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EDITORIAL

Macrophages and tumor angiogenesis

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Angiogenesis plays a crucial role in the cancerogenesis, growth and progression of human solid and hematological tumors.[1,2] The passage from the preangiogenic phenotype to the angiogenic phenotype, referred to as the 'angiogenic switch', allows the formation of a neovasculature that is indispensable for tumor growth and metastatic dissemination.[3]

In 1889, the English surgeon Stephen Paget published his 'seed and soil' explanation of nonrandom pattern of metastasis, and was the first to suggest that interactions between tumor cells and host cells in the microenvironment are critical in regulating tumorigenesis.[4] Certain favored tumor cells (the 'seed'), he said, had a specific affinity for the growth-enhancing milieu within specific organs (the 'soil'), and hence metastasis only occurred when the 'seed' and 'soil' were compatible.[5] The importance of several components of the 'soil' in regulating tumor growth has since been emphasized: (1) the extracellular matrix; (2) stromal cells and their growth factors and inhibitors; (3) microvessels and angiogenic factors; and (4) inflammatory cells.

The tumor microenvironment is a complex system of many cell type, including endothelial cells and their precursors, pericytes, smooth-muscle cells, fibroblasts, neutrophils, eosinophils, basophils, mast cells, T, B and natural killer lymphocytes, and antigen-presenting cells, such as macrophages and dendritic cells, which communicate through a complex network of intercellular signaling pathways that are mediated by surface adhesion adhesion molecules, cytokines and their receptors.

Tumor angiogenesis result not only from the interaction of cancer cells with endothelial cells, but surrounding inflammatory cells have also a crucial role in directing the neoformation of blood vessels.

Macrophages are derived from CD34-positive bone marrow progenitors that continually proliferate and shed their progeny in the bloodstream as promonocytes. They then develop into monocyte and extravasate into tissues where they differentiate into a specific type of 'resident' tissue macrophage.[6] Metchnikoff was the first person in 1893 to use the term 'macrophage' to describe a large cell able to take up microorganisms.[7] The phenotype of these fully differentiated, resident macrophages can vary markedly within tissues, from that of microglial cells in the brain, Kupffer cells in the liver, alveolar macrophages in the lung and Langerhans cells in the skin. Resident macrophages share a set of common functions, including their ability to intervene against microbial infections, to regulate normal cell turnover and tissue remodeling, and to help repair sites of injury.[6]

Almost any local disturbance of tissue normality, be it infection, normal cell turnover or wounding, immune response or malignancy, caused rapid recruitment of macrophages. Recruited macrophages exhibit many phenotypic differences from resident tissue macrophages. The generic term, 'macrophages activation' is commonly used to describe this process, but the nature of an 'activated macrophage' population depends upon both the nature of the recruiting stimulus and the location.

It is now well established that the functional domain of the macrophage extends far beyond its originally recognized role as a scavenger cell. Its rich array of secretory products, anatomic diversity and functional heterogeneity is unmatched by any other cell type. As a result of this remarkable versatility, the macrophage is able to influence every facet of the immune response and inflammation as well as playing a central role in the etiology and/or pathogenesis of a number of disease processes.

Monocyte differentiated into polarized macrophage subset when exposed to different cytokine milieu.[8] In the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), lipopolysaccharide and other microbial products, monocyte differentiate into M1 macrophage. In the presence of macrophage colony-stimulating factor (M-CSF), interleukin (IL)-4, IL-13, IL-10, immunosuppressive agents (corticosteroids, vitamin D_3, prostaglandins) monocytes differentiate into M2 macrophages, involved in tumor angiogenesis.

Firstly in 1863, Rudolf Virchow noticed the infiltration of leukocytes into malignant tissues and suggested that cancers arise at regions of chronic inflammation.[9] Leukocytes that do reach the tumor often remain localized in the tumor periphery or stroma and are often not able to exert strong antitumor activity.[10] Both mouse and human tumors produce tumor-derived chemotactic factors capable of stimulating monocyte migration.[11]

Tumor-associated macrophages (TAMs) derived from circulating monocytes and are recruited at the tumor site by a tumor-derived chemotactic factor from monocytes, originally described by Bottazzi *et al.*,[12] and later identified as the chemokine CCL2/MCP-1.[13,14] When exposed to vascular endothelial growth factor (VEGF)[15] or to brief ischemia,[16] endothelial cells synthesize MCP-1 and the extent of MCP-1 expression in human cancers correlated with both TAM infiltration and tumor malignancy in human melanoma, in Kaposi sarcoma cell lines and in human tumor cell lines of epithelial origin such as breast, colon and ovary.[17] Moreover, MCP-1 is angiogenic when implanted into the rabbit cornea, where it exerted a potency similar to VEGF.[18] MCP-1 expression has been shown to correlate significantly with levels of VEGF, tumor necrosis factor-α (TNF-α) and IL-8.[19–21]

The expression of CCL5/RANTES is elevated in breast tumor cells synergistically by IFN-γ and TNF-α, regulating monocyte migration into tumor sites and stimulate them to secrete matrix metalloproteinase (MMP)-9 and MMP-19.[22,23]

CSF and GM-CSF are commonly produced in a range of different tumor types and are chemotactic for macrophages *in vitro*.[24] Transplanted mouse tumors transfected with the GM-CSF gene exhibit increased TAM infiltration[25] and genetic deletion of CSF-1 in the PyMT mouse model of breast cancer significantly decreased TAM infiltration and attenuated tumor progression to metastasis.[26] Increased expression of GM-CSF has been found in human breast, endometrial and ovarian tumors[27] and high GM-CSF expression is associated with high TAM accumulation in breast carcinomas.[28] Also VEGF is chemotactic for monocytes via VEGFR-1.[29,30]

TAM expresses and releases epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2),[31,32] transforming growth factor-α and -β (TGF-α and -β),[33–35] VEGF,[36] TNF-α[37] IL-1,[38] IL-6, IL-8,[39] platelet-activating factor[40] platelet-derived growth factor (PDGF)[41] G-CSF and GM-CSF.[42] thymidine phosphorylase[43] and chemokines, such as CCL2.[44] TAMs produce, besides, angiogenic factors, angiostatic molecules such as thrombospondin-1,[45] IL-12,[46] IL-18[47] and MMP-12.[48] TAMs can produce angiogenesis regulators and may also induce tissue remodeling by producing various proteinase activators and inhibitors that may destroy the integrity of the basement membrane and extracellular matrix, liberating matrix-bound factors, including MMP-2, MMP-9, MMP-12 and cyclooxygenase-2 (COX-2).[49–51] TAM production of MMP-9 has been shown to be crucial for angiogenesis in a human papillomavirus-16-induced model of cervical carcinogenesis. In this model, inhibition of MMP-9 in macrophages blocked the release of VEGF and thereby inhibited angiogenesis and tumor growth.[52] Inhibition of CSF-1 function in human tumors xenografted into immunocompromised mice reduced their growth and this was correlated with poor macrophage recruitment and reduced angiogenesis due to a depletion of VEGF.

TAMs accumulate in hypoxic regions of tumors and hypoxia triggers a proangiogenic program in these cells. TAM adaptation to hypoxia, which is achieved by the increased expression of hypoxia-inducible and proangiogenic genes, such as VEGF, FGF-2 and CXCL8, as well as glycolytic enzymes, whose transcriptions are controlled by the transcription factors hypoxia-inducible factors 1 and 2 (HIF-1 and -2).[53] Increased number of TAMs in hypoxic regions may promote tumor progression in part by stimulating levels of angiogenesis, such as occurring in breast carcinoma.[54]

White *et al*.[55] used adenoviral infection to overexpress HIF-2 in human macrophages and found it to be the primary inducer of genes encoding angiogenic cytokines in these genes. Macrophages also upregulates VEGF and other proangiogenic factors in response to hypoxia. TAMs express VEGF almost exclusively in avascular and perinecrotic areas of human breast carcinoma.[56]

Macrophages also synthesize increased levels of MMP-7 when exposed to hypoxia *in vitro* and in an avascular area of human tumors.[57] MMP-7 is known to stimulate endothelial cell proliferation and migration.[58]

A complementary DNA array study has identified upregulation of messenger encoding >30 proangiogenic genes in primary macrophages exposed to hypoxia, including CXCL8, angiopoietin, COX-2 and other factors.[55]

Bingle *et al*.[59] found that when macrophages are co-cultured *in vitro* with human tumor spheroids, they infiltrate deep into the central, hypoxic areas of these structures. The release of VEGF by macrophage-infiltrated spheroids was significantly higher than that seen for noninfiltrated spheroids. This increase translated into a significant stimulation of angiogenesis *in vivo* when implanted into microcirculation window chambers on the flags of nude mice for 3 days.[59]

Evans[60,61] has shown that mice depleted of macrophages by whole-body X-irradiation or azathioprine administered before or after implantation of a syngeneic fibrosarcoma showed a delay in the appearance of tumors, and a marked reduction in tumor vascularization. Mostafa *et al*.[62,63] and Stenzinger *et al*.[64] showed that vascularization of several human tumor cell lines grown on the chorioallantoic membrane of the chick embryo or subcutaneously in nude mice occurred coincidentally with mononuclear cell infiltration at the tumor site.

Polverini and Leibovich[65] isolated macrophages from a transplantable rat fibrosarcoma and examined them and their serum-free conditioned media for angiogenic activity in rat corneas. Results showed that TAM and their conditioned media were potently angiogenic *in vivo* and stimulated proliferation of bovine aortic endothelial cells in culture. Moreover, when TAMs were combined with tumor cells at a concentration equivalent to the number of macrophages originally present in the tumor, there was a marked enhancement of tumor neovascularization and growth. Polverini and Leibovich[66] reported that hamsters bearing chemical carcinogen-induced squamous-cell carcinomas showed a marked reduction in the thymidine incorporation by endothelial cells and neovascularization of tumors when treated with low doses of steroids and anti-macrophage serum.

In the mouse model of breast cancer caused by the mammary epithelial cell restricted expression of the Polyoma middle T oncoprotein (PyMT mice) infiltration of TAM in primary tumors is positively associated with tumor progression to malignancy.[26] Depletion of macrophages in this model severely delayed tumor progression and reduced metastasis, whereas an increase in macrophage infiltration remarkably accelerated these processes. By using the PyMT-induced mouse mammary tumors, Lin *et al*.[67] have characterized the development of the vasculature in mammary tumors during their progression to malignancy. They have shown that both angiogenic switch and the progression to malignancy are regulated by infiltrated macrophages in the primary mammary tumors. Moreover, inhibition of the macrophage infiltration into the tumor delayed the angiogenic switch and malignant transition, whereas genetic reduction of the macrophage population specifically in these tumors rescued the vessel phenotype. Finally, premature induction of macrophage infiltration into premalignant lesions promoted an early onset of the angiogenic switch independent of tumor progression.[67]

De Palma *et al*.[68] have shown that a subset of monocytes that express the angiopoietin receptor Tie-2 are inducers of angiogenesis in both spontaneous and orthotopic tumor models. Knockout of these Tie-2-expressing cells *in vivo* markedly reduced angiogenesis in human glioma xenografts and prompted tumor regression.

A relationship between the macrophage content of tumors, the rate of tumor growth and the extent of their vascularization has been shown in several tumors, including breast carcinoma where TAM presence focally in large numbers correlates with a high level of angiogenesis and with poor prognosis, decreased relapse-free and overall survival of the patients,[69,70] malignant uveal melanoma,[71] glioma,[72] squamous-cell carcinoma of the esophagus,[73] bladder carcinoma[74] and prostate carcinoma.[75] In lung cancer, TAM may favor tumor progression by contributing to stroma formation and angiogenesis through their release of PDGF in conjunction with TGF-β-1 production by cancer cells.[76]

Monocytes/macrophages display a high degree of plasticity, as shown by their ability to transdifferentiate into endothelial cells *in vitro* and *in vivo*.[77–85] $CD14^+$ mononuclear cells have been used as the starting population for cultivation of endothelial progenitor cells (EPCs).[77] Cultivated EPC grown from different starting populations, including peripheral blood mononuclear cells, have been shown to express endothelial markers such as von Willebrand factor, VEGFR-2, VE-cadherin, CD156 and CD31.[86] Monocytes coexpress endothelial lineage markers such as VEGFR-2 and AC133 and have the capacity to differentiate into adherent endothelial cells and to form cord-like structures in Matrigel.[80,87]

Kamihata *et al*.[88] and Shintani *et al*.[89] have shown that bone marrow mononuclear cells not only contain EPC but also

angiogenic factors and cytokines and that implantation of bone marrow mononuclear cells into ischemic tissues augments collateral vessel formation.

Elsheikh *et al*.[81] have shown that peripheral blood monocytes $CD14^+$ and VEGFR-2^+ exhibited the potential to differentiate *in vitro* into cells with endothelial characteristics. Moreover, these cells transduced by a lentiviral vector driving expression of green fluorescent protein (GFP) and transplantation of these cells into ballon-injured femoral arteries of nude mice significantly contributed to efficient re-endothelialization. Rehman *et al*.[78] reported that peripheral-blood endothelial-like cells are derived from monocytes/macrophages and secrete angiogenic factors.

Maniotis *et al*.[90] described a new model of formation of vascular channels by human melanoma cells and called it 'vasculogenic mimicry' to emphasize the *de novo* generation of blood vessels without the participation of endothelial cells and independent of angiogenesis. The word 'vasculogenic' was selected to indicate the generation of the pathway *de novo* and 'mimicry' was used because tumor cell pathways for transporting fluid in tissues were clearly not blood vessels.

Recently, we have demonstrated that multiple myeloma bone marrow TAM exposed to VEGF and FGF-2 develop a number of phenotypic properties similar to those of paired bone marrow endothelial cells, and form capillary-like structures overlapping morphologically those produced by endothelial cells.[91] At ultrastructural level, multiple myeloma TAMs exhibit numerous cytoplasmic extroversions arranged in tube-like structures and these data suggest that TAMs contribute to build neovessels in multiple myeloma through a vasculogenic mimicry.

TAMs can influence angiogenesis by releasing angiogenic cytokines directly, or indirectly by secreting extracellular matrix-degrading enzymes that release angiogenic factors that have been sequestered by the matrix. TAMs are found in abundance in many tumor types and once the monocytes have entered the tumor environment they are activated by factors peculiar to the tumor microenvironment and migrate toward the areas of hypoxia perhaps following an oxygen gradient or chemokines released by surrounding tumor cells or other inflammatory cells. TAMs then release factors that initiate angiogenesis into the area and their production of extracellular matrix-degrading enzymes facilitates the growth of endothelial cells and loosens the fibrous network of the extracellular matrix thus allowing potentially metastatic tumor cells increased mobility.

With the seemingly central role that TAM could play in tumor angiogenesis, the macrophage itself becomes an appealing target for future antiangiogenic therapeutic strategies through two approaches: (1) compounds that suppress secretion of angiogenic substances by macrophages; and (2) compound that inhibit macrophage infiltration into the tumor mass.

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References

1 Ribatti D, Vacca A, Dammacco F. The role of vascular phase in solid tumor growth: a historical review. *Neoplasia* 1999; **1**: 293–302.
2 Vacca A, Ribatti D. Bone marrow angiogenesis in multiple myeloma. *Leukemia* 2006; **20**: 193–199.
3 Ribatti D, Nico B, Crivellato E, Roccaro AM, Vacca A. The history of the angiogenic switch concept. *Leukemia* 2007; **21**: 44–52.
4 Paget S. The distribution of secondary growths in cancer of the breast. *Lancet* 1889; **i**: 571–573.
5 Ribatti D, Mangialardi G, Vacca A. Stephen Paget and the 'seed and soil' theory of metastatic dissemination. *Clin Exp Med* 2006; **6**: 145–149.
6 Ross JA, Auger MJ. The biology of the macrophage. In: Nurke B, Lewis CE (eds), *The Macrophage*, 2nd edn. Oxford University Press: Oxford, 2002, pp 45–77.
7 Tauber AI, Chernyak L. *Metchnikoff and the Origins of Immunology: From Metaphor to Theory. Monographs on the History and Philosophy of Biology*. Oxford University Press: New York, 1991, pp 1–155.
8 Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. *Eur J Cancer* 2006; **42**: 717–727.
9 Virchow R. *Die Krankenaften Geschwulste*. August Hirschwald: Berlin, 1863, pp 1–255.
10 Dvorak HF. Tumors: wounds than not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986; **315**: 1650–1659.
11 Graves DT, Valente AJ. Monocyte chemotactic proteins from human tumor cells. *Biochem Pharmacol* 1991; **41**: 331–337.
12 Bottazzi B, Polentarutti N, Acero R, Balsari A, Boraschi D, Ghezzi P *et al*. Regulation of the macrophage content of neoplasms by chemoattractants. *Science* 1983; **220**: 210–212.
13 Matsushima K, Larsen CG, Dubois GC, Oppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J Exp Med* 1999; **169**: 1485–1490.
14 Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J, Leonard EJ. Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. *J Exp Med* 1989; **169**: 1449–1459.
15 Marumo T, Schini-Kert VB, Busse R. Vascular endothelial growth factor activates nuclear factor-kappa B and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells. *Diabetes* 1999; **48**: 1131–1137.
16 Lakshminarayanan V, Lewallen M, Frangogiannis NG, Evans AJ, Wedin KE, Michael LH *et al*. Reactive oxygen intermediates induce monocyte chemotactic protein-1 in vascular endothelium after brief ischemia. *Am J Pathol* 2001; **159**: 1301–1311.
17 Ueno T, Toi M, Saji H, Muta M, Bando H, Kuroi K *et al*. Significance of macrophages chemoattractant protein-1 in macrophage recruitment, angiogenesis and survival in human breast cancer. *Clin Cancer Res* 2000; **6**: 3282–3289.
18 Goede V, Brogelli L, Ziche M, Augusting HG. Induction of inflammatory angiogenesis by monocyte chemoattractant protein-1. *Int J Cancer* 1999; **82**: 765–770.
19 Bingle L, Brown NJ, Lewis CE. The role of tumor-associated macrophages in tumor progression: implications for new anticancer therapies. *J Pathol* 2002; **196**: 254–265.
20 Varney ML, Olsen KJ, Mosley RL, Bucana CD, Talmadge JE, Singh RK. Monocyte/macrophage recruitment, activation and differentiation modulate interleukin-8 production: a paracrine role of tumor-associated macrophages in tumor angiogenesis. *In Vivo* 2002; **16**: 471–477.
21 Liss C, Fekete MJ, Hasina R, Lam CD, Lingen MW. Paracrine angiogenic loop between head-and-neck squamous-cell carcinomas and macrophages. *Int J Cancer* 2001; **93**: 781–785.

22 Locati M, Deuschle U, Massardi ML, Martinez FO, Sironi M, Sozzani S *et al*. Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and the lipopolysaccharide in human monocytes. *J Immunol* 2002; **168**: 3557–3562.
23 Aznenshtein E, Luboshits G, Shina S, Neumark E, Shabbazian D, Weil M *et al*. The CC chemokine RANTES in breast carcinoma progression: regulation of expression and potential mechanisms of promalignant activity. *Cancer Res* 2002; **62**: 1093–1102.
24 Dorsch M, Hock H, Kunzendorf U, Diamantstein T, Blankenstein T. Macrophage colony-stimulating factor gene transfer into tumor cells induces macrophage infiltration but not tumor suppression. *Eur J Immunol* 1993; **23**: 186–190.
25 Heike Y, Sone S, Yano S, Seimiya H, Tsuruo T, Ogura T. M-CSF gene transduction in multidrug-resistant human cancer cells to enhance anti-P-glycoprotein antibody-dependent macrophage-mediated cytotoxicity. *Int J Cancer* 1993; **54**: 851–857.
26 Lin EY, Nguyen AV, Russell RG, Polard JW. Colony-stimulating factor-1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001; **193**: 727–740.
27 Kacinski BM. CSF-1 and its receptor in ovarian, endometrial and breast cancer. *Ann Med* 1995; **27**: 79–85.
28 Tang R, Beuvon F, Ojeda M, Mossseri V, Pouillart P, Lidereau R. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *J Natl Cancer Inst* 1994; **86**: 120–126.
29 Clauss M, Gerlach M, Gerlach H, Brett J, Wang F, Familletti PC *et al*. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J Exp Med* 1990; **172**: 1535–1545.
30 Leek RD, Hunt NC, Landers RJ, Lewis CE, Royds JA, Harris AL. Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. *J Pathol* 2000; **190**: 430–436.
31 Baird A, Mormède P, Bohlen P. Immunoreactive fibroblast growth factor in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. *Biochem Biophys Res Commun* 1985; **126**: 358–364.
32 Joseph-Silverstein J, Moscatelli D, Rifkin DB. The development of quantitative RIA for basic fibroblast growth factor using polyclonal antibodies against the 157 amino acid form of human bFGF. The identification of bFGF in adherent elicited murine peritoneal macrophages. *J Immunol Methods* 1988; **110**: 183–192.
33 Matdess DK, Raines EW, Sakariassen KS, Assoian AK, Sporn MB, Bell GI *et al*. Induction of transforming growth factor-α in activated human alveolar macrophages. *Cell* 1988; **53**: 285–293.
34 Rappolle DA, Mark D, Banda MJ, Werb Z. Wound macrophages express TGF-α and other growth factors *in vivo*: analysis by mRNA phenotyping. *Science* 1988; **241**: 708–712.
35 Assoian RK, Fleurdelys BE, Stevenson HC, Miller PJ, Matdes DK, Raines EW *et al*. Expression and secretion of type *β* transforming growth factor by activated human macrophages. *Proc Natl Acad Sci USA* 1987; **84**: 6020–6024.
36 Berse B, Brown LF, Van de Water L, Dvorak HF, Senger DR. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. *Mol Biol Cell* 1992; **3**: 211–220.
37 Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nusier N. Macrophages-induced angiogenesis is mediated by tumor necrosis factor-α. *Nature* 1987; **320**: 584–588.
38 Roberts NJ, Prill Jr AH, Mann TN. Interleukin 1 and interleukin 1 inhibitor production by human macrophages exposed to influenza virus or respiratory syncytial virus. Respiratory syncytial virus is a potent inducer of inhibitory activity. *J Exp Med* 1986; **163**: 511–519.
39 Koch AE, Polverini PJ, Kunkel SL, Harlow LA, Di Petro LA, Elner VM *et al*. Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* 1992; **258**: 1798–1801.
40 Seo KH, Ko HM, Choi JH, Jung HH, Chun YH, Choi IW *et al*. Essential role for platelet-activating factor-induced NF-κB activation in macrophage-derived angiogenesis. *Eur J Immunol* 2004; **34**: 2129–2137.
41 Martinet Y, Bitterman PB, Mornex JF, Grotendorst GR, Martin GR, Crystal RG. Activated human monocytes express the c-sis proto-oncogene and release a mediator showing PDGF-like activity. *Nature* 1986; **319**: 158–160.
42 Sullivan R, Gans PJ, Mc Carroll LA. The synthesis and secretion of granulocyte-monocyte colony-stimulating activity (CSA) by isolated human monocytes: kinetics of the response to bacterial endotoxin. *J Immunol* 1982; **130**: 800–807.
43 Hotchkiss KA, Ashton AW, Klein RS, Lenzi ML, Zhu GH, Schwartz EL. Mechanisms by which tumor cells and monocytes expressing the angiogenic factor thymidine phosphorylase mediate human endothelial cell migration. *Cancer Res* 2003; **63**: 527–533.
44 Vicari AP, Caux C. Chemokines in cancer. *Cytokine Growth Factor Rev* 2002; **13**: 143–154.
45 Di Pietro LA, Polverini PJ. Angiogenic macrophages produce the angiogenic inhibitor thrombospondin-1. *Am J Pathol* 1993; **143**: 678–684.
46 Inoue Y, Najkayama Y, Mingawa N, Katsuki T, Nagashima N, Matsumoto K *et al*. Relationship between interleukin-12-expressing cells and antigen-presenting cells in patients with colorectal cancer. *Anticancer Res* 2005; **25**: 3541–3546.
47 Belardelli F, Ferrantini M. Cytokines as a link between innate and adaptive antitumor immunity. *Trends Immunol* 2002; **23**: 201–208.
48 Kerkela E, Ala-Aho R, Jeskanen L, Rechardt O, Grenman R, Shapiro SD *et al*. Expression of human macrophage metalloelastase (MMP-12) by tumor cells in skin cancer. *J Invest Dermatol* 2000; **114**: 1113–1119.
49 Sunderkotter C, Goebeler M, Schulze-Osthoff K, Bhardwaj R, Sorg C. Macrophage-derived angiogenesis factor. *Pharmacol Ther* 1991; **51**: 195–216.
50 Lewis CE, Leek R, Harris A, Mc Gee JO. Cytokine regulation of angiogenesis in breast cancer: the role of tumor-associated macrophages. *J Leukoc Biol* 1995; **57**: 747–751.
51 Klimp AH, Hollema H, Kempinga C, van AG, de Vries EG, Daemen T. Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. *Cancer Res* 2001; **61**: 7305–7309.
52 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.
53 Talks KL, Tyrley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ *et al*. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* 2000; **157**: 411–421.
54 Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer* 1999; **79**: 991–995.
55 White JR, Harris RA, Lee SR, Craigon MH, Binley K, Price T *et al*. Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. *Genomics* 2004; **83**: 1–8.
56 Lewis JS, Landers RJ, Underwood JC, Harris AL, Lewis CE. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. *J Pathol* 2000; **192**: 150–158.
57 Burke B, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L *et al*. Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. *Am J Pathol* 2003; **163**: 1233–1243.
58 Nishizuka I, Ichikawa Y, Ichikawa T, Kamiyama M, Hasegawa S, Momiyama N *et al*. Matrylisin stimulates DNA synthesis of cultured vascular endothelial cells and induce angiogenesis *in vivo*. *Cancer Lett* 2001; **173**: 175–182.
59 Bingle L, Lewis CE, Corke K, Reed MW, Brown NJ. Macrophages promote angiogenesis in human breast tumour spheroids *in vivo*. *Br J Cancer* 2005; **94**: 101–107.
60 Evans R. Effect of X-irradiation on host cell infiltration and growth of murine fibrosarcoma. *Br J Cancer* 1977; **35**: 557–566.
61 Evans R. The effect of azathioprine on host-cell infiltration and growth of a murine fibrosarcoma. *Int J Cancer* 1977; **20**: 120–128.
62 Mostafa LK, Jones DB, Wright DH. Mechanism of the induction of angiogenesis by human neoplastic lymphoid tissue: studies on the chorioallantoic (CAM) of the chick embryo. *J Pathol* 1980; **132**: 91–105.
63 Mostafa LK, Jones DB, Wright DH. Mechanism of the induction of angiogenesis by human neoplastic lymphoid tissue: studies

employing bovine aortic endothelial cells *in vitro*. *J Pathol* 1980; **132**: 207–216.
64 Stenzinger W, Bruggen J, Macher E, Sorg C. Tumor angiogenic activity (TAA) production *in vitro* and growth in the nude mice by human malignant melanoma. *Eur J Cancer Clin Oncol* 1982; **19**: 649–656.
65 Polverini PJ, Leibovich SJ. Induction of neovascularization *in vivo* and endothelial proliferation *in vitro* by tumor associated macrophages. *Lab Invest* 1984; **51**: 635–642.
66 Polverini PJ, Leibovich SJ. Effect of macrophage depletion on growth and neovascularization of hamster buccal pouch carcinomas. *J Oral Pathol* 1987; **16**: 436–441.
67 Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA *et al.* Macrophage regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006; **66**: 11238–11246.
68 De Palma M, Venneri MA, Galli R. 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.
69 Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996; **56**: 4625–4629.
70 Leek RD, Harris AL. Tumor-associated macrophages in breast cancer. *J Mammary Gland Biol Neoplasia* 2002; **7**: 177–189.
71 Makitie T, Summanen P, Tarkkanen A, Kivela T. Tumor infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma. *Invest Ophthalmol Vis Sci* 2001; **42**: 1414–1421.
72 Nishie A, Ono M, Shono T, Fukushi J, Otsubo M, Onoue H *et al.* Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. *Clin Cancer Res* 1999; **5**: 1107–1113.
73 Koide N, Nishio A, Sato T, Sugiyama A, Miyagawa S. Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. *Am J Gastroenterol* 2004; **99**: 1667–1674.
74 Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. *Int J Urol* 2000; **7**: 263–269.
75 Lissbrandt IF, Stattin P, Wilkstrom P, Damber JE, Egevad L, Bergh A. Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. *Int J Oncol* 2000; **17**: 445–451.
76 Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002; **23**: 549–555.
77 Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML *et al.* Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* 2000; **65**: 287–300.
78 Rehman J, Li J, Orschell CM, March KL. Peripheral blood 'endothelial progenitor cells' are derived from monocytes/macrophages and secrete angiogenic growth factors. *Circulation* 2003; **107**: 1164–1169.
79 Urbich C, Heeschen C, Aicher A, Dernbach E, Zeiher AM, Dimmeler S. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation* 2003; **108**: 2511–2516.
80 Schmeisser A, Garlichs CD, Zhang H, Eskafi S, Graffy C, Ludwig J *et al.* Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res* 2001; **49**: 671–680.
81 Elsheikh E, Uzunel M, He Z, Holgersson J, Nowak G, Sumitran-Holgersson S. Only a specific subset of human peropheral-blood monocytes has endothelial-like functional activity. *Blood* 2005; **106**: 2347–2355.
82 Fujiyama S, Amano K, Uehira K, Yoshida M, Nishiwaki Y, Nozawa Y *et al.* Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate neoendothelization as endothelial progenitor cells. *Circ Res* 2003; **93**: 980–989.
83 Iba O, Matsubara H, Nozawa Y, Fujiyama S, Amano K, Mori Y *et al.* Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. *Circulation* 2002; **106**: 2019–2025.
84 Nowak G, Karrar A, Holmen C, Nava S, Uzunel M, Hultenby K *et al.* Expression of vascular endothelial growth factor receptor-2 or Tie-2 on peripheral blood cells defines functionally competent populations capable of reendothelialization. *Circulation* 2004; **110**: 3699–3702.
85 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**: 2426–2431.
86 Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M *et al.* Transplantation of *ex vivo* expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci USA* 2000; **97**: 3422–3427.
87 Schmeisser A, Graffy C, Daniel WG, Strasser RH. Phenotypic overlap between monocytes and vascular endothelial cells. *Adv Exp Med Biol* 2003; **522**: 59–74.
88 Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R *et al.* Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001; **104**: 1046–1052.
89 Shintani S, Murohara T, Ikeda H, Ueno T, Sasaki K, Duan J *et al.* Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001; **103**: 895–897.
90 Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LM, Peer J *et al.* Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am J Pathol* 1999; **155**: 739–752.
91 Scavelli C, Nico B, Cirulli T, Ria R, Di Pietro G, Mangieri D *et al.* Vasculogenic mimicry by bone marrow macrophages in patients with multiple myeloma. *Oncogene* 2007 (accepted for publication).