from the bone marrow [24]. To date, the precise origin of myofibroblasts and pericytes, the molecules involved in their directed migration, and their fate within tumors remain unknown.

Proangiogenic bone marrow-derived mononuclear cells within the tumor bed

The contribution of bone marrow cells to angiogenesis remains a controversial issue. While many reports provide precise numbers and locations of genetically engineered bone marrow cells within the tumor bed, others fail to identify bone marrow-derived cells among intratumoral endothelial and smooth muscle cells [42–44]. Interestingly, however, there is mounting evidence that a population of mononuclear cells may directly or indirectly control tumor-induced angiogenesis. Lyden *et al.* first described a population of VEGFR1-positive hematopoietic cells that are co-mobilized with VEGFR2-positive endothelial precursors and are critical for tumor angiogenesis; they appear to confer stability to neovessels [30]. De Palma *et al.* have identified Tie2-expressing mononuclear (TEM) cells of hematopoietic origin which selectively home to angiogenic sites and support neovascularization *via* paracrine effects [23, 42]. Although TEMs and VEGFR1⁺ bone marrow-derived cells share features of proangiogenic monocytes, their exact relation to each other is unknown. Besides supplying growth factors to vascular cells monocytes/macrophages may play a more profound role during tumor angiogenesis. Recently, it was shown that an intimate association of monocytes/macrophages with progenitor cells is required for the formation of new capillary-like structures [45]. Moreover, monocytes/macrophages themselves may possess the potential to develop an endothelial phenotype upon angiogenic stimulation [46].

The “endothelial capacity”

It is well documented that bone marrow-derived circulating cells have the capacity to migrate into tumors and contribute to neovascularization. The precise nature of bone marrow-derived stromal cell subsets in tumors, their relationship to each other and their functional contribution to neovessels, however, remains puzzling. Explanations for the differences in findings among studies include variety of tumor types examined, variations in experimental setup, and limited resolution of vascular components when using immunohistochemistry. However, these differences may also reflect an oversimplified appreciation of the complex regulatory systems. The tumor environment most likely determines what cell

types are recruited from the periphery and when this recruitment occurs [24, 34]. During multistage tumor progression, stromal components are modified to acquire new tumor-specific characteristics which, in turn, influence release of growth factors and recruitment of progenitor cells. Hence, progenitor cells found in tumor stroma may vary depending on tumor type, location and stage. Moreover, there is accumulating *in vitro* evidence that bone marrow-derived mononuclear cells can transdifferentiate into cells of various lineages [47–49]. In addition, cells with mixed phenotypes, displaying vascular endothelial cell and leukocyte markers, exist within tumors and have the capacity to generate functional blood vessels *in vivo* [50]. Although speculative at this stage, a possible scenario is that pluripotent circulating progenitors are recruited into the tumor bed, associate with the vasculature, and provide angiogenic factors, but also retain the capability to transdifferentiate into endothelial cells, myofibroblasts, or pericytes depending on tumor conditions at a specific time during its progression.

Tumor therapy *via* progenitor cells

The homing of progenitor cells to tumor neovasculature suggests new ways to block a tumor's blood supply. This might be achieved by interfering with the homing of hematopoietic stem cells and endothelial progenitors as described by Lyden *et al.* [30]. Alternatively, hematopoietic stem cells or embryonic endothelial progenitor cells could be genetically modified to deliver suicide genes or angiogenesis inhibitors and used as cellular vehicles for tumor targeting [42, 51, 52]. These potential strategies have been applied to transplantation models and, to some extent have delayed tumor growth. Given the variability in progenitor numbers found in different tumor models and the selective accumulation of these progenitor cells in advanced tumor stages [34], targeting vasculogenesis may represent only one aspect of anti-cancer therapy, and anti-vasculopenic therapy likely needs to be combined with other treatment modalities, for effective cancer therapy. However, assessing circulating progenitor cell frequencies as a diagnostic marker in cancer patients is an attractive concept and awaits evaluation in the clinic [53].

Concluding remarks

The crucial role of the stroma during malignant progression is undeniable. Moreover, tumor stroma is critically altered during tumorigenesis and the tumor itself generates a self-supporting environment [54, 55]. Emerging evidence, however, shows that stroma not only passively responds to tumor signals, but also drives proliferation and angiogenesis in a self-perpetuating fashion [24, 34]. Moreover, this active role of the tumor stroma involves the recruitment of a diverse range of circulating progenitor cells into the tumor bed. The plasticity and transdifferentiation potential of these progenitor cells within the tumor environment is largely unknown. Part of the complexity results from poor definition of circulating stem cells and variable surface marker expression. To understand the relative contribution of diverse bone marrow cells *in vivo* will be a major step forward in modulating the neovasculature and the pathological stromal environment.

Acknowledgement

Herbert Spring is acknowledged for providing confocal microscopy pictures of AlbTag tumor vessels.

References

1. **Folkman J.** Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* 1972; 175: 409–16.
2. **Folkman J, Watson K, Ingber D, Hanahan D.** Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989; 339: 58–61.
3. **Hanahan D, Folkman J.** Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; 86: 353–64.
4. **Ryschich E, Schmidt J, Hammerling GJ, Klar E, Ganss R.** Transformation of the microvascular system during multistage tumorigenesis. *Int J Cancer* 2002; 97: 719–25.
5. **Ryschich E, Lizdenis P, Itrich C, Benner A, Stahl S, Hamann A, Schmidt J, Knolle P, Arnold B, Hammerling GJ, Ganss R.** Molecular fingerprinting and autocrine growth regulation of endothelial cells in a murine model of hepatocellular carcinoma. *Cancer Res* 2006; 66: 198–211.
6. **Bergers G, Benjamin LE.** Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; 3: 401–10.

7. **Jain RK.** Molecular regulation of vessel maturation. *Nat Med.* 2003; 9: 685–93.
8. **Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM.** Openings between defective endothelial cells explain tumor vessel leakiness. *Am J Pathol.* 2000; 156: 1363–80.
9. **Abramsson A, Lindblom P, Betsholtz C.** Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J Clin Invest.* 2003; 112: 1142–51.
10. **Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM.** Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol.* 2002; 60: 985–1000.
11. **Benjamin LE, Hemo I, Keshet E.** A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development* 1998; 125: 1591–8.
13. **Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D.** Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest.* 2003; 111: 1287–95.
14. **Hirschi KK, Rohovsky SA, D'Amore PA.** PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate. *J Cell Biol.* 1998; 141: 805–14.
15. **Bhowmick NA, Neilson EG, Moses HL.** Stromal fibroblasts in cancer initiation and progression. *Nature* 2004; 432: 332–7.
16. **Chambers RC, Leoni P, Kaminski N, Laurent GJ, Heller RA.** Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. *Am J Pathol.* 2003; 162: 533–46.
17. **Walter-Yohrling J, Pratt BM, Ledbetter S, Teicher BA.** Myofibroblasts enable invasion of endothelial cells into three-dimensional tumor cell clusters: a novel *in vitro* tumor model. *Cancer Chemother Pharmacol.* 2003; 52: 263–9.
18. **Kunz-Schughart LA, Schroeder JA, Wondrak M, van Rey F, Lehle K, Hofstaedter F, Wheatley DN.** Potential of fibroblasts to regulate the formation of three-dimensional vessel-like structures from endothelial cells *in vitro*. *Am J Physiol Cell Physiol.* 2006; 290: C1385–98.
19. **Schlingemann RO, Rietveld FJ, Kwaspen F, van de Kerkhof PC, de Waal RM, Ruiter DJ.** Differential expression of markers for endothelial cells, pericytes, and basal lamina in the microvasculature of tumors and granulation tissue. *Am J Pathol.* 1991; 138: 1335–47.
20. **Coussens LM, Werb Z.** Inflammation and cancer. *Nature* 2002; 420: 860–7.
21. **Balkwill F, Charles KA, Mantovani A.** Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell.* 2005; 7: 211–7.
22. **Pollard JW.** Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004; 4: 71–8.
23. **De Palma M, Veneri MA, Galli R, Sergi LS, Politi LS, Sampaolesi M, Naldini L.** Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell.* 2005; 8: 211–26.
24. **Orimo A, B. GP, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AJ, Weinberg RA.** Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005; 121: 335–48.
25. **Wagers AJ, Sherwood RI, Christensen JL, Weissman IL.** Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 2002; 297: 2256–9.
26. **Shi Q, Rafii S, Wu MH, Wijelath ES, Yu C, Ishida A, Fujita Y, Kothari S, Mohle R, Sauvage LR, Moore MA, Storb RF, Hammond WP.** Evidence for circulating bone marrow-derived endothelial cells. *Blood* 1998; 92: 362–7.
27. **Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Wagner M, Isner JM, Asahara T.** Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med.* 1999; 5: 434–8.
28. **Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM.** VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 1999; 18: 3964–72.
29. **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: 826–35.
30. **Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, Rafii S.** Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med.* 2001; 7: 1194–201.
31. **Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, Fuks Z, Kolesnick R.** Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science* 2003; 300: 1155–9.
32. **Ruzinova MB, Schoer RA, Gerald W, Egan JE, Pandolfi PP, Rafii S, Manova K, Mittal V, Benezra R.** Effect of angiogenesis inhibition by Id loss and the contribution of bone-marrow-derived endothelial cells in spontaneous murine tumors. *Cancer Cell.* 2003; 4: 277–89.
33. **Li H, Gerald WL, Benezra R.** Utilization of bone marrow-derived endothelial cell precursors in spontaneous prostate tumors varies with tumor grade. *Cancer Res.* 2004; 64: 6137–43.
34. **Spring H, Schuler T, Arnold B, Hammerling GJ, Ganss R.** Chemokines direct endothelial progenitors into tumor neovessels. *Proc Natl Acad Sci USA.* 2005; 102: 18111–6.
35. **Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, Antin JH, Myerson D, Hamilton SR, Vogelstein B, Kinzler KW, Lengauer C.** Contribution of bone marrow-derived endothelial cells to human tumor vasculature. *Nat Med.* 2005; 11: 261–2.
36. **Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore**

- MA, Werb Z, Rafii S. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 2002; 109: 625–37.
37. Hammerling GJ, Ganss R. Vascular integration of endothelial progenitors during multistep tumor progression. *Cell Cycle*. 2006; 5: 509–11.
38. Vajkoczy P, Blum S, Lamparter M, Mailhammer R, Erber R, Engelhardt B, Vestweber D, Hatzopoulos AK. Multistep nature of microvascular recruitment of *ex vivo*-expanded embryonic endothelial progenitor cells during tumor angiogenesis. *J Exp Med*. 2003; 197: 1755–65.
39. Rajantie I, Ilmonen M, Alminäite A, Ozerdem U, Alitalo K, Salven P. Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. *Blood* 2004; 104: 2084–6.
40. Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G. PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. *Nat Cell Biol*. 2005; 7: 870–9.
41. Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulson R, Oukrif D, Alison MR, Wright NA. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. *Cancer Res*. 2004; 64: 8492–5.
42. 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: 789–95.
43. Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, Schaper W. Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res*. 2004; 94: 230–8.
44. Gothert JR, Gustin SE, van Eekelen JA, Schmidt U, Hall MA, Jane SM, Green AR, Gottgens B, Izon DJ, Begley CG. Genetically tagging endothelial cells *in vivo*: bone marrow-derived cells do not contribute to tumor endothelium. *Blood*. 2004; 104: 1769–77.
45. Anghelina M, Krishnan P, Moldovan L, Moldovan NI. Monocytes/macrophages cooperate with progenitor cells during neovascularization and tissue repair: conversion of cell columns into fibrovascular bundles. *Am J Pathol*. 2006; 168: 529–41.
46. Schmeisser A, Garlich CD, Zhang H, Eskafi S, Graffy C, Ludwig J, Strasser RH, Daniel WG. Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res*. 2001; 49: 671–80.
47. Zhao Y, Glesne D, Huberman E. A human peripheral blood monocyte-derived subset acts as pluripotent stem cells. *Proc Natl Acad Sci USA*. 2003; 100: 2426–31.
48. Yeh ET, Zhang S, Wu HD, Korblyng M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells *in vivo*. *Circulation*. 2003; 108: 2070–3.
49. Annabi B, Naud E, Lee YT, Eliopoulos N, Galipeau J. Vascular progenitors derived from murine bone marrow stromal cells are regulated by fibroblast growth factor and are avidly recruited by vascularizing tumors. *J Cell Biochem*. 2004; 91: 1146–58.
50. Conejo-Garcia JR, Buckanovich RJ, Benencia F, Courreges MC, Rubin SC, Carroll RG, Coukos G. Vascular leukocytes contribute to tumor vascularization. *Blood* 2005; 105: 679–81.
51. Davidoff AM, Ng CY, Brown P, Leary MA, Spurbeck WW, Zhou J, Horwitz E, Vanin EF, Nienhuis AW. Bone marrow-derived cells contribute to tumor neovasculation and, when modified to express an angiogenesis inhibitor, can restrict tumor growth in mice. *Clin Cancer Res*. 2001; 7: 2870–9.
52. Wei J, Blum S, Unger M, Jarmy G, Lamparter M, Geishauser A, Vlastos GA, Chan G, Fischer KD, Rattat D, Debatin KM, Hatzopoulos AK, Beltinger C. Embryonic endothelial progenitor cells armed with a suicide gene target hypoxic lung metastases after intravenous delivery. *Cancer Cell*. 2004; 5: 477–88.
53. Shaked Y, Bertolini F, Man S, Rogers MS, Cervi D, Foutz T, Rawn K, Voskas D, Dumont DJ, Ben-David Y, Lawler J, Henkin J, Huber J, Hicklin DJ, D'Amato RJ, Kerbel RS. Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; Implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell*. 2005; 7: 101–11.
54. Tlsty TD, Hein PW. Know thy neighbor: stromal cells can contribute oncogenic signals. *Curr Opin Genet Dev*. 2001; 11: 54–9.
55. Bissell MJ, Radisky D. Putting tumours in context. *Nat Rev Cancer*. 2001; 1: 46–54.